The use of cryopreservation and assisted reproductive techniques to aid colony management

Stuart Newman

Transgenic Coordinator, Biological Services, King's College London

Introduction to Cryopreservation

Why Cryopreserve a line?

- Allows future recovery if a line is no longer required as a live resource (3Rs) facilitating quick shut down if material is already frozen.

- Allows a “reset” point back to the date of freezing (to maintain genetic integrity or for business continuity)

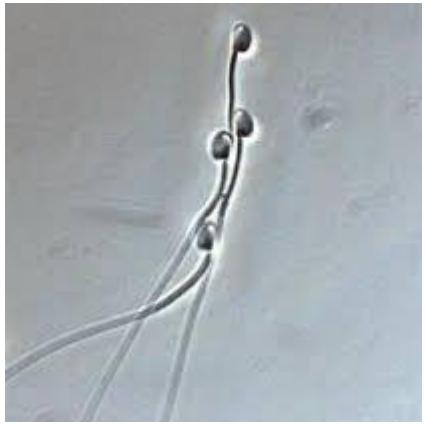
- Permits efficient and ethical distribution of lines around the world.

Methods

How are mouse lines normally cryopreserved?

Primary

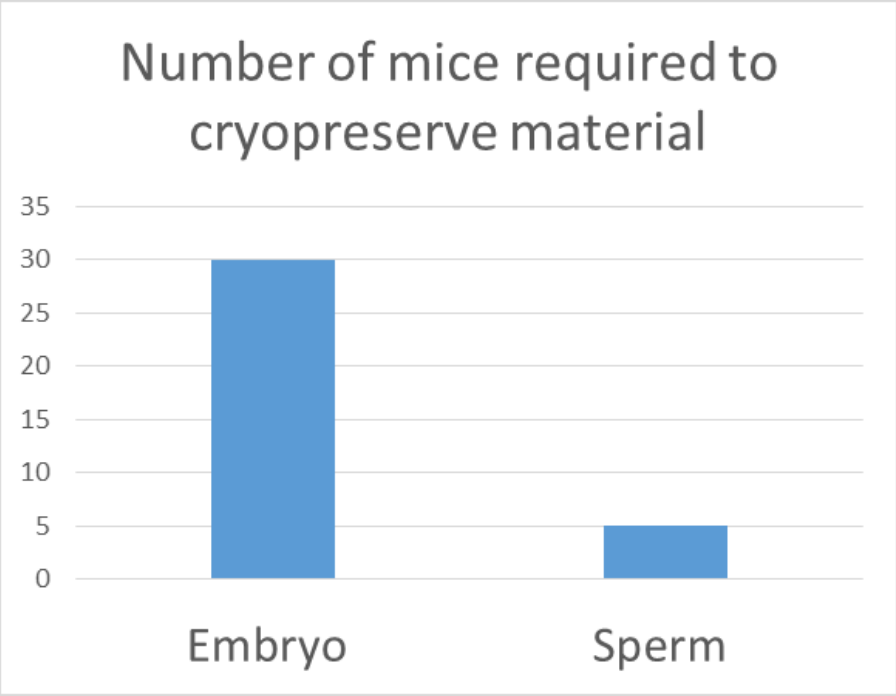
- Preimplantation embryo
- Sperm



Additional

- Oocyte
- Ovary

Embryo Vs Sperm Cryopreservation



	Embryo	Sperm
Time	1-2 months	~2 weeks
Number of mice	~ 30	~4.5
Recovery attempts	~10	20-40
…Price	££££	££

Sperm cryopreservation

Significant progress over the past decade making the method reliable.

Cryoprotectant (R18S3)

- Skimmed milk powder (3%)
- Raffinose (18%)

- Monothioglycerol 477uM (**mCPA**)

Ostermeier, G. C., Wiles, M. V, Farley, J. S., & Taft, R. A. (2008). Conserving, distributing and managing genetically modified mouse lines by sperm cryopreservation. *PloS One*, *3*(7), e2792.

