Derivation of Autologous Embryonic Stem Cell Lines for Stem Cell Therapy in an equine model of cartilage repair.

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INTRODUCTION: Recently, two groups have derived horse embryonic stem (ES)-like cell lines from in vivo-derived embryos [1, 2]. However, it is currently not known whether it is possible to obtain autologous ES cell lines from in vitro-derived horse parthenogenetic or cloned embryos. Our aim is to optimize culture conditions for the establishment of autologous ES lines from for cell therapy studies in an equine model.

METHODS: Equine in vivo-derived, and in vitro-derived parthenogenetic and cloned blastocysts were classified according to their age, developmental status and morphology of their inner cell mass (ICM). ICMs were isolated either mechanically, with trypsin or with pronase. ICMs were seeded either on equine embryonic fibroblasts (EEFs), equine umbilical cord matrix cells (EUCMCs), or bovine umbilical cord matrix cells (BUCMCs). For each condition, formation of primary ES cell colonies was evaluated. Molecular and biochemical characterization of the primary colonies and a parthenogenetic cell line was conducted to confirm the pluripotent stem cell identity.

RESULTS: Ratios of 66%, 48% and 48% of primary colonies were obtained from in vivo, parthenogenetic and cloned embryos, respectively. In all three groups, a higher ratio of primary colonies was obtained if day 6.0 to 7.5 embryos were used. In all groups, early blastocysts and expanded blastocysts gave rise to a higher ratio of primary colonies than late blastocysts. A significantly higher proportion of primary colonies was observed from blastocysts in which the ICM was clearly visible upon morphological examination in all three groups. For in vivo embryos, a greater proportion (72%) of ICMs dissected with trypsin gave rise to primary colonies, whereas for parthenogenetic and cloned embryos, higher ratios of primary colonies were obtained when ICMs were dissected with pronase (41% and 47%). No significant difference was observed when EEFs, EUCMCs or BUCMCs feeder layers were used for derivation. Importantly, primary ES colonies display alkaline phosphatase activity and express molecular markers of pluripotency Oct4, Nanog, and Sox2. ES-like cell lines were maintained for several passages, and display high nucleus-to-cytoplasm ratio, alkaline phosphatase activity, Oct4, Nanog and Sox2 expression, but also Cdx2 expression. Immunohistochemical analysis in equine embryos indicates the presence of Oct4 in the trophectoderm as well as in the ICM.

DISCUSSION & CONCLUSIONS: This study shows that it is possible to derive primary ES cell colonies from in vitro-derived equine parthenogenetic and cloned embryos. Both the use of early stage blastocysts with a clearly visible ICM, and the use of pronase to dissect the ICM allow the derivation of a higher proportion of primary ES cell colonies from these embryos. However, our work indicates that markers currently available to characterise horse ES cells are not adequate, and further knowledge of ES cell biology in the horse is required. This work represents an important first step toward the production of autologous equine ES cells for pre-clinical cell therapy studies on large animal models.

REFERENCES: [1] Saito et al., FEBS Lett. 2002 Nov;531(3):389-96. [2] Li et al., Stem Cells Dev. 2006 Aug; 15(4):523-31.

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