Sex-specific primary cell-line of porcine umbilical vein endothelial cells

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Background and Aim

The **pig** is a very convenient animal for different pre-clinical applications. Their **physiological similarities to humans**, particularly in terms of **cardiovascular systems**, make them the ideal **models for various biomedical research studies** [1].

This similarity extends to processes such as **angiogenesis**, where pigs serve as effective models for studying vascular growth and development, critical for understanding and treating cardiovascular diseases [2]. Therefore, establishing **porcine umbilical vein endothelial cell (PUVECs)** lines is not just a need but a pressing requirement [3].

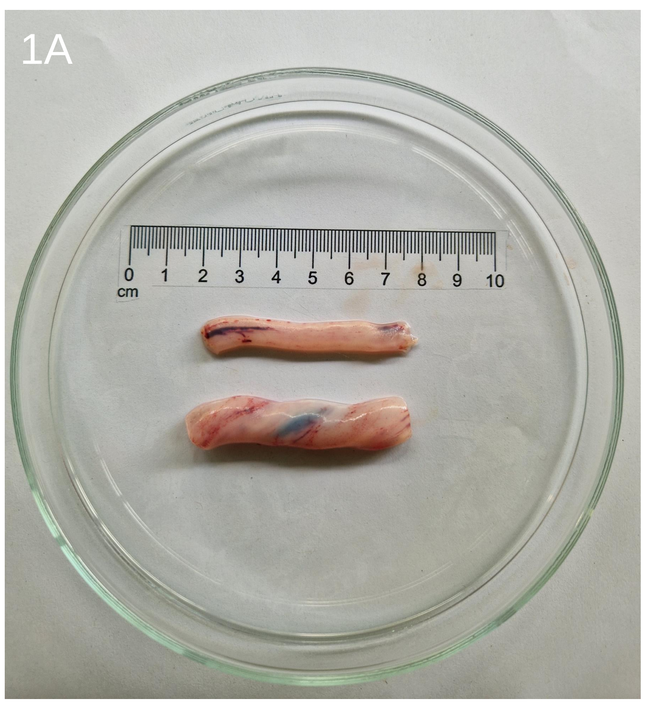
Sex as a biological variable has been absent from most *in vitro* work [4]. Reporting the sex of biological material is critical for transparency and reproducibility in science and should be consistently reported. Sex chromosomes in cells **can potentially affect protein expression and molecular signaling pathways** [5].

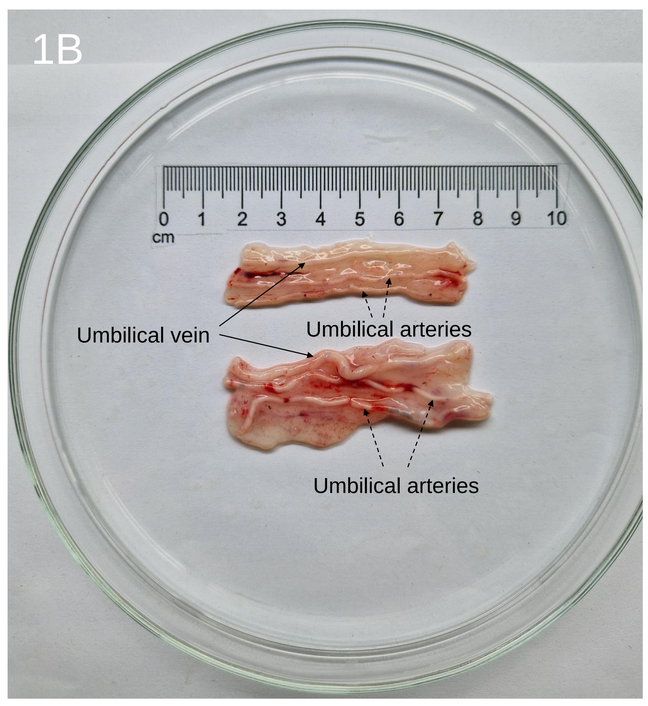
This study aims to set-up a PUVEC cell line with a discrimination between sexes in order to evaluate the angiogenesis capacity of both sexes.

