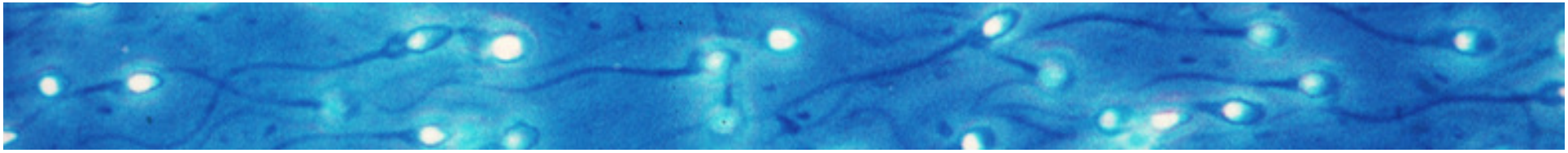

# Role of pre and post-surgical sperm cryopreservation


## Sheena E. M. Lewis

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**s.e.lewis@qub.ac.uk**

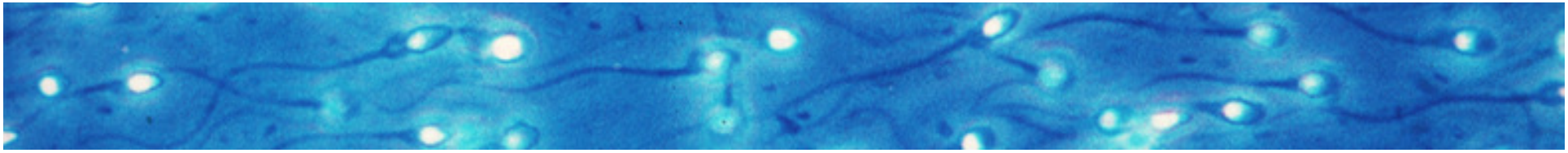

Queen's University Belfast


***ESHRE, Treviso, Italy, Oct 2010***

# Uses of Sperm Cryopreservation

## Freezing provides people with future fertility potential

- **Pre-treatment for Cancer**
- **Pre- operative  or vasectomy insurance**
- **Post surgical retrieval of testicular sperm for ART**



# 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)***

# Alterations in plasma and mitochondrial membrane potentials

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

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

# 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)*

# Cryopreservation and Genome

- **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**
  *(de Paula et al, 2006; Gandini et al, 2006; Amann et al, 1999 Ward et al, 2004, Evenson et al, 1991-2010)*

