Review Article

The dynamics of energy metabolism in the tick embryo

A dinâmica do metabolismo energético em embriões de carrapato

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Abstract

The cattle tick *Rhipicephalus (Boophilus) microplus* is an ectoparasite capable of transmitting a large number of pathogens, causing considerable losses in the cattle industry, with substantial damage to livestock. Over the years, important stages of its life cycle, such as the embryo, have been largely ignored by researchers. Tick embryogenesis has been typically described as an energy-consuming process, sustaining cell proliferation, differentiation, and growth. During the embryonic stage of arthropods, there is mobilization of metabolites of maternal origin for the development of organs and tissues of the embryo. Glycogen resynthesis in late embryogenesis is considered as an effective indicator of embryonic integrity. In the cattle tick *R.(B. (B.) microplus,* glycogen resynthesis is sustained by protein degradation through the gluconeogenesis pathway at the end of the embryonic period. Despite recent advancements in research on tick energy metabolism at the molecular level, the dynamics of nutrient utilization during *R. (B.) microplus* embryogenesis is still poorly understood. The present review aims to describe the regulatory mechanisms of carbohydrate metabolism during maternal-zygotic transition and identify possible new targets for the development of novel drugs and other control measures against *R. (B.) microplus* infestations.

Keywords: Energy metabolism, embryogenesis, *Rhipicephalus (B.) microplus*.

Resumo

O carrapato bovino *Rhipicephalus (B.) microplus* é um ectoparasita capaz de transmitir diversos patógenos, sendo responsável por grandes perdas na pecuária pelos danos causados ao gado. Atualmente, muitos estudos têm negligenciado fases importantes do ciclo de vida deste parasita, como a fase embrionária. A embriogênese é classicamente descrita como um processo que demanda um consumo de energia, possibilitando a proliferação celular, diferenciação e crescimento. Além disso, em artrópodes, o estágio da embriogênese é caracterizado pela mobilização de metabolitos de origem materna para o desenvolvimento de novos tecidos e órgãos. A ressíntese de glicogênio no final da embriogênese tem sido descrita em diversas espécies de artrópodes, sendo considerada um indicador de integridade do embrião. No caso do *R. (B.) microplus* a ressíntese de glicogênio é sustentada pela degradação de proteínas durante a gliconeogênese, no terço final da embriogênese. Apesar dos recentes avanços, no estudo molecular e do metabolismo energético, os mecanismos envolvidos na dinâmica da utilização de diferentes substratos energéticos durante a embriogênese do carrapato *R. (B.) microplus* ainda é pouco entendido. Diante deste panorama, estudos que descrevam a regulação destes mecanismos e da associação do metabolismo de carboidratos com a transição materno zigótica, pode auxiliar na busca de novos alvos para o desenvolvimento de novos acaricidas e outras intervenções para o controle infestações de *R. (B.) microplus*.

Palavras-chave: Metabolismo energético, embriogenese e *Rhipicephalus (B.) microplus*.

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Introduction

Oviparous animals face embryogenesis in the absence of exogenous nutrient supply. In this case, maternal nutrients are packaged into the female oocytes during oogenesis (FAGOTTO, 1990; SAPPINGTON & RAIKHEL, 1998). In arthropods oogenesis the oocytes exhibit fast growth, accumulate carbohydrates, lipids and proteins that will meet the regulatory and metabolic needs of the developing embryo (CHIPPENDALE, 1978).

The current literature provides ample information regarding metabolic events during larval and adult phases of several arthropods (BRIEGEL et al., 2003; GOLDSTROHM et al., 2003; ZHOU et al., 2004a,b; BROWN et al., 2008). Nevertheless, aspects concerning energy metabolism such as the activity of central metabolic pathways in arthropod embryogenesis or the determination of energy reserves to be used are scarce. In the fruit fly *Drosophila melanogaster* an increase in glycogen content strongly correlated with protein levels in follicles and young embryos has been described (SHIOMI & KITAZUME, 1956; MEDINA & VALLEJO, 1989; GUTZEIT et al., 1993). Histochemical studies reveal that glycogen is the predominant form of carbohydrate storage in *D. melanogaster* eggs (YAMAZAKI & YANAGAWA, 2003). Furthermore, changes in protein content occur in an opposite direction to that determined for the carbohydrate content (GUTZEIT et al., 1993).

