



Přírodovědecká
fakulta
Faculty
of Science

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

STATEMENT OF THE BACHELOR THESIS REVIEWER

Name of the student: Sarah Schnabellehner

Thesis title: The role of Csf1 and Zfp101 in preimplantation mouse development
Supervisor: Alexander W. Bruce, Ph.D.

Reviewer: RNDr. Alena Krejčí, Ph.D.

Reviewer's affiliation: Faculty of Science, University of South Bohemia

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		19
(2) PRACTICAL REQUIREMENTS		
Clarity and 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 (absence of discussion with the literature is not acceptable)	0-3	2
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	3

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Formal requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)		
	48	(44) ²

Suggestions and questions, to which the student has to answer during the defense:

- In relation to the Zfp116 immunostaining results, one antibody gave a signal while the second did not. Moreover, the signal derived was unexpectedly on the apical membrane in addition to the nucleus. How could one check the specificity of this antibody, in relation to the protein it recognises?
- In relation to the Csfl knockdown study and the lack of effect on cell number by the blastocyst stage, how could you confirm if the RNAi strategy you were using had successfully reduced Csfl mRNA levels in the embryo?

Eventual additional comments of the reviewer on the student and the thesis:

The bachelors thesis of Sarah Schnabellehner focuses on the initial characterisation of two cell fate influencing candidate genes, previously identified in the Bruce lab from a bioinformatics based screen, in the context of the preimplantation stages of mouse embryo development. These genes are a) the transcriptional coactivator, Zinc-finger protein multitype1/ Zfp116 and b) the cytokine, Colony stimulating factor 1/ Csfl.

During her project she used confocal based immunofluorescent staining techniques to assay the protein expression pattern of Zfp116 (using two different antibodies) at the 8-cell, 16-cell and blastocyst stages, testing the hypothesis expression of the protein would be restricted to progenitor of or the matured trophectoderm/ ICM lineages. While her data refused this hypothesis, suggesting Zfp116 is expressed in all cell nuclei at the 8- and 16-cell stages but is absent in the blastocyst, she did uncover unexpected localisation of the protein at the apical surface of 8- and outer-residing 16-cell, that could be of potential significance for inherited cell fate of daughter cells after asymmetric cell divisions. However, it should be noted that she only detected a signal with one of the antibodies used.

She also attempted to functionally assay any potential cell fate influencing role of cytokine Csfl, and specifically Csfl produced by the embryo itself that could then act in an auto/paracrine manner, as opposed to the provision of maternal Csfl that is known to contribute to trophectoderm cell proliferation *in vivo*. To this end, she employed a microinjection based strategy to introduce a long dsRNA specific to Csfl mRNA into the 1-cell stage zygote embryo to knockdown expression of the Csfl gene throughout all subsequent cells of the developing embryo in *in vitro* culture. She then compared the number of cells, and specifically trophectoderm cells that had been immunofluorescently stained for the specific marker protein Cdx2, in Csfl-KD embryos to control microinjections in early blastocyst stage embryos, by confocal microscopy. The results of these experiments showed that there were no statistically significant differences in total cell or trophectoderm cell number between the Csfl knockdown and control groups, although the efficacy of the Csfl knockdown was not confirmed

² Enter the number of points awarded.

The structure and logical flow of the thesis was good. It was easy to read and understand and except from a couple of spelling mistakes and the odd wording, the grammar was also good. The introduction was substantial, perhaps a little longer than necessary, and demonstrated a good and clear understanding of the preimplantation embryo field. The methods were clearly described and the results sections well presented and understandable. The discussion of the results, and their relevance in the wider context, was a little brief, as is often the case with Bachelor's level theses. Overall, this thesis and the project described represented a good and solid effort.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade **excellent** .³

In Ceske Budejovice date 14/6/2016

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signature

³ You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted.