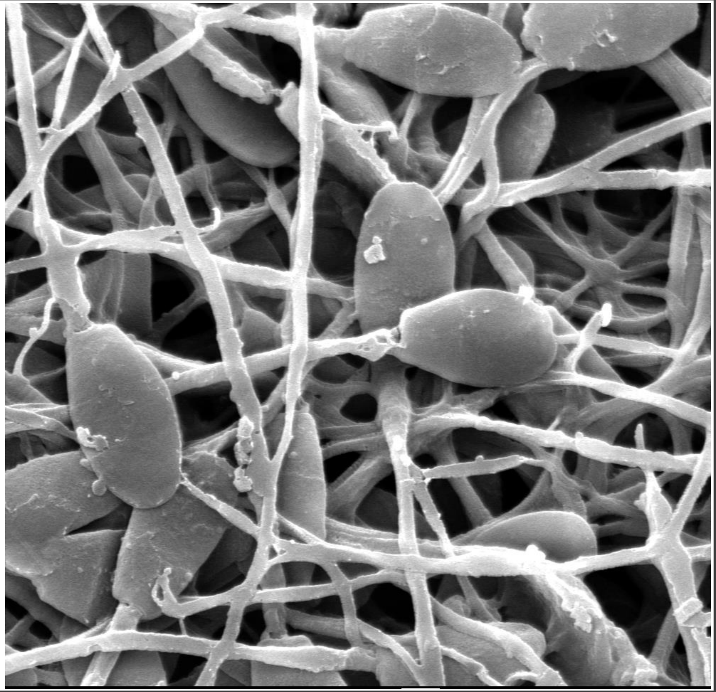


How modern technology can safeguard genetic diversity and biodiversity.

Tullis Matson

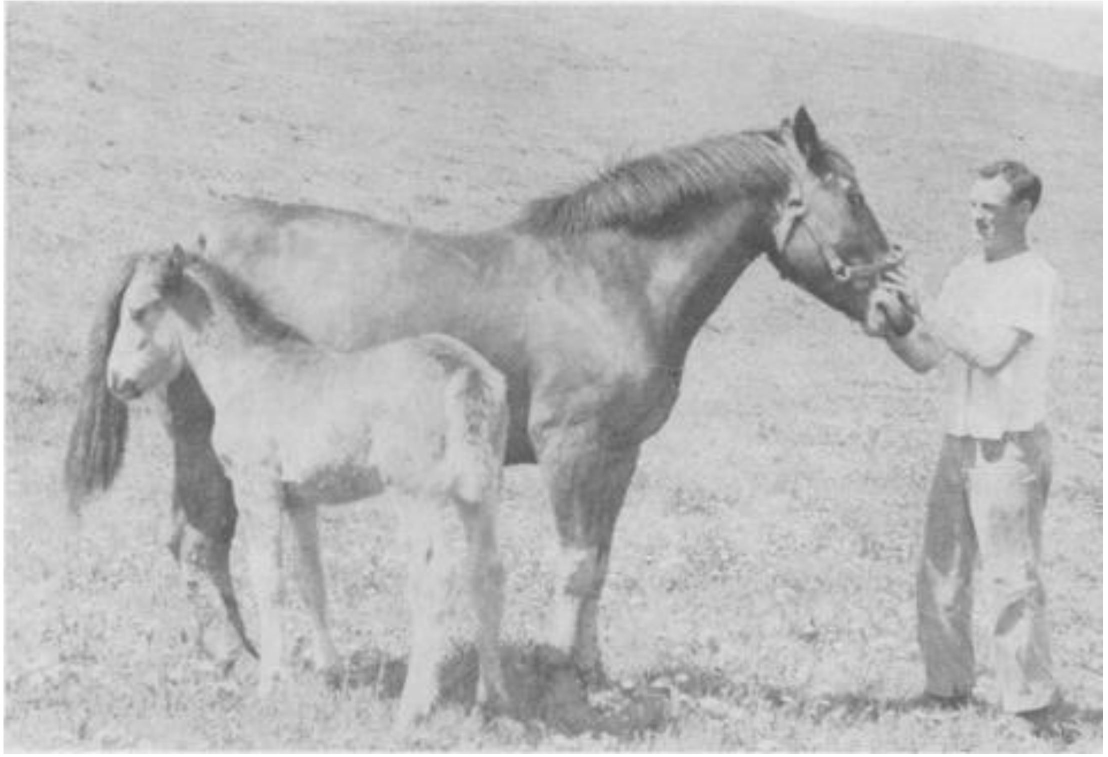
Stallion AI Services

Contents

- History of AI
- Biobanking our genetic diversity
- Assisted Reproductive Technologies (ART)
- Genomics & Future Breeding Technologies

History of AI

- <1300 First references in Arabic Text
- 1700 L. Spillanzani first documented scientific research
- 1903 E. I Ivanoff first insemination station
- 1955 Saw the first foal from frozen semen



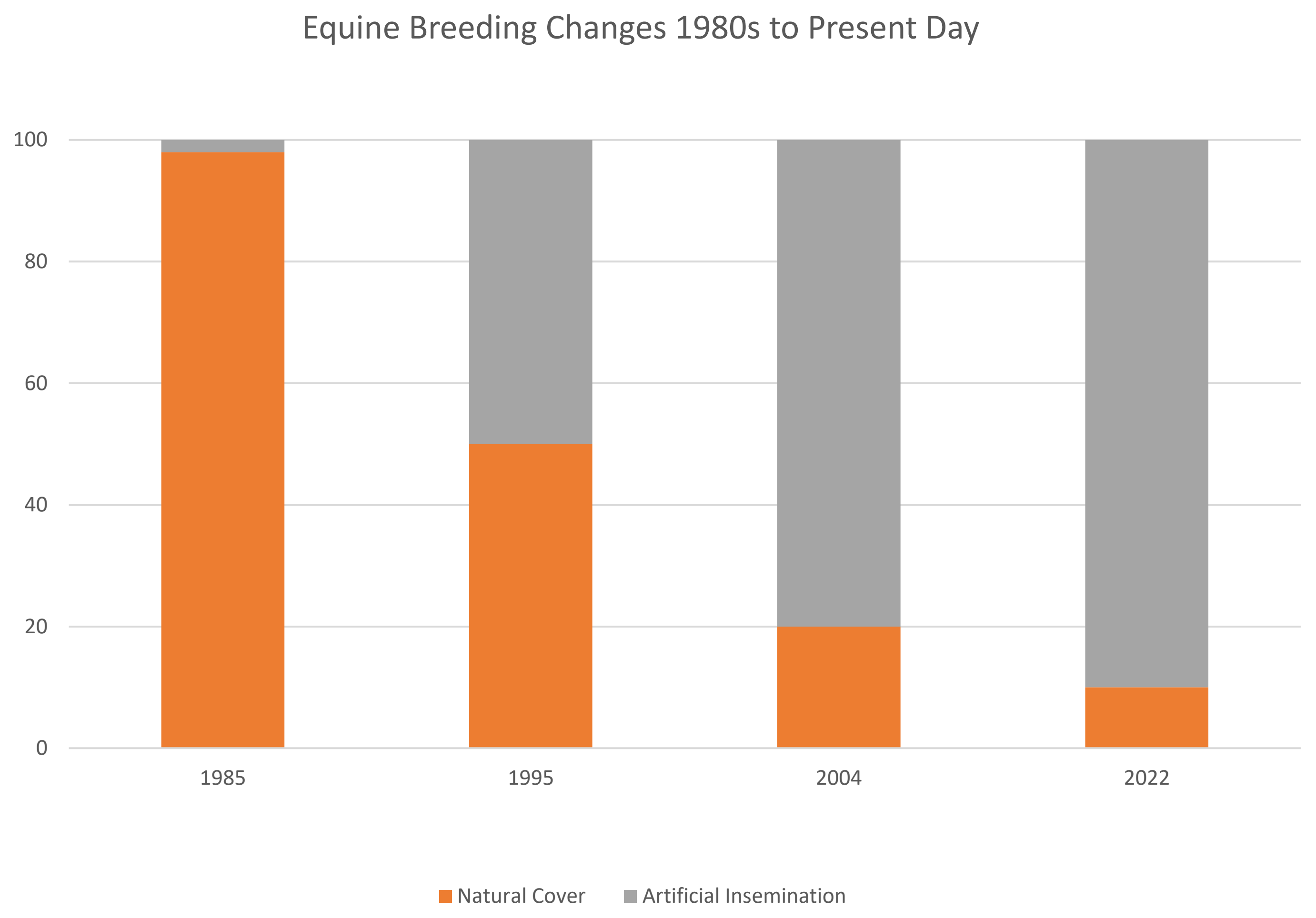
History of Equine Breeding

- 1985

98% of mares bred by natural cover

- 2022

37 years later in the present day 90% sport horses via AI



Biobanking Our Genetic Diversity

- Collection of biological and genetic material for future use

- Future proofing and future security against the unknown

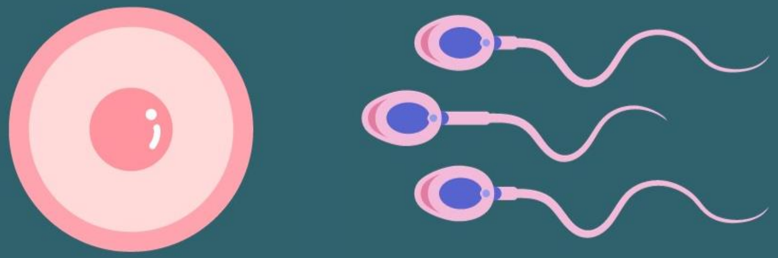
- Safeguarding genetic diversity

Our Biobanking Model

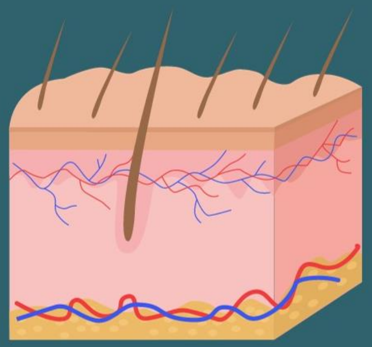
Multiple sample types

Regeneration

Ovarian & Testicular Tissue



Eggs & Sperm



Skin Samples

Cryo-Conservation & A Living Biobank

- Cryo-conservation
- Preservation of biological resources for conservation purposes
- Retention of a ‘living’ format
- Ability to regenerate when needed

- 196 Storage

- 80 Storage

Ice Crystal Formation

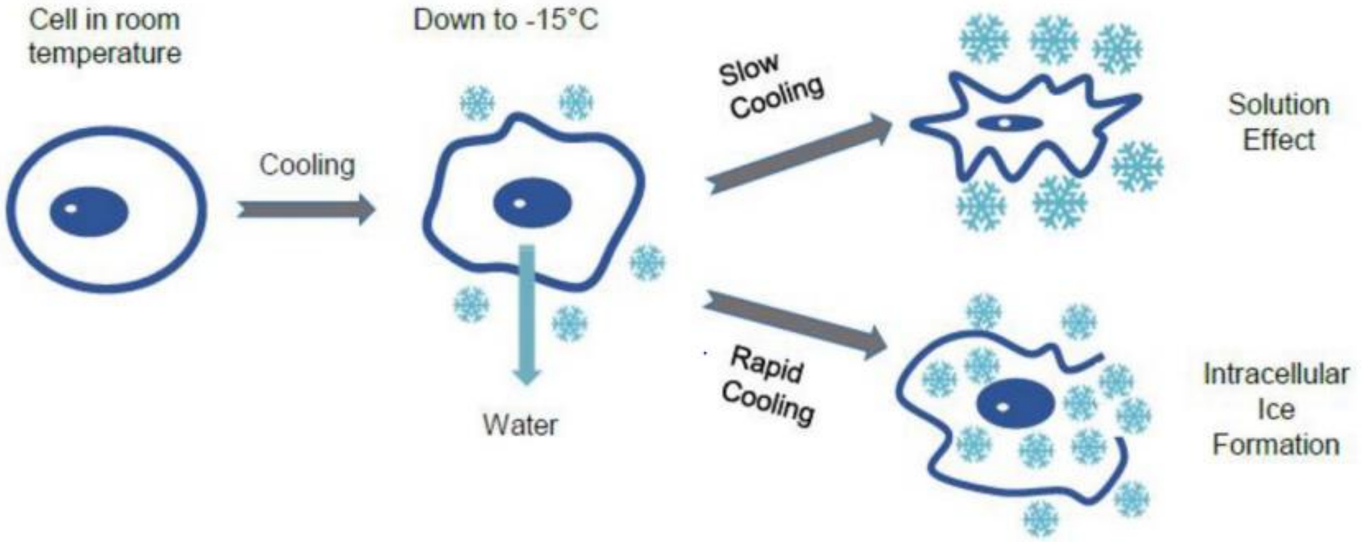
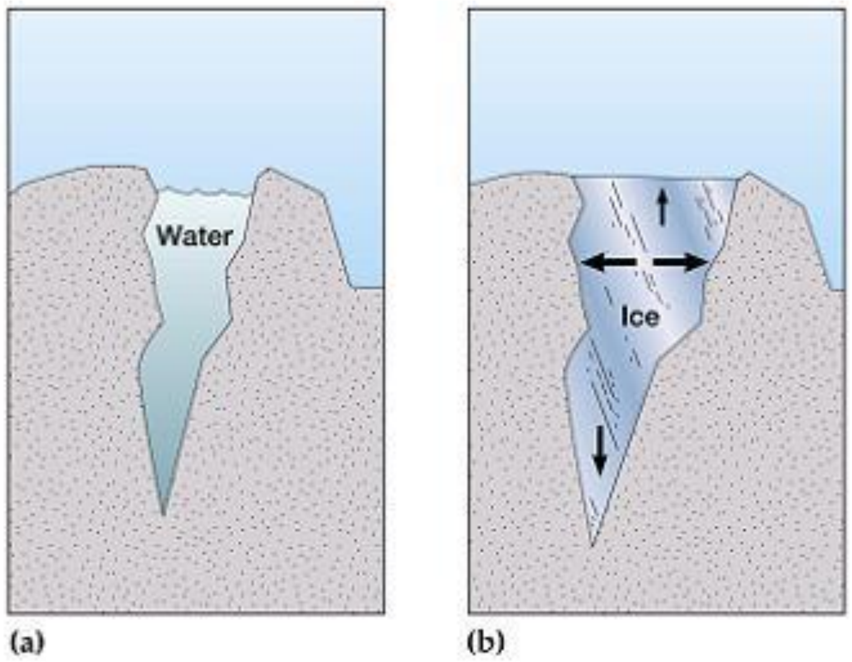


Figure 1. Physical changes in the cells under different cooling rates: slow and rapid.



Our Work With Rare Breed Equines

- Support of rare breeds since 2002
- Temporal trends in stallion sperm quality
- Cryopreservation of challenging semen types
- Driven our developments in cryopreservation


RBST Gene Bank
Rare Breeds Survival Trust

The Rare Breeds Survival Trust is the only charity dedicated to the conservation and development of the UK's native breeds of farm livestock and equines. Our Gene Bank is an extremely important asset and a key part of our work.

We save native farm breed genetics in our Gene Bank by collecting genetics in the form of semen and embryos from farm livestock and equines. Of the 6 species we work with we collect genetic material from all except poultry.

Traditionally RBST has collected mainly semen but since 2015 advances in cryogenic technology have enabled us to collect embryos. We have already successfully collected embryos from cattle, sheep and goats. Although we are currently unable to freeze embryos from other species, work is ongoing to achieve this.

To carry out our semen and embryo work we use specialist technicians across the United Kingdom. The material collected is split across sites where possible to minimise the risk of genetic material being lost.