Thus, the regulation of glucose metabolism pathways and the dynamics of nutrient utilization in embryogenesis often differ from species to species. Therefore, studying these events in different arthropods allow us to understand the regulatory mechanisms of energy metabolism, finding potential targets for the development of vaccines and drugs against these diseases vectors.

Embryogenesis in arthropod model

The fruit fly *D. melanogaster* has been used as a model organism for the understanding of the molecular mechanisms that orchestrate arthropod development (OBER et al., 2012). There are many molecular and genetic tools available for studying the events involved in cell differentiation of *D. melanogaster* embryos and gene regulatory networks that control this process which makes this Diptera the best known multicellular organism (ROY et al., 2010). The understanding of the complex embryo gene regulatory networks and gene interactions may unravel the hidden secrets of insect evolution (DAVIS & PATEL, 2002; LYNCH & DESPLAN, 2003).

The pre-gastrulation embryo should be defined as a two-dimensional Cartesian coordinate system (NÜSSLEIN-VOLHARD & WIESCHAUS, 1980) with bilateral symmetry and clear anterior-posterior (AP) and dorsal-ventral (DV) axes which are independent but develop simultaneously. Predetermined embryonic cell fate is possible if maternal mRNA is deposited in oocytes providing positional information that can be interpreted and refined by downstream target genes (NÜSSLEIN-VOLHARD et al., 1987). Combined approaches have been used to understand the transitions that occur in embryonic patterning strategies during the

evolution of insects (PRPIC et al., 2008). One particular approach has generated greater understanding of insect embryogenesis. Comparing hemimetabolous and holometabolous insects with a short-germ type of development with those insects with a long-germ type of development such as *Drosophila* may reveal how gene regulatory networks evolved in insects and may also unravel common strategies that would be linked to the transition from short-germ type to long-germ type of embryogenesis (DAVIS & PATEL, 2002).

During the early stages of its development, the embryo undergoes maternal-to-zygotic transition (MZT), which is a stage in embryonic development characterized by extensive mRNA degradation followed by the activation of zygotic transcription. During oocyte formation, the female lays eggs containing mRNA, protein, and nutrients necessary for zygotic development (TADROS & LIPSHITZ, 2009). After fertilization, the genetic material deposited by the female in the oocyte controls the majority of the early events of embryogenesis (DE RENZIS et al., 2007).

MZT occurs in a variety of organisms including echinoderms, nematodes, insects, fish, amphibians, and mammals. The MZT of *Drosophila melanogaster* is completed in about 2.5 hours. In the mouse MZT occurs within approximately 22 hours after fertilization (SIMPSON & WIESCHAUS, 1990). In *D. melanogaster*, maternal transcript destabilization consists of two major events. The first one is exclusively maternal and occurs after oocyte activation even in the absence of zygotic products, while the second one takes place after zygotic activation (TADROS & LIPSHITZ, 2009). The RNA binding protein Smaug (SMG) is involved in the maternal transcript destabilization of *D. melanogaster* (TADROS et al., 2003; SEMOTOK et al., 2005; SEMOTOK et al., 2008).

