Mouse Colony Management through Cryopreservation and Assisted Reproductive Technologies (ARTs).

2009 CCR Mouse Research Models/Mouse 101 Course

G. Charles Ostermeier, Ph.D.

SAIC-Frederick, Inc.

Laboratory Animal Science Program.

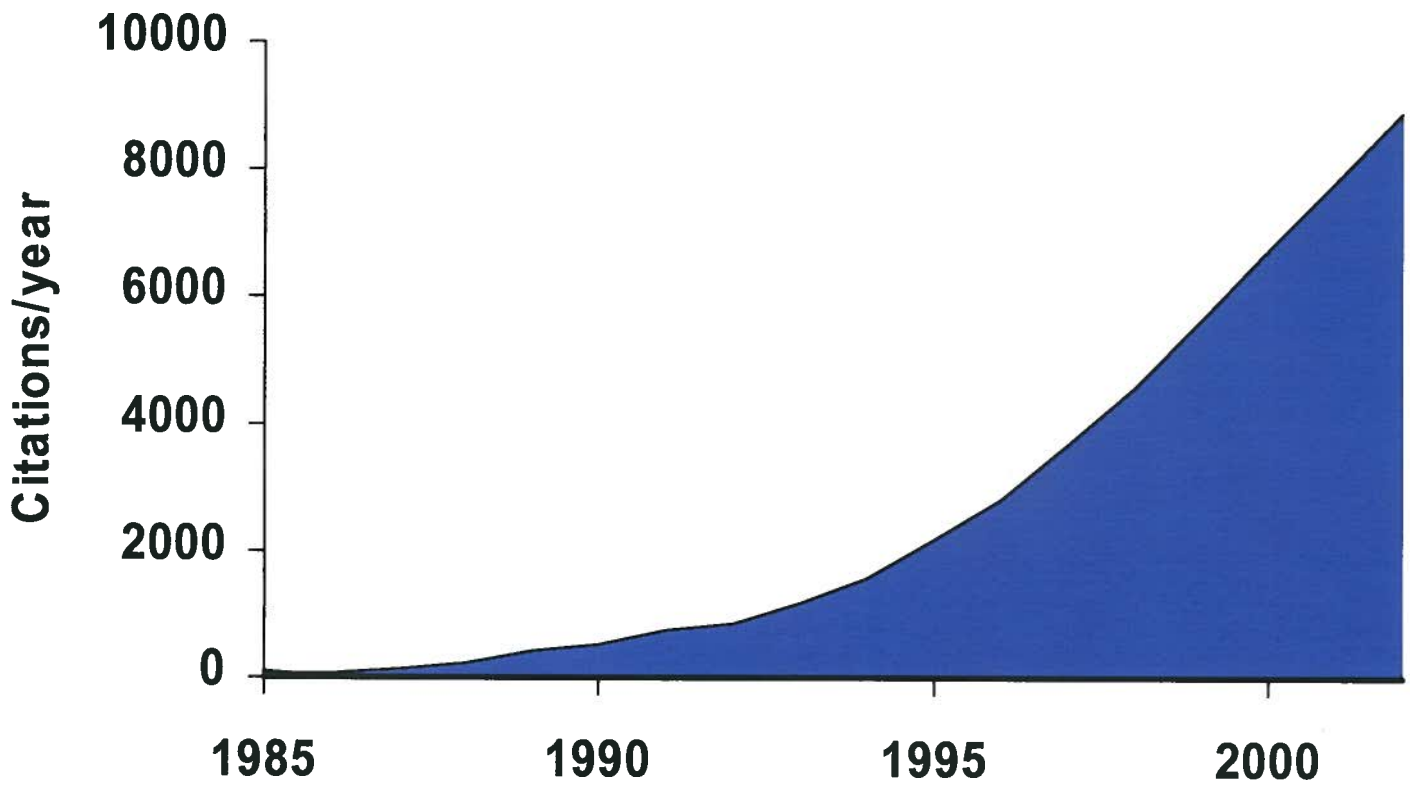
The Cryopreservation and Assisted Reproductive Technologies Laboratory

- Cryopreservation
  - Embryo
  - Ovary
  - Sperm
- Assisted reproductive technologies
  - Colony expansion
  - Rederivation
  - Strain rescue