OR

- L-glutamine 1.0mM (**gCPA**)

Takeo, T., & Nakagata, N. (2010). Combination medium of cryoprotective agents containing L-glutamine and methyl-{beta}-cyclodextrin in a preincubation medium yields a high fertilization rate for cryopreserved C57BL/6J mouse sperm. *Laboratory Animals*, *44*(2), 132–7.

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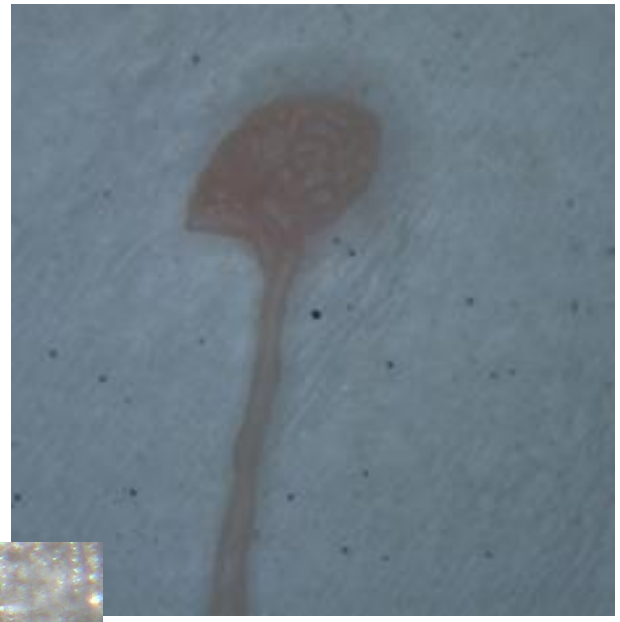
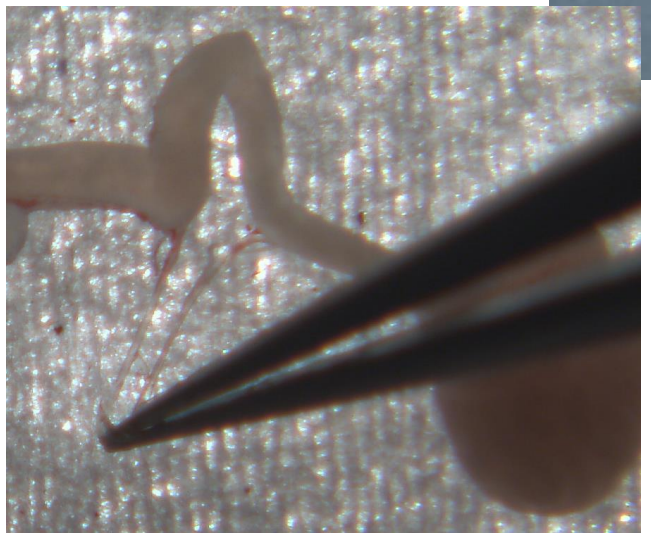
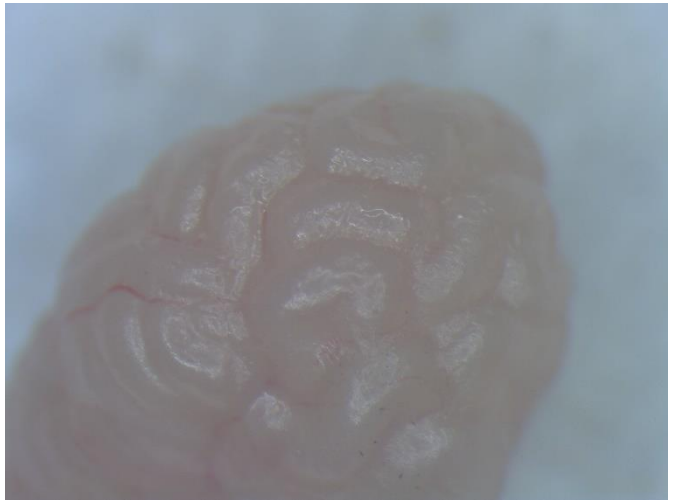
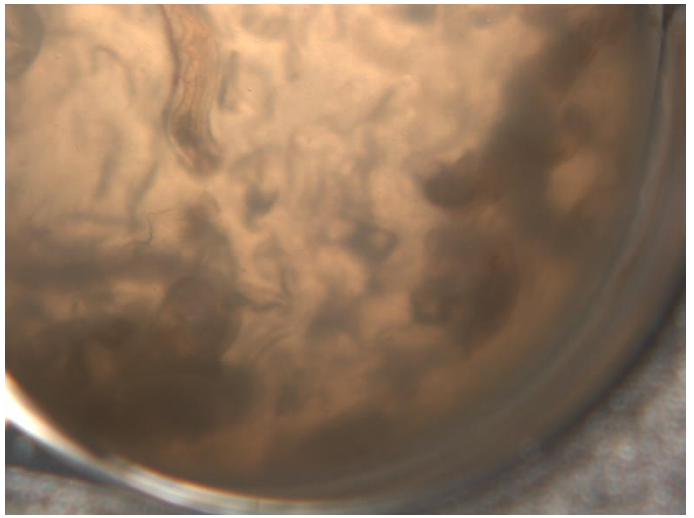








