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Pavlína Černá joined my laboratory as a Masters student in the Autumn of 2018 and worked closely on a project with our group's post-doctoral researcher Lenka Gahurová (who also acted as Pavlína's official co-supervisor). After concluding her active research in our group in late 2020, she passed her final state exams in January 2021 and immediately took up employment as a "university/academic worker" at the "IVF (*in vitro* fertilisation) Pronatal Repro Clinic" here in České Budějovice (more details to follow). In the time since then until now, Pavlína has been juggling the competing demands of writing up her Masters' thesis and becoming professionally established in her role at the IVF clinic (which she has successfully achieved). Indeed, it might be fair to say that she may have under-estimated just how challenging it was to write up her thesis whilst also working full-time in a demanding and exciting job. Consequently, we decided to suspend her studies between January 2022 and April 2022, to enable her to submit her Masters' thesis in this current Spring 2022 round (a difficult decision but one on which all concerned parties agreed was ultimately correct).

Regarding her research project, Pavlína was set to work on a project in the laboratory investigating the potential role of regulated translation, under the control of the mTOR protein (mammalian Target Of Rapamycin), in the differential spatial positioning of early mouse embryo blastomeres resulting from the 8- to 16-cell stage transition; the 16-cell stage being the first developmental stage individual daughter blastomeres can occupy opposing positions in the embryo that correlate with their ultimate cell fate by the peri-implantation stage (*i.e.* by either retaining cell-contactless domains and residing on the outside of the embryo and acting as a progenitor pool of trophectodermal cells, or by becoming completely surrounded by other cells and encapsulated within the embryo and contributing to the inner cell mass blastocyst lineages of the epiblast and primitive endoderm). As mentioned, her work was part of a wider project and contributed to preliminary findings that pharmacological inhibition of mTOR using the compound TORIN1, specifically around the late 8-cell stage entry into mitosis, resulted in the generation of statistically fewer inner cells at the 16-cell stage; a result we reasoned from other reported cell models was related to mis-regulated translation of specific mRNA transcripts, defined by the presence of pyrimidine tract sequences in their 5'-UTR regions known as TOP-motifs, requiring enhanced availability of the 7-methyl-guanosine-cap binding complex (eIF4F) for their efficient translation into protein. Hence, in this context Pavlína was set three research goals:

i. To confirm (or refute) the mTOR inhibition induced phenotype of reduced numbers of inner 16-cell stage blastomeres using the alternative mTOR inhibitor Rapamycin (potentially being able to discriminate mTOR function in either the mTORC1 or mTORC2 containing complexes).
ii. Screen a panel of pharmacological inhibitors, with specificity against other intra-cellular signalling pathways reported to interact with mTOR, in similar 8- to 16-cell transition culture experiments; assaying for phenocopies of mTOR inhibition elicited reductions in 16-cell stage inner cell generation and potentially identifying functionally relevant upstream mTOR inputs regulating spatial positioning of cells in the embryo.
iii. To assay the effect on 16-cell stage inner cell generation by clonally over-expressing wild-type or dominant negative mutant versions of the mTOR substrate 4EBP1; itself an inhibitor of functional eIF4F complex formation, and protein translation, when in its non-phosphorylated state. Furthermore to assay the expression of endogenous phosphorylated 4EBP1, using varied anti-sera, around the mTOR inhibition phenotype generating sensitive window at the 8- to 16-cell transition.

Without wishing to pre-empt the findings of Pavlína's thesis, nor her pending presentation defence, she was able to achieve these experimental goals and has generated a large amount of valuable data that has been incorporated into a still growing pre-submission draft manuscript we intend to publish this year (Gahurová *et al.,* 2022). Therefore, I have a very positive view of Pavlína's time in our group, one that I know is also shared by her co-supervisor.

Accordingly, during her time in our group I always found Pavlína to be a very dedicated and conscientious worker. She very quickly assimilated the basic skills of animal husbandry, mouse embryo recovery, *in vitro* embryo culture/ manipulation, embryo fixation and (immuno-) staining and confocal microscopic imaging/ analysis. The rapid adoption of these skills was a real plus point, as they often represent a lengthy lag period before novice research students can find themselves in a position to generate any project relevant data. Having mastered these fundamental skills and techniques, and after individual experimental designs had been discussed and agreed upon, Pavlína exhibited a careful and methodical approach to her experimentation. This is her great strength and resulted in the generation of a large quantity of high quality and informative data (some of which will be shortly published). Pavlina was engaged in the theory of the experiments she was conducting (increasingly so, as her project and thesis writing progressed) but I think it is fair to say that it was the 'cut and thrust' of her daily practical embryology that she so obviously enjoyed the most. In the course of such work, Pavlína worked diligently and was often engaged in long hours in the lab and at highly unsociable times; primarily relating to the fact the 8- to 16-cell embryonic stages she was studying arise in the early hours of the morning. At such times when Pavlína came up against technical issues relating to her project (admittedly not so often), she invariably demonstrated dogged determination to successfully resolve them; something she was mainly successful in achieving with minimal fuss but she was also not afraid to consult with her supervisors. Her only real weaknesses seem to derive from her natural shyness and charming self-deprecation and her initial reluctance to write her thesis and orally present her data in English, rather than Czech. Notwithstanding, it is my view that Pavlína exhibited an especially high level of technical competence (that she has obviously taken into her new IVF-related career) and professionalism during her time in our group (again sentiments I am confident her co-supervisor would echo; made all the more remarkable as Lenka was under a period of maternity leave for most of 2020).

In summary, I regard Pavlína's time in our laboratory as being very successful. Undeniably, I have a high opinion of her as a research scientist and I tried (and failed) to retain her skills in the lab as a Ph.D. student. However, what is experimental mouse embryology's loss is certainly the gain of clinical assisted reproductive medicine. Indeed, on reflection I am now very happy that one of our lab's research student *alumni* is now so successfully and practically engaged (for example, by performing intra-cytoplasmic sperm injection/ ICSI of human oocytes, with her own hands) in the overwhelmingly meaningful and rewarding endeavour of creating new children, parents and families; replacing what was once only disappointment and despair with hope, joy and happiness! Therefore, for all the stated reasons and notwithstanding her charming, relaxed and friendly personality, I commend Pavlína's Masters' degree thesis to the examining committee.

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