Cryopreservation of semen: necessary or just useful?

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Sperm and testicular tissue banking

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Overview of freezing

- Why freeze
- What to freeze
- The process of freezing
- Effect of freezing on sperm parameters
- Use of frozen samples

Systemic malignancies affect fertility before treatment

- increased catabolic state
- malnutrition
- hypothalamic dysfunction *Vigersky et al 1977*
- stress hormone secretion *Schenker et al 1992*
- testicular dysfunction *Fitoussi et al 2000*

Impact of cancer therapy on spermatogenesis

Table 1 The impact of radiation therapy or systemic chemotherapy agents on spermatogenesis in patients with cancer

Agent	Cumulative dose	Azoospermia	Additive effect with other chemotherapy drugs	Comments
Radiation				
Gonads	2.5 Gy/0.6 Gy	Permanent/temporary	Yes	3 to 7 weeks courses worse than single dose. 0.15 Gy decrease count
Total body	8 Gy Single 12 Gy fract.	Permanent	Yes	
Chemotherapy				
Cyclophosphamide	19 gm/m^2	Yes		
Chlorambucil	1.4 g/m^2	Yes		
Cisplatin	500 mg/m^2	Yes		
Procarbazine	4 g/m^2	Yes		
Carboplatin	>2 g/m^2	Likely		
Nitrosoureas				
Busulfan	>600 mg/kg	Likely		
Ifosfamide	>30 g/m^2	Likely	+cyclophosphamide	
Carmustine	1 g/m^2	Likely		
Lomustine	500 mg/m^2	Likely		
Nitrogen mustard		Unknown		Used with other highly gonadotoxic agents
Malphalan		Unknown		Same
Actinomycin D		Unknown		Same
Doxorubicin	770 mg/m^2	Temp oligo (alone)	Yes	Azoo in combo
Cytosine arabinoside	1 g/m^2	Temp oligo (alone)	Yes	Azoo in combo
Vinblastine	50 g/m^2	Temp oligo (alone)	Yes	Azoo in combo
Vincristine	8 g/m^2	Temp oligo (alone)	Yes	Azoo in combo; Less toxic than vinblastine
Pacliatxel		Unknown		
Docetaxel		Unknown		
Gemcitabine		Unknown		
Trastuzumab		Unknown		
Irinotecan		Unknown		
Oxaliplatin		Unknown		

Abbreviations: Azoo = azoospermia, Combo = combination, Oligo = oligospermia, Temp = temporary.

Male fertility and cancer treatment

Male fertility preservation and cancer treatment

Table 2 Gonadal toxicity of selected chemotherapy regimens used as systemic therapies in patients with potentially curable cancers

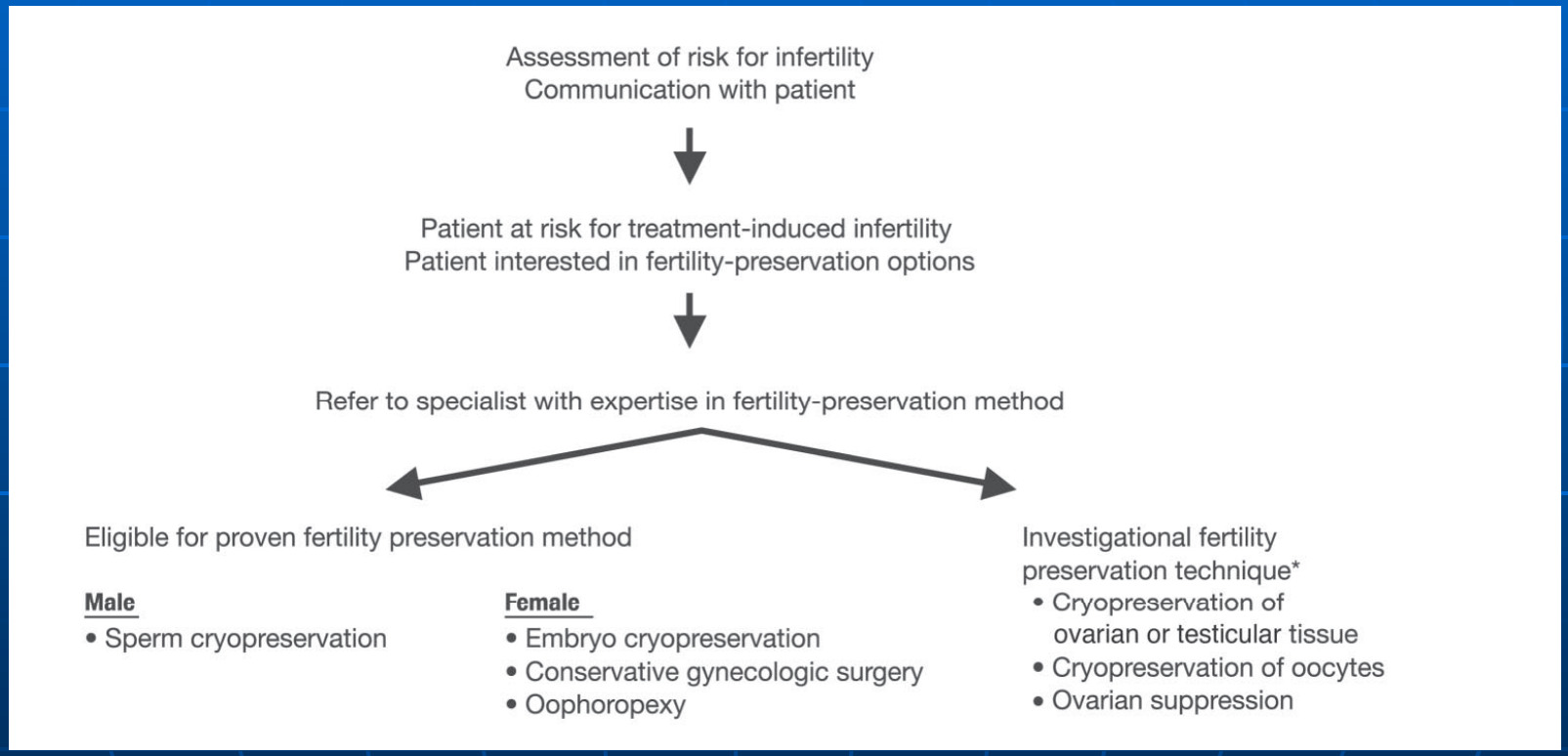
Disease/regimen	Potential for azospermia (%)	Ref.
Hodgkin's disease		
MOPP or MVPP	85	34,35
ABVD	0	23,36
ChlVPP	87	37,38
MOPP/ABVD	100	39
Non-Hodgkin's lymphoma		
CHOP-Bl	17 to 53[a]	40
COPP	0 to 67	41,42
Sarcomas		
CyVADIC or CyADIC	30 to 90[a]	43
Testicular cancer		
PVB	14 to 28[b]	44,45

