Unit II – Cryopreservation of gametes

Why cryopreservation of gametes?

- Zootechnics
- Reproductive medicine
- Laboratory animals
- Biodiversity

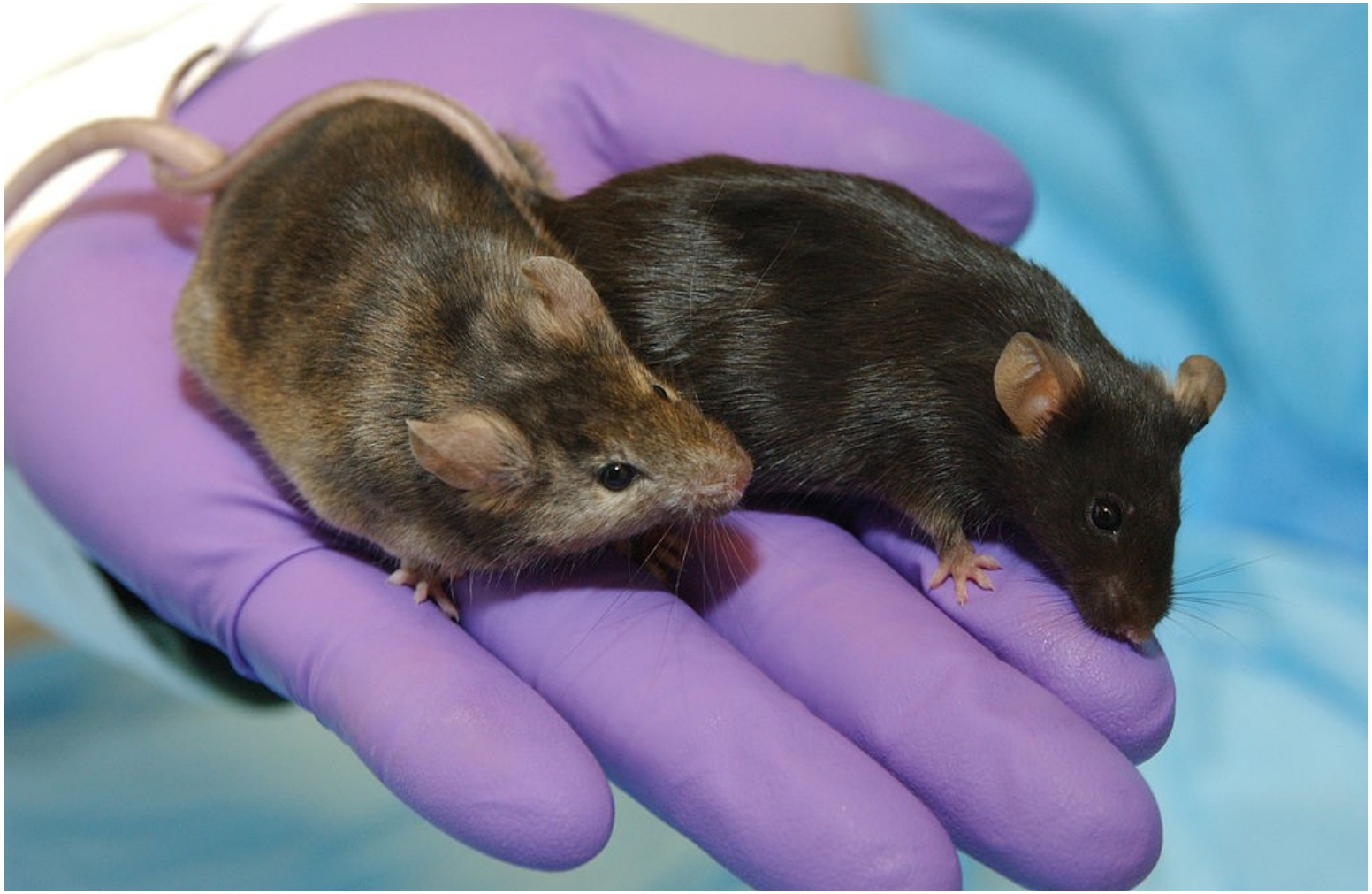
Not only cows…

ART

Indication for gametes cryopreservaton in human ART

- Chemoterapy, radiotherapy
- Diabetes and autoimmune disorders
- Infectious diseases
- MESE/TESE.
- Semen collection distress

Laboratory animals



Biodiversity

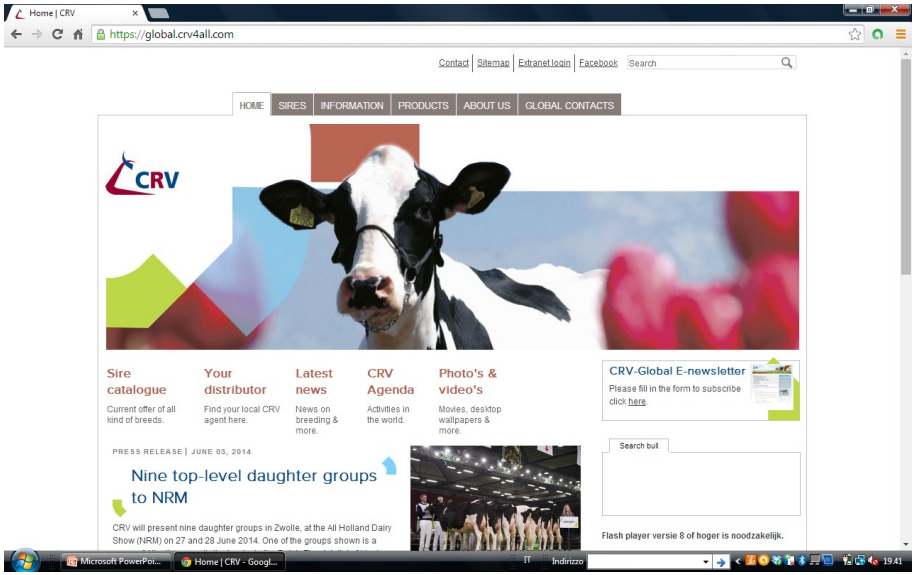
Interesse di diverse categorie

- zootechnicians
- physicians
- Veterinarians
- Farmers
- Association for biodiversity protection/conservation
- Patients

…

It means …


Cryobiology, Inc. Sperm b
cryobio.com
Cryobiology
The best gifts come in small packages.
When you hold your baby in your arms, you are holding a blessing. A blessing that first began with today's choice, tomorrow's future.
Welcome to Cryobiology - the leader in reliable, high quality, tested and quarantined, private donor sperm samples.
Donor
Donor
Services
About
FAQ/
Prices
Cryobiology, Inc. Sperm bank - Google Chrome


Home | CRV
https://global.crv4all.com
Contact
Sitemap
Extranet login
Facebook
Search
HOME
SIRES
INFORMATION
PRODUCTS
ABOUT US
GLOBAL CONTACTS
CRV
Sire catalogue
Current offer of all kind of breeds.
Your distributor
Find your local CRV agent here.
Latest news
News on breeding & more.
CRV Agenda
Activities in the world.
Photo's & video's
Movies, desktop wallpapers & more.
CRV-Global E-newsletter
Please fill in the form to subscribe click here.
PRESS RELEASE | JUNE 03, 2014
Nine top-level daughter groups to NRM
CRV will present nine daughter groups in Zwolle, at the All Holland Dairy Show (NRM) on 27 and 28 June 2014. One of the groups shown is a
Search bul
Flash player versie 8 of hoger is noodzakelijk.

Cryopreservation of spermatozoa

problems

- Non spherical shape
- Highly dynamic citoschkeleton
- Highly dynamic membranes
- Need of economic protocols
- High differencies among subjects, breeds, species, …

Methods

IMPORTANT: it is believed that, due to the low water content and high concentration of citoplamsmic proteins, in spermatozoa of intracellular ice crystals are not formed , regardless of the used freezing protocol.

Morris GJ, Actona E, Murray BJ, Fonseca F. Freezing injury: The special case of the sperm cell. Cryobiology 2012;64: 71-80.

Metodologie applicate

Slow Freezing: 2-4h.
example:
fom RT to 5°C -> cooling rate 0.5 – 1 °C/min
from 5°C to -80°C -> " " 1 – 10 °C/min
Then in N_2 at -196°C.

- Manually
- Programmable freezers

Rapid Freezing: direct contact of sample with N_2 vapors.

It requires 8-10 minutes.

https://www.corning.com/media/worldwide/cls/documents/t_cryoanimalcc.pdf

Cryopreservation of small numbers of spermaozoa

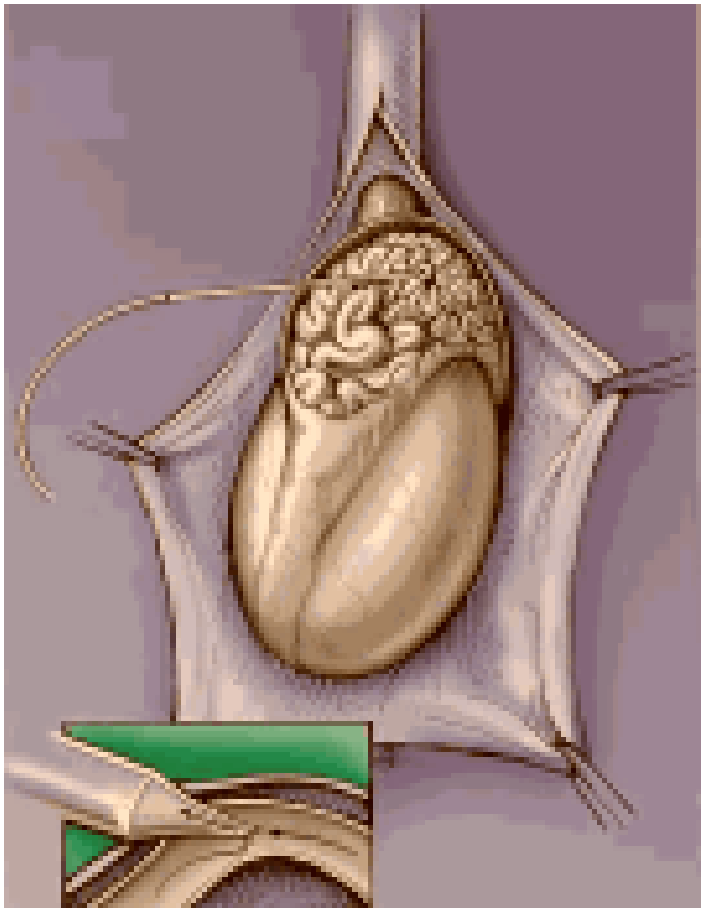
Useful in several pathological conditions.

It allows the availability for long times of gametes.

