Abstract #: 150

LEME
Laboratory of Embryonic Metabolism and Epigenetics

Changes in pyruvate metabolism alters the epigenetic and molecular maturation of bovine oocytes

J. V. A. Silva*,[1,2], J. Ispada[1], A. M. Fonseca Junior[1], E. C. dos Santos[1], C. B. de Lima[1], H. C. da Rocha[1], M. P. Milazzotto[1]

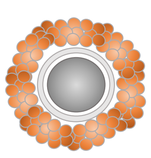
1 Center of natural and Human Science – Federal University of ABC, 2 University of Mogi das Cruzes.

INTRODUCTION

During *in vitro* maturation (IVM), bovine oocytes undergo important metabolic, epigenetic, and transcriptional changes for the acquisition of developmental competence. Pyruvate, the end-product of glycolysis, is produced by cumulus cells, and is destined into the mitochondria of both cumulus cells and oocyte as a master fuel input for tricarboxylic acid cycle (TCA), contributing to the generation of important substrates for oxidative phosphorylation and ATP synthesis. The citrate generated in the TCA cycle can also be directed to the cytoplasm and converted back to acetyl-CoA, being driven to lipid synthesis or, still, be used as substrate for histones acetylation. In this sense, metabolic changes that alter the availability of cytoplasmic acetyl-CoA, may alter the epigenetic profile of the oocyte, with consequences for correct molecular maturation. Thus, the hypothesis of this work is that the modulation of the pyruvate metabolism induces metabolic changes in oocyte and cumulus cells, with consequences to histone acetylation and transcriptional control. The aim of this work was to verify the impact of pyruvate metabolism modulation on histone acetylation and RNA transcription in bovine cumulus oocyte complexes.

RESULTS

Cumulus–oocyte complexes (COCs) *in vitro* matured in the presence of sodium dichloroacetate (DCA), a pyruvate to acetyl-CoA conversion stimulator, had an increase in mitochondrial activity in CC and oocytes, while those IVM in the presence of sodium iodoacetate (IA), a glycolysis inhibitor, presented lower MA in CC whereas oocytes had the opposite profile (Figure 2 and 3, respectively). These changes in mitochondrial activity are an indicative that TCA cycle and consequently the generation of acetyl-CoA varied among groups. Indeed, distinct dynamics of histone acetylation were observed in both CC and oocytes (Figures 4 and 5, respectively), with consequences to the global RNA synthesis in CC (Figure 6).



Cumulus cells

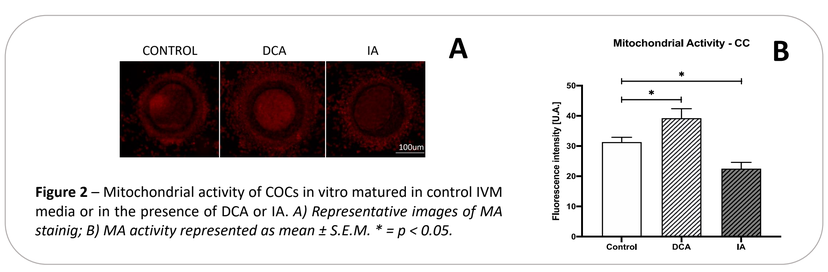
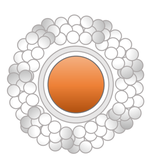


Figure 2 – Mitochondrial activity of COCs in vitro matured in control IVM media or in the presence of DCA or IA. *A) Representative images of MA stainig; B) MA activity represented as mean ± S.E.M. * = p < 0.05.*



Oocyte

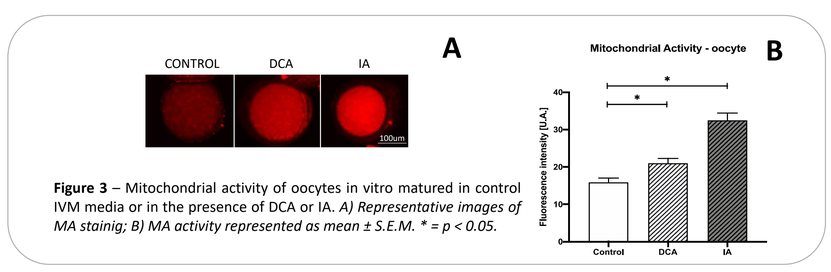


Figure 3 – Mitochondrial activity of oocytes in vitro matured in control IVM media or in the presence of DCA or IA. *A) Representative images of MA stainig; B) MA activity represented as mean ± S.E.M. * = p < 0.05.*