Puscheck E. Cancer Treat Reviews (2004) 30, 173–180

Cancer treatment can affect the genetic material of the offspring

- Single-gene mutations and chromosomal aberations in spermatogonia *Witt and Bishop 1996*
- Persistense of mutation (early vs late stage of spermatogenesis) *Meistrich et al 1993*

American Society of Clinical Oncology Recommendations on Fertility Preservation in Cancer Patients



Stephanie J. Lee et al, J Clin Oncol vol 24 (18) 2006

Conservation of fertility

- Sperm banking
- Testicular tissue banking
- Spermatogonial stem cell banking and in vitro maturation?

Sperm banking

‘‘FUNDAMENTAL CRYOBIOLOGY’’ = understanding the effects of low temperature on cellular systems.

- Sample mixed with cryoprotectant, then undergo slow-freezing, and then maintained at -196º C, in liquid nitrogen tanks for many years
- Later use in IUI, IVF, ICSI

Differences between species *(W.V. Holt, Theriogenology 53, 47–58, 2000)*

Freeze-drying of sperm ? M.A. Ward, *(Biol. Reprod. 69 (2003) 2100–2108)*

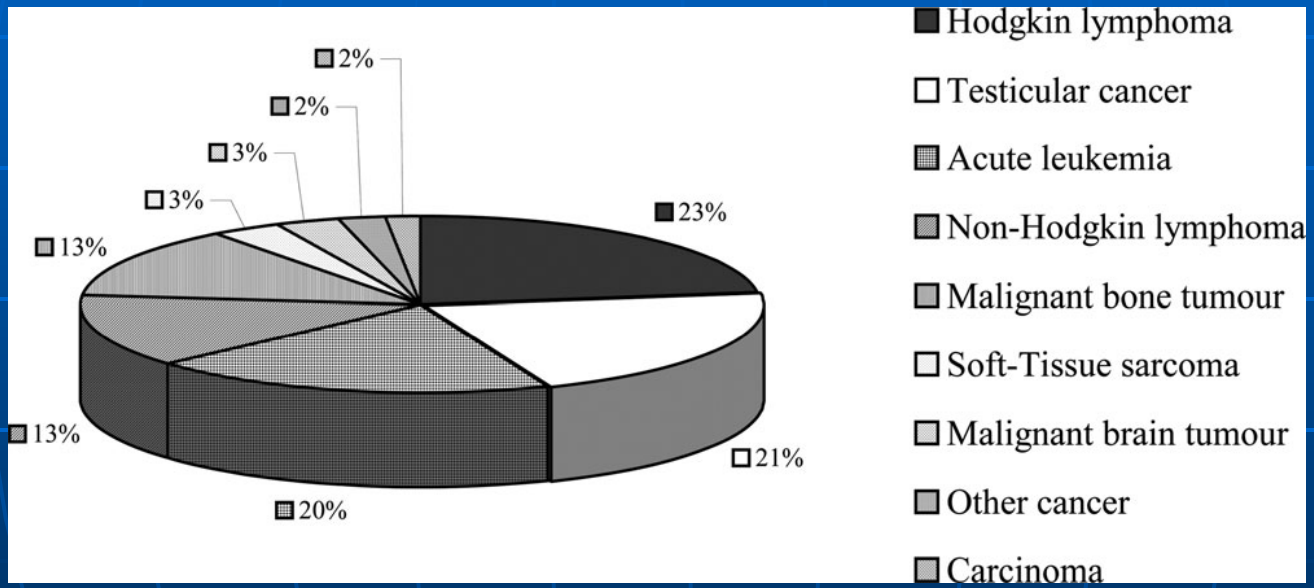
Distribution (%) of malignant disease

Table 2
Types of cancer

Testicular cancer	42 (26%)
Hodgkin's lymphoma	35 (21%)
Leukemia	23 (13%)
Gastrointestinal	15 (9%)
Prostate	8 (5%)
Non-Hodgkin's lymphoma	8 (5%)
Brain	7 (4%)
Genito-urinary	5 (3%)
Sarcoma	5 (3%)
Lung	3 (2%)
Thyroid	1
Melanoma	1
Aplastic	1
Breast	1
Liver	1
Thymus	1
Spine	1
Unspecified	6 (4%)

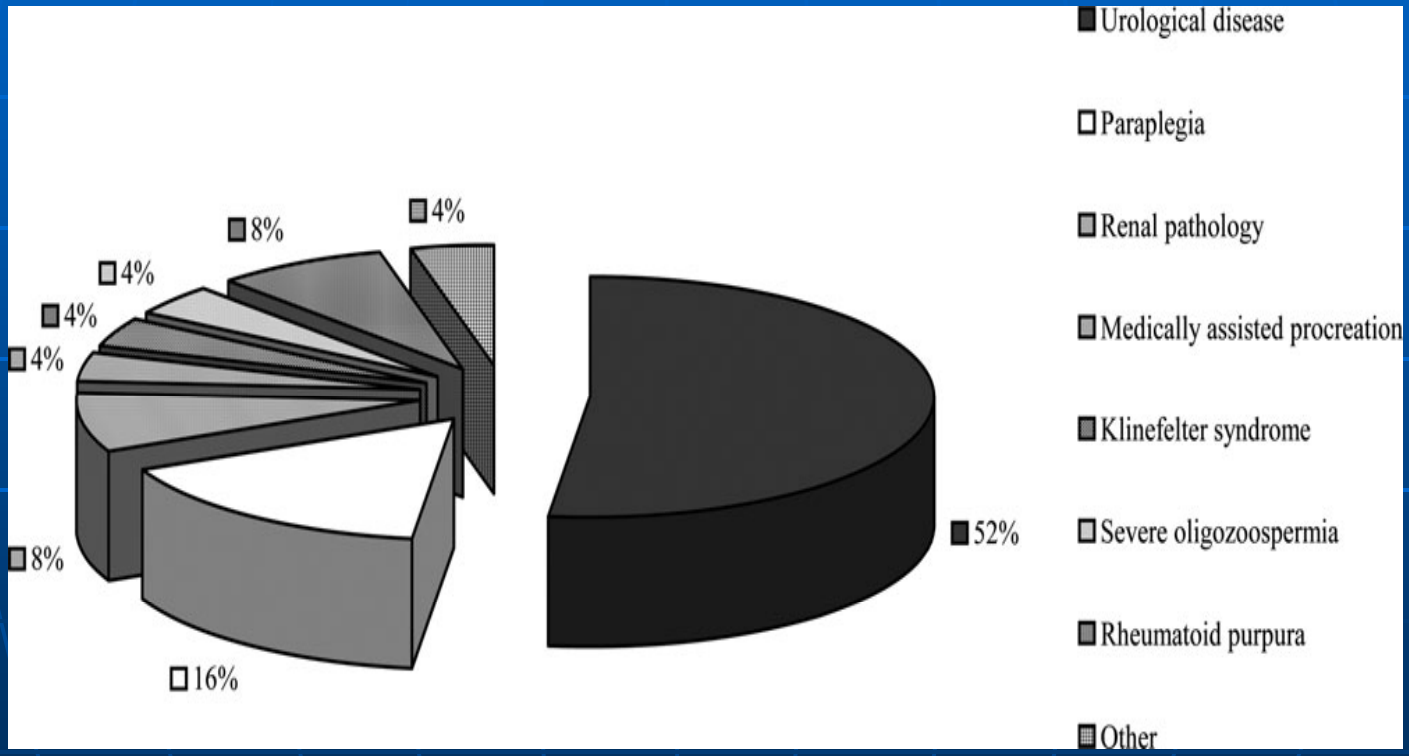
- *K. Chung et al. European Journal of Obstetrics & Gynecology and Reproductive Biology 113S (2004) S7–S11*

Distribution (%) of malignant disease.