Development of mouse models

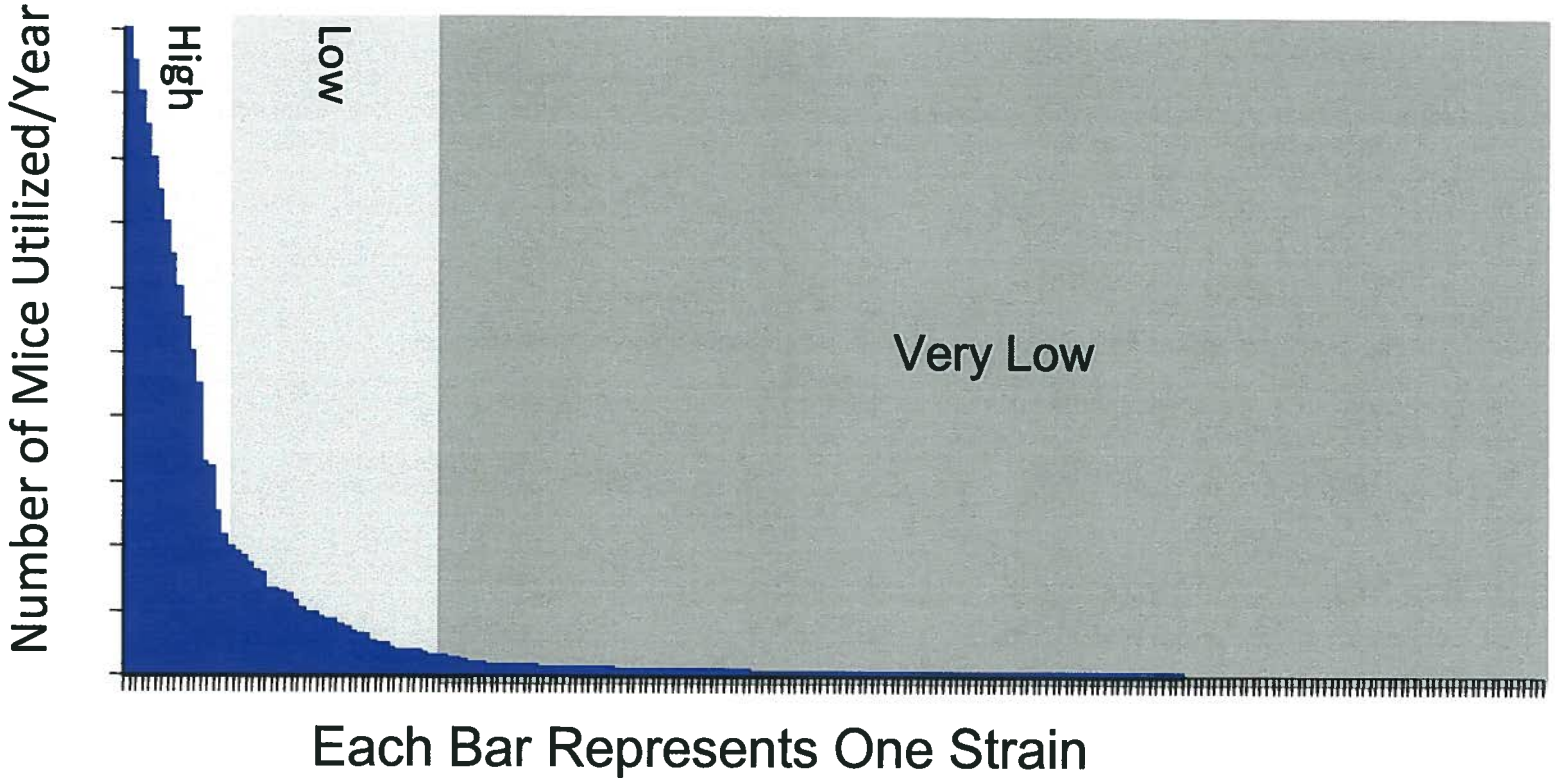
Cancer Genome Atlas Project

- KOMP
- EUCOMM
- NORCOMM



~40,000 genetically modified ES cells becoming publicly available by 2010

Mouse Model Usage



The True Cost of Low Demand Strains

Time

Genotyping

Space

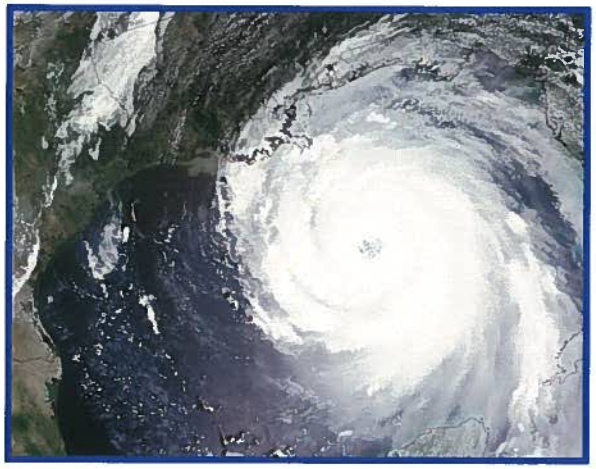
Colony Management

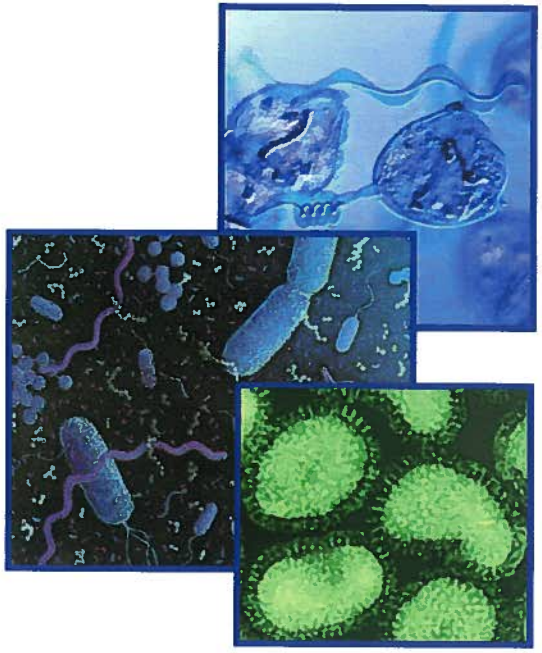
Per Diem $$$

Veterinary care

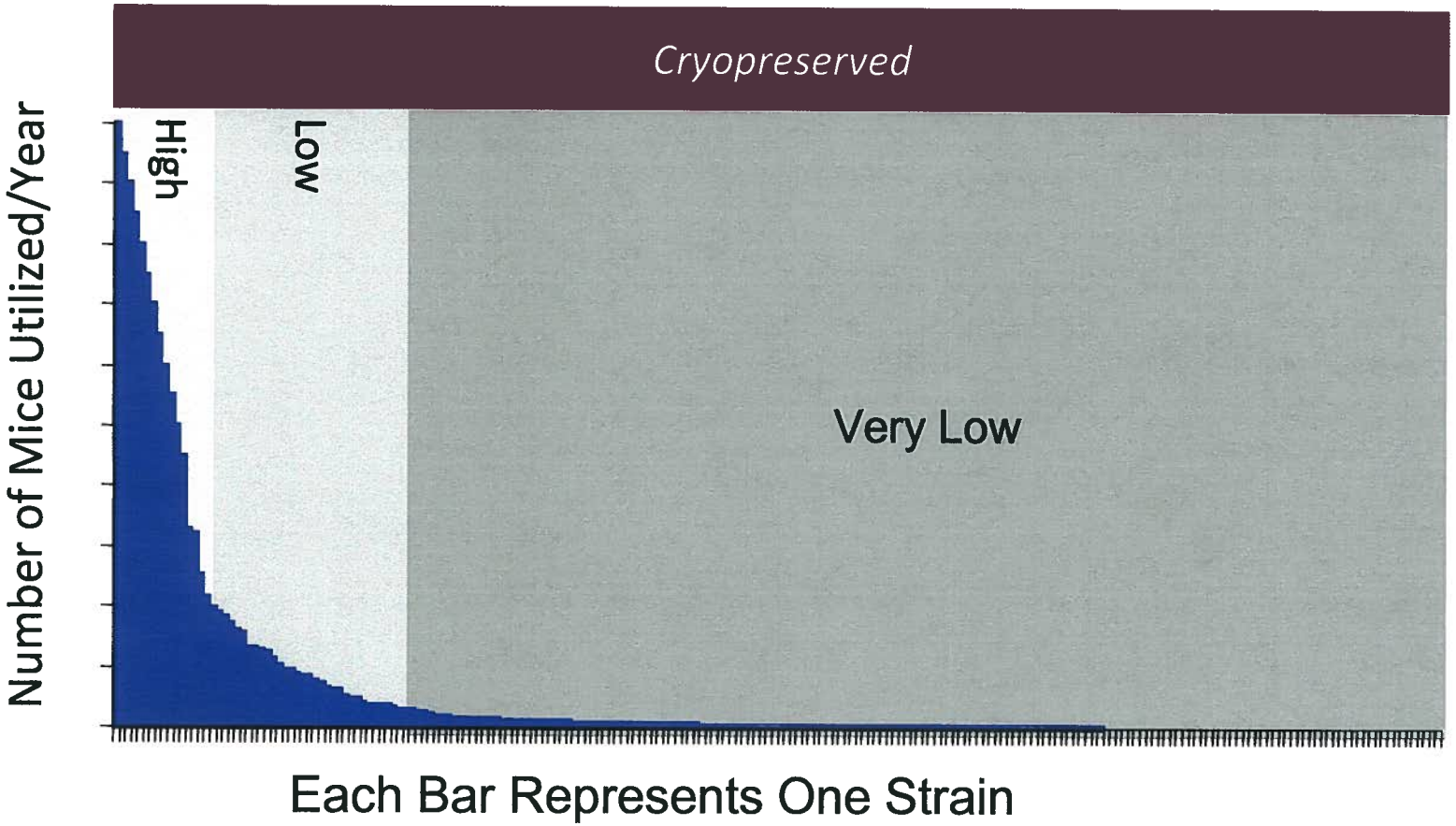
Cryopreservation, An Ideal Alternative

- $ Control
  - Saves space
  - Reduces housing & genotyping costs
  - Allows rapid deployment - distribution
  - Projects sleep; i.e. can be revived
- Risk Control
  - Contamination
  - Disease
  - Mutation
  - Other Disasters …





Mouse model usage



What is Cryopreservation?