Objectives of a Gene Bank

- To ensure future genetic diversity
- For current conservation breeding to produce new breeding lines
- To store genetics that may otherwise be lost
- Act as an insurance policy, genetic materials can be used to revive a breed if extinction occurs

How can you help?

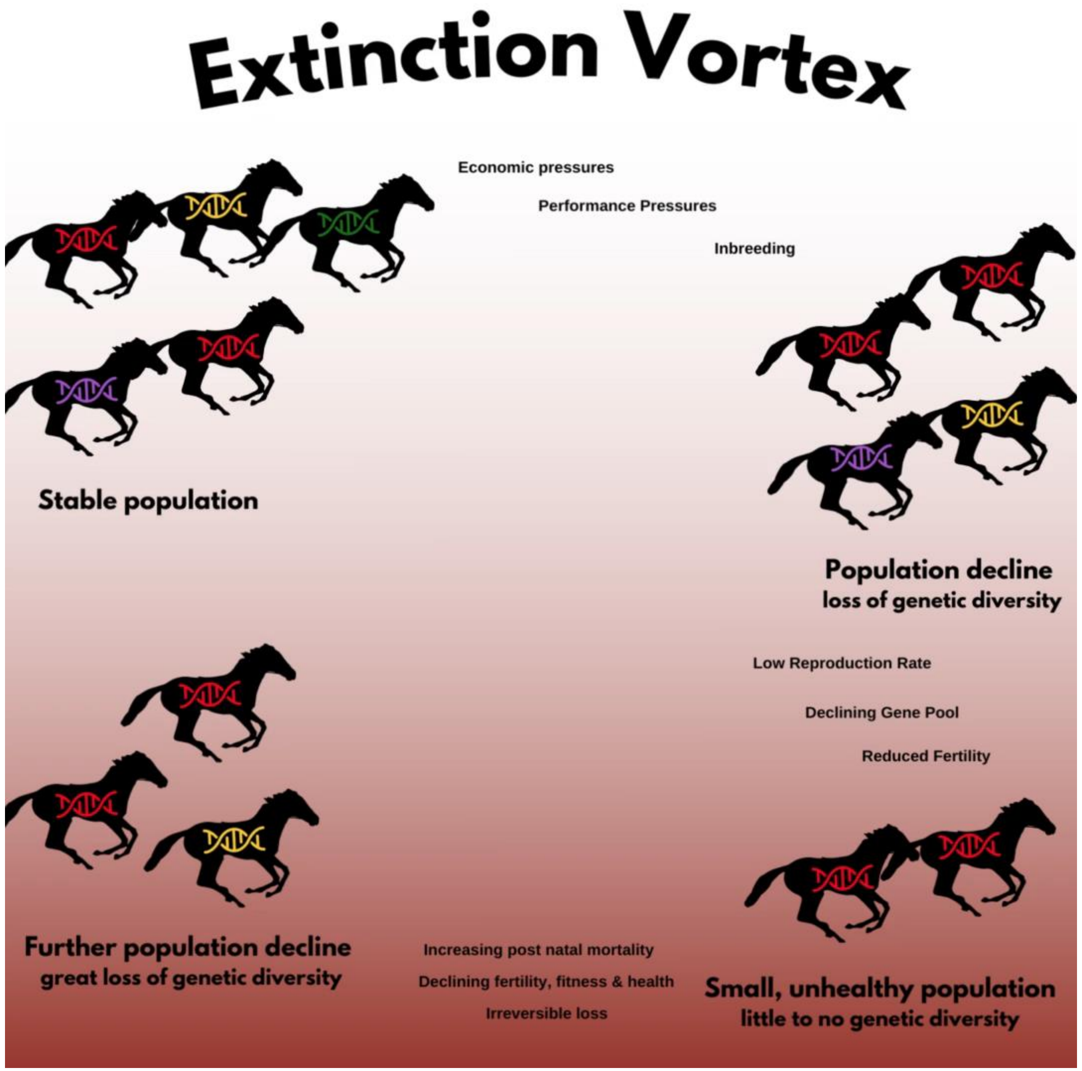
In order for the RBST to achieve our objectives with the Gene Bank we depend on support in a number of forms.

- Nominating an animal for collection
- Donation of semen or embryos that have already been collected
- Funding for collections

The cost of collection varies depending on species:

The data shown in the tables are for our archive collections and for each species the number of straws that make up one archive collection varies. For embryos we require 8 per full collection. We have highlighted the additions to the Gene Bank in the past 2 years.

The Extinction Vortex



Breed	Average Inbreeding
Suffolk Punch	11%
Eriskay Pony	15%
Cleveland Bay	22%
Clydesdale	26%
Shire	28%

Current Topics in Equine Breeding

- Inbreeding references of 10 - 13.28%
- Approximately 5%–10% of confirmed equine pregnancies fail before 65 days of gestation, with a further 7.3% failing before the end of the first day of life. (general population)
- Study found that pregnancies lost in mid and late gestation, from Thoroughbred mares in the UK, had significantly higher inbreeding metrics than UK adult thoroughbred horses

Received: 13 March 2023 | Accepted: 29 December 2023
DOI: 10.1111/evj.14057
Equine Veterinary Journal WILEY
ORIGINAL ARTICLE

Does inbreeding contribute to pregnancy loss in Thoroughbred horses?

Jessica M. Lawson[1] | Charlotte A. Shilton[2] | Victoria Lindsay-McGee[3] | Androniki Psifidi[3] | D. Claire Wathes[1] | Terje Raudsepp[4] | Amanda M. de Mestre[2]

Risks of Inbreeding

- Inbreeding associated with an increased risk of inherited mutations
- Reduced fertility (foal loss & lower semen quality)
- Retained placenta
- Performance?? 10% increase in inbreeding (FROH) is associated with a 7% lower probability of ever racing. (Hill *et al.,* 2022

Semen Quality

- Temporal trends in stallion sperm quality
- Initial findings

  - Significant 10% decline in raw semen motility 2000-2020

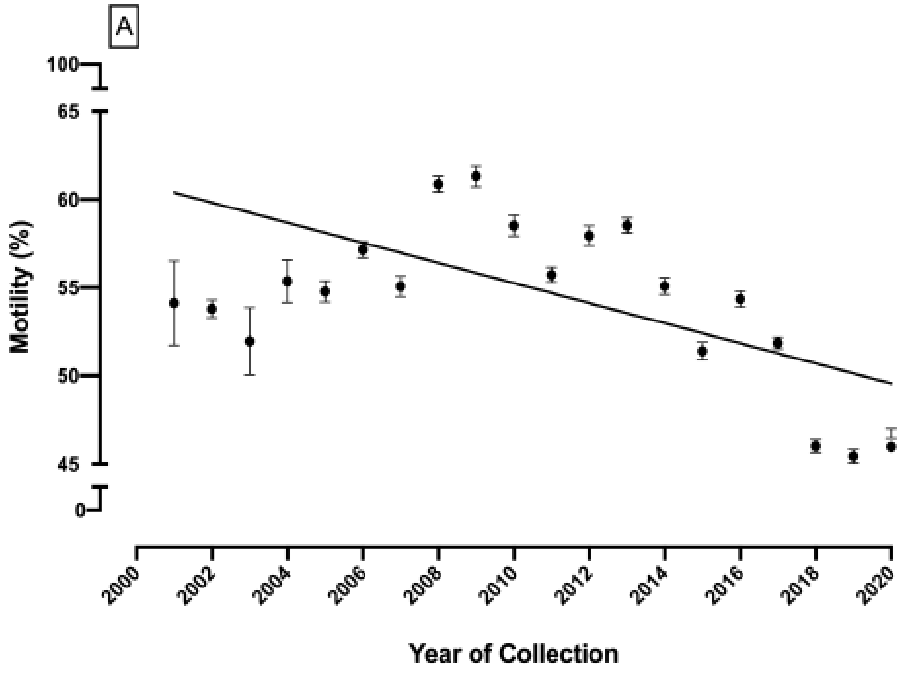
  - multiple possible causes including inbreeding
  - further research will look at the influence of environmental chemicals on the declining trend

- 11,387 ejaculates
- 1,036 Stallions

4. Results

4.1 Raw Sperm Motility

A significant decline of ≈10% (B= -0.663 (p≤0.001))



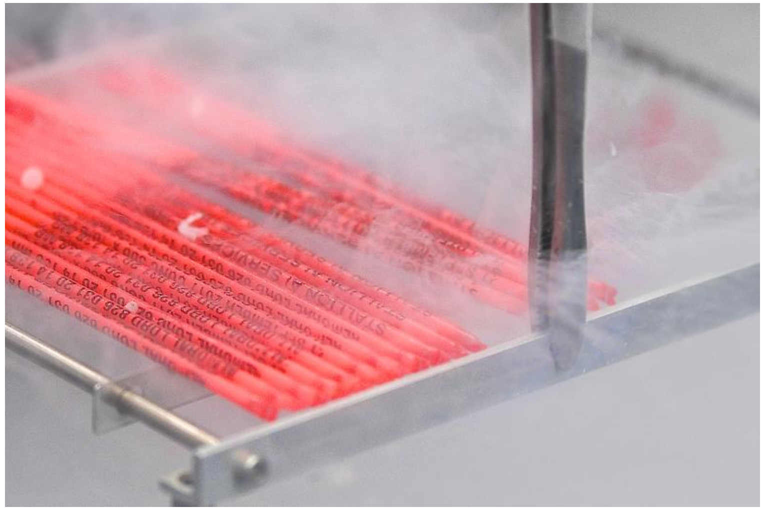
- PhD – Imogen Harris, Hartpury University

Advanced AI Technologies Used To Help Safeguard Genetic Diversity

- Semen Freezing
- Post Castration Semen Extraction
- Semen Sexing
- Oocyte Recovery
- ICSI
- Cloning
- Genomics
- Digital DNA

Frozen Semen

- For semen banking for future use
- Indefinite preservation of semen
- Allows the capture and preservation valuable genetics
- Future use beyond the lifespan of the horse
- Protection against the unexpected
- -196 degrees Celsius storage
- Geographical spread of genetics



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Frozen Semen Pregnancy Results

STALLION
AI SERVICES

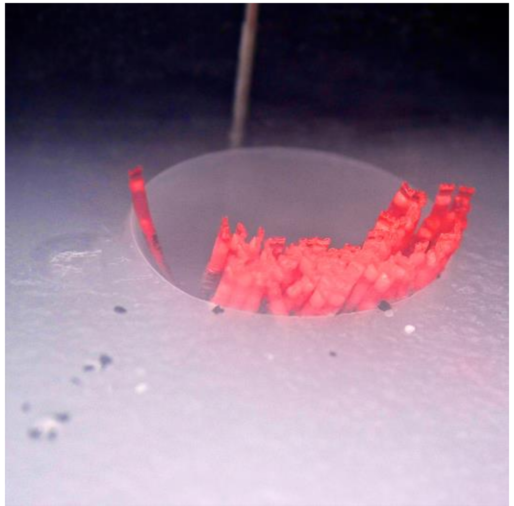
- Some stallions, frozen semen results can be higher than chilled
- Improvements in semen freezing techniques, mediums and cryoprotectants

	Maiden mares ≤ 11years (n=178)	Maiden mares >11years (n=182)
Fresh	81%	55%
Chilled	46%	24%
Frozen	53%	34%

<u>Overall per cycle rates</u>

<u>(over 200 different stallions)</u>

- Frozen semen: 48.6%
- Chilled semen: 43%
- Fresh semen: 63%*
- (n=1023 cycles 578 mares)

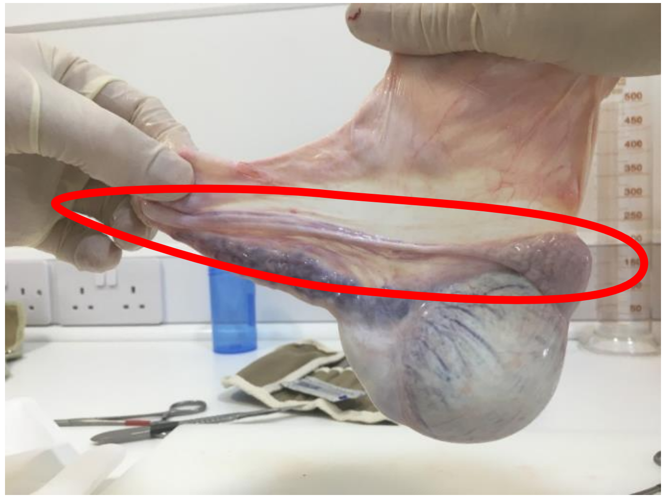


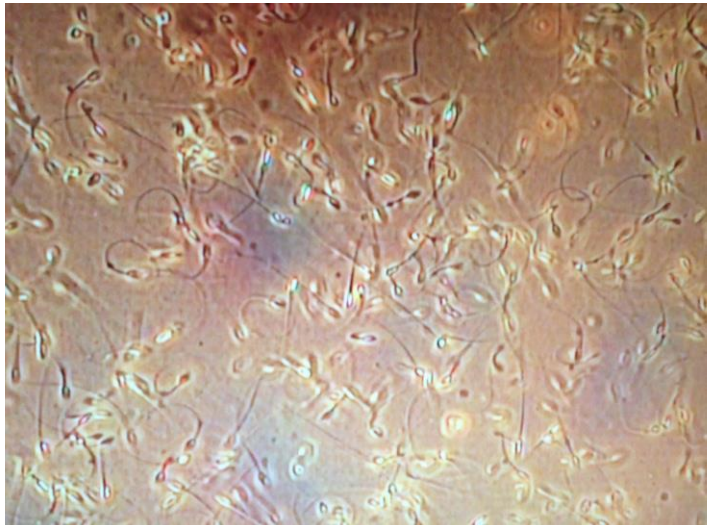
Present day freezing extenders

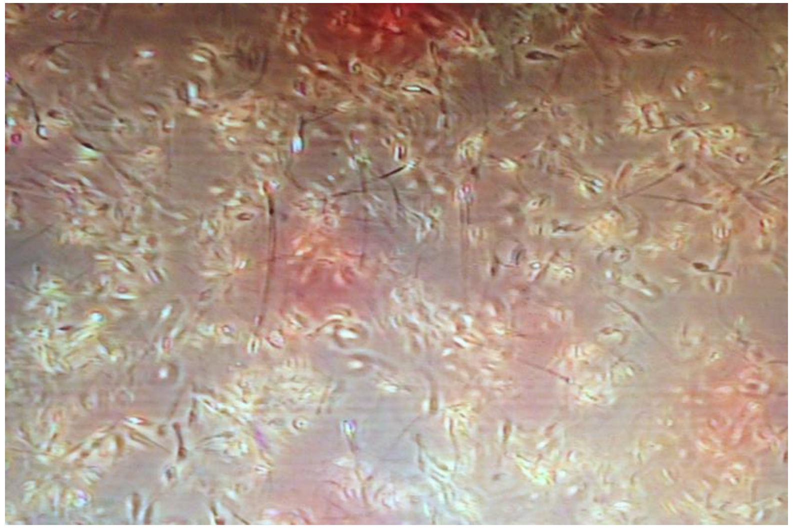
Data courtesy of Twemlows Hall Stud and Niamh Lewis MRCVS

Post Castration Epididymal Semen Extraction

- Trauma
- Routine castration
- Preservation of genetic bloodlines
- +350 equine extractions
- Non-equine species – approx. 75 (2017-2022)