- *Menon S. et al, Hum Reprod, Vol.24, 37 – 44, 2009*

Distribution (%) of non-malignant disease.



- *Menon S. et al, Hum Reprod, Vol.24, 37 – 44, 2009*

Tissue banking

- cryobiological complications arise due to the high tissue compartmentalization
- variation in cell types, somatic cells (i.e., Leydig, Sertoli cells) and germ cells
- protection of cell-to-cell interactions

Picton H.M et al, Br. Med. Bull. 56 (2000) 603–615.
Res U., et al , Hum. Reprod. 15 (2000) 861–864.

Spermatogonial stem cell banking

- Isolation of various pre-meiotic cells of the germ line
- In vitro maturation and cell expansion

Avarbock M.R. et al, Nat. Med. 2 (1996) 693–696.

- Tissue drafting

Schlatt S.et al , Biol. Reprod. 68 (2003) 2331–2335.
Honaramooz A, et al Nature 15 (2002) 778–781.

Attitudes of Physicians and Parents Towards Discussing Infertility Risks and Semen Cryopreservation With Male Adolescents Diagnosed With Cancer

. . . because of the private character of sexuality and the potentially inadequate maturity assessment by parents, semen cryopreservation should be discussed separately with adolescent and parents...

de Vries et al, Pediatr Blood Cancer 2009;53:386–391

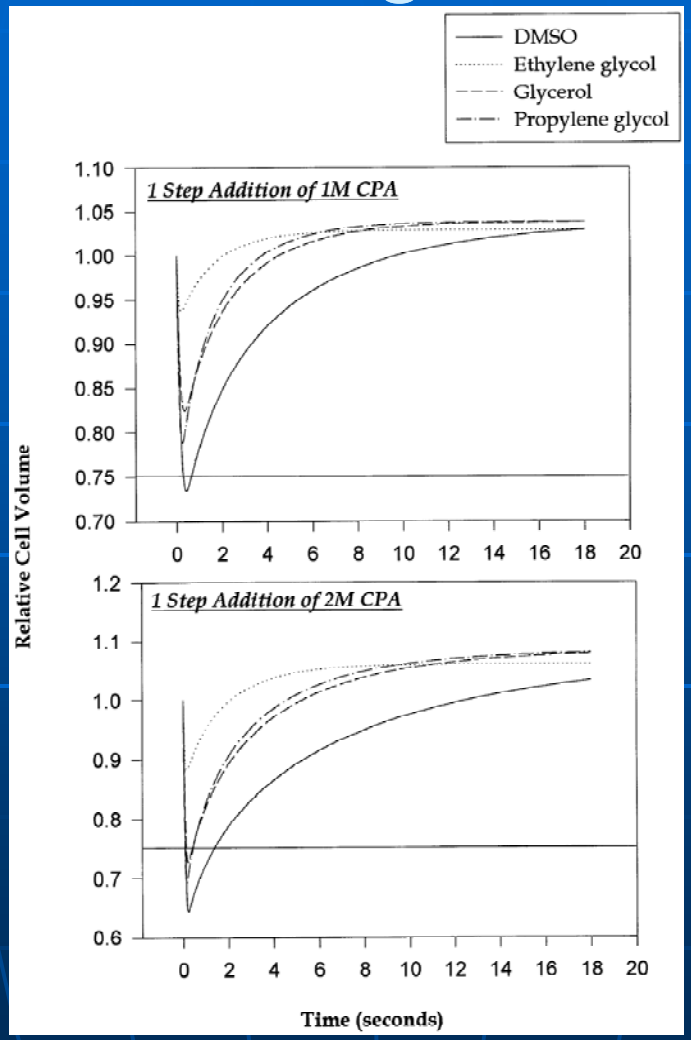
Process of freezing-thawing

- Addition of cryoprotectant
- Freezing : dehydration and shrinkage of the cell
- Thawing : rehydration and swelling upon removal

- This shrinkage and or swelling is capable of causing damage or even cell death
- Potentially damaging intracellular ice will form

