



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Il cane quale modello di studio della terapia con cellule stromali mesenchimali, vescicole extracellulari e secretoma per l'ostruzione/ipoplasia delle arterie polmonari in età pediatrica

The dog as a study model of mesenchymal stromal cell, extracellular vesicle and secretome therapy for pediatric pulmonary artery obstruction/hypoplasia

Angelita Capone, 1st year

XXXIX Cycle (A.A. 23-24)

Curriculum: Scienze cliniche

Posizione: PNRR ex DM 118/23

Supervisor: Prof. Iacono E.

Co-Supervisors: Prof. Giardino L., Prof. Merlo B.

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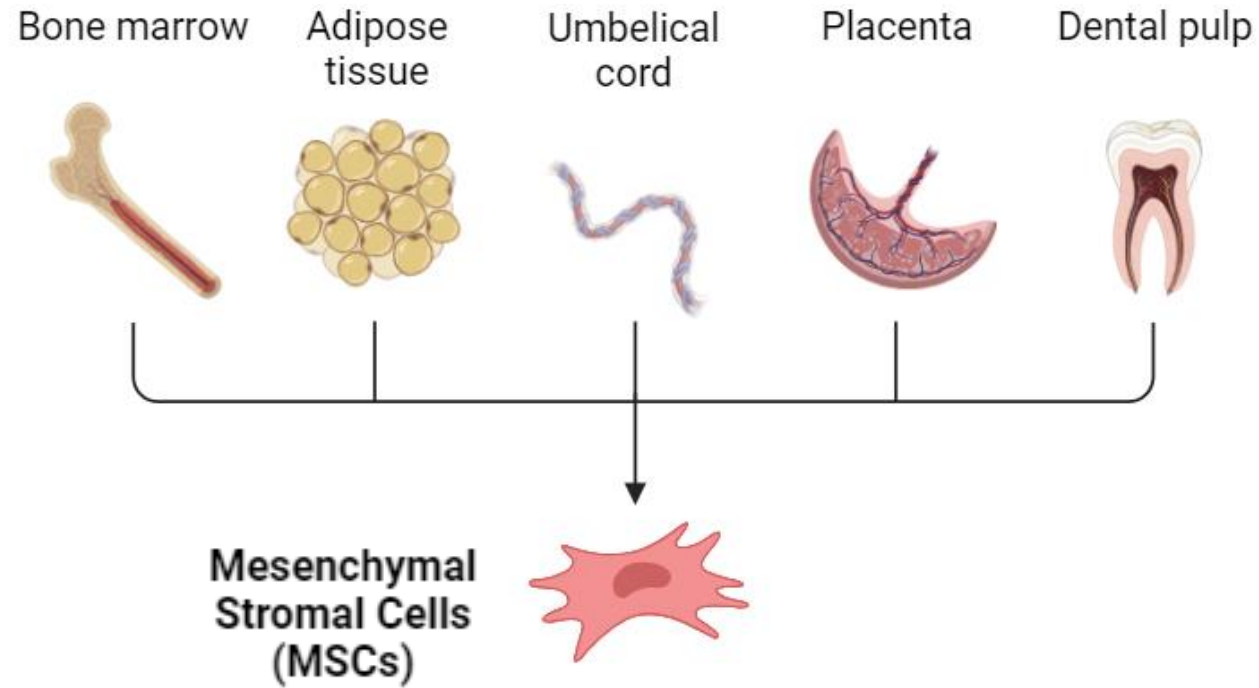
TECNOPOLO
BOLOGNA ~ OZZANO
EMILIA-ROMAGNA 