# 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

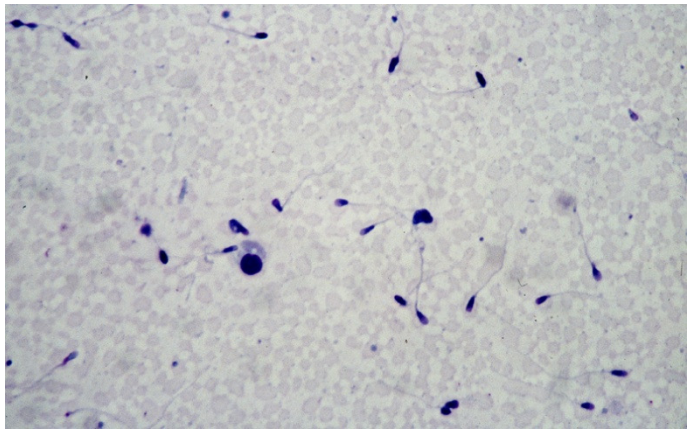
*(Watson, 1995)*

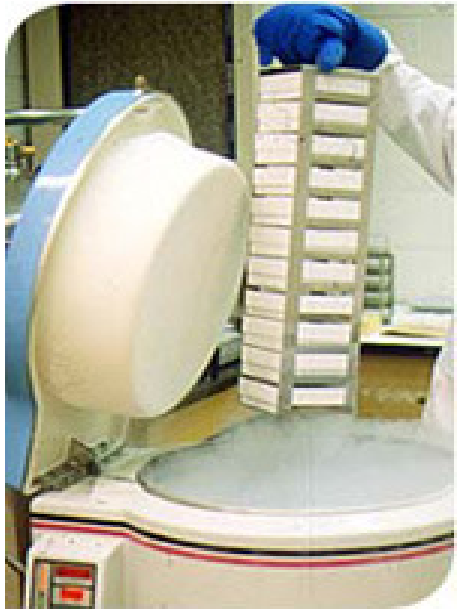
## Not during storage

- 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)*

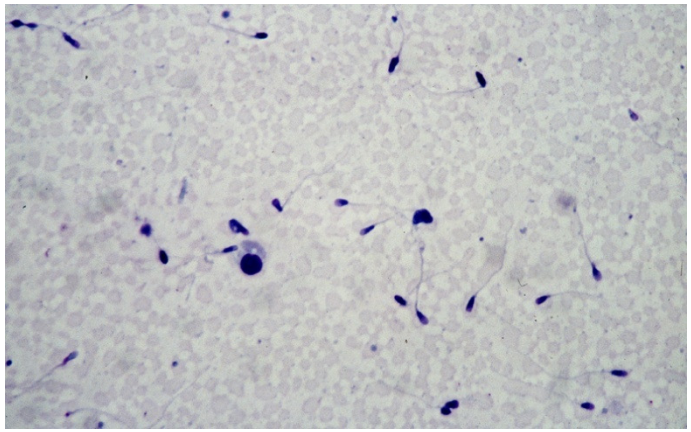
- **IVF and ICSI - ↓ FR, IR, CPR**

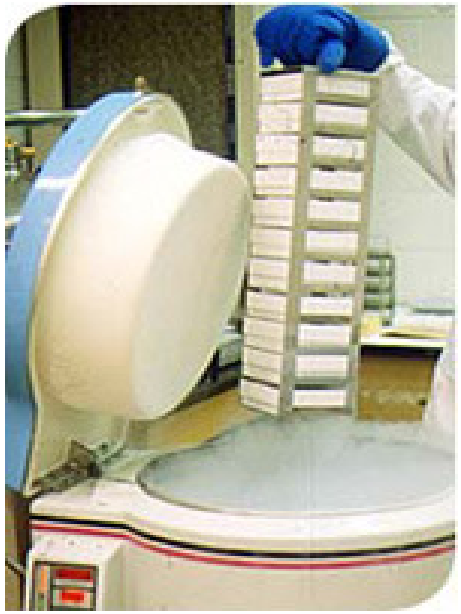
  *(Critser et al, 1987; Crabbe et al, 1999; Hammadeh et al, 1999)*

- **Conversely-**
- **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





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# Comparison of the effects of two methods of cryopreservation on testicular sperm DNA

→ **No difference in sperm DNA fragmentation when f-t in suspension or in biopsy**

*E. Kristine Steele, B.Sc., M.R.C.O.G.,[a] Neil McClure, M.D., M.R.C.O.G.,[a,b] and Sheena E. M. Lewis, E.M., Ph.D.[a]*

*The Queen's University of Belfast, Institute of Clinical Science, Belfast, Northern Ireland*

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RBMOnline - Vol 7. No 4. 449–455 Reproductive BioMedicine Online; www.rbmonline.com/Article/957 on web 8 September 2003

Article

## Effects of cryopreservation on testicular sperm nuclear DNA fragmentation and its relationship with assisted conception outcome following ICSI with testicular spermatozoa

Dr Sheena Lewis is Reader in Obstetrics and Gynaecology at Queen's University of Belfast in Northern Ireland. She is also Scientific Director of the Andrology Laboratory at The Royal Maternity Hospital in Belfast. She has published more than 60 research papers in the field of male infertility and has been invited to speak around the world. She reviews for many of the major journals and funding bodies in her field. Her special interests are the influences of sperm nuclear and mitochondrial DNA on assisted reproductive outcome, cryopreservation and antioxidant status, comparisons of semen profiles and assault susceptibility of spermatozoa from fertile and infertile men.

*Dr Sheena Lewis*

MEM Thompson-Cree[1], Neil McClure[1,2], Eilish T Donnelly[1], Kristine E Steele[1], Sheena EM Lewis[1,3]
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[3]Correspondence: Tel: +44 28 90633987; Fax: +44 28 90328247; e-mail: s.e.lewis@qub.ac.uk

**Table 1.** Effects of freeze–thawing on sperm DNA fragmentation. The test for homogeneous subsets drew a clear distinction between the group that was plunge frozen and then thawed at room temperature.

| *Group* | n | *DNA fragmentation (%)* | *P-value (group versus fresh)* |
|---|---|---|---|
| Fresh | 76 | 13.94 ± 0.86 | n/a |
| Plunge freeze–thaw at RT | 56 | 17.80 ± 0.89 | <0.001 |
| Plunge freeze–thaw at 37°C | 34 | 14.51 ± 0.78 | NS |
| Programmed freeze–thaw at RT | 35 | 14.34 ± 0.91 | NS |
| Programmed freeze–thaw at 37°C | 37 | 14.48 ± 0.73 | NS |

NS = not significant; n/a = not applicable.

**Table 2.** Relationship between DNA fragmentation and assisted conception success.

| | *Successful cycles* | *Unsuccessful cycles* | *P-value* |
|---|---|---|---|
| *n* | 11 | 23 | |
| DNA fragmentation (%) | 11.28 ± 1.30[a] | 18.44 ± 1.96[a] | <0.001 |

[a]Significantly different.