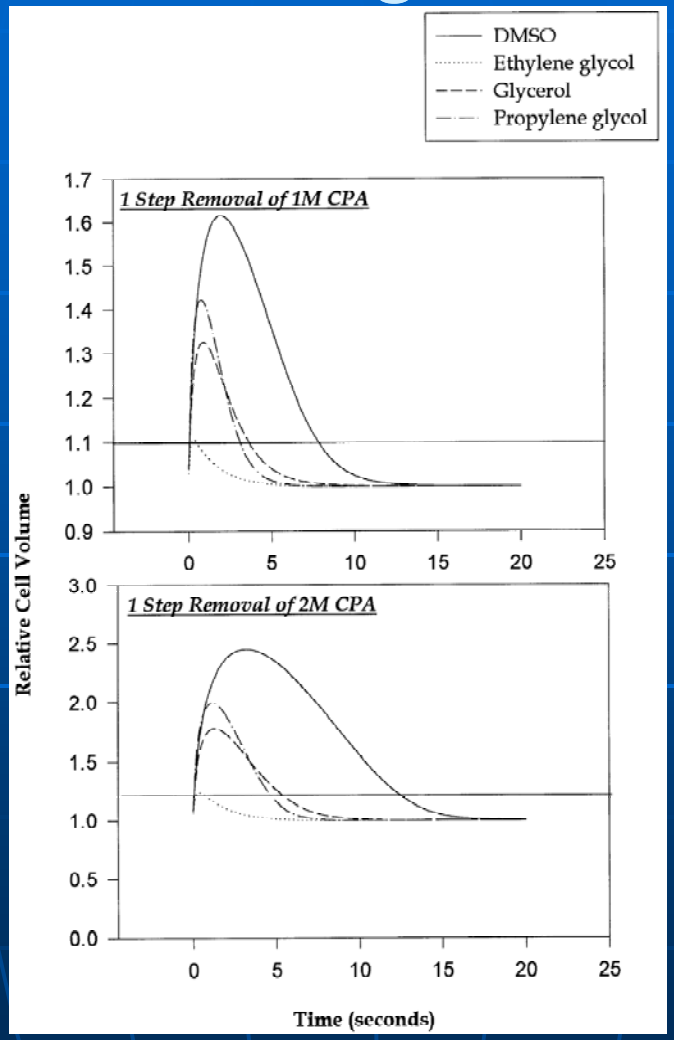
D.Y.Gao et al Hum Reprod vol.10 no.5 pp.1109-1122, 1995

Effect of freezing on cell volume



Gilmore J.A. et al Hum Reprod, *vol 12 , 112–118, 1997*

Effect of thawing on cell volume



J.A.Gilmore et al Hum Reprod, *l.12 , 112–118, 1997*

Cryoprotectans

- **Glycerol**

Polge C et al, Nature 164 (1949) 666–676

- **Dimethyl Sulfoxide**

Lovelock J.E. and Bishop M., Nature 183 (1959) 1394–1395.

- Other

Effect of type of cryoprotectant on sperm viability and tail swelling

TABLE 4

Effects of multistep (protocol A) and single-step (protocol B) treatment by different CPAs on human sperm viability and swelling status (coiled, stump, or straight sperm tails).

	% Live		Δ % Coiled tail[a]		Δ % Stump tail[a]		% Straight tail	
Treatment	Protocol A	Protocol B	Protocol A	Protocol B	Protocol A	Protocol B	Protocol A	Protocol B
PD3	1.00 ± 0.02	0.94 ± 0.03	40.4 ± 7.6	56.5 ± 7.3	8.1 ± 3.4	8.9 ± 2.4	0.54 ± 0.09	0.36 ± 0.08
GLY	1.03 ± 0.03	0.92 ± 0.02	26.7 ± 6.1	43.3 ± 6.7	5.7 ± 2.0	8.8 ± 1.8	0.66 ± 0.08	0.45 ± 0.07
EG	0.95 ± 0.03	0.94 ± 0.02	40.0 ± 7.7	53.6 ± 1.7	6.1 ± 1.7	12 ± 1.5	0.53 ± 0.08	0.33 ± 0.02
DMSO	0.88 ± 0.03	0.88 ± 0.04	45.5 ± 4.4	63.6 ± 6.0	13 ± 3.9	6.7 ± 0.8	0.50 ± 0.05	0.29 ± 0.05
EHP	0.87 ± 0.10	0.98 ± 0.05	19.8 ± 7.9	42.7 ± 10.1	0.2 ± 0.2[b]	12 ± 4.8	0.63 ± 0.19	0.54 ± 0.11

Note: The CPAs tested were PD3, GLY, EG, DMSO, and EHP. Values (mean ± SEM and n = 7 for each CPA, except for EHP, where n = 5) are expressed as ratios of control in the same experiments (see Table 2 for original control values), except where stated otherwise.

[a] Differences in percentage points from control values.

[b] Significantly different between protocols B and A for the same parameter.

Widiasih. Cryoprotectant addition to human spermatozoa. Fertil Steril 2009.