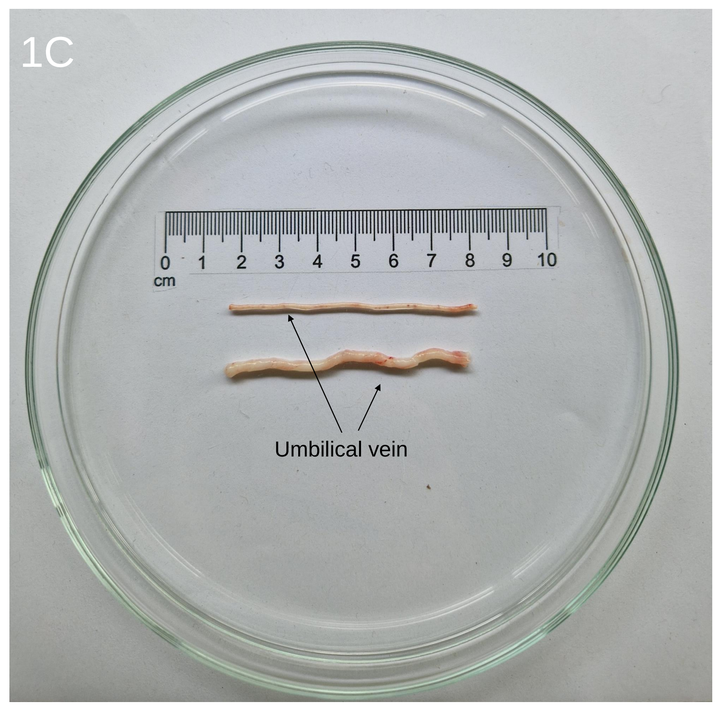
Materials and Methods

1. Umbilical cords (Fig. 1A) from both sexes were collected from pigs immediately after birth.

2. Within 6 hours after harvesting, umbilical cords were dissected (Fig. 1B), the umbilical vein (Fig. 1C) was identified and placed with the lumen towards the bottom of a 0.1% gelatine in DPBS coated 24-well plate.

