

Mouse Sperm Cryopreservation at JAX Current & *Future Challenges*

(& ES cell only germ cell transmission via Perfect Host blasts)

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Directed Research / Technology Evaluation and
Development

The Jackson Laboratory
USA

Our Cryo' Mission @ JAX

To provide a reliable service to all comers/backgrounds

Where mouse strains can be cryopreserved reliably, strain independently and at a reasonable cost (& distribute)

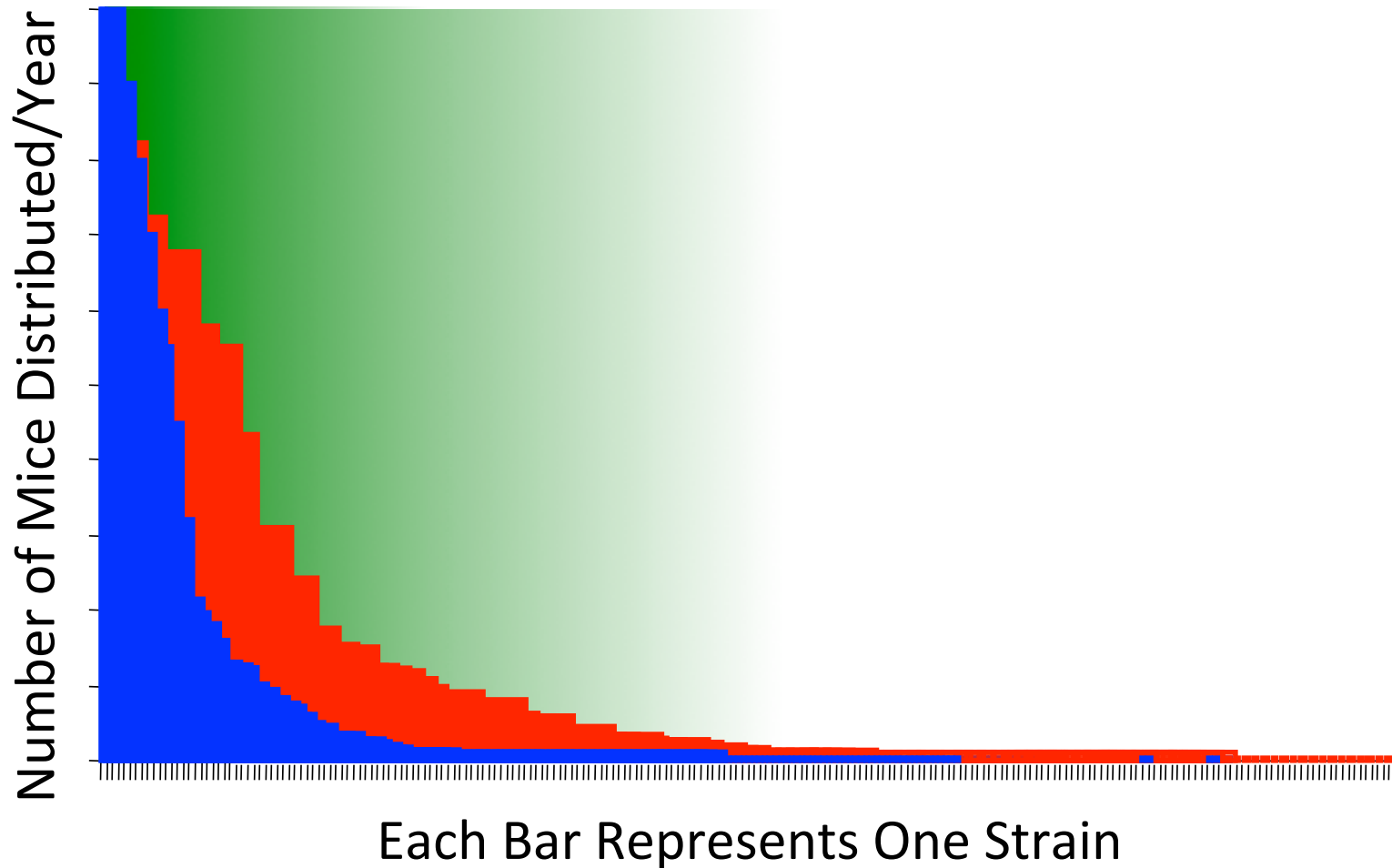
Our internal aims are the same – plus we need *high throughput & robustness* to be economic

To publish & assist others to duplicate and use our work

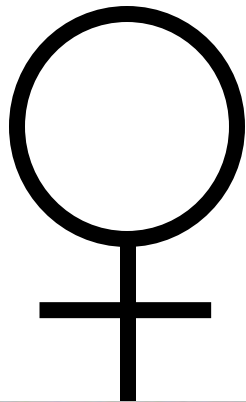
... building on the work of many before us
(many are here at this meeting) - thank you