The first zygotic genes activated during *D. melanogaster* embryogenesis display variants of sequences termed "TAGteam" approximately 500 bp upstream of its transcriptional start sites (TEN BOSCH et al., 2006). Studies have identified that the protein Zelda, which is encoded by the zinc-finger gene *zld,* binds to the TAGteam promoting its transcription (LIANG et al., 2008). In the *D. melanogaster* embryo, two zygotic clusters are activated during mitotic cycles 8 and 14, and their zygotic mRNA targets are mostly transcription factors (DE RENZIS et al., 2007). Some of these genes are responsible for establishing the anteroposterior (AP) and dorsoventral (DV) axes during the embryonic syncytial and cellular blastoderm stages, while others possibly induce zygotic genome activation (SATIJA & BRADLEY, 2012). The role of *zld* during MZT has been recently shown to be conserved in different insect species including a role in posterior embryonic patterning (RIBEIRO et al., 2017).

The majority of the studies on the *D. melanogaster* embryo have focused on transcription factors and signaling molecules during early development. In contrast, much less is currently known about the regulation of the metabolic status during these stages, as a limited number of studies have been conducted in order to investigate this particular aspect of the insect embryogenesis (FRAGA et al., 2013) (Figure 1).
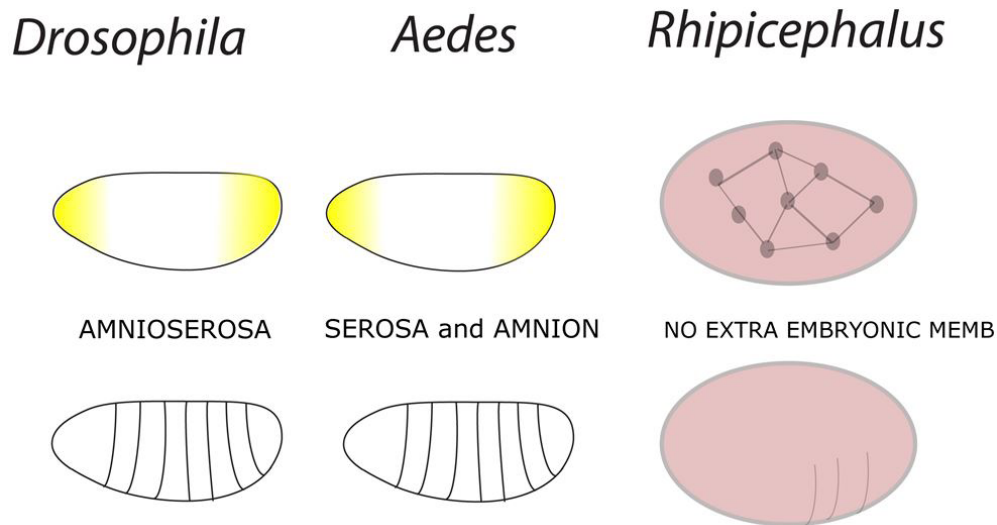
Figure 1. Representation of *Rhipicephalus (B.) microplus, Aedes aegypti, Drosophila melagnogaster* embryonic development. While chelicerates such as *R. (B.) microplus* do not display extra-embryonic membranes, presumably are cellularized at early stages of development and perform posterior segmentation via a secondary process (SANTOS et al., 2013), mosquitoes and fruit flies are Diptera with long-germ development, where the embryo occupies most of egg length already at early stages of development.

Dynamics of energy metabolism in arthropod vectors

The blood-sucking arthropod disease vectors are responsible for major epidemics throughout human history such as malaria, yellow fever, plague and Tifo. These arthropods display a great challenge for biological control, since they are responsible for thousands of deaths, causing great harm on public health in the world. Among these arthropod vectors, the mosquito *Aedes aegypti* is responsible for the largest disease transmission rates in tropical and subtropical countries. This mosquito transmits chikungunya, zika, dengue and yellow fever virus, both of particular importance in Brazil, where it is estimated that 50 million dengue infections occur annually (BRASIL, 2017). Other important vectors of diseases are the ticks, affecting both human and animal population in these countries, transmitting babesiosis, ricketisiosis and anaplasmosis (SILVA & SILVA, 1999; CORSON et al., 2004; SONENSHINE et al., 2006). Ticks cause considerable losses to the cattle industry worldwide and incur substantial damage to livestock (GRISI et al., 2014). Altogether the economic losses caused by *R. (B.) microplus* to livestock industry and the cost of tick control in Brazil, are estimated at 3 billion U.S. dollars per year (GRISI et al., 2014).