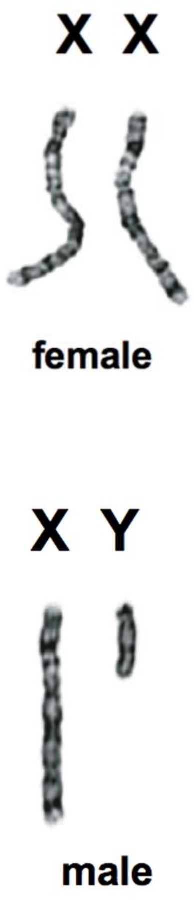


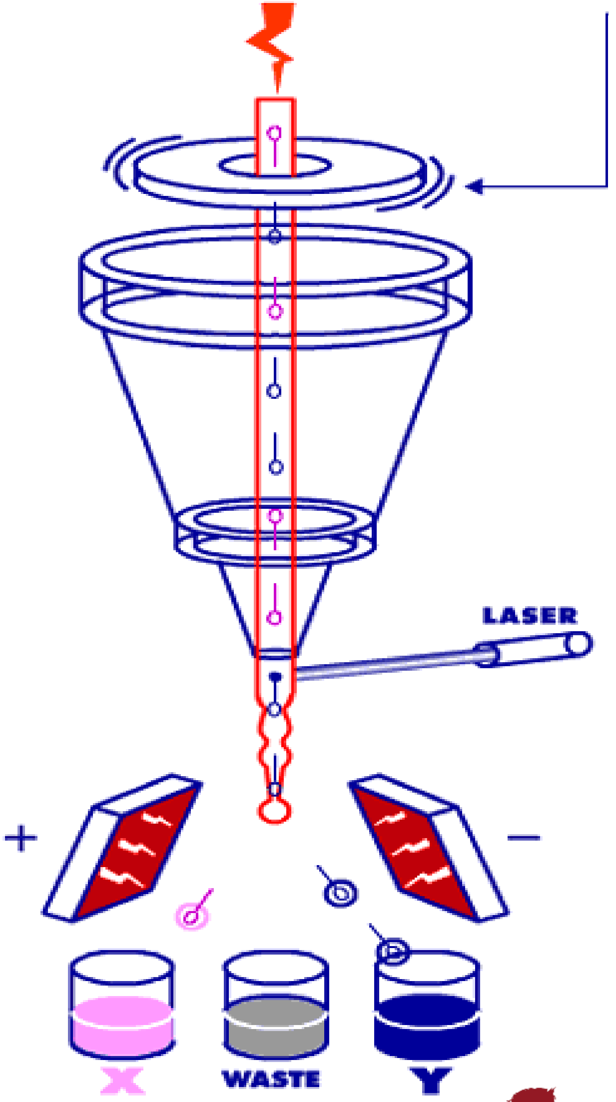




Semen Sexing

- Based on the difference in DNA content between X & Y bearing sperm
- X bearing sperm 2.8-4.2% more DNA than Y bearing)
- 60% sorted; 90% accuracy
- Insemination fresh dose 40 million PMS





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Stallion AI Services

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Semen Sexing

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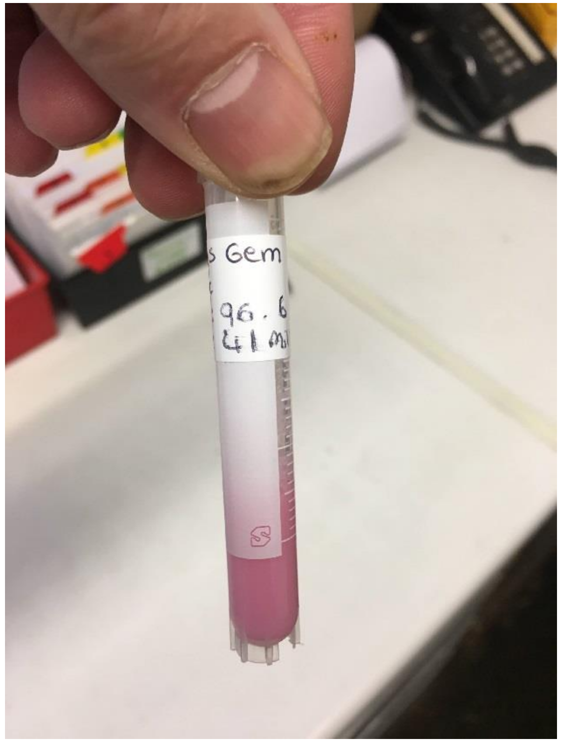

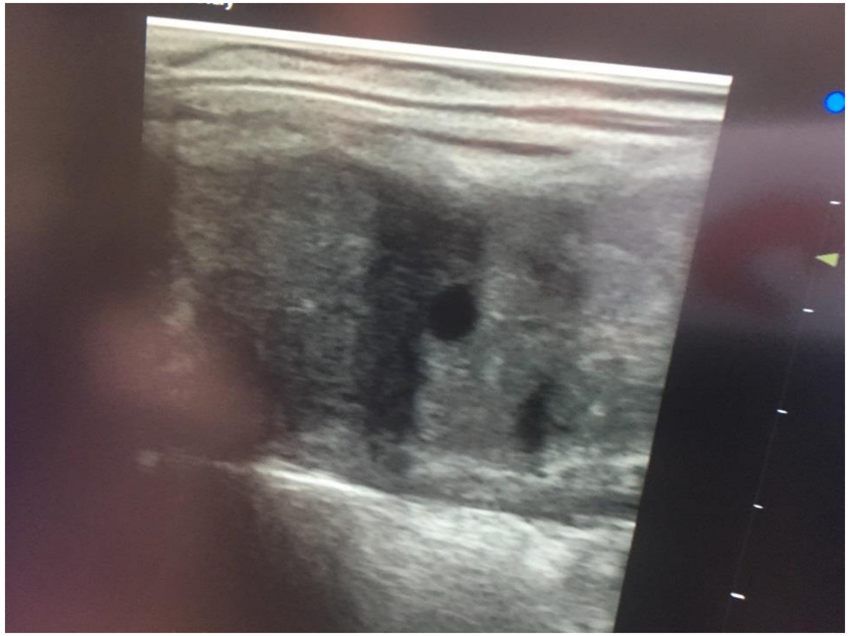

First sexed semen confirmed pregnancy in the UK 2018

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First Sexed Semen Rare Breed Foal 2019

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"The project demonstrates the viability of using new techniques for selecting female foals to increase the breeding population more rapidly than could be achieved through traditional methods. We hope it will prove to be a model for more projects in future."

RBST chief executive Christopher Price

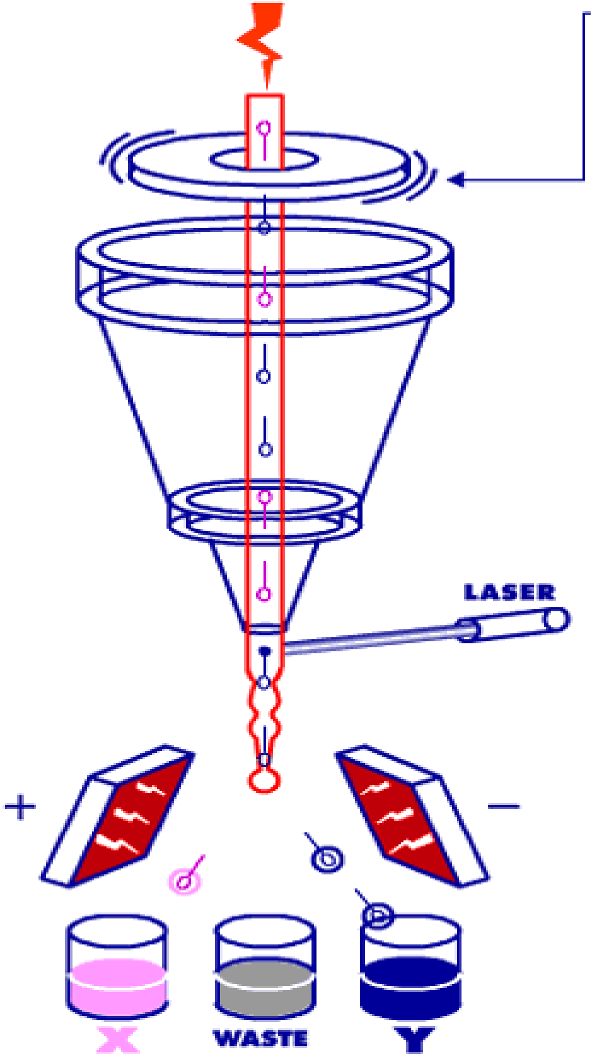
NOTTINGHAM TRENT UNIVERSITY | TWEMLOWS STUD FARM | cogent | THE SUFFOLK HORSE SOCIETY | Holbeache Suffolk Punches | RBST | STALLION AI SERVICES

2023 World First Frozen Sexed Semen

- Frozen sexed semen now available
- For use in combination with ICSI
- Offers greater potential for wider use than fresh semen

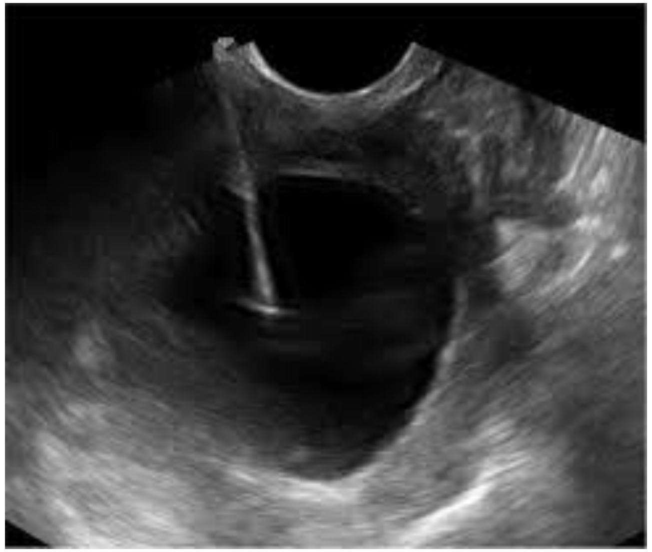
Rhino Semen Sexing

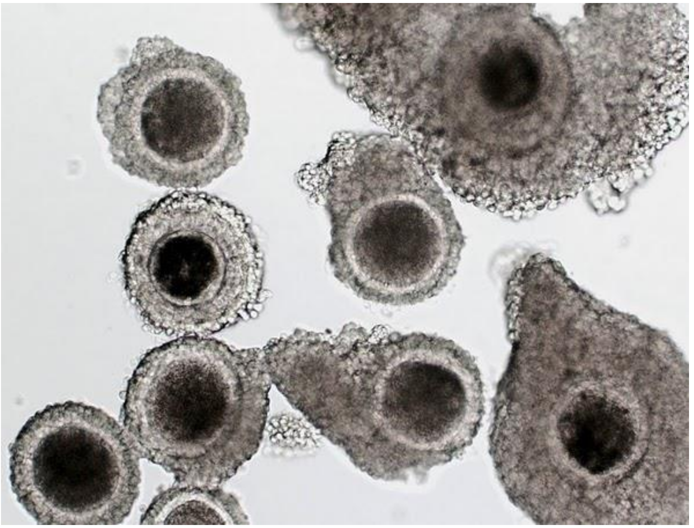
- Work started in the US



Oocyte Recovery & Ovum Pick Up

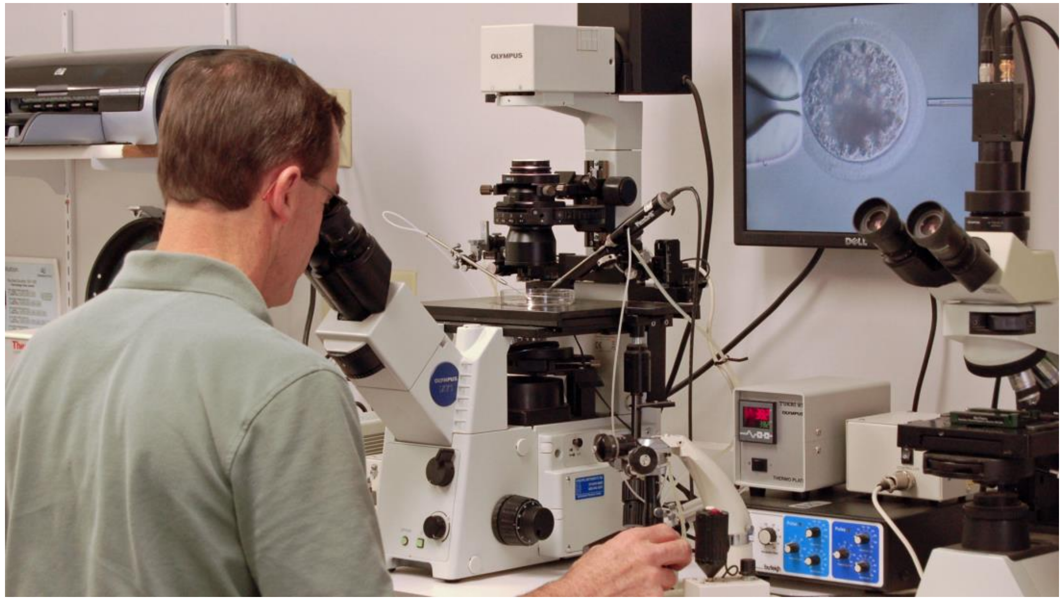
- Post mortem oocyte recovery
- Live mare recovery
- Rapid uptake 2021 breeding season
- Used alongside ICSI

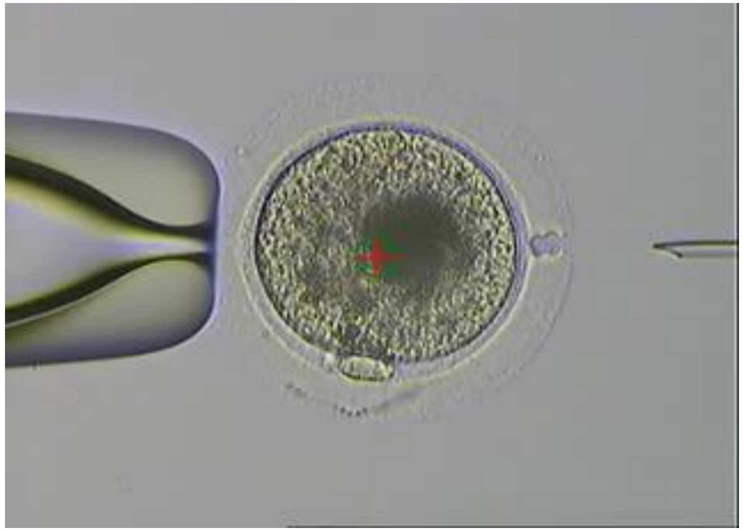


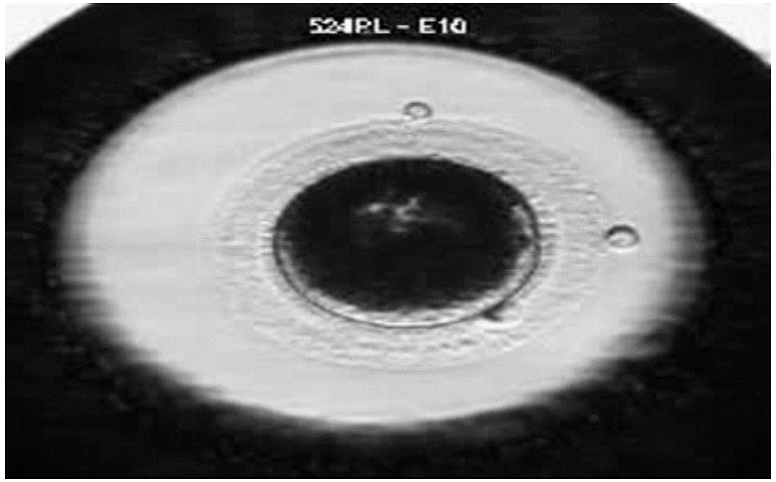


OPU & Intra Cytoplasmic Sperm Injection

- First equine ICSI = 1996 (USA)
- Avantea, Italy –one of the largest ICSI centres