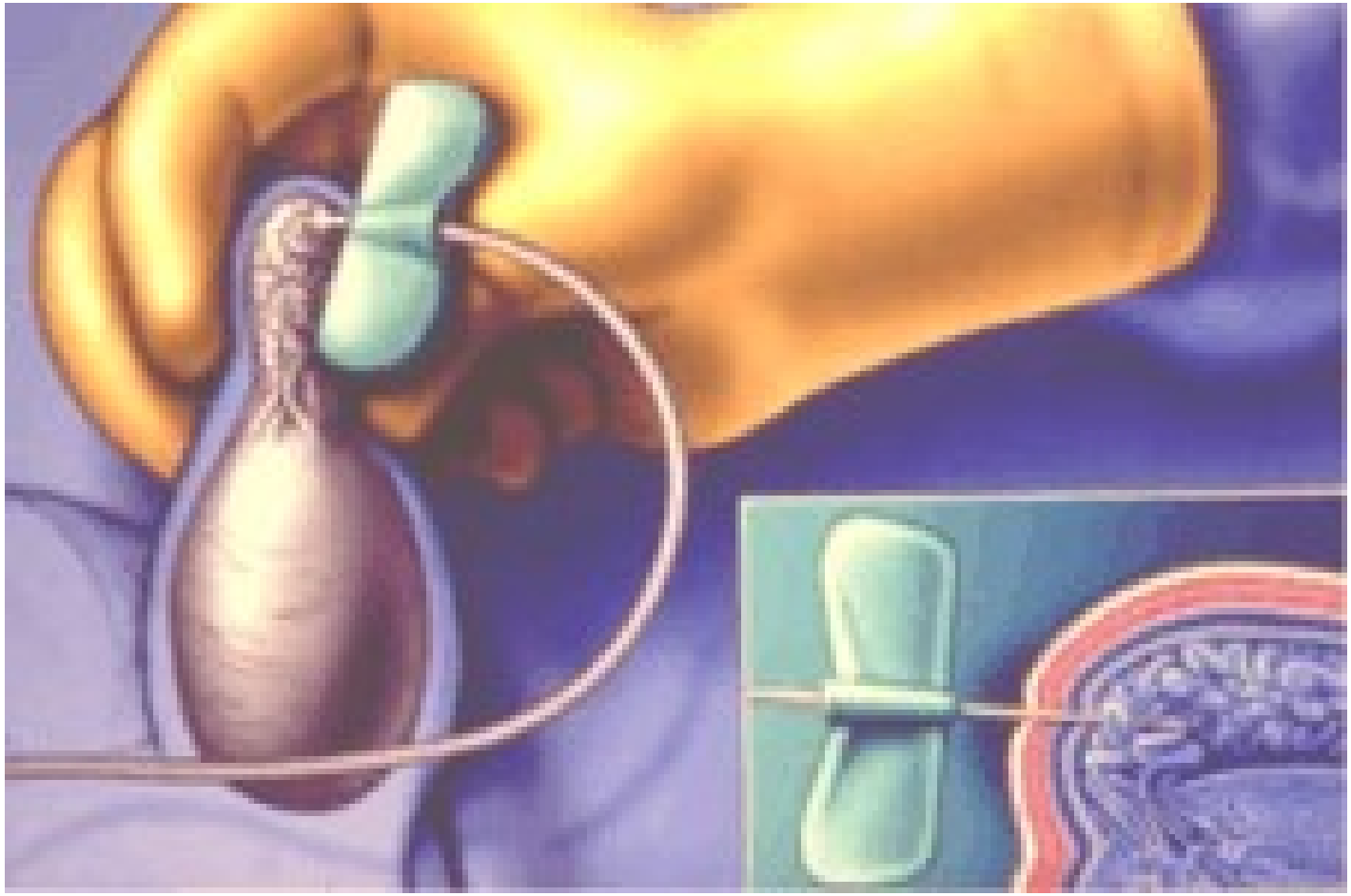
It is often used in combination with other technologies

- 1. MESA, *Microsurgical epididymal sperm aspiration*
- 2. PESA, *Percutaneous epididymal sperm aspiration*
- 3. TESE, *Testicular sperm extraction*
- 4. TESA, *Percutaneous testicular sperm aspiration*

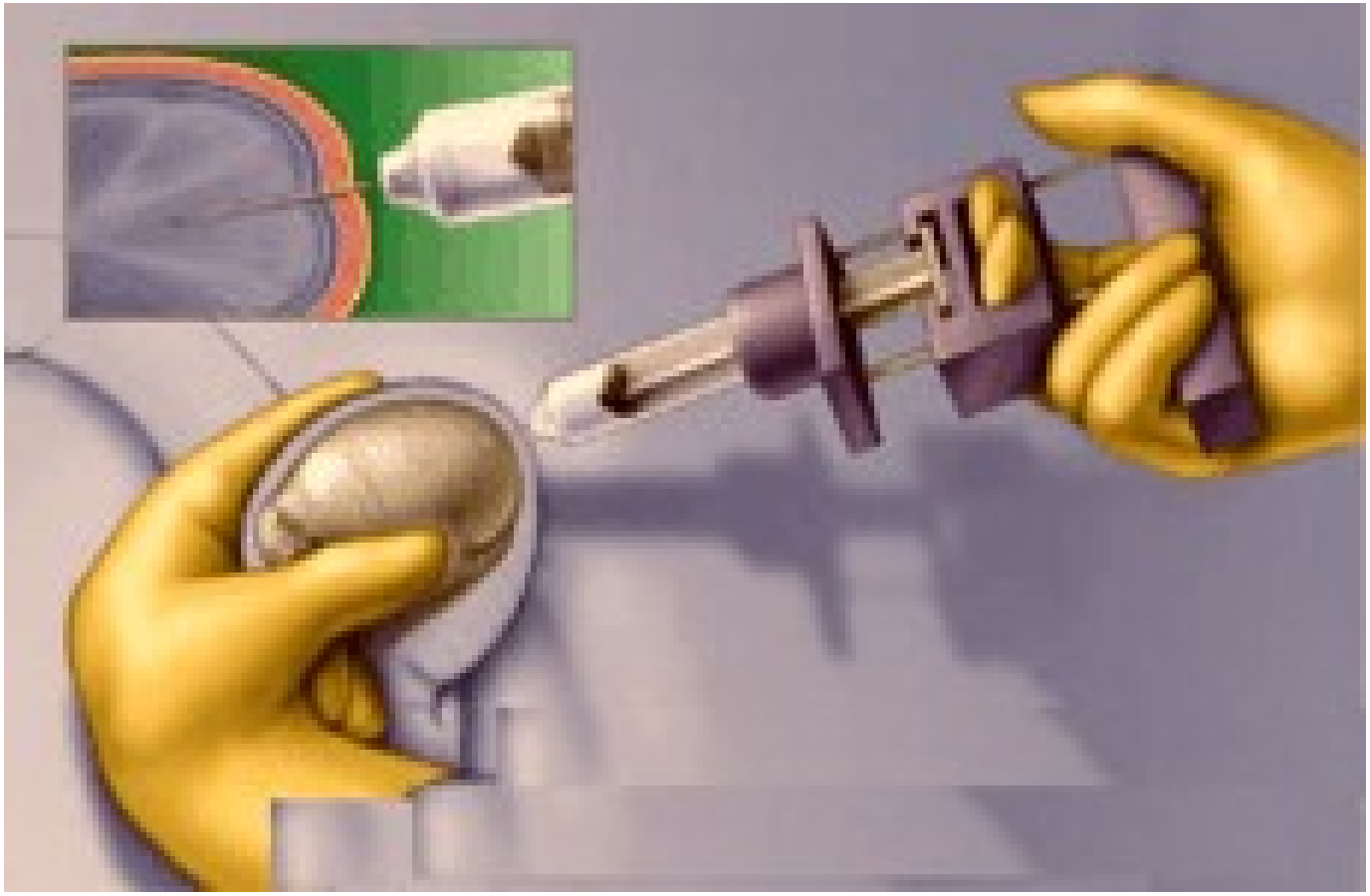
Microsurgical Epididymal Sperm Aspiration



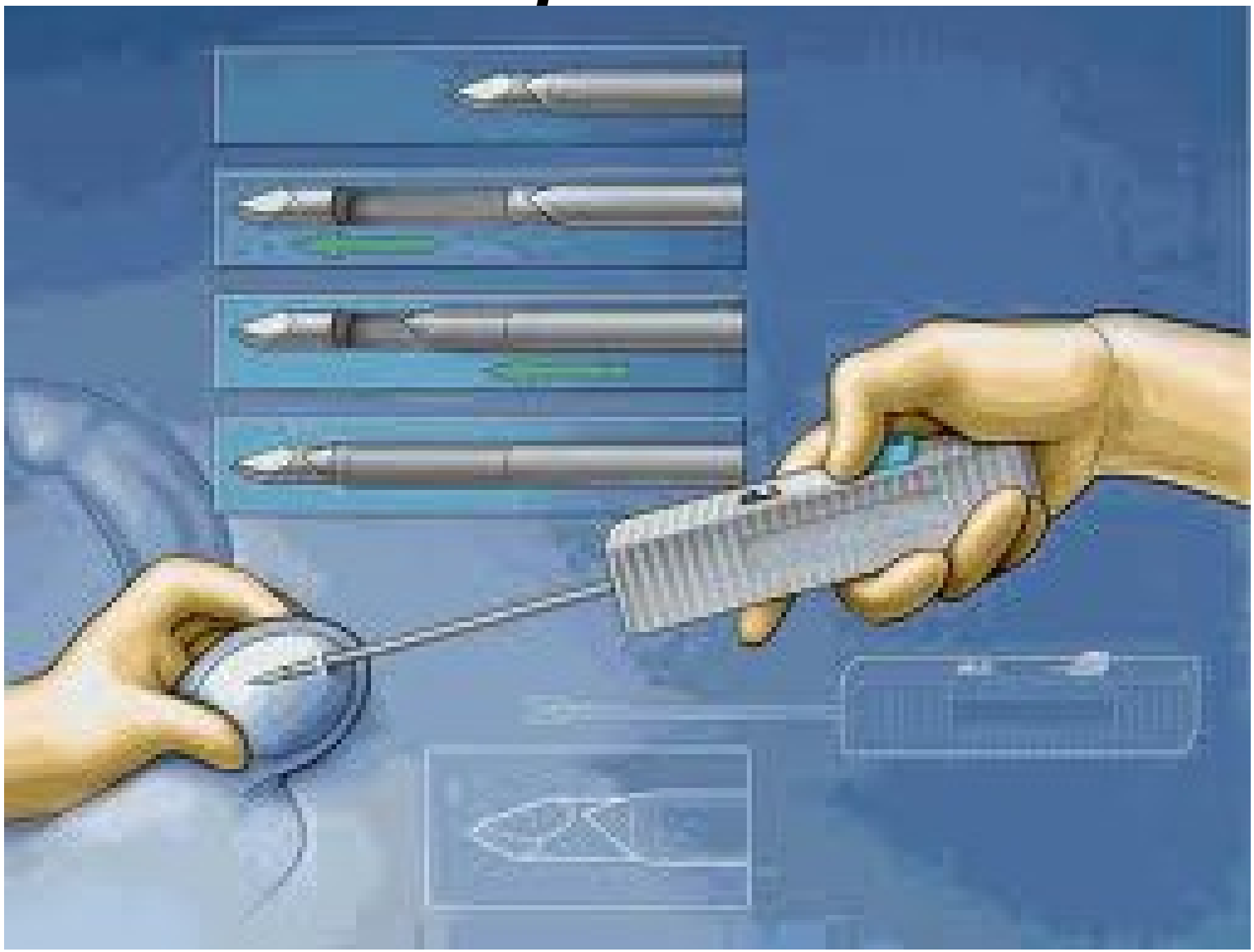
Percutaneous Epididymal Sperm Aspiration



