A300E Cloning, transgenesis, and stem cells

Effect of Estradiol and Progesterone on ovine Amniotic Epithelial Cells

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Keywords: oAEC, estradiol, progesterone.

This study was designed to clarify Estradiol (E2) and Progesterone (P4) steroid effects on ovine Amniotic Epithelial Cells (oAECs) that has a conserved plasticity and highly self-renewable capacity (Parolini et al., Stem Cells, 26(2), 300–311, 2008; Barboni et al., Stem Cell Rev Rep, 10:725–741, 2014). Based on their conserved immunomodulatory properties, oAECs are suitable for allo and xeno-transplantation (Barboni et al., Cell Transplant, 21(11), 2377–2395, 2012; Muttini et al., Res Vet Sci, 94(1),158–169, 2013). To date, no information is present on the effects of prolonged steroid exposition on AECs. oAECs were cultured as previously reported (Barboni et al., Cell Transplant. 21(11), 2377–2395, 2012) and treated with 12.5µM and 25µM of E2 or P4 (Sigma-Aldrich, Milan, Italy), alone and in both combinations, for three passages. Untreated cells were marked control (CTR). At 70% confluency, cells were detached for doubling time (DT) evaluation. Cells at fourth passage were differentiated for 21 days in osteogenic media (DM) (Mattioli et al., Cell Biol Int 36(1):7-19, 2012) without steroid. Alizarin Red and Alcian-Blue (Sigma-Aldrich, Milano, Italy) stainings were performed. RNA and cDNA were obtained as previously reported (Barboni et al., Cell Transplant. 21(11), 2377–2395, 2012). Real Time for *NANOG*, *SOX2* ,*OCT4* stemness genes expression were performed by SensiFast SYBR (Bioline, Aurogene, Rome, Italy) using specific primers (Mattioli et al., Cell Biol Int. 36(1):7-19, 2012).The protocol was: 5 min at 95°C, 30 cycles at 95°C for 15 sec, 60°C for 30 sec, 72°C for 15 sec. Comparative Ct $2^{-\Delta\Delta C(t)}$ normalization to *GAPDH* was applied. IHC analyses were carried out for Cytokeratin 8 and αSMA expression as previously reported (Barboni et al. PLoS ONE 7(2): e30974, 2012). Data expressed as mean (±SD), compared by one-way ANOVA followed by Tukey's test (GraphPad Prism 5). Significant values for $P < 0.05$. Steroids treated ovine AECs proliferate with significant differences between concentrations. While P4 treated cells showed cuboidal shape and Cytokeratin expression until third passage, CTR and E2 treated cells showed a rapid downregulation of Cytokeratin and increased αSMA expression. oAECs with E2+P4 showed both cell type morphology. Steroids modified stemness genes based on the concentration. 12.5 µM E2, 25µM P4 and 25µM of both E2+P4 treatments maintained higher *OCT4*, *NANOG* and *SOX2* expressions in treated cells despite their progressive downregulation in the CTR. Moreover, compared to CTR, after Alizarin staining, steroid pretreated cells suffered morphological changes under DM acquiring Alcian Blue-positive chondrogenic-like morphology. AECs stemness properties and plasticity can be modified by prolonged steroidal treatment. These data improve our knowledge, opening new prospective on oAEC use in stem cell-based therapy.
Acknowledgments. Research supported by H2020-MSCA ITN EJD-REP BIOTECH 675526.