Cryopreservation of mouse lines: embryo and sperm freezing

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Australian BioResources

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- Specified Pathogen Free (SPF) facility
- Provide standard mice for medical research
- Manage and care for researcher's GM mouse colonies
- Users throughout NSW and beyond

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Biobubbles

- Soft, transparent walled independent breeding rooms
- 1,400 cage capacity per bubble
- 2 technicians per bubble
- 80 air changes per hour

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Scientific Services at ABR

- Rederivation
- Microinjection
  (generation of novel GM lines)
- DNA prep
  (as part of the Mouse Genotyping Service)
- Cryopreservation
  (embryo or sperm freezing)

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Why cryopreserve a line?

- End of experimental usage
- Ethical considerations (reduction)
- Cost reduction
- Security against:
  - Genetic drift
  - Human error
  - Loss of gene of interest
  - Natural disasters
- Readily available for transport
- Future rapid expansion of colony

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Embryos or sperm, which is better to freeze?

Depends on circumstance - factors to consider:

- Cost
- Nature of GM
- Background of line
- **Validation**
- Likelihood of reanimation

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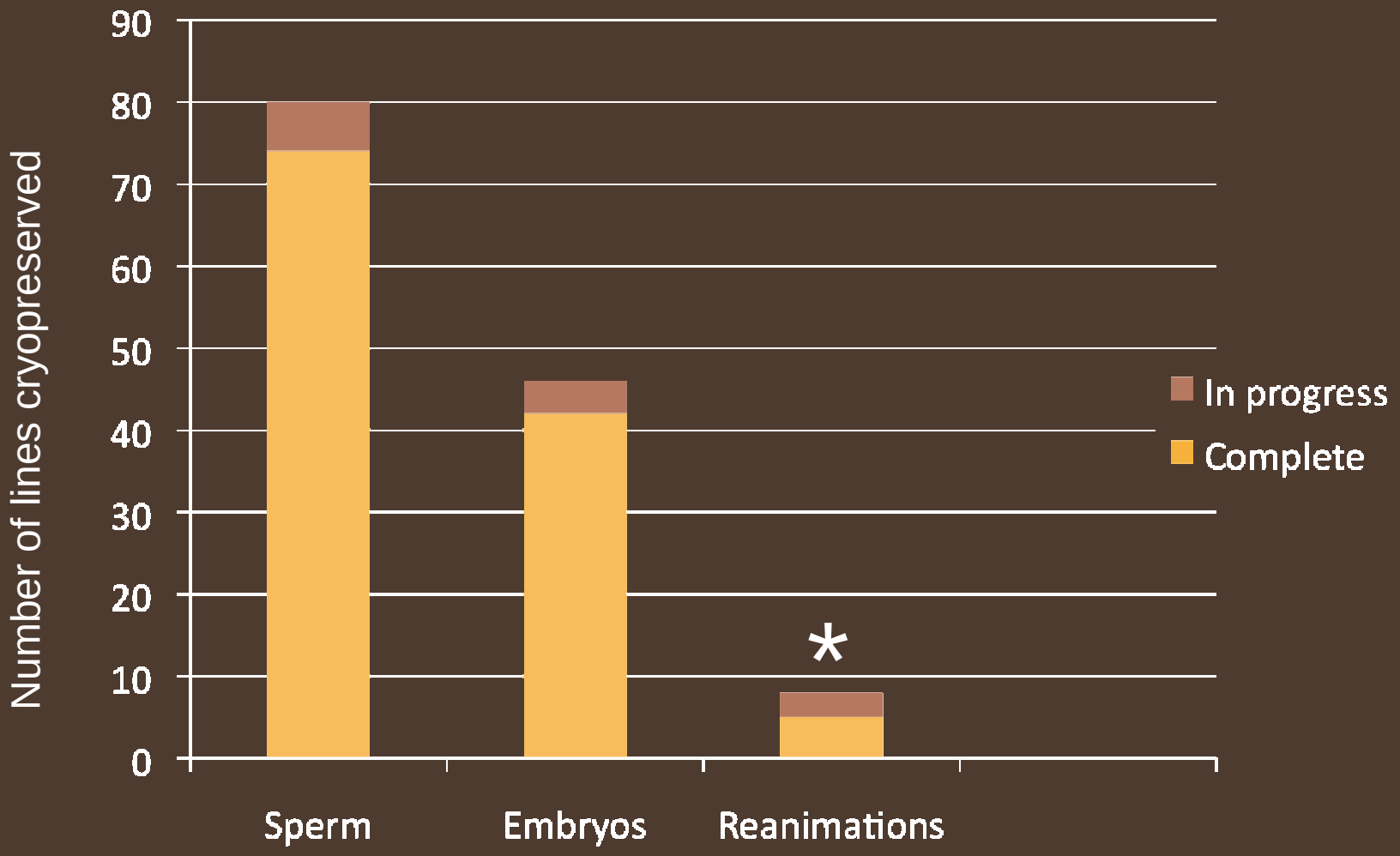
Cryopreservation - method comparison

	Sperm	*Embryos*
Completion time	**Faster**	**Slower**
Cost of freezing	**Lower**	**Higher**
Mice required	**Few (4 males)**	**Many**
Number frozen	**Millions**	**Few hundred**
Genetic material	**Haploid**	**Diploid**
Recovery	**Involved (IVF)**	**Simple**
Background	**Only half preserved**	**Fully preserved**