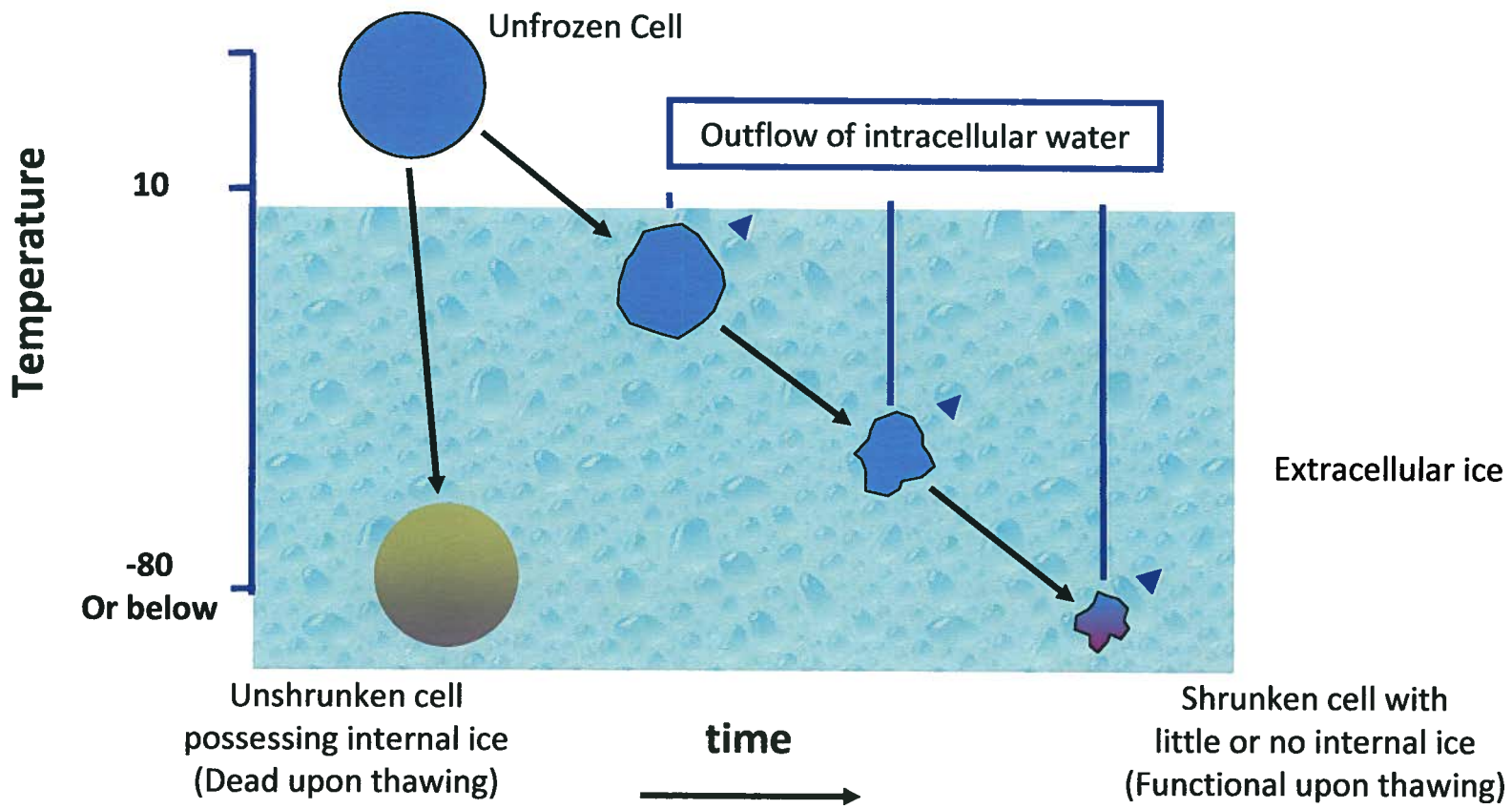
- Metabolic rate is dependent on temperature
- Each depression of 10°C below body temperature reduces metabolic activity by 50%
- Bringing cells from 37°C to 2°C reduces metabolic activity to 5% of the value at 37°C
- A temperature colder than -80°C ensures that metabolic processes are essentially stopped

What is Cryopreservation?

- Cryopreservation – A procedure that prepares and then conveys cells and(or) tissues into long term cold storage; introduction of a cryoprotectant, cooling, storage at a temperature colder than -80°C, warming to body temperature and removal of cryoprotectant.

- Cryoprotectant – a solvent or solute incorporated into the cells and(or) tissue that alters the nature or extent of changes that occur both within the cells and suspending medium and thereby increases the percentage of cells that survive cryopreservation.

Physics of Cryopreservation



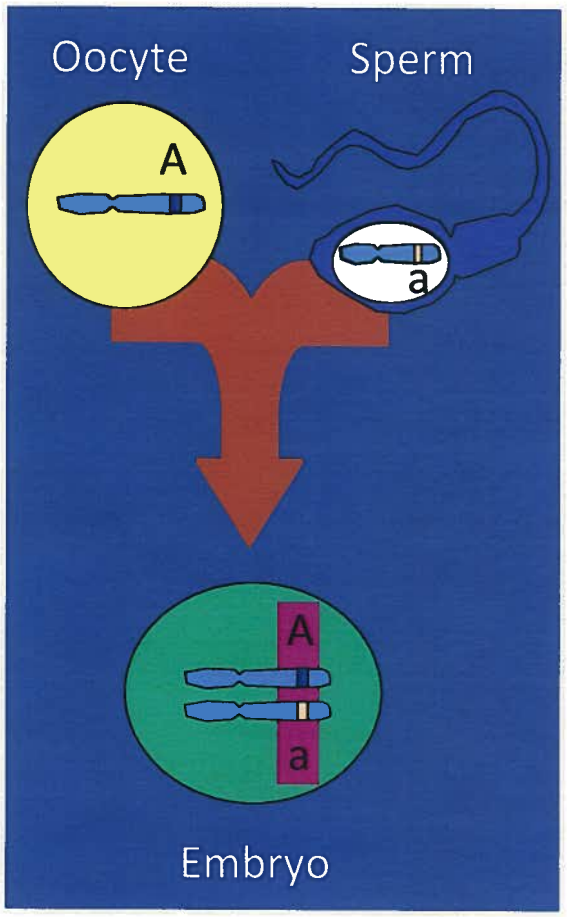
Glenister, PH and WF Rall 1999. Cryopreservation and rederivation of embryos and gamates. In Mouse Genetics & Transgenics: A Practical Approach, 2nd Edition, Oxford University Press, Oxford, pp27-59.

Considering Cryopreservation as a Colony Management Tool

- How will samples be used in the future
- Type of strain
- Quantity of material needed to reliably recover
- Genetic background

Selecting Samples for Cryopreservation

- **Embryos**
  - What you freeze is what you get back
  - Homozygous, multiple unlinked loci of interest: Inbred, outbred, congenic, etc.
- **Ovaries**
  - Progeny produced are heterozygotes
  - Single mutations on a common inbred background: most transgenics and knockouts
- **Sperm**
  - Progeny produced are heterozygotes
  - Single mutations on a common inbred background: most transgenics and knockouts

