



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

# Canine Adult and Fetal Adnexa-Derived MSCs and Their Secretome: A Veterinary and Translational Perspective toward Pulmonary Vascular Regeneration

**Angelita Capone, 2<sup>nd</sup> year**

XXXIX Cycle (A.A. 24-25)

**Curriculum:** Clinical Sciences

**PhD Position:** NRRP ex DM 118/23

**Supervisor:** Prof. Iacono E.

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

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

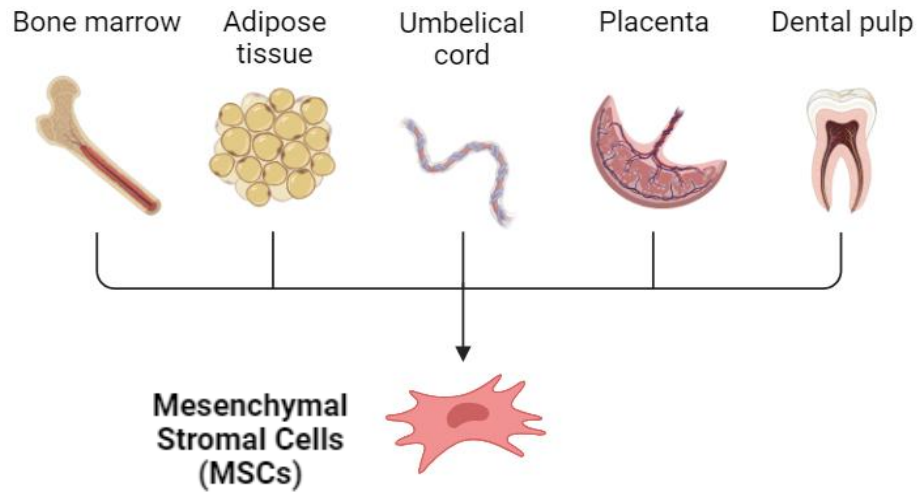


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# Background and Rationale



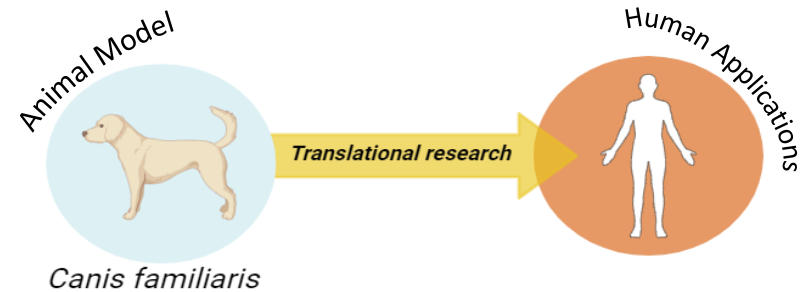
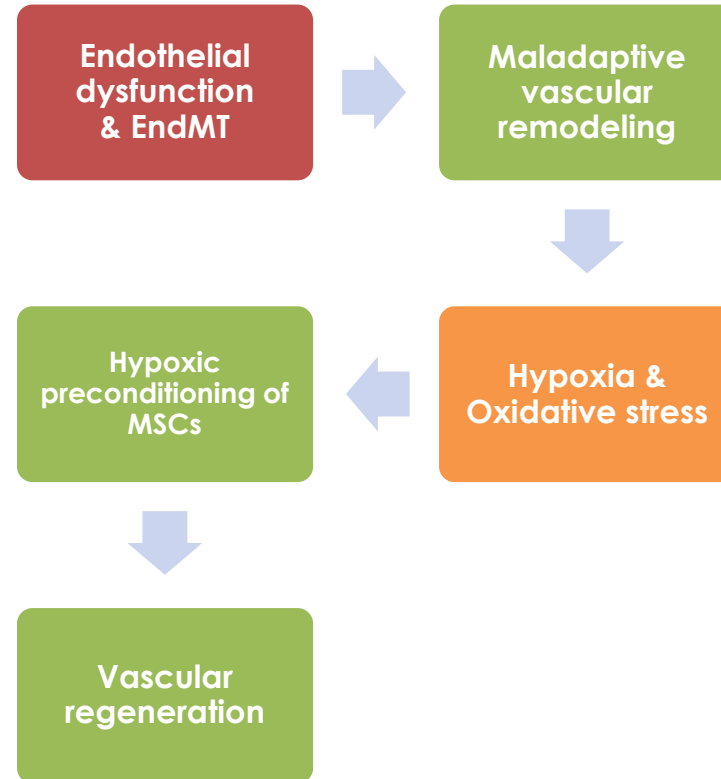
Multiple differentiation capacity