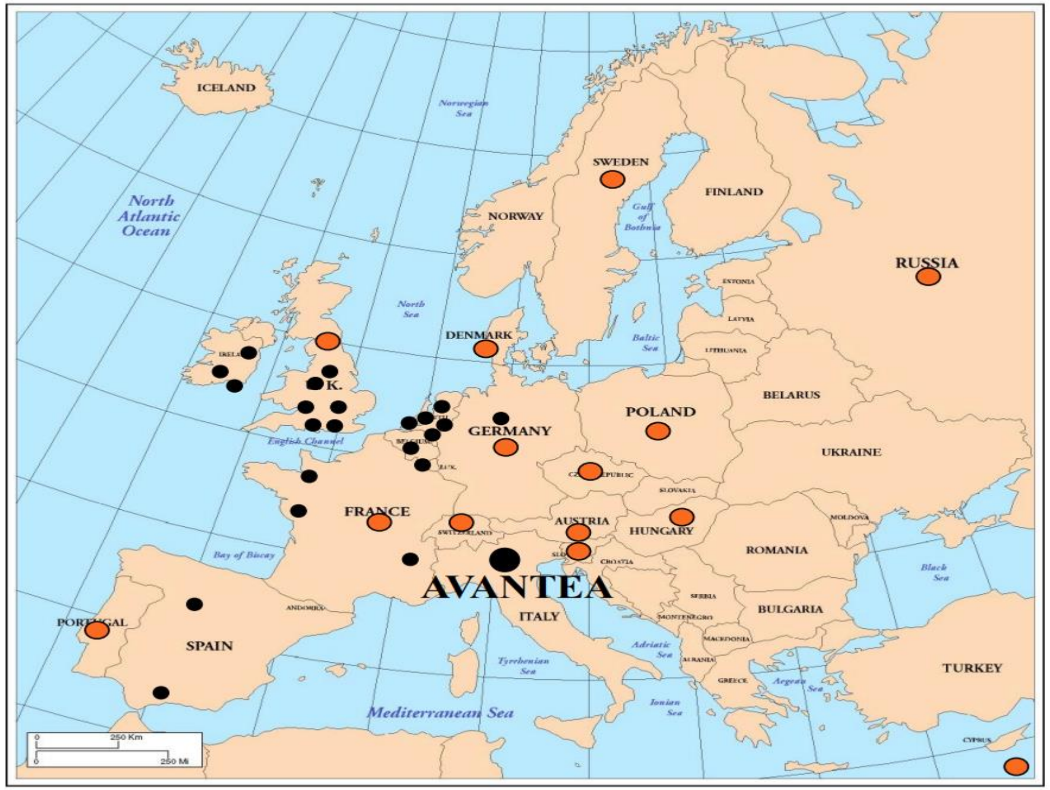




Intra Cytoplasmic Sperm Injection (ICSI)

Avantea Equine OPU-ICSI activity in Europe

EUROPE



Produced by the Cartographic Research Lab University of Alabama

AVANTEA, Cremona, Italy

Satellite centres shipping OPU oocytes to Avantea for ICSI

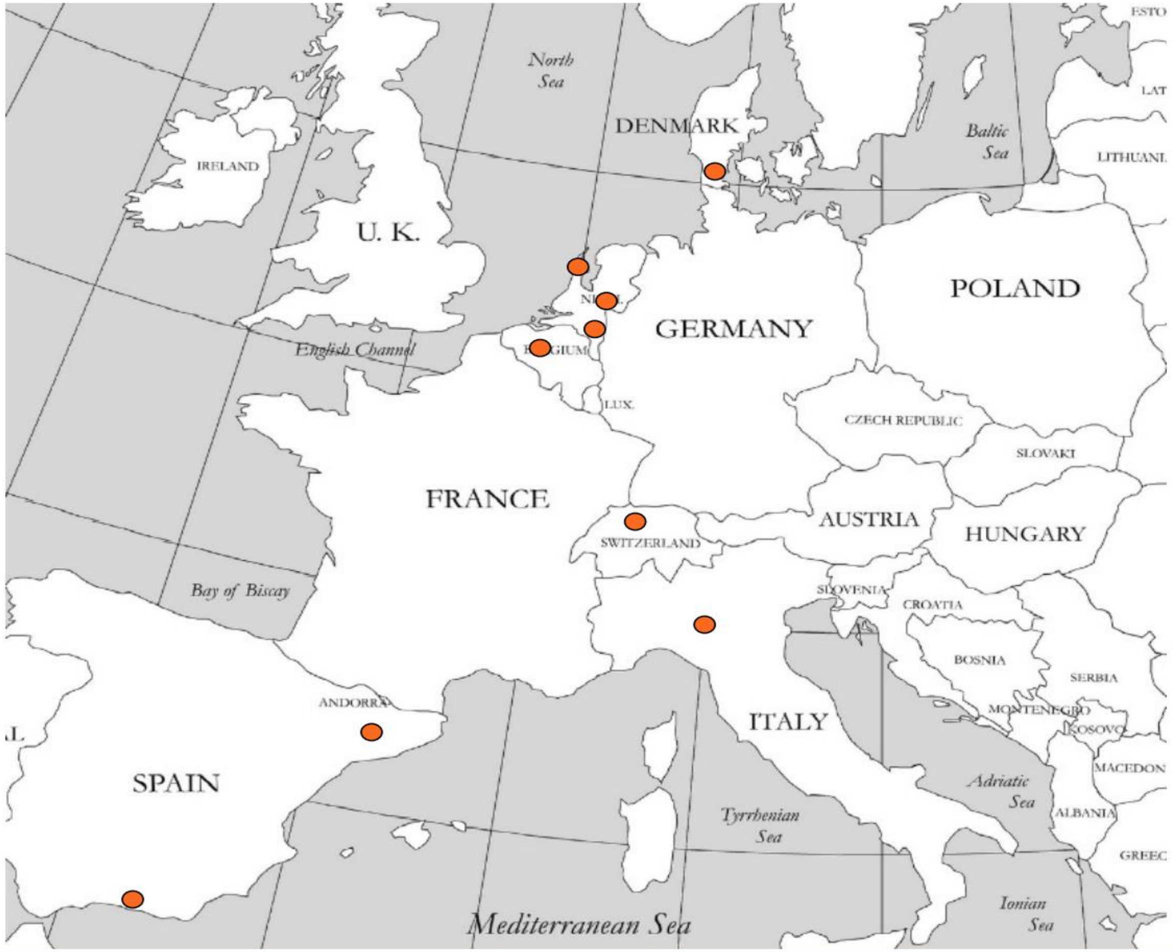
Countries (Clients/ veterinarians) driving /sending donors to Avantea for OPU and ICSI

Middle East Countries

AVANTEA

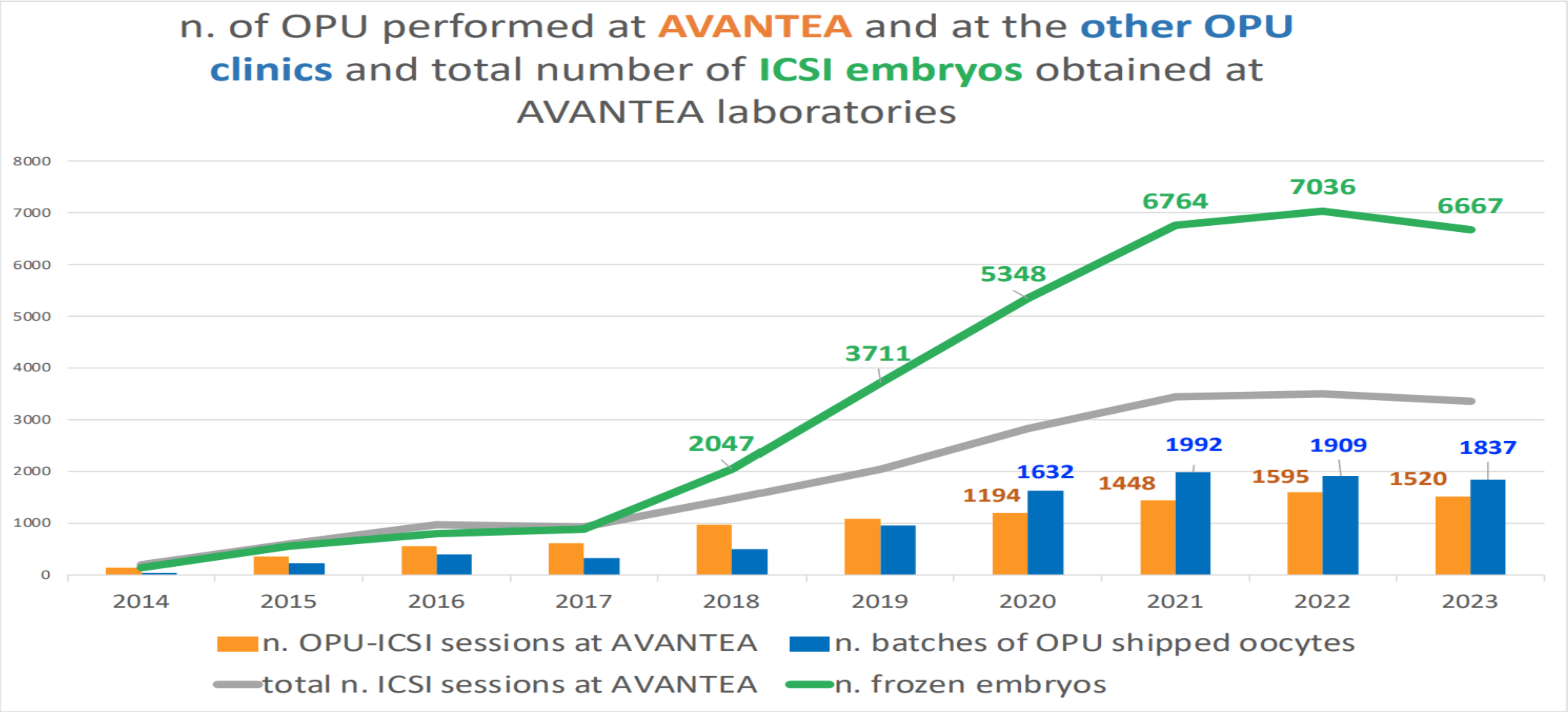
Intra Cytoplasmic Sperm Injection (ICSI)

Current landscape of Equine ICSI lab in Europe



- 3 Holland
- 2 Spain
- 1 Belguim
- 1 Switzerland
- 1 Denmark
- 1 Italy

Intra Cytoplasmic Sperm Injection (ICSI)



Lazzari et al 2020, J Equine Vet. Sci. and updates

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Embryo Freezing

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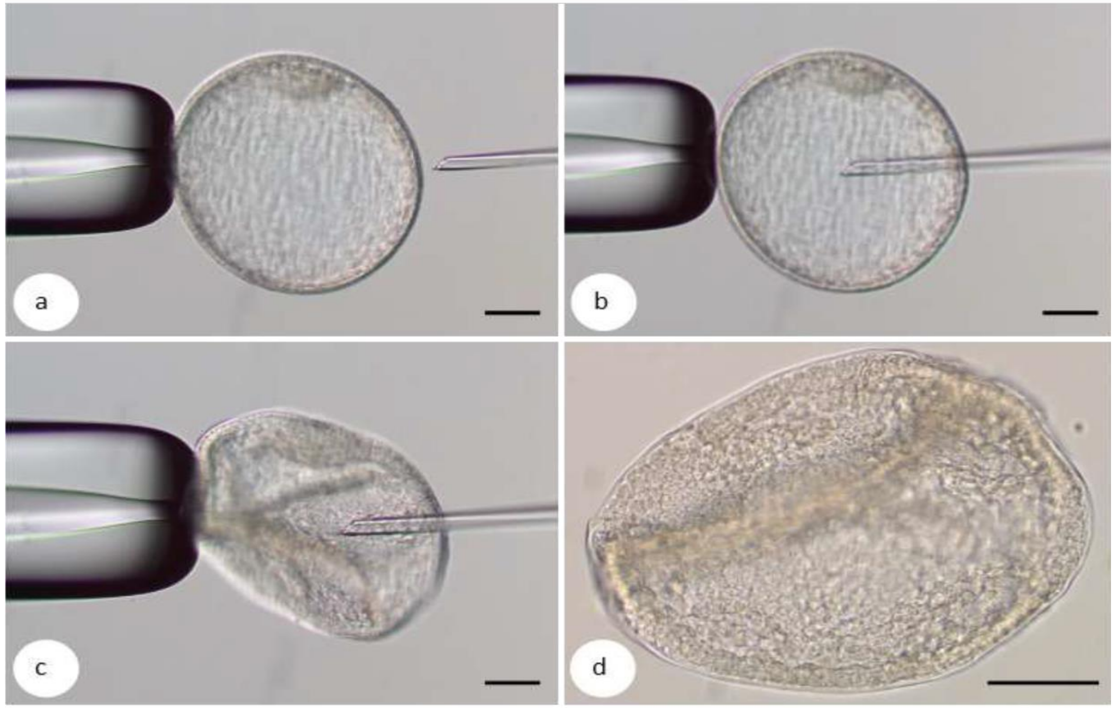
- Embryos flushed after day 6 = blastocysts >300µm
- Day 8 = >500µm & large blastocoele cavity
- Vitrification can assist in embryo freeze ability

All Embryos	Pregnancy Rate (%)
Punctured only (n=22)	10/22 (45%)
Punctured & aspirated (n=28)	21/28 (75%)



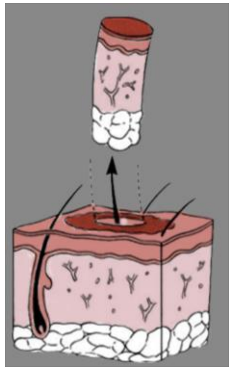
Source: Vitrification of Equine Expanded Blastocysts (Wilsher et al., 2018)

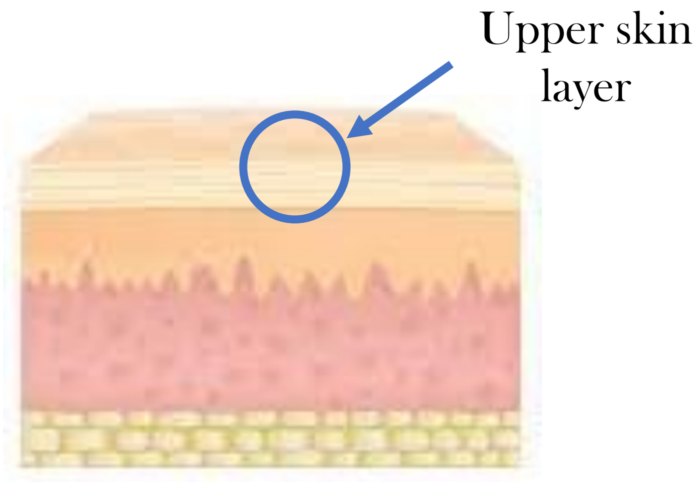
Skin Sample Genetic Preservation

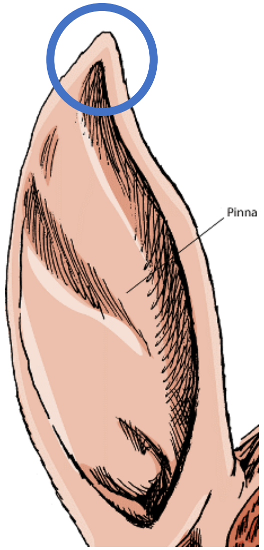
- Preservation of DNA for future use
- Unique preservation that permits future regeneration of the DNA
- And so facilitates animal regeneration technologies e.g. cloning
- Elite sport horses
- Biobanking for conservation