Widiasih et al,Fertil Steril 2009;92:382–9. 2009

Effect of multistep and single-step addition of cryoprotectant

TABLE 1

Effects of single- and multistep treatment by CPAs on human sperm viability, motility, mucus penetration (number of spermatozoa at 1 and 4.5 cm), and swelling status (coiled, stump, or straight sperm tails).

Treatment	% Live	% Motile	% Progressive	Mucus 1 cm	Mucus 4.5 cm	% Coiled[a]	% Stump[a]	% Straight
Control (protocol C)	81 ± 1	71 ± 2[b]	84 ± 3[b]	193 ± 3[b]	70 ± 9[b]	1.5 ± 0.3[b]	0.5 ± 0.1[b]	98.0 ± 0.3[b]
Single step (protocol B)	0.93 ± 0.01	0.61 ± 0.04[c]	0.78 ± 0.05	0.55 ± 0.06[c]	0.12 ± 0.03[c]	53.1 ± 2.8[c]	9.5 ± 0.9[c]	0.38 ± 0.03[c]
Multistep (protocol A)	0.94 ± 0.02	0.76 ± 0.04	0.83 ± 0.05	0.69 ± 0.06	0.30 ± 0.06	35.9 ± 3.1	7.0 ± 1.3	0.56 ± 0.04

Note: Values (mean ± SEM, n = 33) given for controls (protocol C) are raw values, and those for treatment by CPAs are expressed as ratios of controls in the same experiments, except when stated otherwise (superscript a).

[a] Values in C are raw data, and values in B and A are differences in percentage points from C.

[b] Significantly different from protocols B and A for the same parameter.

[c] Significantly different between protocols B and A for the same parameter.

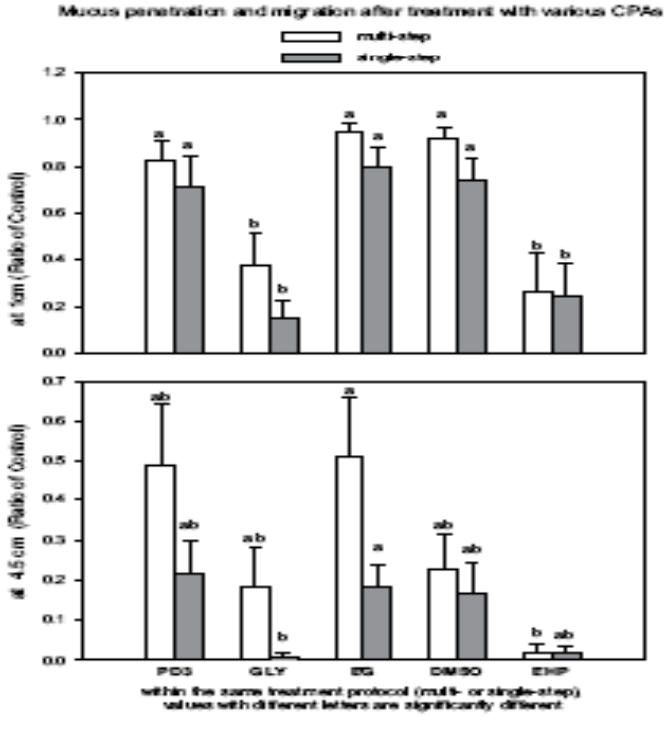
Widiasih. Cryopreservation addition to human spermatozoa. Fertil Steril 2009.

Widiasih et al,Fertil Steril 2009;92:382–9. 2009

Effect of cryoprotectant on sperm function

FIGURE 2

Mucus penetration (number of spermatozoa in the treated group as a ratio of that of the control) at 1 cm (upper panel) and at 4.5 cm (lower panel; ordinate) of spermatozoa treated with various CPAs (abscissa) in multistep (open columns) and one-step (gray columns) procedures. For either protocol, columns bearing different superscripts are significantly different.



Widiasih. *Cryoprotectant addition to human spermatozoa. Fertil Steril 2009.*

Widiasih et al,Fertil Steril 2009;92:382–9. 2009

Freezing small quantities of sperm

- empty zona pellucida as the sperm carrier *(Walmsley et al., 1998; Fusi et al., 2001)*
- microdroplets on ice ‘pills’ *(Gil-Salom et al., 2000)*
- open straws *(Koscinski et al., 2007)*
- cryoloops *(Nawroth et al., 2002; Schuster et al., 2003; Isachenko et al., 2004b, 2005)*

Survival of micro-organism during cryostorage

- Chlamydia
- Ureaplasma urealyticum and Mycoplasma hominis
- Yeasts
- Cytomegalovirus

Vapor phase storage

...no direct evidence of cross contamination in a cryobank....