*osteocytes, chondrocytes & adipocytes*

Low immunogenic profile

Secretome enriched in:

*Immunomodulatory, pro-angiogenic, and anti-apoptotic molecules*

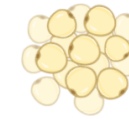


# PhD Project – Main Objectives

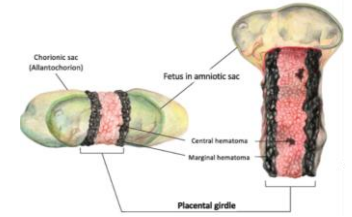
## 1. Isolation and characterization of Canine Adult and Fetal Adnexa-MSCs

- PDT analysis, CFU assays, Spheroid Formation, Wound Healing assay (Scratch assay), tri-lineage differentiation potential, surface marker expression (RT-PCR)

Adipose Tissue (AT)



Umbilical Cord (UC)



Amniotic Membrane (MA)

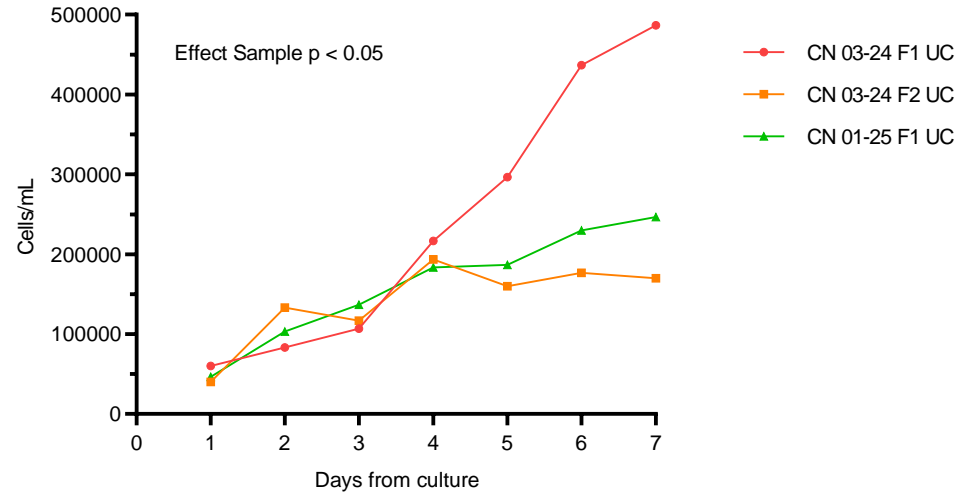
## 2. Evaluation of pro-angiogenic potential of cells and CM (Normoxia vs Hypoxia 5% & 0% O<sub>2</sub>)

- MTT assay, H<sub>2</sub>DCFDA staining, JC-1 staining, Tube Formation assay
- Co-culture system with canine pulmonary ECs

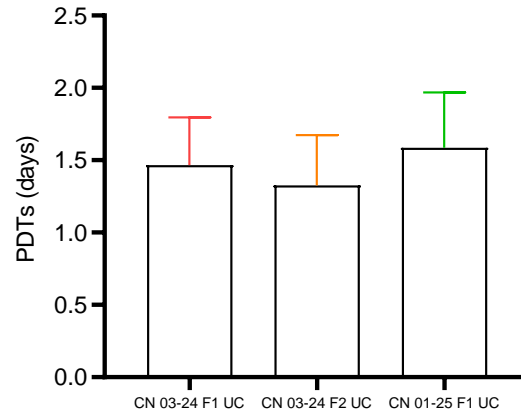
## 3. Characterization of the Canine MSCs-Secretome & Potency assays

