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Effects of a polylactic acid, 3-D printed scaffold, on bovine embryo development in vitro

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The development of 3D printers and the advances in materials science and tissue engineering in the last years have allowed the implementation of this technology in several research areas. So, it has been used as a tool to create a 3D-oviduct-on-a-chip with poly(dimethylsiloxane) that mimics de physiological environment, which has proved to be useful in reducing the differences between in vitro and in vivo produced embryos (Ferraz et al, Nat commun, 9(1), 1-14, 2018). As an alternative, we suggested polylactic acid (PLA) as a great candidate to generate engineered 3D scaffolds due to its high biocompatibility and mechanical properties (Chi et al, BMC chem, 14(1), 1-12, 2020). Our goal was to evaluate for the first time, the feasibility of PLA scaffolds printed by the fused filament fabrication method to support IVF, by means of resulting blastocyst rates and total cell number. IVF was performed under 3 different conditions (N=4 replicates): i) Conventional IVF (Parrish et al., Biol Reprod, 38(5), 1171-1180, 1988) (control group, N=360) ii) IVF in a medium conditioned by the scaffold for 24h (rinse group, N=215) and iii) IVF inside the scaffold used for the rinse group (scaffold group, N=190). Before IVF, the scaffolds were sterilized in 70% ethanol for 1h, washed 4 times for 5 min in PBS and air-dried at room temperature. For IVF, in vitro matured oocytes were washed and cultured in Fert-TALP medium (Parrish, Theriogenology, 81(1), 67-73,2014) for 22h with frozen-thawed bull sperm ($1x10^6$spz/ml) selected by Bovipure gradient (Nidacon, Sweden). Putative zygotes were cultured in microdrops of SOF medium (Holm et al, Theriogenology, 52(4), 683-700, 1999) supplemented with 0.3% BSA (w/v) covered with paraffin oil (Nidoil, Nidacon). Cleavage (48h) and blastocyst rates (day 8) were evaluated. At day 8, blastocysts were fixed in glutaraldehyde and stained with Hoechst 33342 to assess their cell number by fluorescence microscopy. The parameters were analyzed by Kruskal-Wallis one-way ANOVA test when the distribution was not normal and by one-way ANOVA when it was. Differences were considered significant when $p<0.05$. Regarding cleavage rate, it was significantly higher in the control group (79.4±2.1%^{a}) than in the rinse and scaffold groups (30.7±3.2%^{b} and 52.6±3.6%^{c}, respectively). Furthermore, the control group showed a higher blastocyst rate (25.6±2.3%^{a}), than the scaffold and rinse groups (14.1±2.5%^{b} and 2.3±1.0%^{c}, respectively). As for the total cell number, no significant differences were found (78.2±3.6 for control, 89.6±22.3 for rinse, and 79.4±7.0 for scaffold). It's known that the incubation of PLA in PBS causes a rapid drop in pH on the first day, probably due to the release of lactic acid, a known PLA degradation byproduct (Diomede et al, Stem Cell Res Ther, 9(1), 1-21, 2018). This fact could explain the differences between groups, since the rinse group contained the medium that had been in contact with PLA for 24 hours. Further experiments controlling the post-culture pH could corroborate such hypothesis. In conclusion, the current data demonstrates that PLA does not seem te be applicable to use for 3D scaffolds for IVF. Supported by Fundación Séneca reference 21651/PDC/21.

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