Tomlinson and Sakkas, Hum Reprod 15, 2460-63, 2000

- Cost of vapor phase
- Temperature stability

Vapor phase storage

- New pathohens
- Incubation period of pathogens
- Liquid nitrogen is a sourse
- Microorganisms found in both types
- Temperature stability
- Cost of vapor phase

Cryoinjury

Damage on chromatin

- chromatin condensation (*Hammadeh et al 1999*)
- chromatin structure assay (*Spano et al 1999*)

Damage induced on membranes

- lipid peroxidation of unsaturated fatty acids by free radicals (*Bell et al.,1993*)
- acrosome reaction *(Esteves et al, 2000)*

Cryoinjury

Damage induced on chromosomes

……..no alteration in the
frequency of chromosomal abnormalities or sex ratio in human spermatozoa after freezing……..

(*Chernos and Martin 1989, Martin et al., 1991)*

Effect of freezing on sperm parameters

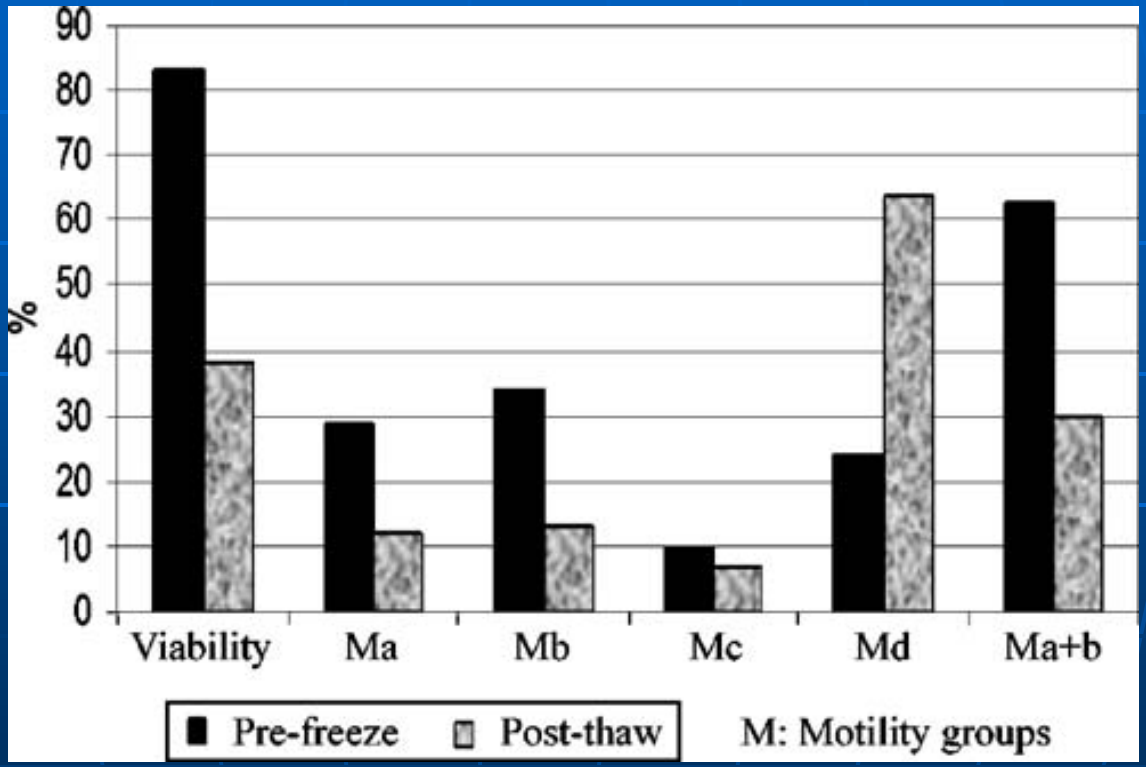
Table II Pre-freeze and post-thaw forward motility (mean ± SEM)

	Pre-freeze forward motility (*A*: %)	Post-thaw forward motility (*B*: %)	Variation of forward motility (*C*: %)	Comparison (*P*-value)
HL	29.30 ± 2.50	10.94 ± 1.73	66.66	$P < 0.0001$
TC	35.70 ± 2.38	13.31 ± 1.78	62.72	$P < 0.0001$
AL	23.30 ± 1.66	8.80 ± 2.00	62.23	$P = 0.0015$
NHL	33.45 ± 1.70	16.33 ± 2.09	51.18	$P = 0.0006$
MBT	31.79 ± 4.55	15.47 ± 2.66	51.34	$P = 0.002$
All patients ($n = 156$)	29.88 ± 1.15	12.41 ± 0.79	58.46	$P < 0.0001$

HL, Hodgkin's lymphoma; TC, testicular cancer; AL, acute leukaemia; NHL, non-Hodgkin's lymphoma; MBT, malignant bone tumour; Non-cancer, 'non-cancer' group; *C*, $[(A - B)/A] \times 100$; *n*, number of patients.