# Preliminary Results (I/III)

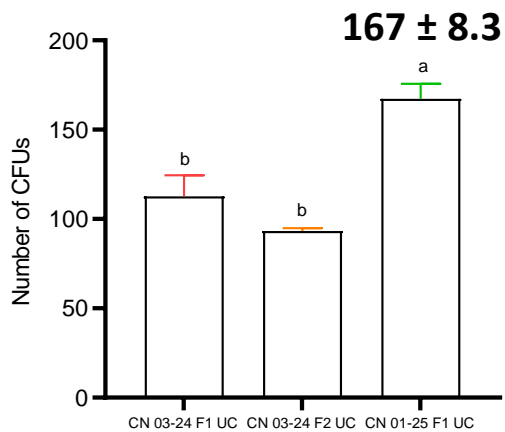
**Growth curves**



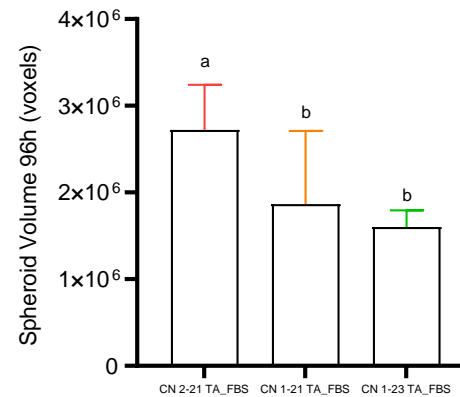
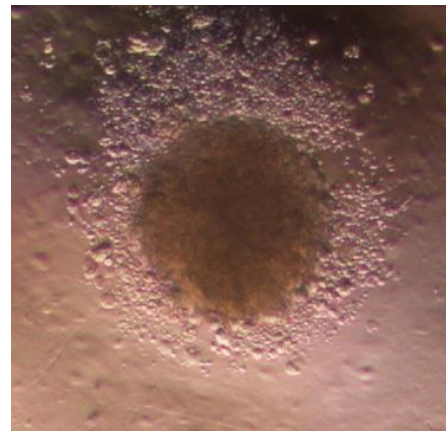
Two-way ANOVA: Days from culture x Sample



Housekeeping	GAPDH	+
MSC markers	CD90, CD44	+
Hematopoietic markers	CD34, CD45	-
MHC II markers	DLA-DRA1,	-
	DLA-DQA1	-; +/-



One-way ANOVA with Tukey's post-hoc test (letters)



Spheroid volume was reconstructed and quantified using dedicated 3D image analysis software (ReViSP)

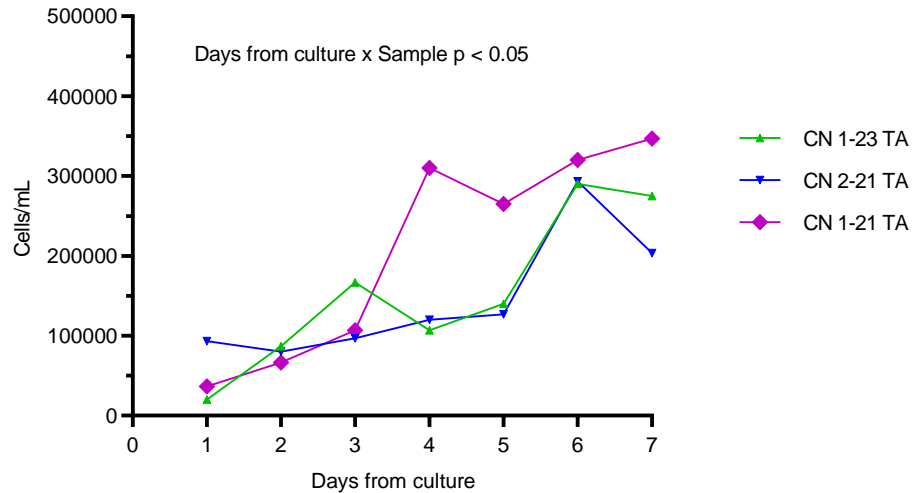
**High migratory capacity:**  
 Canine UC-MSCs achieved complete wound closure (100%) within 24 hours

