

Cryopreservation in Australia

The Australian Phenome Bank

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APB Curator

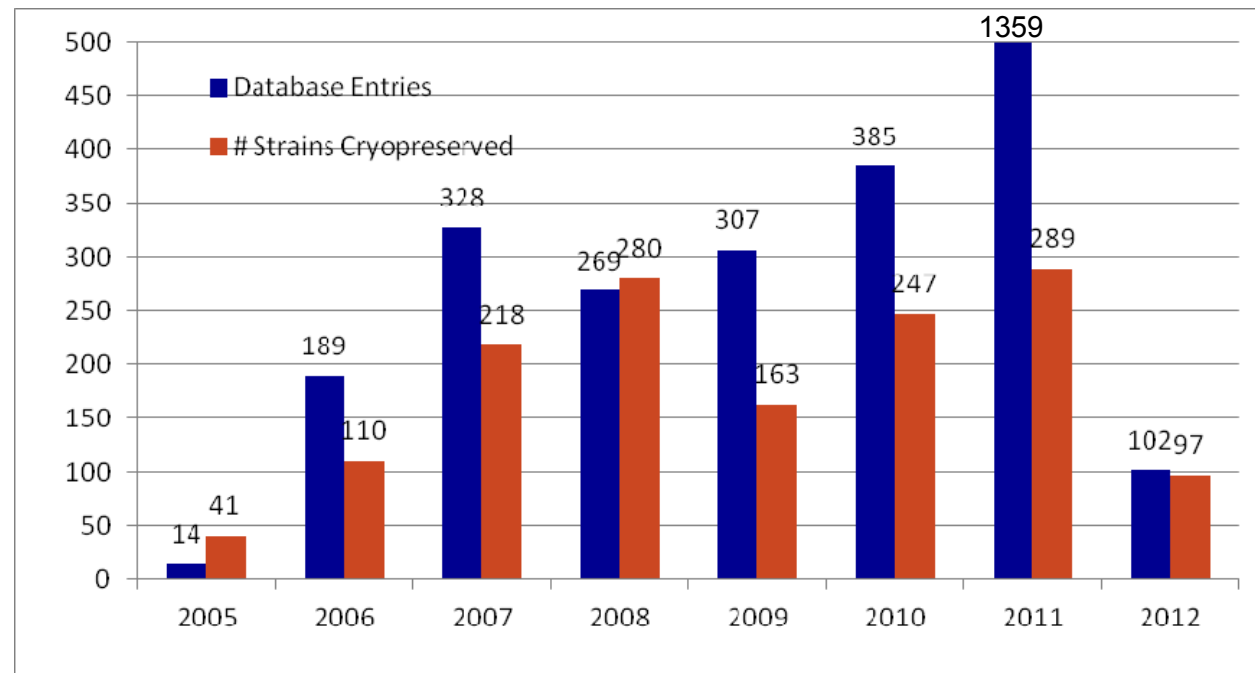
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Australian Phenome Bank

Established in 2005 to provide:

- Centralised sperm cryopreservation service and
- Centralised strain database.
- Techniques used standardised across all nodes
- Techniques used established prior to 2005



Mouse Models – Created, Characterised, Cryopreserved

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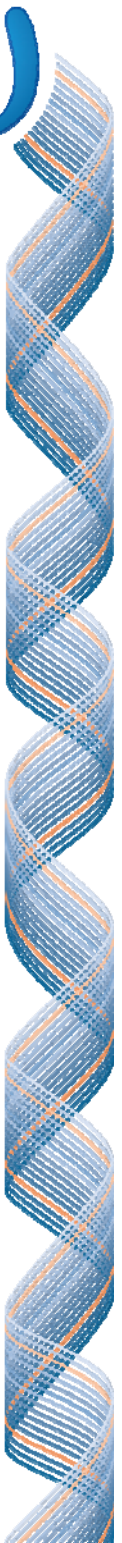
Sperm Cryopreservation – storage