Genetic Preservation

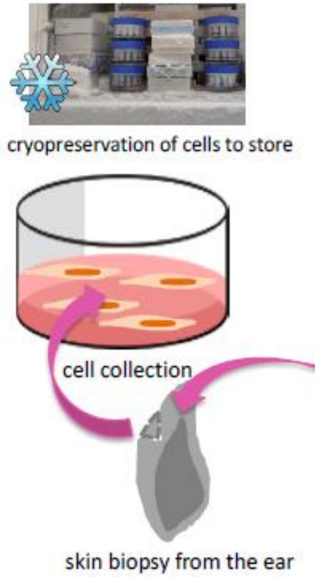
- Before or after death
- Sample received within 5 days of passing / 5 days of taking
- Kept chilled; never frozen
- Whole genetic profile – non gamete cell







DNA techniques in action



These are fibroblast cell lines grown in October from an ear sample cryopreserved in 2019

One vial of skin sample can produce up to six vials of fibroblast cells

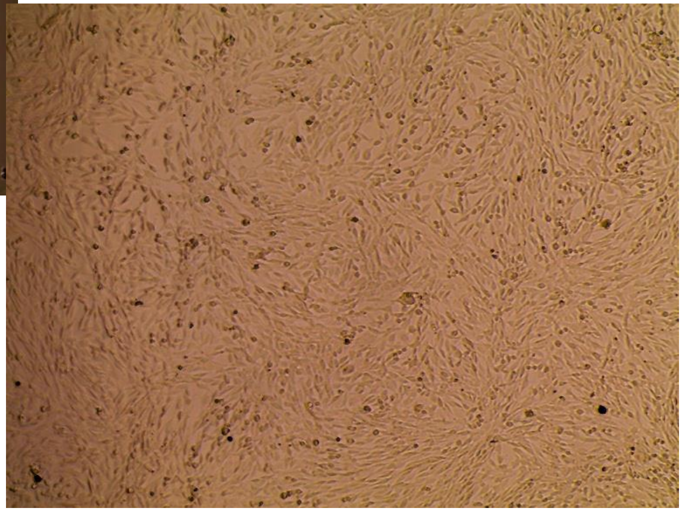
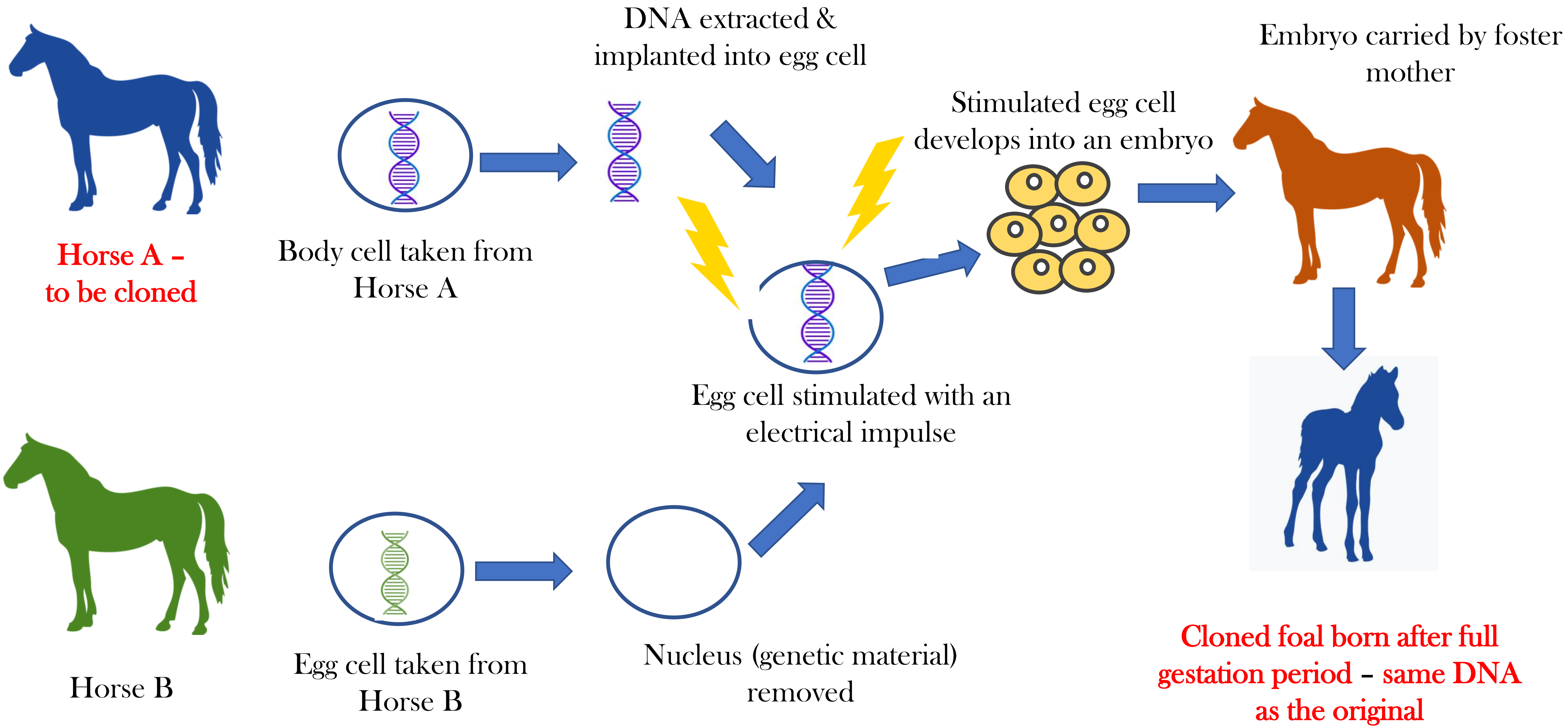


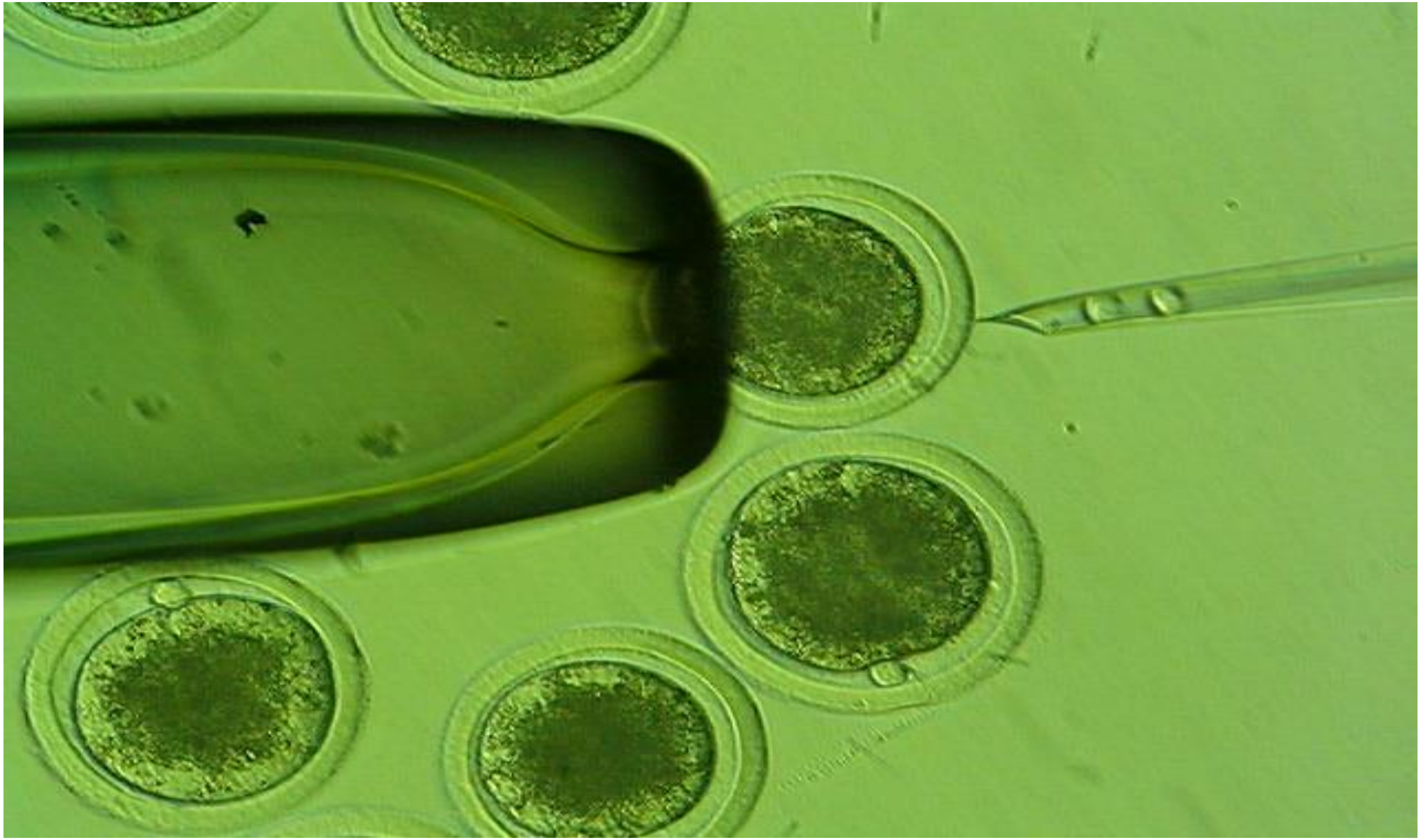
Photo © Chester Zoo 2021

Cloning Process



Egg Enucleation

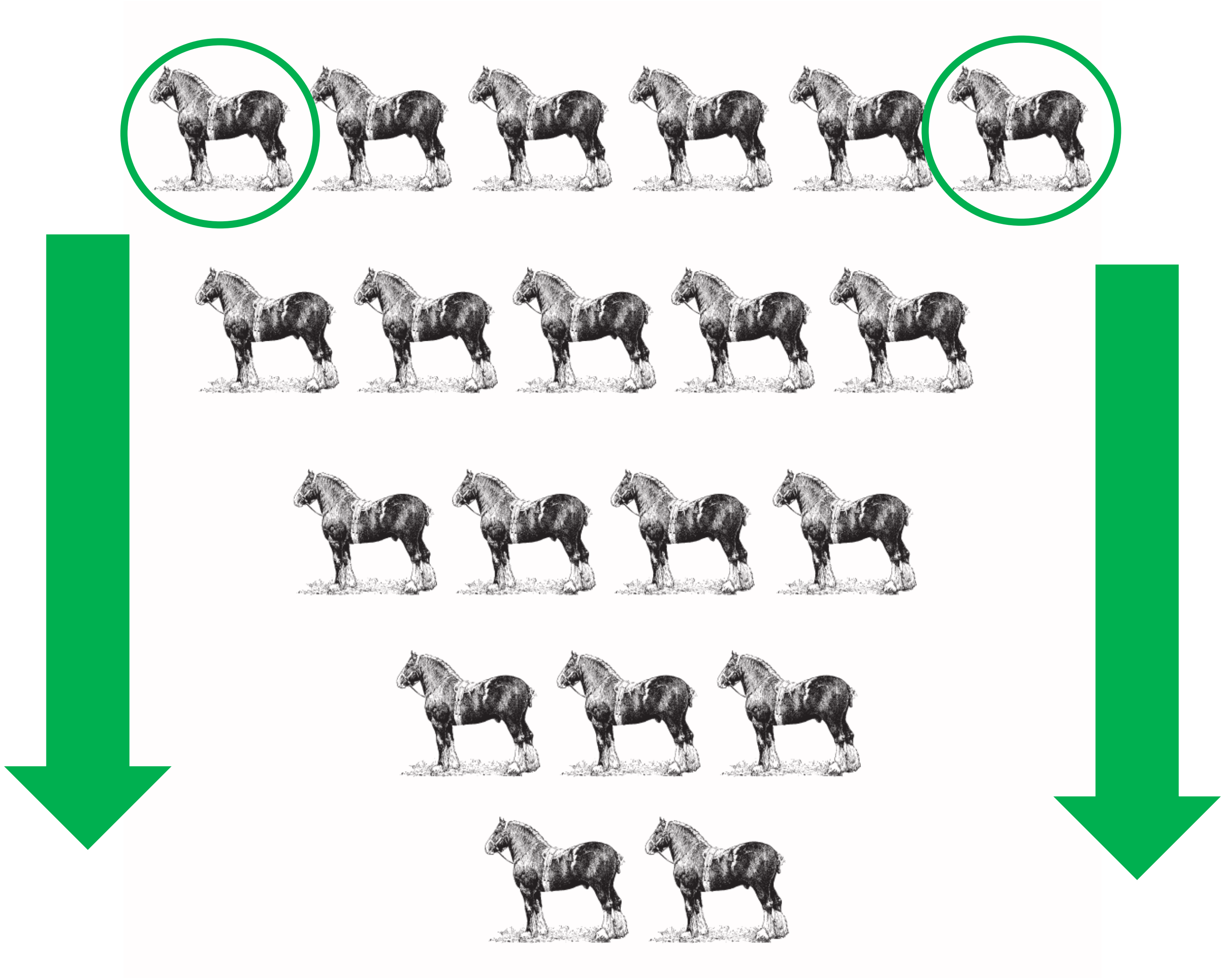
Egg Reconstruction



Skin Sample Preservation For Conservation

- Skin samples are likely to play a crucial role in biodiversity preservation in the future across all species
- Preservation of the 'pillars of the population'
- 50 different genetic lines

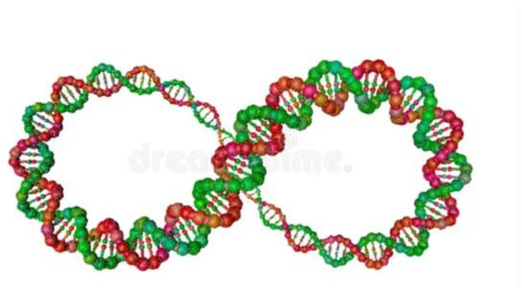
Skin Sample Preservation For Conservation

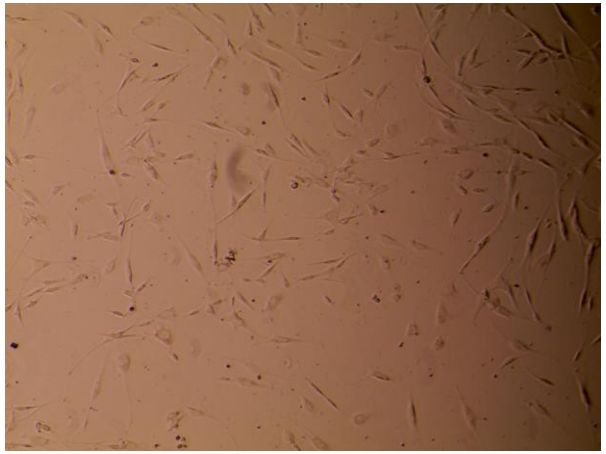


- Minimum of 50 different genetic lines
- Preserving a wider genetic pool as possible

Benefits of Using Skin

- Whole genetic profile – 100% DNA profile – gametes = 50%
- Preservation of all genders including castrated
- Infinite resource
- Possible easier adaptation to future requirements





VIAGEN
PETS & EQUINE

Przewalski's Horse

GEMINIGENETICS

- Thankfully, San Diego Zoo in the US had the foresight to bank skin samples
- Began officially collecting samples 45 years ago
- Some sporadic collection beforehand
- Over 400 samples in store for this species
- Oldest sample from an individual born 68 years ago

San Diego Zoo

Utilisation of Skin Samples - Genetic Rescue

- Skin samples preserved by San Diego Zoo
- 40-year-old sample utilized in 2020 to re-introduce genetic diversity to the closed population

Cloned Przewalski's Horse, Kurt, born August 2020
https://reviverestore.org/projects/przewalskis-horse/