The Challenge - The Long Tail

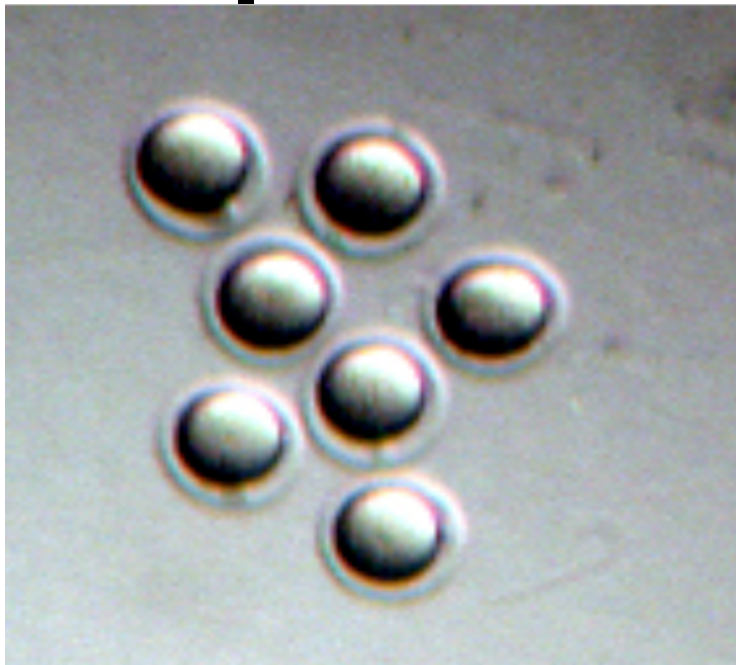
A Future of Providing Less of More



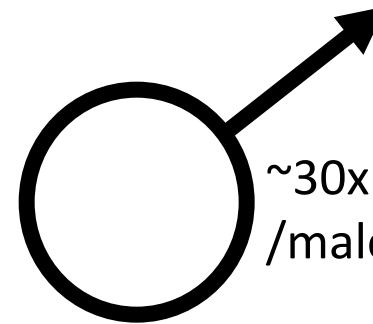
Some Basic Biology - Gamete Possibilities