**Table 3.** Effects of freeze–thawing of spermatozoa on assisted conception success.

| | *Fresh* *Successful outcome* | *Unsuccessful outcome* | *Freeze–thawed* *Successful outcome* | *Unsuccessful outcome* | *P-value (fresh versus freeze–thawed)* |
|---|---|---|---|---|---|
| Fertilization rate | 0.75 ± 0.04 (*n* = 14) | 0.80 ± 0.03 (*n* = 32) | 0.70 ± 0.05 (*n* = 11) | 0.66 ± 0.04 (*n* = 31) | NS |
| Blastomere no. at embryo transfer | 5.5 ± 0.5 | 5.7 ± 0.3 | 6.7 ± 0.8 | 5.5 ± 0.3 | NS |
| Cumulative embryo score | 39.1 ± 4.2 (*n* = 11) | 35.6 ± 3.2 (*n* = 27) | 44.3 ± 5.5 (*n* = 11) | 35.7 ± 2.5 (*n* = 23) | NS |
| Clinical pregnancy rate | 30% (*n* = 14) | 70% (*n* = 32) | 26% (*n* = 11) | 74% (*n* = 31) | NS |
| Take-home babies | 6 boys; 2 girls; 3 sets of twin boys | | 6 boys; 3 girls; 1 set of twin girls | | NS |

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

- **FR, IR and CPR significantly reduced**

  *(Nicopoullos et al, 2003,Dr Croo et al, 1998; Wood et al, 2002)*

- **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;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**

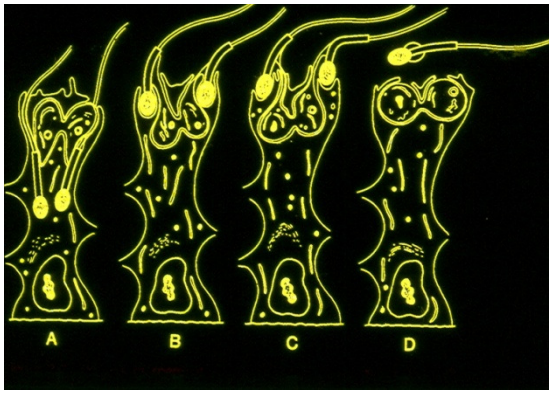
  (*meta- analysis Nicopoullos et al, 2004)*

- **↓ 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




A testicular sperm

Compaction of DNA by crosslinkage in epididymis

An ejaculated sperm

# Overnight incubation of post-thaw testicular sperm

- routine clinical practice
- reasons: convenient

  non invasive

  quick viability test

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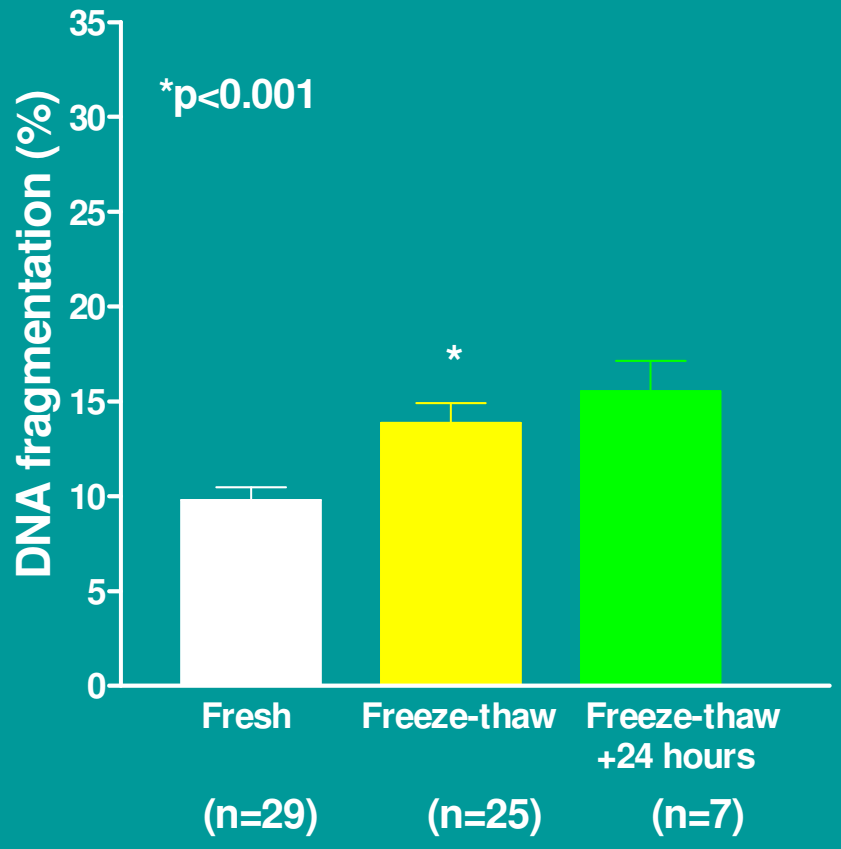
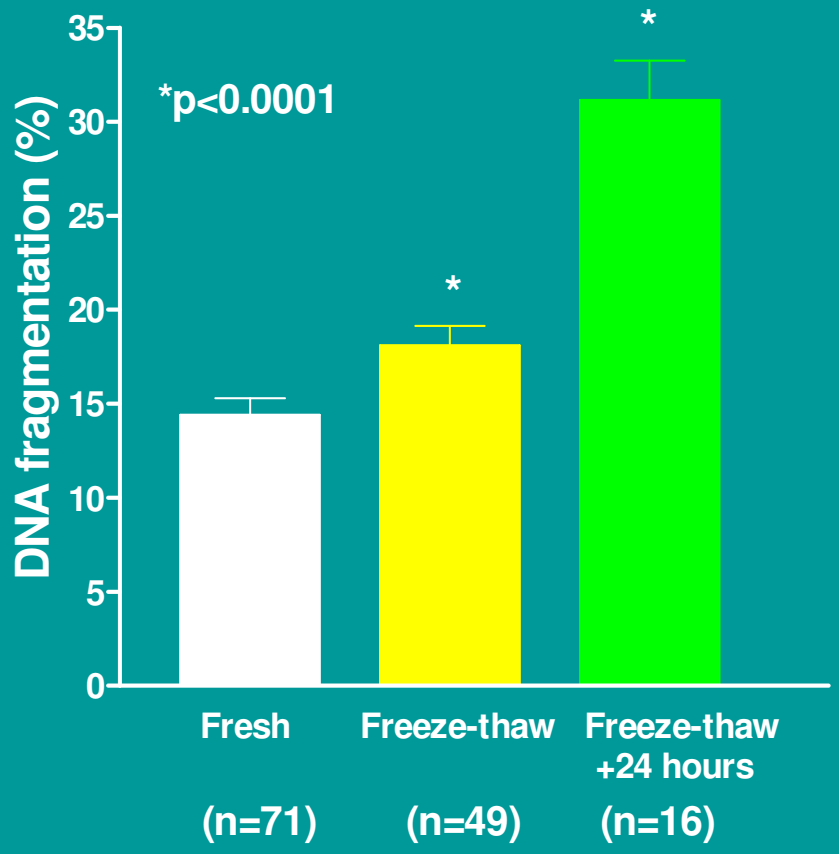
VOL. 79, SUPPL. 3, JUNE 2003


