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EFFECT OF PULSED ELECTROMAGNETIC FIELD ON THE DEVELOPMENTAL COMPETENCE, QUALITY AND GENE EXPRESSION IN BUFFALO EMBRYOS PRODUCED BY HAND-GUIDED CLONING

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This study was carried out to i) standardize the frequency of pulsed electromagnetic field for treatment of cloned embryos, ii) study the effect of pulsed electromagnetic field on the developmental competence and quality of cloned embryos, iii) compare the expression level of some important genes in treated and untreated cloned, and IVF embryos. Cumulus cells, isolated from *in vitro* matured oocytes and established in culture, were exposed to different dosages (0, 10 µT, 15 µT, 30 µT or 60 µT) of Pulsed Electromagnetic Field (PEMF) for 1 or 3 h for optimizing the effective dosage of PEMF. The relative cell viability and cell proliferation was higher (P<0.05) at 15 µT and 30 µT dosage of PEMF than the controls, both at 1h and 3h. Then, the cumulus cells were subjected to PEMF treatment at 15 µT for 1 h or 30 µT for 3h and were then incubated for 10h. The relative expression level of *OCT4, SOX2, NANOG, BCL2, BAX, DNMT1, DNMT3A, DNMT3B, GLUT1, GSK3B, CCNB1, P53, SOD1, GPX1 and HSP70* but not that ofCASPASE3 and CATALASEwas found to differamong the treatment groups (15 µT-1h or 30 µT-3h) and untreated controls.Following PEMF treatment of embryos produced by Hand-guided cloning, the blastocyst rate was higher (P<0.05) than the controlsat 15µT dosage for 1h,(47.2±1.23% vs 43.6±1.23 %)and at 30µT dosage for 3h(51.4±1.36%vs 42.8±1.29%). Following TUNEL assay,total cell number of blastocysts of treatment groups 15 µT-1h and30 µT-3h,and untreated controlswas found to be similar. The apoptotic index, which was similar in blastocysts of 30 µT-3hand 15 µT-1hgroups and IVF blastocysts was lower (P<0.001) than in blastocysts of untreated control group.Differential staining revealed that the ratio of ICM:trophectoderm cells was higher (P<0.001) in IVF blastocysts than in cloned treated and untreated blastocysts.The global level of H3K18ac, which was similar in blastocysts of PEMF-treated (30 µT-3h) and untreated control groups, was lower (P<0.001) than the IVF blastocysts whereas, that of H3K27me3, was higher (P<0.001) in IVF blastocysts than in PEMF-treated cloned embryos which, in turn, washigher (P<0.001) than in untreated control blastocysts. The expressionlevel of *OCT4, SOX2, NANOG, CDX2, BCL2, BAX, DNMT1, DNMT3B, HDAC1 GLUT1, GSK3B, CCNB1, P53,* and HSP70was different amongthe PEMF-treated (30 µT-3h) and control blastocysts and those produced by IVF.In conclusion, the results of this study demonstrate thatPEMF treatmentof cumulus cells increases the relative cell viability and proliferation rate and alters the relative expression level of pluripotency-, epigenetics-, cell cycle-, stress- and apoptosis-related genes in cumulus cells. In addition, these results also show that PEMF treatment of cloned embryos at selected dosages (15 µT for 1h or 30 µT for 3h) increasesthe blastocyst rate, lowers the apoptotic index, changes the epigenetic status and alters the expression level of pluripotency-, cell cycle-, metabolism-, stress- and apoptosis-related genes in cloned blastocysts.

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