DIPARTIMENTO DI SCIENZE MEDICHE VETERINARIE - DIMEVET
LABORATORIO DI RIPRODUZIONE E BIOTECNOLOGIE ANIMALI - LRBA



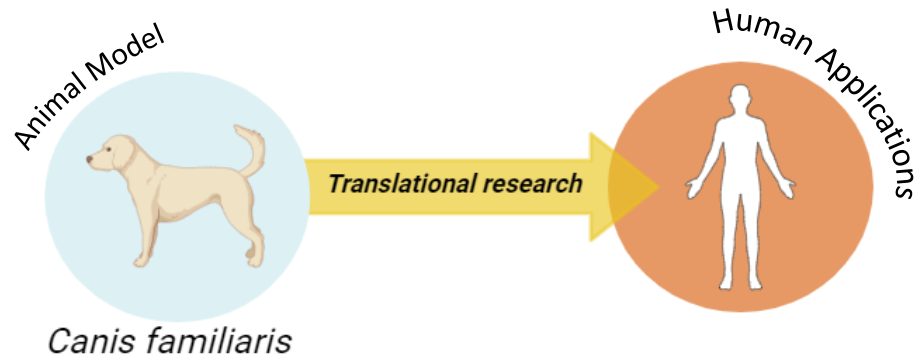
Introduction



Multiple differentiation abilities

Low immunogenicity

Angiogenic and antiapoptotic properties



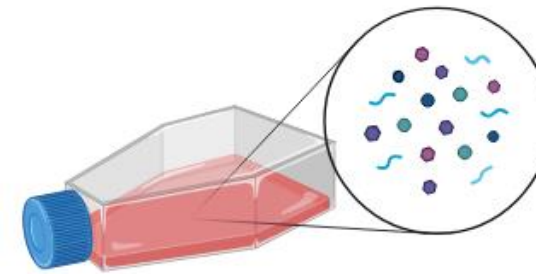
Aim of the project

Investigate the pro-angiogenic properties of **canine MSCs**

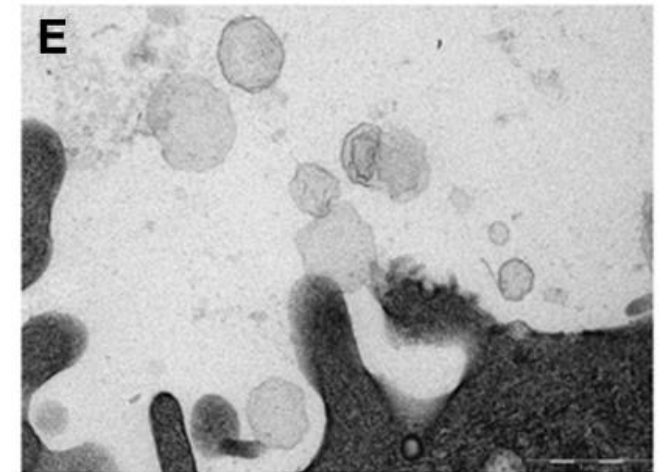
- Fetal and adult **MSCs**
 - Secretome and EVs

for their potential use in **lung vascular bed regeneration**

- Physiological conditions
 - Hypoxic conditions
(0% O₂, 95% N₂, 5% CO₂)



- Cytokine
- Lipid mediators
- Growth factors
- Hormones
- Exosomes
- Microvesicles

