NON-INVASIVE DELIVERY OF DSRNA INTO DE-WAXED TICK EGGS BY ELECTROPORATION

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Introduction: RNA interference-mediated gene silencing was shown to be an important functional genomics tool, specially for validation of candidate targets that may become anti-tick vaccine components. In this work, we improved this approach in the validation of components of molecular signaling cascades, such as the Protein Kinase B (AKT)/Glycogen Synthase Kinase (GSK) axis during tick embryogenesis, due to its importance in carbohydrate metabolism, cell survival and embryonic development. Objectives: Silencing AKT and GSK by electroporation for improved the delivery was removed the wax of eggs using heptane and hypochlorite, the effects of silencing in embryo tick metabolism was evaluated the glycogen levels and hatching rate of silenced eggs.

Material and methods: Nucleotide sequences encoding AKT and GSK were used to design primers for dsRNA delivery by electroporation and knockdown confirmation (qRT-PCR). Moreover, the hatching rates were determined in silenced and control eggs. To assess whether electroporation is capable of transporting molecules into the embryo, eggs were electroporated with dye fluorescent DAPI. Discussion and Results: It was shown that heptane and hypochlorite treatments of tick eggs can remove wax, affects integrity of corium with slight effects on embryo’s, but improved the entry of dsRNA into 7-day-old eggs. In addiction the electroporation was enabled to deliver the dye fluorescent into the eggs. Overall, the suppression of AKT and GSK transcripts was approximately 50% in both genes. Additionally, the effect of AKT silencing on embryo was evaluated by decreased of glycogen levels. Interestingly, GSK silencing in 7-day-old eggs caused a 50% reduction in hatching in comparison with eggs treated with unrelated dsRNA (dsMal control). Conclusions: We showed that electroporation can deliver DAPI to the embryos. These data demonstrate that electroporation of de-waxed R. microplus eggs could be used for gene silencing in tick embryos, and improve the knowledge about arthropod embryogenesis. Acknowledgements: FAPERJ, CNPq, INCT-EM, CNPq-CAPES

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