Freezing and reanimation at ABR



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Maintenance breeding

•Recommended: 2 breeding pairs
2 cages of stock

•Assume: 2 active breeding pairs
6 litters per year
8 mice born per litter

<u>Around 100 animals per year</u>

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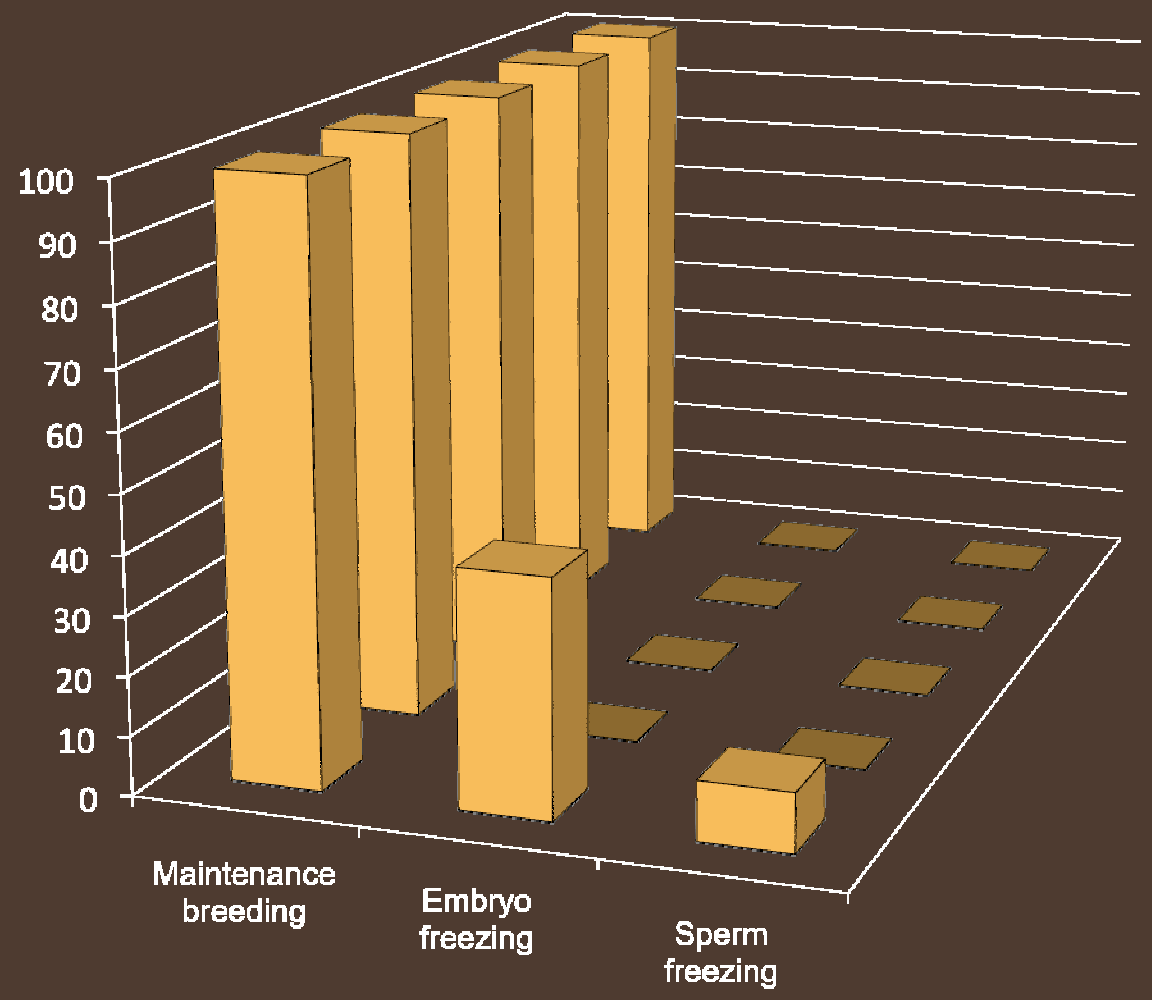
Number of animals used