50 or less oocytes
/female



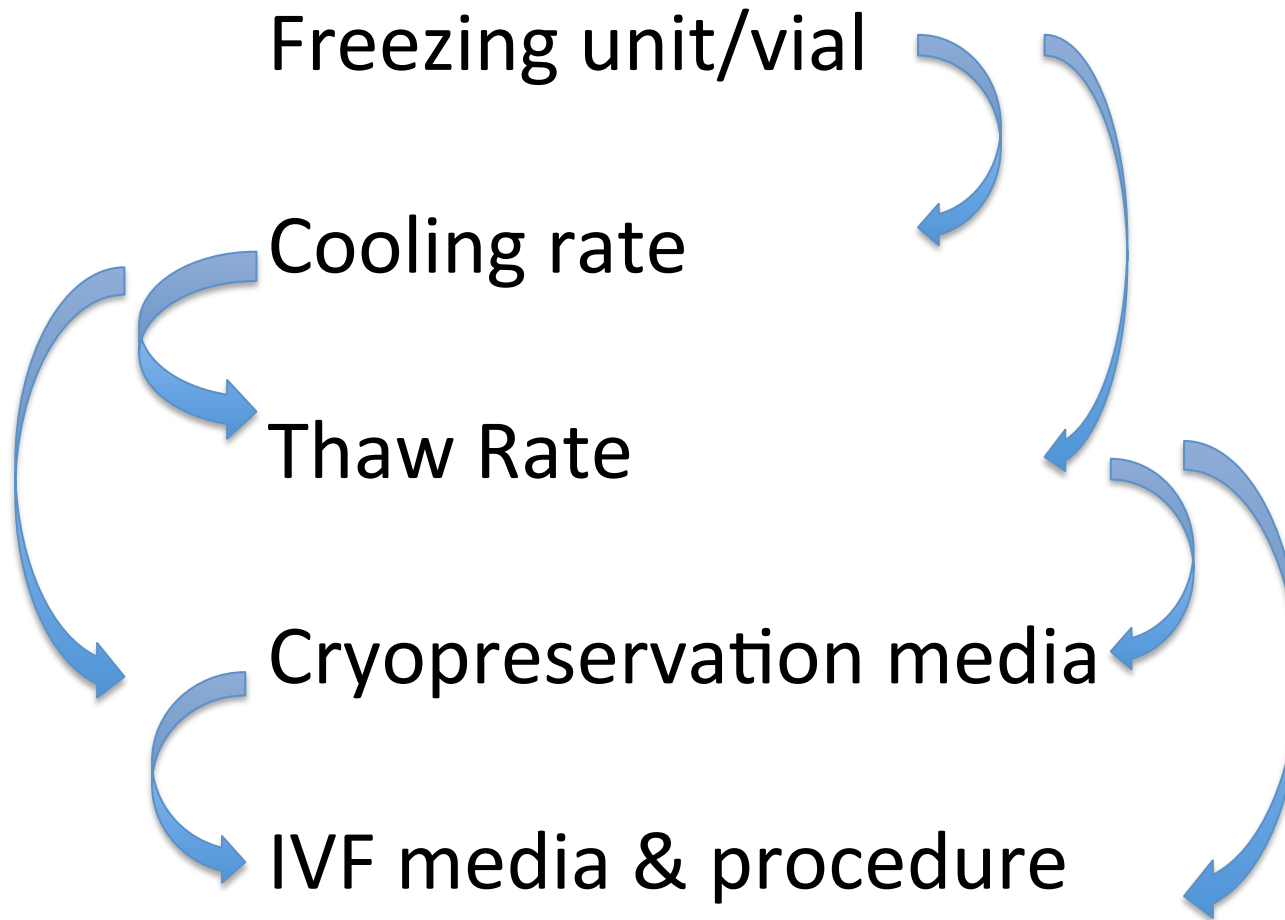
C57BL/6



$\sim 30 \times 10^6$ sperm
/male



*Factors examined and optimized**

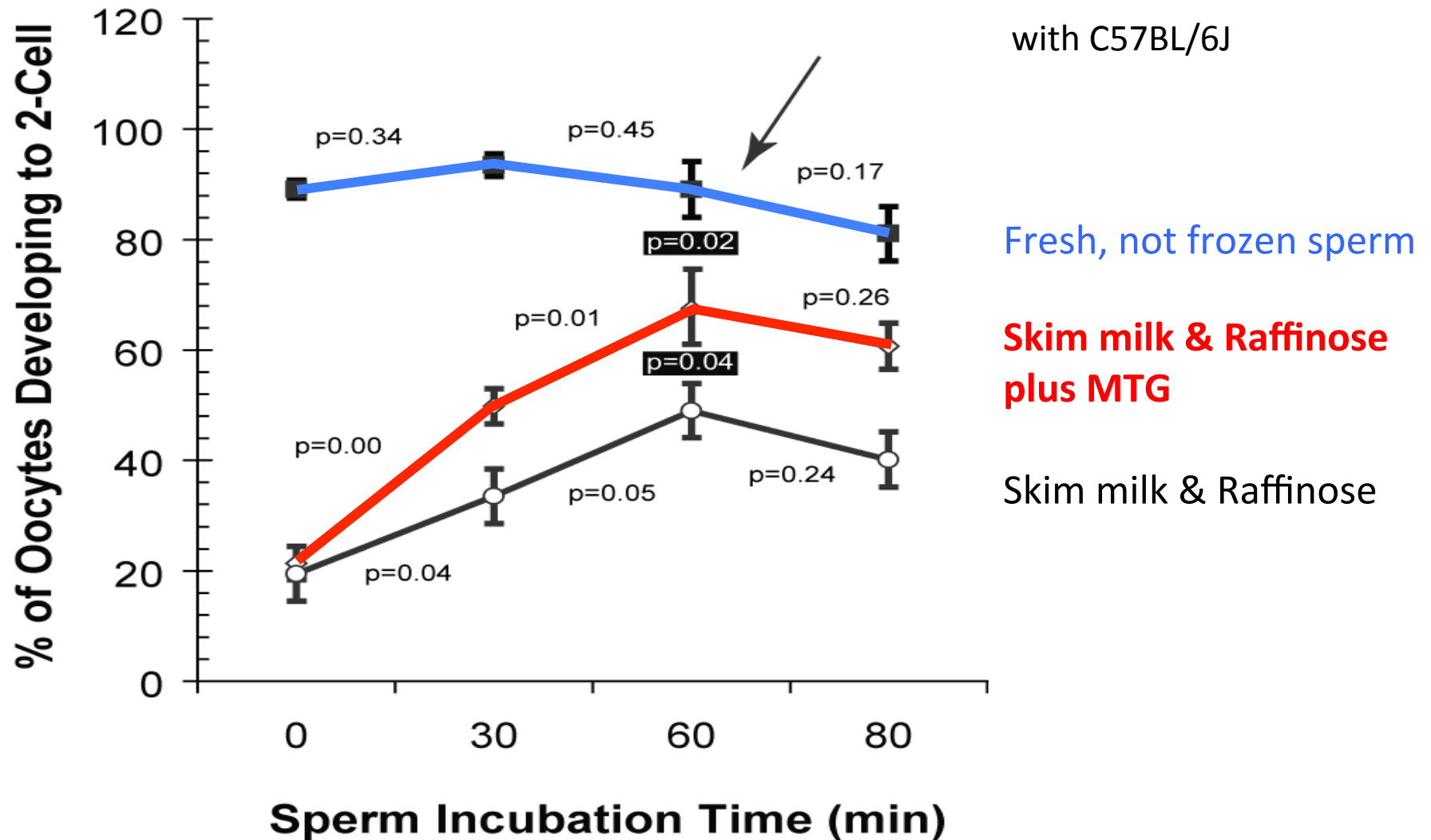


End point ?