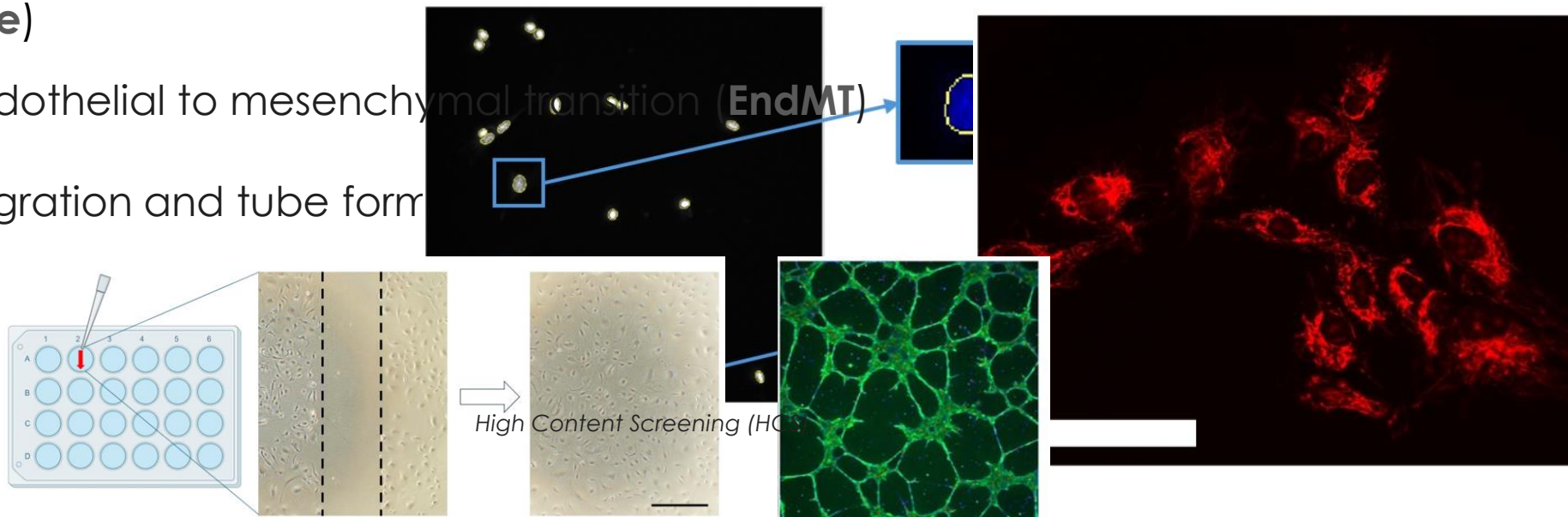


Iacono, E. et al. (2018) *Veterinary research communications*



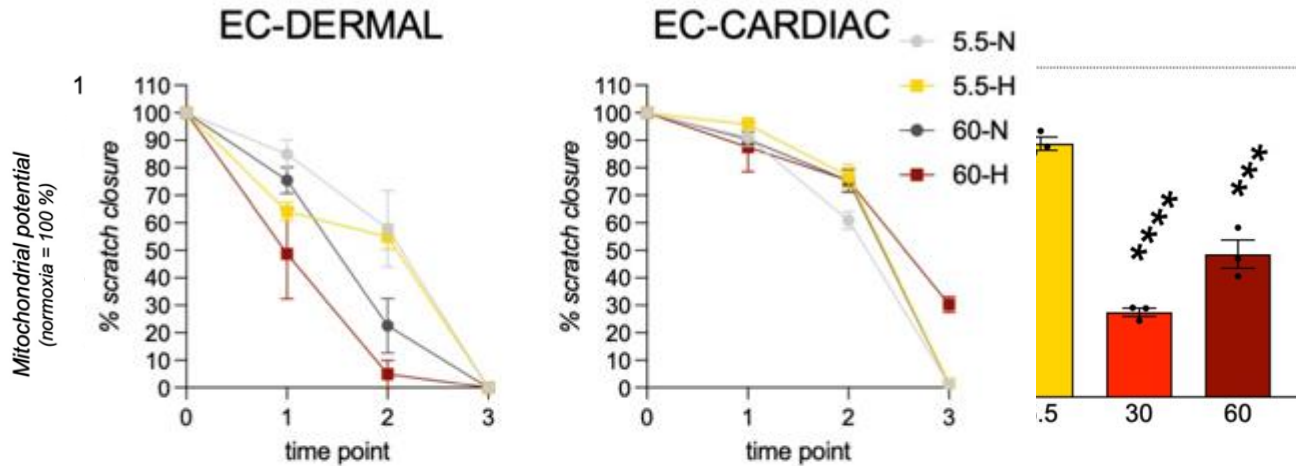
Supporting experiments

- Set a **cellular platform** using **cardiac and dermal endothelial cells (Ecs)**
- Define experimental conditions (Hyperglycemia and **Hypoxia**) and vulnerability
 - Cell viability (**MTT assay and pycnotic nuclei analysis**)
 - Mitochondrial function (**MitoTracker Orange vital dye**)
 - Endothelial to mesenchymal transition (**EndMT**)
 - Migration and tube formation





Development of a cellular platform to identify biomarkers of risk and neuroischemic complications in diabetes

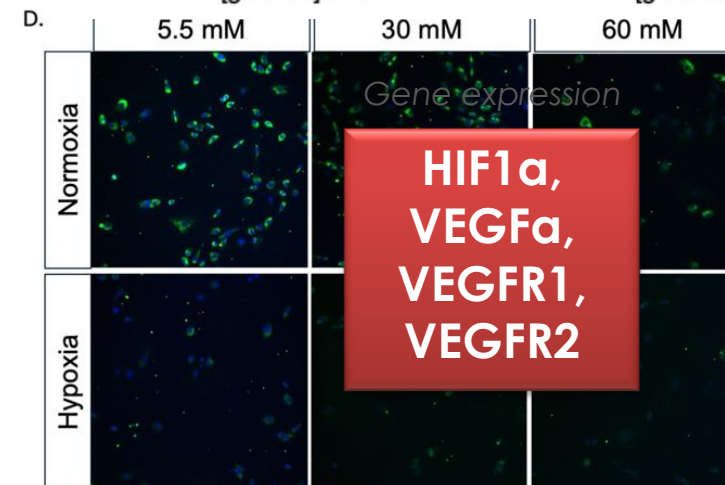
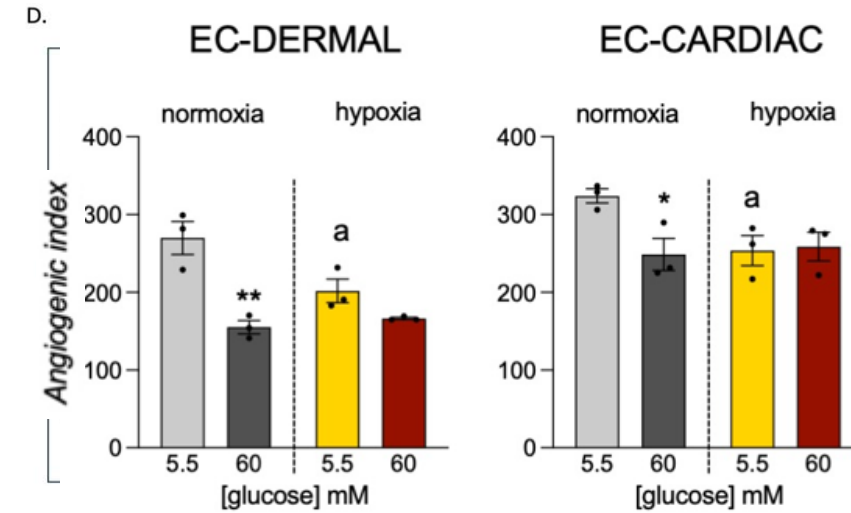
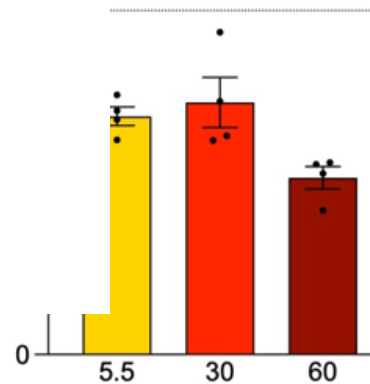
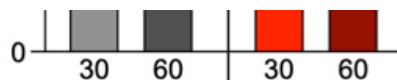