Research groups around the world have studied the disease vectors energy metabolism in order to find possible targets to control these arthropods. Nutrient synthesis and degradation are the basis of life, allowing growth and reproduction, maintaining their structures to correctly respond to their environment. This set of reactions is called metabolism, which must be finely adjusted in order to maintain the energy integrity of the organism. Therefore, the understanding of arthropod dynamic energy metabolism and its nutrients utilization contribute to effective drug and vaccine development to control these vectors. However, despite the current literature provide extensive information on the

metabolic events during larval and adult stage of arthropod vectors (CHANDRA, et al., 2008; SERVICE, 1983; RAGHAVENDRA & SUBBARAO, 2002) other important life cycle stages, such as embryogenesis have been largely neglected.

Embryogenesis in Arthropods

Embryogenesis has been typically described as an energy consuming process (THOMPSON & STEWART, 1997; VLECK & HOYT, 1991). In such context, energy metabolism needs to be finely regulated since the egg constitutes a closed system which relies solely on yolk contents for embryo energy maintenance and organ development (FAGOTTO, 1990; YAMAMOTO & TAKAHASHI 1993, LOGULLO et al., 1998). Once embryonic development is complete, hatching occurs. Yolk remains inside the midgut of the larval tick (Taheri et al., 2014). During the starvation period, ticks are sustained by reserve substances stored in their midguts (LOGULLO et al., 1998). Larval stages of the Lone Star Tick *Amblyomma americanun* can survive starvation for approximately 8 months whereas the nymphal and adult stages are able to survive starvation for 6 months and 19 months, respectively (GODDARD & VARELA-STOKES, 2009) (Figure 1).

Other tick species can survive longer periods without feeding such as the adult stages of *Ornithodoros erraticus* that under natural conditions can withstand starvation for several years (PALMA et al., 2012). Larvae of the tick *Amblyomma* spp. can survive starvation for 6 months while nymphs of this tick species can survive up to 12 months of starvation (DIAMANT & STRICKLAND, 1965). In extreme climatic conditions, larval stages of the tick *R. (B.) microplus* can survive over 6 months without feeding (GONZALES et al., 1974). A dynamic regulation of energy metabolism is required for ticks to survive without feeding for prolonged periods of time.

Energy metabolism in the arthropod embryo

Our research group has investigated the mechanisms underlying energy metabolism during the embryonic development of the cattle tick *R. (B.) microplus*. Our results have provided insights into the dynamics of nutrient utilization during tick embryogenesis. Information generated from our studies may help finding candidate antigens for the development of vaccines against ticks (MORAES et al., 2007; LOGULLO et al., 2009; FABRES et al., 2010; ABREU et al., 2013).

The study of molecules that participate in intracellular pathways of the developing tick embryo could reveal the complex networks that regulate metabolism during embryonic development in various organisms. Thus, research characterizing control mechanisms and identifying key enzymes involved in the energy metabolism of *R. (B.) microplus* may help in the identification of potential new vaccine target antigens in order to develop novel immunotherapeutic strategies against the cattle tick and implement efficient tick eradication and control measures (CAMPOS et al., 2006; MORAES et al., 2007).