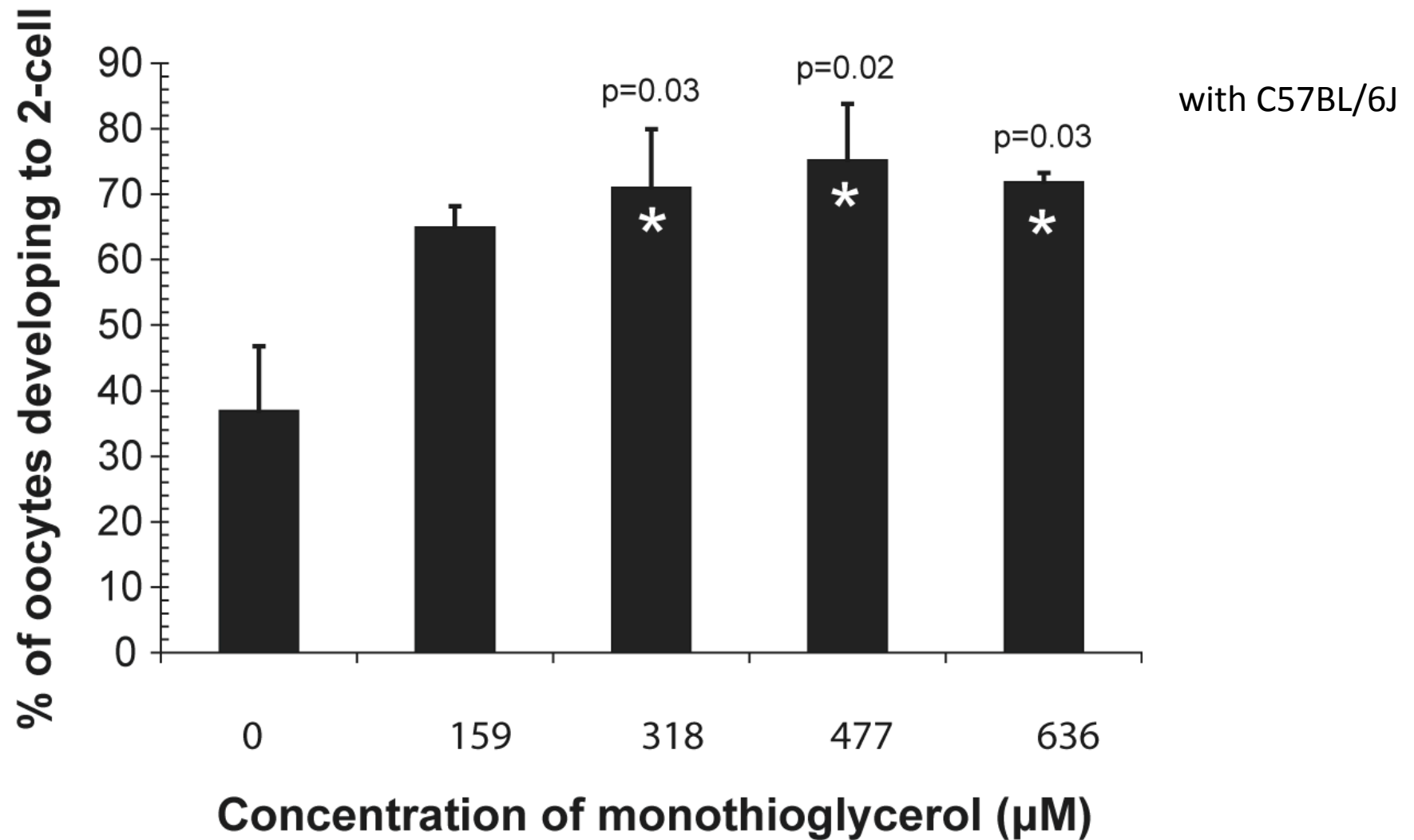
Motile sperm ?
2 cell ?
blasts' ?
live born ?

*Note, most data shown used C57BL/6J sperm & oocytes - NOT F1's

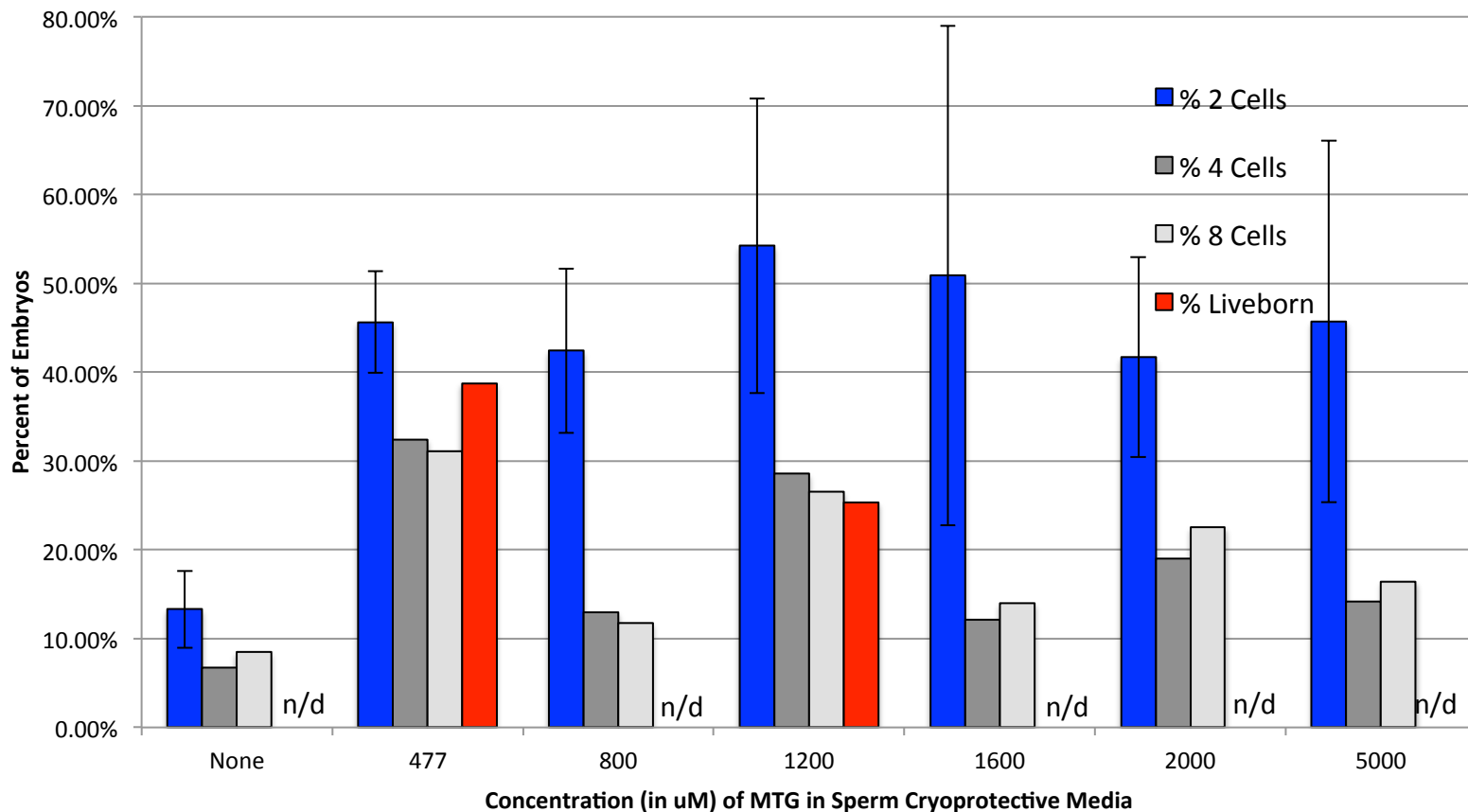
Cryo' Sperm - Incubation Time & MTG effect on IVF eff.