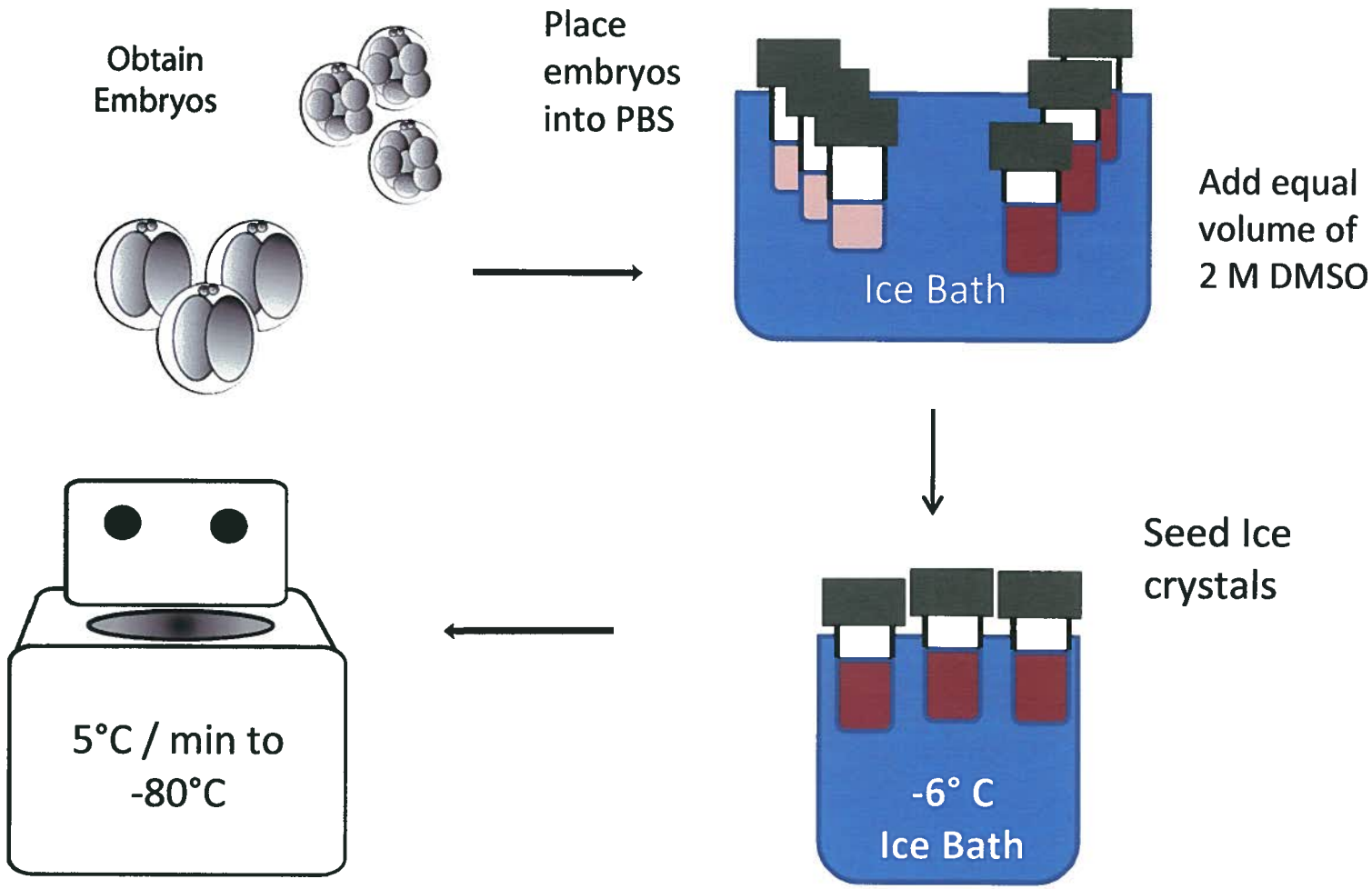


Embryo Cryopreservation

- **Advantages:**
  - Donor genotype preserved
  - Reliable
  - Embryo transfers are inexpensive and simple
  - Biosafety
  - Homozygous, multiple unlinked loci of interest: Inbred, outbred, congenic, etc.

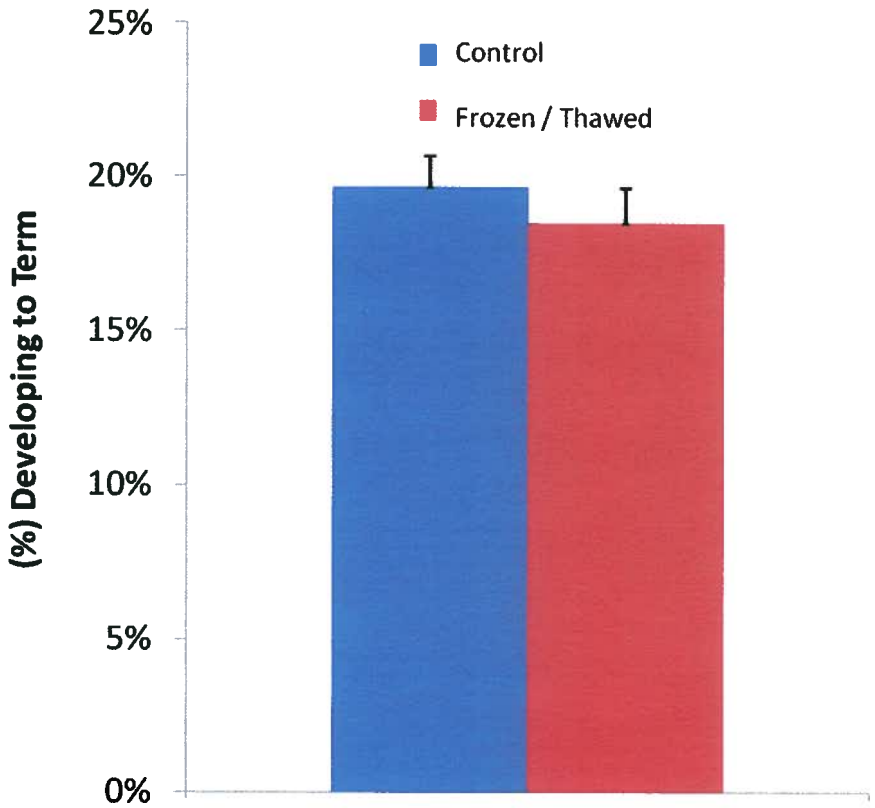
- **Disadvantages:**
  - Cost / time to freeze
  - Yield / Animal
  - Requires 5 breeding pair that are 8 weeks of age.

Embryo Cryopreservation Protocol



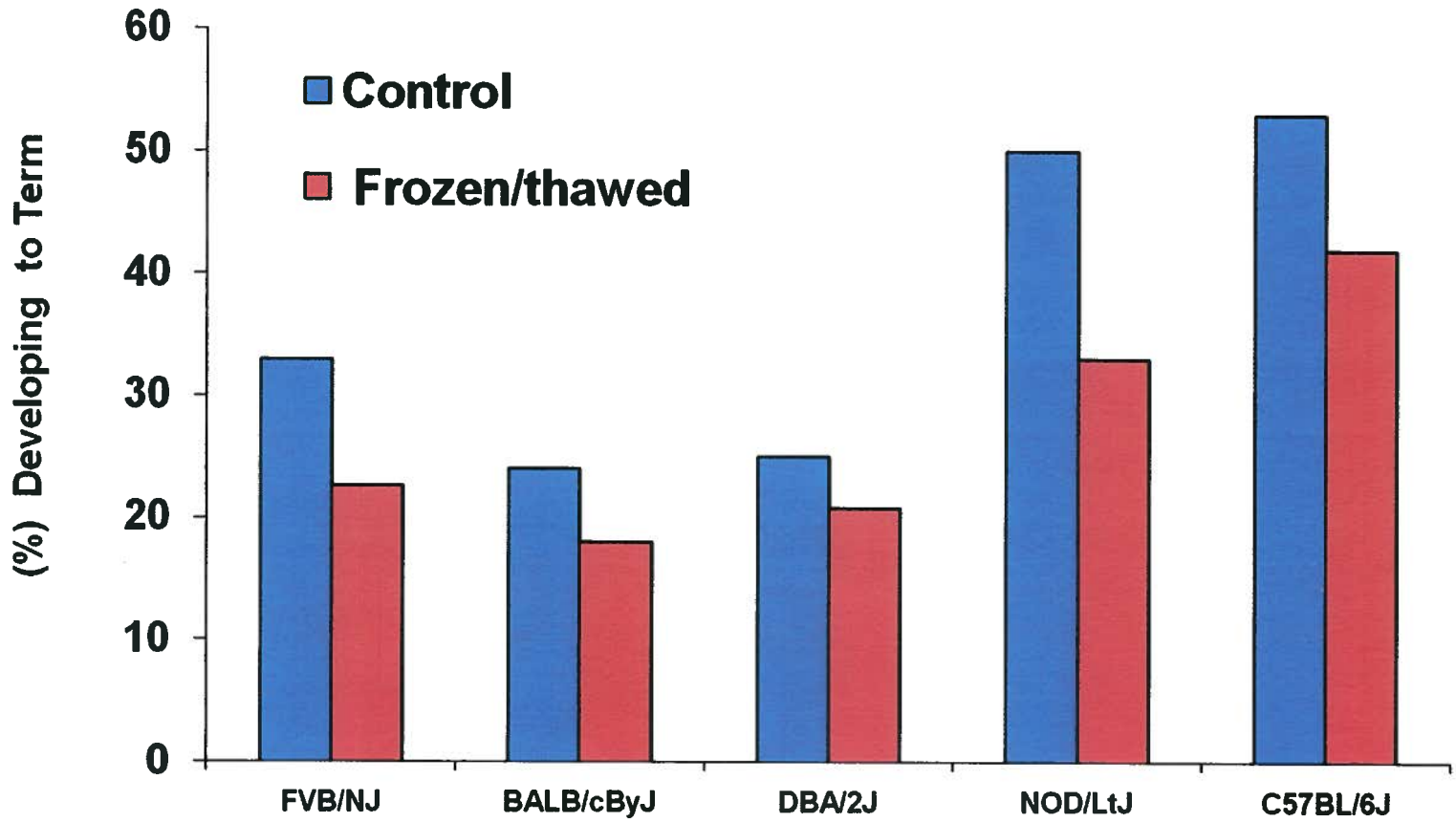
MoBraaten LE. Mouse Embryo Cryobanking. *Journal of in Vitro Fertilization and Ernblyo TransJer, 1986:3(1);28-32*