Our findings indicate that there is mutual regulation among major enzymes of glucose metabolism at the transcriptional and enzymatic levels (SILVA et al., 2015). In our metabolomics research, metabolite levels from metabolic routes in *D. melanogaster* were measured. This approach furthered our understanding of substrates involved in carbohydrate catabolism and anabolic pathways of insects (AN et al., 2014). These findings have expanded our knowledge about the physiology of hematophagous arthropods and energy metabolism of ticks (MORAES et al., 2007; LOGULLO et al. 2009; SILVA et al., 2015). Furthermore, the investigation of key components of these metabolic pathways has provided invaluable information for the development and improvement of vaccines and drugs to control important disease vectors. Although these events are primarily studied in embryonic cells or during embryogenesis (ABREU et al., 2013; AN et al., 2014), some of the aspects of glucose metabolism have also been investigated in adult arthropod tissues and organs before or after a blood meal (VITAL et al., 2010).

There is a relationship between blood ingested and the number of eggs laid in hematophagous arthropods (Table 1). The female adult tick must have a blood meal in order to complete oogenesis and consequently their reproductive success since all the nutrients required for oogenesis are present in the ingested blood (GRIMES, 1980). Table 1 shows the following information about the feeding habits of three different species of hematophagous arthropods - *Rhipicephalus (B.) microplus, Rhodnius prolixus*, and *Aedes aegypti*:

the weight gain of the blood-feeding arthropod after a blood meal; the number of eggs laid by these females after a blood meal; and the volume blood ingested by these females during one blood meal. These classical studies often investigate the hormonal signaling that regulates the synthesis and transport of nutrients from the fatty body to the ovaries, and consequently their deposition in the oocytes in response of the blood meal. Therefore, these studies did not focus on the maternal-zygotic transition or on the energy metabolism of the embryo itself.

In arthropods, classical studies on embryonic metabolism have demonstrated that glycogen reserves are important to sustain the development of the embryo (FAGOTTO, 1990; MORAES et al., 2007, CAMPOS et al., 2006, LOGULLO et al., 2009; VITAL et al., 2010). During *Drosophila melanogaster* oogenesis, glycogen is predominantely accumulated in mature ovarian follicles and is a major storage form of carbohydrate in eggs (GUTZEIT et al., 1993). In the early stages of *Drosophila* embryogenesis, protein metabolism and glycogen metabolism are negatively correlated since total protein levels decline as glycogen levels increase (SHIOMI & KITAZUME, 1956; MEDINA AND VALLEJO, 1989; GUTZEIT et al., 1993). Other studies have shown that glycogen levels vary during *Drosophila* embryogenesis, and that abundant glycogen stores are present during the late stages of the fly embryogenesis (YAMAZAKI & NUSSE, 2002; YAMAZAKI & YANAGAWA, 2003).

Glycogen metabolism during the embryonic development of the tick *R. (B.) microplus* is similar to that of the fly *D. melanogaster*. During early development of the tick embryo, glycogen degradation occurs while during the late stages of embryogenesis glycogen reserves are resynthesized and protein degradation ensues. Additionally, the proteomic profiles of sequential stages of *R. (B.) microplus* ovaries have been studied and showed the temporal dynamics of the proteins during ovary development. It was observed a significant variation in the levels of proteins involved in carbohydrate metabolism, especially enzymes involved in glycogen metabolism (XAVIER et al., 2018).

During the final stage of tick embryogenesis, gluconeogenesis is intensified to provide the embryo with an adequate glucose supply. Amino acids play an important role as substrates for gluconeogenesis and subsequent glycogen resynthesis during the final stages of embryogenesis in these arthropods (MORAES et al., 2007). During the embryogenesis of *Rhipicephalus (B.) microplus,* lipids, carbohydrates and proteins are used as major energy sources (Figure 2), Our studies show that gluconeogenesis makes a significant contribution to maintain the energy balance in the late stages of

Table 1. Comparison between three different species of hematophagous arthropods - *Rhipicephalus (B.) microplus, Rhodnius prolixus*, and *Aedes aegypti* - with regard to their feeding habits: the volume blood ingested by a female during one blood meal; the weight gain of the blood-feeding arthropod after a blood meal, and the number of eggs laid by these females after a blood meal.