The Reducing agent MTG enhances cryo'ed sperm IVF to 2 cell



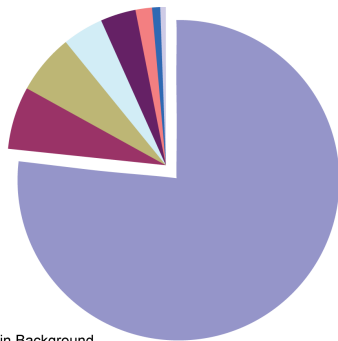
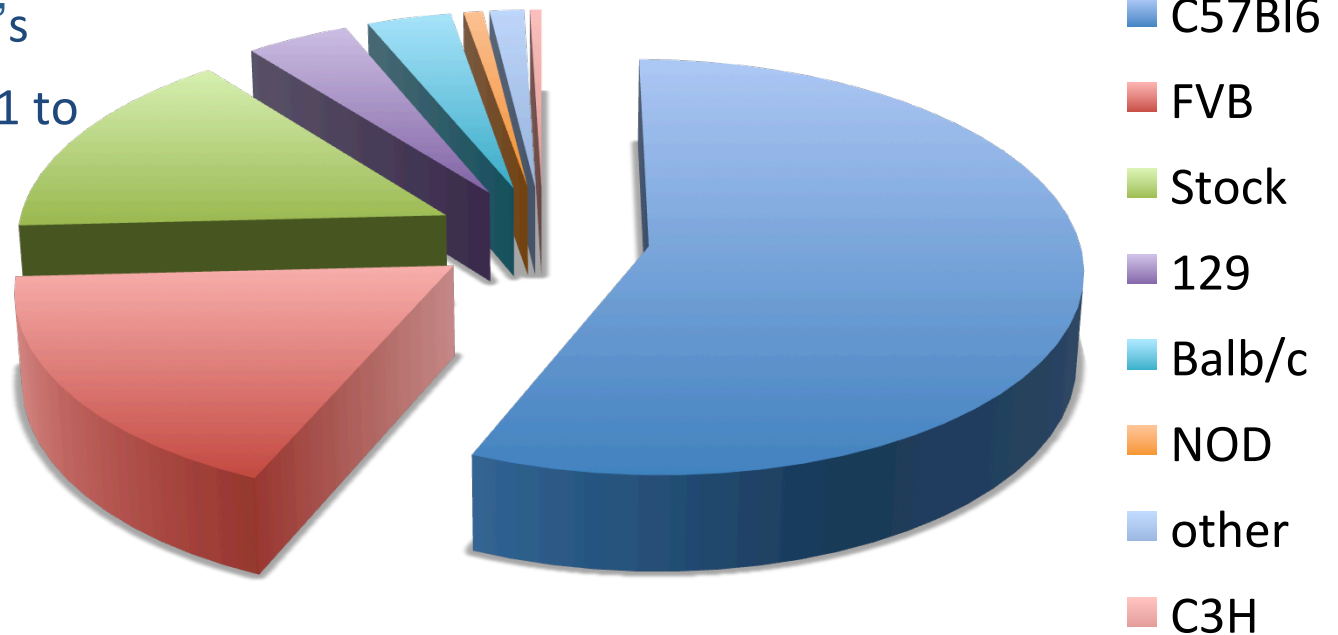
% embryo development post IVF vs. MTG conc. in Sperm Cryopreservation Media