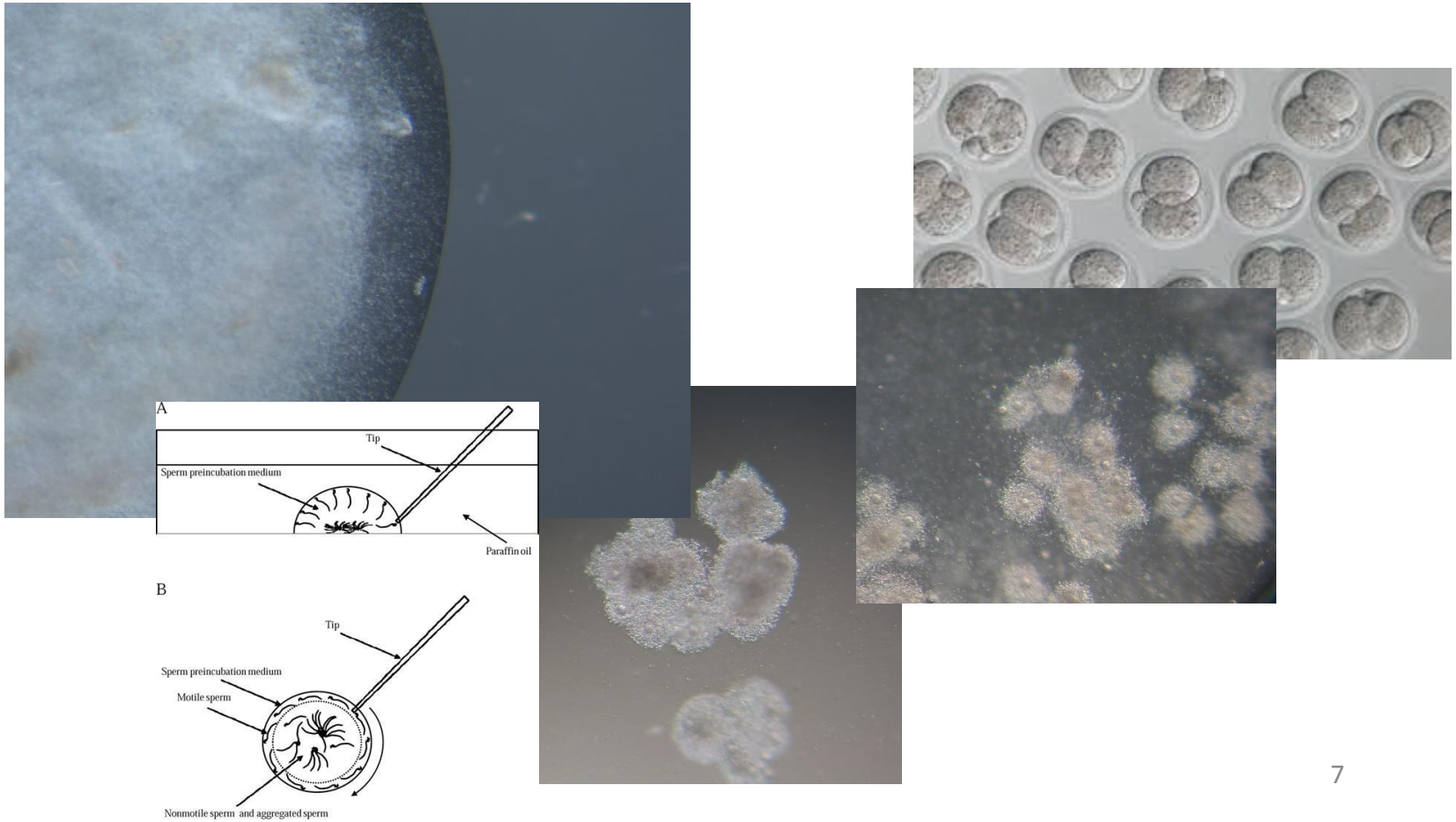
Assisted reproductive techniques

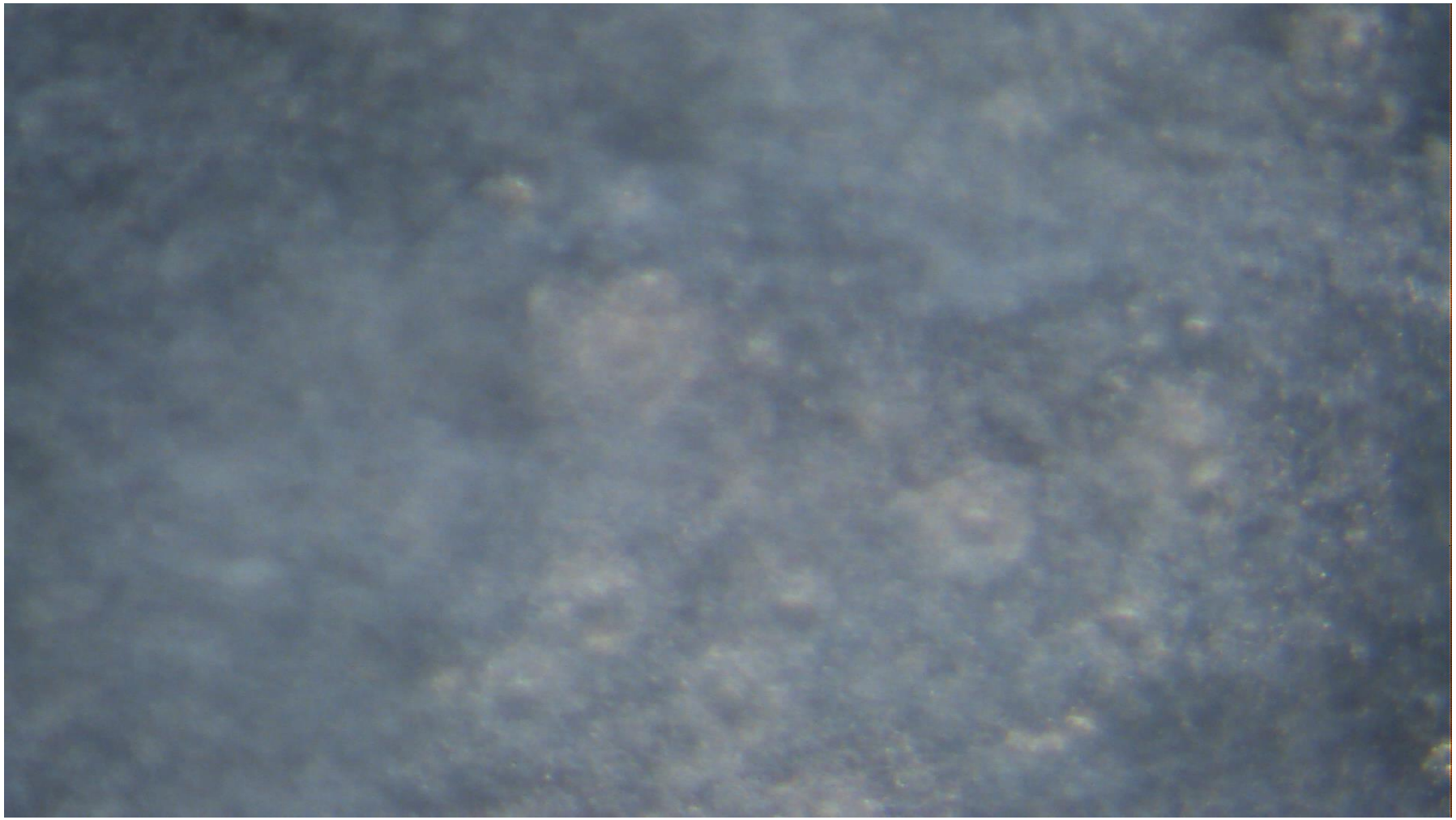
IVF

Massively improved over the past few years, in line with sperm cryopreservation

Process

Day -3	PMS @1700
Day -1	HCG @1700
Day 0	Sperm thaw/harvest ~0800
	Oocyte harvest ~0830
	Insemination ~0900
Day+1	Embryo transfers
	Cryopreservation





Preincubation Media

- Facilitates sperm capacitation (prepares for fertilisation)