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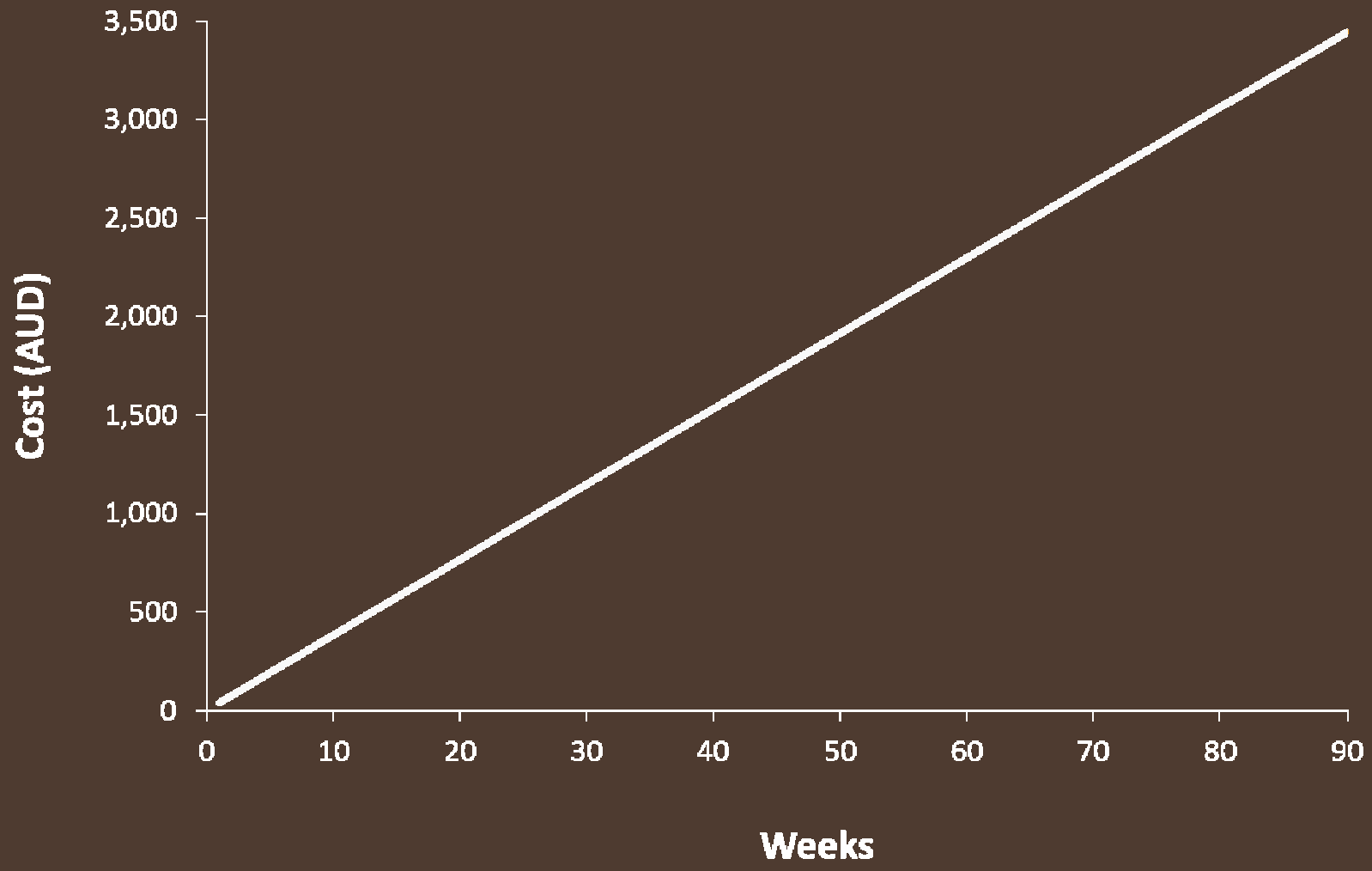
Cumulative cost of ongoing breeding under maintenance conditions



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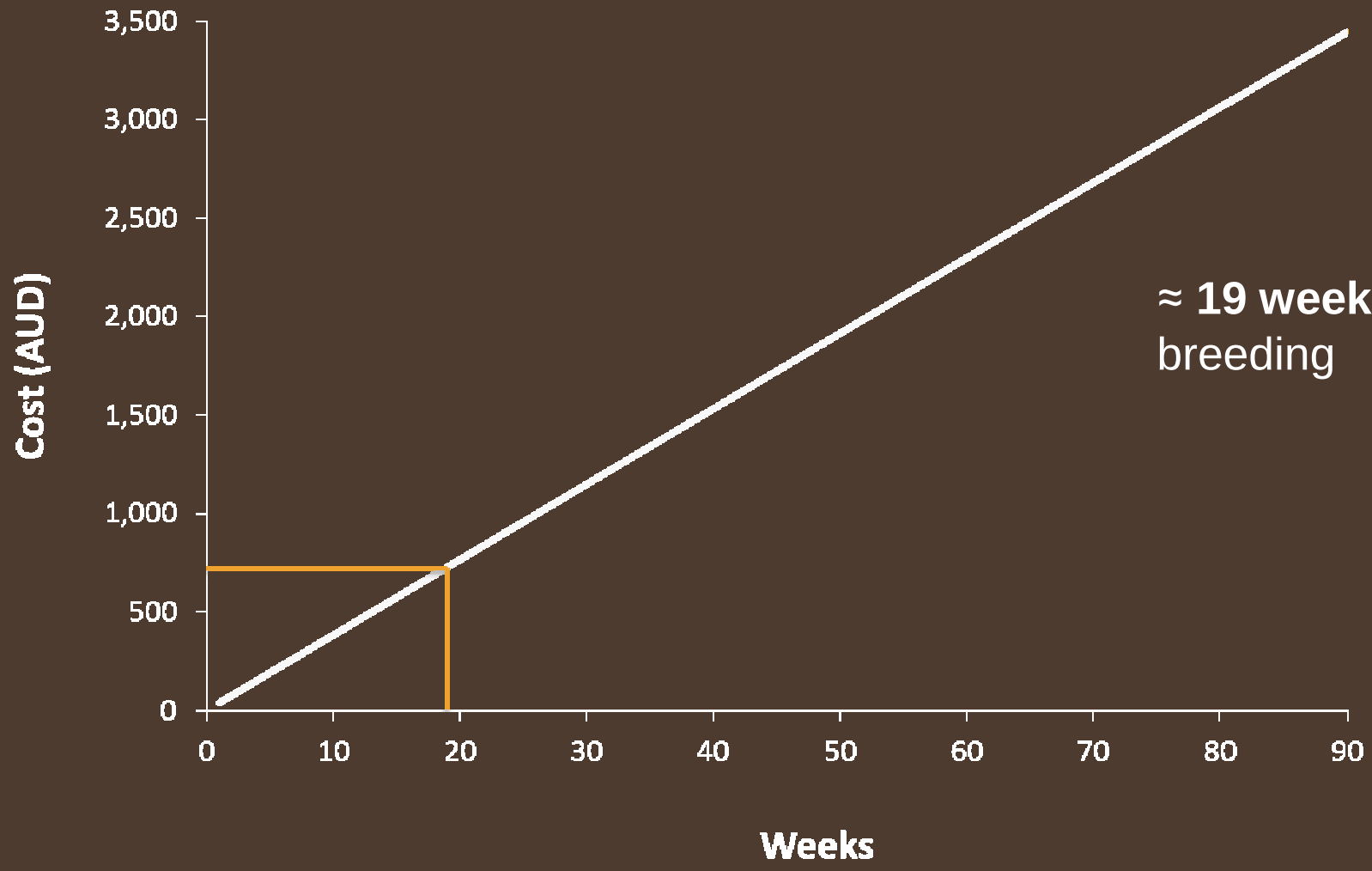
Sperm freezing with basic validation