	Rhipicephalus (B.) microplus	*Rhodnius prolixus*	*Aedes aegypti*
Blood volume ingested	300-500 µl	297 µl**	2,5 µl
Weight gain after a blood meal	150-200 mg	236,8 mg	3 mg
Number of eggs laid*	3000	574	75

*The number of eggs laid is mean values per female; **The volume of blood is the average flow rate of 0,33 µl per second.Sources: (BUXTON, 1930; WOKE et al., 1956; DAVEY et al., 1986; MADEIRA et al., 2002; ARÉVALO et al., 2007; RUEGG & DAVEY, 2012; REY & O'CONNELL, 2014).
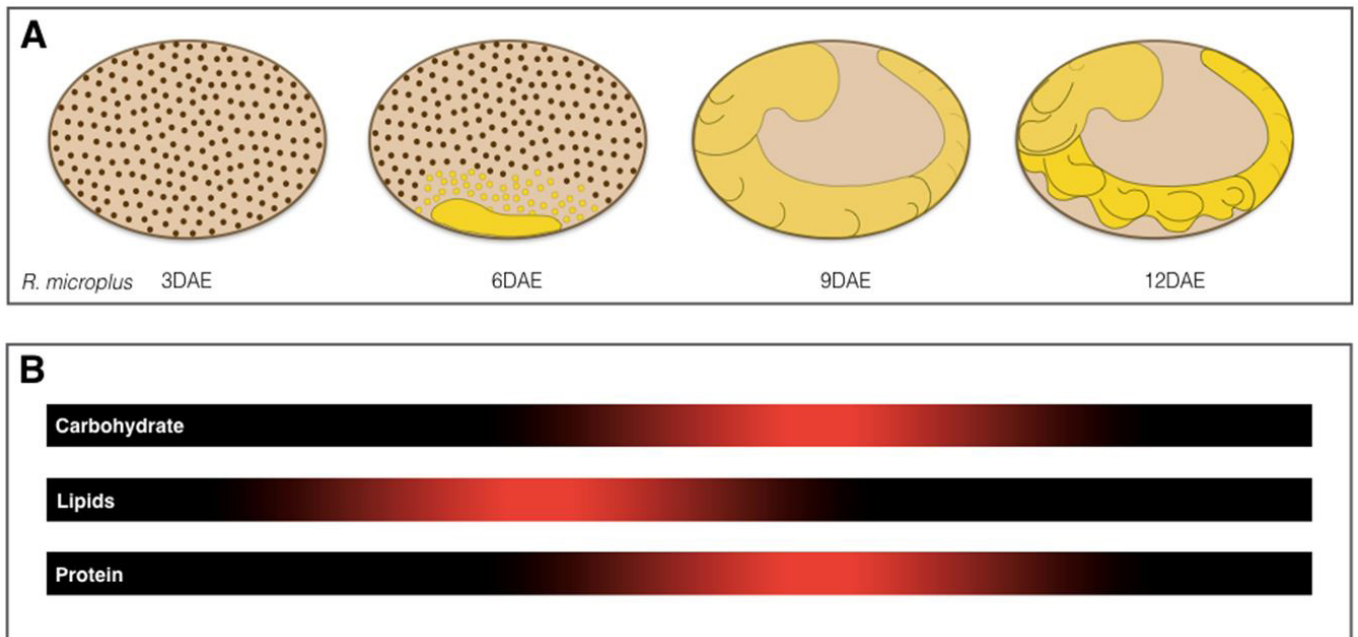
Figure 2. Dynamic of energy source during embryogenesis of *R. (B.) microplus*. In early development. The description of energy source utilization (B) during *R. (B.) microplus* embryogenesis (A) shows that the first energy source mobilized is lipids, in middle and late of development carbohydrate and protein are recruited and glycogen is resynthesized. DAE (days after eggs laying).

