Controlled Rate Embryo Freezing Past, Present, Future

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- Successful cryopreservation of mouse embryos using equilibrium freezing
  - Whittingham, Leibo, Mazur
  - Wilmut

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- Is it effective?
- Is it reliable?
- Is it safe?
- Will viability be retained long-term?
- Will it be useful?

Effectiveness/Reliability

Table 1. Viability of frozen eight-cell mouse embryos of two inbred strains after storage at −196°C for up to 8 months and aerial transportation

Mouse strain	*Length of storage at −196°C (days)*	*No. of embryos frozen*	*No. of embryos recovered*	*No. of embryos developing to blastocysts in vitro*	*No. of blastocysts transferred to pseudopregnant recipients*	*No. of fetuses*	*No. of liveborn*
C57BL/6J	2	48	48	48	—	—	—
	189	38	38	35	35	11	5
	222	26	25	22	22	10	3
Total		112	111	105	57	21	8
BALB/cWt	2	66	61	39	—	—	—
	189	11	10	3	3	—	3
	222	10	10	5	5	4	—
Total		87	81	47	8	4	3

Whittingham and Whitten 1974

Long term viability

Table 1. Survival and development *in vitro* of frozen–thawed 8-cell mouse embryos after exposure to various doses of γ-irradiation during storage at −196°C

Radiation dose (cGy)	No. of ampoules* thawed	No. of embryos recovered (%)	No. of embryos normal at recovery (%)	No. of morulae and blastocysts after 24 h in culture (%)
0	8	225 (93·8)	135 (56·3)	160 (66·7)
10	8	223 (92·9)	142 (59·2)	151 (62·9)
50	8	222 (92·5)	147 (61·3)	157 (65·4)
100	8	223 (92·9)	155 (64·6)	172 (71·7)
200	8	217 (90·4)	144 (60·2)	154 (64·2)

* Each ampoule contained 30 8-cell embryos.
All percentages calculated from total no. of embryos originally frozen.

Glenister et al 1984

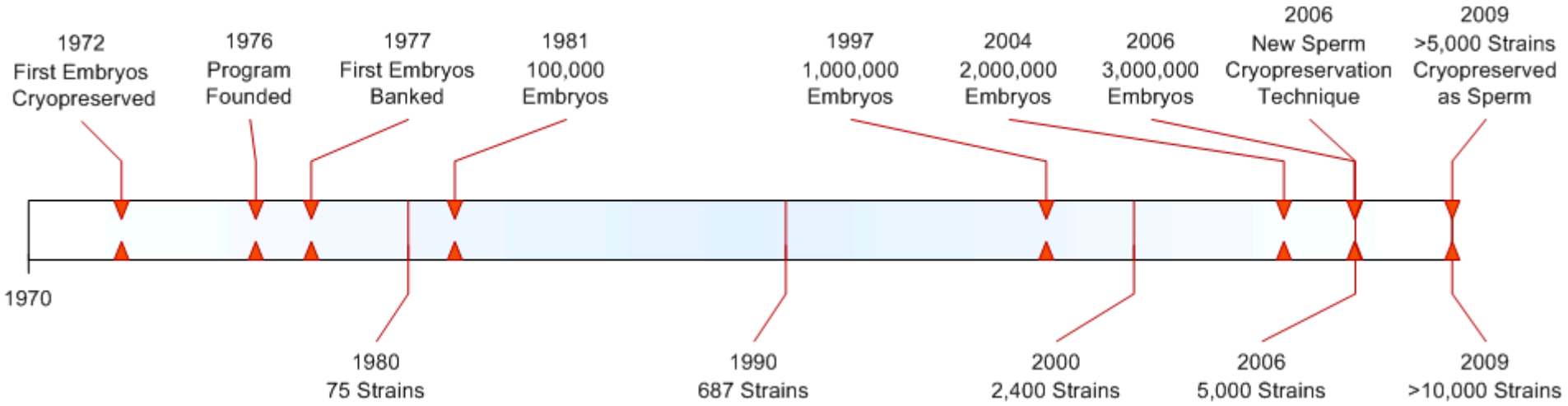
Utility

- **Banking** of inbred strains, mutations, recombinant inbred strains- proposed by 1974
- Use of cryopreservation as **protection against loss**- proposed by 1974
- Facilitating **standardization** and **international distribution** of strains- demonstrated in 1974
- Tool for reducing rate of **genetic drift**- proposed by 1977