Set up Requirements



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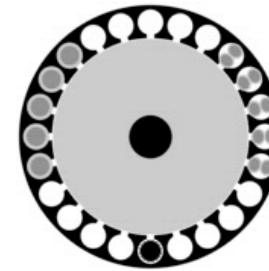
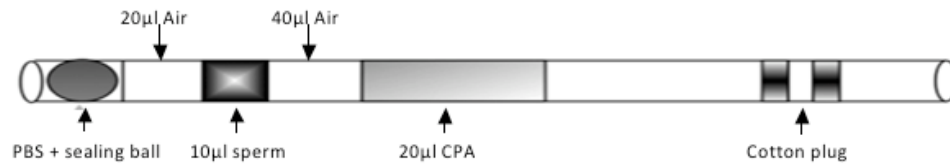


Sperm Cryopreservation

Load sperm into straws (11 per mouse)

Incubate at -155°C (3mins)

Plunge into Liquid N_2



From Cryologic, Vic, Dr Gabriel Sanchez-Partida (Monash Uni) involved in development

IVF Procedure Comparison

	Oocyte Donor Age	Cryoprotectant	Media	# oocytes	# 2-cell embryos	% 2-cell embryos
Group 1a	3 – 4 weeks	APB	APB	61	0	0
Group 1b	3 – 4 weeks	EMMA	EMMA	75	21	28
Group 1c	3 – 4 weeks	EMMA	FERTIUP	22*	18	82
Group 2a	8 – 12 weeks	APB	APB	71	4	6
Group 2b	8 – 12 weeks	EMMA	EMMA	25	6	24
Group 2c	8 – 12 weeks	EMMA	FERTIUP	18*	16	89

* oocyte numbers were low due to insufficient donors being available.

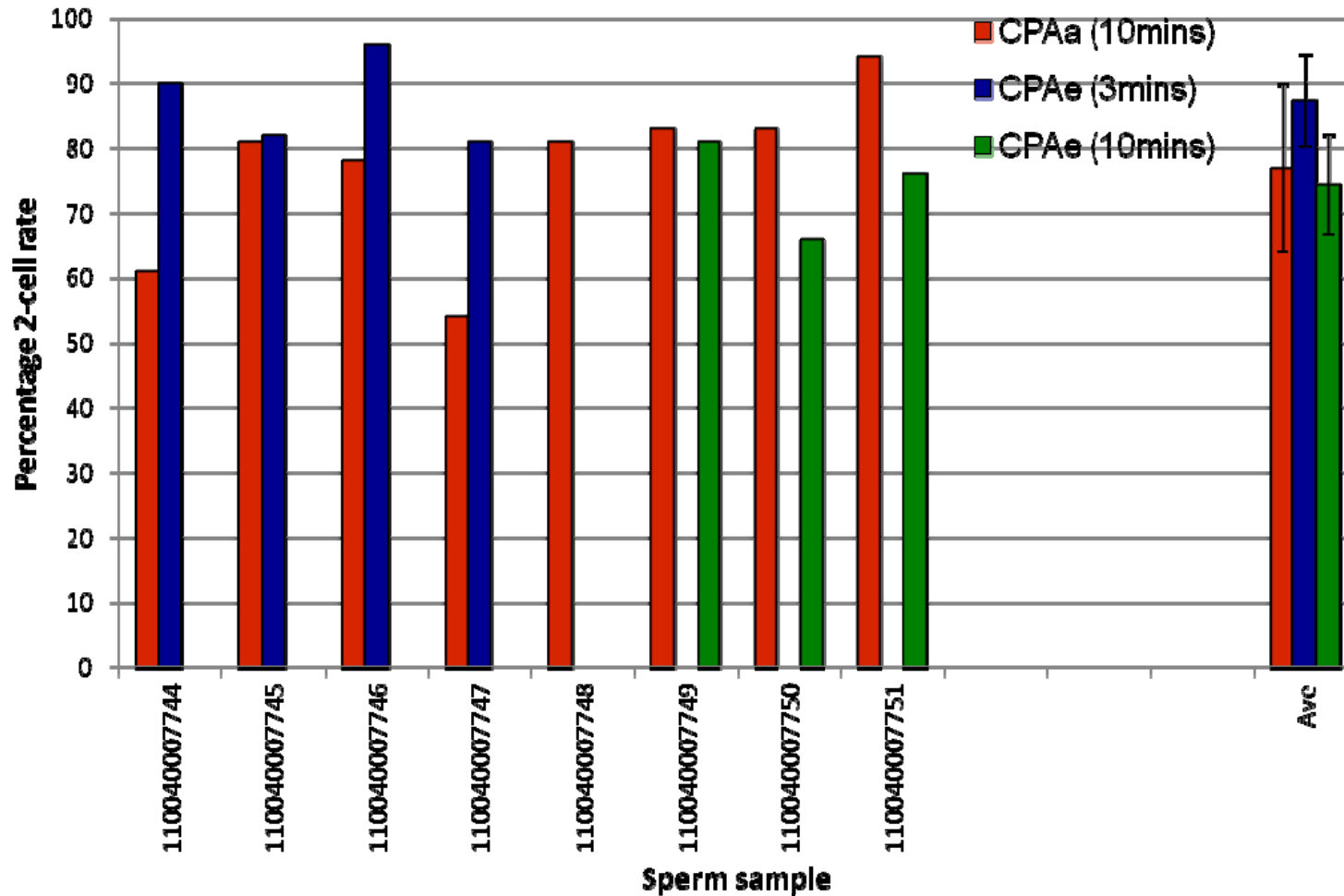
Experiments performed by Cassandra du Boulay and Naomi Williams

