Melatonin improves porcine oocyte maturation through regulation of F-actin associated mitochondrial fission *in vitro*

Seul-Gi Yang[1,2]; Hyo-Jin Park[2,3]; Deog-Bon Koo[1,2,3]

1. Department of Companion Animal Industry, Daegu University, 201 Daegudae-ro, Jillyang Gyeongsan, Gyeongbuk, 38453, Republic of Korea
2. DU Center for Infertility, Daegu University, 201 Daegudae-ro, Jillyang, Gyeongsan, Gyeongbuk, 38453, Republic of Korea
3. Department of Biotechnology, Daegu University, 201 Daegudae-ro, Jillyang, Gyeongsan, Gyeongbuk, 38453, Republic of Korea

Mitochondria play a crucial role in regulating cell survival and apoptosis by facilitating mitochondrial fission through the action of the dynamin-related protein (DRP1) during mammalian meiotic maturation and early embryonic development. Additionally, F-actin supports the migration and fission of mitochondria, essential for energy metabolism, maturation, proliferation, and development in mammalian cells. In our previous study, we demonstrated that melatonin enhances spindle assembly-related functions by reducing mitochondrial-derived superoxide during the maturation of porcine oocytes. In this study, we investigated the unclear relationship between the regulation of F-actin by antioxidants and mitochondrial fission. We confirmed a significant reduction ($p < 0.05$) in the expression levels of proteins related to mitochondrial fission, as well as decreases in mitochondrial activation and fragmentation, following Drp1 knockdown. Afterward, we found that the expressions of genes associated with mitochondrial dynamics, oxidative stress, and cytoskeleton were altered by Drp1 siRNA treatment during meiotic maturation, as revealed by mRNA-sequencing analysis. Changes in genes associated with the response to oxidative stress were particularly notable. Also, the expressions of tubulin formation, DNA repair, actin binding, and responses to oxidative stress-related genes significantly changed ($p < 0.05$), and the rate of abnormal spindle assembly and mitochondrial superoxide production increased ($p < 0.05$) in Drp1 siRNA treated oocytes. Based on these findings, we administered melatonin and MIT-001 to the Drp1 siRNA-treated group to mitigate oxidative stress during porcine oocyte maturation. In the groups treated with melatonin and MIT-001 following Drp1 siRNA administration, we observed a reduction in the expression of mitochondrial fission-related proteins, and the abnormalities in mitochondrial activation and morphology were restored to levels comparable

to those in the untreated group ($p < 0.05$). Furthermore, there was a decrease in the rate of abnormal spindle assembly and mitochondrial superoxide production ($p < 0.05$). Additionally, the expression of genes related to tubulin formation, DNA repair, actin binding, and responses to oxidative stress were largely restored ($p < 0.05$). In conclusion, we suggest that melatonin mediates the regulation of mitochondrial fission by reinstating the cytoskeleton in porcine oocytes with Drp1 knock-down. This cytoskeletal restoration is crucial for the enhancement of porcine oocyte maturation.
Keywords: Melatonin, Drp1, Cytoskeleton, Meiotic maturation, Porcine oocyte

Funding

This research was supported by the National Research Foundation of Korea (NRF) funded by the Korea government (MSIT) (NRF-2021R1C1C2009469 and NRF-2022R1A2C1002800) and the Basic Science Research Program through the NRF funded by the Ministry of Education (RS-2023-00246139), Republic of Korea.