Cryopreservation of human spermatozoa: the process of sperm freezing

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The History of Semen Cryopreservation

- 1776: Spallanzani
- 1866: Montegazza- soldier could beget a legal heir with stored semen
- 1930-1940: some sperm survived after freezing
- 1949: first cryoprotectant- glycerol (Polge *et al* , 1949)
- 1950: glycerol- egg yolk- citrate $\rightarrow \uparrow$ animal semen use
- 1963: Freezing in liquid Nitrogen at -196^0C
- Optimization of protocols (reviewed by Brotherton, 1990; Storey et al, 1998)
- Last 30 years - $\uparrow$ 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 future fertility potential

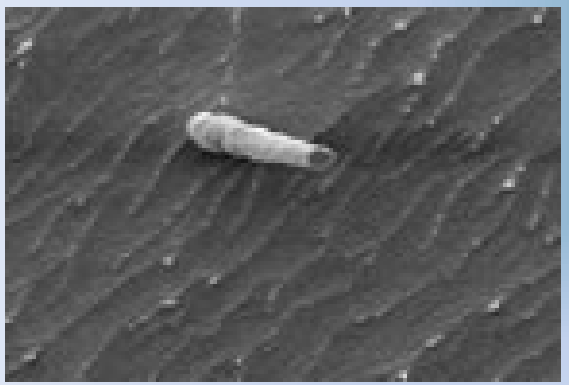
- Cancer and Multiple Sclerosis
- ART - patient
- - donor
- Pre- operative insurance
- Vasectomy insurance
- Post-mortem sperm retrieval

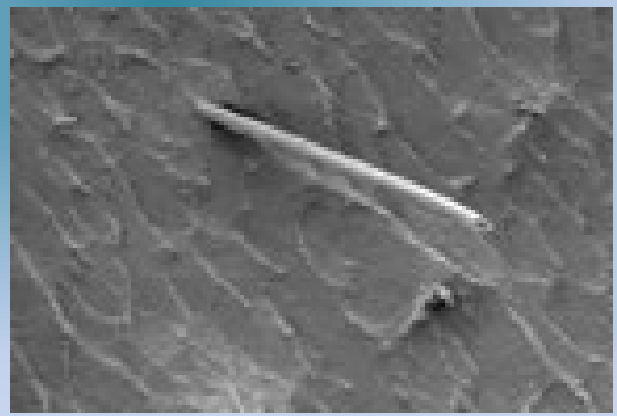
THE EFFECTS OF CRYOPRESERVATION

- Ice crystallization
- Osmotic and chilling injury
- Cytoplasmic fractures
- Effects on cytoskeleton or genome-related structures

Sperm Cryopreservation - Freeze fracture electron micrographs of cryopreserved human sperm.

Head Fracture





Tail fractures

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The effects of cryopreservation on sperm morphology, motility and mitochondrial function

M.O'Connell[1], N.McClure[1,2] and S.E.M.Lewis[1,3]

Table II. The effects of cryopreservation on sperm motility

Parameter	Fresh semen	Freeze–thawed semen	% Change	*P*-value
Motile (%)	40.2 (12.0–66.0)	24.8 (7.0–42.0)[a]	–33	0.0001
Progressively motile	22.9 (7.0–37.0)	13.6 (2.0–22.0)[a]	–41	0.0001
VAP (μm/s)	51.2 (46.9–58.4)	35.3 (27.8–46.4)[a]	–31	0.0001
VSL (μm/s)	42.1 (39.4–45.6)	29.8 (22.4–35.1)[a]	–29	0.0001
VCL (μm/s)	75.5 (67.9–84.1)	51.5 (42.1–64.3)[a]	–32	0.0001
ALH (μm)	3.8 (3.2–4.6)	3.9 (2.4–4.0)[a]	0.1	0.05
BCF (Hz)	19.1 (17.1–21.2)	15.6 (12.5–18.4)[a]	–18	0.001
LIN (%)	57.3 (52.0–62.0)	47.7 (47.0–54.0)[a]	–16	0.01

Values are medians (± IQR), $n = 50$.
[a]Significance $P < 0.05$, Wilcoxon matched pairs test.
VAP = average path velocity; VSL = straight line velocity; VCL = curvilinear velocity; ALH = amplitude of lateral head displacement; BCF = beat cross frequency; LIN = linearity.

Table III. The effects of cryopreservation on sperm morphology

Parameter	Fresh semen	Freeze–thawed semen	% Change	*P*-value
Normal morphology (%)	8.2 (4.0–13.0)	5.2. (2.0–8.0)[a]	–37	0.001
Amorphous (%)	12.0 (7.0–18.0)	18.0 (13.0–22.0)[a]	+50	0.001
Megalo (%)	7.5 (4.0–11.0)	10.5 (9.0–13.00)[a]	+28	0.001
Elongated (%)	6.5 (2.0–9.0)	9.0 (6.0–12.0)[a]	+27	0.001
Midpiece abnormalities (%)	10.0 (8.0–12.0)	19.0 (15.0–22.0)[a]	+46	0.001
Cytoplasmic droplet (%)	7.2 (2.0–13.0)	3.0 (0.0–5.0)[a]	–58	0.001
Loose heads and tails (%)	8.0 (3.0–12.0)	11.3 (6.0–19.00)[a]	+29	0.001
Tail defects (%)	6.0 (4.0–12.0)	9.3 (4.0–16.0)[a]	+35	0.001

Values are medians (± IQR), $n = 50$.
[a]Significance $P < 0.05$, Wilcoxon matched pairs test.

Table IV. The relationship between mitochondrial function and motility before and after freeze–thawing

	Before freeze–thawing				After freeze–thawing			
	R123 Uptake (%)		R123 Intensity (AU)		R123 Uptake (%)		R123 Intensity (AU)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
P.motile (%)	0.62	0.005	0.46	0.05	0.57	0.05	0.54	0.05
VAP (μm/s)	0.84	0.0001	0.52	0.05	0.33	NS	0.39	NS
VSL (μm/s)	0.79	0.0001	0.51	0.05	0.38	NS	0.37	NS
VCL (μm/s)	0.69	0.002	0.47	0.05	0.41	0.06	0.34	NS
ALH (μm)	0.43	0.03	0.39	NS	0.31	NS	0.53	0.01
BCF (Hz)	0.37	NS	0.40	NS	0.40	NS	0.33	NS
LIN (%)	0.88	0.01	0.34	NS	0.72	0.001	0.32	NS
R123 uptake (%)	0.77	0.001	0.40	NS				

Significance $P < 0.05$, $n = 50$, r = correlation coefficient (stepwise linear regression).
P.motile = progressively motile; VAP = average path velocity; VSL = straight line velocity; VCL = curvilinear velocity; ALH = amplitude of lateral head displacement; BCF = beat cross frequency; LIN = linearity; NS = not significant.

Cryo-injury is expressed as

Structural Damage-

- loss of membrane integrity
- ↓ intact acrosomes
- cytoplasmic vacuolation
- mitochondrial distortion

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