Testicular Percutaneous Sperm Aspiration/Fine Neddle Aspiration



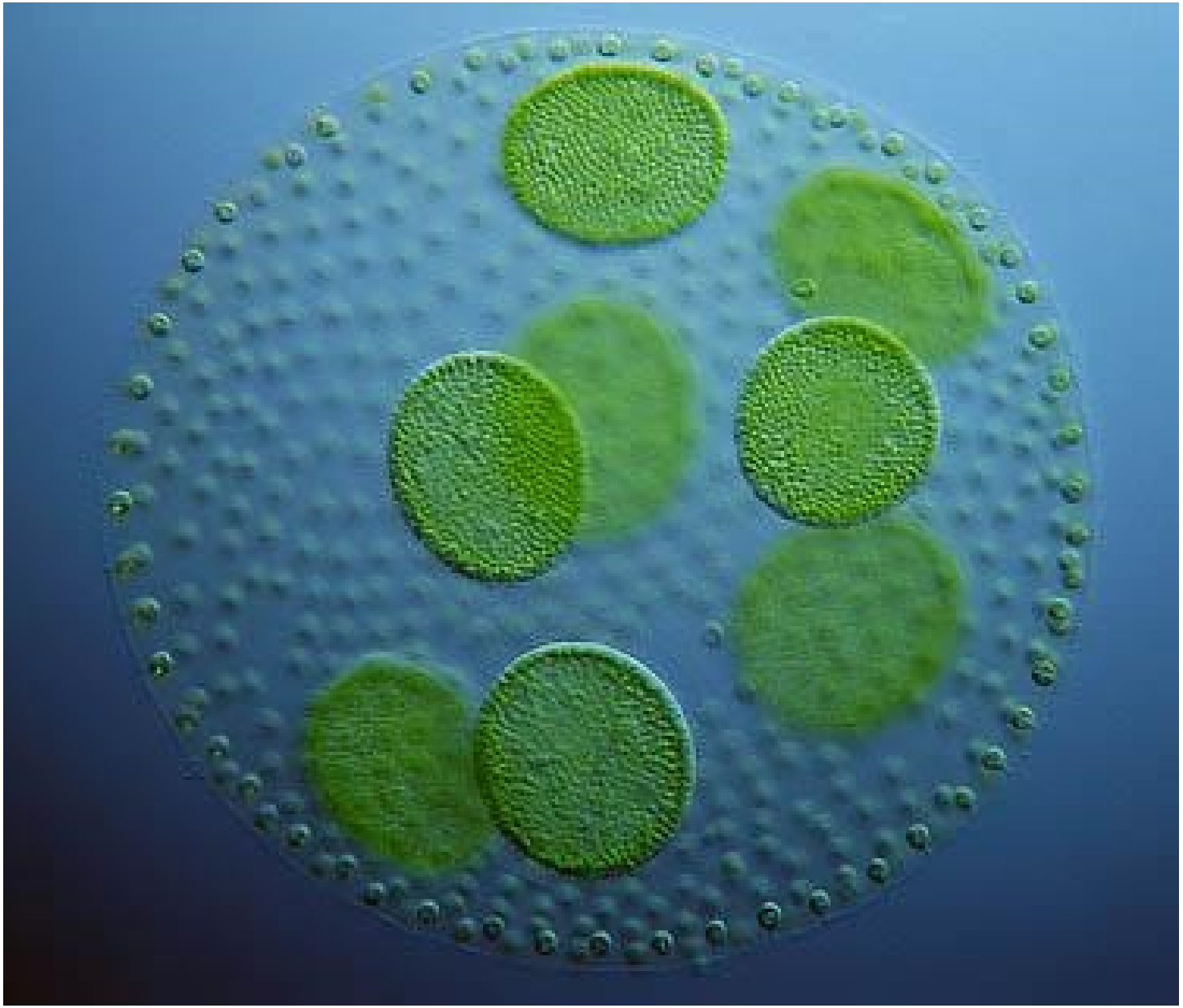
Testicular Sperm Extraction



Methods

Table 1: Approaches to cryopreserve limited number of spermatozoa.

Cryopreservation techniques	Authors	Principle	Main advantages	Main disadvantages
Empty zona pellucida	Borini et al. [69] Cohen et al. [70] Walmsley et al. [71] Montag et al. [72] Hsieh et al. [73] Liu et al. [74] Levi-Setti et al. [75] Cesana et al. [76] Hassa et al. [77]	Storage of individual spermatozoa in animal or human empty zona pellucida.	Avoid waste of time in screening to locate motile sperm; cryoprotectants can be added and removed without loss of spermatozoa sequestered in the zona	Risk of biological contamination
Microdroplets	Gil-Salom et al. [78] Sereni et al. [79] Quintans et al. [80] Bouamama et al. [81]	Storage of droplets of sperm/cryoprotectants mixture on the surface of dry ice and directly plunged into liquid nitrogen	Avoid sperm loss through adherence to the vessel	Risk of cross-contamination; shape and size of dishes make difficult to handle and store in conventional freezers and liquid nitrogen tanks
ICSI pipette	Gvakharia et al. [82] Sohn et al. [83]	Storage of spermatozoa in ICSI pipettes	Sterile, simple, and convenient system	Not practical for long-term storage; fragility of ICSI pipettes; risk of cross-contamination
Volvox globator spheres	Just et al. [84]	Storage of sperm into spheres of *Volvox globator*	Significant postthaw recovery of motile sperm	Exposure to genetic material from the algae; constant source of algae
Alginate beads	Herrler et al. [85]	Microencapsulation in alginate beads	Inert nature of alginate beads	Decrease sperm motility with encapsulation
Cryoloop	Nawroth et al. [86] Schuster et al. [87] Isachenko et al. [42] Isachenko et al. [42] Desai et al. [88] Desai et al. [89]	Individual spermatozoa deposited directly on cryoprotectant film covering the nylon loop and immersed in liquid nitrogen	Excellent vessel for vitrification; no additional preparation	Open system: risk of cross-contamination
Agarose microspheres	Isaev et al. [90]	Storage of sperm loaded in agarose microspheres	Nonbiological carrier	Clinical value of this approach not evaluated
Straws	Desai et al. [91] Isachenko et al. [92] Koscinski et al. [93]	Sperm/cryoprotectants loaded into the ministraw	Sterile, simple, and convenient system	Not ideal for severely impaired specimens; sperm loss due to adherence to the vessel



Sperm cryopreservation and DNA damage

Table 2: (a)–(c) Evaluation of DNA integrity after cryopreservation: description of the experimental design and conclusions.

(a)

Authors	Test to evaluate DNA integrity	Number of samples	Cryopreservation method	"Does the freezing-thawing procedure induce sperm DNA damage?"
Hamamah et al. [94]	Acridine orange staining and Feulgen-DNA quantitative microspectrophotometry	10	Unspecified	Yes
Spanò et al. [44]	SCSA + Acridine orange staining	19	Equilibration at 37°C, freezing in liquid nitrogen vapour at −80°C and then storage in liquid nitrogen at −196°C	Yes
Hammadeh et al. [95]	Acridine orange staining	59	Computerized slow-stage freezer + static liquid nitrogen vapour	Yes
Donnelly et al. [6]	COMET assay	40	Equilibration at 37°C, freezing in liquid nitrogen vapour at −80°C and then storage in liquid nitrogen at −196°C	Yes
Gandini et al. [96]	Acridine orange staining	19	Equilibration at 37°C, freezing in liquid nitrogen vapour at −80°C and then storage in liquid nitrogen at −196°C	Yes
de Paula et al. [40]	TUNEL assay	77: (I) 30 normozoospermic (II) 47 oligozoospermic	Use of freezer at −20°C, freezing in liquid nitrogen vapour, then storage in liquid nitrogen −196°C	Yes
Petyim and Choavaratana [43]	Acridine orange staining	50	Freezing with liquid nitrogen vapour + computerized program freezer	Yes
Nagamwuttiwong and Kunathikorn [97]	Acridine orange staining	20	Freezing with liquid nitrogen vapour	Yes
Dejarkom and Kunathikorn [98]	Acridine orange staining	20	Computerized controlled rate freezing	Yes
Thomson et al. [46]	TUNEL assay	60	Use of programmable freezer	Yes
Thomson et al. [46]	TUNEL assay	320	Sample frozen with and without cryoprotectant by slow-controlled-rate method using a programmable freezer	Yes
Zribi et al. [45]	TUNEL assay	15	Equilibration at 37°C, freezing in liquid nitrogen vapour at −80°C, then storage in liquid nitrogen at −196°C	Yes

(b)

Authors	Test to evaluate DNA integrity	Number of samples	Cryopreservation technique	"Does the freezing-thawing procedure induce sperm DNA damage?"
Donnelly et al. [6]	COMET assay	57: (I) 17 fertile (II) 40 infertile	Equilibration at 37°C, freezing in liquid nitrogen vapour at −80°C, then storage in liquid nitrogen at −196°C	Yes, but semen from fertile men appears to be more resistant to freezing damage
Kalthur et al. [49]	COMET assay + Acridine orange staining	44	Equilibration at 37°C, static cooling at 4°C, cooling vapour phase, then storage in liquid nitrogen at −196°C	Yes, but morphologically abnormal sperms seems to be less resistant to freezing damage
Ahmad et al. [99]	COMET assay	196: (I) 30 normospermic (II) 166 infertile	Freezing with static-phase vapour cooling procedure	Yes, but the sperm DNA integrity of frozen samples of fertile men is higher

(c)

Authors	Test to evaluate the DNA integrity	Number of samples	Cryopreservation technique	"Does the freezing-thawing procedure induce sperm DNA damage?"
Høst et al. [100]	Immunoperoxidase detection of digoxigenin-labelled genomic DNA	53: (I) 20 fertile (II) 33 infertile	Conventional cryopreservation	No
Steele et al. [101]	COMET assay	21: (I) 9 control (II) 12 with obstructive azoospermia	Freezing in liquid nitrogen vapour	No
Duru et al. [41]	TUNEL assay + annexin V	21	Equilibration at 37°C, freezing in liquid nitrogen vapour at −80°C, then storage in liquid nitrogen at −196°C	No
Isachenko et al. [42]	COMET assay	18	Programmable slow freezing + vitrification	No
Paasch et al. [50]	TUNEL assay + flow cytometric kit for apoptosis	84	Freezing at −20°C, freezing in liquid nitrogen vapor at −100°C, then storage in liquid nitrogen at −196°C	No