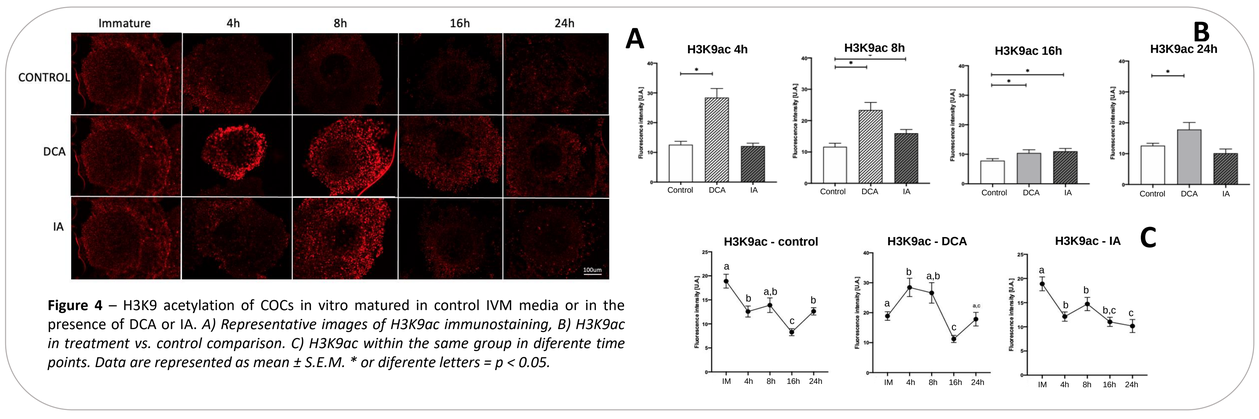


Figure 4 – H3K9 acetylation of COCs in vitro matured in control IVM media or in the presence of DCA or IA. *A) Representative images of H3K9ac immunostaining, B) H3K9ac in treatment vs. control comparison. C) H3K9ac within the same group in diferente time points. Data are represented as mean ± S.E.M. * or diferente letters = p < 0.05.*

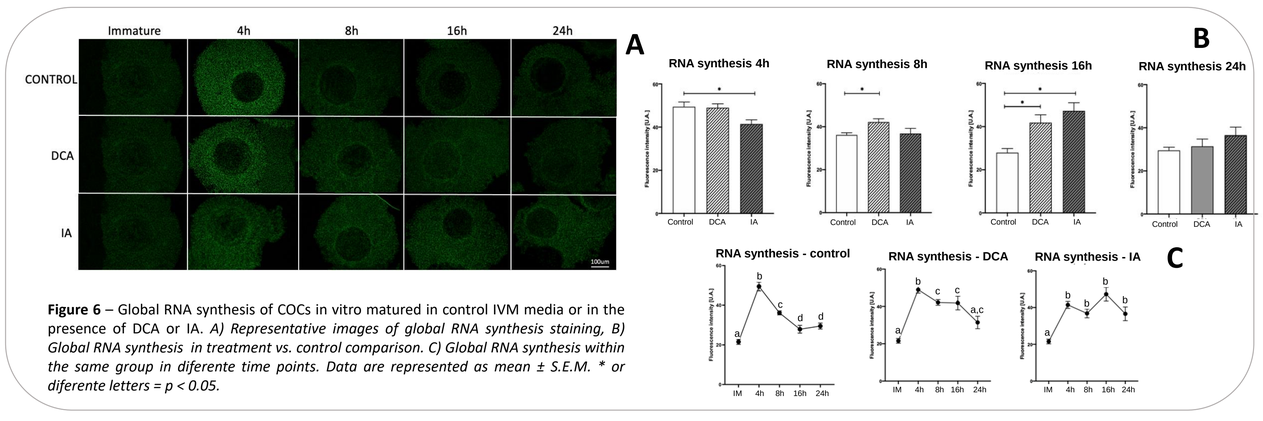


Figure 6 – Global RNA synthesis of COCs in vitro matured in control IVM media or in the presence of DCA or IA. *A) Representative images of global RNA synthesis staining, B) Global RNA synthesis in treatment vs. control comparison. C) Global RNA synthesis within the same group in diferente time points. Data are represented as mean ± S.E.M. * or diferente letters = p < 0.05.*

