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Non-thermal DBD plasma treatment improves chicken sperm motility via the regulation of demethylation levels

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The quality of avian semen is an important economic trait in poultry production. The present study examines the *in vitro* effects of non-thermal dielectric barrier discharge plasma on chicken sperm to determine the plasma conditions that can produce the optimum sperm quality. Exposure to 11.7 kV of plasma for 20 s is found to produce maximum sperm motility by controlling the homeostasis of reactive oxygen species and boosting the release of adenosine triphosphate (ATP) and respiratory enzyme activity in the mitochondria. However, prolonged exposure or further increase in plasma potential impairs the sperm quality in a time- and dose-dependent manner. Optimal plasma treatment of sperm results in upregulated mRNA and protein expression of nuclear factor erythroid 2-related factor 2, peroxiredoxin 4, *ATP5A1*, and mammalian target of rapamycin by increasing their demethylation levels, but downregulated mRNA and protein expression of kelch-like ECH associated protein 1 and adenosine monophosphate-activated protein kinase  $\alpha 2$  by decreasing their demethylation levels. However, 27.6 kV of plasma exerts significant adverse effects. Thus, our findings indicate that appropriate plasma exposure conditions improve chicken sperm motility by regulating demethylation levels of genes involved in antioxidant defense and energetic metabolism; this finding might be beneficial to elevate the fertilization rate in poultry breeding.

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