≈ 19 weeks
breeding

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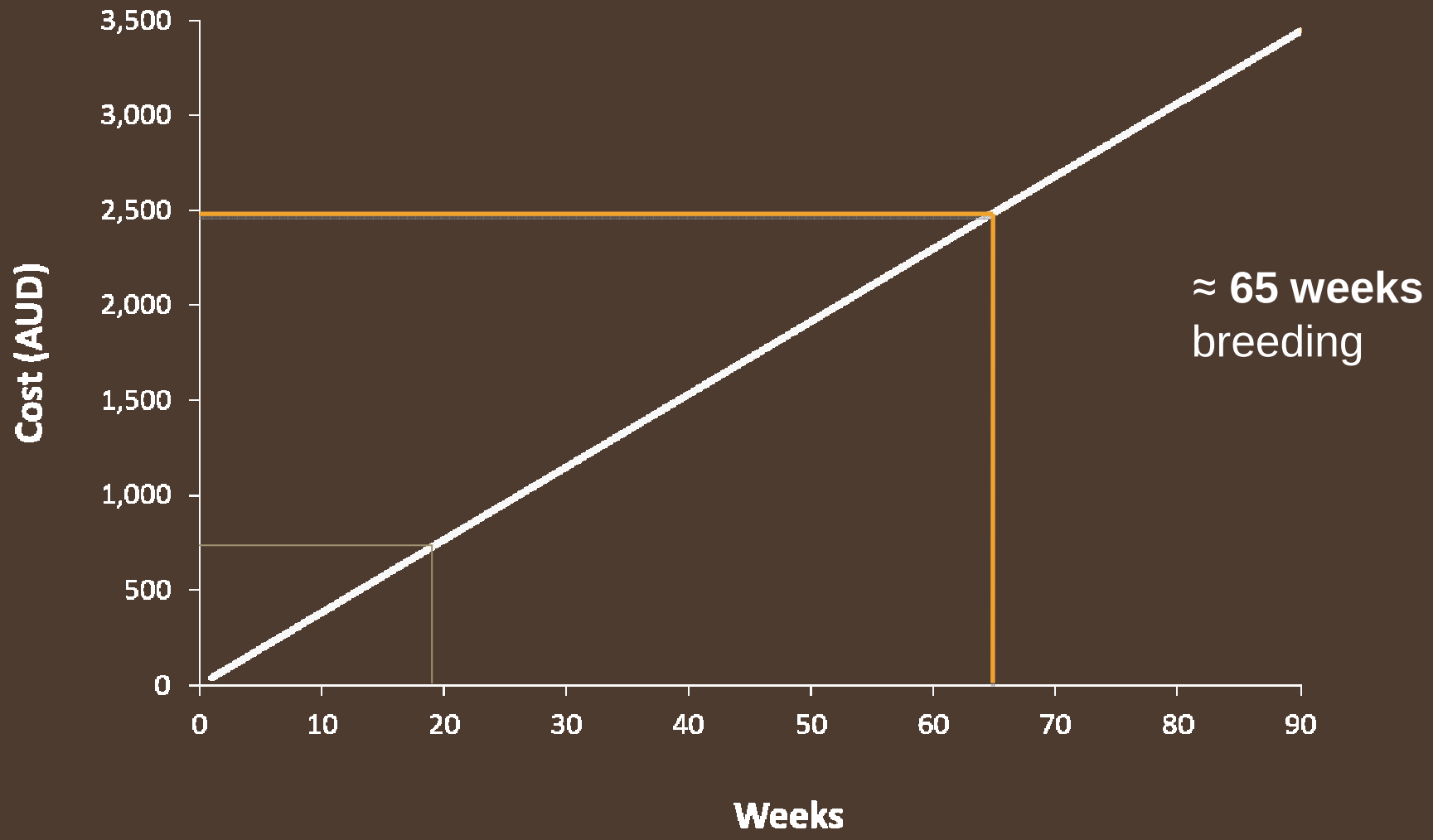
Embryo freezing with basic validation



≈ 65 weeks
breeding

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Conclusion

- Many lines are kept breeding long after experiments are finished
- Replace breeding stock with cryopreserved material
- Reduce number of animals used per year
- Reduce ongoing costs to researchers
- Refinements to technique (better freezing and thawing methods) allow fewer animals to be used as donors
- Frozen material is easy to store and transport – can avoid difficulty of transporting live animals

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Cryopreservation - cost comparison

	Sperm	*Embryos*
Freezing (with validation by culture)	**$725**	**$2,458**
Freezing (with validation by live pup testing)	**$1,418**	**$2,793**
Reanimation	**$1,611**	**$1,289**
Annual storage (per line)	**$26**	**$26**

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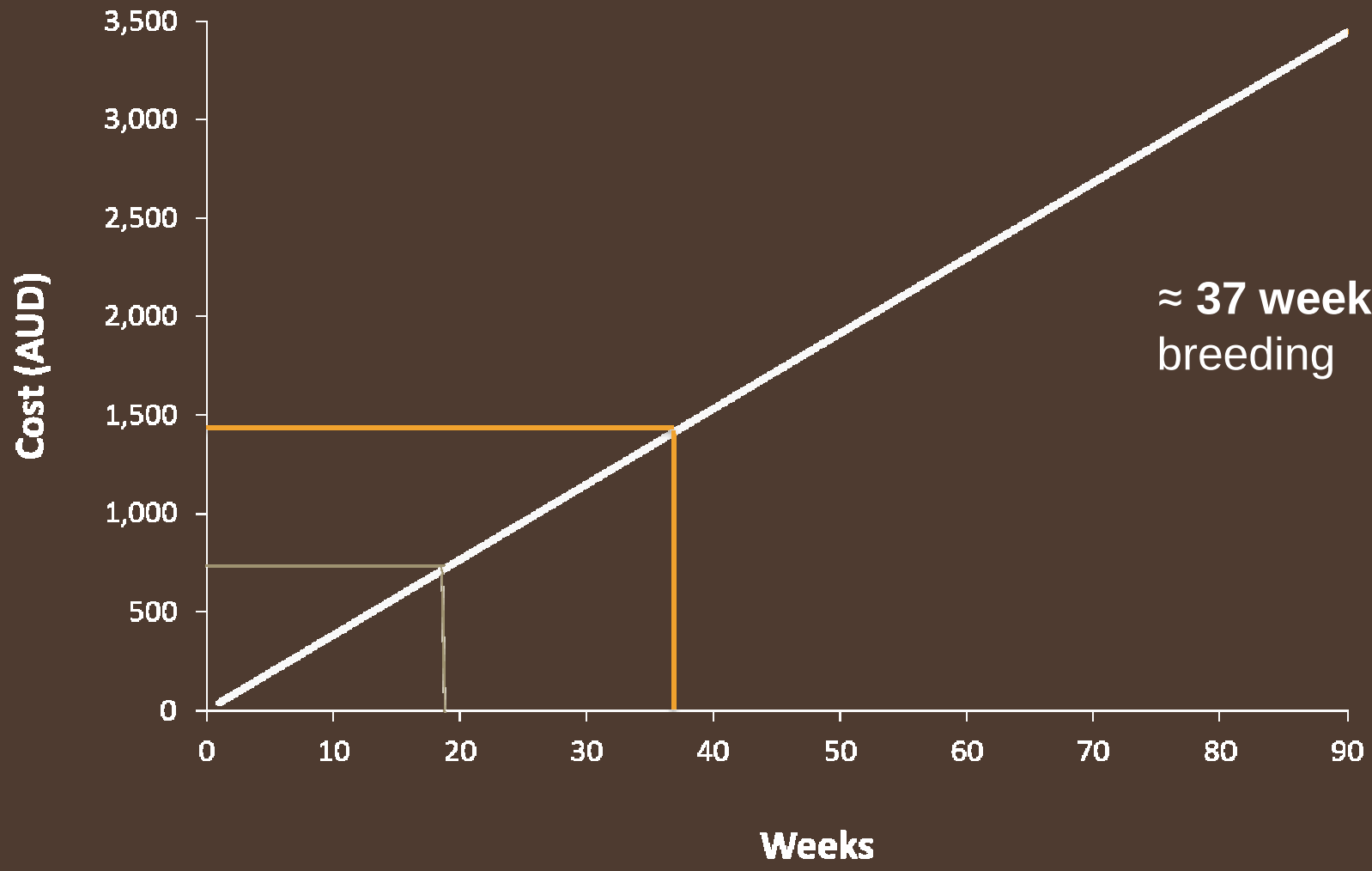
Sperm freezing with full validation



≈ 37 weeks
breeding

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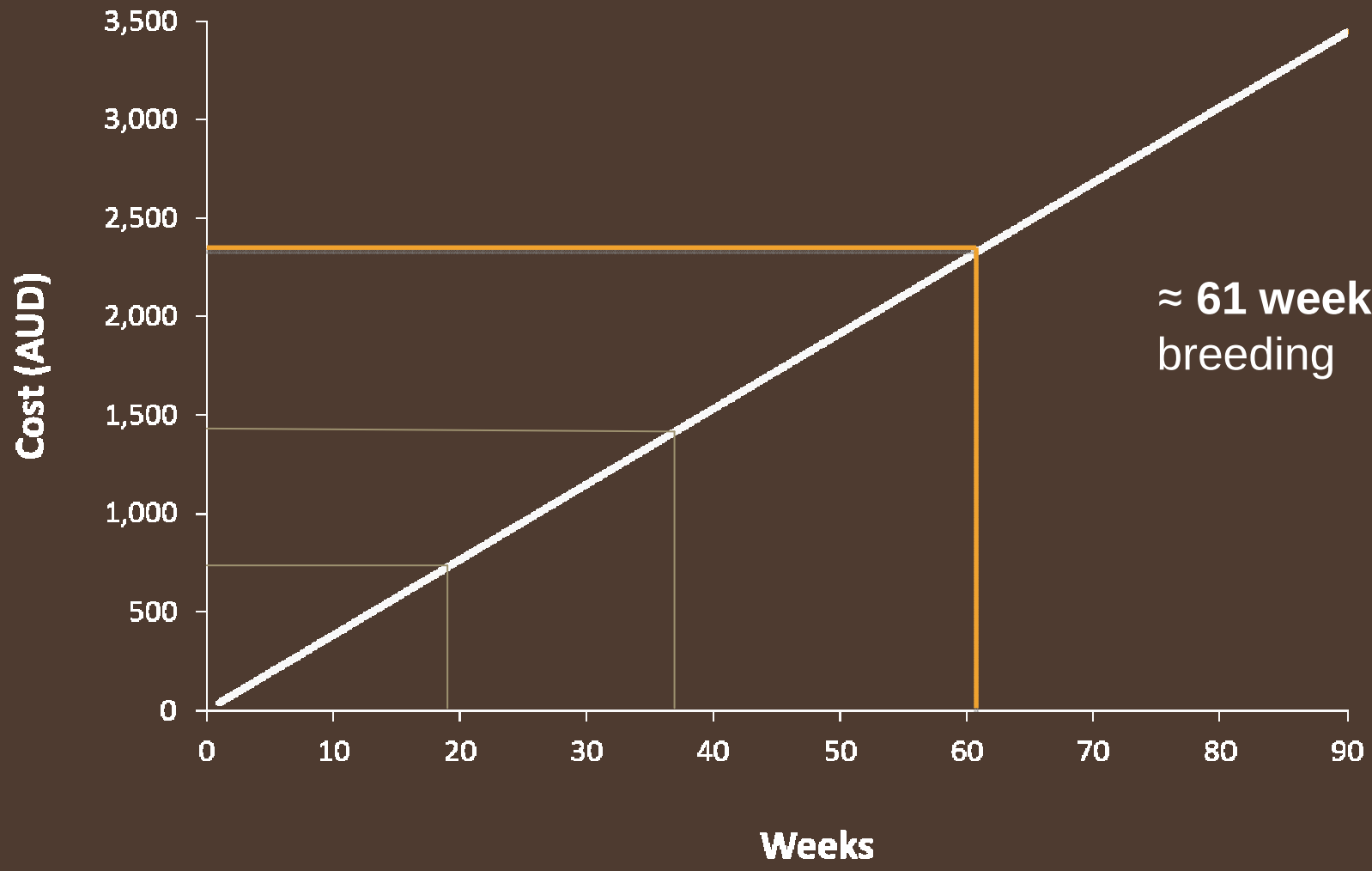
Sperm freezing with basic validation & reanimation



