University of Cyprus
Department of Biological Sciences

## Ph.D. Thesis Defense

# Student Presentation

**Wednesday, 10 June 2026 at 11:00**
**Building ΧΩΔ01 (CTF 01), Room 002, Panepistimioupoli Campus**

***This seminar is open to the public***


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## "New insights into the biology of trophoblast stem cells and trophoblastic influences on early embryo patterning: a cellular and molecular investigation."

This project investigated unknown aspects of the biology of mouse trophoblast stem (TS) cells and their *in vivo* analogue tissue, the extraembryonic ectoderm (ExE) trophoblast. ExE, which forms during the early post-implantation period at around embryonic day 5.0 (E5.0), is the undifferentiated and proliferative progenitor tissue of all the trophoblast cell types that make up the placenta, and its differentiation potential (potency) is restricted to these cell types. TS cells are a powerful stem cell model for studying trophoblast differentiation, can be derived from ExE, have indefinite self-renewal (indefinite proliferation without loss of cell identity) and the same potency as ExE.

TS cells are routinely derived and maintained (kept proliferative and undifferentiated without losing their identity or potency) as flat one-cell thick epithelial colonies in undefined culture with added Activin and FGF4 (or FGF2), mostly in serum-containing conditions or in serum-free conditions on Matrigel-coated surfaces. However, serum and Matrigel are undefined components that contain many signalling and extracellular matrix (ECM) molecules. Consequently, it is currently unclear whether Activin and FGF are sufficient or necessary for TS cell maintenance and whether the ECM molecules in these cultures have a role in both maintenance and TS cell attachment to culture surfaces. Furthermore, TS cells could not be derived using the serum-free Matrigel culture, as mouse embryo fibroblasts (MEFs) were also needed. There is only one published culture for TS cell maintenance that is completely defined and serum free:

Activin, FGF2, XAV (inhibitor of canonical Wnt signalling) and ROCKi (inhibitor of RhoA signalling) on fibronectin. Although the latter culture suggests that Activin/FGF may not be sufficient, there is evidence that fibronectin is not the physiological ECM that ExE binds to *in vivo*, thereby leaving open the possibility that TS cells cultured on a more physiologically relevant ECM may behave differently than they do on fibronectin. To address these TS cell issues, we produced several findings including the following. (1) We developed a novel serum-free and completely defined culture for TS cell maintenance and derivation, using the fewest and most physiologically relevant signalling and ECM molecules to date: (a) AFL culture (Activin and FGF4 or FGF2 on laminin) for maintenance, and (b) AFL culture with transient use of ROCKi for derivation. (2) The global transcriptome of TS cells derived and maintained in AFL culture is very similar to that of TS cells derived and maintained in serum-containing (conventional) culture, but importantly it is more similar to the *in vivo* analogue of TS cells, the ExE, than conventional TS cells. (3) We used AFL culture to provide evidence that FGF4/2 and Activin are collectively sufficient and individually necessary for TS cell maintenance and that the role of the ECM molecule laminin is only to allow TS cell attachment. (4) We validated that TS cells cultured on fibronectin with added Activin/FGF2 also need XAV for their maintenance, whereas this is not required in AFL culture. (5) We derived for the first time TS cells on Matrigel with added Activin/FGF in the absence of MEFs, by transiently adding ROCKi during their derivation. (6) We provide the first evidence that TS cell colonies cultured for prolonged periods without passaging in conventional TS cell maintenance culture display self-patterning similar to that observed in the ExE *in vivo*, thereby indicating for the first time that TS cells can also be used for studying early trophoblast pattern formation, rather than only trophoblast proliferation and differentiation.

The role of ExE in early post-implantation/pre-gastrulation embryos at around E5.5, is not only as the progenitor of the trophoblastic component of the placenta, but also to signal the adjacent embryonic region [the epiblast (progenitor of the newborn) and its associated visceral endoderm (embryonic VE)] for establishing early asymmetries (patterning) along the embryonic proximo-distal (P-D) and anterior-posterior (A-P) axes. However, it is unclear whether the E5.5 ExE functions to: (a) restrict the formation of distal VE (DVE, the earliest morphological asymmetry along the embryonic P-D axis and an important signalling centre for establishing this axis) to the distal part of the embryonic region or to inhibit the proximal expansion of DVE after it has formed, (b) promote the anterior displacement of DVE to become anterior VE (AVE; signalling centre for the establishment of the A-P axis), and (c) solely responsible for BMP signalling within the proximal epiblast. We provide evidence including that: (1) ExE is required for BMP signalling within the epiblast, (2) BMP signalling within the epiblast is the earliest to date molecular asymmetry within the epiblast (found in proximal epiblast at pre-DVE stages), (3) ExE-derived BMP signalling is required at pre-DVE stages for restricting the formation of DVE distally, whereas at DVE stages it is required for the anterior displacement of DVE to become AVE.

Overall, our findings provide new insights about the molecular basis of TS cell derivation and maintenance and identify stage-dependent inductive roles for ExE on patterning the embryonic region.