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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Electroporation preceding *in vitro* fertilization of *in vitro* matured oocytes: Implications for bovine embryo development

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Electroporation (EP) offers promise for delivering exogenous molecules into cells and embryos for gene editing purposes. However, mosaicism poses a common challenge in genetic modification. It arises from incomplete genetic editing in embryo cells, influenced by the timing of gene editing component delivery into the zygote cytoplasm, coinciding with DNA replication onset at the one cell stage. We have assessed EP before *in vitro* fertilization (IVF) to potentially reduce mosaicism. However, voltage strengths and embryo development post-EP have remained unreported. To enlighten this topic, *in vitro* matured *cumulus*-oocyte complexes were randomly assembled in the following groups: A) Control A (n=149): IVF of oocytes surrounded by *cumulus* cells; B) Control B (n=171): IVF of denuded oocytes; C) EP plus IVF (EP of denuded oocytes followed by IVF; n=358); D) EP minus IVF (EP of denuded oocytes without IVF; n=339). The oocytes of groups C and D were randomly distributed into the following voltages: 3, 5, 10, 15, and 20 V/mm. EP was performed using the Nepa21 electroporator system (Nepagene, Japan) and the presumptive zygotes were cultured for seven days, with at least three replicates. Data was analyzed using the mixed linear model of the SAS Software, and presented as mean±SEM. Voltages of 3 and 5 V in Group C (EP with IVF) resulted in cleavage (74.7±5.2% and 69.4±3.7%, respectively) and blastocyst (33.3±5% and 35.1±5.9%, respectively) rates similar (P>0.05) to control groups A (81.5±3.6% and 43.5±8.3) and B (71.4±5.1% and 37.1±3.8%). There was no difference (P>0,05) between groups A and B. However, voltages of 10, 15 and 20 V resulted in cleavage (52.8±13.2%, 37.8±4% and 22.1±3.9%, respectively) and blastocyst (7.33±3.7%, 3.3±2.3% and 2.8±1.5%, respectively) rates lower (P<0.05) than voltages of 3 and 5 V and than control groups A and B. When IVF after EP was omitted (group D), all tested voltages resulted in cleavage (20.8±6.2%, 40.0±22.7%, 44.6±19.9%, 28.3±10.9% and 10.8±5.5% for 3, 5, 10, 15 and 20 V, respectively) and blastocyst (3.4±1.7%, 0%, 2.3±2.3%, 3.4±1.7% and 0% for 3, 5, 10, 15 and 20 V, respectively) rates lower (P<0.05) than control groups A and B. In conclusion, EP preceding IVF impairs the production of blastocysts when voltages of 10 V/mm and above are applied. Also, EP alone can induce the cleavage of in-vitro matured oocytes that eventually can develop up to blastocyst stage at very low rates. Voltages of 3 and 5 V/mm hold promise in delivering gene editing components before fertilization to reduce mosaicism, but further investigation is warranted to evaluate their efficacy and potential implications for gene editing. This study provides valuable insights into optimizing parameters for EP preceding IVF, demonstrating embryo potential to reach the blastocyst stage.
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