Background Usage - Frequency

620 mouse strains submitted to

The Jackson Laboratory's
Repository January 2011 to
January 2012



Inbred Strain Background

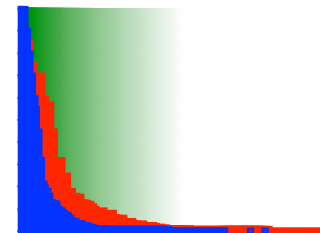
C57BL/6J
BALB
B6129F1/J

NOD
FVB/NJ
C3H

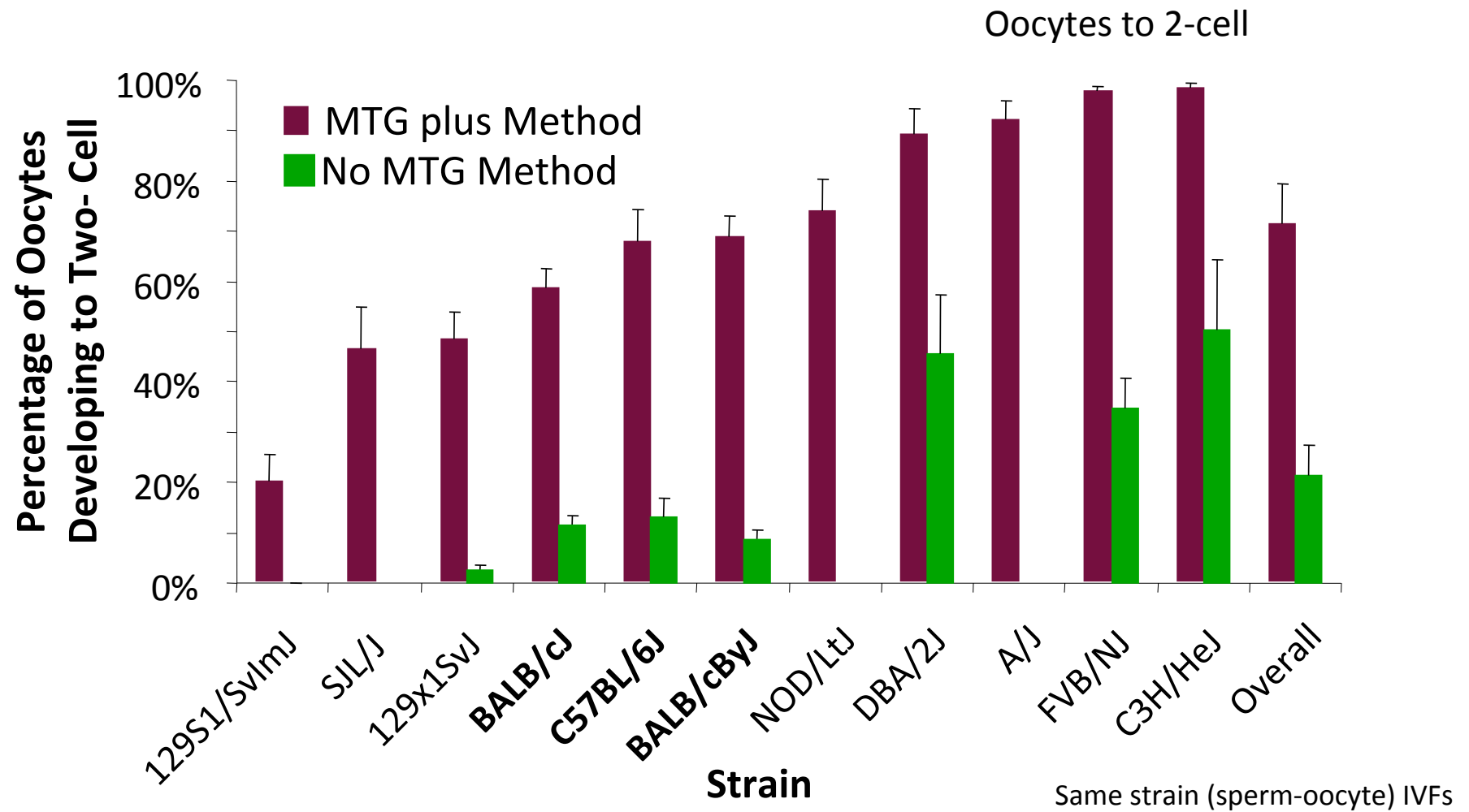
DBA
CBA

670 mouse strains

January 2004 to January 2006



Cryo'd Sperm IVF Recovery vs. Background



Simplicity – Sperm Cryo Kit “do it yourself”

Collect sperm in buffer
(epididymal or ejaculate)



+
2x Cryoprotectant !!
(CPM)



Sperm in CPM is loaded into “straws”



Or do it all yourself -
Wiles & Taft,
(2010)
The Sophisticated
Mouse – Protecting a
Precious Reagent
*In Mouse Models for
Drug Discovery, in:
Methods in Molecular
Biology*
Vol 602:23-36



Long Term
Storage in LN₂



QC and
storage



LN₂ vapour on
float >15 mins
then plunged into
LN₂

Some “Metrics”

~6,700 strains cryopreserved (and QC’ed) using the
“MTG approach” by JAX Reproductive Services

~2100 strains cryopreserved in the last 12 months

~1500 strains recovered in the last 12 months
(*allows rederivation*)

0 cases where JAX sperm cryo-recovery IVF
process has failed (*with sperm which functions in a conventional IVF*)

The Challenge of

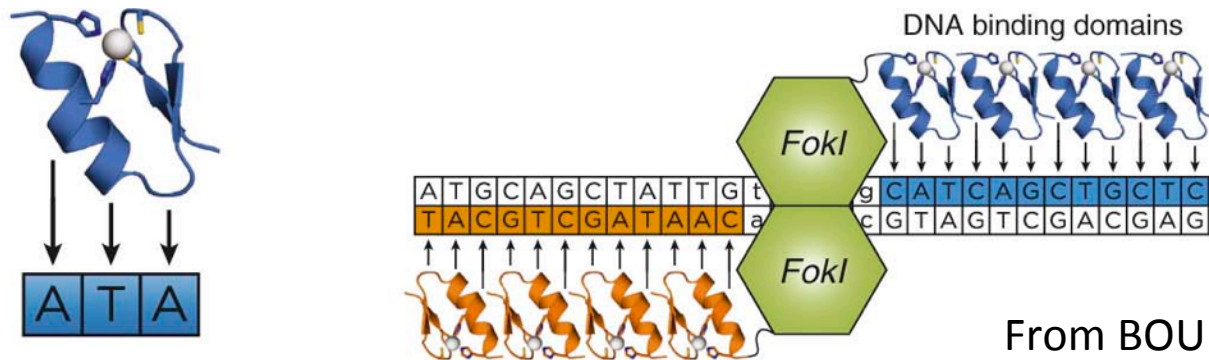
Things to Come ... ?

i.e.