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IVF Procedure Comparison

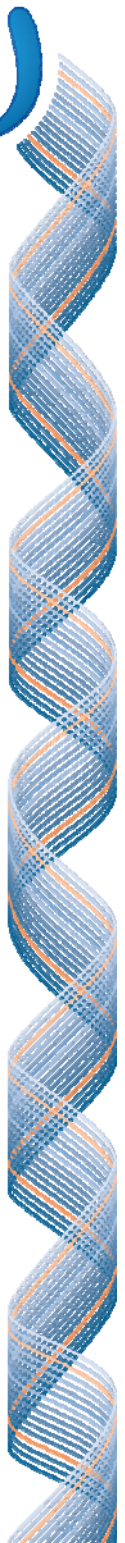
C57BL/6 mice treated with ENU



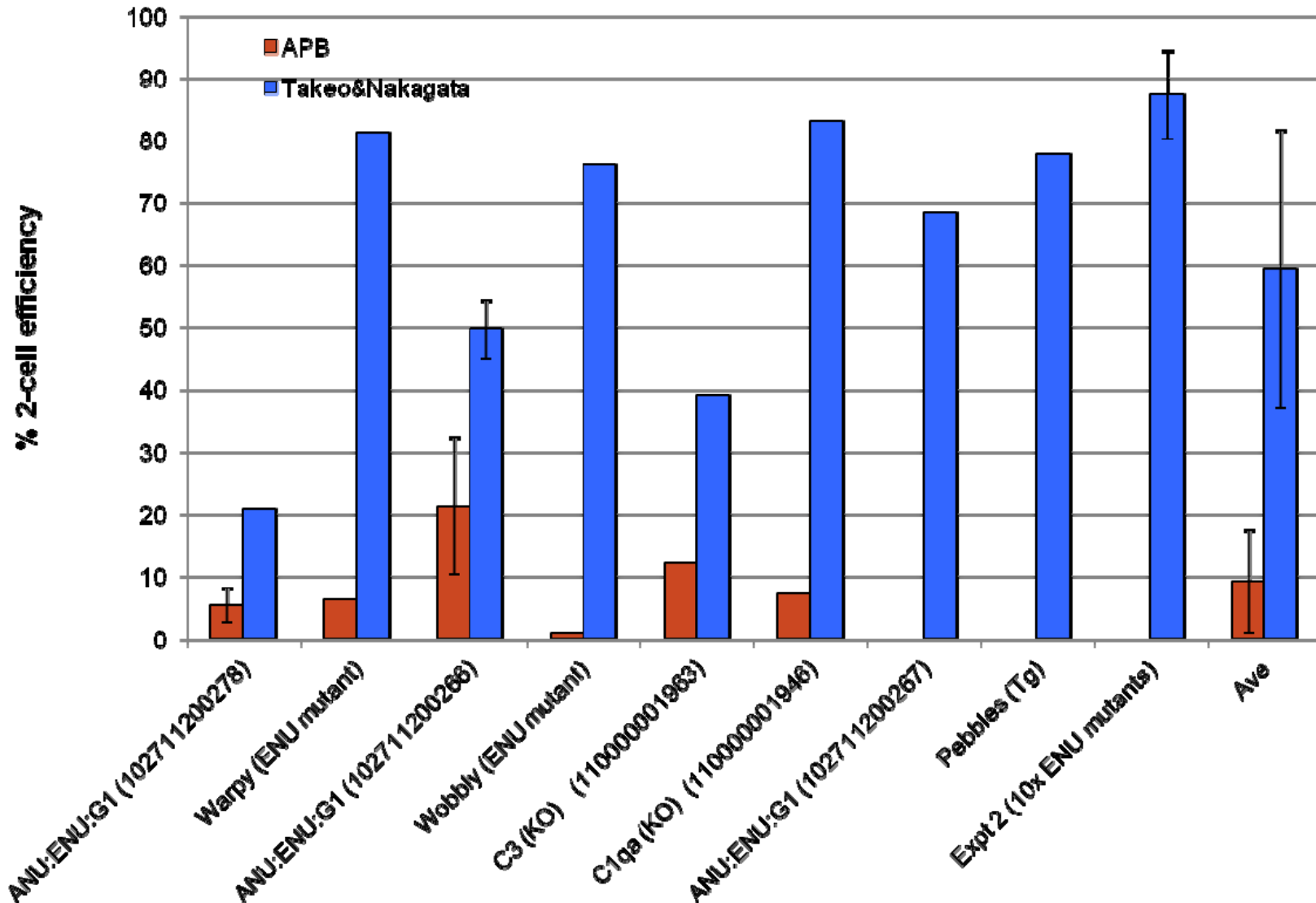
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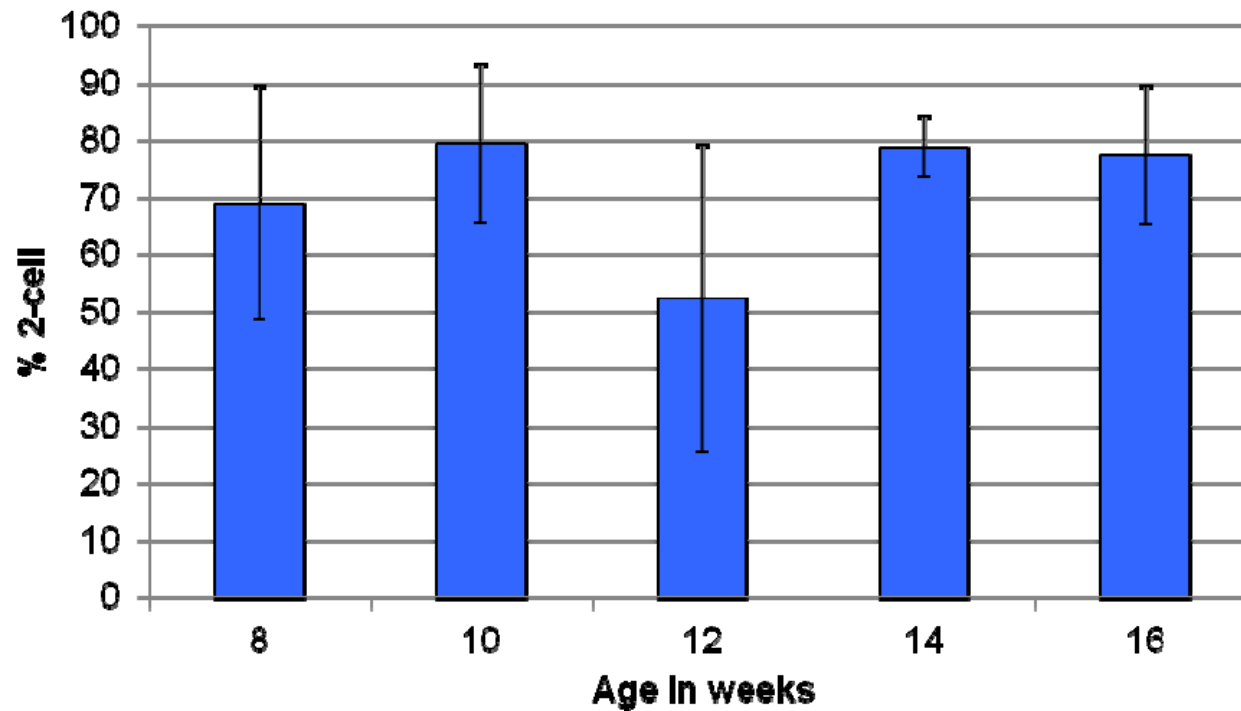
IVF Procedure Comparison