**Tri-lineage differentiation potential**  
 (adipogenic, chondrogenic & osteogenic)

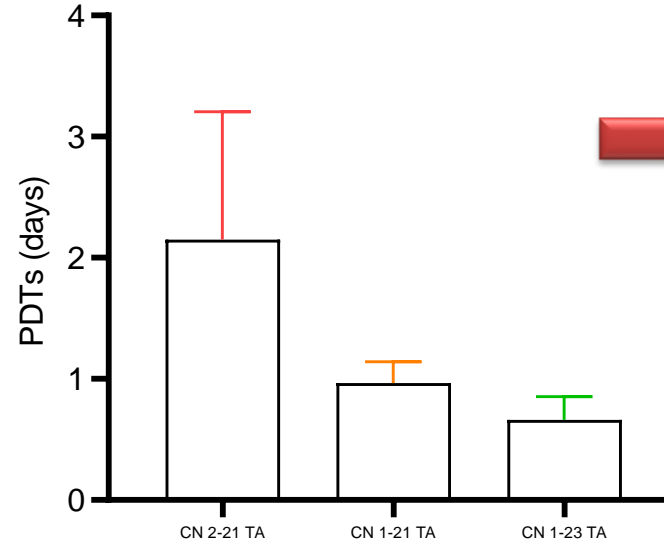


# Preliminary Results (II/III)

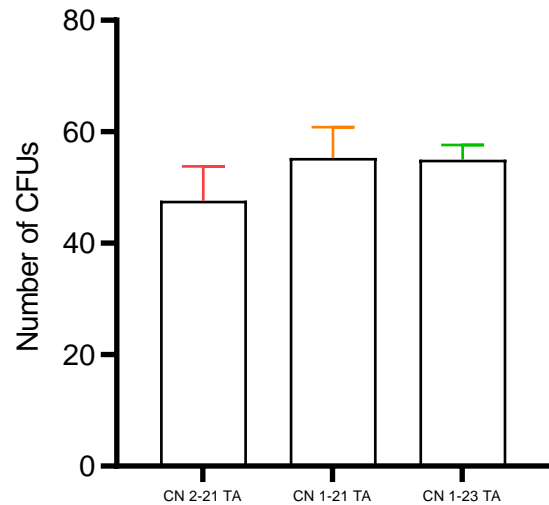
**Growth curves**



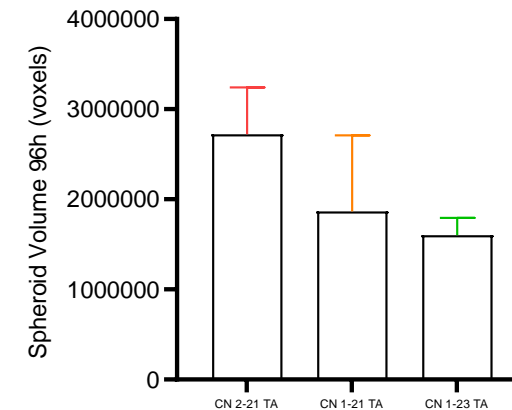
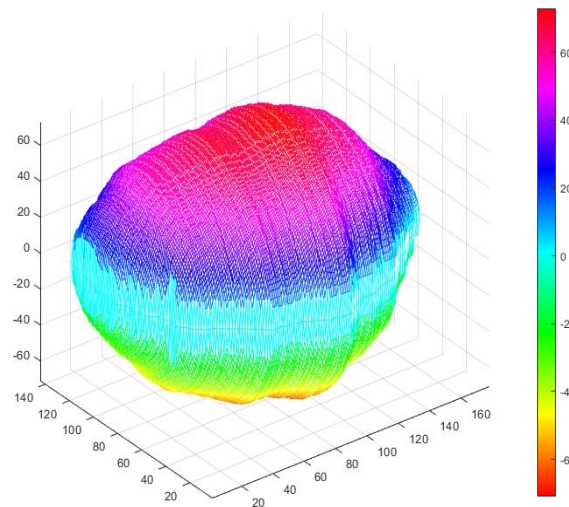
Two-way ANOVA: Days from culture x Sample



