



**Institute
of Experimental
Medicine, CAS**

EU Centre of Excellence

RNDr. Mária Hovořáková, PhD.
Institute of Experimental Medicine
Czech Academy of Sciences
Videňská 1083
142 20 Prague 4
Czech Republic

**Referee report for the doctoral thesis:
“The study of *Xenopus tropicalis* testis-derived stem cells”
Ph.D. study program: Cell and Developmental Biology**

Candidate: M.Sc. Nguyen Thi Minh Xuan.

The submitted thesis “**The study of *Xenopus tropicalis* testis-derived stem cells**” prepared by **M.Sc. Nguyen Thi Minh Xuan** introduces *Xenopus tropicalis* testis-derived stem cells (XtiSCs) as a novel model for the investigation of epithelial – mesenchymal transition (EMT) signalling and the stemness acquisition of EMT-shifted cells.

On the basic knowledge that pre-Sertoli cells possess mesenchymal phenotype and must undergo a mesenchymal-epithelial transition (MET) to become mature cells M.Sc. Nguyen Thi Minh Xuan studied the reverse process of EMT and conversion of Sertoli cell precursors or of immature Sertoli cells back to their stem cell-like stage. Interestingly the experiments performed in frame of the present study clearly showed that XtiSCs possessed characteristics of both, mesenchymal and epithelial cells.

Concerning the formal aspects of the work, the thesis is written clearly in an understandable manner, with only minor imprecisions in the text (eg. minor defective numbering of pages – pages Nr. 67-68 are doubled). The text is segmented in chapters, accompanied with a high number of figures of a high quality documenting the research, which are also summarized in the separate list of figures. The Aims are clearly formulated and they were sufficiently fulfilled and answered properly in the Conclusions. The summary in Czech and English is available. The used methodology is relevant and properly described in the Materials and Methods section. The results are presented and documented clearly in Chapter 3. (Results) and they are appropriately discussed in the Discussion chapter in relation to the recent knowledge in the field. The references are complete, current and accurately listed in the References section.

The results form the basis of three original publications (one of them already published, two recently under revision, one with minor revisions) in international

Videňská 1083
142 20 Prague 4
Czech Republic

phone: +420 241 062 230

iem@iem.cas.cz
www.iem.cas.cz



journals with IF (two of them with a major contribution of M.Sc. Nguyen Thi Minh Xuan as the first author). These papers clearly demonstrate the candidate's good understanding of the implications of her work in a broad scientific context as well as her contribution to the existing knowledge in the research field.

I would like to ask the candidate to answer the following questions:

1. In the context of the information in Chapter 2. General background, the general description of the germ cells proliferating and entering meiosis stopped in diplotene stage after birth until puberty mentioned on page 6 is rather confusing. I would like to ask the candidate to explain the fate of the germ cells and compare the situations in males and females. Do you think the germ cells enter meiosis initiating oogenesis and spermatogenesis at the same time-point?
2. I would like to ask the candidate to specify the prospective potential or advantages of the *Xenopus tropicalis* testis derived stem-cells in comparison to the pluripotent stem cells derived from mouse or human testes. What about the general perspectives of the usage of XtiSCs?
3. It has been shown recently that retinoic acid (RA) may play important functions in the differentiation of Sertoli cell precursors and primitive spermatogonia. Did you think about experimentally influencing RA signalling or maybe manipulating the mammalian target of rapamycin (mTOR) kinase signalling pathway in the case of XtiSCs?

Summarizing, M.Sc. Nguyen Thi Minh Xuan - the candidate for PhD degree has performed a large amount of insightful research and has obtained new original results that broaden our understanding of Sertoli cells formation and function, as well as of EMT mechanisms and of general stemness acquisition. She is fully capable of accomplishing creative and valuable scientific work and thus she is deserving of a doctoral degree after a successful defence of her thesis.

I recommend here the thesis of M.Sc. Nguyen Thi Minh Xuan for a defence.

In Prague May 21st 2019

RNDr. Mária Hovořáková, PhD.