In Pilsen on 4th of September, 2020

Review to PhD thesis

Edgar del Llano

Age-related differences in translation of mammalian oocytes

General comments

The thesis describes cellular and molecular events in oocytes underwent to aging. The thesis consists of an introduction, well-describing scientific issue, leading to the hypothesis, and followed by paper comments. All results are discussed comprehensively and summarized in conclusions.
Based on the shape of the thesis (compilated articles), there is obvious relevance of the thesis, valuable for above mentioned medical implications. Undoubtedly, the author is capable to perform serious experiments, using highly advanced approaches, as well as analyzing and introduce these data for a purpose of a talk and, in particular, for a manuscript. Not at least, the ability of experiment designing and manuscript writing is elucidated due to a key manuscript, attached to this PhD thesis and being *in press* recently.
Due to all facts included in the thesis, several questions raise as follows:

i) Fully-grown oocyte is considered as transcriptionally inactive cell. Could you briefly explain the mechanism of how gene silencing is occurred? Is there any evidence of basal transcription even pre-NEBD oocytes? How mitochondrial genome (mtDNA) does contribute on this phenomenon – is mtDNA silenced too in fully-grown oocytes, due to anterograde signaling and partial mtDNA transcription dependence on the nuclear genome?

ii) You described 4E-BP1 phosphorylation after NEBD as a mark of increased translation. Is there any idea about translational products on the poles after BP1 phosphorylation? Moreover, is 4E-BP1 still adjacent to mRNA transcript when it is phosphorylated? Does the localization of p4E-BP1 at the spindle poles correlate with mRNA, belonging to it, or it is rather an inheritance of mRNA translated and gone away yet?

iii) Non-disjunction and premature separation are mentioned as modes of oocyte-born aneuploidy. In addition to cohesin, are there any other age-related factors responsible for increased risk of aneuploidy incidence? Besides the age, is there another crucial factor leading to aneuploidy?

iv) As you wrote on page No. 23, „No significant changes were detected in the polysomal profiles of both oocyte groups“, your findings indicate maternal mRNA non-polysomal

pool rather destined for the fertilization and pre-EGA embryonic development than oocyte maturation. Are there any candidate maternal age-related factors, non bounded to polysomes in the matured oocyte, leading to embryo rescue during early embryonic development?

Minor comments to individual chapters

Introduction: Ovarian and post-ovulatory aging is not compared, although both mean serious issue for reproductive medicine. Similarly, the embryonic capability of aneuploidy reparation through the elimination of aneuploidy blastomeres is not mentioned.
Material & methods are missed, although a brief list of them would be suitable. On the other hand, methodological part is included in the Discussion and, therefore, rather discussed than explained.
Results are described precisely with respect to the format of the thesis, while complete data are included in single papers.
Discussion is well-written, although medical implications are not discussed. As aforementioned, the impact of maternal mRNAs for embryonic development is not mentioned at all.
Conclusions summarize all achieved findings comprehensibly and clearly.

Final consideration

Experimental work on behalf of the thesis is worthwhile and significant piece of research of female reproduction, through the study of the oocyte, in general, being an essential cell for female reproductive health and fertility. In the light of reproductive medicine, the ovarian and oocyte research is extremely important for further progress in prevention of infertility and/or infertility healing. For these reasons, I appreciate the thesis as an effort to improve current knowledge with many possible implications into the reproductive medicine and assisted reproductive approaches.
Based on my best knowledge, the manuscript has been submitted and accepted for publication in Ageing Cell, highly-ranked scientific journal (IF 7.238, Q1). With respect to the rules at Charles University, I can recommend MSc. Edgar Del Llano to be promoted to PhD, due to the significance of the manuscript as well as the contribution of the first author.

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