Embryo Cryopreservation is Robust



- **Used since early 70's**
- **Preserves entire genome**
- **Easy to recover**
- **Excellent biosafety**

Influence of Genetic Background on Embryo Cryopreservation Success

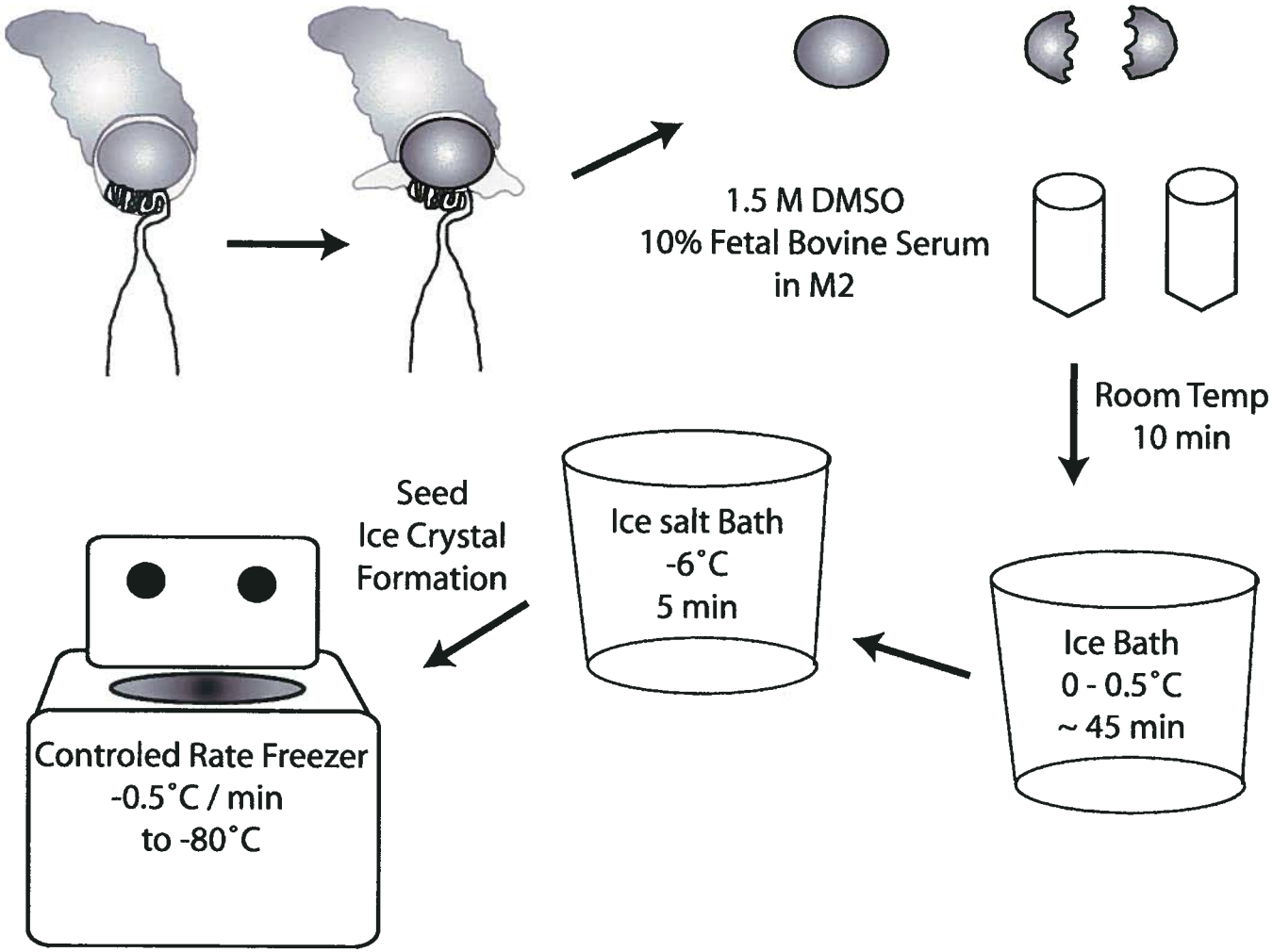


Byers *et al.* Performance of ten inbred mouse strains following assisted reproductive technologies (ARTs). Theriogenology. 2006:65(9);1716-1726

Ovary Cryopreservation

- **Advantages:**
  - Cost efficient
  - Relatively quick and simple
  - Single mutations on a common inbred background: most transgenics and knockouts
- **Disadvantages:**
  - Preserves only one half the genome
  - Long time investments
  - Yield / Animal low (~10 - 20 live born / donor)
  - Limited biosafety
  - The reliability of recovery is questionable

Ovary Cryopreservation



Sztein, J., et al. Cryopreservation and orthotopic transplantation of mouse ovaries: New approach in gamete banking. Biol of Repro. 1998:58 (4);1071-1074

Ovarian Cryopreservation results

Parameter	Frozen / thawed ovaries	Non-frozen Ovaries
Number of recipients	23	30
Number of successful ovary transplant takes (%)	13 (57%)	21 (70%)
Mean number of donor-type pups per recipient (SE)	3.2 (±0.4)	11.2 (±2.1)*
Mean number of litters per recipient (SE)	1.5 (±0.3)	2.4 (±0.4)
Mean interval (days) to first litter (SE)	40.1 (±4.9)	45 (±2.5)

Sztein, J., et al. Cryopreservation and orthotopic transplantation of mouse ovaries: New approach in gamete banking. Biol of Repro. 1998:58 (4);1071-1074

Sperm Cryopreservation

- **Advantages:**
  - Lots of material (10^7 sperm / male)
  - Simple AND efficient
  - Single mutations on a common inbred background: most transgenics and knockouts
  - Requires only 3 males (12 to 24 weeks of age)
- **Disadvantages:**
  - Preserves only one half the genome
  - Embryos must be created
  - Recovery of sperm from some strains can be problematic

Sperm freezing Protocol

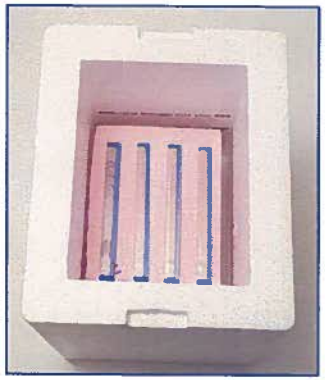
sperm collected into
3% skim milk; 18% raffinose;
477µM MTG

