Title: Ferritin 1 silencing effect in Rhipicephalus sanguineus sensu lato (Acari: Ixodidae) oogenesis during Ehrlichia canis interaction

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Abstract

Introduction: Ticks are able to act as vectors of different pathogens, such as viruses, protozoa, bacteria and filarial nematodes. The brown dog tick *Rhipicephalus sanguineus* has a worldwide distribution, being particularly frequent in tropical and subtropical regions. This tick is known to act as main vector of *Ehrlichia canis*, the causative agent of canine monocytic ehrlichiosis (CME), a potentially fatal tick-borne disease of dogs. Vaccines have been shown as a promising tick control method, however identification of tick protective antigens remains a limitative step in vaccine development.

Aim: The main objective of this study was to evaluate, by functional analysis, the role of the gene that encodes for the protein ferritin 1 on the ovaries and egg development.

Methods: For this, a two-month-old male German shepherd dog was inoculated I.V. with 4.5 mL of *E. canis* purified from DH82 cells. The transcript correspondent to *ferritin 1* was selected from a previously reported *R. sanguineus* sialome database. Thirty adult females *per* group were nanoinjected between the coxa and trochanter with double-stranded RNA (*ds*RNA) for *ferritin 1*. The control group was injected with the same volume of elution buffer. After, injected female ticks were incubated overnight and then placed into separated alimentary chambers attached to the dog dorsum, together with the same number of male ticks to



stimulate co-feeding, and allowed to feed until the drop-off. Ovaries were then dissected to evaluate the effect of gene silencing by electronic microscopy, histological analysis and histochemistry.

Results: Results have shown that in the control group the ovaries had a larger amount of oocytes in advanced development stage, when compared to ovaries of the silenced group. Furthermore, ovaries from the latter group presented mature oocytes in a degenerative and/or reabsorption process. It has been reported that ferritins proteins play a role in tick reproduction, among other functions related with iron metabolism during the blood meals. **Conclusions:** Overall results revealed that this protein affects oogenesis in this tick species, consequently the reproduction ability, and that further investigation is worth pursuing to elucidate the mechanisms by which this action occurs.