Hindawi Publishing Corporation
Advances in Urology
Volume 2012, Article ID 854837, 12 pages
doi:10.1155/2012/854837

Review Article

Human Sperm Cryopreservation: Update on Techniques, Effect on DNA Integrity, and Implications for ART

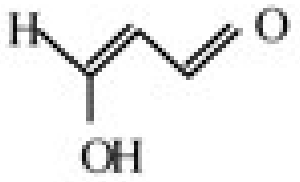
Marlea Di Santo, Nicoletta Tarozzi, Marco Nadalini, and Andrea Borini

Cryopreservation damages

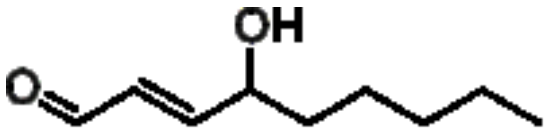
lipoperoxidation

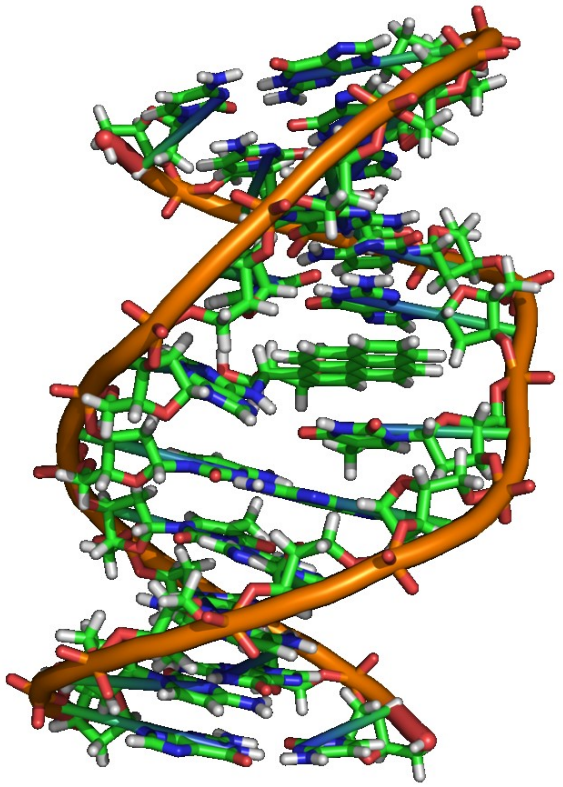
As terminals products are formed reactive aldehydes, including the malonilaldeide (MDA) and the hydroxynonenal (HNE). Possible formation of adducts with DNA

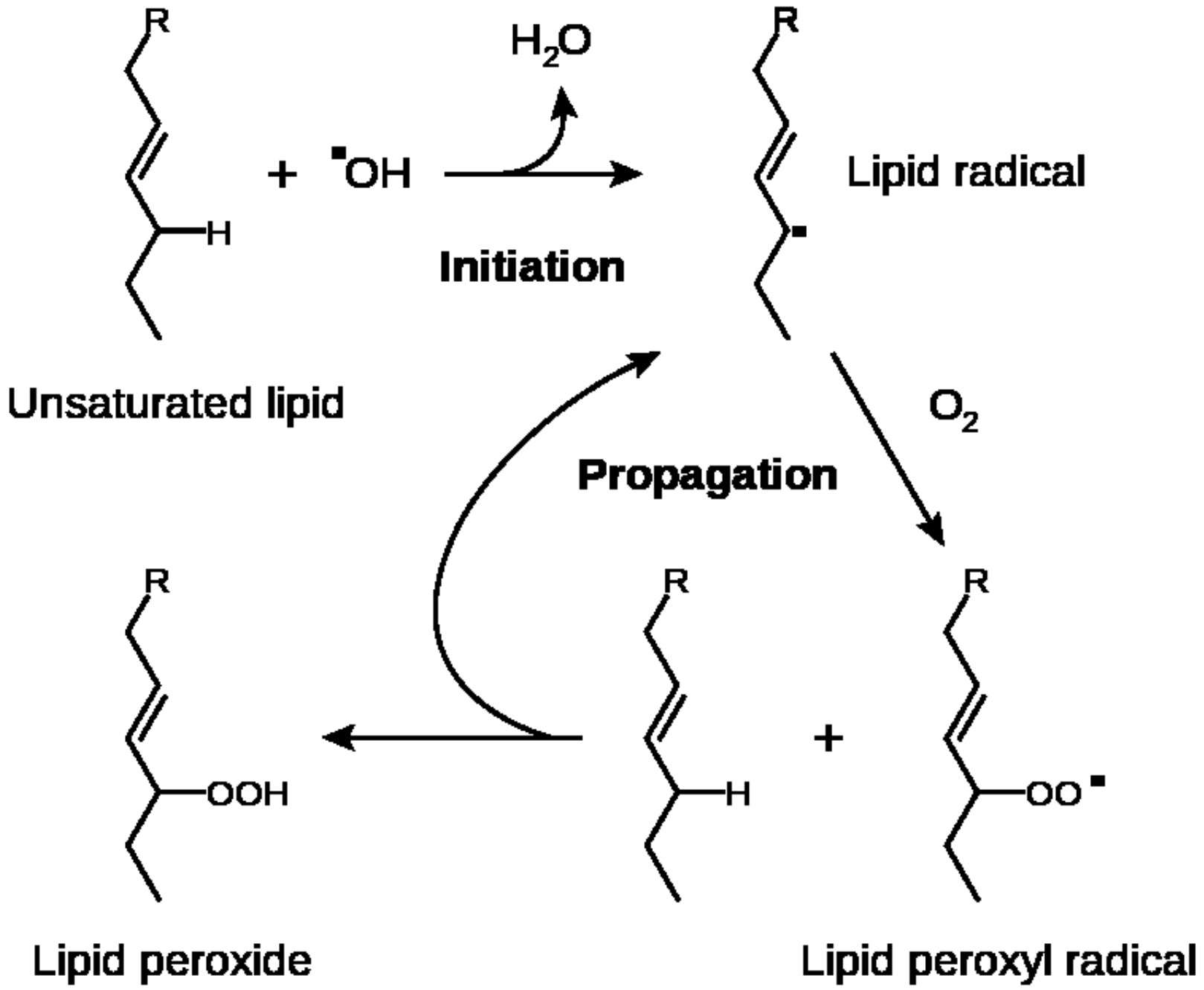
MDA



HNE






R
H
+
•OH
H2O
Initiation
R
•
Lipid radical
Unsaturated lipid
O2
Propagation
R
OOH
Lipid peroxide
R
H
+
R
OO•
Lipid peroxyl radical

Differencies among individuals and breeds

- It is a very important issue, for instance, in swine.

- The use of cryopreserved spermatozoa causes the reduction of the number of newborns (1 – 3).
- It is likely due to the high levels of polyunsaturated fatty acids DPA; 22:5, n-6 and DHA; 22:6, n-3.

REPRODUCTION
RESEARCH

Within and between breed differences in freezing tolerance and plasma membrane fatty acid composition of boar sperm

K E Waterhouse[1,2], P O Hofmo[3], A Tverdal[4] and R R Miller Jr[5]

[1]Team Semin, PO Box 8146 Dep., N-0033 Oslo, Norway, [2]The Norwegian School of Veterinary Science, Department of Production Animal Clinical Sciences, PO Box 8146 Dep., N-0033 Oslo, Norway, [3]Norsvin (Norwegian Pig Breeders Association), PO Box 504, N-2304 Hamar, Norway, [4]The Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, PO Box 8146 Dep., N-0033 Oslo, Norway and [5]Hillsdale College, Biology Department, 33 E College, Hillsdale, Michigan 49242, USA

Table 1 Percentages of live and live acrosome intact (LAI) sperm at different storage temperatures for Norwegian Landrace ($n = 12$) and Duroc ($n = 12$) boars. Values are presented as means (S.D.).

	18 °C[a]	5 °C[b]	38.5 °C[b]	Post-thaw[b]
Live sperm (%)				
Landrace	95.5 (1.5)	92.4 (1.1)	87.3 (2.9)	48.8 (10.1)
Duroc	94.3 (2.6)	92.8 (2.0)	87.6 (3.6)	51.3 (11.0)
LAI sperm (%)				
Landrace	94.4 (1.7)	91.1 (1.5)	63.5 (18.9)	44.7 (10.5)
Duroc	92.5 (4.4)	91.9 (2.2)	71.7 (12.8)	45.0 (8.8)

[a]Sperm diluted and stored in BTS; [b]sperm diluted and stored in freezing extender containing 20% egg yolk and 4% glycerol.

Table 2 Percentage gram fatty acids found in phospholipids of sperm plasma membrane from Norwegian Landrace ($n = 12$) and Duroc ($n = 12$) boars. Values are presented as means (S.D.).

Fatty acid	Landrace	Duroc
Saturates, total	37.3 (7.15)	37.4 (6.0)
12:0	0.35 (0.52)	0.05 (0.17)
14:0	2.15 (3.30)	1.11 (0.51)
16:0	18.35 (4.03)	18.93 (3.10)
18:0	16.44 (4.12)	17.31 (3.46)
Monounsaturates, total	12.4 (3.7)	13.4 (3.0)
14:1	0.67 (1.18)	0.21 (0.59)
16:1	nd	0.48 (0.84)
18:1, n-9	11.75 (3.67)	12.71 (3.48)
Polyunsaturates, total	50.3 (7.1)	49.1 (5.9)
18:2, n-6	6.32 (2.16)	6.83 (2.43)
18:3, n-3	1.59 (4.55)	0.30 (0.89)
20:4, n-3	6.21 (4.53)	8.76 (10.36)
22:3, n-6	1.55 (2.17)	0.30 (0.70)
22:4, n-6	1.87 (2.63)	0.75 (1.16)
22:5, n-6	15.40 (6.01)	13.92 (5.41)
22:5, n-3	0.45 (0.86)	0.26 (0.43)
22:6, n-3	16.91 (6.25)	18.03 (5.95)
Fatty acid ratios		
Unsaturates/saturates	1.76 (0.46)	1.73 (0.38)
22/rest of fatty acid	0.60 (0.23)	0.52 (0.18)
22:6, n-3/22:5, n-6	1.20 (0.43)	1.39 (0.61)

nd, not detected.

Table 3 Correlation coefficients between percentages of live sperm after freezing and thawing and fatty acid ratios of frozen-thawed sperm from Norwegian Landrace ($n = 12$) and Duroc ($n = 12$) boars.

Ratio	Live sperm (%), Landrace	Live sperm (%), Duroc
Unsaturates/saturates	0.42	−0.04
22/rest of fatty acid	0.64*	0.67*
22:6, n-3/22:5, n-6	−0.52	−0.24

*$P < 0.05$.