↓

sperm loaded into
prelabeled straws and cassette

↑

static
vapor
freezing



storage
-196°C

↓

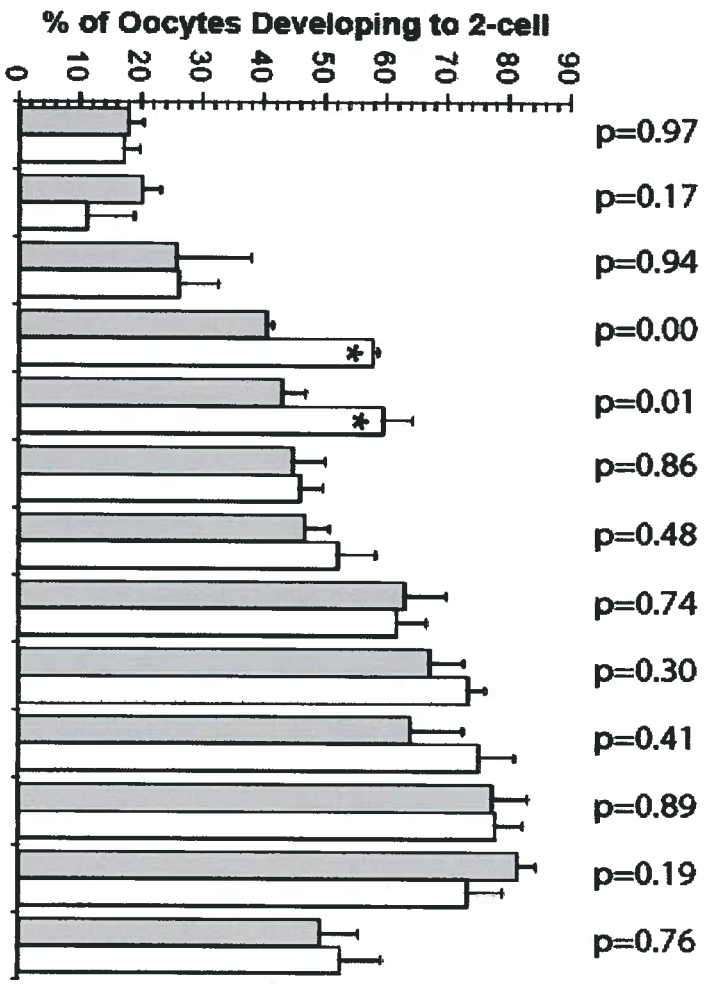
thawing

in vitro
fertilization

Ostermeier GC, Wiles MV, Farley J, Taft RA. Conserving, distributing and managing genetically modified mouse lines by sperm cryopreservation. PLoS ONE. 2008;3(7): e2792.

Fertilizing Capacity with GM strains

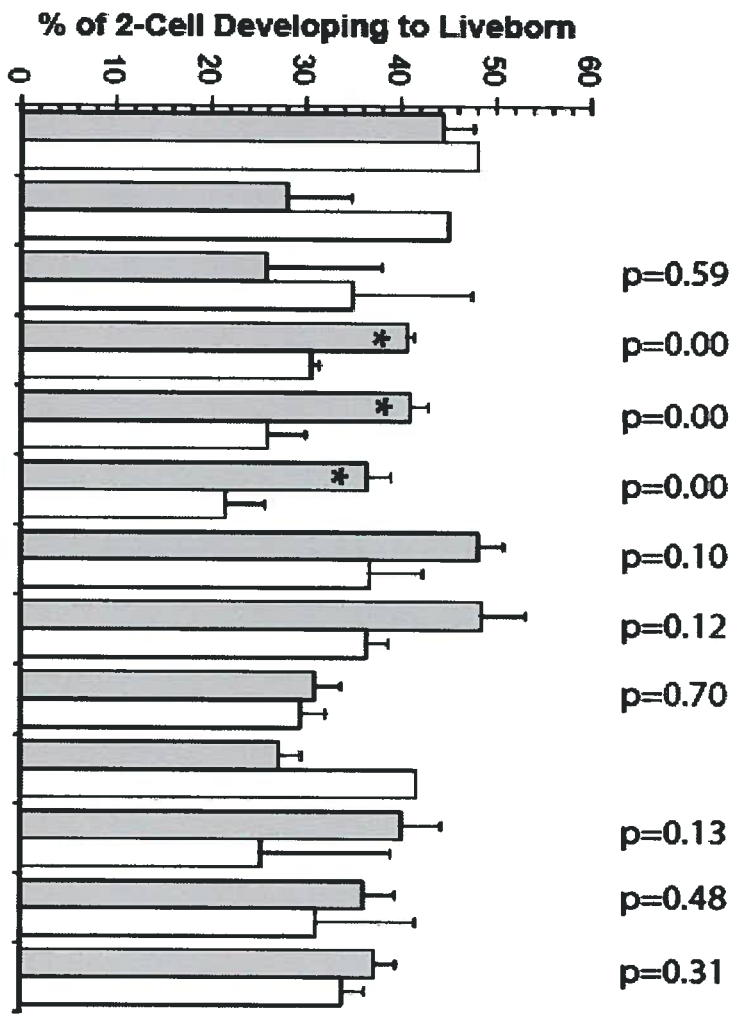
Genetic background of sperm donor lines	# of GM sperm donor lines	Sperm cryo status	# females	# IVF oocytes
129S1/SvImJ	29	Frozen	395	8,770
	19	Fresh	750	19,744
C57BLKS/J	5	Frozen	45	677
	3	Fresh	120	985
129X1/SvJ	5	Frozen	60	1,206
	6	Fresh	375	6,764
C57BL/6J	527	Frozen	3,439	103,716
	643	Fresh	20,353	616,318
BALB/cByJ	39	Frozen	420	7,289
	30	Fresh	1,645	28,320
BALB/cJ	25	Frozen	321	4,061
	28	Fresh	1,871	25,751
B6129SF1/J	33	Frozen	302	7,215
	17	Fresh	597	14,091
NOD/ShiLtJ	11	Frozen	109	1,981
	21	Fresh	567	11,957
FVB/NJ	30	Frozen	397	5,318
	65	Fresh	2,696	39,142
C3H/HeJ	5	Frozen	48	635
	3	Fresh	180	2,331
C3HeB/FeJ	15	Frozen	296	2,856
	5	Fresh	175	2,418
DBA/2J	11	Frozen	100	2,365
	7	Fresh	179	3,504
Overall	735	Frozen	5,932	146,089
	847	Fresh	29,508	771,325



Ostermeier GC, Wiles MV, Farley J, Taft RA. Conserving, distributing and managing genetically modified mouse lines by sperm cryopreservation. PLoS ONE. 2008;3(7): e2792.

Embryo development

Genetic background of sperm donor lines	# of GM sperm donor lines	Sperm cryo status	Embryo cryo status	# 2-cell embryos trans
129S1/SvimJ	26	Frozen	Fresh	730
	2	Fresh	Frozen	486
C57BLKS/J	5	Frozen	Fresh	130
	1	Fresh	Frozen	60
129X1/SvJ	5	Frozen	Fresh	1,206
	3	Fresh	Frozen	384
C57BL/6J	418	Frozen	Fresh	13,315
	201	Fresh	Frozen	18,886
BALB/cByJ	39	Frozen	Fresh	1,208
	13	Fresh	Frozen	2,026
BALB/cJ	23	Frozen	Fresh	717
	10	Fresh	Frozen	1,420
B6129SF1/J	24	Frozen	Fresh	784
	9	Fresh	Frozen	1,628
NOD/ShiLtJ	9	Frozen	Fresh	300
	6	Fresh	Frozen	459
FVB/NJ	21	Frozen	Fresh	938
	26	Fresh	Frozen	5,422
C3H/HeJ	6	Frozen	Fresh	180
	2	Fresh	Frozen	104
C3HeB/FeJ	10	Frozen	Fresh	402
	3	Fresh	Frozen	176
DBA/2J	11	Frozen	Fresh	420
	3	Fresh	Frozen	826
Overall	597	Frozen	Fresh	20,330
	279	Fresh	Frozen	31,877



Ostermeier GC, Wiles MV, Farley J, Taft RA. Conserving, distributing and managing genetically modified mouse lines by sperm cryopreservation. PLoS ONE. 2008;3(7): e2792.

