A142 Embryology, developmental biology and physiology of reproduction

# Influence of apical domain formation on the segregation of cell lineages in early development bovine embryos

**Marcelo Demarchi Goissis[1], Samuel Augusto Aguiar dos Anjos[2,1], Mayra Elena Ortiz D'Ávila Assumpção[1], José Antonio Visintin[1]**

[1]VRA - FMVZ - USP - Department of Animal Reproduction, College of Veterinary Medicine and Animal Science, University of Sao Paulo, Cidade Universitária, São Paulo, SP; [2]IB - USP - Institute of Biosciences, University of Sao Paulo, São Paulo, SP, Brasil.

The first event of cellular differentiation in mammals consists in the segregation between the inner cell mass (ICM) and the trophectoderm (TE). Biological processes that comprise this event are not yet clear during bovine embryo development and studies in mouse suggest that cellular contractility and formation of an apical domain plays a role in this event. In this study, we tested the hypotheses that blocking cellular contractility would block apical domain and inhibit TE formation or that direct inhibition of apical domain formation would inhibit TE formation in bovine embryos. First, we evaluated the presence of an apical domain during bovine embryo development by immunofluorescence of apical domain proteins PARD6B (Novus Biologicals, Littleton, CO USA) and EZR (Abcam, Cambridge, MA, USA) in IVP embryos. We observed that EZR is present since 8-cell stage while PARD6B becomes apically localized at the blastocyst stage. To test the effect of cellular contractility on TE formation we treated IVP embryos with blebbistatin (Bb), a myosin light chain kinase inhibitor. We assessed embryos at 90 hours post-insemination (90hpi) and those at 8-cell stage or further ahead in development were submitted to the following treatments: control, 25μM Bb (+)- and 25μM Bb (-)- (Cayman Chemical, Ann Arbor, USA). Embryos were kept in treatments until 186hpi when development rates (blastocysts/treated embryos) were assessed and embryos fixed with paraformaldehyde (Merck KGaA, Darmstadt, Germany). Developmental rates were analyzed by ANOVA followed by Tukey's adjustment for comparison of means after 5 replicates. Unexpectedly, no statistical difference ($p<0.05$) was observed considering developmental rates among all three groups: control 47.24±7.30% (45/96), Bb (+)- 60.24±7.30% (58/96) and Bb (-)- 49.50±7.30% (46/96). Immunofluorescence revealed that EZR was practically abolished in Bb (+)- treated embryos while present in the other groups. YAP (Abcam), a HIPPO-pathway related protein, was nearly undetected in Bb (+)- treated embryos while visible in other treatments. Also, CDX2 (Abcam), a commonly used marker for TE cells, was not observed in Bb (+)- embryos. To confirm these results, we used the same experimental design and statistical analysis to test if inhibition of apical domain establishment blocks TE formation. Embryos were submitted to following treatments: Control, vehicle (DMSO, Merck) and 7.5μM U73122 (Cayman Chemical), a phospholipase C inhibitor. No statistical difference was observed considering developmental rates among all three groups: control 41.8±3.27% (51/122), vehicle 35.29±3.27% (44/124) and U73122 35.27±3.27% (45/128). Combined, these results led us to conclude that inhibition of contractility or inhibition of the apical domain do not block formation of the TE in bovine embryos, suggesting that different biological processes are involved in ICM/TE segregation in bovine embryos. Funded by FAPESP grants 2017/09576-3, 2017/25574-0, 2018/08285-8.