**High migratory capacity:**  
 Canine AT-MSCs achieved complete wound closure (100%) within 24 hours



One-way ANOVA with Tukey's post-hoc test (letters)



Spheroid volume was reconstructed and quantified using dedicated 3D image analysis software (ReViSP)

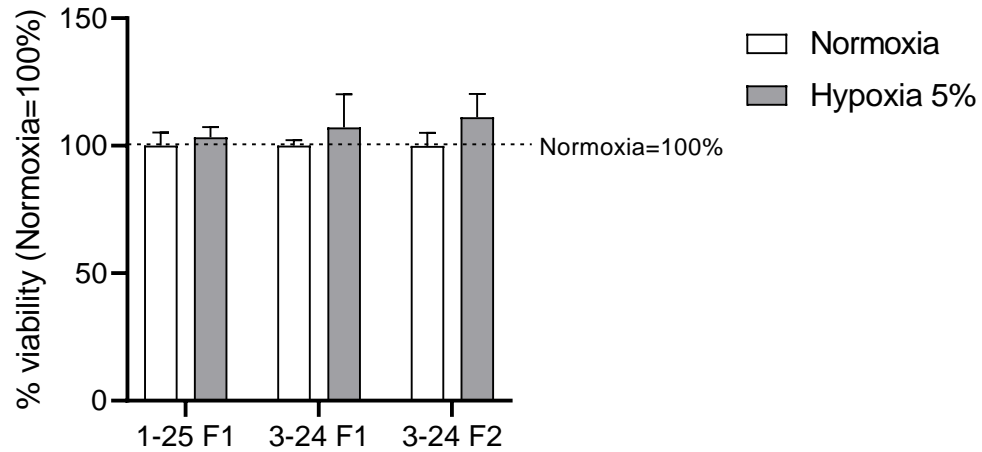


# Preliminary Results (III/III)

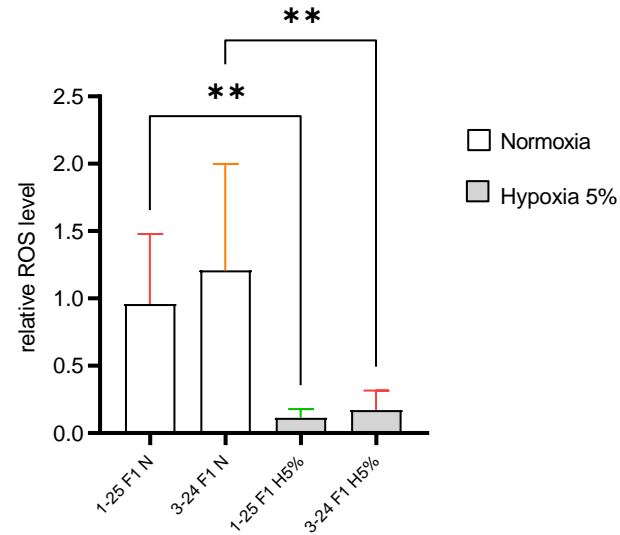
Canine UC-MSCs

5% O<sub>2</sub>

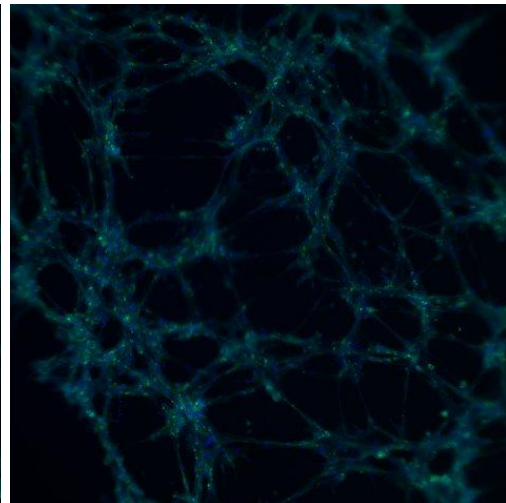
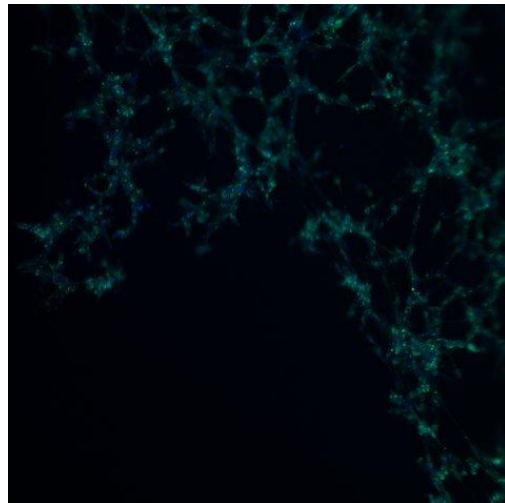
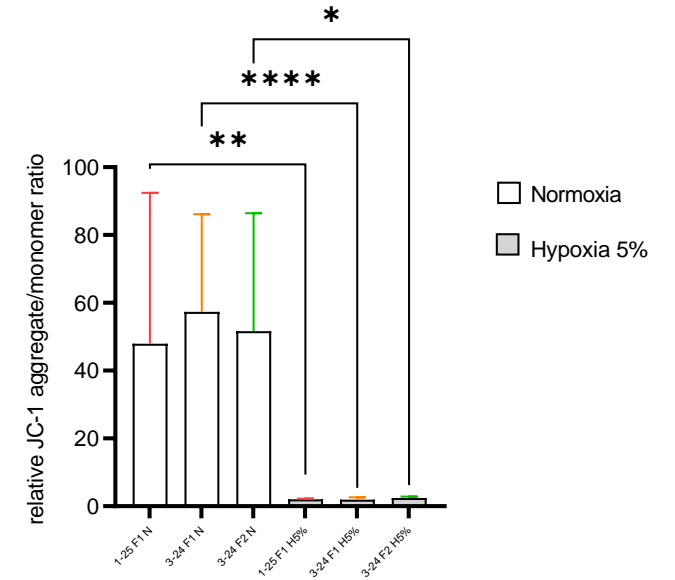
MTT\_24h



H<sub>2</sub>DCFDA Staining\_24h



JC-1 Staining\_6h



Tracing marker:  
Phalloidin

