Culture Conditions and Cumulus-Oocyte Complex Integrity Affect the Program of Oocyte Maturation in Higher-Order Mammals

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In oocytes, resumption and completion of meiosis I are driven by post-transcriptional mechanisms on a background of repressed mRNA transcription. However, there is a lack of in-depth understanding of the regulation of translation in maturing oocytes, particularly in higher-order mammals, primarily due to methodological constraints in isolating polysome-associated mRNAs. Thus, we aimed to shed light on this process and how it can be affected by oocyte in vitro maturation (IVM). This objective was achieved through three sequential steps. First, we applied a bioinformatics approach to gain information on mRNA polysome association (GSE56603 and GSE196484) and polyadenylation (GSE61717) in immature and in vitro matured bovine oocytes. The GEO-retrieved datasets were re-analyzed to determine differentially polyadenylated/polysome-associated genes using edgeR. While the overlap of differentially polyadenylated/polysome-associated genes between datasets was limited when comparing mature oocytes), all comparisons conducted in immature oocytes were substantial. These findings indicate that the experimental approaches potentially yield comparable results, but that this homogeneity is lost with IVM, denoting that culture conditions contribute to changes in mRNA translation patterns.
Next, we tested whether the EGF-like growth factors regulate the translation of some transcripts by activating the oocyte PI3K/AKT/mTOR axis in bovine oocytes, as demonstrated for mice. For this, the previously mentioned datasets were compared with polysome-associated transcripts of mouse oocytes exposed to the EGF-like growth factor, amphiregulin (GSE46640). Notably, one bovine dataset showed a significant correlation with EGF network activation, supporting, at least in part, the observation that IVM conditions might affect mRNA translation.
To experimentally confirm the role of the EGF network in the bovine, we monitored oocyte AKT during IVM by western blots and immunofluorescence and observed a significant, transient phosphorylation with amphiregulin, indicative of the pathway activation. To understand if AKT activation affected translation, we investigated polyadenylation in target transcripts using two approaches. First, we attempted to indirectly catch differences in the retrotranscription efficiency in association with short or long poly(A) tail. The mRNA was split in half and retrotranscribed using random hexamers or oligo(dT). This assay did not highlight differences between immature and mature oocytes or IVM conditions. However, when the poly(A) tail length was directly assayed, a differential extent of polyadenylation in target genes related to cell cycle progression and protein localization to the centrosome was observed in

amphiregulin-supplemented IVM. Furthermore, with this assay a starting length of the poly(A) tail was set at approximately 200 bp in immature oocytes, providing an explanation for the inability of observing differences using random hexamers and oligo(dT).
Since the extension of the poly(A) tail is necessary for polysome association and translation, these results confirm that, despite differences in timing and initial tail length, also in higher-order mammals translation of genes crucial for proper oocyte maturation are regulated by the EGF network, likely through PI3K/AKT/mTOR.
To further understand if the impossibility to activate the EGF network during IVM could affect downstream signaling, AKT phosphorylation was monitored during rescue IVM of denuded human immature oocytes obtained after controlled ovarian stimulation. As predicted by our model, AKT failed to activate, providing a putative explanation for the low quality achieved after rescue IVM in humans.
This study highlights the importance of providing an appropriate cultural environment and maintaining the structural integrity of the cumulus-oocyte complex for a proper execution of the maturation program.
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