



Přírodovědecká  
fakulta  
Faculty  
of Science

Jihočeská univerzita  
v Českých Budějovicích  
University of South Bohemia  
in České Budějovice

## STATEMENT OF THE BACHELOR/~~DIPLOMA~~\* THESIS SUPERVISOR

Name of the student: Tamara Gajovská  
Study program: Biology  
Department/Institute: Department of Molecular Biology & Genetics, Faculty of Science.  
Thesis title: *Effects of mTOR and AMPK pathways on specification of inner cell mass (ICM) cell lineages during preimplantation development.*  
Supervisor: doc. Alexander W. Bruce, Ph.D.  
Co-Supervisor: Mgr. Lenka Gahurová, Ph.D.  
Supervisor`s affiliation: Faculty of Science, University of South Bohemia

	Point scale <sup>1</sup>	Points
<b>(1) FORMAL REQUIREMENTS</b>		
Formal and graphical quality of the thesis	0-3	3
Ability to work with literature	0-3	2
Language and stylistics	0-3	2
Formal requirements – points in total		7
<b>(2) PRACTICAL REQUIREMENTS</b>		
Fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of results and their discussion with the literature	0-3	3
Logic in the plan of the experimental work	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Contribution of the thesis to the knowledge in the field and the possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		17
<b>POINTS IN TOTAL (MAX/AWARDED)</b>	<b>27</b>	<b>24</b>

Overall classification: 3-excellent

\* Choose one

<sup>1</sup> Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

### **Eventual additional comments of the (co-)supervisor on the student and the thesis:**

It was the pleasure of the co-supervisor (Lenka Gahurová – a post-doc in my group) and I to have Tamara working in the laboratory. She was/ is well liked by myself and other members of the research group. During her time in the laboratory Tamara proved herself to be an extremely diligent and careful worker and certainly met our collective expectations of what is required of a Bachelors' level research student. She was able to work very independent and ultimately required very little direct supervision. Moreover, she presented her draft thesis well in advance of the submission deadline and responded very well to the suggestions provided by myself and her co-supervisor.

Without wishing to pre-empt the contents of Tamara's thesis and defence presentation, she was set to work in close collaboration with my post-doc, Lenka Gahurová, on an offshoot from her main project related to AMPK/mTOR signalling (regulators of homeostatic cellular metabolism) in the developing preimplantation stage mouse embryo; focussing upon the maturation of the blastocyst and the specification of the inner cell mass lineages (ICM - *i.e.* differentiating primitive endoderm and pluripotent epiblast) prior to uterine implantation. The hypothesis was pharmacological activation of the AMPK pathway would provide a phenocopy of mTOR inhibition, whereby blastocyst development would become developmentally diapaused and ICM cells would remain unspecified in terms of cell-fate (this hypothesis was based on phenocopies of similar AMPK activation and mTOR inhibition observed by Lenka Gahurová at an earlier developmental stage). Tamara's actual results were much more interesting and unexpected. Whilst she confirmed the known mTOR inhibition phenotype, she found AMPK activation actually selectively impairs specification of the primitive endoderm, with minimal effects on epiblast specification. This result is significant as it is an actual phenocopy of chemical inhibition of p38-MAPK (shown in numerous previous publications from our group) to regulate protein translation during primitive endoderm specification differentiation; thus, opening a new avenue of potential research.

Tamara's work involved learning to collect and *in vitro* culture preimplantation stage mouse embryos under control and pharmacologically supplemented conditions, fix and immunofluorescently stain blastocysts for ICM cell lineage markers, acquire confocal embryo micrographs and analyse cell-lineage specific numbers (with attendant statistical analysis). In this regard, Tamara demonstrated a very good laboratory technique and was able to troubleshoot any problems she encountered (as stated above, with quite minimal assistance after initial training).

Throughout Tamara's time in the laboratory, we were always confident that she fully understood both the theory and practice of what she was doing. Indeed, we think Tamara made a very good job of introducing preimplantation mouse embryo development, and within the context of AMPK/mTOR signalling, in her written thesis. Additionally, we think she also made a good job of compiling a relevant Discussion section and well placed her own results in the wider context; something that we would argue is traditionally lacking in most Bachelor's level theses.

Overall, we were more than satisfied Tamara received a solid foundation in experimental design, execution, interpretation and presentation. Moreover, that she would be able to successfully draw on these skills in her future career. We understand she has applied to continue studying in active Masters' degree research, at the Institute of Entomology, Biology Centre of the Czech Academy of Sciences (under the guidance of prof. Ivo Šauman, Ph.D.) – what is the gain of insect chronobiology, is simultaneously the loss of early mammalian embryology! We have no hesitation in recommending Tamara's Bachelors project thesis is accepted as successfully defended.

### **Conclusion:**

**In conclusion, we,**

**Alexander W. Bruce & Lenka Gahurová**

recommend / ~~do not recommend~~\*

In: České Budějovice

Date: 14<sup>th</sup> April 2021

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