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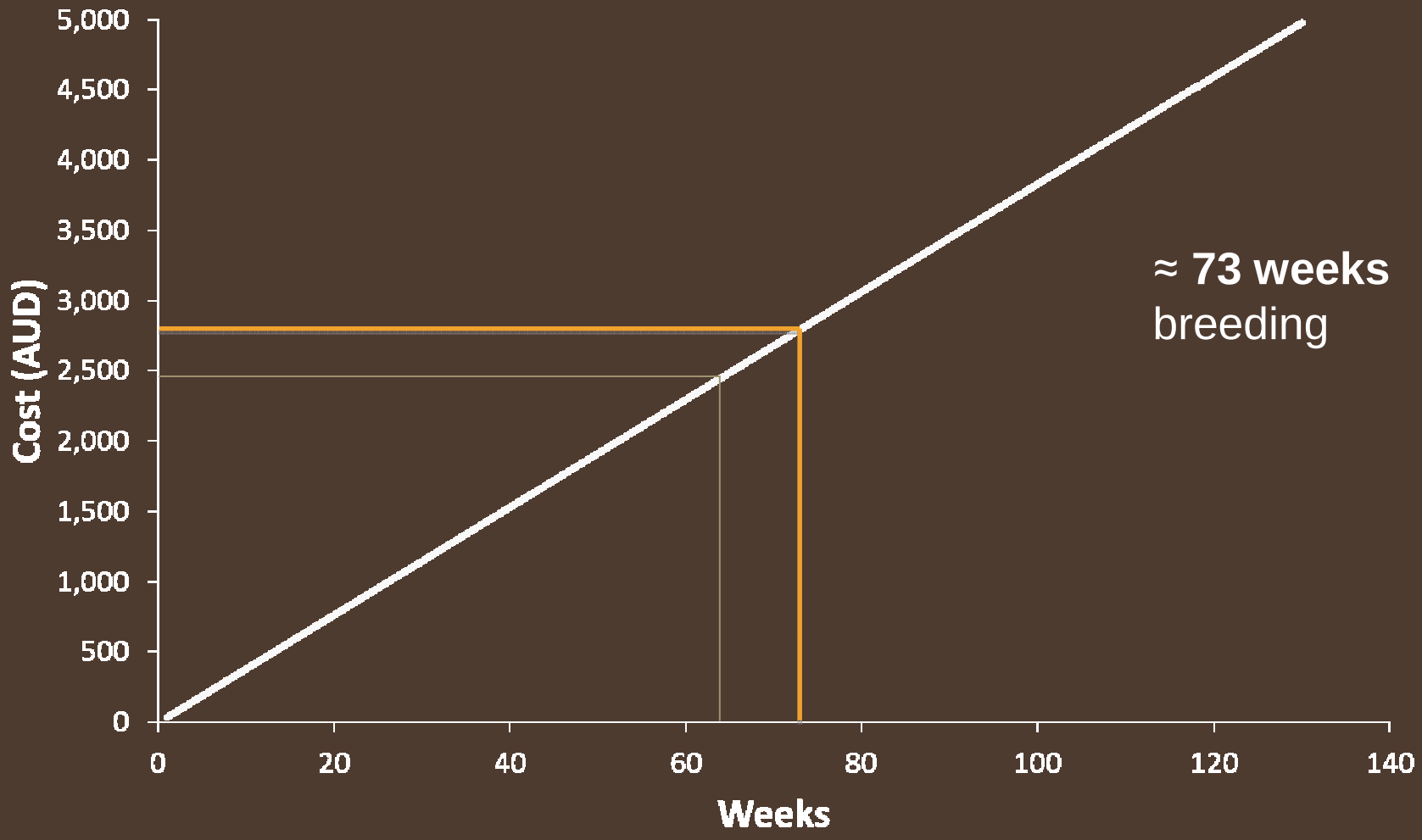
Embryo freezing with full validation



≈ 73 weeks
breeding

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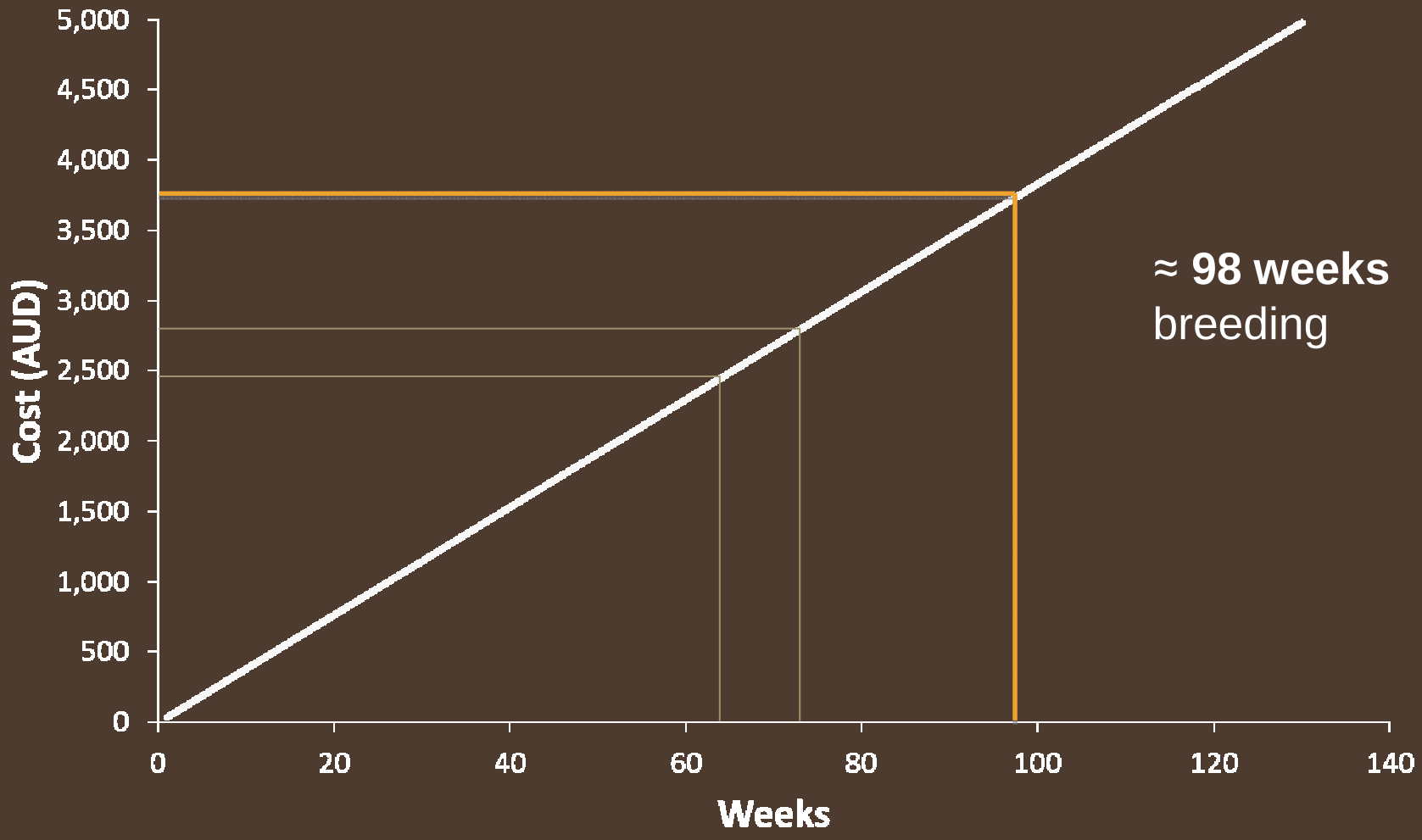
Embryo freezing with basic validation & reanimation



