CRISPR/dCas9-Tet1-mediated targeted DNA demethylation of MTNR1A in porcine cumulus-oocyte complexes enhances oocyte maturation

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Oocyte maturation is a crucial aspect of in vitro embryo production (IVEP), as it directly impacts the subsequent fertilization and growth of porcine embryos. Failure to perform maturation accurately and under ideal conditions can negatively affect embryo development. Several studies have demonstrated the positive effects of melatonin on oocyte maturation and subsequent embryonic development in various animal species such as pigs. Melatonin receptors play a critical role in triggering physiological responses through different signal transduction pathways, such as the AKT and ERK pathways, which aid in nuclear maturation and cumulus layer expansion around oocytes. Therefore, overexpression of melatonin receptors could potentially affect the oocyte maturation process in vitro. In this study, we successfully used CRISPR/dCas9-Tet1 to demethylate melatonin receptor 1A (MTNR1A) in porcine oocytes as a novel strategy for in vitro oocyte maturation (IVM). We introduced 1 μL of both sgRNA and dCas9 into a carefully selected group of porcine oocytes in maturation media by transfection. The diameter of the cumulus-oocyte complexes (COCs) was assessed following a 22-hour incubation at a temperature of 39°C and a carbon dioxide concentration of 5% in wells containing hormones (equine chronic gonadotropin; eCG and hcG). The diameter of cumulus-oocyte complexes (COCs) was significantly increased in the groups treated with dCas9 and sgRNA (491.516 μm) compared to the control group (419.186 μm) ($p < 0.0001$). In addition, following an incubation period of 18-20 hours in wells without hormones, the group treated with dCas9 and sgRNA exhibited a greater proportion of grades 3 and 4 (48.173 %) when assessing cumulus cell expansion using a scoring system (Grade 1-4) compared to the control group (37.493 %) ($p < 0.01$). The CRISPR/dCas9-Tet1 system demonstrated efficient demethylation of endogenous MTNR1A in cumulus cells surrounding oocytes. Following in vitro fertilization (IVF) using fresh swine spermatozoa, the rate of nuclear maturation was compared to the percentage of oocytes in the MII phase, which was higher in the treatment group (67.90 % vs 62.78 %; $P < 0.01$). The cleavage and blastocyst rates on days 2 and 7, respectively, were compared between the treatment and control groups using in vitro culture (IVC) conditions of 38 °C, 5% CO2, and 5% O2. The results showed that both rates were greater in the treated group (Cleavage rate: 77.87 % vs 71.43 %; Blastocyst rate: 22.45 % vs 17.39 %; $P < 0.05$). In addition, during our assessment of the expression of genes related to cumulus cell expansion, we observed considerable upregulation of several genes involved in the AKT and ERK pathways in the treatment group. Notably, demethylation of MTNR1A using CRISPR/dCas9-Tet1 improved the maturation of oocytes damaged by reactive oxygen species (ROS). These findings suggest the potential use of the CRISPR/dCas9-Tet1 system to enhance the success rate of both normal and damaged oocytes during IVM.