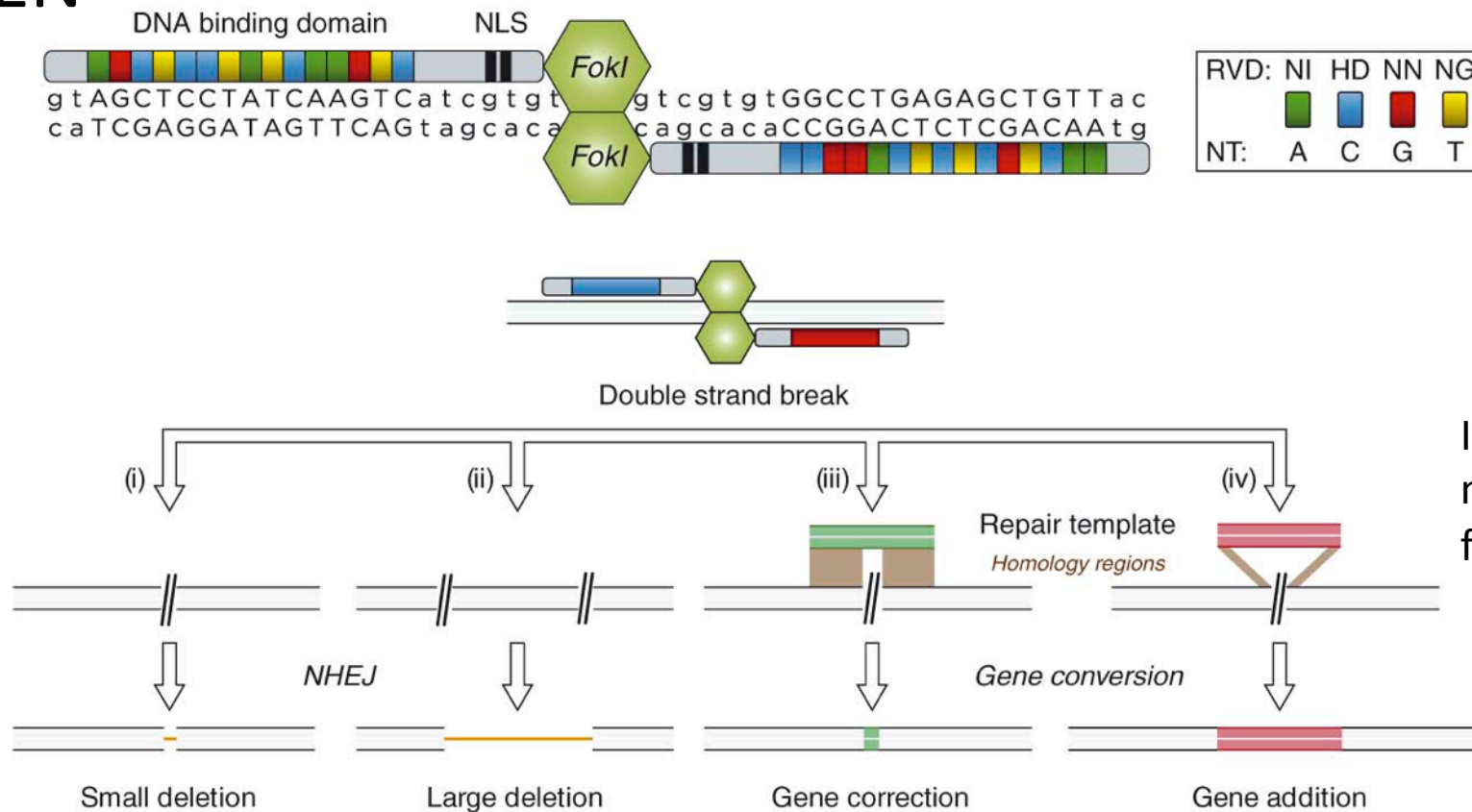
More, Faster, Complexity
(with less \$'s)

Direct Genetic Modification in the Zygote – *any Zygote*

ZFN's



TALEN



It's all a matter of frequency

.. The Future – Homozygous Freeze

The Challenge

– to freeze a strain down using ≤ 10 animals for $< \$750$ (£460, €570, ¥60k, CNY4.7k)

The Delivery

Provide ≥ 4 *reliable* *homozygous* recoveries

Background independent

(use sperm as final backup – i.e. het recovery)

Perfect ES cell Host embryo

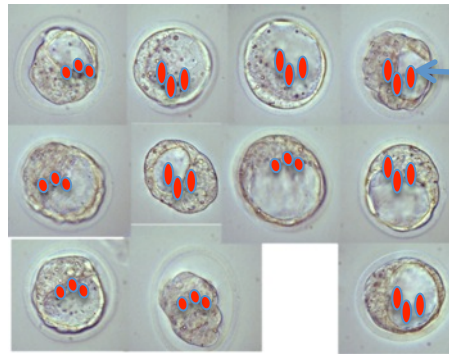
Flox'ed stop, release
gives DTA expression