Equilibrium freezing of embryos today

- Effective, reliable, safe
- Used routinely to
  - Bank inbred strains, mutations and recombinant inbred strains
  - Protect strains against loss
  - Facilitate standardization and international distribution
  - Reduce genetic drift
- Widely used by repositories and core facilities
  - Taught in courses

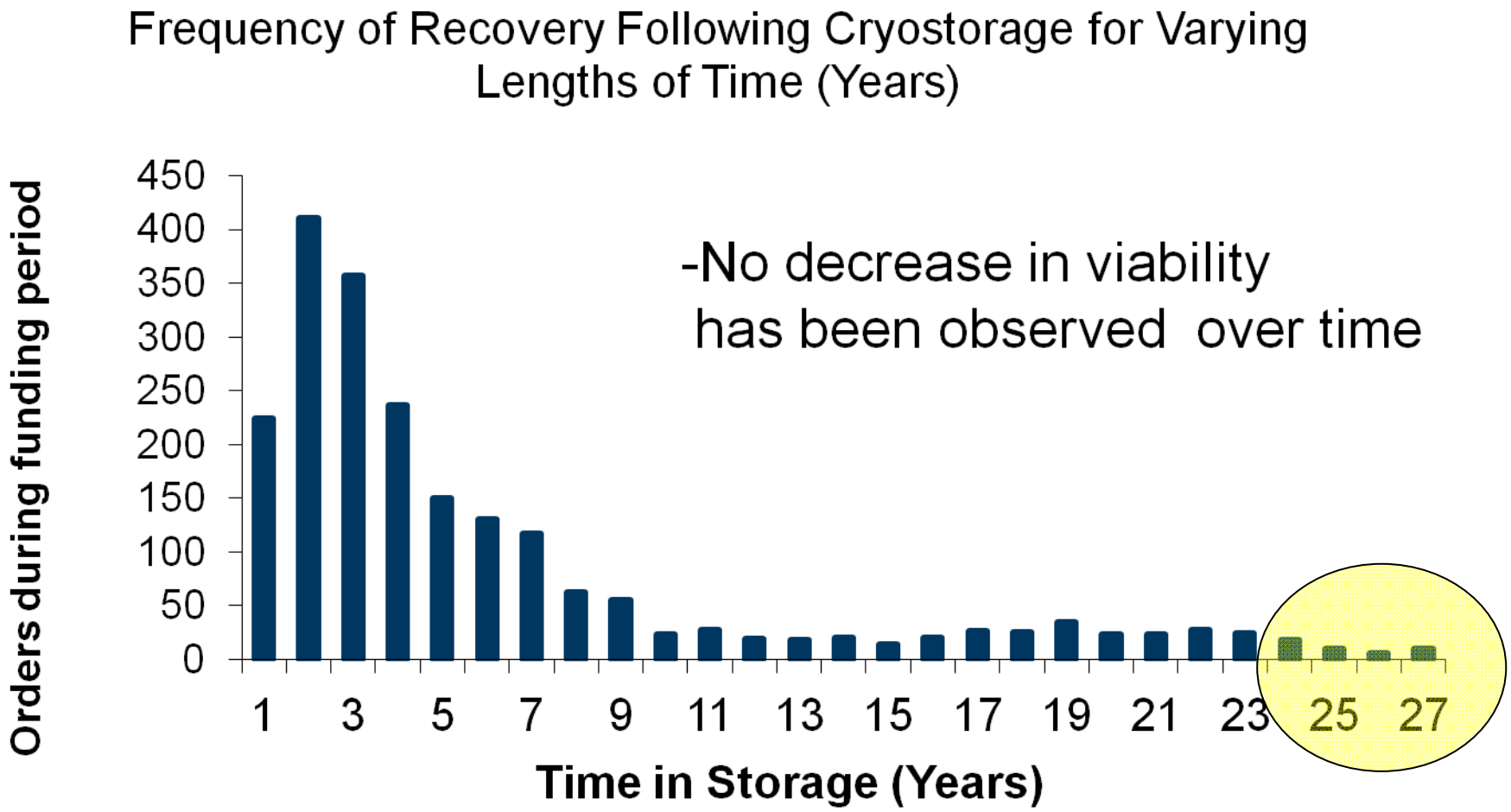
JAX Repository perspective



Today:

- 6,000 strains
- 3,900,000 embryos cryopreserved
- 1,000,000 embryos thawed or shipped