# Effects of 24-hour incubation after freeze-thawing on DNA fragmentation of testicular sperm from infertile and fertile men

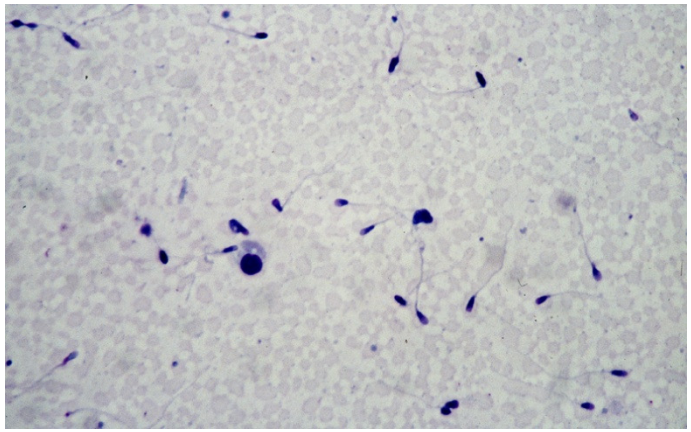
## Fertile

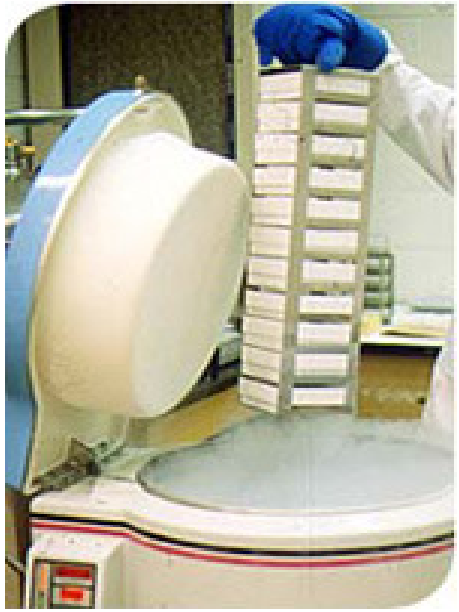



## Infertile



# A better way to freeze sperm





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Vol. 67, No. 1, January 1997
Printed on acid-free paper in U. S. A.

# Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men

Sheena E. M. Lewis, Ph.D.*†
E. Samantha L. Sterling, M.Med.Sci*
Ian S. Young, M.D.‡
William Thompson, M.D.*

*The Queen's University of Belfast, Belfast, Northern Ireland, United Kingdom*

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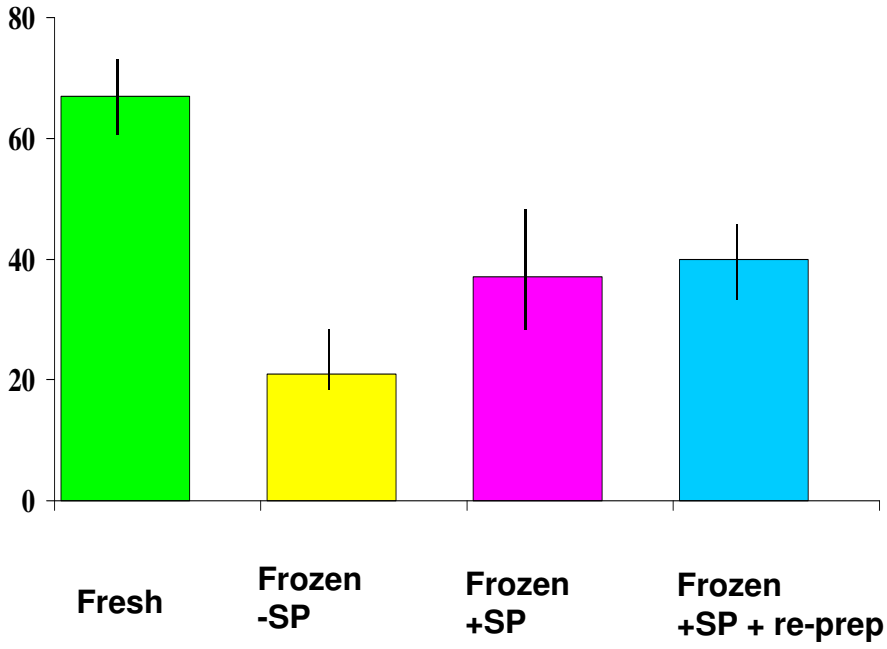
# Cryopreservation of human semen and prepared sperm: effects on motility parameters and DNA integrity

