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# Non-thermal DBD plasma regulates chicken Sertoli cell proliferation via AMPK-mTOR signaling pathway

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**Abstract**

Non-thermal dielectric barrier discharge (DBD) plasma treatments are being investigated for multiple biological applications. Our previous work have found non-thermal plasma treatment can improve chicken sperm quality [1,2]. The number of Sertoli cells (SCs) decides sperm production. However, whether plasma treatment can regulate SC proliferation is unclear.

This study investigated the effects of non-thermal DBD plasma on immature chicken SC proliferation and the regulation mechanism. Our results showed that double exposure to 2.4 W of plasma for 30 s with an interval time of 6 h produced maximum SC viability, cell growth, and cell cycle progression. However, exposure time longer than 1 min or treatment with higher potentials significantly inhibited SC proliferation. Therefore, intensity and exposure time of DBD plasma should be optimized for promoting immature SC proliferation.

Adenosine monophosphate-activated protein kinase (AMPK)-mammalian target of rapamycin (mTOR) signaling pathway plays a crucial role in the regulation of SC proliferation via mitochondrial metabolism and biogenesis [3]. Our results suggested that chicken SC proliferation-promoting effect of optimum plasma treatment was regulated by increasing mitochondria, respiratory enzymes activities, and ATP production via AMPK-mTOR signaling pathway.

Optimum plasma treatment increased miR-7450 expression and led to a decrease in AMPKα1 level. On the other hand, miR-100 expression was reduced and led to an increase in mTOR level 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. AMPK-mTOR signaling can be regulated by miRNAs in a post-transcriptional level [4] in the regulation of SC proliferation. Therefore, our findings indicated that optimum plasma treatment improved chicken SC proliferation via the regulation of miR-7450 and miR-100 on AMPK-mTOR signaling pathway.

Taken together, our study suggests non-thermal DBD plasma treatment improves chicken SC proliferation via AMPK-mTOR signaling pathway, this findings will benefit for sperm production.

**References**

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**Figures**

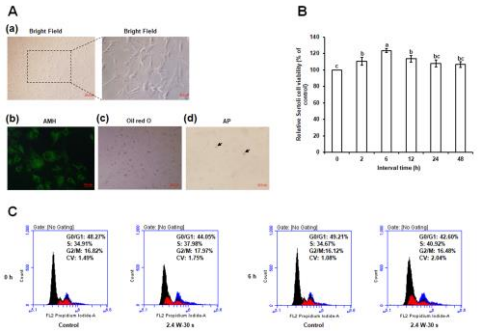

Figure 1. Effect of double plasma treatment with different intervals on SC viability and cell cycle.

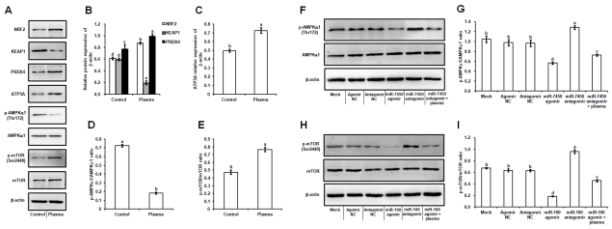

Figure 2. Effect of plasma, miRNA agomir and antagomir on chicken SC protein expression.

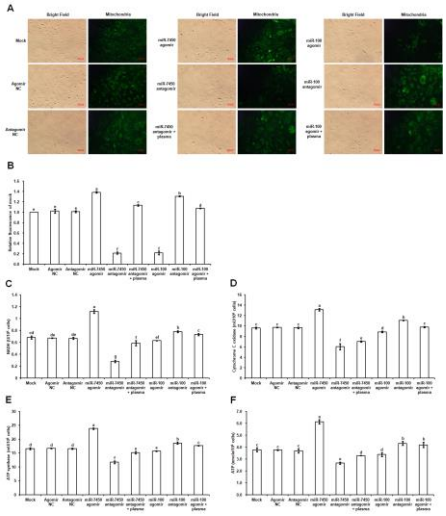

Figure 3. Effect of miRNA agomir and antagomir on mitochondria, mitochondrial respiratory enzymes, and ATP levels in SCs.