R. (B.) microplus embryo development (MORAES et al., 2007; LOGULLO et al., 2009; FABRES et al., 2010).

During gluconeogenesis, glucose is generated from non-glycosidic compounds. This metabolic pathway is regulated by phosphoenolpyruvate carboxykinase (PEPCK) which is an enzyme that catalyzes the initial steps of gluconeogenesis (BALLARD & OLIVER 1963; BALLARD et al., 1967). When glucose levels are low, as during the early stages of tick embryonic development, the rate of gluconeogenesis increases. In this scenario, PEPCK activity in gluconeogenesis is increased. Transcription of the gene for this enzyme is controlled by several hormones and is tissue-specific (CHAKRAVARTY et al., 2005).

In one study about glucose metabolism during the embryogenesis of *R. (B.) microplus* Moraes et al. (2007) found that PEPCK activity increases as glycogen is re-synthesized. These findings suggest that glucose may form from other substrates through this pathway ensuring glycogen re-synthesis. PEPCK dynamics, activity and regulation during *Aedes aegypti* embryogenesis are different from those of *R. (B.) microplus* embryonic development. During the early stages of embryogenesis of this mosquito, PEPCK activity is low. PEPCK activity increases as embryogenesis progresses until it reaches high levels which are kept high until hatching (VITAL et al., 2010). These results suggest that, in addition to PEPCK activity, gluconeogenesis may also have an important role in maintaining the embryo viability.

Embryo proteins as targets for tick control

The identification and characterization of molecules involved in embryonic development support to a better understanding of the tick parasite physiology and can help to select potential targets to

the development of new tick control methods. With this objective, various enzymes have been tested as targets to immunological or chemical controls. Immunization of bovines with an egg-yolk aspartic endopeptidase (*Boophilus* Yolk pro-cathepsin (BYC) (SILVA VAZ et al., 1998; LEAL et al., 2006) or a *R. (B.) microplus* cysteine endopeptidase (VTDCE) (SEIXAS et al., 2008), enzymes involved in the digestion of vitellin, interfered with the reproductive process of the tick, interfering with embryo development. Effects in embryogenesis also were observed by immunization of rabbits and cattle with a ferritin, an iron-storage protein (HAJDUSEK et al., 2010). Also, hamster immunized with *Ixodes persulcatus* CDK10 (a cyclin-dependent kinase, involved in cell cycle control showed a partial protective immune response against tick infestations (GOMES et al., 2015).

Other tick proteins important in embryogenesis have been studied as target for control. A *R. (B.) microplus* triosephosphate isomerase (TIM), an fundamental enzyme in the energy metabolism during embryogenesis was be inhibited by antibodies (SARAMAGO et al., 2012) and by sulfhydryl reagents that interfere in essential cysteine residues of the BmTIM (MORAES et al., 2011). Also, a catalase inhibitor (3-amino-1,2,4-triazole) induced deleterious effects to *R. (B.) microplus*, interfering in oogenesis and oviposition (CITELLI et al., 2007). Also, the interference in the physiology function of Hq05 and Bm05br, two homologous proteins involve, respectively, in *Haemaphysalis qinghaiensis* (GAO et al., 2009) and *R. (B.) microplus* (ALZUGARAY et al., 2017) reproduction induced alteration in tick embryo development. Taken together, these results suggest that various tick proteins with important role in embryogenesis can be promising targets to development of new methods for the parasite control.

Conclusion

The metabolic requirements of the developing tick embryo and the mechanisms regulating embryogenesis in arthropods have been extensively studied by many researchers over the years and are now well-established and well-characterized. However, some aspects of the tick embryo physiology remains unclear. Glucose metabolism plays an essential role during embryonic development and viability of various species. There is an increasing body of knowledge gained from research regarding glucose metabolism in the tick embryo. Information generated in these studies will give us a comprehensive view of the dynamics of this metabolic pathway during tick embryogenesis.

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