**** time (t)
n.s. oxygen (o)
* [D-glu] (g)

n.s. t x g
* t x o
n.s. g x o
n.s. t x o x g

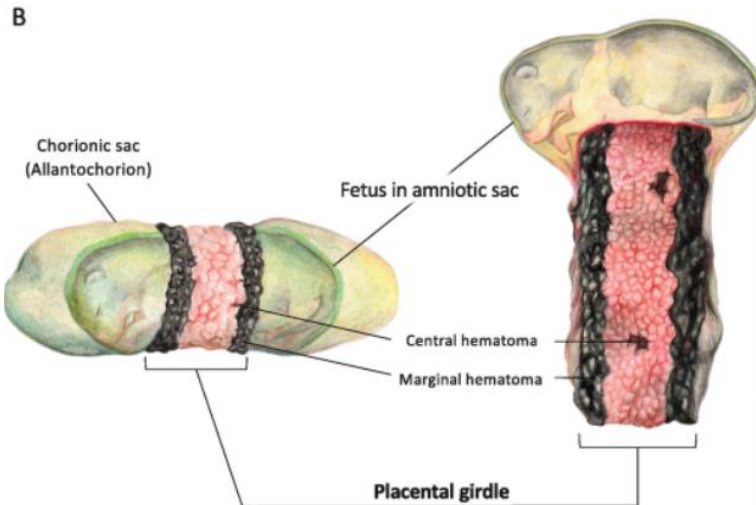
**** time (t)
* oxygen (o)
n.s. [D-glu] (g)

** t x g
* t x o
n.s. g x o
** t x o x g



Main steps

- Literature review
 - Collection of canine biological samples & MSCs isolation
 - Characterization of isolated MSCs
 - Evaluation of the pro-angiogenic potential
 - Development of an in vitro co-culture system of canine MSCs and endothelial cells (ECs) and culture of ECs in conditioned medium
 - Evaluation of the translational potential of the canine *in vitro* model

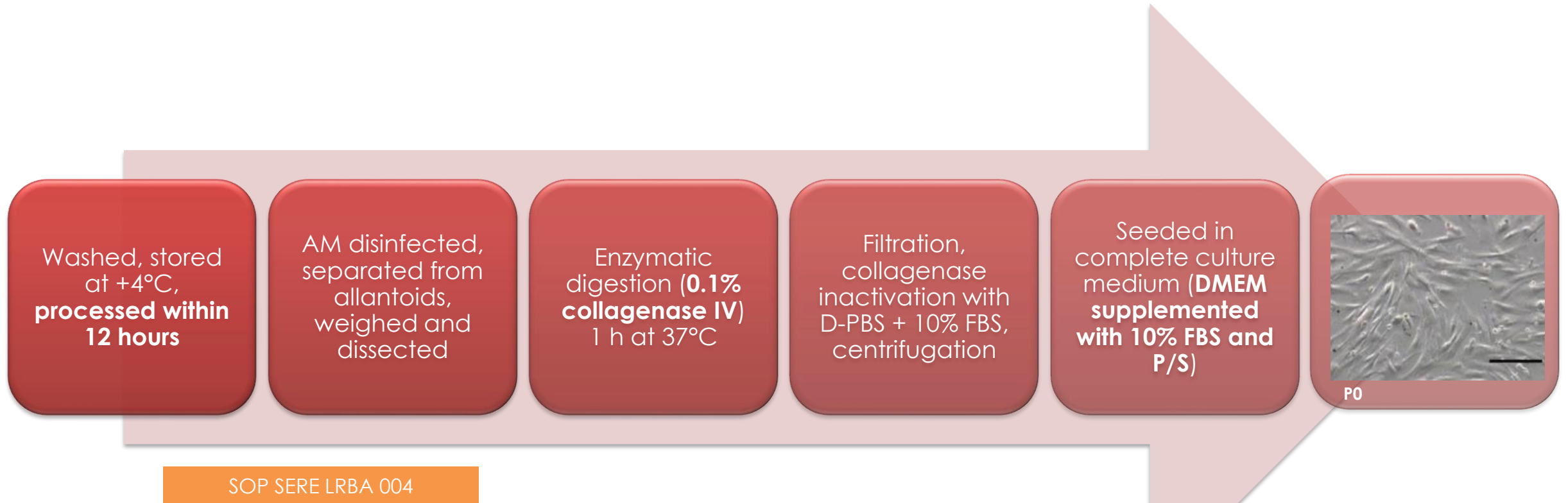


Umbilical Cord (UC)



