Sperm

SZILARD

Procedure for Freezing Human Semen

The fresh sample is extended with four times its volume of a solution prepared by homogenizing fresh egg white in a buffer of the following composition.

NaCl	5.00 g./l
KCl	0.20 g./l
$CaCl_2$	0.20 g./l
$MgCl_2 \cdot 6H_2O$	0.10 g./l
$NaH_2PO_4 \cdot H_2O$	4.00 g/l.
$NaHCO_3$	2.00 g/l.
glucose	1.00 g/l
ion-exchange H_2O	to make 1000 ml.

P_{H^+} = 6.5

The extender is made by adding this buffer to 25 cc fresh egg white until the vol. = 100 ml. The P_{H^+} of the extender is ≈ 7.4 - 7.6

Na ham

The extended sperm sample is then slowly diluted with a solution containing 20 g. glycerol/100 ml. The solution is added stepwise ($\frac{1}{5}$ vol. of extended semen / 6 min) until the volume is doubled. The final concentration of glycerol = 10 g/100 ml extended semen.

The glycerol carrier is made by adding a buffer of the following composition to 20 gm glycerol until the volume = 100 ml.

$NaCl$: 5.030 g
KCl : 0.20 g
$CaCl_2$: 0.20 g
$MgCl_2 \cdot 6H_2O$: 0.10 g
$NaH_2PO_4 \cdot H_2O$: 0.5 g
$NaHCO_3$: 5.00 g
glucose : 1.00 g
H_2O to make 1000 ml
(P.H. 7.4)

The extended ~~sample~~ semen (sample with 10% glycerol is then dispensed into ampoules. Ordinary wasserman tubes 11-12 mm ID have been used. An ampoule made by plugging a 16 mm ID test tube with a Wasserman tube to give an annular space of 1.5 - 2.5 mm. affords more even cooling. The ampoules are sealed in the oxygen - gas flame, taking care that the sample is not heated. Except for the brief operation of sealing the samples are protected from rapid temperature fluctuations by immersion in a room temp [25-28° C] water bath.

An equilibration time of 30 min is allowed after the last glycerol addition. Then the ampoules are immersed in a freezing mixture of the proper temperature. The baths used were the following:

- −160°C = ~~Eutectic mixture of EtOH-H_2O~~ liquid-solid isopentane
- −80°C = CO_2 − EtOH
- −53°C = Eutectic mixture EtOH-H_2O (by weight 72% EtOH 28% H_2O)
- −23°C = Liquid solid − CCl_4

After reaching the bath temperature, ampoules are quickly transferred to −80°C bath, stored 24 hrs.

Selected Experimental Results:

Wasserman tube ampoules

	Immersion bath	% recovery	Original motility
I	−190° (liq N_2)	9	30%
	−79°C	57	30%
	Cooled 1°/min.	25%	30%
II	−160°C	8%	25%
	−79°C	51%	25%
	−23°C	22%	25%

~~III~~ Annular Ampoules:

III:

Immersion bath	% Recovery	Original motility
−160°C	7%	47%
− 80° C	18%	47%
− 53° C	22%	47%
− 23° C	13%	47%

Speed Measurements. 25°-24° C

No. moving sperm counted	Sample	Speed	mean dev.
260	II (control)	31 μ/sec	± 2
150	II (frozen, thawed)	29 μ/sec	± 2
365	III (control)	24 μ/sec	± 0.1
135	III (frozen, thawed	21 μ/sec	± 2
275	I (control)	28 μ/sec	± 1.5
160	I (frozen, thawed)	25 μ/sec	± 2

Dear Szilard, June 30, 1953

Marcus repeated the freezing expt. I give data below.

Assay before freezing	9.1×10^8
Mutant frequency before freezing	
T4	$147/10^8$
T5	$7.1/10^8$
T6	$14.2/10^8$

	Control	Expt
Assay after freezing	—	11.2×10^8
Regrew 10cc sample in 1 liter broth to about same density.		
Mutant frequency after regrow		
T4	$267/10^8$	$306/10^8$
T5	$19.3/10^8$	$16.7/10^8$
T6	$23.9/10^8$	$22.0/10^8$

Human sperm protocol was used.
I think the experiment was done as well as possible and I can think of no improvements

Aaron