sent at the time of fertilization. The experimental design was based on two principles; (i) The receptors responsible for the recognition of gametes in the oocyte membrane appear exposed in the ovulated egg and disappear once a single sperm has fertilized the egg (zygote), to facilitate the prevention of polyspermy. (ii) These interactions are usually found to be highly transient.
Materials and Methods: A comparison of the proteomics profile from membrane fractions of oocytes and zygotes will be analysed. For this purpose, 400 *in vitro* matured bovine oocytes and 400 zygotes were produced in 3 replicates. Briefly, cumulus-oocyte-complexes were selected for *in vitro* maturation. At the end of the maturation period, oocytes were split into two groups, (i) vortexed to remove cumulus cells, washed in PBS, snap frozen and stored at −80 °C, or ii) prepared for *in vitro* fertilisation (IVF). Bull spermatozoa were selected by swim-up and added at a final concentration of 1×10^f spermatozoa/mL. After 22 h of coculture, zygotes were gently stripped of cumulus cells by vortexing, washed twice in PBS, snap frozen and stored at −80 °C. For cell membrane purification, cells were washed in PBS, suspended in a hypotonic lysis buffer, incubated in an ice bath, disrupted using a dounce homogenizer with a tight-fitting pestle, and centrifuged to collect the cell membranes. Later, samples were further processed and analysed by mass spectrometry by nLC coupled to an ion trap mass spectrometer equipped with a Captive source. Three different runs were carried out per sample. Once proteins were identified, gene ontology analysis was carried out filtering on membrane proteins.
Results: From all identified proteins (216 oocyte proteins, 295 zygote proteins), a total of 109 and 188 were identified exclusively in the oocyte and zygote samples respectively, and 107 were common to both groups, from which gene ontology analysis revealed 49 and 33 proteins overexpressed in oocytes and zygotes, respectively.
Conclusions: The proteomic analysis show turnover of the membrane proteins from the oocytes to zygotes. Further detailed study of the proteins exclusively detected in the oocyte membrane may undelved new candidates involved in gamete binding and fusion.
Acknowledgements: PLEC2021-00773, PID2020-114109GB-I00 funded by MCIN/AEI/10.13039/501100011033.

doi: 10.1016/j.anscip.2023.03.048

048
A meta-analysis suggests that the culture environment affects mRNA translation in bovine oocytes

M. Ladrón de Guevara [a], M.B. Rabaglino [b], M.A. Sirard [c], F. Franciosi [a]

[a] Reproductive and Developmental Biology Laboratory, Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy
[b] School of Agriculture and Food Science, University College Dublin, Dublin, Ireland
[c] Centre de recherche en reproduction, développement et santé intergénérationnelle, Département des Sciences Animales, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Québec City, Canada

Presenting author.
Magdalena Ladrón de Guevara. E-mail: magdalena.ladrondeguevara@unimi.it

Application: Refining culture environment to support mRNA translation may improve egg quality.
Introduction: Upon removal from antral follicles, fully-grown oocytes resume meiosis and fertilize *in vitro*. However, embryo development is lower compared to *in vivo* matured oocytes. Such loss of performance is likely caused by two main factors: (1) skipping final differentiation, also known as 'oocyte capacitation', occurring during follicular dominance and (2) inability of fully recreating a microenvironment that supports oocyte maturation. Given that resumption and completion of meiosis I is largely driven by post-transcriptional mechanisms and that the epidermal growth factor (EGF) network partially regulates maternal mRNA translation in mice, we conducted a meta-analysis in the attempt of better elucidating how the culture environment affects translation in bovine oocytes.
Materials and Methods: Isolation of polysome-associated mRNAs requires high amount of starting material. Therefore, we exploited deposited datasets to gain information on (1) mRNAs polysome association in immature (GV) and mature (MII) bovine oocytes (GSE56603); (2) extent of amplification of polyadenylated mRNAs in GV and MII bovine oocytes (GSE61717); (3) mRNAs polysome association in MII mouse oocytes upon activation of the EGF network (GSE46640). A comparison between the datasets was conducted to identify translation patterns that are affected by maturation and by EGF-like growth factors. Since there was no suitable dataset on bovine oocytes to inform on the latter, a mouse dataset was used. GEO-retrieved datasets were re-analyzed using R-Studio. Differential expression was determined using edgeR (Bioconductor – Software packages). AdjP < 0.05 and LogFC > 2 were considered.
Results: Twenty-seven transcripts were differentially associated to the polysomes in MII compared to GV bovine oocytes, and only one was common to the 320 transcripts overexpressed in response to EGF network. Therefore, we included a second bovine dataset (GSE61717), which preferentially identifies polyadenylated, and therefore translated, mRNAs. However, also in this case the overlap between maturation-induced and EGF network-induced differences was minimal. To test if the failure to detect overlap was due to inter-specificity, we compared intraspecifically the polysome-associated and polyadenylated transcripts. Notably, while the overlap was still limited for MII oocytes, all the mRNAs preferentially associated to the polysome in GV were also overexpressed in the polyadenylated dataset at the same stage, indicating that the two experimental approaches yield comparable results for immature oocytes, but this homogeneity is somehow lost with *in vitro* maturation (IVM).
Conclusions: This meta-analysis represents indirect evidence that IVM may lower egg quality by disrupting the oocyte translational program.
Acknowledgements: Supported by MSCA-ITN-ETN 2019 EUROVA n. 860960.

doi: 10.1016/j.anscip.2023.03.049