Amniotic membrane (AM)

Methods – Canine AM-MSCs isolation



Parallel Research Activities (I)



Isolation and characterization of equine MSCs derived from colostrum

PAPER



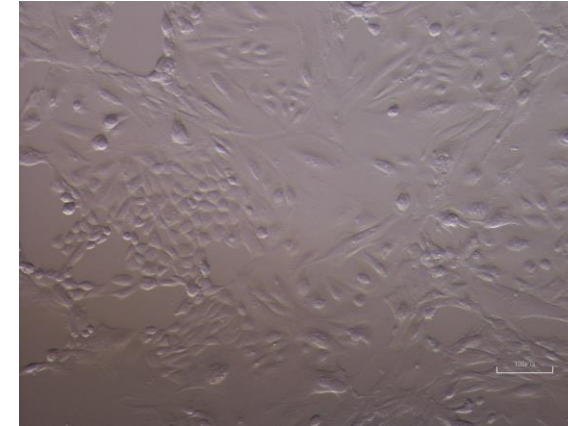
in progress



CD90
CD73

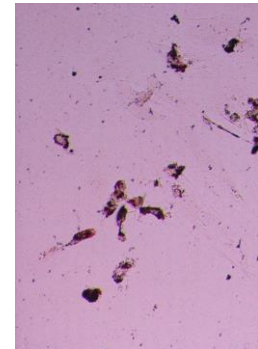


CD34
CD45
MHCII

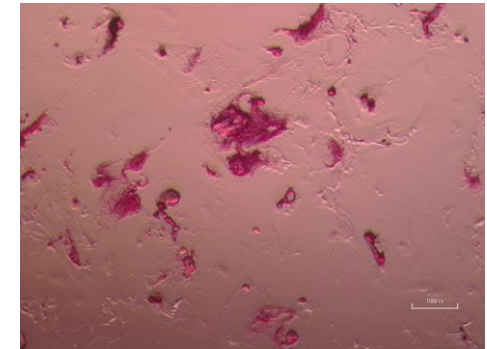


MSCs spheroid

Adipogenic



Osteogenic

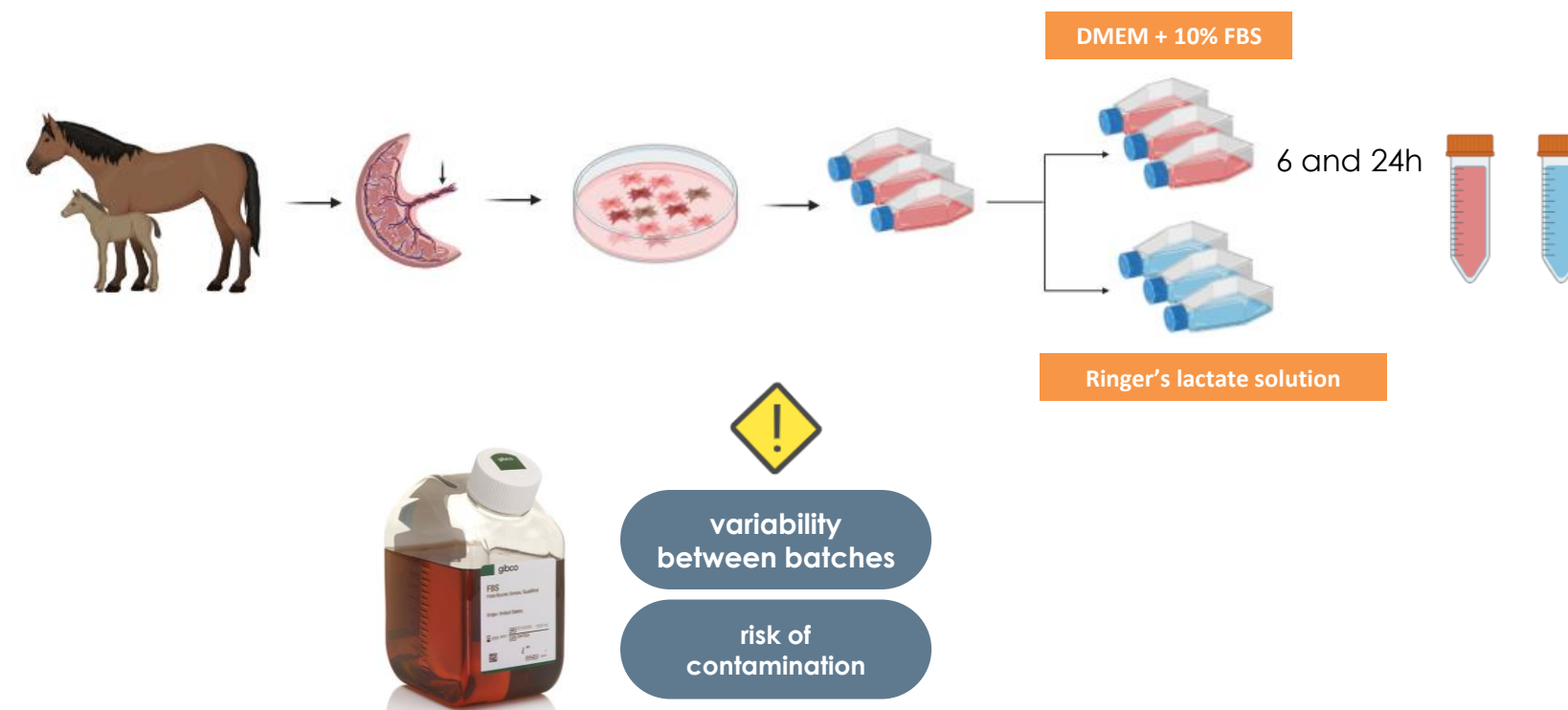


- Adherence to plastic in standard culture condition
- Robust proliferative capacity (***Doubling time assay***) and self-renewal capacity (***Colony Forming Unit assay***)
- Surface marker expression
- Tri-lineage *in vitro* differentiation
- Migration and adhesion capacity (***scratch test, spheroid formation assay***)

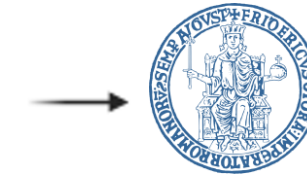
Parallel Research Activities (II)



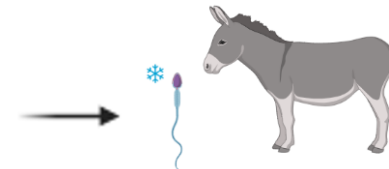
Conditioned medium production from Equine Wharton's jelly-derived MSCs