In conclusion, the results of our study indicate that it is the individual male and not the breed that is decisive for the survival rate, measured as plasma membrane integrity, after freezing and thawing of boar sperm. Furthermore, the male-to-male differences in sperm survival after freezing and thawing seem to be partially related to the amounts of long-chain PUFAs in the plasma membrane after freezing and thawing. Future work will compare initial and frozen–thawed fatty acid composition of the sperm plasma membranes and will study the relationship with survival rates after cryopreservation.

BIOLOGY OF REPRODUCTION **66,** 545–554 (2002)

Identification of Amplified Restriction Fragment Length Polymorphism Markers Linked to Genes Controlling Boar Sperm Viability Following Cryopreservation[1]

Lisa M. Thurston,[2,3,4,5] Ken Siggins,[6] Alan J. Mileham,[6] Paul F. Watson,[5] and William V. Holt[4]

Department of Biochemistry and Molecular Biology,[3] Royal Free & University College Medical School, London NW3 2PF, United Kingdom
Institute of Zoology, Zoological Society of London,[4] Regent's Park, London NW1 4RY, United Kingdom
Veterinary Basic Sciences,[5] Royal Veterinary College, Camden, London NW1 0TU, United Kingdom
PIC International Group,[6] Department of Pathology, Cambridge University, Cambridge CB2 1QP, United Kingdom

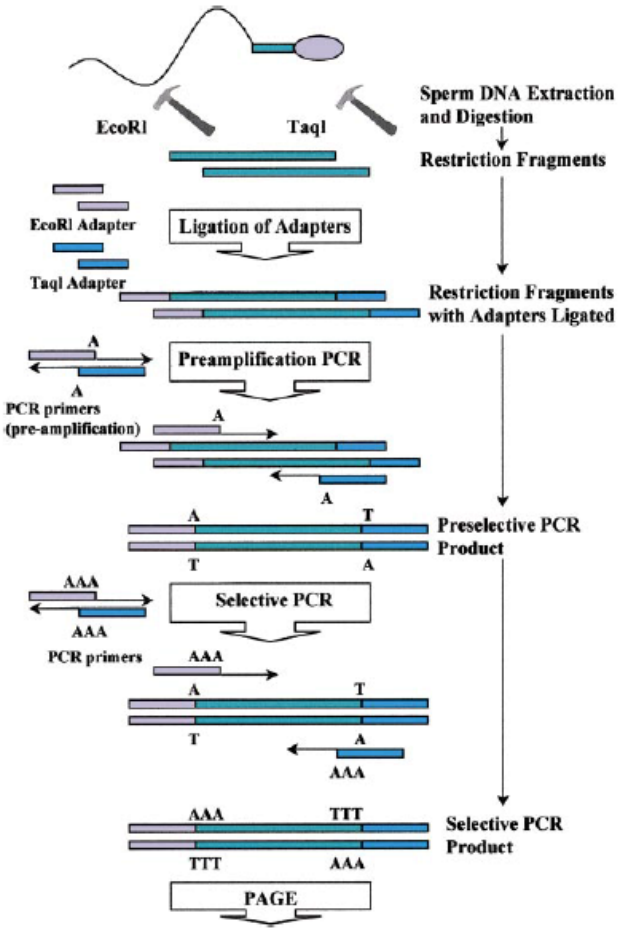


FIG. 1. Schematic outline of the AFLP technique.

TABLE 5. Mean (±SD) sperm viability measurements for each of the PATN-derived classifications of good, average, and poor postthaw recovery (frezability).

Freezability	No. boars	Motility (%)	Membrane intact (%)	Acrosome intact (%)	Active motility (%)
Poor	42	17.65 (5.12)	17.22 (9.97)*	88.45 (4.32)	3.01 (1.98)*
Average	63	22.69 (4.83)	28.67 (7.29)*	82.76 (6.66)	5.27 (2.34)*
Good	24	54.12 (12.32)*	37.19 (10.60)*	90.82 (7.14)	12.41 (4.45)*

* Viability parameters are significantly different between PATN-derived classifications of semen freezability (poor, average, and good) ($P < 0.05$).

TABLE 6. Variation in the number of boars classified with good, average, and poor semen freezability from each of three breeds.

	No. boars			
Breed	Good n (%)	Medium n (%)	Poor n (%)	Total
Large White	9 (7)	25 (19)	13 (10)	47 (36)
Landrace	13 (10)	17 (13)	4 (3)	34 (26)
Duroc	2 (2)	21 (16)	25 (19)	48 (37)
Total	24 (19)	63 (49)	42 (33)	129 (100)

TABLE 7. Logistic regression analysis of the presence or absence of AFLP markers with classifications of good or bad freezability and semen quality assessments.

Marker	Variable*	Regression coefficient	SEM	Significance of variables (*P*)	Significance of logistic model (*P*)
1	G/B	0.599	1	0.005	0.005
2	G/B	0.643	1	0.005	0.003
3	G/B	0.650	1	0.005	0.004
4	G/B	0.740	1	0.001	0.0007
5	G/B	0.589	1	0.005	0.005
6	G/B	0.759	1	0.001	0.0001
7	G/B	0.639	1	0.005	0.005
7	SYBR14 (%)	0.775	0.1	0.0009	
8	G/B	0.639	1	0.005	0.005
8	SYBR14	0.436	0.1	0.04	
9	G/B	0.641	1	0.005	0.005
9	SYBR14 (%)	0.752	0.07	0.001	
10	G/B	0.663	1	0.005	0.003
11	G/B	0.662	1	0.004	0.004
11	Motile (%)	0.503	0.12	0.02	
11	Progressive motility (%)	0.436	0.41	0.04	
11	SYBR14 (%)	0.639	0.06	0.005	
12	G/B	0.600	1	0.003	0.005
13	G/B	0.662	1	0.006	0.005
14	G/B	0.797	1	0.0008	0.005
15	G/B	0.727	1	0.001	0.004
16	G/B	0.752	1	0.001	0.0008

* G/B are classifications of good and bad freezers, SYBR14 (%) refers to the percentage of spermatozoa with intact plasma membranes in the thawed ejaculate.

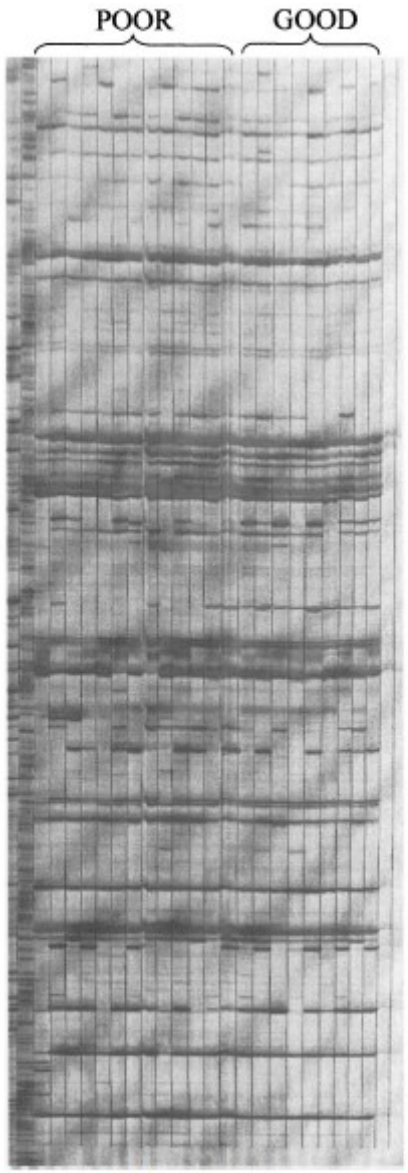
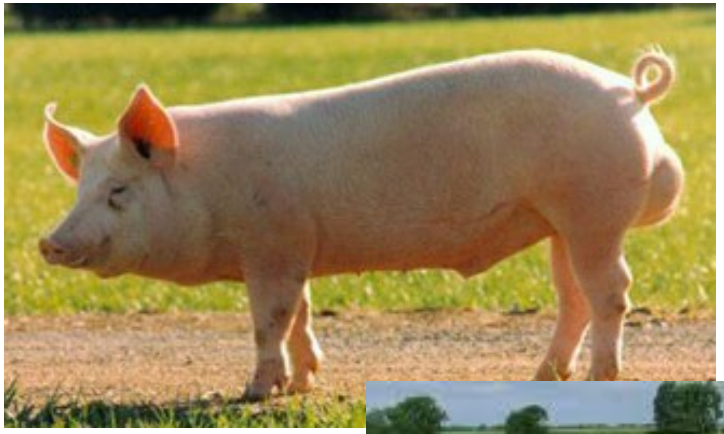


FIG. 2. Example of an AFLP profile for a specific primer com
The arrow indicates a potential freezability marker. Each lane
DNA restriction fragments amplified from a specific boar classi
good or poor freezer based on the quality of its semen after free



Available online at www.sciencedirect.com
SCIENCE DIRECT®
Theriogenology
Theriogenology 63 (2005) 370–382
www.journals.elsevierhealth.com/periodicals/the

The significance of cooling rates and animal variability for boar sperm cryopreservation: insights from the cryomicroscope

William V. Holt[a,*], Alfredo Medrano[b], Lisa M. Thurston[c], Paul F. Watson[c]

[a]*ZSL Institute of Zoology, Regent's Park, London NW14RY, UK*
[b]*Facultad de Estudios Superiores-Cuautitlan, National Autonomous University of Mexico, 54740 Cuautitlan Izcalli, Mexico*
[c]*Royal Veterinary College, London NW10TU, UK*

Like any other experiments, semen cryopreservation studies require replication with samples from different individuals for the estimation of variance, so that the experimental data can be evaluated using formal statistical analysis. This rightly engenders an approach in which the evaluation of treatment effects is the priority, significant between-replicate variance being something of a nuisance. However, critical evaluation of much of the semen cryopreservation literature suggests that this approach actually hides much that is of interest, especially in terms of species and individual variation.

Pazienti oncologici

Sperm (and oocyte) cryopreservation could be useful for oncologic patients as well as those with several other pathologies

Human Reproduction, Vol.26, No.11 pp. 2935–2943, 2011
Advanced Access publication on August 26, 2011 doi:10.1093/humrep/der281

human reproduction

REVIEW *Andrology*

Sperm storage for cancer patients in the UK: a review of current practice

Vinay Sharma*

The Leeds Centre for Reproductive Medicine, Seacroft Hospital, Leeds LS146UH, UK

*Correspondence address. E-mail: vinay.sharma@leedsth.nhs.uk

Submitted on June 1, 2011; resubmitted on July 26, 2011; accepted on August 1, 2011

In the UK in the period 1979-2008 about 75% of cancer patients were over 65 years old and about 1% were aged under 14 years. About 24% of tumors hit patients between 15 and 64 years.