Viability is maintained over time



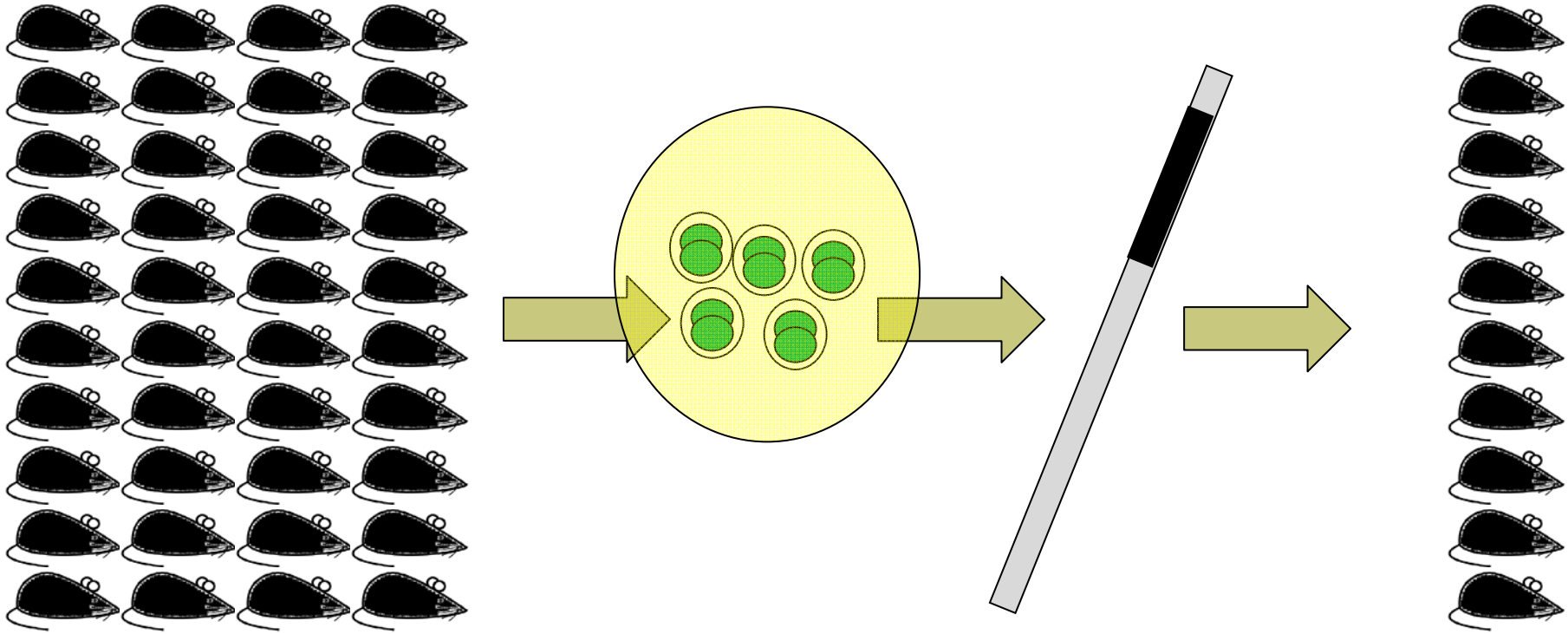
Looking ahead

http://commons.wikimedia.org/wiki/File:NY_429_from_Upper_Mountain_Road_overpass.jpg