Utilisation of Skin Samples - Genetic Rescue

VIAGEN PETS & EQUINE

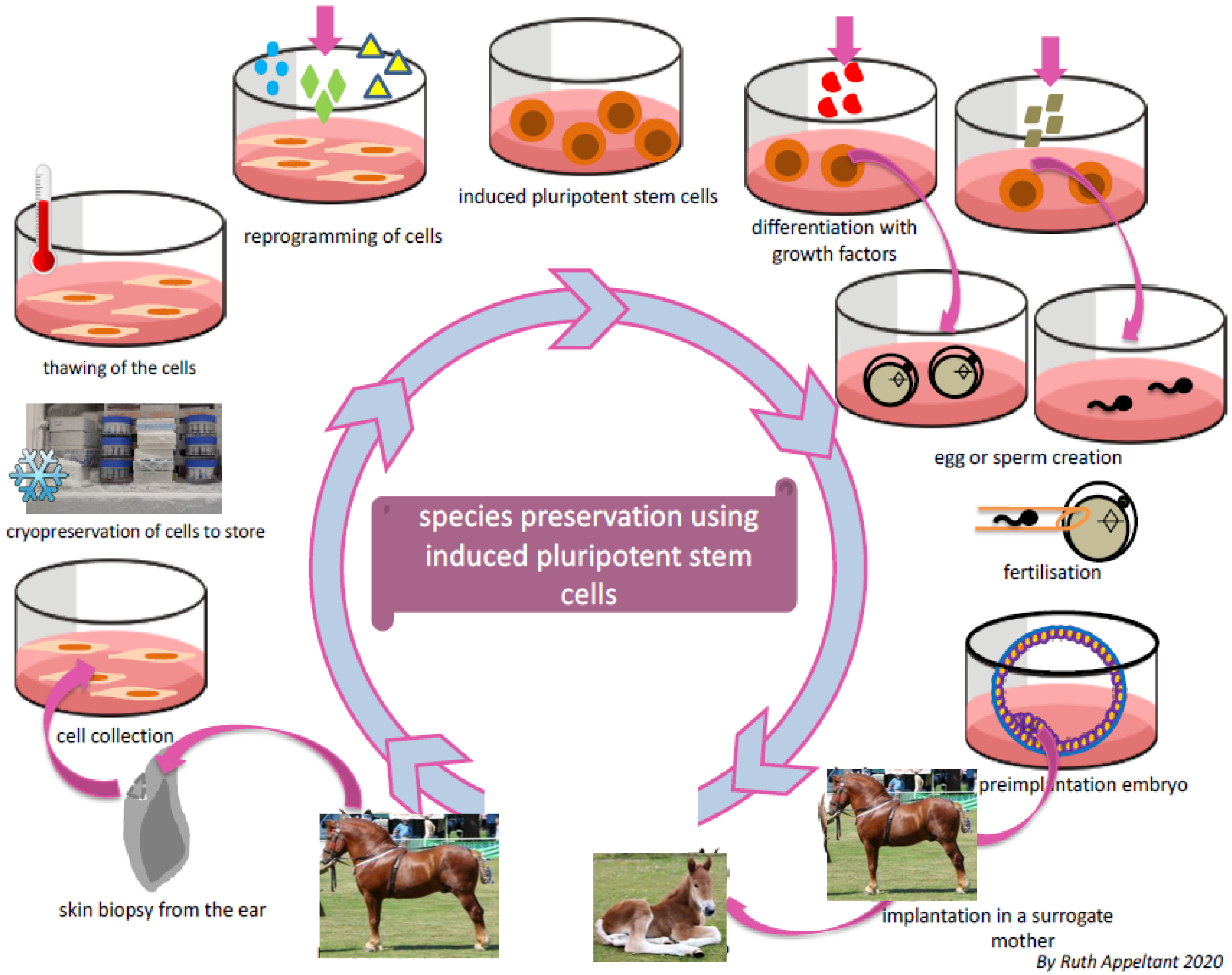
GEMINIGENETICS

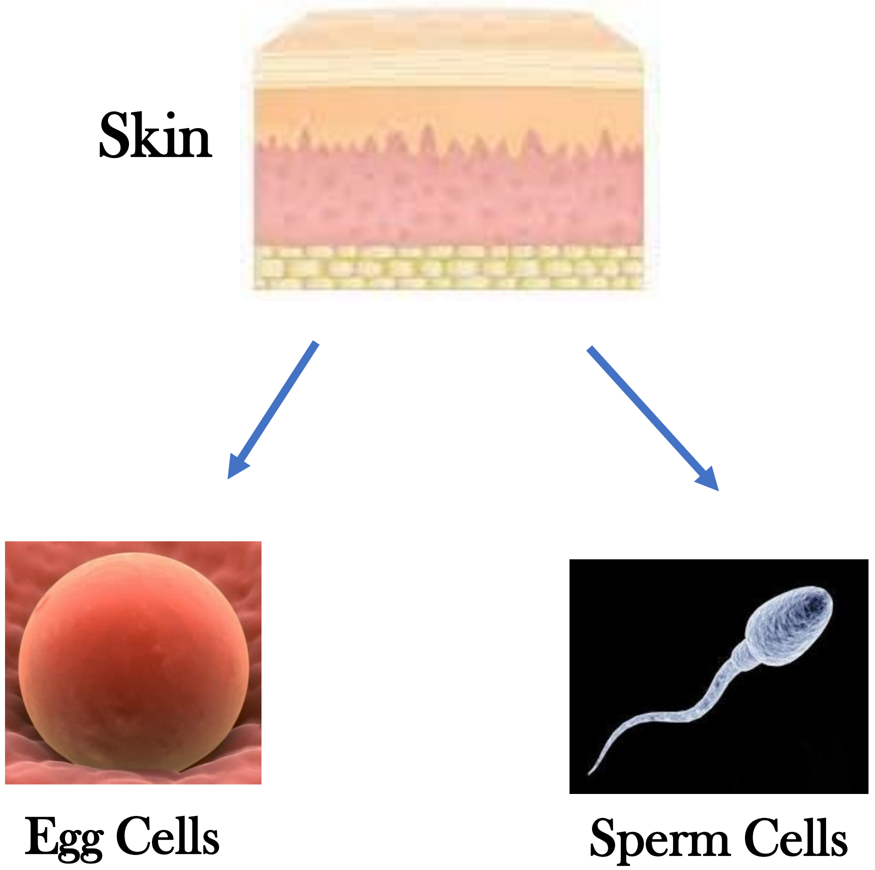
- Endangered black-footed ferret
- Few founder individuals
- Notable disease challenges
- Skin samples preserved 30 years ago
- Clone born 10th December 2020 to re-introduce genetic diversity to the closed population

Cloned Black Footed Ferret, Elizabeth Ann, born 10th Dec 2020

https://reviverestore.org/projects/black-footed-ferret/

Other Uses- IPSCs





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STALLION
AI SERVICES

Knowledge Transfer and Laboratory Set-ups

Mexico November 2023

India December 2023

Japan February 2024

Finland March 2024

Iceland April 2024

Faroe Islands August 2024

Conservation Success in Iceland

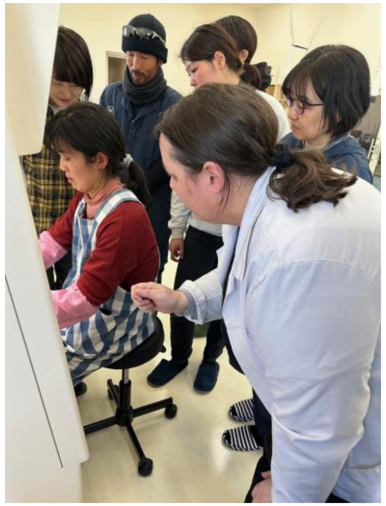
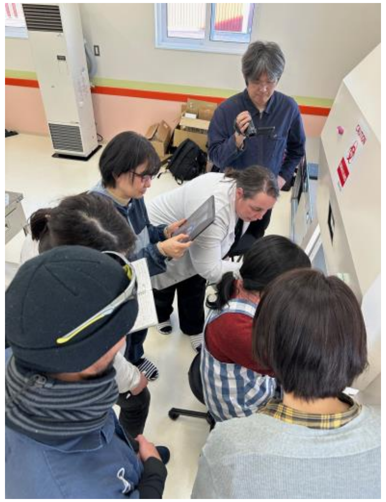
1st equine pregnancy via frozen semen – confirmed May 10th 2024

Conservation Work In The Faroe Islands

- Critically endangered Faroese Horse
- Only 87 left in total; 23 stallions
- Smaller than an Icelandic Pony but bigger than a Shetland – considered to be a descendant of both
- Biobanking via semen harvest August 2024
- Biobanking via skin samples (all horses) - 2025

Bio-Banking Knowledge Transfer


Biobank in India
December 2023

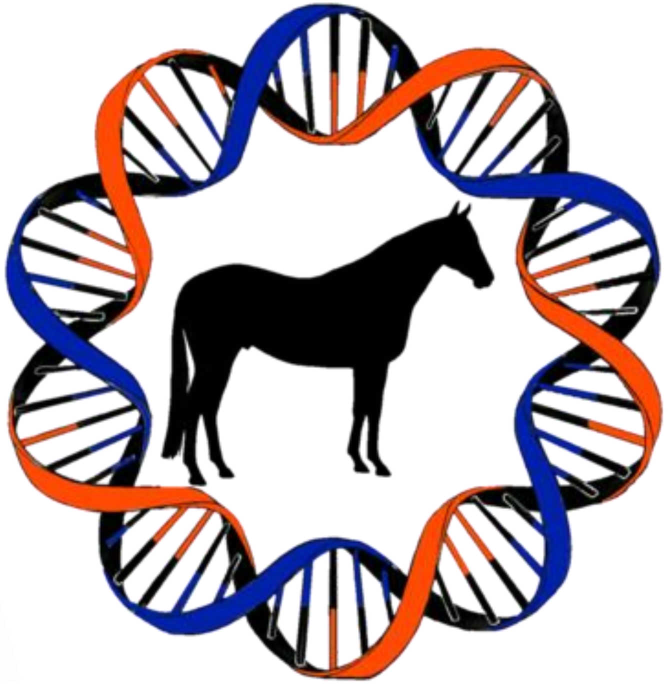
Biobank in Japan
February 2024

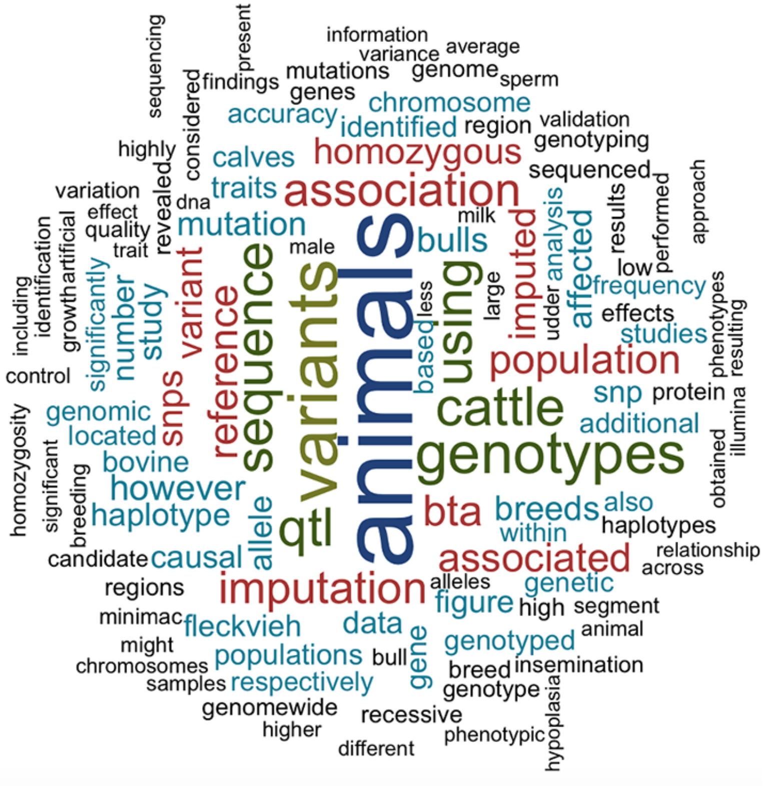
Our Vision

- Aims to roll out our cryopreservation technologies globally to create multiple centres of expertise within new living biobank hubs

A Look Towards the Future …….

Next Developing Technologies – Genomics, semen genetic testing & gene editing





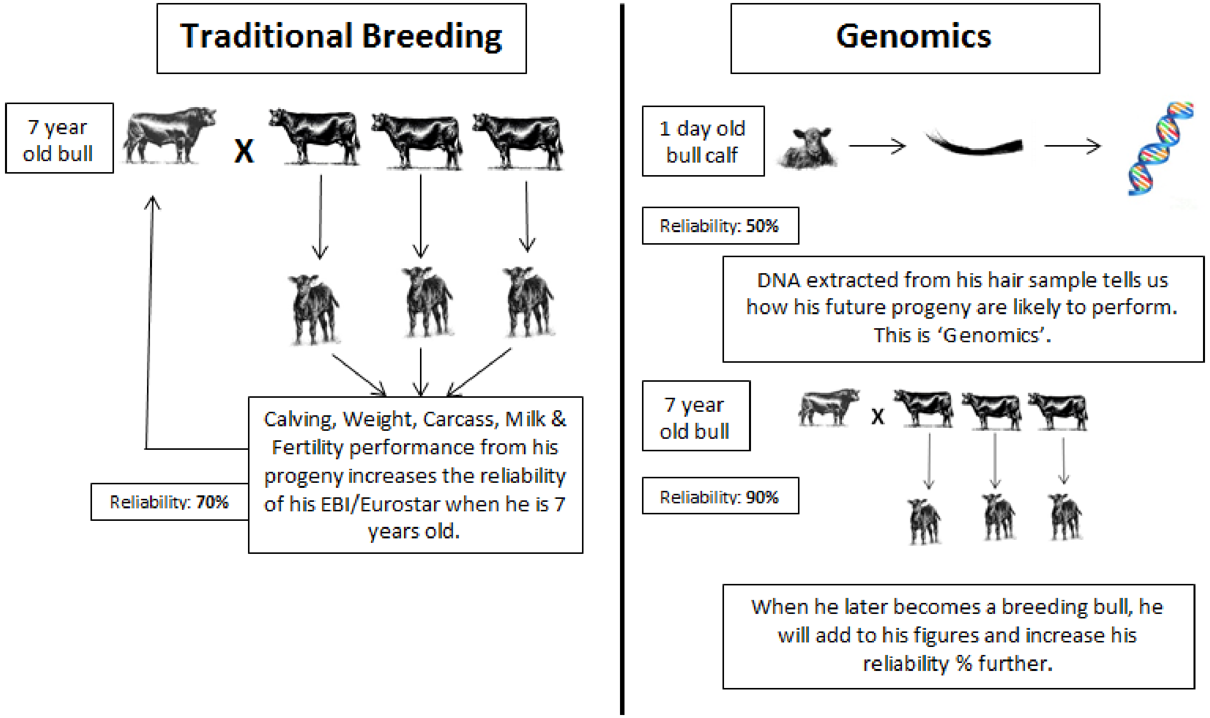
Tullis Matson
Gemini Genetics

Genomics & Equine Breeding

- Using DNA in animal breeding
- the identification of the genomic architecture of traits of interest
- Revolutionized the dairy industry, reducing generation interval, improving breeding efficiency and overall breeding productivity

Genomics & The Bovine Industry

- Genomics uses a young animal's DNA to estimate their genetic potential
- Gives dairy farmers confidence to make informed breeding decisions with their youngstock.
- And much earlier than traditional breeding



