ALTERED EXPRESSION OF PLURIPOTENCY AND EARLY LINEAGE SEGREGATION GENES IN IVP EQUINE EMBRYOS

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Compared to their in vivo counterparts, in vitro produced (IVP) horse embryos are smaller, have fewer cells, show developmental delay and, while pregnancy rates are comparable, may be more prone to pre-implantation failure (1,2). During mouse blastocyst formation, Oct4 and Cdx2 are essential for inner cell mass (ICM) and trophectoderm delineation respectively, while differential expression of Nanog and Gata6 determines which ICM cells form pluripotent epiblast or primitive endoderm respectively (3). Expression of these genes and other pluripotency markers (DPPA4, ESRRB, GDF3, SALL4, SOX2 and TERT) was used to examine the effects of IVP on early horse embryo development. Morulae, early and expanded blastocysts were recovered non-surgically from mares, or produced in vitro by ICSI of oocytes from slaughtered mares (n = 5, 7 and 9 per group at the respective stages). Gene expression was quantified by real-time PCR (4). Expression of most pluripotency genes decreased during the morula-to-early blastocyst (ESRRB and GDF3) or early-to-expanded blastocyst (DPPA4, NANOG, OCT4 and SOX2) transitions in vivo; consistent with a decreasing proportion of pluripotent (epiblast) cells. Expanded blastocysts showed reduced GATA6 and increased CDX2 expression presumably reflecting completion of primitive endoderm segregation, and trophectoderm proliferation. In IVP embryos, GDF3, TERT and GATA6 expression was undetectable, and SALL4 reduced. IVP morulae had undetectable CDX2 expression and reduced OCT4 compared to in vivo morulae. Additionally, the expected down-regulation of OCT4 and up-regulation of CDX2 during blastocyst expansion was not observed in vitro, while down-regulation of some pluripotency genes was delayed (ESRRB) or advanced (NANOG and SOX2). In summary, IVP embryos show an altered expression pattern for genes associated with cell lineage segregation. Whether this primarily reflects developmental retardation or indicates changes in the proportion of cells entering the 3 cell lineages remains to be investigated, as does the significance for developmental competence.