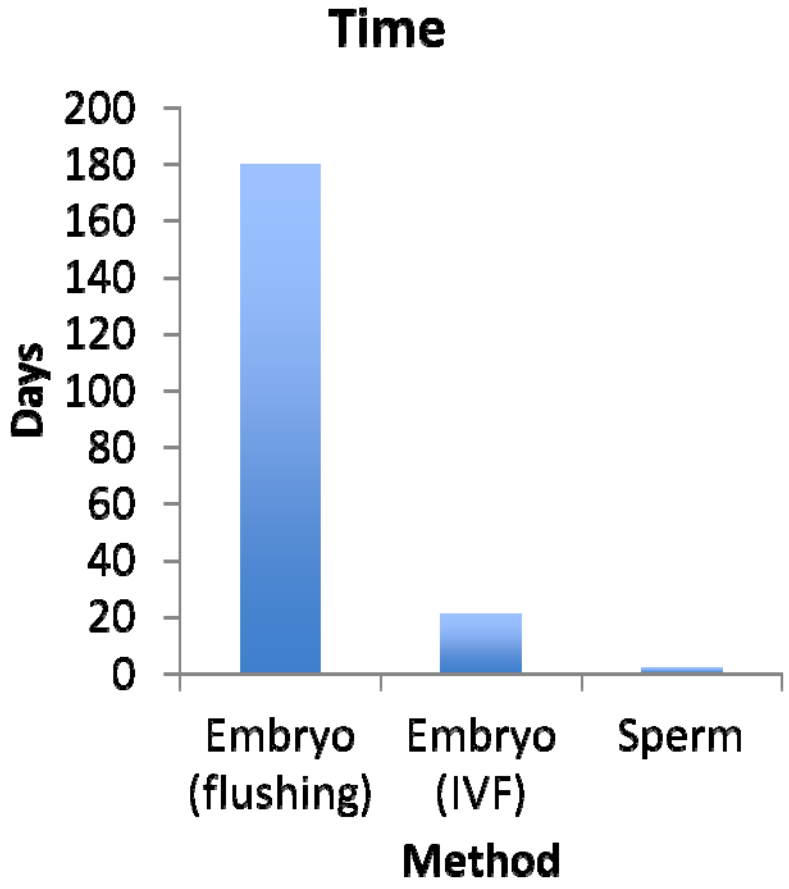
Alternative methods

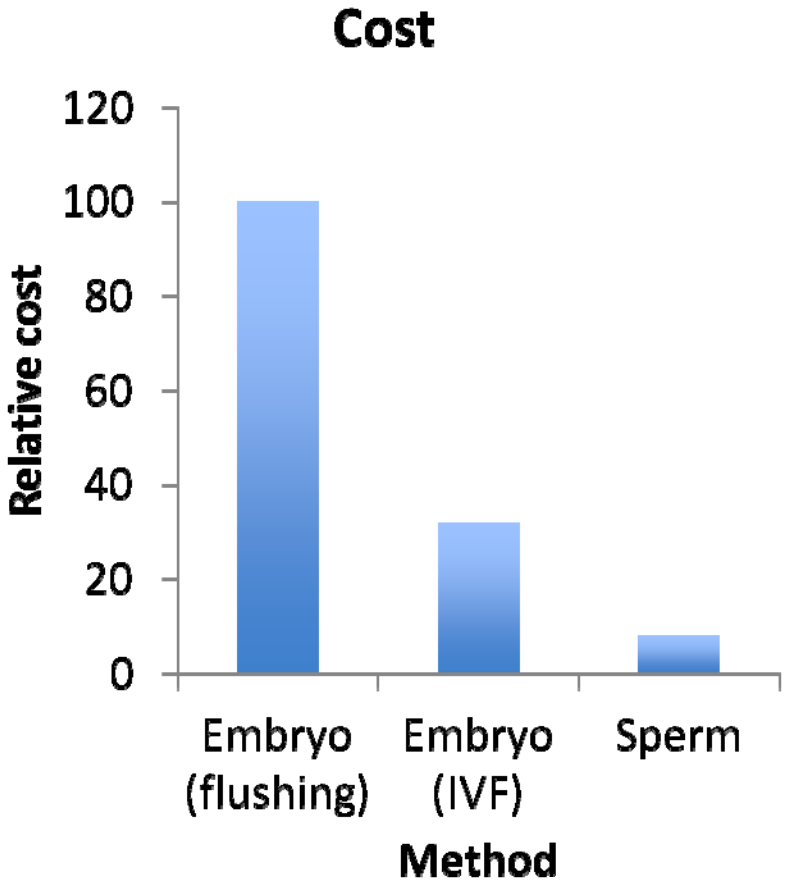
- Vitrification- Rall and Fahy, 1985
  - Effective
  - Less Investment required