  - Cholesterol efflux from plasma membrane.

αMEM

Cook

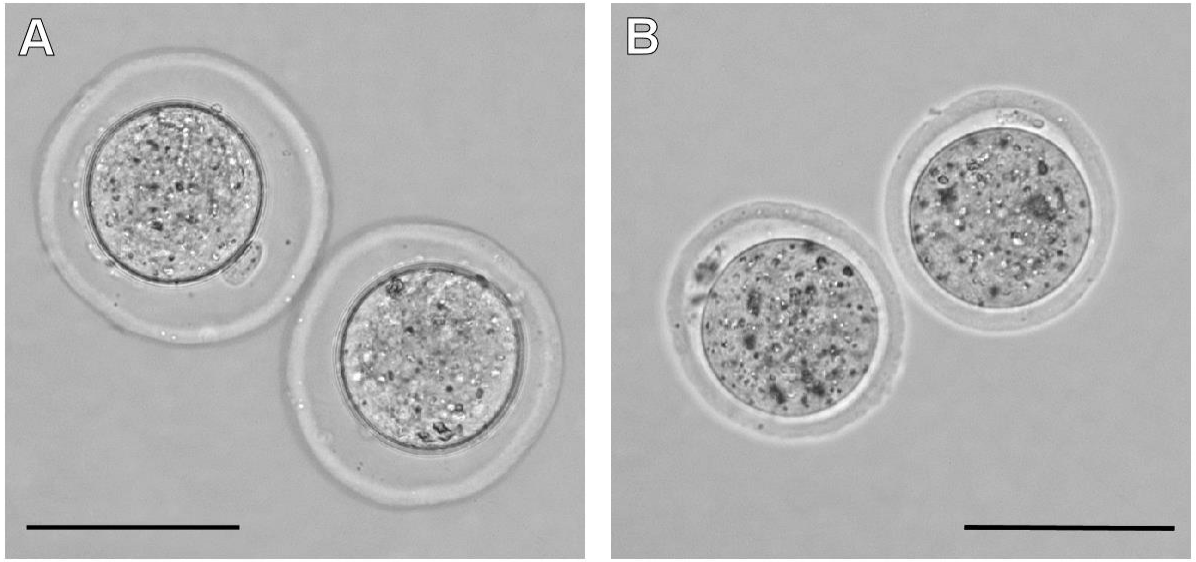
Modified Krebs Ringer Bicarbonate solution (TYH) plus MBCD

TYH & MBCD

Takeo, T., Hoshii, T., Kondo, Y., Toyodome, H., Arima, H., Yamamura, K., … Nakagata, N. (2008). Methyl-Beta-Cyclodextrin Improves Fertilizing Ability of C57BL/6 Mouse Sperm after Freezing and Thawing by Facilitating Cholesterol Efflux from the Cells. *Biology of Reproduction* , *78*(3), 546–551.

Fertilisation Media

- Creates a good environment for fertilisation to occur



- **High Calcium HTF (human tubal fluid)**

- **Reduced Glutathione ~1.0mM (GSH)** (Vary according to fresh, cold or frozen sperm)

Takeo T, Nakagata N. Reduced glutathione enhances fertility of frozen/thawed C57BL/6 mouse sperm after exposure to methyl-Betacyclodextrin. Biol Reprod. 2011 Nov; 85(5):1066-72.