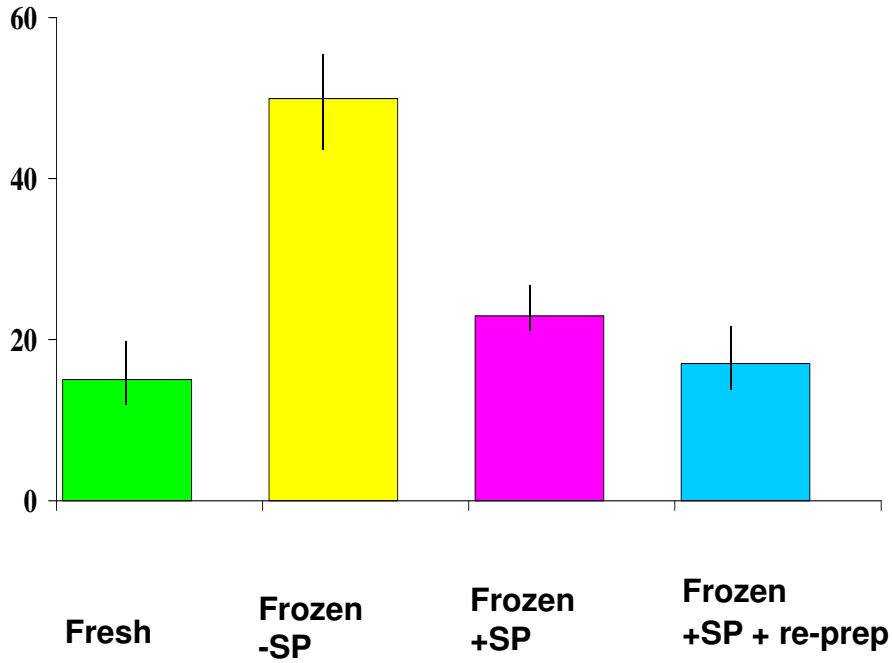
*Eilish T. Donnelly, Ph.D., Neil McClure, M.D., and Sheena E. M. Lewis, Ph.D.*

*Department of Obstetrics and Gynaecology, The Queen's University of Belfast, Belfast, Northern Ireland*

## Progressive motility (%)




## DNA fragmentation (%)



# 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)*

# Vitrification or Ultra Rapid Freezing

- Process involves solidifying liquids without crystallization
- Blastocysts and births from slow rate frozen embryos from vitrified oocytes with rapid plunge frozen sperm

***Merlo et al, 2008; Wang et al, 2008; Chen et al, 2008***

- Embryo cryopreservation by vitrification

***Shaw et al, 1991; Stehlik et al, 2005; Zhou et al, 2005, Libermann and Tucker, 2004;***

in $LiqN_2$

*Yavin et al, 2008*

- Semen Vitrification > 30 000$^0$C/min

v <100$^0$C/min vapour and $LiqN_2$ plunge

# Vitrification of Sperm

Rapidly cooled human sperm: no evidence of intracellular ice formation

***GJ Morris, 2006***

Acromosomal status and mitochondrial activity of human sperm vitrified with sucrose

***Isachenko et al, 2008***

Human Reproduction Vol.19, No.4 pp. 932–939, 2004
Advance Access publication March 11, 2004
DOI: 10.1093/humrep/deh194

# DNA integrity and motility of human spermatozoa after standard slow freezing versus cryoprotectant-free vitrification

**E.Isachenko[1,5], V.Isachenko[2], I.I.Katkov[3], G.Rahimi[1], T.Schöndorf[1], P.Mallmann[1], S.Dessole[4] and F.Nawroth[1]**

[1]Department of Obstetrics and Gynecology, University of Cologne, Kerpener Str. 34, D-50931 Cologne, [2]Department of Gynecological Endocrinology and Reproductive Medicine, University of Bonn, Bonn, Germany, [3]Cancer Center, University of California at San Diego, La Jolla, CA, USA and [4]Department of Obstetrics and Gynecology, University of Sassari, Sassari, Italy

