Effects of WNT-inhibition and Activin A on Developmental Competency and Pluripotency Markers of Bovine Haploid Parthenogenetic Embryos.

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Haploid embryonic stem cell lines (ESC) from haploid parthenogenetic embryos (HPE) have been obtained in mice and humans. However, most bovine HPE (bHPE) undergo developmental arrest before reaching the blastocyst stage, which precludes their use for deriving ESC. Therefore, the present study aimed to investigate whether supplementing the in vitro culture IVC medium with CHIR99021 or Activin A (AA) affects the developmental competence and pluripotency markers of bHPE. To do this, bHPE embryos were produced by incubation with 5 µM of ionomycin for 5 min followed by culture for 5 h in cycloheximide (CHX). A group of diploid parthenogenetic embryos cultured in 6-DMAP instead of CHX was included as a control group. Treatments with CHIR99021 (3 µM, GSK3B-inhibitor) and Activin A (20 ng/mL, AA) were performed from Day 5 onward using a serum-free culture medium (SFCM) system. Morulas cultured in 0.001% of DMSO were used as the vehicle control. Embryo development was assessed at 48 h (cleavage), 120 h (morula), and 192 h (blastocyst). Morphological quality and immunostaining analysis of total cells and pluripotency markers (CDX2, SOX2, GATA2, and NANOG) were performed at the blastocyst stage. Our results showed that the SFCM did not affect the developmental potential or cell allocation into the inner cell mass (ICM) and trophectoderm (TE) of diploid parthenogenetic embryos. Although there was a tendency ($p>0.05$) for a higher blastulation rate of bHPE with AA (77%) compared to the control DMSO group (55%), all groups of bHPE showed similar blastulation rate (range: 55%-77%) and levels of pluripotency markers ($p>0.05$). These data show that GSK3B inhibition and AA do not improve blastulation and expression of markers associated with embryonic pluripotency of bovine haploid parthenogenetic embryos. Further studies will be focused on evaluating the effects of these small molecules on derivation of haploid embryonic stem cell lines. Grant support: ANID – FONDECYT INICIACION 11230091.