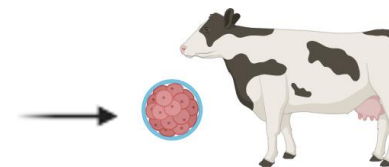
Characterization



Equine mares suffering from **post-insemination endometritis**

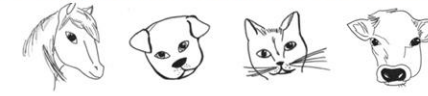


Donkey semen cryopreservation

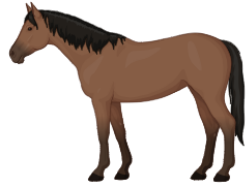


In vitro production of **bovine embryos**

Parallel Research Activities (III)



Evaluation of an **essential oil-based phytotherapy** on **endometrial epithelial cells** for the treatment of **post-partum endometritis** in mares



0.25% Trypsin-EDTA



DMEM F12 + **2.5 % FBS** + Anti-Anti 1x + **EGF**

DMEM F12 + **10 % FBS** + Anti-Anti 1x

0.1% collagenase type I solution in DPBS

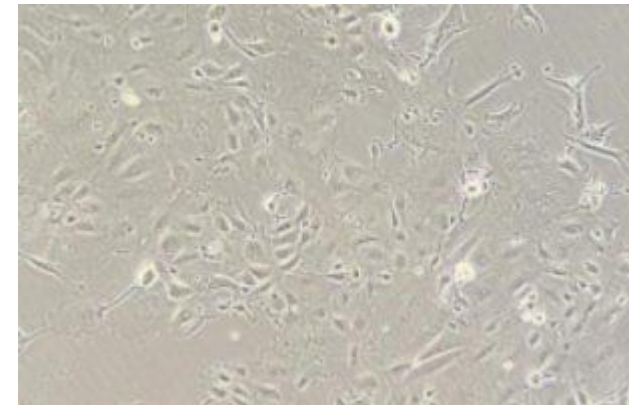


DMEM F12 + **2.5 % FBS** + Anti-Anti 1x + **EGF**

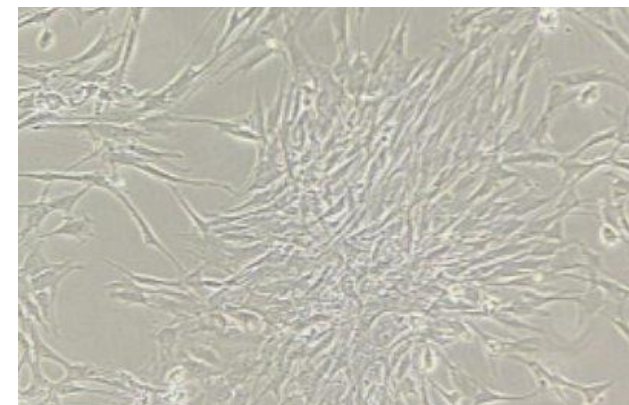
DMEM F12 + **10 % FBS** + Anti-Anti 1x

- *Calendula officinalis*
- *Harpagophytum procumbens*
- *Echinacea angustifolia*