[5]To whom correspondence should be addressed. E-mail: jeniaisachenko@yahoo.de

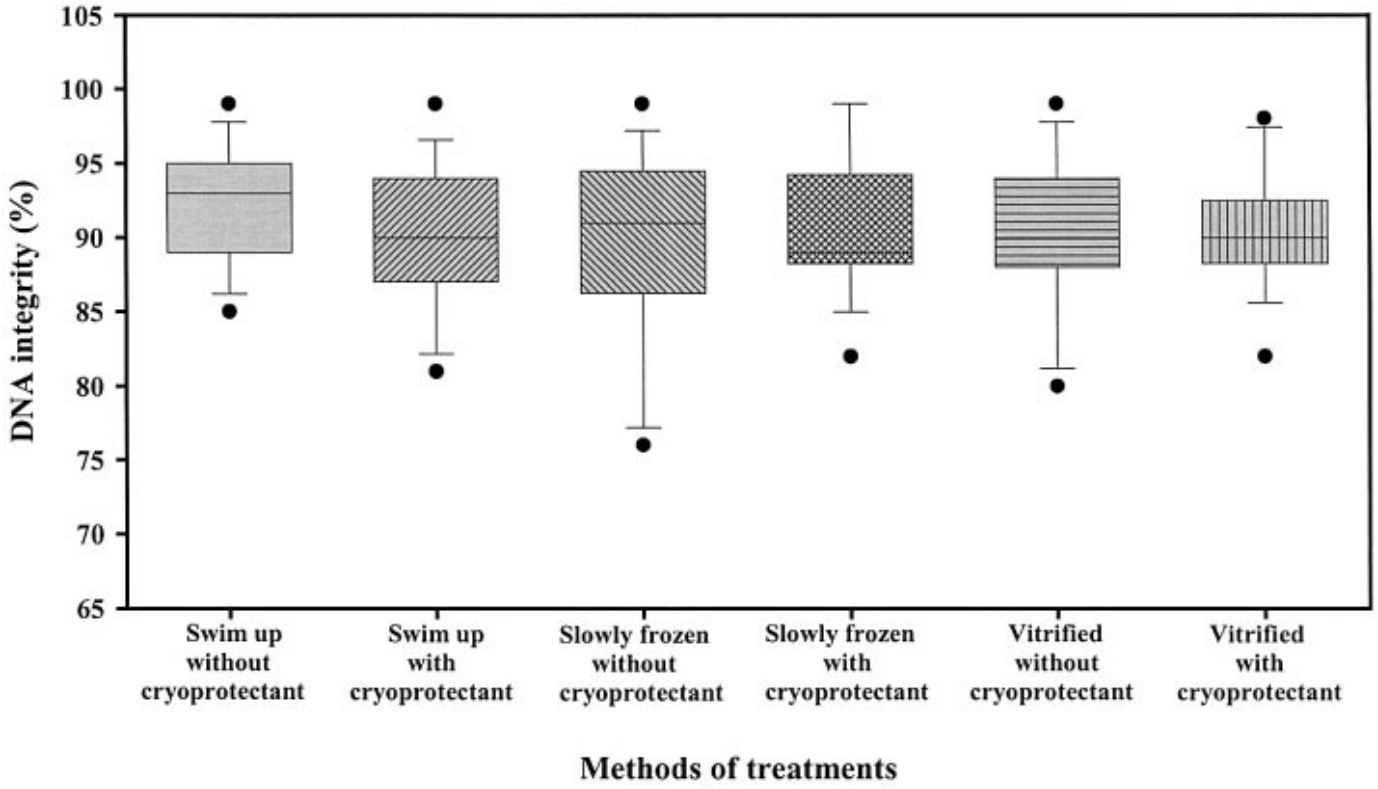



Figure 2. DNA integrity of spermatoza according to treatment and cryopreservation method. Each bar represents the median, 25th and 75th percentile, minimum and maximum values. Bars with different letters within each treatment group indicate a signi®cant difference ($P < 0.05$).

# Maintenance of genetic integrity in frozen and freeze-dried mouse spermatozoa

Hirokazu Kusakabe*†, Monika A. Szczygiel*‡, David G. Whittingham*, and Ryuzo Yanagimachi*§

*Institute for Biogenesis Research, University of Hawaii Medical School, Honolulu, HI 96822; and ‡Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected on May 1, 2001.

Contributed by Ryuzo Yanagimachi, September 28, 2001

## Mouse sperm freeze dried for ICSI only

**sperm plunged into Liq$N_2$ for 20s then freeze- dried for 4h**

- **stored at $4^0$C**
- **↓ zygotes with normal karyotypes (96% v 75%)**
- **↓embryos developing into fetuses ( 58% v 35%)**
- **no further deterioration with time**

**•normal live offspring were born after 1.5yr**

**•Benefit of storage and shipping at RT**

BIOLOGY OF REPRODUCTION 69, 2100–2108 (2003)
Published online before print 20 August 2003.
DOI 10.1095/biolreprod.103.020529

**Long-Term Preservation of Mouse Spermatozoa after Freeze-Drying and Freezing Without Cryoprotection[1]**

Monika A. Ward,[2,4] Takehito Kaneko,[4] Hirokazu Kusakabe,[3,4] John D. Biggers,[5] David G. Whittingham,[4] and Ryuzo Yanagimachi[4]

