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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.

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