Genomics & Equine Health

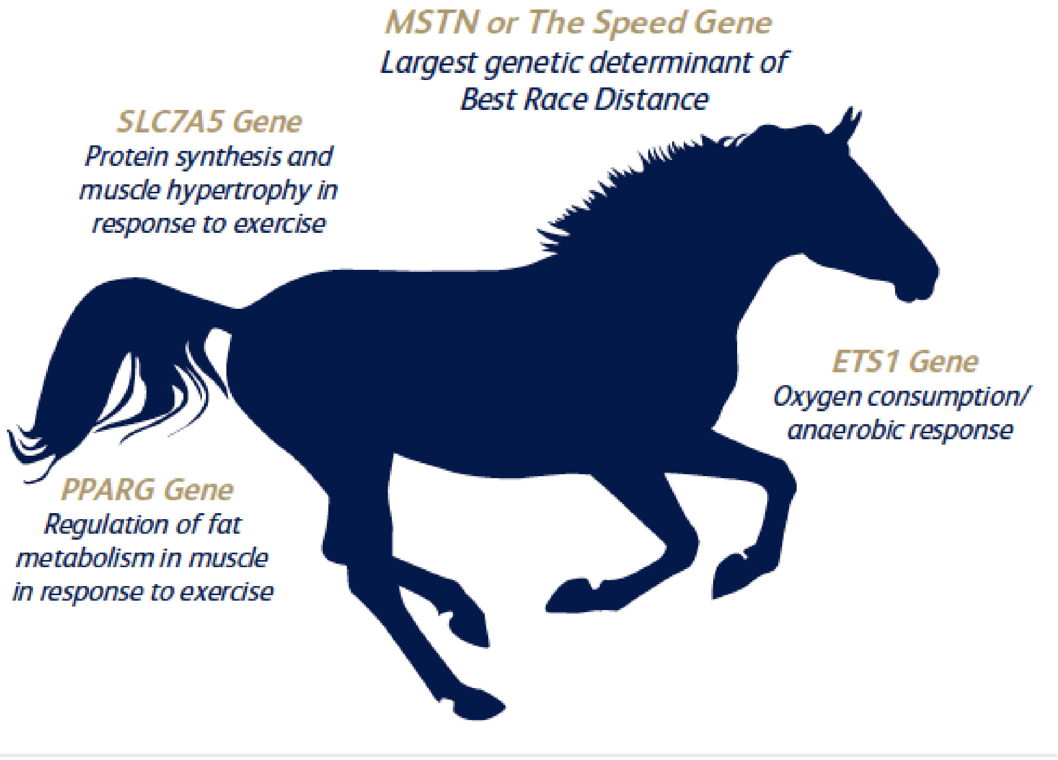
- Several genetic tests available for assessing for carrier status of disease-causing genetics
- Increasing trend and awareness for disease testing
- Examples –

  - Warmblood Fragile Foal Syndrome (WFFS)

  - Polysaccharide Storage Myopathy (PSSM)

Animal Genetics

Genomics & Performance Breeding



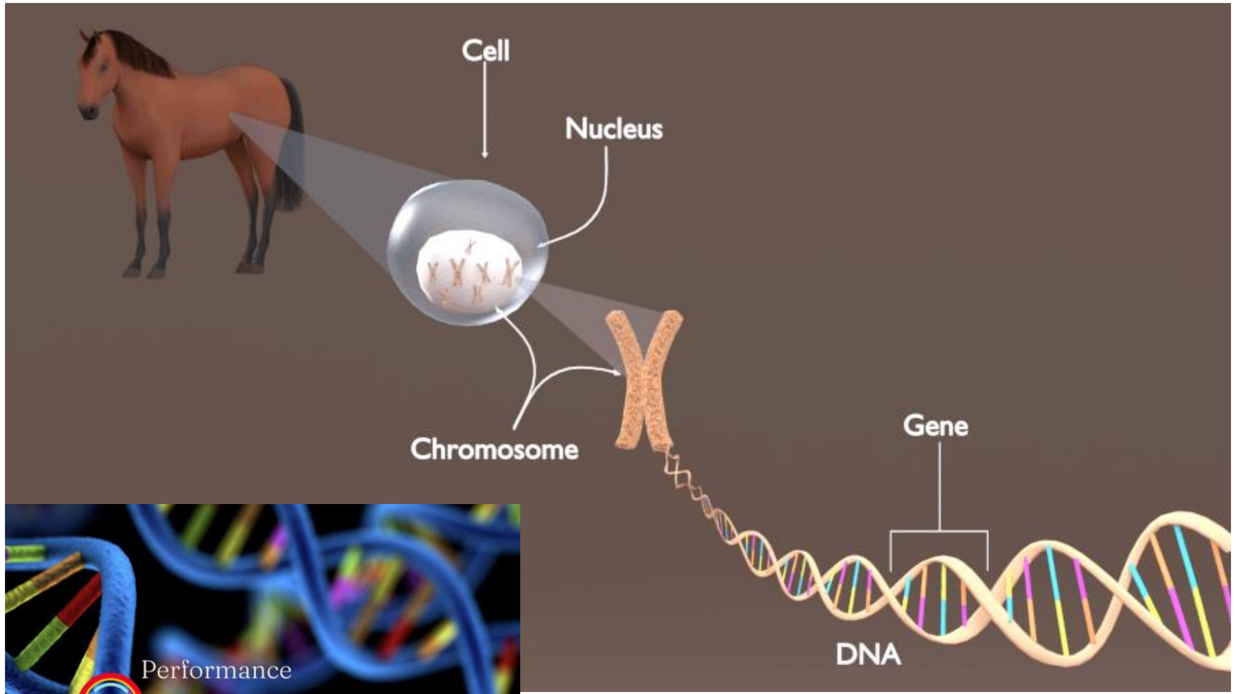
- Applications to equine performance
- E.g., equine speed gene
- Identification and quantification of the % of each trait that is due to genetics, and the % due to the environment.

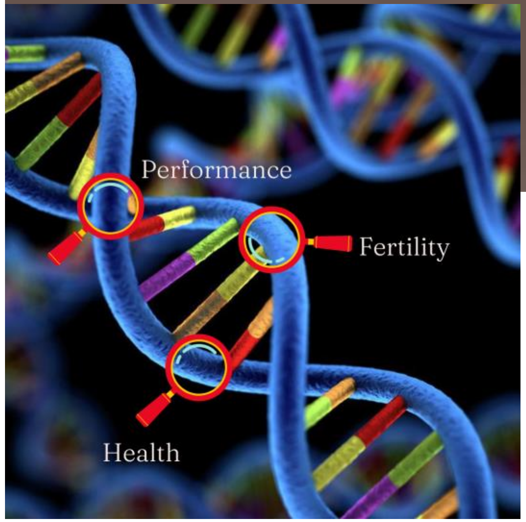
WBFSH
WORLD BREEDING FEDERATION FOR SPORT HORSES

The Future of Equine Breeding

STALLION
AI SERVICES

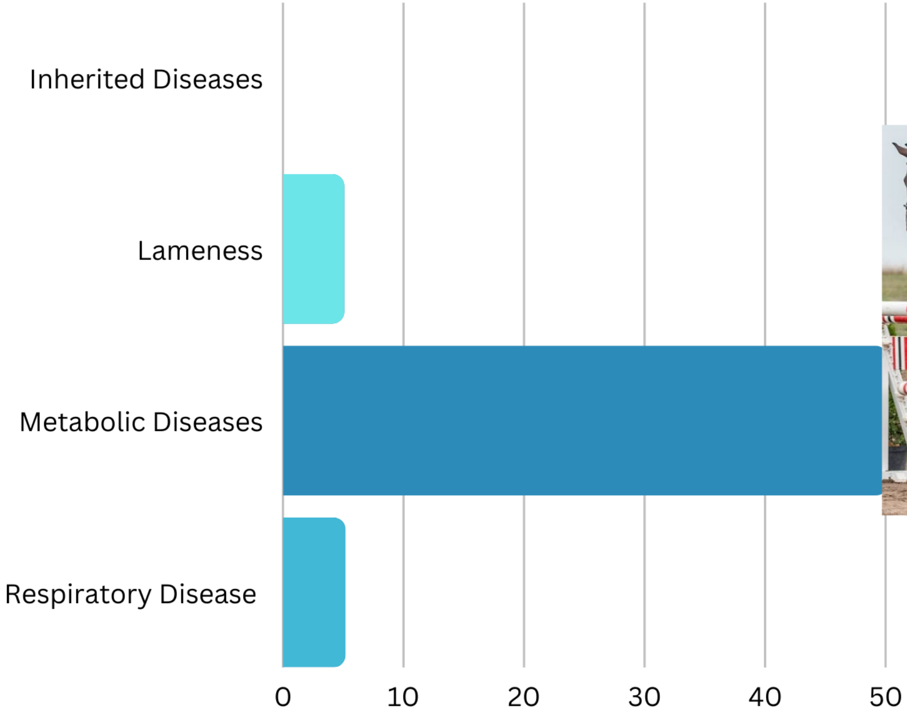
- Genomics may revolutionize the way we breed, buy, sell or purchase our horses
- Allow for more accurate breeding
- Reduce ‘wastage’ & improve health & welfare
- Targeted breeding towards the characteristics we want



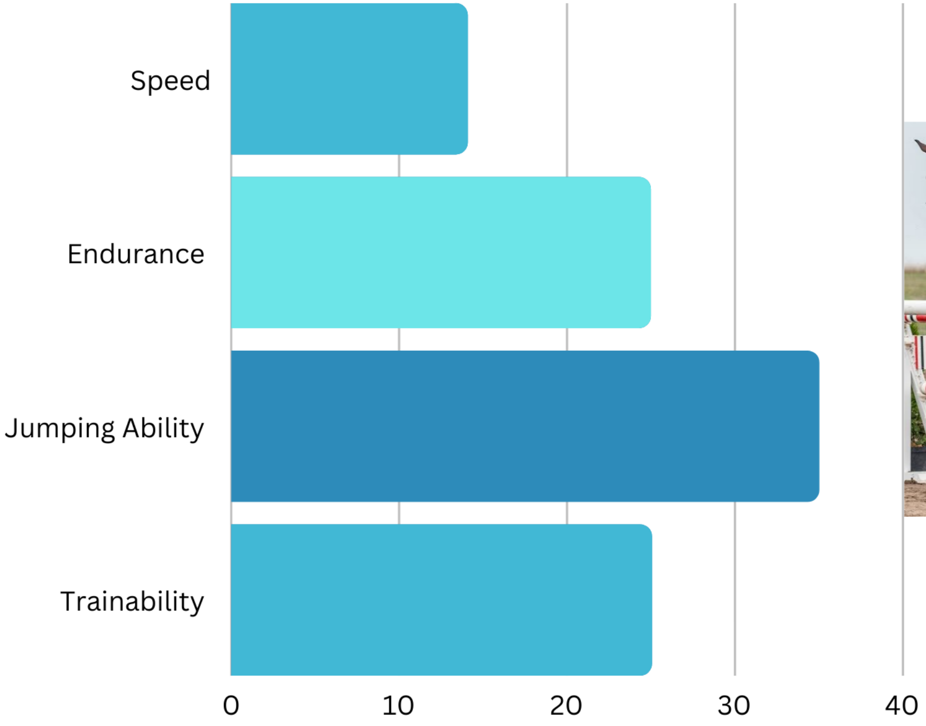


The Future of Equine Breeding

Health



Performance

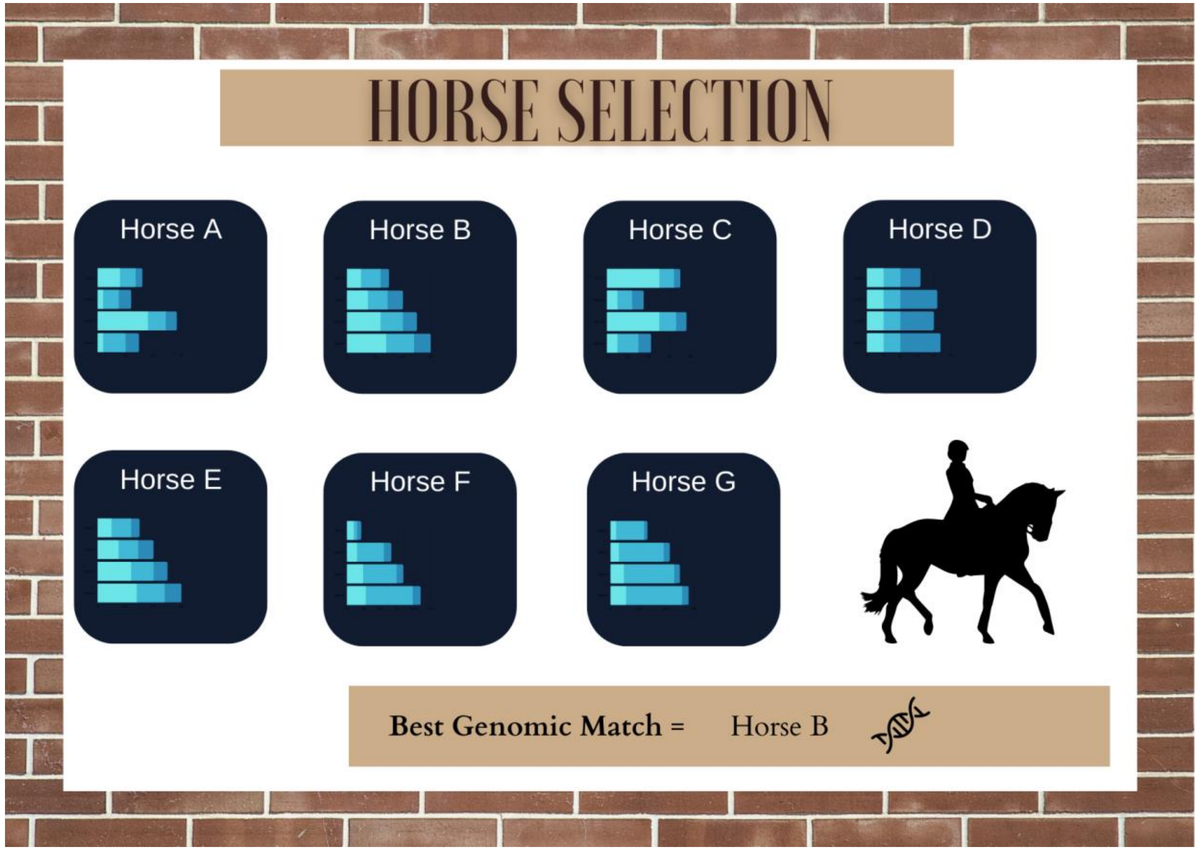


The Future of Equine Breeding

- Future horse selection may be based on genomic potential charts

- Such charts may be available from foal age or even possible before birth as a predicted outcome of a mating

