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This study investigated the effects of non-thermal dielectric barrier discharge plasma on immature chicken Sertoli cell (SC) proliferation and the involvement of microRNAs in this process. Results showed that double exposure to 11.7 kV of plasma for 30 s with an interval time of 6 h produced maximum SC viability, cell growth, and cell cycle progression. This was possible through controlling reactive oxygen species homeostasis and improving energy metabolism by boosting the production of adenosine triphosphate and respiratory enzyme activity in the mitochondria. Optimum plasma treatment increased miR-7450 expression and led to a decrease in adenosine monophosphate-activated protein kinase α 1 (AMPK α 1) levels. On the other hand, miR-100 expression was reduced and led to an increase in mammalian target of rapamycin (mTOR) levels in SCs. A single-stranded synthetic miR-7450 antagomir and a double-stranded synthetic miR-100 agomir significantly inhibited SC proliferation. However, this can be ameliorated by an optimum plasma treatment. Our findings confirmed that miR-7450 and miR-100 are involved in the regulation of plasma-induced SC proliferation through targeting of the AMPK-mTOR signaling pathway; this finding might provide a potential safe strategy in the control of SC number and sperm production of cocks *in vivo*.

This research was supported by the R&D Program of “Plasma Advanced Technology for Agriculture and Food (Plasma Farming)” through the National Fusion Research Institute of Korea (NFRI) funded by the Government.