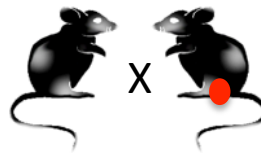
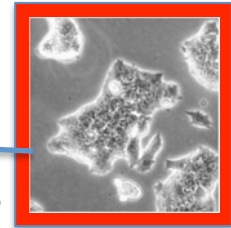
Vasa promoter
driven Cre



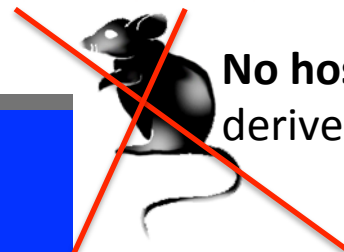
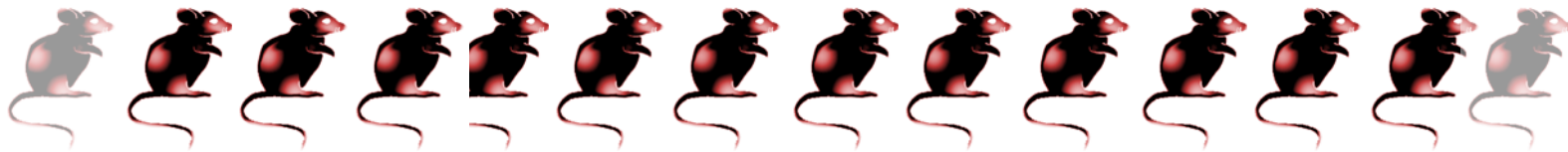
Perfect Host embryos
have *no* endogenous
germ cells after ~E14.4



Host Blastocysts
Microinjected with
GM ES cells



any fertile chimeras can **only** have
gametes from the introduced ES cells



**No host embryo
derived offspring**

Testes from Wild Type and Perfect Host Mice



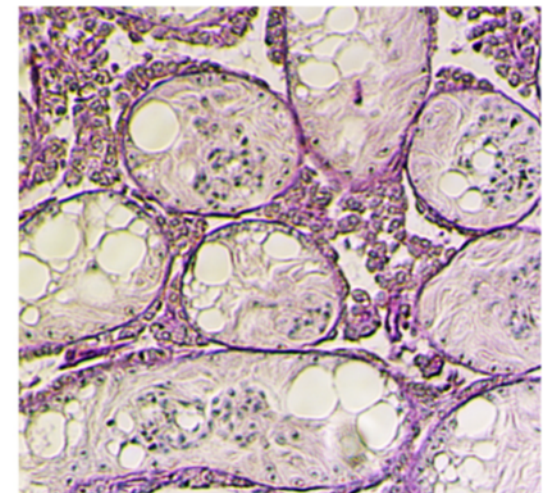
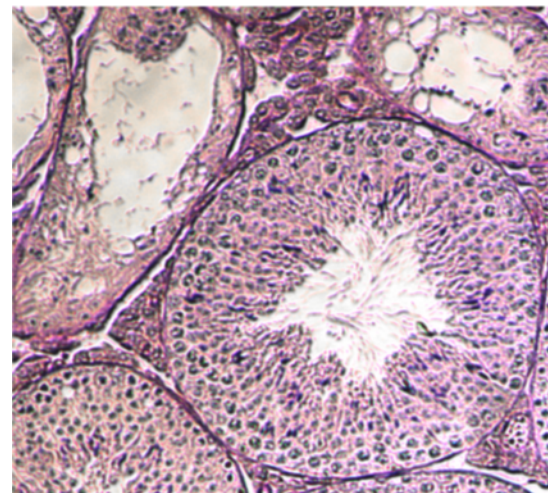
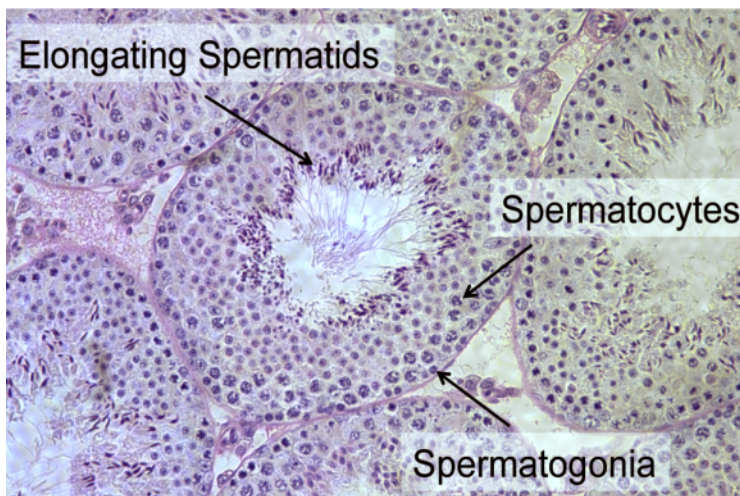
Wild type



Perfect Host
Colonized



Perfect Host
-ve control



Summary of Perfect Host



- ES can transmit
 - No *host* germline leakage detected
 - Option for IVF and massive rapid expansion of Hets as sperm can *only come* from ES
 - If ES fails to transmit, provides *closure*
- Applied to *KOMP* (*in progress*)

Acknowledgements

- Ben Low
- Cindy Avery
- In collaboration with Dr. Rob Taft
Jane Farley
Shannon Byers

and .. if you have a *mouse* with No *Where* To Go see us at:

www.jax.org/donate-a-mouse