- Predetermining performance genetic potential

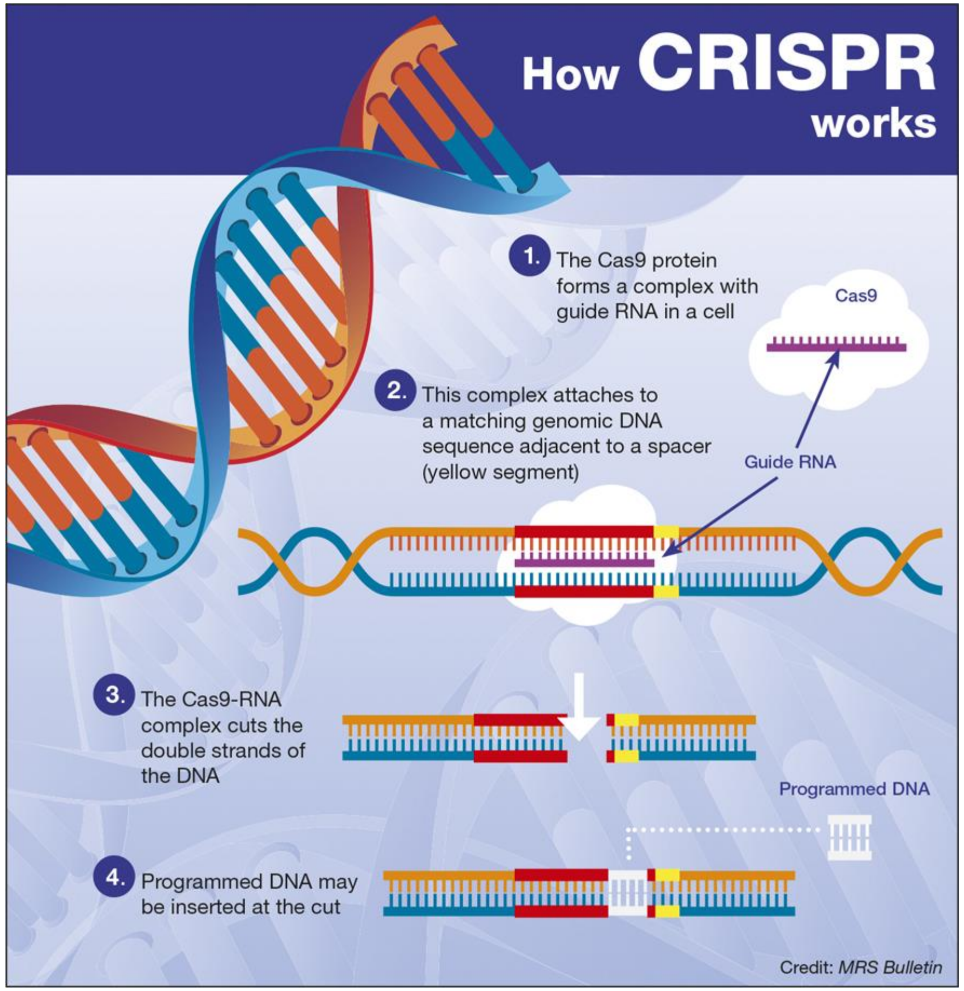


Genomic Limitations & Challenges

- May lead to all aiming for the same 'type of horse' / same characteristics
- Need to monitor inbreeding risk
- Equally needs to accommodate different tastes within the equine industry
- Time and a lot of data to establish accurate genomic information for the range of characteristics needed per discipline

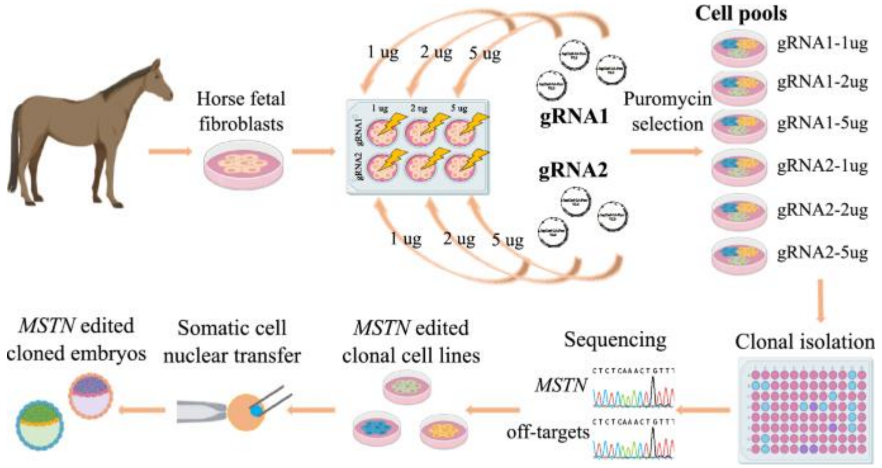
CRISPR Technologies & Gene Editing

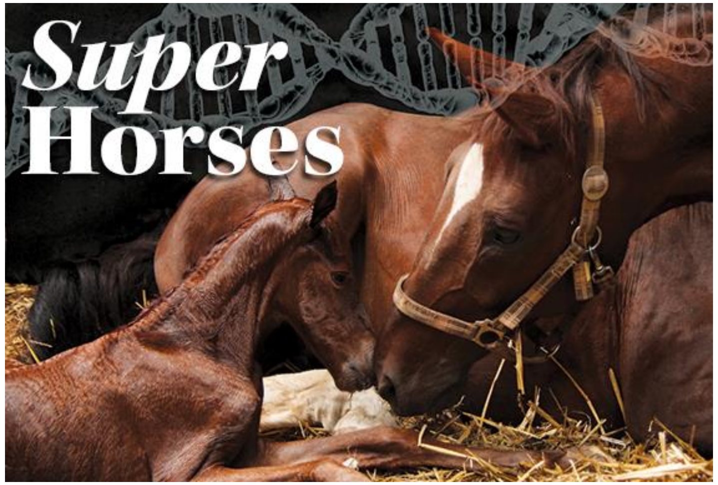
- CRISPR is a technology that can be used to edit genes
- Essentially a way of finding a specific bit of DNA inside a cell
- After that, the next step in CRISPR gene editing is usually to alter that piece of DNA
- Or turning genes on or off without altering their sequence.



Equine Gene Editing

- Researchers in Argentina used CRISPR to “knock out” the myostatin gene
- This gene regulates muscle development
- Chose to knock out the myostatin gene as a proof of concept.



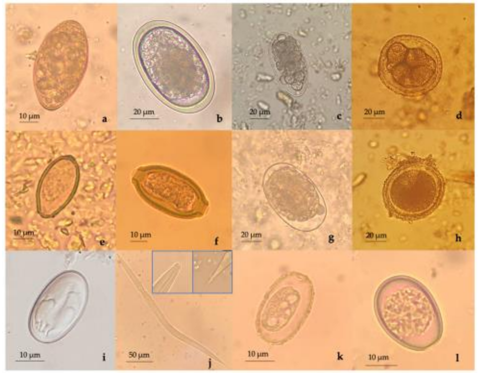


Equine Gene Editing

- As well as performance, there are also potential applications in health and disease

- Editing out disease causing genes

- Or adding in genes to benefit health, provide genetic immunity or increase genetic resilience e.g. to climate challenges, endemic diseases etc.



3D Digital DNA Printing

- Digital DNA
- Future possibility
- Digital characterisation & storage of animal genetic profiles
- 3D printing of DNA for breeding & utilisation
- Prof. Philippe B. Wilson, NTU School of Animal Rural & Environmental Sciences

Equine & Endangered Species

- We stand at a pivotal time in sustainability and survival for many of the worlds rare and endangered animals

- Increasingly seen that there are comparable challenges and solutions to population loss between the domestic and wild animal sectors

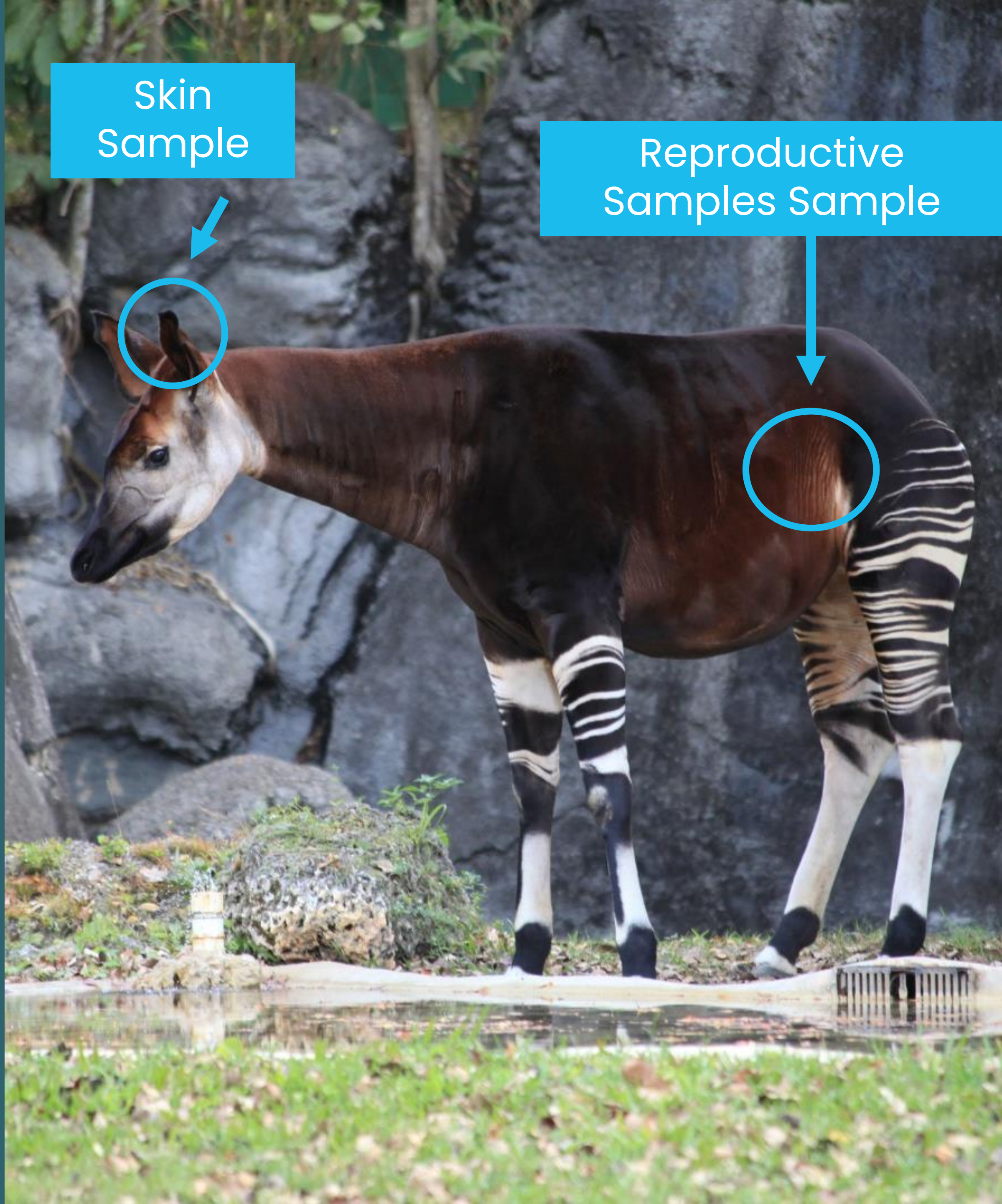
- Transfer of equine breeding technologies to endangered species conservation and vice versa

NATURE'S SAFE
SAVING ANIMALS FROM EXTINCTION
Charity No 1192876

We also collect several samples per animal

So, we have robust resources for future uses

Reproductive samples plus skin



NATURE'S SAFE IN ACTION

SAMPLES	802
INDIVIDUALS	628
SPECIES	274

Equine, Zoo and Farm Species

- Working with equine semen, in particular rare breed semen, has driven many developments and improvements to protocols and technologies
- We can now transfer that knowledge, experience and expertise Livestock conservation

UK National Livestock Biobank

UK National Livestock Biobank

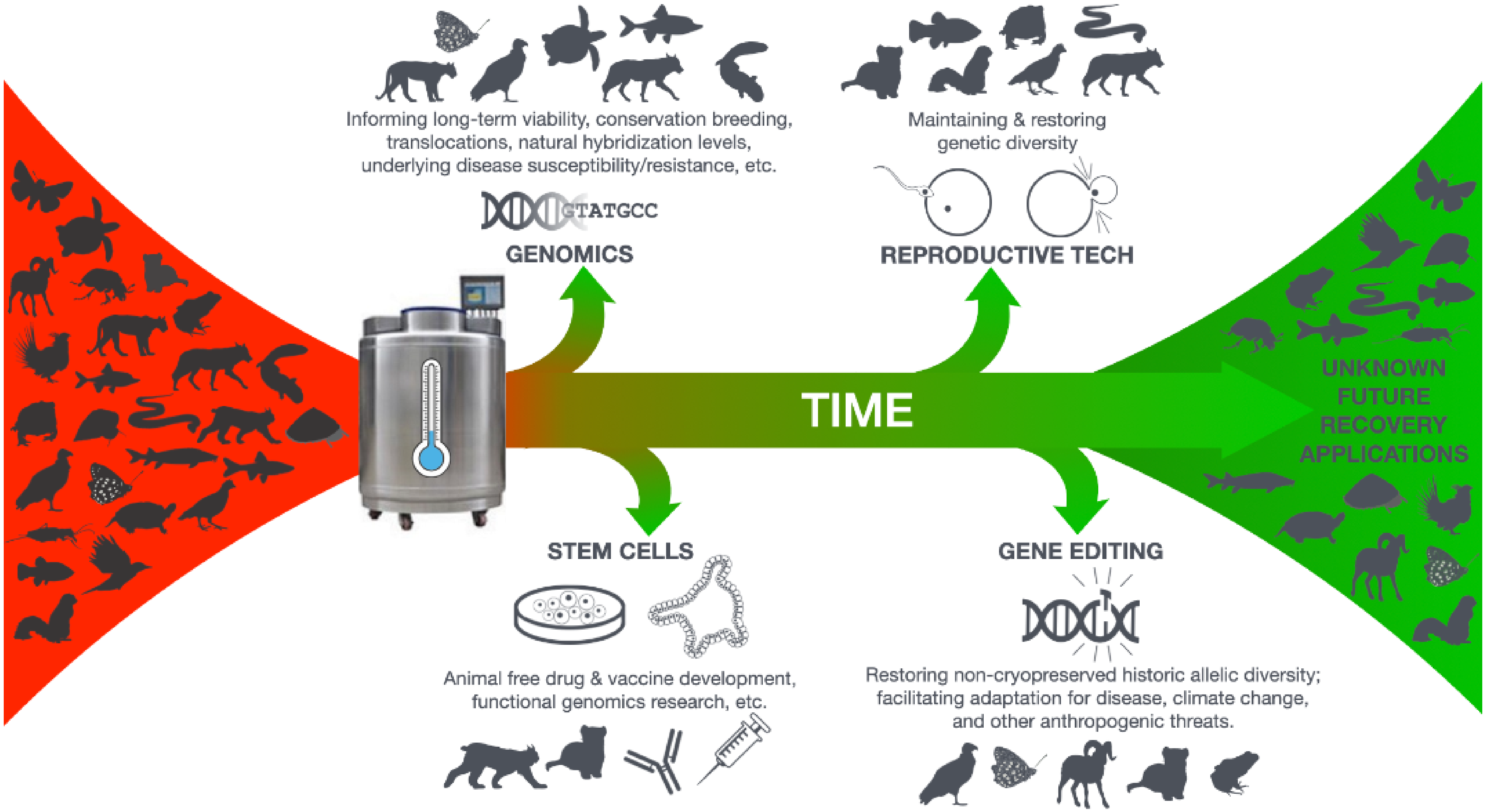
- Nordic Genetic Resource Centre
- Biobank for farm animal breed and species security
- For UK & global food security
- Skin plus reproductive samples
- International partners e.g. NordGen, Norway

NordGen

- Nordic Genetic Resource Centre
- Nordic countries' joint gene bank
- Mission to preserve and promote sustainable use of the diversity of domestic animals, forests and plants that are important to Nordic agriculture.

NordGen

Informed Biobanking



[Biobanking - Revive & Restore (reviverestore.org)](https://reviverestore.org)

Conclusion

- Modern technology offers a powerful toolkit for safeguarding genetic diversity and biodiversity
- Through biobanking and genetic advancement, we can protect genetic material and potentially address genetic issues or even resurrect extinct species
- While challenges remain, such as ethical considerations and the need for continued research and development, modern technology offers a promising path toward a more sustainable future
- **We need to preserve what is available to us today, to ensure a sustainable and adaptable tomorrow.**

Thank You For Listening

THE QUEEN'S AWARDS FOR ENTERPRISE: INNOVATION 2022

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