Ishizuka, Y., Nishimura, M., Matsumoto, K., Miyashita, M., Takeo, T., Nakagata, N., … Anzai, M. (2013). The influence of reduced glutathione in fertilization medium on the fertility of in vitro-matured C57BL/6 mouse oocytes. *Theriogenology*, *80*(5), 421–6.

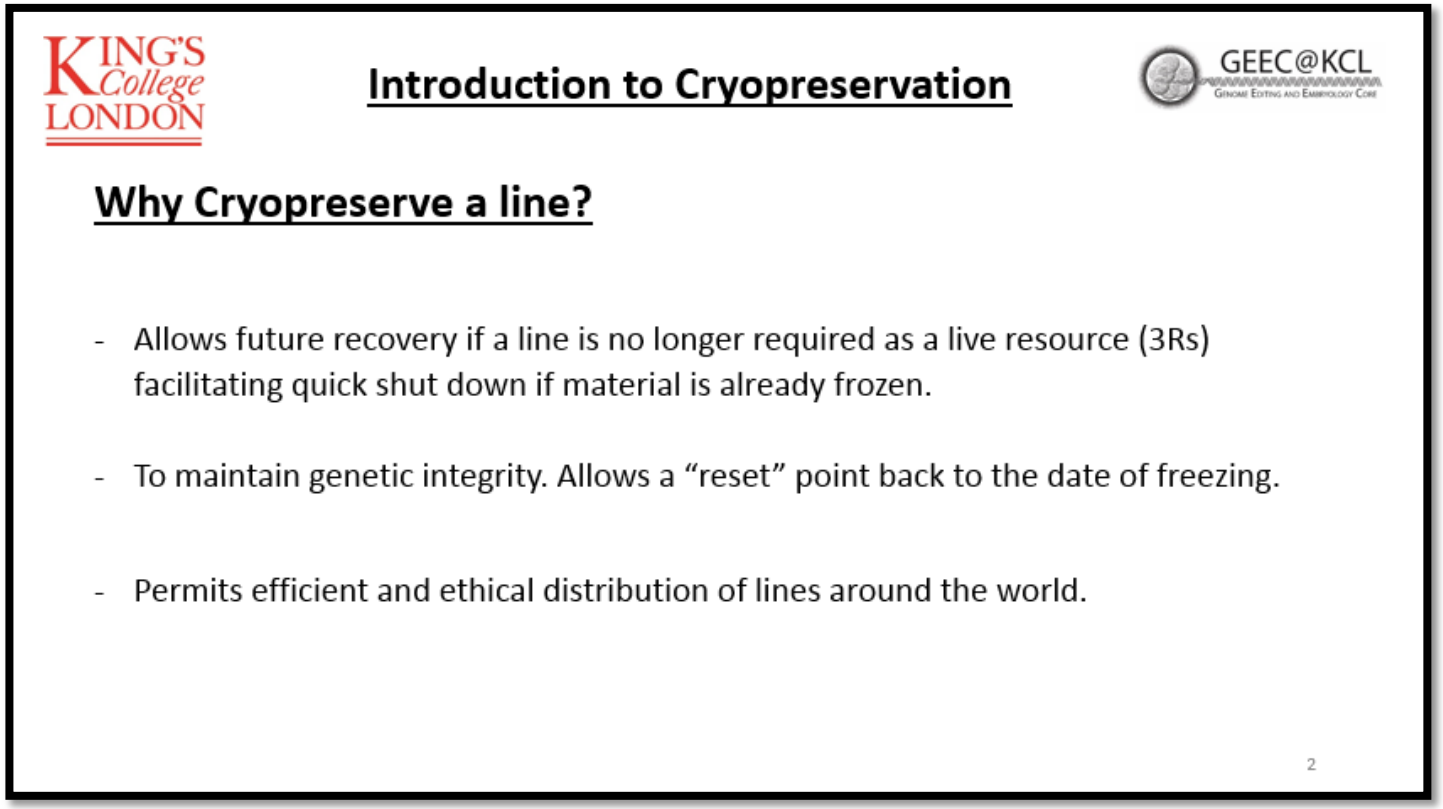
Controlling all IVF variables

- superovulation times/volumes (genetic background?)
- Batches of media *
- Plastic ware/components
- Use by dates on consumables
- Consistent criteria when cryopreserving sperm (SOP's, selection process)
- Accurately recording information (GLP?)