*Institute for Biogenesis Research,[4] University of Hawaii Medical School, Honolulu, Hawaii 96822*
*Department of Cell Biology,[5] Harvard Medical School, Boston, Massachusetts 02115*

# Mouse sperm frozen without cryoprotectant

- sperm kept on surface of $LiqN_2$ for 10 min then plunged into $LiqN_2$ , thawed at RT for 5 min
- ↓ zygotes with normal karyotypes (87% v 75%)
- ↓embryos developing into fetuses ( 45% v 35%)
- no further deterioration with time
- normal live offspring were born after 1.5yr

# Benefits of antioxidant addition during cryopreservation

- **Catalase maintains motility**

  ***( Foote, 1967, Bilodeau et al, 1999)***

- **∝- tocopherol and ascorbate → ↑ viability**
- **SOD and Catalase → ↑ embryo numbers**

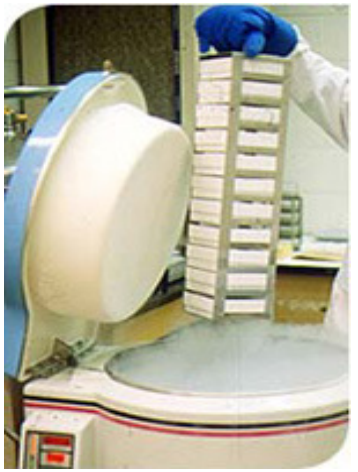
  ***(Roca et al, 2005)***

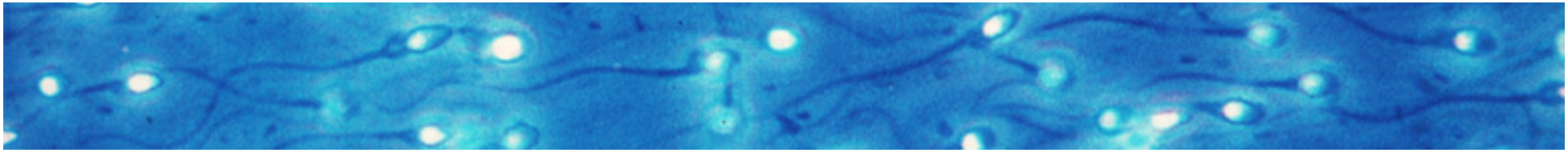
- **Ascorbate → ↑ hamster egg penetration**

  **→ ↑ implantation in cows**

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

# The need for sperm cryopreservation for cancer patients





- In UK, 5,000 men diagnosed/yr with NHL- an increase of 40% between 1987-2006
- 2000 new cases of testicular (T) cancer were registered (increasing 1-6%/yr)
- Caucasian populations seem at particular risk- **lets sperm bank!**
- But is there an increase in congenital abnormalities or cancer incidence of future?
- Cryobanking is expensive service, (£200/sample/yr)
- >10% of samples are used
- Not all oncologists are supportive of cryobanking (48% do not offer service)

*(Schover et al, USA survey, 2002)*

- due to lack of time to inform and counsel, perceived high costs, lack of knowledge or facilities, poor return rates
- We need to develop guidance for oncologists to make this standard practice

*(Am Soc Clin Oncologists Recomendations 2006)*

# As survival rates improve.....

- 80-90% of patients can now be cured
- Children of survivors are set to become a larger part of Society
- Survival highlights greater need to protect fertility and protect gametes from DNA damage

European Association of Urology

Review – Andrology

# Semen Quality in Men with Malignant Diseases before and after Therapy and the Role of Cryopreservation

*Matthias Trottmann *, Armin J. Becker, Thomas Stadler, Julia Straub, Irina Soljanik, Boris Schlenker, Christian G. Stief*

*Department of Urology, University Hospital Grosshadern, Ludwig Maximilians University of Munich, Munich, Germany*

- Disease process influences spermatogenesis
- Oligozoospermia is present in 28% of men presenting with testicular, 25% of Hodgkin's, 57% of leukaemia
- DNA integrity and compaction are impaired in testicular and Hodgkins
- Pre treatment parameters are most NB predictor of recovery

## Sperm DNA integrity in cancer patients: the effect of disease and treatment

O. Ståhl,*† J. Eberhard,*† E. Cavallin-Ståhl,* K. Jepson,† B. Friberg,†‡ C. Tingsmark,† M. Spanò§ and A. Giwercman†

*Department of Oncology, Lund University Hospital, Lund, †Department of Reproductive Medicine, Malmö University Hospital, Malmö, ‡Department of Obstetrics and Gynaecology, Lund University Hospital, Lund, Sweden, and §Section of Toxicology and Biomedical Sciences, BAS-BIOTEC-MED, ENEA Casaccia Research Center, Rome, Italy

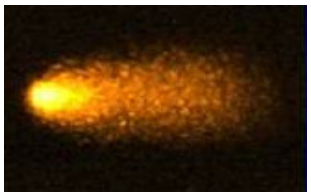
- N=121 (GCC and HL) v  137 controls
- 3 year follow up
- SCSA and DFI
- Pre treatment + 8% DFI ( 95% CI 3.2-8.8)
- no increase in DFI between pre and post treatment regardless of treatment
- pre and post cryo 0% for donors and 4% patients