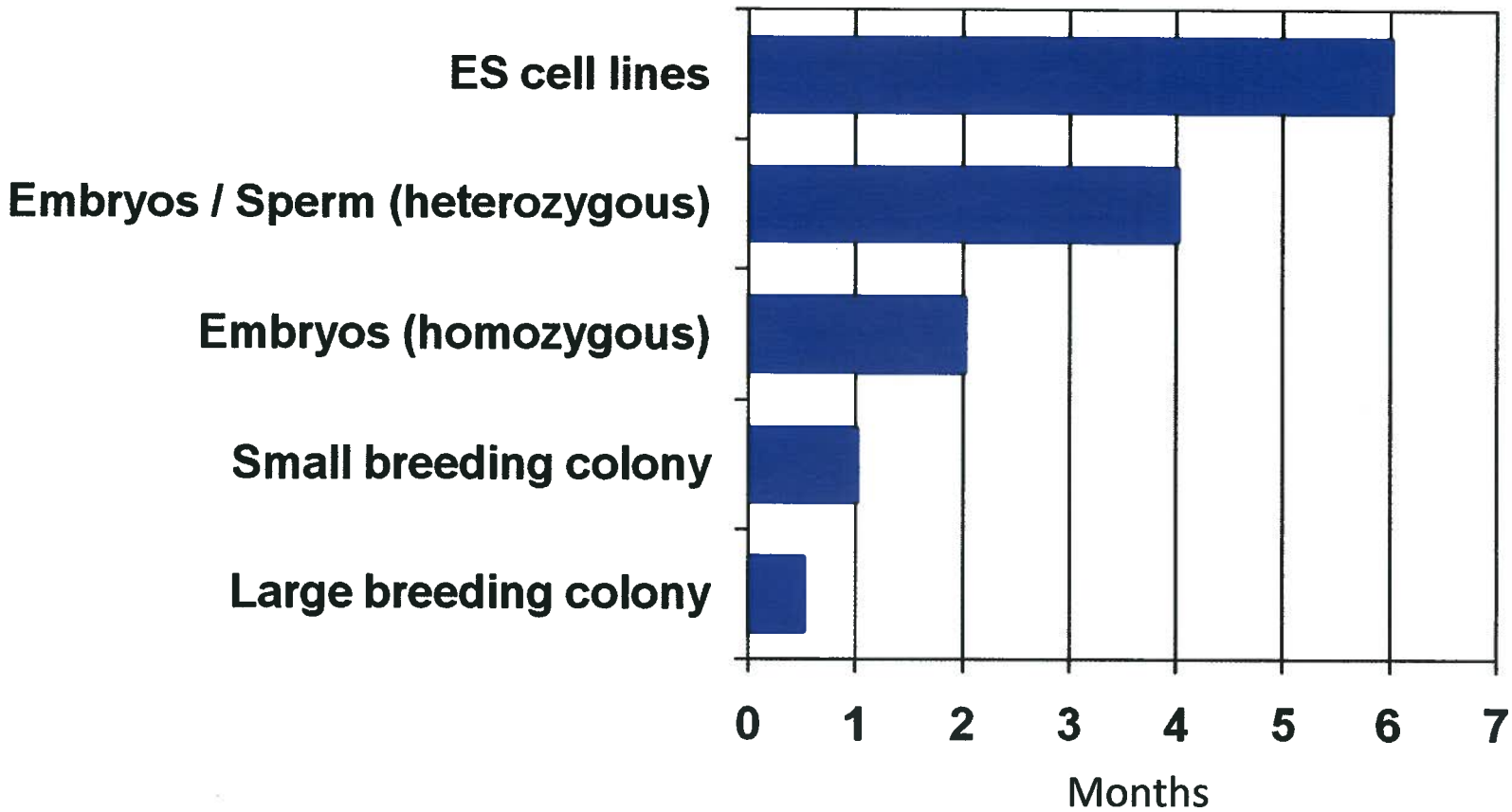
Recovery of cryopreserved sperm

Genetic background of sperm donor line	% of oocytes to 2-cell	Std Err	% of embryos to live born	Std Err	# of GM sperm donor strains
Balb/CAnCr	19	8	9	9	2
C57BL/6J	19	3	15	4	6
129S1/svmJ/Cr	27	N/A	25	N/A	1
C57BL/6J albino	50	14	20	8	3
C57BL/6NCr	56	3	19	1	66
FVB/NCr	68	4	20	3	26
FVB/NJ	89	4	23	2	6
B6D2F1/J	76	8	18	4	7
pten-/-p53-/-	93	N/A	50	N/A	1
OVERALL	51	8	21	3	9
Fresh sperm / Frozen embryos	60	8	18	1	11

Strain recovery and expansion

# Sperm Doses	# 2-cells using 5 ♀s or ~80 oocytes / IVF ~50% to 2-cell	# Pups born ~20% to liveborn
1	40	8
2	80	16
3	120	24
4	160	32
5	200	40
6	240	48
7	280	56
8	320	64
9	360	72
10	400	80
100	4,000	800

Minimum Restoration Time for Homozygous Mutants



Cryopreservation Options

	Embryos	Ovaries	Sperm
Pros	Reliable Inexpensive recovery Entire genome	Quick and simple	Simple Abundant Reliable
Cons	Heavy upfront resource Slow build	Very slow build Half genome	Half genome Create embryos

The Cryopreservation and Assisted Reproductive Technologies Laboratory

- Cryopreservation
  - Embryo
  - Ovary
  - Sperm
- Assisted reproductive technologies
  - Colony expansion
  - Rederivation
  - Strain rescue

Colony expansion through IVF

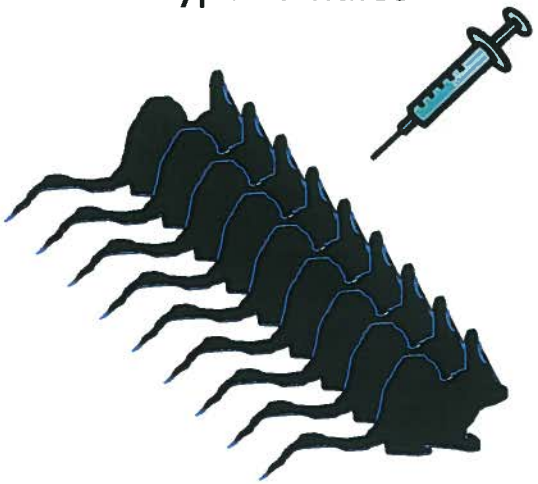
Need dozens of mice -

all of the same age -

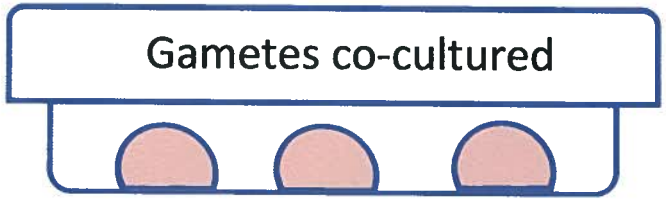
fast?