**Angiogenic Index** (% of the image area covered by connected tubes): from **40.33** (Normoxia) to **88** (Hypoxia %5 O<sub>2</sub>)

# PhD Project – Main Objectives



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## 1. Angiogenic, Immunomodulatory and inflammatory profiling

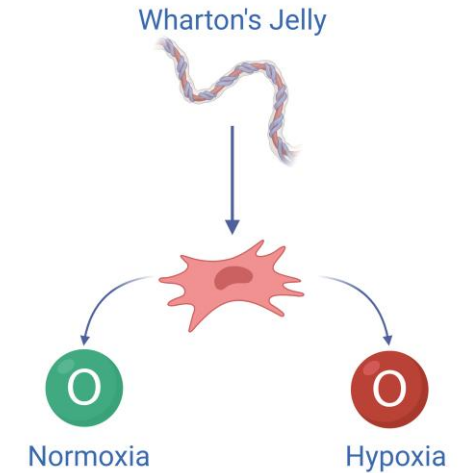
- VEGF-A, PDGF-C, FGF2, FGFR-1, Ang-1, HIF-1 $\alpha$ , IL-1 $\beta$ , IL-6, TGF- $\beta$ , PGE2, TNF- $\alpha$ , COX-2, IDO
- ELISA on CM and qPCR (cMSCs)

## 2. Lipidomic profiling (DESI-MS)

- Different MSCs **sources** (AT vs UC),
- Different **culture conditions** (20% FBS vs Serum Replacement) and **oxygen tension** (Normoxia vs Hypoxia)
- DH82+cMSCs-**Secretome**