≈ 98 weeks
breeding

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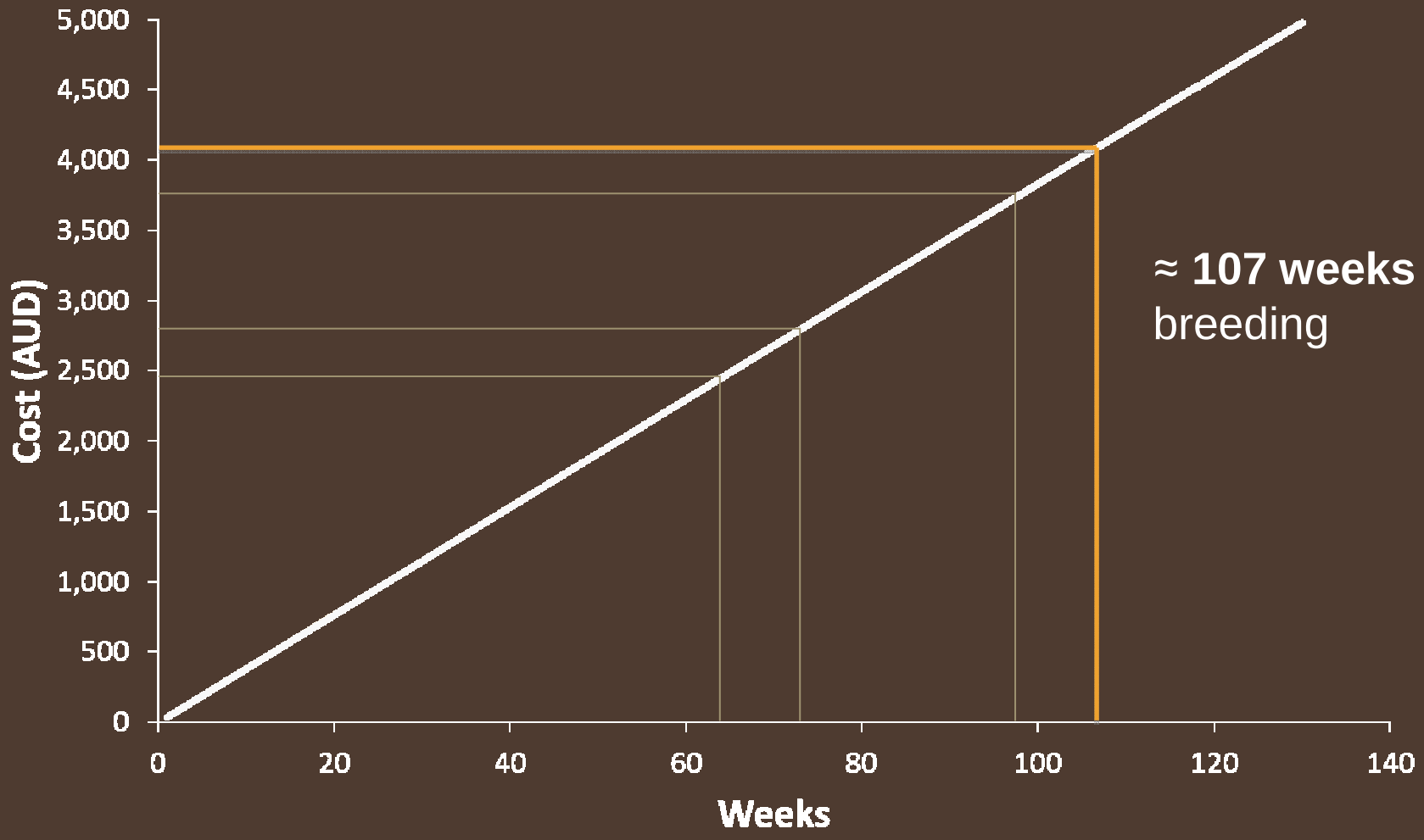
Embryo freezing with full validation & reanimation



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