25	50	60*	45	25	16	74*	39	60*	Embryos transferred
5	26	2	20	10	11	12	13	11	Pups born
3	13	^{fostered} 13	10	11	12	9	5	5	Pups of correct genotype
141	660	141	98	77	77	147	150	150	Age (days) at collection

Experiments performed by Cassandra du Boulay and Naomi Williams

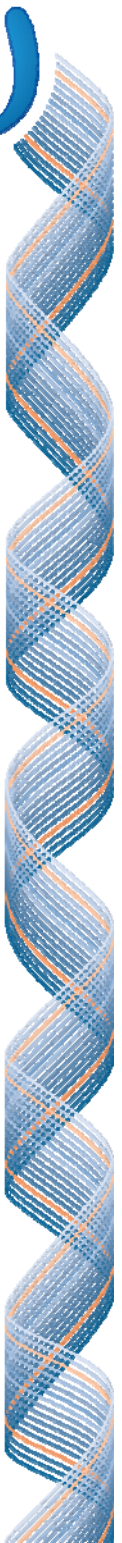
Sperm Donor Age



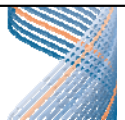
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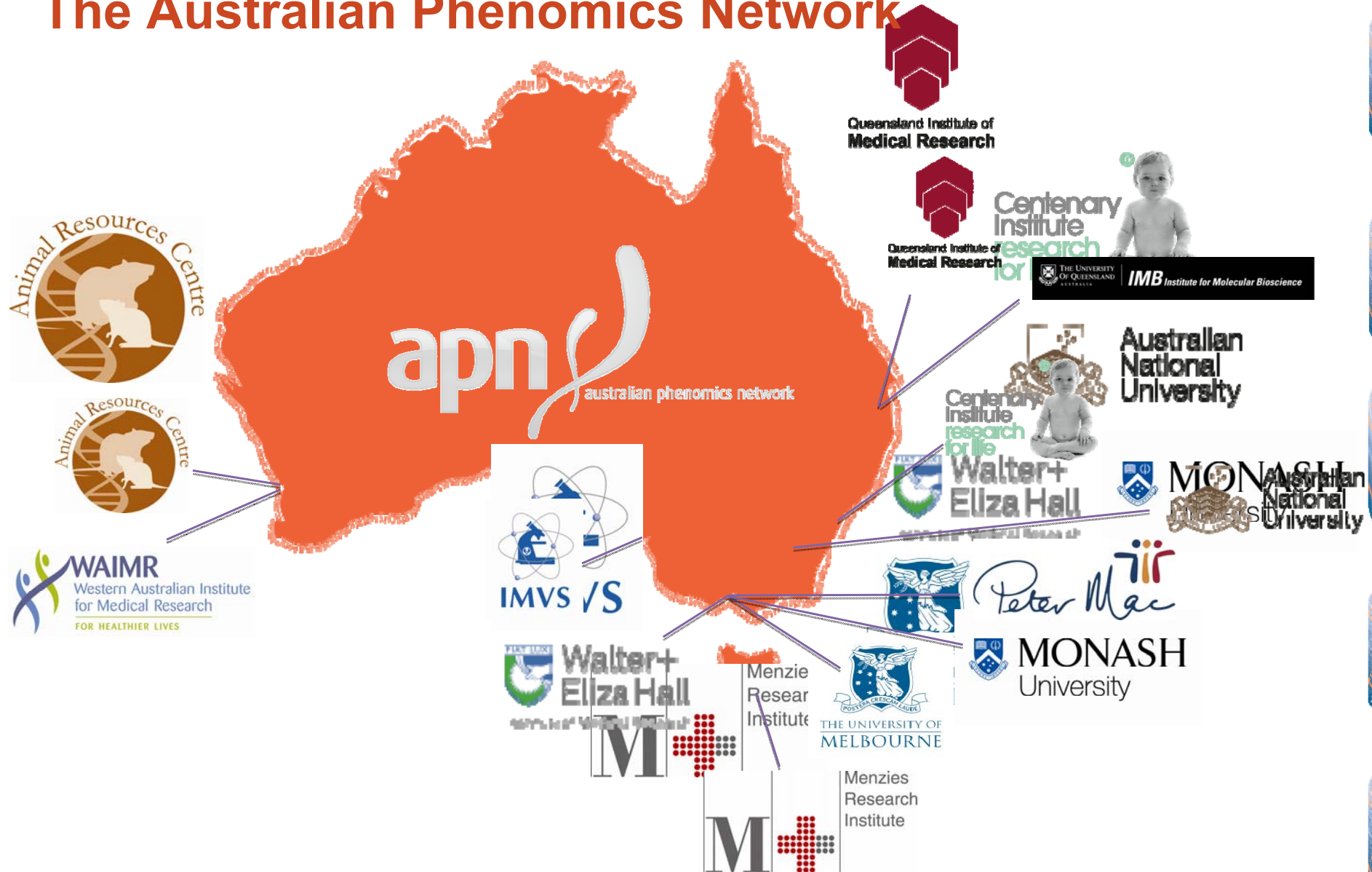
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Procedure Step	Current APB Procedure	Takeo and Nakagata Procedure	Observation / Recommendation
CPA	R18S3	R18S3 + glutamine	Better 2-cell fertilisation rate with Takeo and Nakagata
CPA manufacture	1x 30min incubation	3x 30min incubation + harder spin	Adopt Takeo
CPA shelf life	1 week (4□)	3 months (RT)	
Oocyte donor	3 - 4 weeks old	8 – 12 weeks old	Undecided*
Sperm + CPA incubation	10mins	3mins	Sperm seemed happier when only incubated for 3mins Prevents overlap when processing mice Adopt Takeo
Capacitation	30mins SIM	30min TYH + MBCD Or FERTIUP	Improved capacitation with TYH + MBCD, FERTIUP Adopt Takeo
Oocyte harvest	Hyaluronidase step	Leave in cumulus	Adopt Takeo
Sperm + oocyte	KSOM-HEPES	HTF + GSH Or CARD + GSH	Improved oocyte fertilisation rate with HTF + GSH, CARD + GSH
Sperm + oocyte incubation time	5 – 6 hours	33 – 4 hours	Adopt Takeo
Post incubation wash	10x KSOM-HEPES	6x HTF	10x HTF
Overnight incubation	KSOM-aa	HTF	HTF