(1) possible deterioration or loss of viability of gametes or embryos as a result of storage, handling, freezing, transportation and thawing.
(2) the potential risk of cross-contamination between samples.
(3) the regulations for statutory storage periods for gametes and embryos.
(4) the regulations for extending storage periods including, in the case of embryos, the requirement for both gamete providers to consent to any extension of storage.
(5) the likelihood of a live birth resulting from previously cryopreserved embryos or gametes.
(6) the treatments that may be necessary.
(7) the screening tests to be done.
(8) the cost of these.
(9) the reason for them.
(10) the implications of the tests for the gamete providers.

Effect of chemotherapy on fertility

Table I Long-term fertility prognosis following treatment with different agents

Good	Moderate	Poor
Azathioprine	Thiotepa	Cyclophosphamide (>7.5 g/m^2) (Meistrich *et al.*, 1992)
Fludarabine	Gemcitabine	Ifosfamide (>60 g/m^2) (Williams *et al.*, 2008)
Methotrexate	Cisplatin	Mustine, carmustine
6-mercaptopurine	Oxaliplatin	Busulfan
	Carboplatin	Chlorambucil (>1.4 g/m^2)
Vincristine	Doxorubicin	Melphalan (140 mg/m^2)
Vinblastine	Dacarbazine	Chlormethine
Bleomycin	Cytosine-arabinoside (cytarabine)	Procarbazine (>4 g/m^2) (Bokemeyer *et al.*, 1994)
Actinomycin-D	Daunorubicin	Cisplatin (>600 mg/m^2) (Petersen *et al.*, 1994; Pont and Albrecht, 1997)
Etoposide	Mitoxantrone	Mechlorethamine

Adapted from Meirow and Schenker, 1995; Howell and Shalet, 2001.

Testes cryopreservation

Human Reproduction Update, Vol.16, No.3 pp. 312–328, 2010
Advanced Access publication on January 4, 2010 doi:10.1093/humupd/dmp054

human reproduction update

Options for fertility preservation in prepubertal boys

Christine Wyns, Mara Curaba, Bernard Vanabelle, Anne Van Langendonckt, and Jacques Donnez[1]

Department of Gynecology, Université Catholique de Louvain 1200, Brussels, Belgium

[1]Correspondence address. Department of Gynecology, Université Catholique de Louvain, Cliniques Universitaires St Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium. Tel: +32-2-764-95-01; Fax: +32-2-764-95-07; E-mail: jacques.donnez@uclouvain.be

Table II Indications for immature testicular cryopreservation in case of malignant and non-malignant disease

Malignant	Non-Malignant
• Leukemia • Hodgkin's disease • Non-Hodgkin's lymphoma • Myelodysplastic syndromes • Solid tumors • Soft tissue sarcoma	(1) HSCT in case of: • hematological disorders: thalassemia major, sickle cell disease, aplastic anemia, Fanconi anemia • primary immunodeficiencies • severe autoimmune diseases unresponsive to immunosuppressive therapy: juvenile idiopathic arthritis, juvenile systemic lupus erythematosus, systemic sclerosis, immune cytopenias • osteopetrosis • enzyme deficiency disease: Hurler's syndrome (2) Risk of testicular degeneration • Klinefelter syndrome

HSCT, hematopoietic stem cell transplantation.

Strategies to manage the fertility damage

in vivo protection of SSC:

- obtained or with hormonal therapies (ormonosoppressive or to stimulate spermatogonia) or with the use of anti-apoptotic and / or cytoprotective substances.
- Cryopreservation of immature gametes:
- I) cell suspensions
- II) tissue fragments

III) Whole Testes

To date not encouraging data in humans.

NEW PROSPECTIVES

- Germ cell transplantation

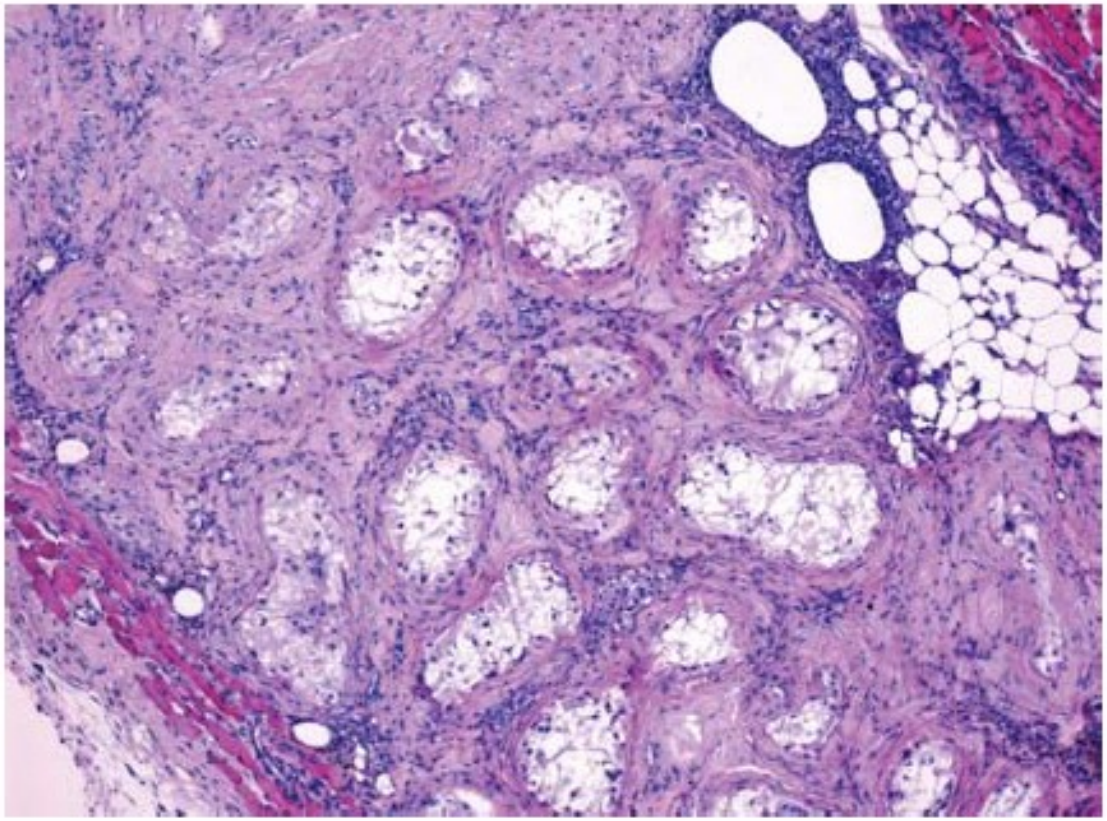


Figure 2 Histological appearance (hematoxylin–eosin sections) of donor testicular tissue from a 44-year-old man after 3 weeks' orthotopic xenografting at x200 magnification. Most tubules show degenerative changes, i.e. sclerosis, while the remaining contain mainly Sertoli cells.

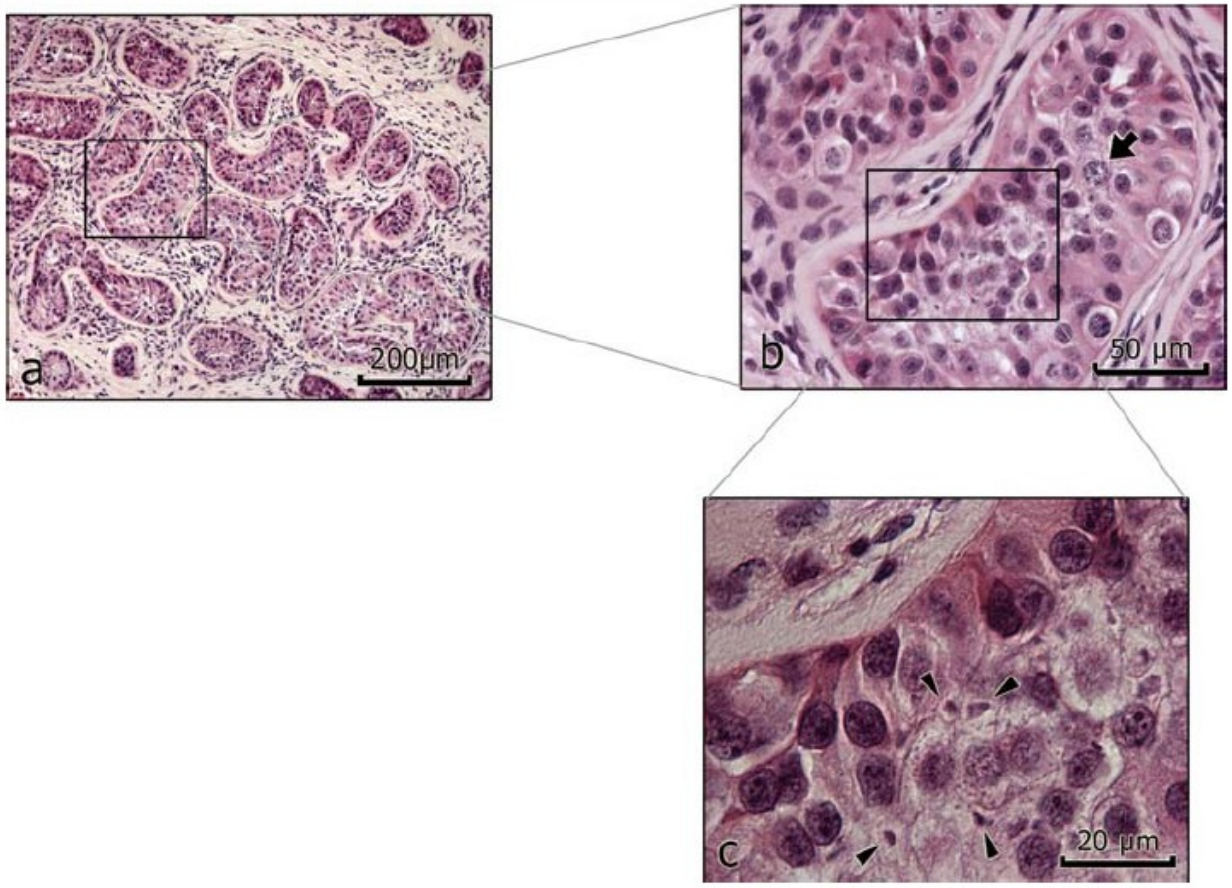
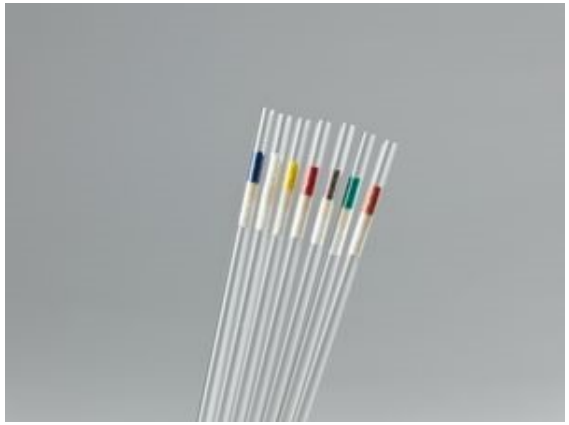
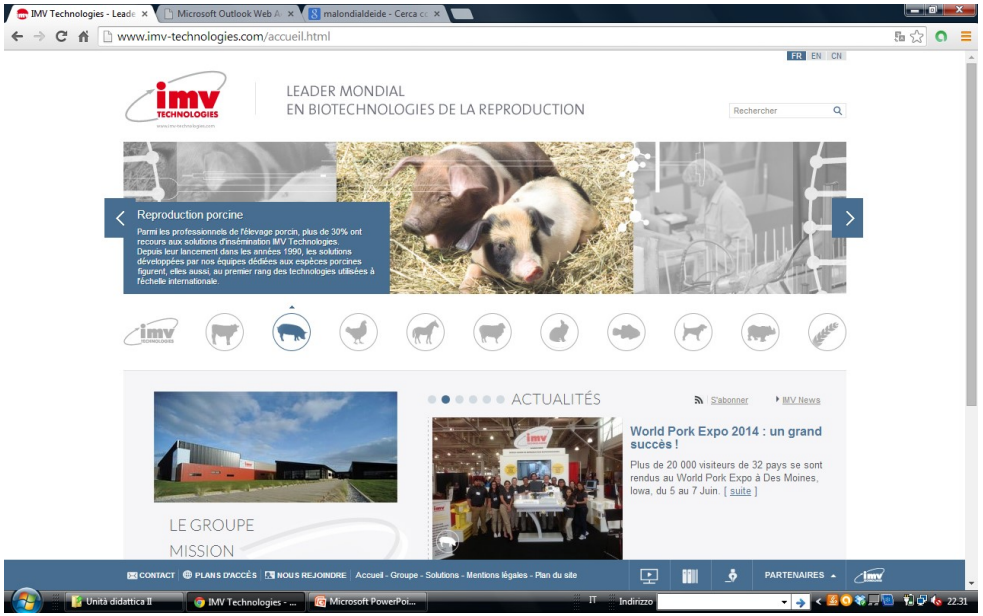


