

Delivery of Pharmacological Inhibitors to the Early Embryo of *R. prolixus* By Ethanol Diffusion Through the Egg Chorion and Detergent-permeabilization of the Extraembryonic Layers

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Most vectors of arthropod-borne diseases produce large eggs with hard and opaque egg shells. In several species it is still not possible to induce molecular perturbations to the embryo by delivery of molecules using microinjections or eggshell permeabilization without losing embryo viability, which impairs basic studies regarding development in these models. Here we test the properties and permeability of the eggshell of *R. prolixus*, a Chagas disease vector, with the aim to deliver dsRNAs, transgenesis vectors and pharmacological inhibitors to the egg cytoplasm and allow controlled molecular perturbations in the embryo.

Using field-emission scanning electron microscopy, we found that the egg is coated by three layers: chorion, vitelline membrane and plasma membrane, and that the pores that allow gas exchange (aeropiles) have an average of $2 \pm 0.2 \mu\text{m}$ in diameter and are found in the rim of operculum at the anterior pole of the egg. Stereoscope-witnessed immersions of the eggs in a droplet of ethanol leads to the penetration of this solvent through those pores, without affecting embryo viability. Immersions of fertilized eggs for 5 min in pharmacological inhibitors, such as sodium azide, potassium cyanide and cycloheximide solubilized in ethanol resulted in impairment of embryogenesis before gastrulation stages in a dose-dependent manner, indicating that these inhibitors could reach the egg cytoplasm. Concentrations of $0.30 \mu\text{M}$ azide, 5mM cyanide, and 35mM cycloheximide were required to induce 100 % abortion of embryogenesis.

Thus, we established that delivery of pharmacological inhibitors to the egg cytoplasm of *R. prolixus* can be performed by submersing the fertilized eggs in ethanol with no need for additional methods such as microinjections or electroporation. We are currently testing protocols to increase the permeability of this protocol by adding detergents and/or electroporation steps to allow the delivery of larger molecules like dsRNAs and transgenesis vectors to the egg.

Key words: Pharmacological Inhibitors, *Rhodnius prolixus* and Permeabilization.
Patronage: FAPESP, CNPq and CAPES