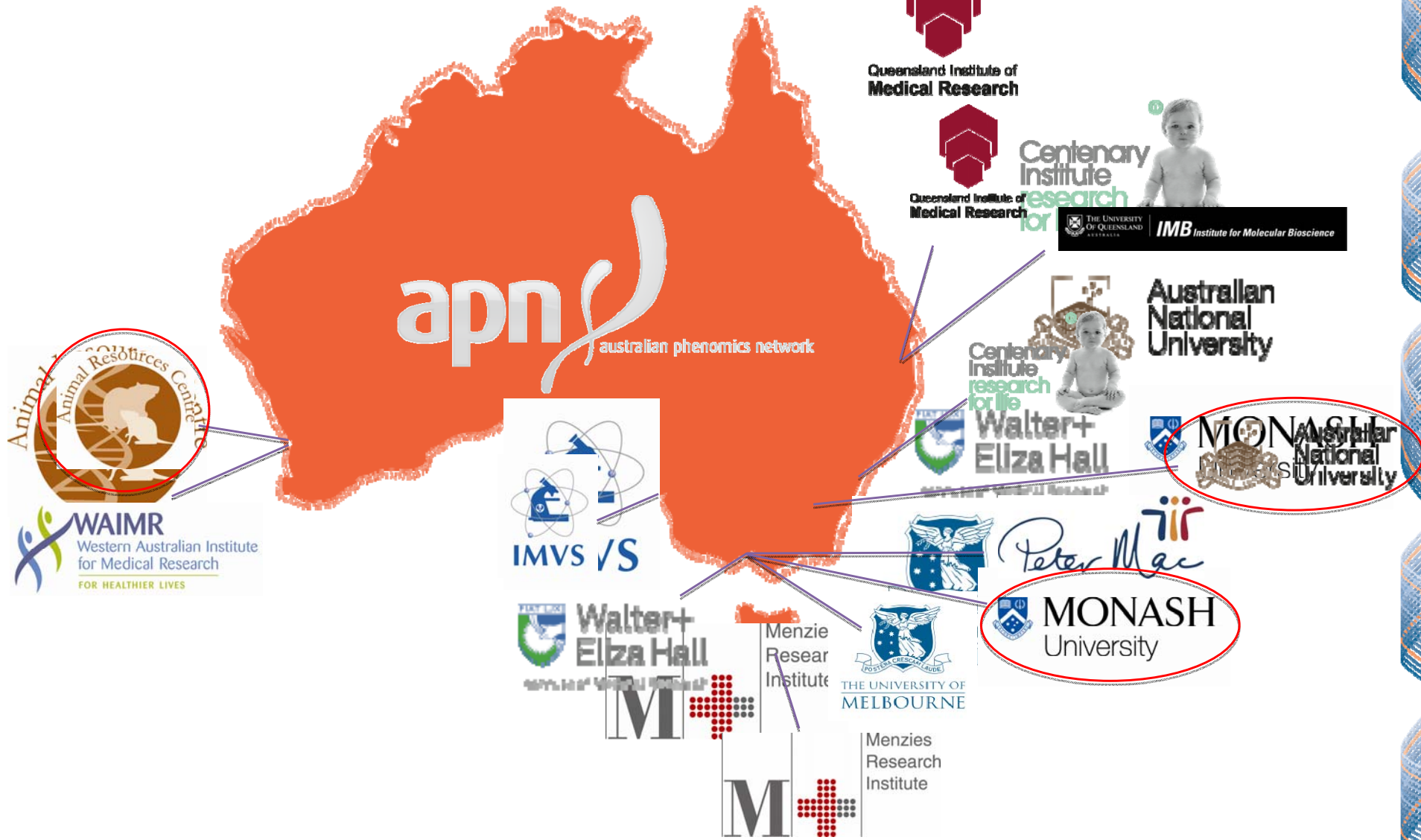
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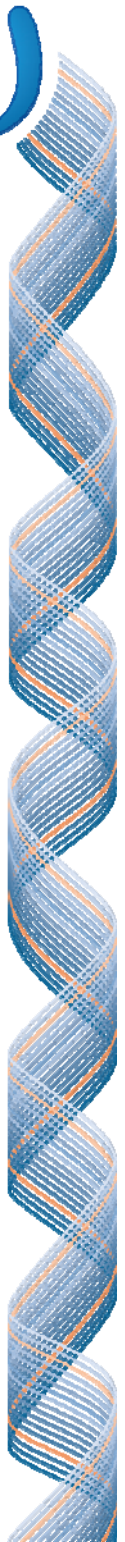
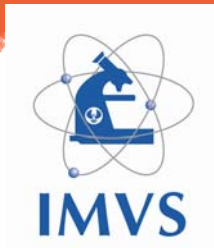
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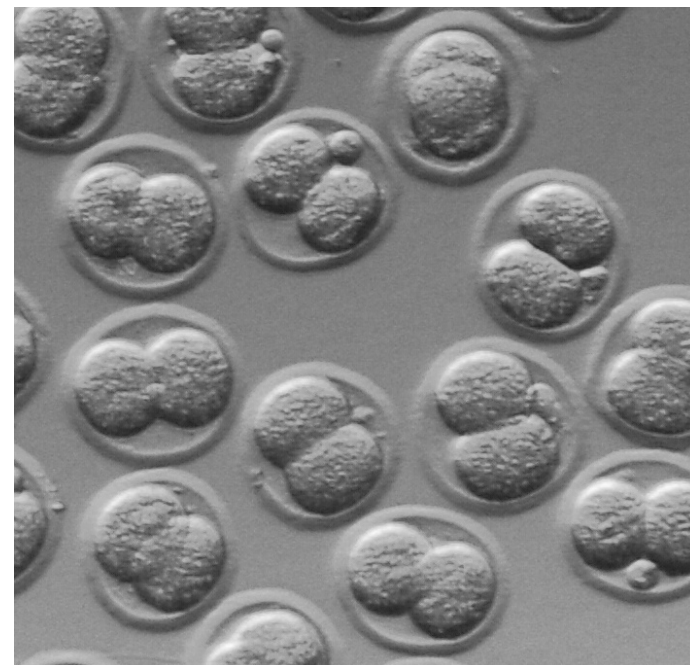
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Federated Australian Phenome Bank



Federated APB Service

- **Training**
 - Establish training packages and convene sessions
- **Quality Assurance**
 - All researchers can have the confidence in service which ever lab they chose
- **Coordinated Technique advancement**
 - Remove duplication of effort



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Acknowledgements

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