



Canadian Mouse Mutant Repository

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Outline

- Services at the CMMR
- The numbers for cryopreservation at CMMR
- Applying best practices for sperm cryo & IVF



CMMR

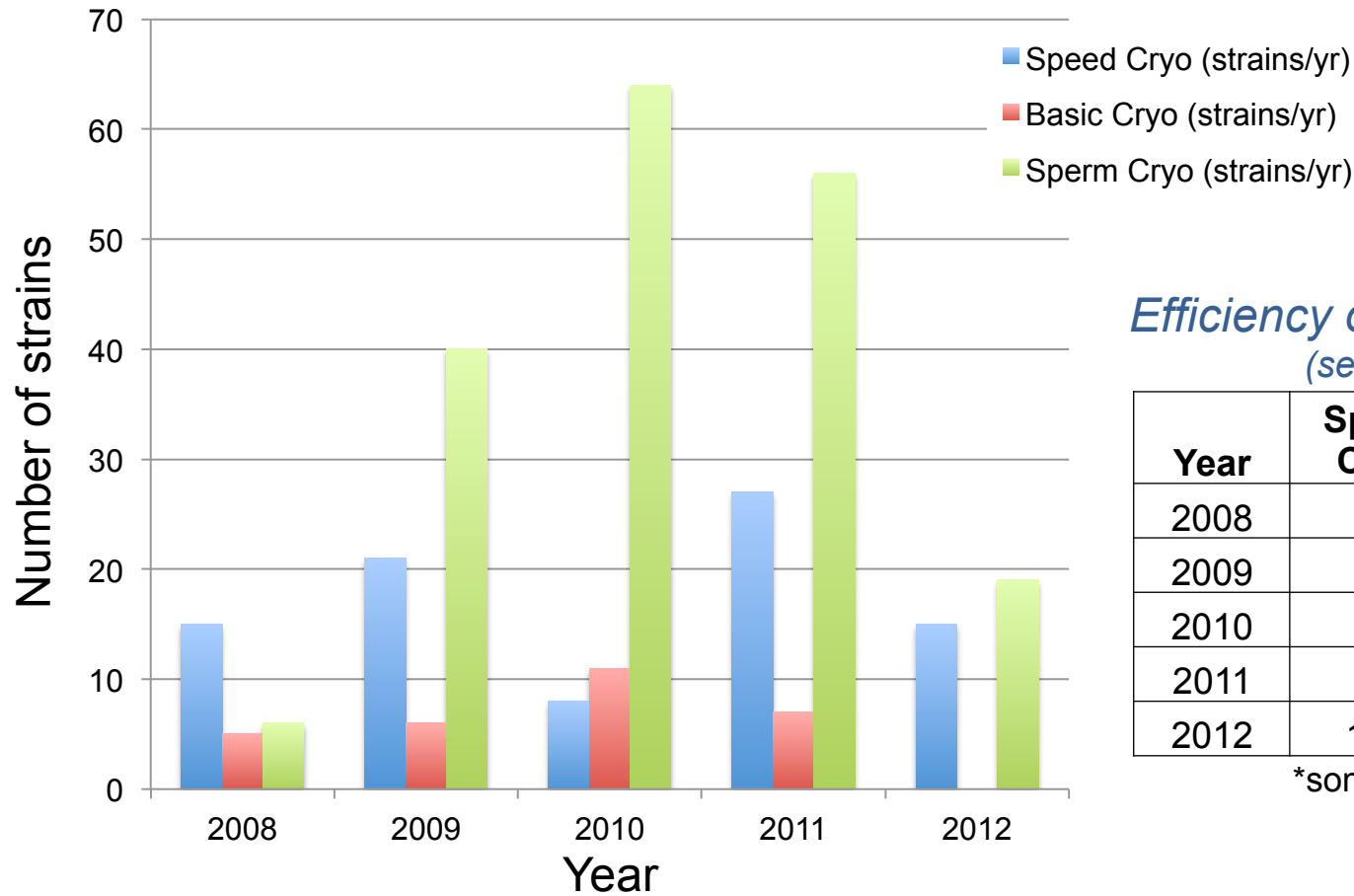
The CMMR

- SickKids program @ TCP
- Repository for
 - CMHD ENU mutagenesis program
 - NorCOMM gene trap and gene targeted ES cells
 - NorCOMM2 IMPC mouse lines
 - fee-for-service for Canadian scientific community
- Public distribution of deposited material ***optional*** for fee-for-service users

Services @ CMMR

- Cryopreservation
 - embryos from natural mating or IVF (speed cryo)
 - sperm
- Cryorecovery
 - embryos
 - sperm
- NorCOMM ES cell distribution
 - gene trap & gene targeted ES cells
 - parental 6NTac-C2 cells
- NorCOMM target vector distribution
- NorCOMM2 mouse lines (when available)

Cryopreservation Service Stats



Efficiency of cryopreservation (sessions/strain)

Year	Speed Cryo	Basic Cryo	Sperm Cryo
2008	1.9	2.2	1.0
2009	1.4	2.5	1.1
2010	1.4	2.2	1.1
2011	1.3	1.6	1.2
2012	1.0*	-	1.1*

*some strains still in progress

Number of deposited strains by service type

CMMR numbers for cryo

Embryo Cryo

- want 200-300 carrier embryos
- 20-25 embryos/straw
- 2 M & 20 F / IVF session
- 34 mice/cryopreserved strain, including QC

Sperm Cryo

- want 3 carrier males
- 14-16 straws/male
- 3 M & 5 F for QC / strain
- 8 mice/cryopreserved strain, including QC

Embryo Recovery

- 20-40 embryos in 1-2 recipients per strain
- 0-20 pups/recovery (mean=5, 23% born)
- 33 lines recovered; 6 failed (~85%)