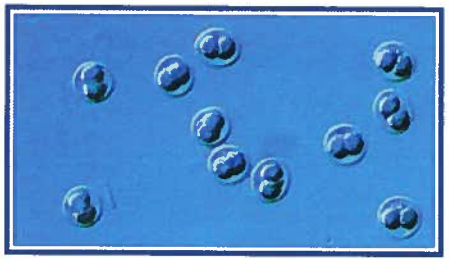
Colony Expansion Through IVF

Superovulation of wildtype females



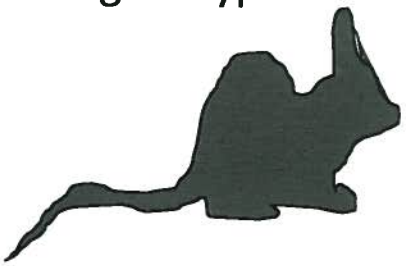
Gametes co-cultured

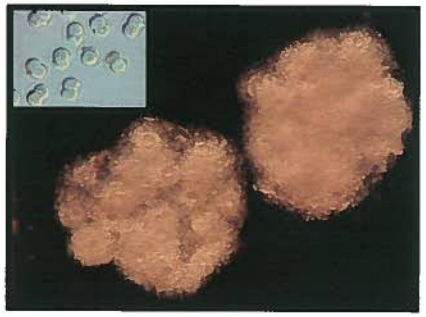


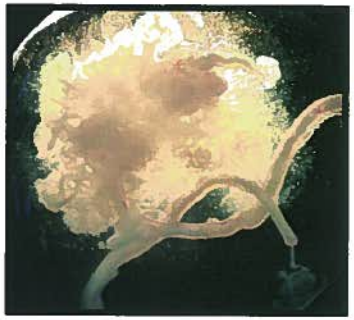


2-cell embryo obtained and transferred

Male of desired genotype







Colony expansion through IVF

- Quickly produces large quantities of same-age, genetically modified mice on a common inbred background.
- Requires only two males from your colony.
- Ideal for strains where males have a short lifespan or breeding window.

Rederivation

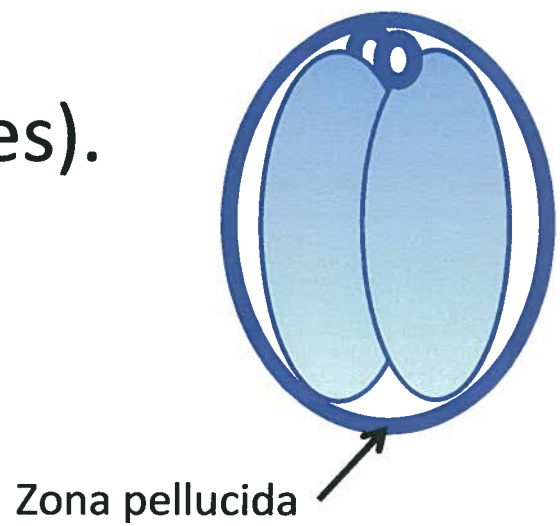
- The presence of adventitious pathogens can alter laboratory mice as research models.
  - If mice are infected with pathogens, their response to that infection may mask genetic changes and/or complicate physiological data.

Rederivation

- Collection of preimplantation embryos from contaminated or donors of unknown status.
- Extensive washes of zona-intact embryos.
- Transfer of washed embryos into "clean" recipients eliminates pathogens

(e.g. viruses, bacteria, and parasites).

- Quarantine of recipients.
- Verify health status of offspring.



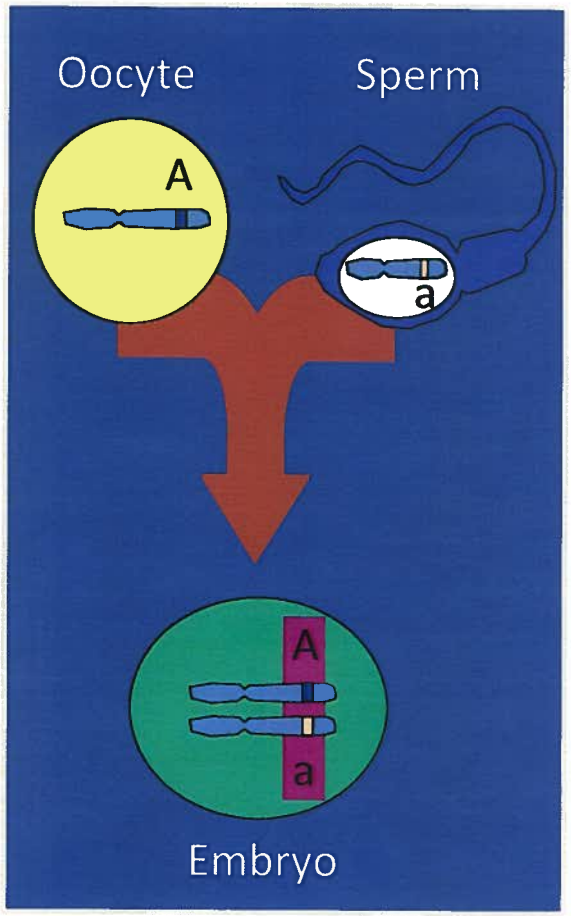
Rederivation

Traditional

- Ideal for strains maintained as homozygotes or have multiple unlinked loci of interest.
- Requires 3 to 5 breeding pair.
- Uses superovulation, followed by traditional mating, embryo flushing and transfer.

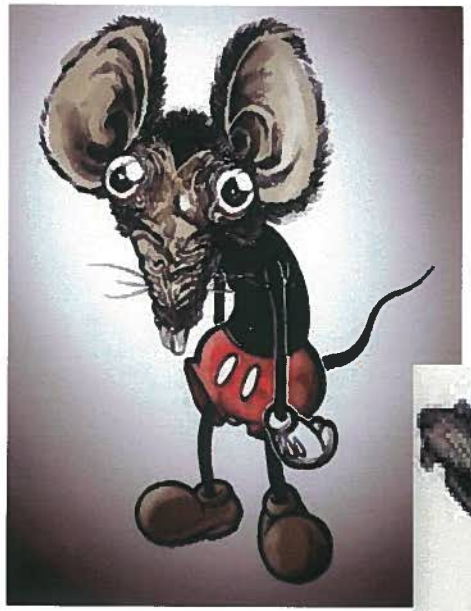
IVF

- Ideal for strains maintained as heterozygotes, such as transgenics, knockouts, or single gene mutant mice on inbred background.
- Requires 2 males from your colony.
- Uses IVF with wild-type oocytes and embryo transfer.



Strain Rescue

Assist in rescuing small colonies threatened by age, breeding cessation, or poor health.



Strain Rescue

Males available from endangered colony

- Sperm collection, sperm cryopreservation
- *IVF*

Females available from endangered colony

- Superovulation
- Ovary cryopreservation/transplant

Call us before it's too late!!!

Colony Management through Cryopreservation and ARTs

- Cryopreservation
  - Embryo
  - Ovary
  - Sperm
- Assisted reproductive technologies
  - Colony expansion
  - Rederivation
  - Strain rescue

The Cryopreservation and Assisted Reproductive technologies laboratory

http://web.ncifcrf.gov/rtp/lasp/intra/cryo/

G. Charles Ostermeier, Ph.D.	Phone: 301-846-6221 Email: ostermeiergc@mail.nih.gov
Roackie Awasthi, M.Sc.	Phone: 301-846-1864 Email: pawasthi@ncifcrf.gov
Anna Moten	Phone: 301-846-5155 Email: motena@ncifcrf.gov
Jennifer Harrison	Phone: 301-846-6227 Email: jharrison@ncifcrf.gov
Misty Hawes	Phone: 301-846-1133 Email: hawesmy@mail.nih.gov