Establishment of an in vitro model to study trophoblast adaptive response during placenta development in sheep

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During embryo implantation (Day 18), ovine trophoblast cells (oTCs)[1] invade the endometrium and differentiate to form the syncytiotrophoblast layer, which is the first "bridge-tissue" between foetus and mother. Normal conceptus development is affected by oTCs metabolism whose dysregulation leads to several gestational impairment, such as early pregnancy loss[2], characterized by inadequate nourishment or oxygen supply for developing foetus[3]. However, placental growth adapts to safeguard foetal survival, but how impaired activity of oTCs may be compensated to allow foetal development is still incompletely characterized[4]. In the early stage of placentation, endometrial histotrophic factors support placenta development through the modulation of specific cellular mechanisms, including Akt/mTOR signalling pathway, to balance the effects of stressful environmental conditions[5]. Therefore, the use of oTCs in vitro model may improve our understanding of placenta adaptive response, associated with pathological complication during pregnancy.

The main goal of this project was to set up an in vitro culture system from early sheep placenta in order to study the cellular development in physiological conditions. Preliminary, the cellular model was used to explore how oTCs regulate their adaptive response occurring in suboptimal environment during placenta development. oTCs functionality was assessed under starvation or with FGF2 supplementation and mTOR inhibitor (rapamycin) treatment in order to mimic adverse situations in impaired pregnancy.

Firstly, primary oTCs from 21 days old sheep placenta collected at the slaughterhouse were cultured in supplemented medium and characterized by cell morphology, immunofluorescence and PCR with established trophoblast markers. Then, oTCs primary cells and oTr cell line were subjected to different treatment (starvation, 50 ng/ml FGF2, 100 nM rapamycin for 24h) to study their effect on cell functionality, (cell proliferation and migration) and on cell characterization (gene and protein expression profile) through qPCR and western blot analyses.

oTCs in vitro model showed mainly mononuclear cells with epithelial cell-like growth and placental morphological properties, such as binucleate cells and multinucleated syncytium-plaques formation expressing peculiar trophoblast markers. FGF2 increased significantly proliferation in both oTCs and oTr ($p< 0.001$). Starvation and FGF2 supplementation stimulated cell migration ($p<0.001$), while rapamycin treatment suppressed it ($p<0.001$) compared to controls. Therefore, the inhibition of mTOR signalling pathway by rapamycin reduced the ability of FGF2 to induce cell migration. Significantly, e-CAD was shown to be up-regulated ($p<0.05$) both in starved and FGF2-treated cells (with or without rapamycin), whereas IFN-τ expression wasn't influenced by 24h treatment.

The results confirm FGF2 induced proliferation and migration activity by phosphorylation of Akt/mTOR in sheep trophoblast cells. Interestingly, the study provides the first evidence that FGF2 up-regulates e-CAD expression in stressful conditions associated with starvation and mTOR-inhibition. As previously shown in vivo experiments, these findings suggest that trophoblast expressed "transitory" epithelial-mesenchymal transition during peri-implantation period.

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