## 3. Functional Potency assays

- T Cell assay
- Macrophage polarization Assay



Surface Marker  
Characterisation



Lipidomics  
Analysis



Macrophage  
Polarisation Assay



T Cell  
Assay

# Parallel Research Activities

Article

## Equine Colostrum-Derived Mesenchymal Stromal Cells: A Potential Resource for Veterinary Regenerative Medicine

Angelita Capone <sup>1,2</sup>, Barbara Merlo <sup>1,3</sup>, Fabiana Begni <sup>1</sup> and Eleonora Iacono <sup>1,3,\*</sup>



### In vitro evaluation of an essential oil-based phytotherapy on endometrial epithelial cells for the treatment of post-partum endometritis in mares

- Thyme, oregano, cinnamon, clove, devil's claw

### Conditioned medium production from Equine Wharton's jelly-derived MSCs to improve bovine oocyte in vitro maturation (IVM)

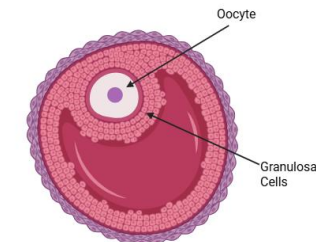
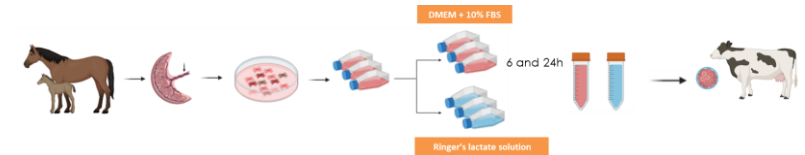
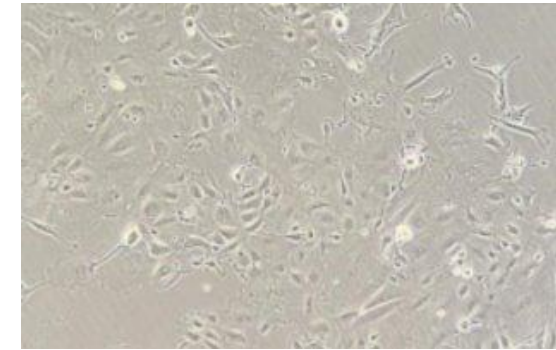
- Attolini, E., Capone, A., Geirola, M., Iacono, E., & Merlo, B. Equine mesenchymal stromal/stem cells secretome enhance nuclear maturation of bovine oocytes [Oral Presentation]. **78° Convegno SISVET**, Giardini Naxos, Italia (10-12 Giugno 2025).

### Isolation and culture of granulosa cells from bovine oocytes, studying the impact of heat stress on reproductive function

- Zambelli, D., Ballotta, G., Cunto, M., Gugole, P. M., Merlo, B., **Capone, A.**, & Iacono, E. Urethral Reconstruction Using a Preputial Mucosa Free Flap and Autologous Mesenchymal Stromal Cells (Mscs) in a Male Alpaca: A Case Report. Available at SSRN 5244906. **Under Revision**
- **Capone A.**, Merlo B., Lanci A., Gugole P.M., Iacono E. Isolation, expansion and characterization of mesenchymal stromal cells from fresh and cryopreserved umbilical cord tissues in horse and dog. **Manuscript in preparation**



Endometrial Epithelial cells (EECs)



## Learning activities, congresses and lectures

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- Transversal skills
- Training of **Animal Biotechnologies internship students** and **PCTO students**
- **Teaching tutor (40h)** for laboratory classes in Animal Biotechnology Degree course
- 6 hour-lecture both *face-to-face* and *hands-on* for **Cornell and Cyprus exchange students** from the **IVSA Association**
- **Annual Meeting of the Italian Mesenchymal Stem Cell Group** in Perugia (May 8–9, 2025) – *Poster presentation*



### *Next Steps...*

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- **Characterization** of the Canine MSCs-**Secretome** & **Potency assays**
- Development of *in vitro* **co-culture systems** between **canine pulmonary ECs** and **MSCs/MSCs-Secretome**
- **Publication of results** obtained during the second year of research



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