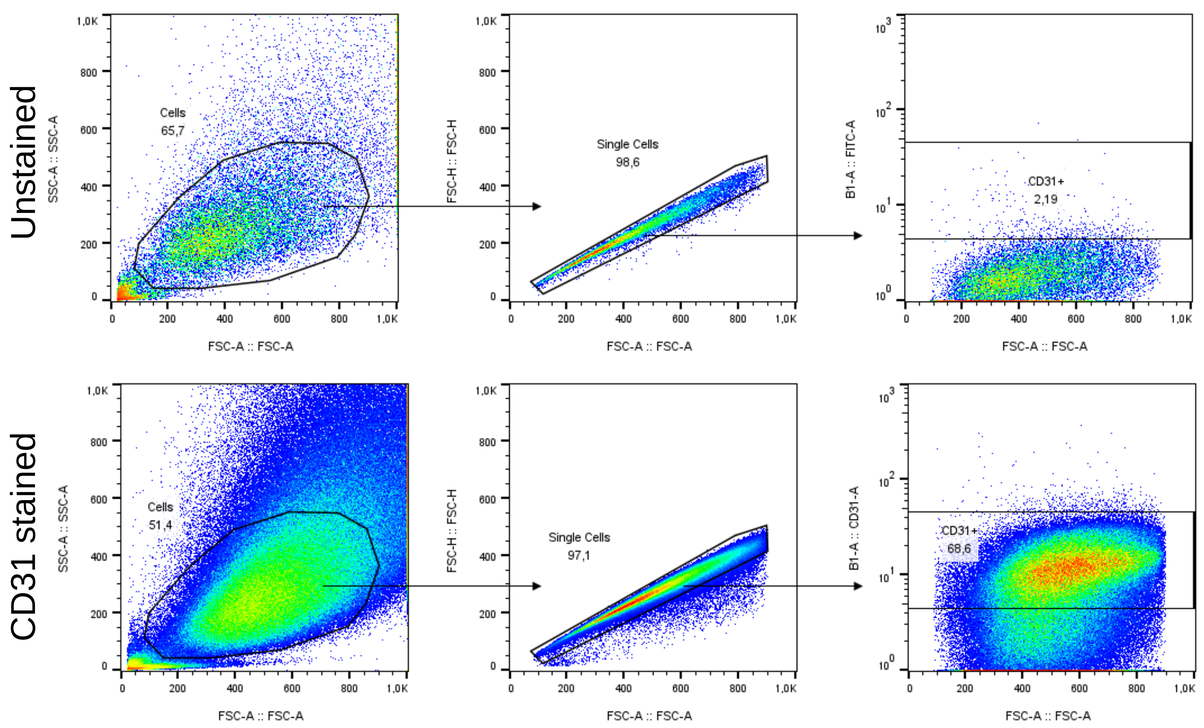


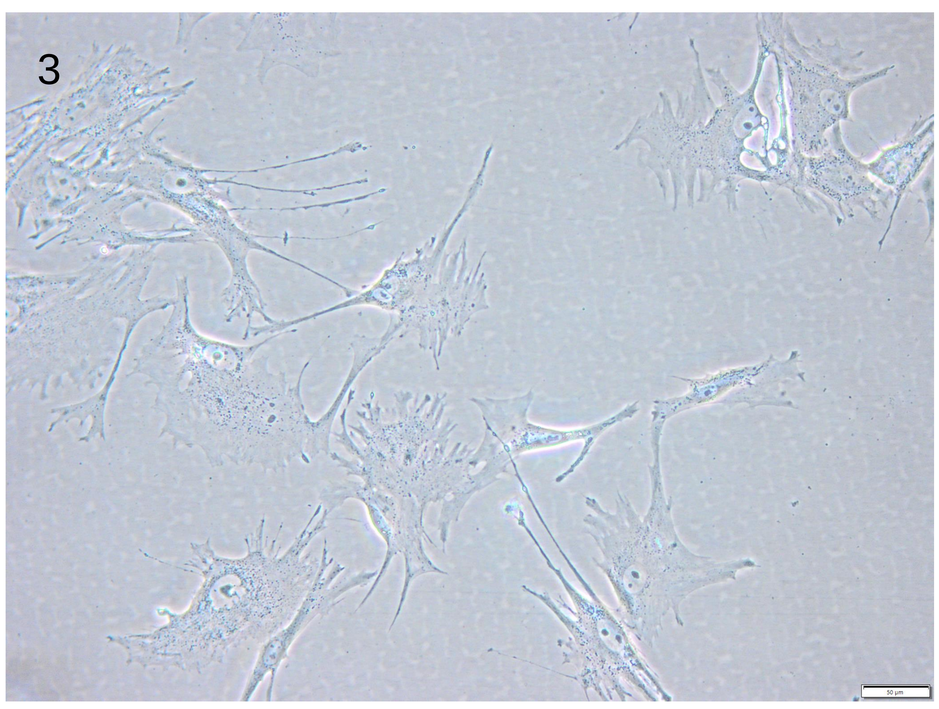




3. Cells migrated from the harvested tissue towards the 24-well plate while incubating at 38°C at 5% CO_2 in a M199/RPMI1640 (50/50) medium supplemented with 5% FBS, 1% L-glutamine, 1 mM Na-pyruvate, 1% Penicillin-streptomycin, 1% NEAA, 15 mM HEPES, 0.5% endothelial supplement.

4. When a monolayer of cells was grown, we selected PUVECs from this co-culture using CD31, an endothelial cell marker, via fluorescence-activated cell sorting with a FITC-CD31 monoclonal antibody (Fig. 2). After sorting, PUVECs were cultivated and evaluated for their typical morphology *in vitro* (Fig. 3).





Results and Conclusions

Collection and **cultivation of PUVECs** as a primary cell line from umbilical veins is **challenging** since cells grow **very slowly** (± 50 days). Furthermore, when a monolayer of cells is grown, a **co-culture** of different cells is created. Fluorescence-activated cell sorting (using CD31) showed that 53.7 ± 10.3% of the cells in culture were $CD31^+$ cells.

Future perspectives

The next step would be to perform a **tube formation assay** to assess the **angiogenesis capacity** of PUVECs from different sexes. These results will give us more **insight into possible sex differences in cardiovascular diseases.**

References

[1] Jia *et al*. (2024)
[2] Nowak-Sliwinska *et al*. (2018)
[3] Chrusciel *et al*. (2011)
[4] James *et al*. (2021)
[5] Vallabhajosyula *et al*. (2020)

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