- Cell viability (**MTT test**)
- Apoptosis
- MMP
- ROS production



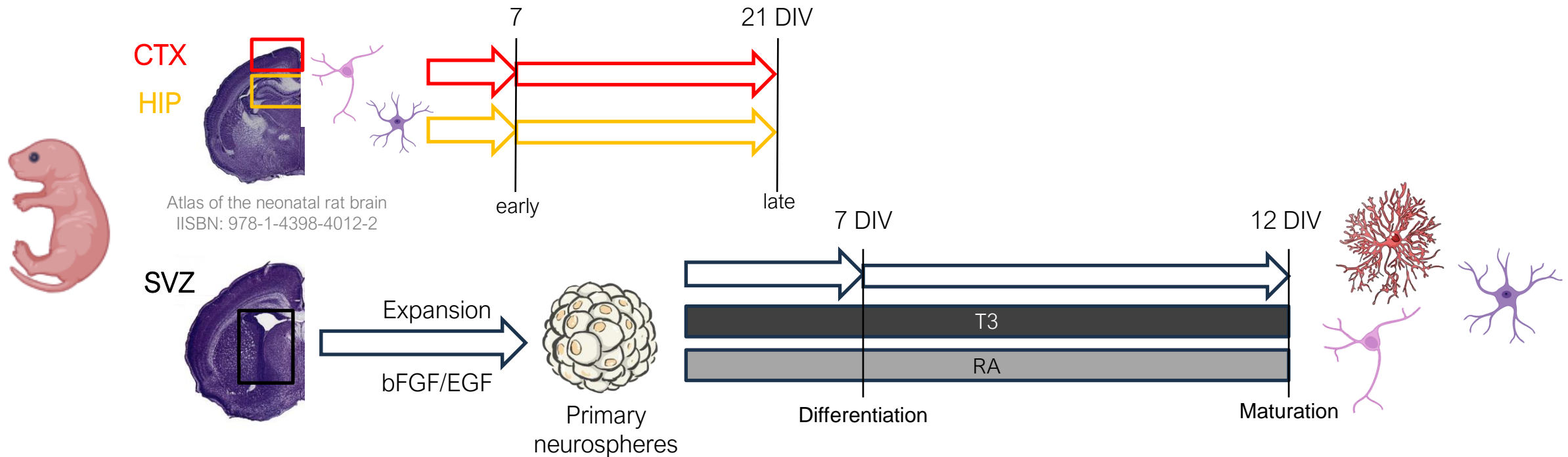
Endometrial Epithelial cells (EECs)



