GENERATION OF EQUINE INDUCED PLURIPOTENT STEM CELLS: A KEY STEP TOWARDS 'ONE MEDICINE' STRATEGIES

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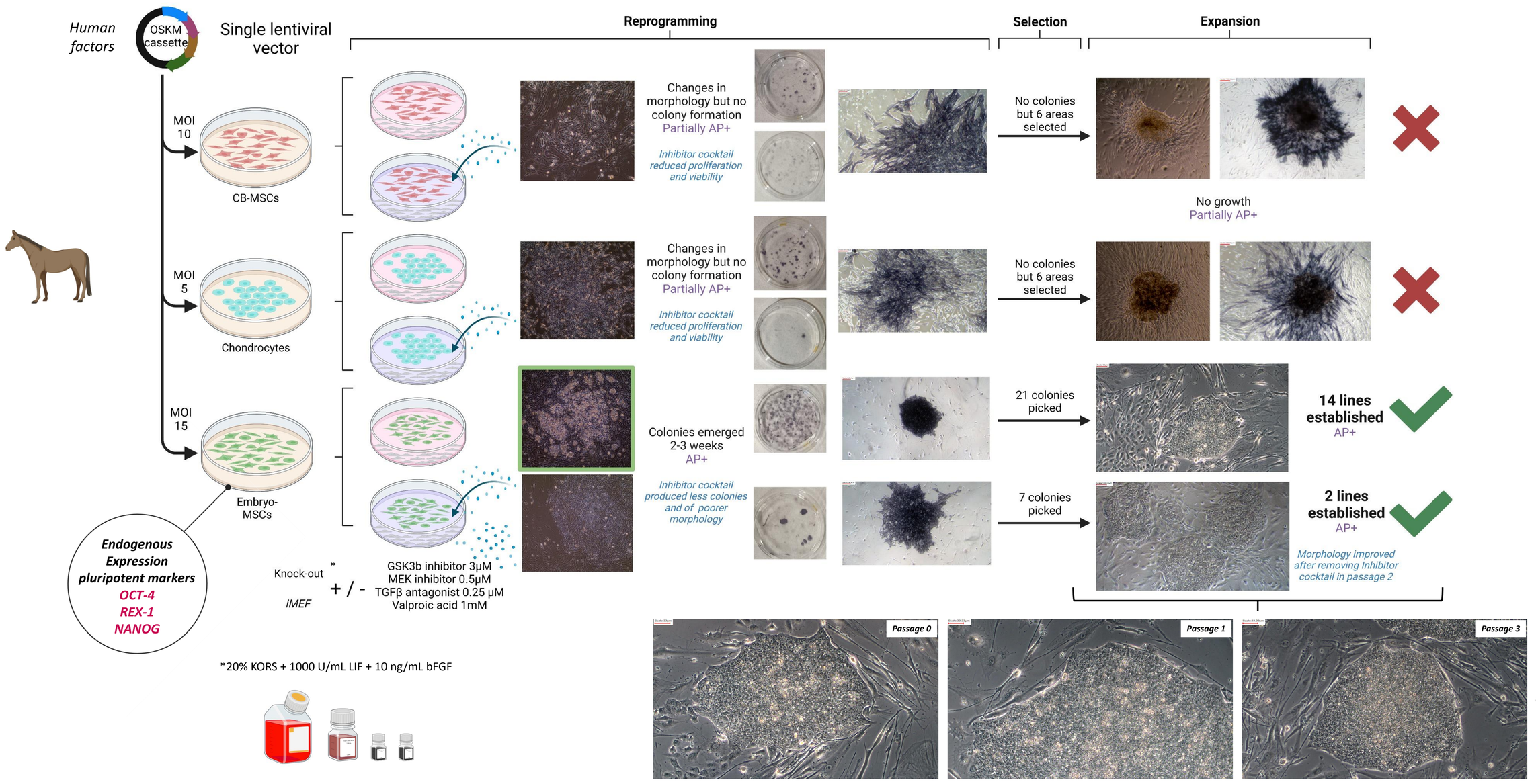
INTRODUCTION & OBJECTIVES

- **Osteoarthritis** is a major contributor to disability in **humans** and to chronic lameness in **horses** → **iPSCs** can be used for **therapy** but **pre-clinical** knowledge is limited to small animals.
- Equine joints better resemble human features (**models**) **+** horses can benefit from cell therapy (**patients**) **=** **One Medicine**
- In order to develop a One Medicine approach for osteoarthritis, our first goal is to establish **equine iPSCs** from new sources with potential **chondrogeneic commitment**:
  - Umbilical cord blood MSCs
  - Articular chondrocytes
  - Embryo-derived MSCs

The varying requirements in different species make it necessary to develop iPSC reprogramming protocols for equine cells, ideally serum-free and feeder-free to facilitate clinical application