Figure 3 Histological appearance (hematoxylin–eosin sections) of donor testicular tissue from a 12-year-old boy after 6 months' orthotopic xenografting at x200 magnification (**a**), showing pachytene spermatocytes (arrow) and spermatid-like cells (inset) at x400 magnification (**b**) and spermatid-like cells at x1000 magnification (**c**).

Devices




IMV Technologies - Leade
Microsoft Outlook Web A
malondialdeide - Cerca c
www.imv-technologies.com/accueil.html
FR EN CN
imv TECHNOLOGIES
LEADER MONDIAL
EN BIOTECHNOLOGIES DE LA REPRODUCTION
Rechercher
Reproduction porcine
Parmi les professionnels de l'élevage porcin, plus de 30% ont recours aux solutions d'insémination IMV Technologies. Depuis leur lancement dans les années 1990, les solutions développées par nos équipes dédiées aux espèces porcines figurent, elles aussi, au premier rang des technologies utilisées à l'échelle internationale.
ACTUALITÉS
S'abonner
IMV News
World Pork Expo 2014 : un grand succès !
Plus de 20 000 visiteurs de 32 pays se sont rendus au World Pork Expo à Des Moines, Iowa, du 5 au 7 Juin. [suite]
LE GROUPE
MISSION
CONTACT
PLANS D'ACCÈS
NOUS REJOINDRE
Accueil - Groupe - Solutions - Mentions légales - Plan du site
PARTENAIRES
Unità didattica II
IMV Technologies - ...
Microsoft PowerPoi...
IT
Indirizzo
22.31


BTS

CRIOMOCROSCOPY

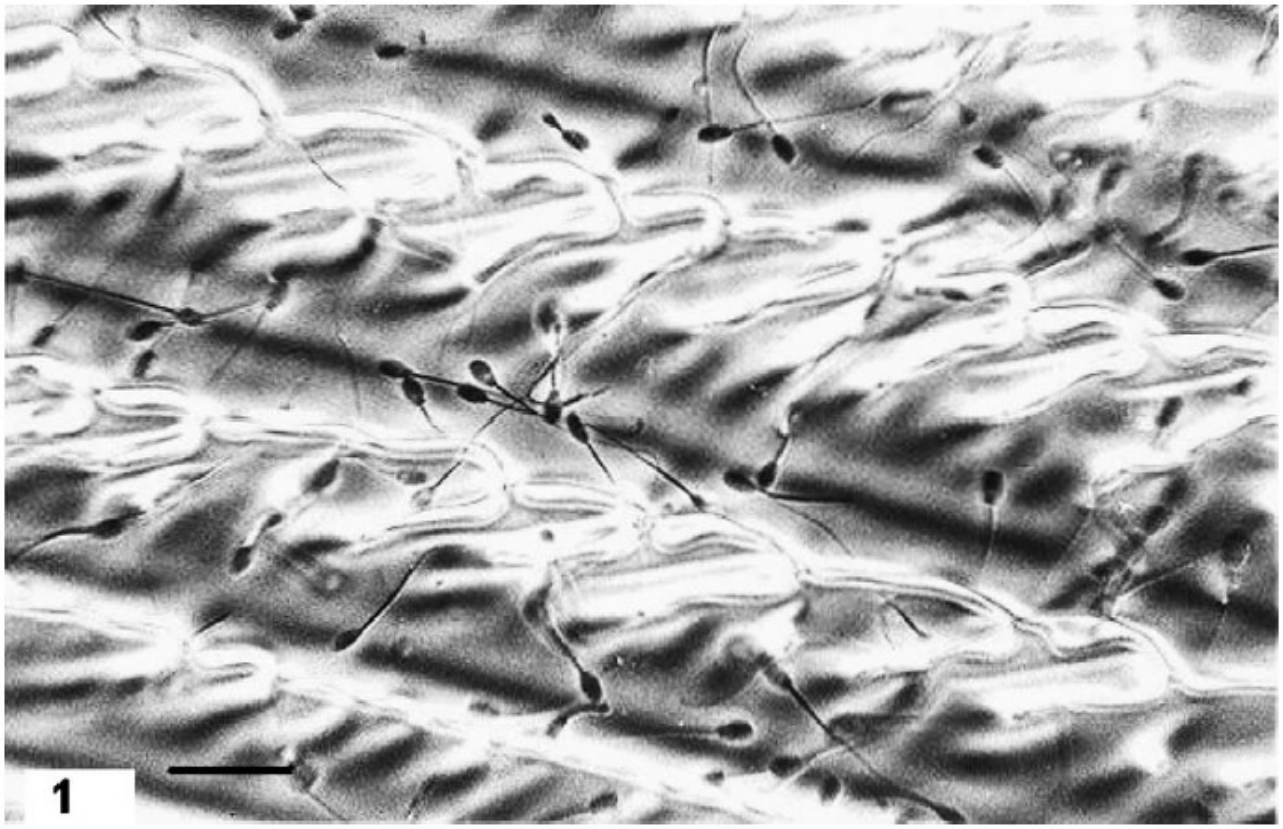


Fig. 1. Photomicrograph of ram spermatozoa diluted in saline media, frozen on the cryomicroscope stage at 10 °C/min and viewed at −15 °C. Dark diagonal regions indicate zones where the solutes have become concentrated during the freezing process. The intervening brightly contrasted regions are ice crystal formations. It is unclear whether spermatozoa are distributed between or within the ice crystals. Horizontal bar = 25 μm.

TESTS

Electronic microspcopy

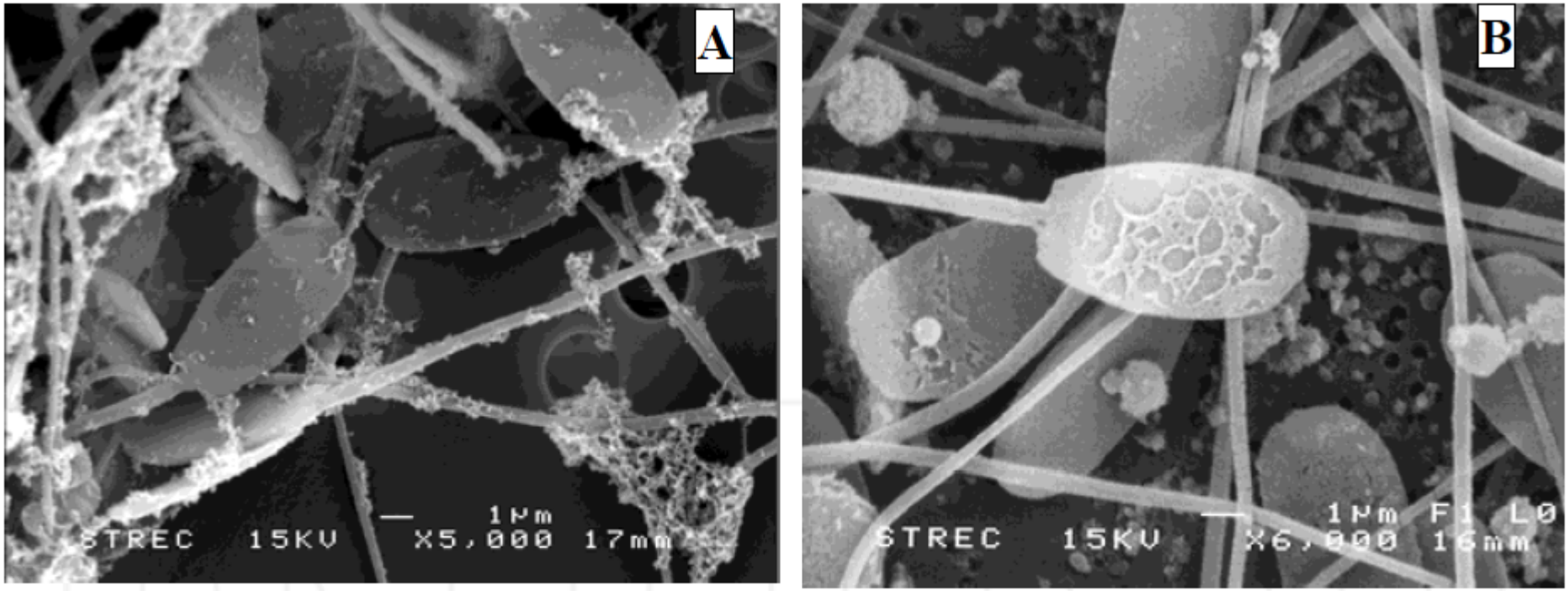


Fig. 2. Scanning electron microscopic (SEM) picture of fresh boar semen with normal plasma membrane (A) as compare with SEM picture of frozen boar semen with plasma membrane damage (B).

