Animal Reproduction

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CLONING, TRANSGENESIS, AND STEM CELLS

A pregnancy of cloned minipigs: IVM, parthenogenesis and SCNT embryo transfer

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Gene-edited pigs are shown to be adequate as biomedical models, and due to their similarities with humans, they are used for research, such as surgeries, disease modeling, and cancer therapy studies. To produce these animals, it is necessary to combine biotechnologies such as gene editing, in vitro production (IVP), and somatic cell nuclear transfer (SCNT). Therefore, this study aimed to produce cloned pigs after SCNT using a minipig model, including optimizing IVM, parthenogenesis, and embryo transfer (ET). For that, cumulus-oocyte complexes (COCs) were aspirated from ovaries collected from postmortem swine females (gilts >150 days and sows >3 years). COCs were selected and in vitro matured in IVM medium (TCM199 B supplemented with 20% porcine follicular fluid, 100µg/mL cysteine, 0.91mM sodium pyruvate, 3.05mM D-glucose, 10ng/mL EGF, 20µg/mL gentamicin, 10µg/mL FSH, 5UI/mL hCG, and 1mM cAMP), in groups of 50 COCs per well in an incubator at 38.5°C, 5% CO2, and humidified air for 44–46 hours. To produce parthenotes, mature oocytes were activated with 15mM Ionomycin, 10µg/mL Cycloheximide, 7.5µg/mL Cytochalasin B, and 10mM Strontium. The parthenotes were in vitro cultured (IVC) for 7 days in a PZM3 medium. Eighteen routines of IVP (~5 months) were necessary to standardize the IVM medium and parthenogenetic activation. The average IVM rate obtained was 72.26% ± 4.93%. Additionally, the average parthenote blastocysts rate was 28.47% ±0.06%, with a total number of cells (measured by DAPI staining) averaging 100.17 ± 3.44 nuclei. Then, two SCNT routines (NT1 and NT2) were performed without Hoechst 33342, instead, 10µg/mL demecolcine was used before the enucleation step. The donor cells were derived from an adult male mini-pig, were in vitro cultured at the 5th passage, and synchronized with 48 hours of confluence (G0/G1). For the reconstruction of zygotes, the activation was the same as parthenotes, and the electrofusion was performed using a single direct current (DC) pulse of 1.6KV/cm with 70µs of duration. In total, 140 cloned embryos were produced (at 2 and 4 cells) and surgically transferred through laparotomy with general anesthesia to one surrogate gilt (>180 days) previously synchronized using 32mg progesterone for 14 days. To increase gestational signaling, 22 parthenotes at 4 cells (Day 2) were co-transferred. The pregnancy diagnosis was performed by ultrasonography (US) at day 25 after embryo transfer (ET) observing the presence of at least 7 embryonic vesicles, and kept at days 35, 45, 55, and 65, with the presence of the fetuses and vascularization. In conclusion, it was possible to obtain pregnancy after two SCNT routines and a single surgical ET. Hence, the SCNT pregnancy rates were 4.32% (7/162), showing the possibility of enhancing the production of cloned pigs. The pregnant female will be evaluated and supplemented with 24mg progesterone daily until delivery, and the cloned offspring will be genotyped to confirm the origin.