METHODS & RESULTS

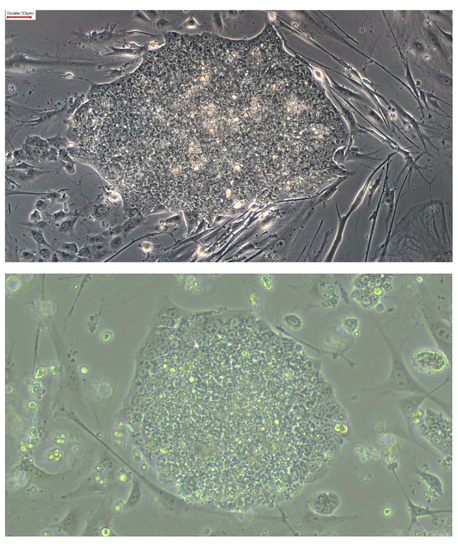
Lentiviral reprogramming



Characterisation of equine iPSCs derived from embryo-MSCs

1. Celular characterisation

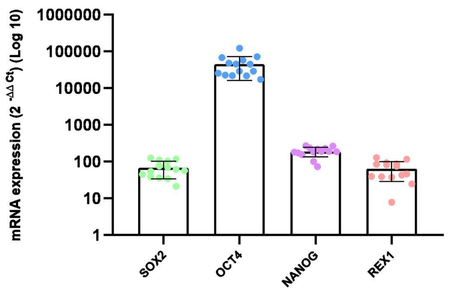
Typical morphology of iPSCs colonies.



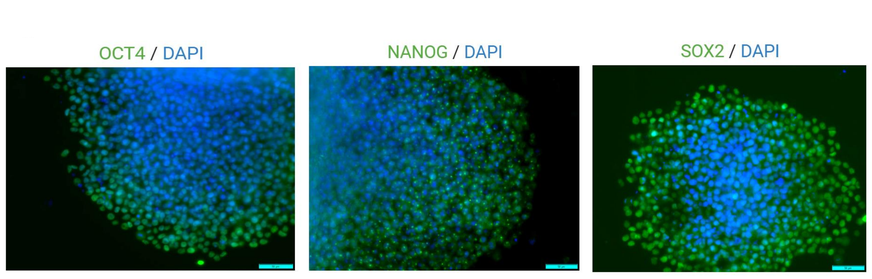
2. Molecular characterisation

Expression of pluripotency markers by reprogrammed cells.

→ qPCR



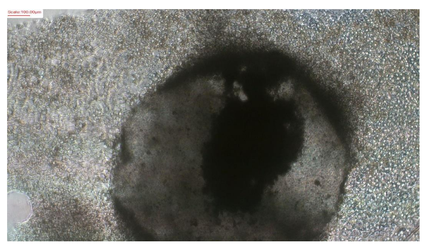
→ Immunofluorescence



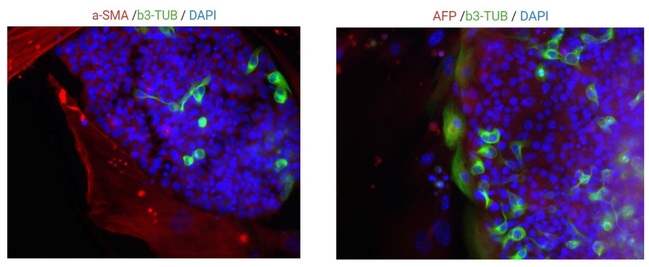
3. Functional characterisation

In vitro differentiation potential.

→ Ability to form EBs



→ Ability of the EBs to differentiate into cells of the three germ layers.



Immunofluorescence: markers of mesoderm (a-SMA), ectoderm (b3-tub) and endoderm (AFP).

Abbreviations: iPSC: induced pluripotent stem cell; MSC: mesenchymal stromal cell; MOI: multiplicity of infection; CB: umbilical cord blood; iMEF: inactivated mouse Embryonic fibroblasts; GSK3b: glycogen synthase kinase-3 beta; TGFβ: transforming growth factor-beta; KORS: knock-out replacement serum; LIF: leukaemia inhibitory factor; bFGF: basic fibroblast growth factor; AP: alkaline phosphatase; EBs: embryoid bodies ; a-SMA: smooth muscle alpha-actin; b3-tub: tubulin beta-III; AFP: alpha-fetoprotein.

CONCLUSIONS

Reprogramming system

- Retroviral reprogramming (single factors) → lower transduction, less control
- Lentiviral reprogramming (cassette) → higher transduction, more control

Culture conditions

- Knock-out media (LIF + bFGF) + feeder cells → works better for equine iPSCs
- Inhibitor cocktail did not enhance reprogramming and decreased proliferation

However, neither the reprogramming system nor the culture conditions seemed to be the most important → **Origin of cells**

- Clear superiority of **embryo-derived cells** over perinatal and adult cells
  → Endogenous expression of pluripotent factors can enhance reprogramming
  → In spite of presenting the lowest transduction efficiency *(intrinsic resistance to virus infection?)*

Good characterisation

- Celular
- Molecular
- Functional

Reprogrammed cells are iPSCs.

This work illustrates the significant challenges associated with the generation of iPSCs in veterinary species
Understanding pluripotency networks in animals is key to provide appropriate conditions for reprogramming

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