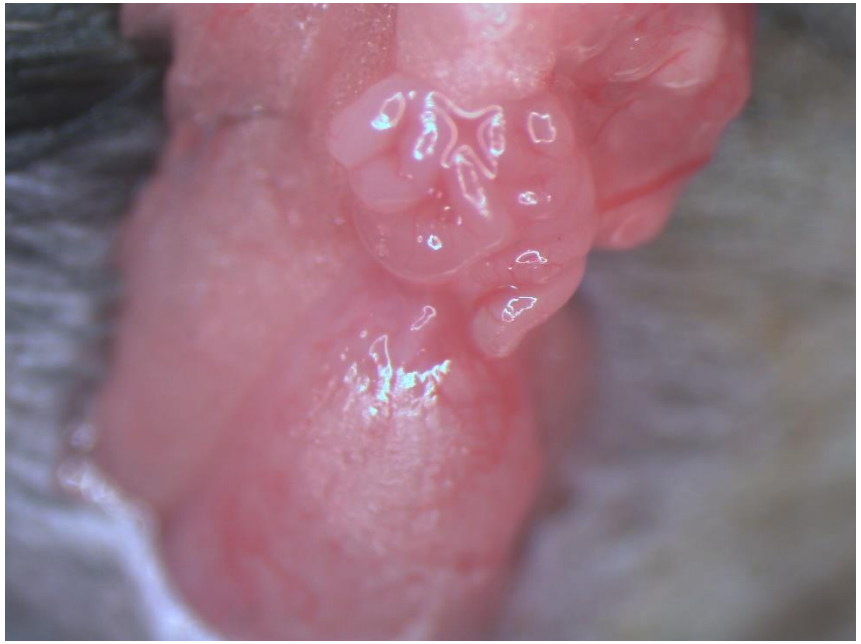
Efficiency and reliability > IVF > tool to aid colony management

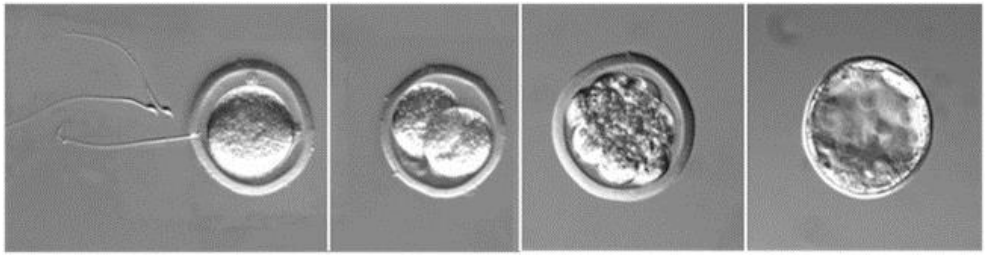
Recap…

Increased efficiency in IVF allows us to use it in the application of colony management too…



- **Efficient embryo generation**
  - Embryo cryopreservation
  - Rederivation
  - Quality control
  - Microinjection





Cohort generation

Generate experimental cohorts straight from archived material (no colony expansion phase)

IVF using GA sperm

specific crosses e.g. to Cre or Flp driver line (don't need to maintain additional colonies) and maintains genetic integrity.

Cell permeable technologies?

Rescue IVF

Where by bad luck or poor management a colony is close to being lost – cryopreserve and recover experimental cohort or FBS.

Recovery IVF e.g. from Chimeric male failing to breed

Harvest sperm > Cryopreserve > Genotype > IVF

Rescue IVF protocol

In case normal IVF protocol fails to recover from frozen material.

- Thaw
- Centrifugation with MTG (reduce damage from ROS)
- Re-suspend in TYH & MBCD
- **BUT** Wouldn't be as efficient if the archive was "normal"

Questions ?