Sperm Recovery

- 1 straw, 10-20 oocyte donors
- 4-178 (mean=73) embryos, 1-5 recipients
- 1-65 pups/recovery (mean=28, 35% born)
- 11 lines recovered; 1 failed (~92%)

The 3Rs for Archiving & Distribution

TOTAL MICE / STRAIN WITH ONE RECOVERY

Speed Cryo → 35-36 mice

Sperm Cryo → 20-33 mice



Animal Use

Lines for distribution → speed cryo

Lines for archiving → sperm cryo

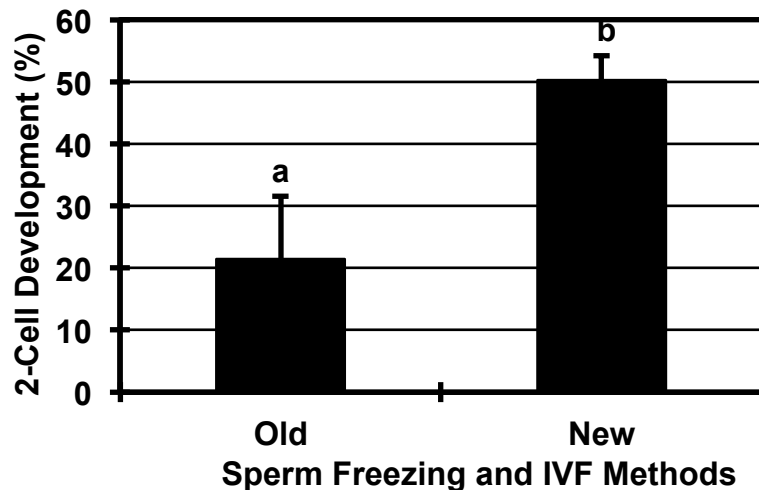


Financial Considerations

Where is majority of cost to be borne, archiving or recovery?

Improving Sperm Cryo & IVF

In vitro fertilization rates CPA vs. mCPA



Liu *et al.*, 2009. J Am Assoc Lab Anim Sci. 48:39-43

mCPA & preincubation

Cryopreservation

- CPA: 3% skim milk, 18% raffinose, 1X MEAA
- 110 μ l CPA/male
- freeze in floating canister

Cryorecovery

- thaw 5-10 min at 37°C
- pre-incubate in 90 μ l MEM + MBCD
- selection of motile sperm
- IVF in 90 μ l MEM drop

mCPA vs. MTG-CPA

JAX MTG-CPA

- 1 ml per male
- incubate sperm in Cook's IVF drop
- add oocytes to sperm
- 500 µl IVF drop in Cook's

Modified MTG-CPA

- 250 µl MTG-CPA/male
- pre-incubate in Cook's & select motile sperm
- add sperm to oocytes
- 250 µl Cook's IVF drop

Effect of CPA volume & pre-incubation on C57BL/6 frozen-thawed sperm in vitro fertilization rates

Pre-incubation & selection	Volume of MTG-CPA/male (µl)	Fertilization rate	Average
-	1000	26%	42%
-	1000	58%	
Cook's	1000	26%	23%
Cook's	1000	27%	
Cook's	1000	15%	
Cook's	250	56%	51%
Cook's	250	35%	
Cook's	250	61%	

Modified JAX method for sperm cryo

In vitro fertilization rates of frozen-thawed sperm from mutant mouse lines

Male strain background	N	Fertilization rate		
		Range	Average	SD
C57BL/6	28	2-85%	34%	±22%
Other inbred strains	12	3-84%	37%	±24%
Outbred & mixed stocks	13	5-93%	42%	±29%

TYH-MBCD & HTF+Ca/GSH for sperm cryo

In vitro fertilization rates with frozen-thawed C57BL/6 sperm cryopreserved in 250 μ l/male MTG-CPA

Pre-incubation medium	IVF medium	Overnight culture	N	Fertilization Rates		
				Range	Average	SD
Cook's	Cook's	Cook's	28	2-85%	34%	\pm 22%
Cook's + MBCD	Cook's + GSH	Cook's	3	8-47%	24%	\pm 17%
TYH + MBCD	HTF+Ca + GSH	HTF+Ca	6	16-71%	51%	\pm 19%

- 250 μ l MTG-CPA / male
- pre-incubation in 90 μ l for 30-60 minutes
- select motile sperm and add to eggs in 90 μ l IVF drop
- co-incubate 3-5 hours
- wash and culture overnight

TYH-MBCD & HTF+Ca/GSH for fresh IVF

In vitro fertilization rates with fresh sperm for speed cryo

Male Strain Background	Preincubation medium	IVF medium	Overnight culture	N	Fertilization Rates		
					Range	Average	SD
C57BL/6	Cook's	Cook's	KSOM	26	5-90%	51%	±26%
	Cook's + MBCD	Cook's + GSH	Cook's	1	68%	-	-
	TYH + MBCD	HTF+Ca + GSH	HTF-Ca	6	86-99%	94%	±4%
Others	Cook's	Cook's	KSOM	10	5-94%	64%	±26%
	Cook's + MBCD	Cook's + GSH	Cook's	1		-	-
	TYH + MBCD	HTF+Ca + GSH	HTF-Ca	3	43-100%	87%	±20%

- pre-incubation in 90 µl for 30-60 minutes
- select motile sperm and add to eggs in 90 µl IVF drop
- co-incubate 3-4 hours
- wash and culture overnight

The tests continue...

- Sperm Cryo
 - continue testing birth rate from post-thaw IVF (53%, N=1)
 - test IVF protocol with previously frozen sperm in mCPA and CPA
- Speed Cryo
 - test birth rate from post-thaw embryos (transfers complete, awaiting birth)

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