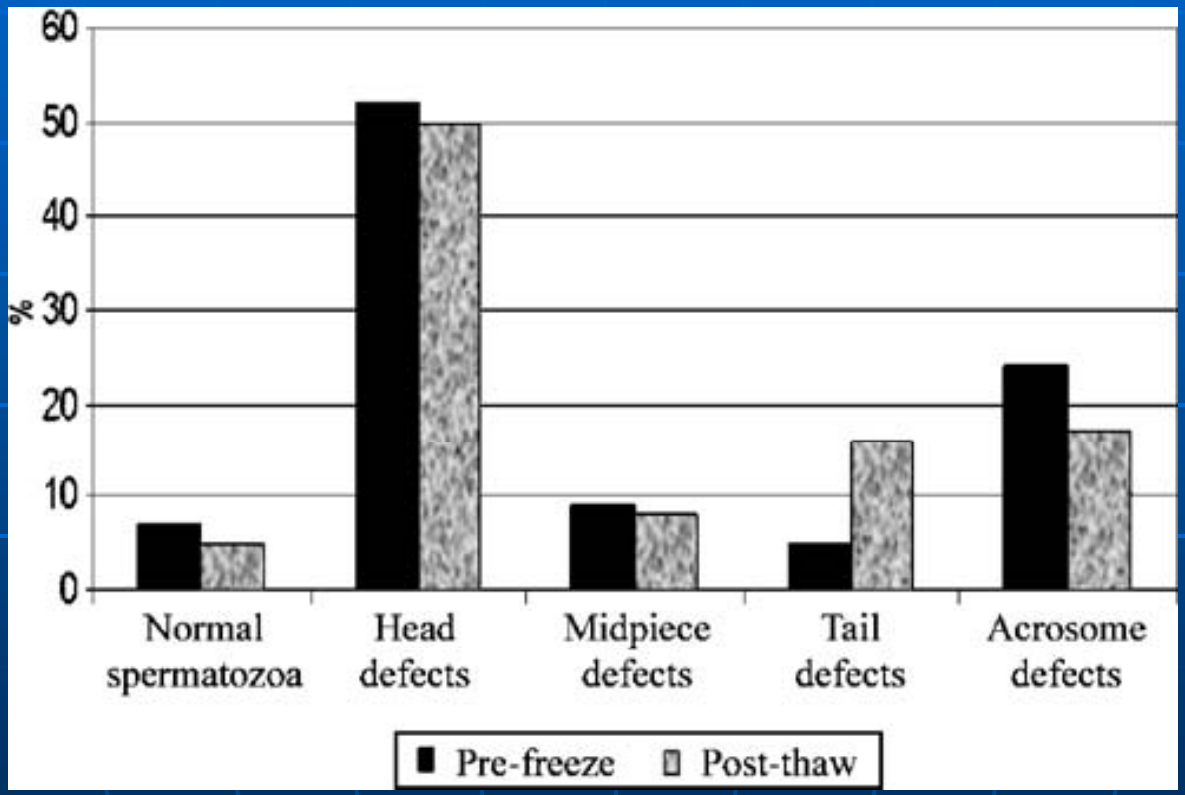
- *Menon S et al, Hum Reprod, Vol.24, 37 – 44, 2009*

Effect of freezing on sperm parameters



- *Ozkavukcu et al, J Assist Reprod Genet (2008) 25:403–411*

Effect of freezing on sperm parameters



- *Ozkavukcu et al, J Assist Reprod Genet (2008) 25:403–411*

Outcome of ART with cryopreserved spermatozoa

TABLE 6

A comparison of ART results before and after ICSI.

	ICSI	IVF	*P*[a]
No. of cycles	169	54	
Mean age of wife, years	34.8	33.4	NS
Fertilization rate, %	77.6	31.5	<.001
Failed fertilization, n (%)	1 (0.6)	6 (11.1)	<.001
Delivery rate, %	50.3 (85/169)	24.1 (13/54)	<.001

[a] *P*-values calculated by Fisher's exact test and unpaired Student's *t*-test.

Hourvitz. ICSI with sperm frozen before cancer treatment. Fertil Steril 2008.

- *Hourvitz, et al Fertil Steril 2008;90:557–63*

- Today, 1 in 700 young adults is a cancer survivor and it is estimated that 1 in every 250 adults will be a childhood cancer survivor in 2010

- In United States, more than 20,000 children and young people of reproductive age are exposed to of chemotherapy and/or radiotherapy every year

Muller J. et al Horm Res 2003;59(Suppl. 1):12–20.
Blatt J. et al Med Pediatr Oncol 1999;33:29–33.
Arnon J, et al Hum Reprod Update 2001;7:394–403.

Necessary !

... our database suggests that sperm cryostorage for fertility preservation in male cancer patients

is under-utilized....

Chung et al. / European Journal of Obstetrics & Gynecology and Reproductive Biology 113S (2004) S7–S11

Thank you !