Human Reproduction Vol.23, No.5 pp. 1044–1052, 2008 doi:10.10
Advance Access publication on March 17, 2008

# Characterization of sperm chromatin quality in testicular cancer and Hodgkin's lymphoma patients prior to chemotherapy

C. O'Flaherty[1], F. Vaisheva[1], B.F. Hales[1], P. Chan[2,4] and B. Robaire[1,3,4,5]

- HL (81%) and TC (37%) patients had normozoospermia
- ? Sperm DNA damage cf healthy men
- No increase in either group with TUNEL
- No increase in HL, but higher in TC with $CMA_3$
- Significantly more damage in both groups with the Comet assay



# Impact of chemotherapeutics and advanced testicular cancer or Hodgkin lymphoma on sperm deoxyribonucleic acid integrity

*Cristian O'Flaherty, Ph.D.,*[a] *Barbara F. Hales, Ph.D.,*[a] *Peter Chan, M.D.,*[b,c] *and Bernard Robaire, Ph.D.*[a,b,c,d]

[a] Department of Pharmacology and Therapeutics; [b] Department of Urology; [c] McGill University and the McGill University Health Centre; and [d] Department of Obstetrics and Gynecology, McGill University and the McGill University Health Centre, Montreal, Quebec, Canada

- Before chemotherapy, DNA damage was higher ( 35% v 15%  in controls)
- 24 months post treatment, TC (67%) and HL (60%) had normal sperm concs and high FSH
- But by 6 mth, sperm DNA had increased and remained elevated at 12 mth and 24 mth

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VOL. 81, NO. 2, FEBRUARY 2004

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**Fertility after cancer: a prospective review of assisted reproductive outcome with banked semen specimens**

*Ashok Agarwal, Ph.D.,[a] Pavithra Ranganathan, M.D.,[a] Namita Kattal, M.D.,[a] Fabio Pasqualotto, M.D.,[a] Jorge Hallak, M.D.,[a] Saba Khayal, M.D.,[a] and Edward Mascha, M.S.[a,b]*

18% of men achieved a spontaneous pregnancy

*Schover et al, 2009*

9 TC, 12 HL and 8 other cancers

87 ART cycles (42 IUI, 26 IVF and 19 ICSI)

18% pregnancy ( 45% IUI, 23% IVF,37% ICSI)

75% resulted in live births

No congenital abnormalities

# Sperm cryopreservation in oncological patients: a 14-year follow-up study

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- Between 1991-1994, 186 cancer patients stored sperm
- 6 months later, 27% had recovered normal spermatogenesis
- 30 ICSI cycles were performed using frozen sperm and 15% pregnancies resulted
- Cost/benefit ratio is favourable

# Effects of alkyating agents on sperm DNA

- they act to create DNA adducts
  modify bases
  form cross linkages
- all of which prevent accurate replication of DNA

- As sperm have no facility for repair, these modifications may be irreversible

# Knowledge and Experience Regarding Cancer, Infertility, and Sperm Banking in Younger Male Survivors

By Leslie R. Schover, Kimberly Brey, Alan Lichtin, Larry I. Lipshultz, and Sima Jeha

*J Clinical Oncology 2002 20 1880-1889*

**Aim: to survey male patients (14-50) recently treated for cancer re future family**

- 77 % were childless at diagnosis
- Despite fear of non- survival and risk to future child's health
- 51% of men wanted children in future
- All felt experience of cancer made this desire greater
- 51% had been offered cryopreservation
- 24% had banked semen
- 25% did not bank due to inadequate information

# Psychological Benefits of Cryobanking

- fear of infertility is a significant stressor upon diagnosis *(Saito et al, 2005)*

- knowledge that their fertility is protected gives them important psychological benefit during their treatment

*(Crawshaw et al, 2008)*

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Review

# Current trends, biological foundations and future prospects of oocyte and embryo cryopreservation

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•Elective SET has brought cryotechnology of oocytes and embryos to the fore

•Oocyte  slow freezing- not successful

•Oocyte vitrification- simpler, more convenient and more effective than slow cooling ***(Vajta and Nagy, 2006)***

• Less ice crystal formation and osmotic shock ***(Lucena et al, 2006)***

•  better meiotic spindles and chromosome  configuration ***( Lane and Gardner, 2001)*** less zona hardening

• no increases in abnormal karyotypes of congenital abnormalities – 16 pregnancies *(Porcu et al, 2000)*

• still only  600-1000 babies worldwide

• More data needed, future directions-

• Sugars, antifreeze proteins and antioxidants

•Freezing immature oocytes

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human reproduction update

# Current achievements and future research directions in ovarian tissue culture, *in vitro* follicle development and transplantation: implications for fertility preservation

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# The latest in female fertility preservation

- Banking of oocytes prior to fertility threatening surgery or therapy
- Orthotopic transplants of fresh or frozen ovarian tissue
- Follicle culture
- Follicle transplantation
- research into more rapid revascularization
  - by vitrification techniques
  - by advanced media with growth factors , EC matrix support
- Immunoassays to evaluate maturation
- Non primate models are essential

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