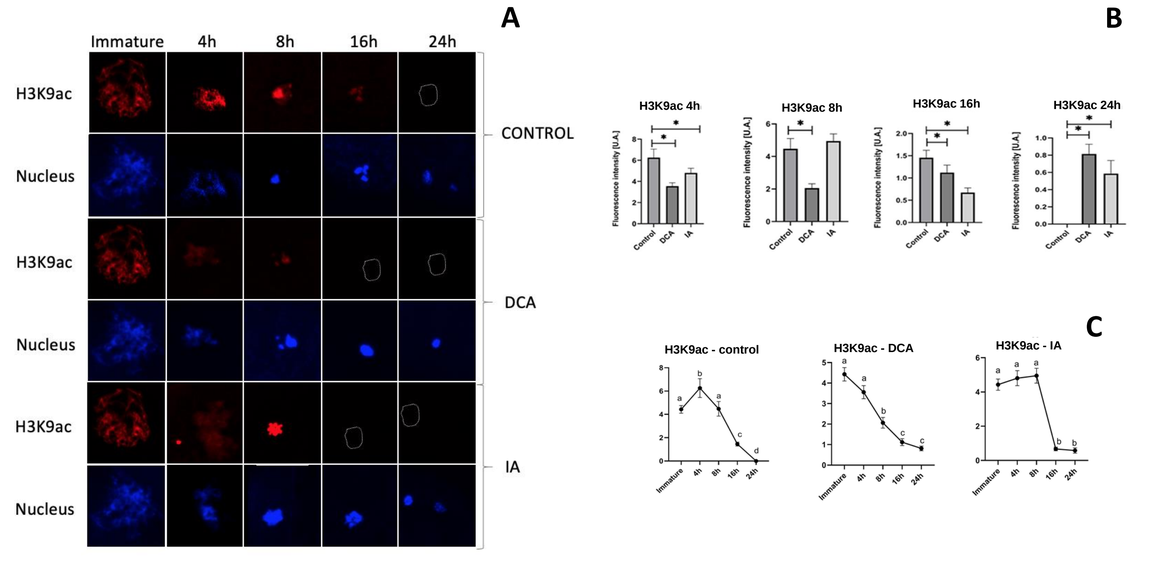


Figure 5 – H3K9 acetylation of oocytes in vitro matured in control IVM media or in the presence of DCA or IA. *A) Representative images of H3K9ac immunostaining, B) H3K9ac in treatment vs. control comparison. C) H3K9ac within the same group in diferente time points. Data are represented as mean ± S.E.M. * or diferente letters = p < 0.05.*

MATERIAL AND METHODS

Cumulus–oocyte complexes (COCs) were IVM in three experimental groups: Control [IVM medium], DCA [IVM medium supplemented with 1.5 mM sodium dichloroacetate] or IA [IVM medium supplemented with 5 µM sodium iodoacetate].

Cumulus cells (CC) and oocytes were analyzed separately at 24 h (mitochondrial activity, MA; MitoTracker Red CMXRos, ThermoFisher Scientific] and at 0, 4, 8, 16, and 24 h of IVM [lysine 9 histone 3 acetylation (H3K9ac immunofluorescence) and global transcript synthesis (only CC; Click-iT® RNA, ThermoFisher Scientific)] (Figure 1).

The images were acquired using a fluorescence microscope and analyzed by Image J software. The results from at least 3 replicates were compared by Student's t-test (treatment vs. control) or by ANOVA followed by Tukey's test (comparison within the same group in different time points) considering P < 0.05.

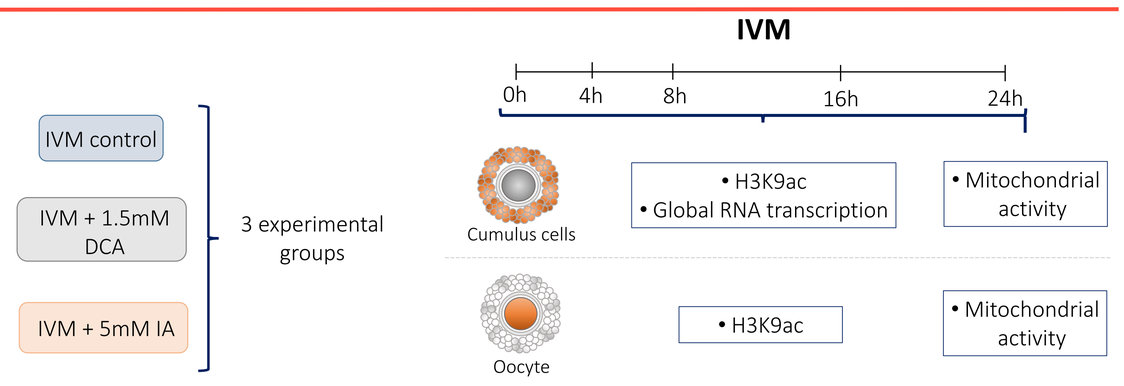


Figure 1 – *Experimental design*

CONCLUSION

In conclusion, changes in pyruvate metabolism caused by manipulation of the IVM system lead to epigenetic and molecular changes in both CC and oocytes, highlighting that the manipulation of in vitro production systems must be more carefully performed in order to avoid possible consequences to the offspring.

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