Effectiveness isn't a limitation- yield is

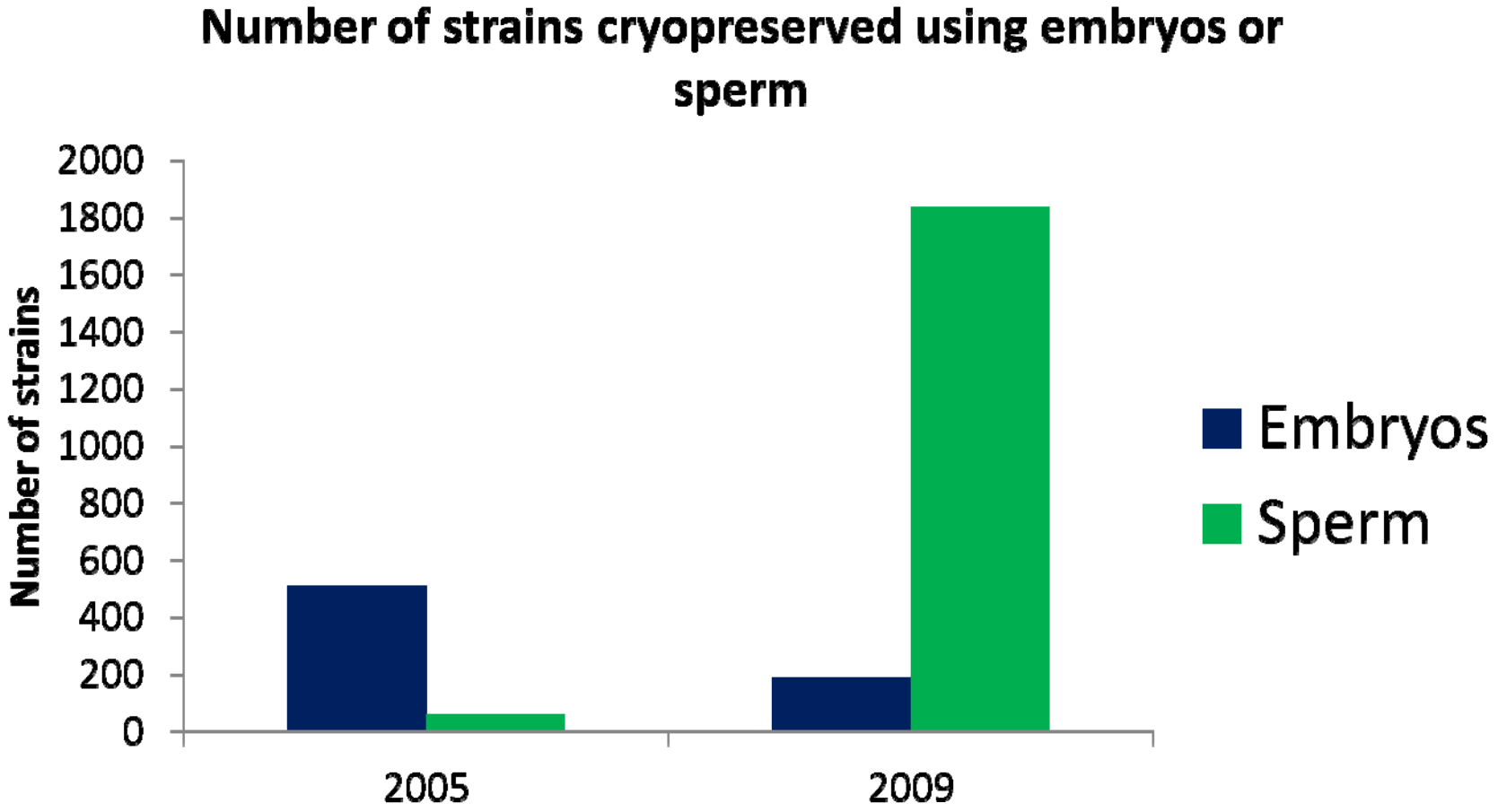


Time and cost of embryo and sperm cryopreservation



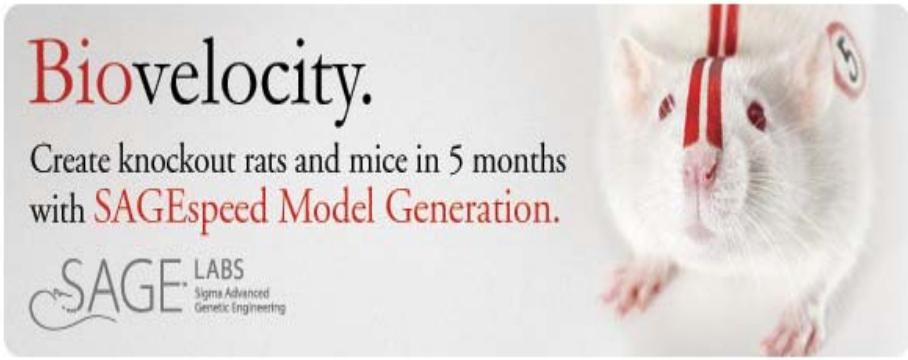


Displacement of embryo cryopreservation by sperm cryopreservation



Zinc Fingers and TAL effectors- Alternatives to conventional strain creation

- Reduced time to make model
- Model can be re-made quickly



TAL effectors RESOURCES

Will it become more cost effective to re-make model instead of preserving it?

Summary- Equilibrium freezing of embryos

- Is effective and reliable
- Has broad utility
- Widely used
- Relatively high cost as made alternatives attractive
- New technologies for making mouse models may impact use
- Mouse models are becoming more complex, creating a growing need to be able to cost effectively preserve entire genome.

Questions?