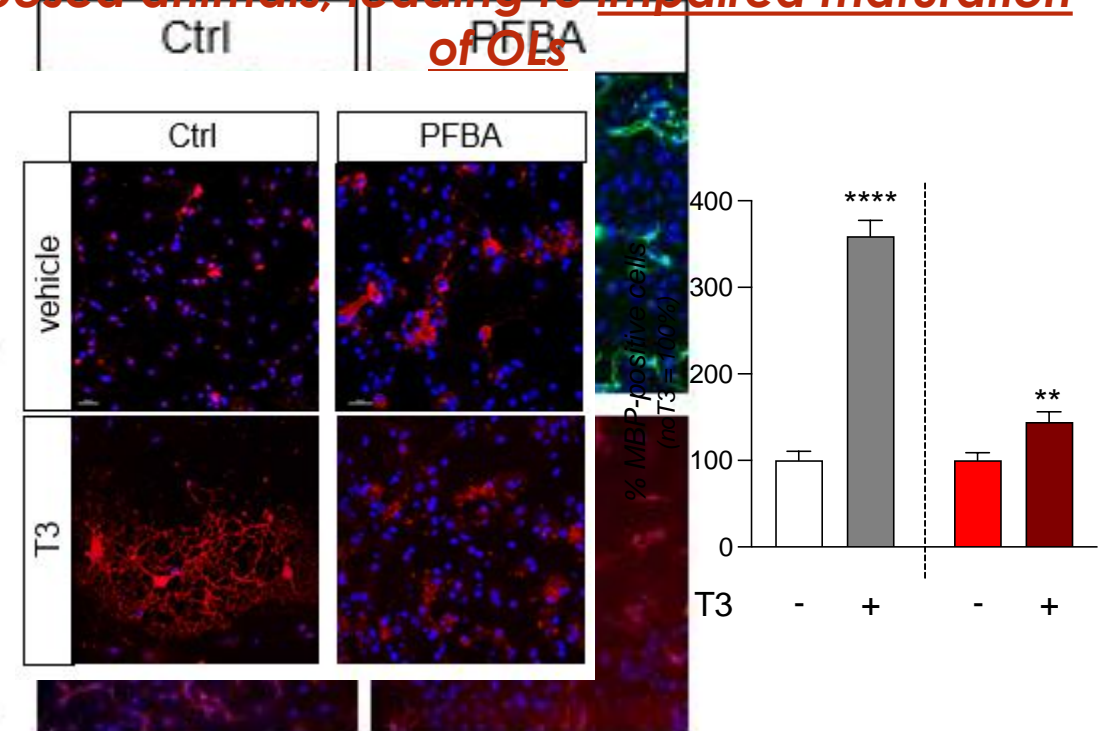
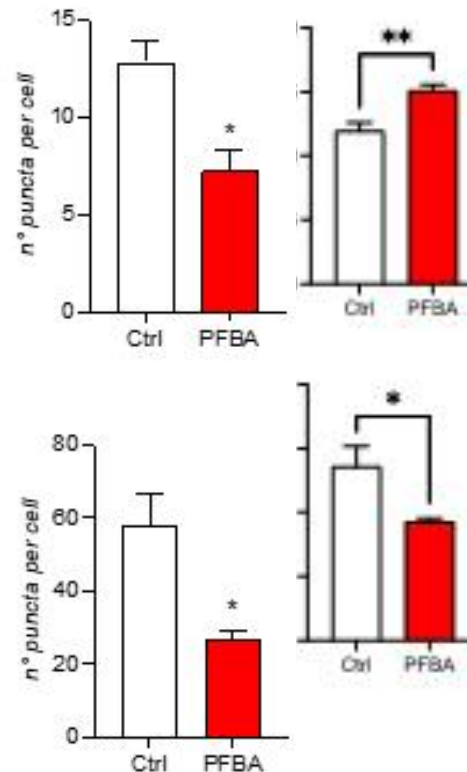
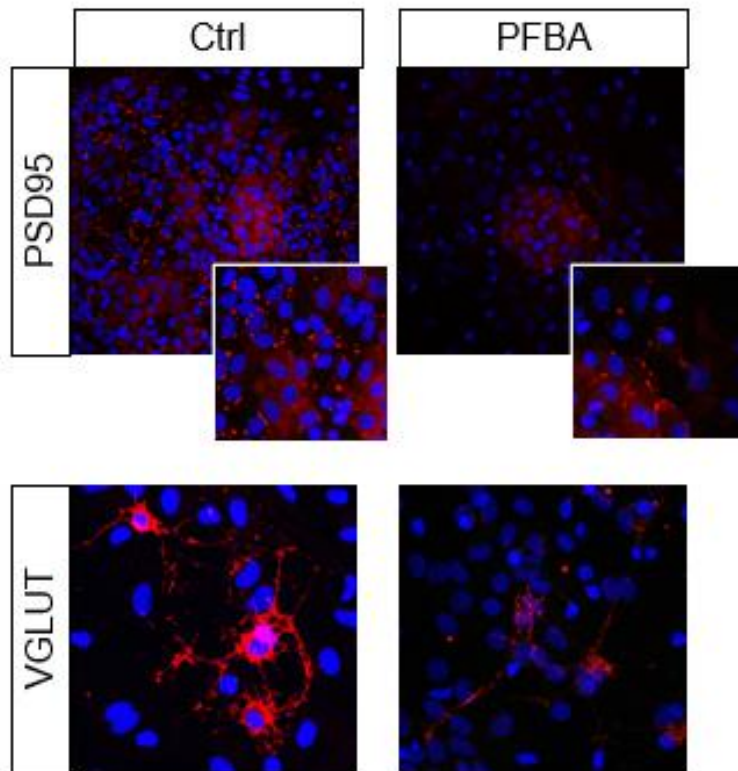
Endometrial Stromal cells (ESCs)

Parallel Research Activities (IV)

Assess the pre-natal and neonatal interference of **short-chain PFASs** exposure with the action of thyroid hormones and Central Nervous System (CNS) development



PFBA exposure reduces the hippocampal neuron maturation in terms of synaptic markers expression
PFBA exposure reduces the hippocampal neuron maturation in terms of synaptic markers expression
neurotrophin is blocked in OPCs derived from PFBA-exposed animals, leading to impaired maturation of OEs



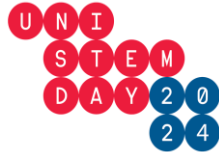
→ endocrine disruptor chemical
(thyroid disruptor)

Learning activities and lectures

- Transversal skills



- Speaker at **UniStem Day**



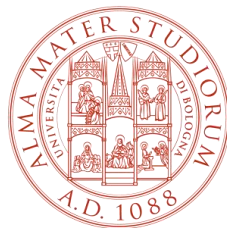
- Training of **Animal Biotechnologies internship students** and **PCTO students**
- 3 hour-lecture both *face-to-face* and *hands-on* for **South African exchange students** from the

IVSA Association



Next Steps...

- **Characterization tests** on isolated canine MSCs (*in vitro* differentiation tests and molecular marker analysis via PCR) and **functional assays** (*scratch assay, spheroid formation, and tube formation assay*)
 - Qualitative and quantitative **analysis of the secretome** and **EVs** (Multiplex xMAP, Western Blot) and development of ***in vitro* co-culture systems** between **MSCs** and **ECs**, as well as EC cultures in **conditioned medium**, to assess the regenerative potential of MSCs
 - **Publication of results** obtained during the first year of research
 - **GISM Annual meeting** (Perugia, Maggio 2025), **TREMIS** (Friburgo, Maggio 2025)
 - Abroad research period at the **Regenerative Medicine Institute**,



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