Kit Live/Dead

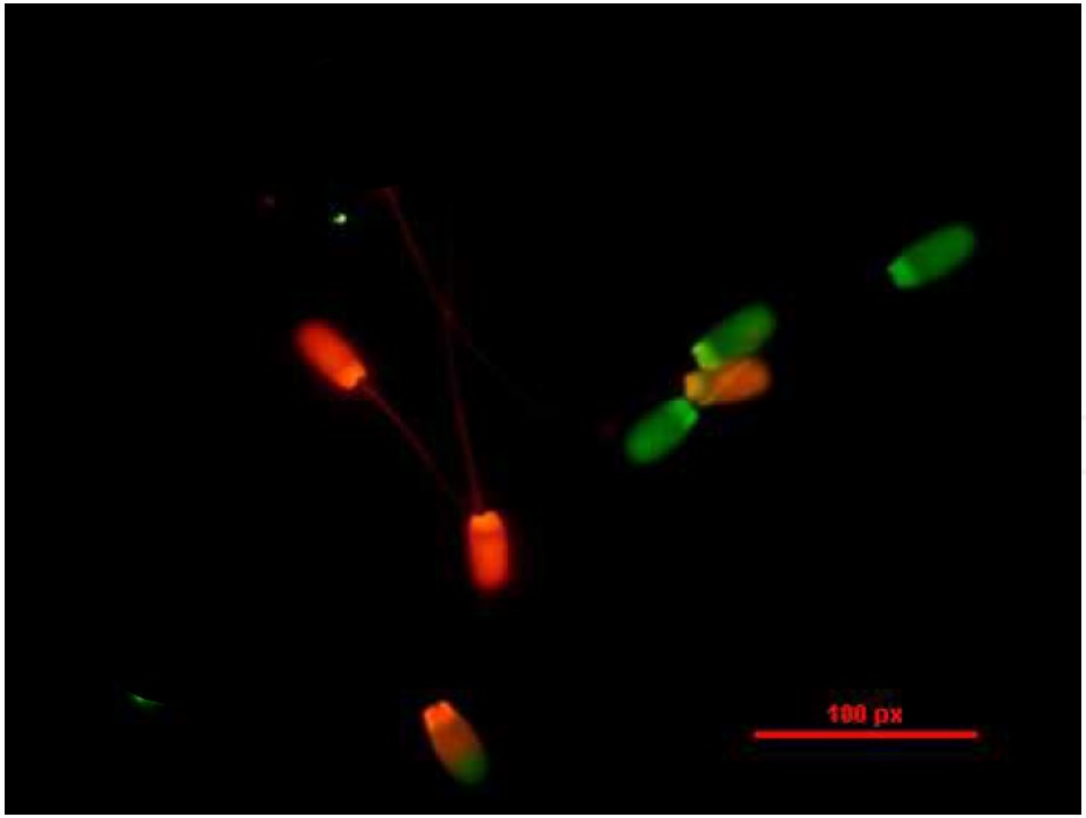
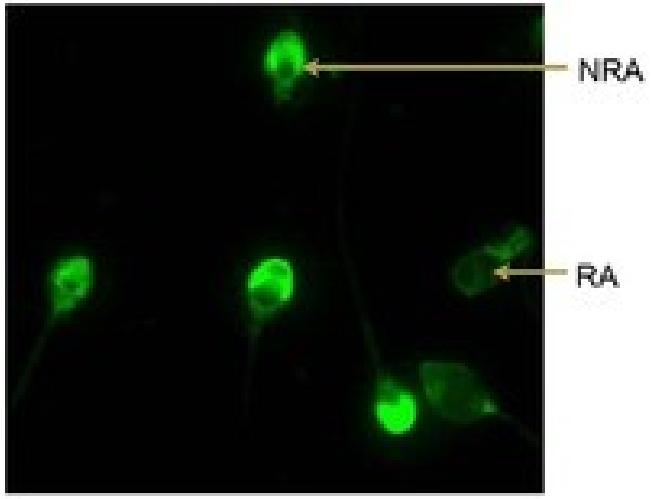


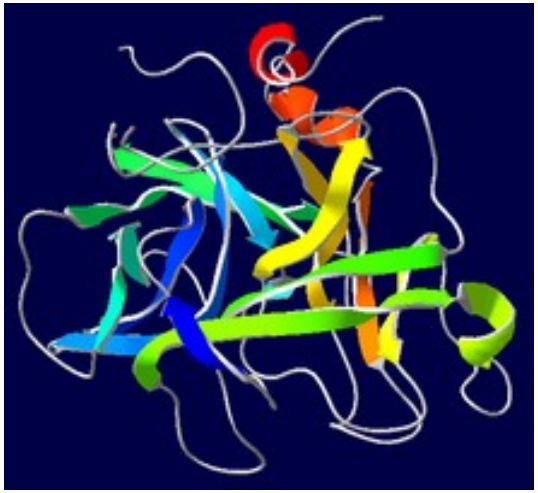
Fig. 3. Spermatozoa stained with SYBR-14/EthD-1 or PI: live spermatozoa stained green with SYBR-14 while dead spermatozoa stained red with EthD-1 or PI.

Acrosome intgrity

- PSA



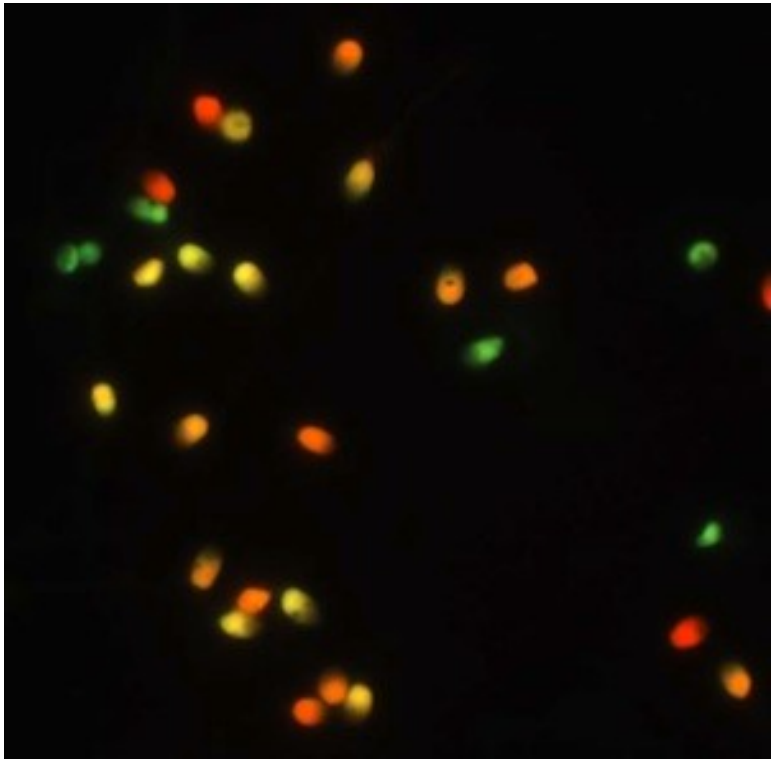
- acrosin

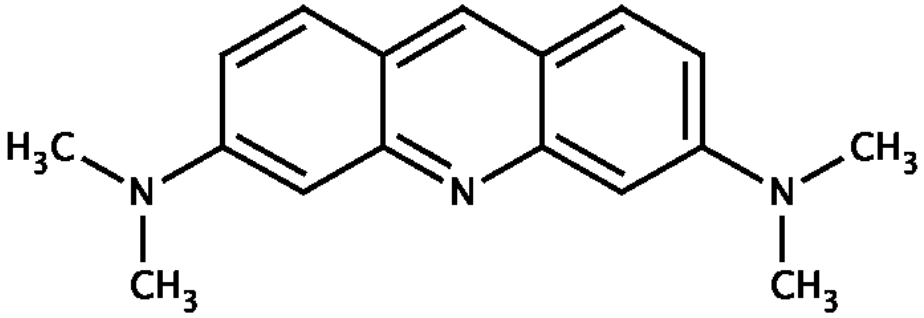


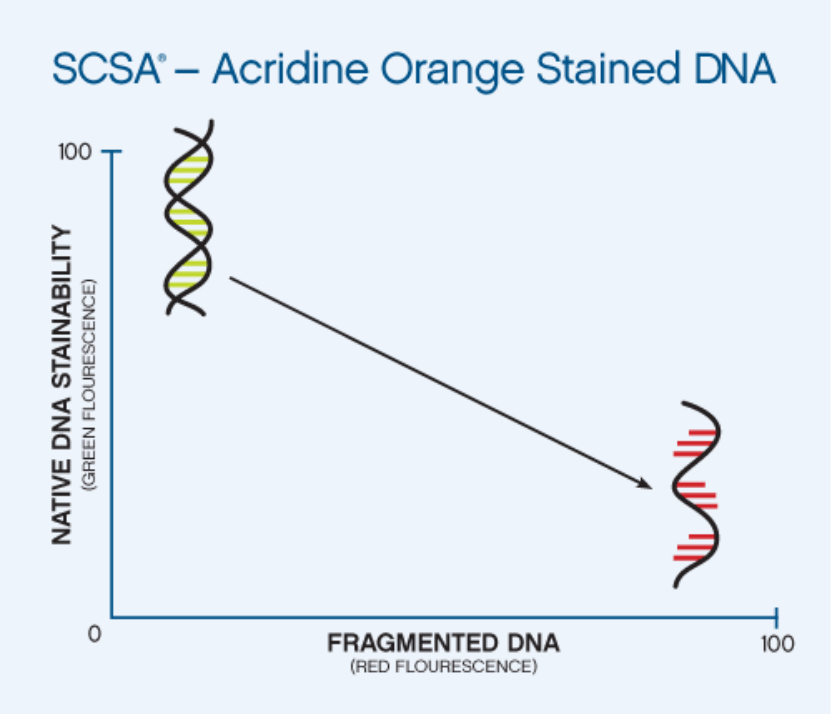
- HOS test

The functional integrity of the sperm plasma membrane will be assessed using a short hypo-osmotic swelling test (sHOST) (Perez-Llano et al., 2001). Spermatozoa are incubated, at 38 °C for 30 min, with 75 mOsm/kg a hypo-osmotic solution that consist of 0.368 % (w/v) Na-citrate and 0.675 % (w/v) fructose (Merck, Germany) in distilled water. Following this incubation time, 200 µl of the semen-hypo-osmotic solution is fixed in 1000 µl of a hypo-osmotic solution plus 5 % formaldehyde (Merck, Germany), for later evaluation. Two hundred spermatozoa are assessed under a phase contrast microscope at 400x magnification. The coiled tail (sHOST positive) spermatozoa found following incubation are functional intact plasma membrane.

Acridine




H3C
N
CH3
N
N
CH3
CH3


SCSA® – Acridine Orange Stained DNA
100
NATIVE DNA STAINABILITY
(GREEN FLOURESCENCE)
0
FRAGMENTED DNA
(RED FLOURESCENCE)
100

TUNEL - COMET