Estudo da Herpetofauna Brasileira

Daiane Patricia Oldiges
(Organizadora)

Atena Editora

Ano 2018

Daiane Patricia Oldiges
(Organizadora)

Estudo da Herpetofauna Brasileira

Atena Editora
2018

2018 *by Atena Editora*

Editora Chefe: Profª Drª Antonella Carvalho de Oliveira
Diagramação e Edição de Arte: Geraldo Alves e Natália Sandrini
Revisão: Os autores

Dados Internacionais de Catalogação na Publicação (CIP)
(eDOC BRASIL, Belo Horizonte/MG)

E82 Estudo da herpetofauna brasileira [recurso eletrônico] / Organizadora Daiane Patricia Oldiges. – Ponta Grossa (PR): Atena Editora, 2018.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
ISBN 978-85-85107-61-1
DOI 10.22533/at.ed.611182310

1. Anfíbios – Ecologia – Brasil. 2. Répteis – Ecologia – Brasil. 3.Zoologia. I. Oldiges, Daiane Patricia.

CDD 591.5

Elaborado por Maurício Amormino Júnior – CRB6/2422

APRESENTAÇÃO

A herpetologia trata do ramo da zoologia responsável pelo estudo de répteis e anfíbios, abordando temas como classificação, fisiologia e comportamento, entre outros. Atuando tanto como presa quanto como predador na complexa rede de interações ecológicas, os répteis e anfíbios são fundamentais para o funcionamento adequado dos ecossistemas - aquático e terrestre.

Dentro da herpetologia, o estudo ecológico de répteis e anfíbios é um campo bastante amplo, no qual são analisadas características como interações sociais, comportamento no ambiente, distribuição e conservação das espécies. Tais animais são capazes de povoar uma ampla gama de ambientes, com grande variedade de concentração de solutos, temperatura e fontes de alimentos. Por serem bastante sensíveis a alterações nos mesmos, em sua grande maioria decorrentes da intervenção humana, e dada a grande área de povoamento se tornam importantes bioindicadores ambientais.

Estudar esses organismos é fundamental para promover sua conservação, e, consequentemente, a manutenção do equilíbrio do ecossistema como um todo. Não devemos, no entanto, esquecer do impacto direto que a pesquisa de répteis e anfíbios exerce sobre o desenvolvimento do estudo científico. Estes animais apresentam um grande potencial biotecnológico, tendo em vista que as secreções por eles produzidas são uma inestimável fonte de novas moléculas, ou mesmo de análogos de moléculas já existentes, que podem auxiliar o desenvolvimento de novos fármacos.

A presente obra se trata de uma coletânea de textos, e apresenta em seus 6 capítulos novas informações na área de herpetologia, tendo como foco a ecologia destes animais e o potencial biotecnológico do estudo dos mesmos. Por fim, esperamos que este livro possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias para esta interessante área de conhecimento.

Daiane Patricia Oldiges

SUMÁRIO

CAPÍTULO 3

EVALUATION OF THE OXIDATIVE STRESS USING BIOMARKER MALONDIALDEHYDE IN ATRETIC EGGS OF BRAZILIAN SNAKES FROM *Bothrops genus*.

Poliana Garcia Corrêa
Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, Brasil
Laboratório de Ecologia e Evolução, Divisão de Desenvolvimento Científico, Instituto Butantan, São Paulo, Brasil
pgcorrea@usp.br;

Giuseppe Puorto
Museu Biológico, Divisão de Desenvolvimento Cultural Instituto Butantan, São Paulo, Brasil

Daniel da Conceição Rabelo
Laboratório de Biologia Molecular- Divisão de Desenvolvimento e Inovação, Instituto Butantan, São Paulo, Brasil

Rosely Cabette Barbosa Alves
Laboratório deVirologia, Divisão de Desenvolvimento e Inovação, Instituto Butantan, São Paulo, Brasil

Durvanei Augusto Maria
Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, Brasil
Laboratório de Biologia Molecular- Divisão de Desenvolvimento e Inovação, Instituto Butantan, São Paulo, Brasil
durvanei.maria@butantan.gov.br

ABSTRACT: Atresia is a process of cell death or apoptosis of the majority of ovarian follicles, known by a variety of morphological or pathological conditions. A large number of atretic eggs was released by diferent groups of captive females snakes, in the Laboratory of Ecologia and Biological Museum, from Instituto Butantan. Lipoperoxidation induces tissue damage, related to the pathogenesis of distinct diseases, which can lead to cell death. We investigated the released atretic eggs doing analysis of lipoperoxidation. Extraction and purification of the lipids were done by the method adapted from Bligh & Dyer. The reaction method of the malondialdehyde biomarker (MDA), a secondary product of lipid peroxidation complexed with thiobarbituric acid (TBA), was used. The Reading was done at 532 nm. Analyzing the results, a great variation in MDA concentration was found in different atretic eggs at the same pit viper specimen. For example: *Bothrops insularis* (from 7.4 to 58 nmols/mL ±13.7 of lipid), *B. jararaca* (from 9.4 to 100 nmols/mL±26), *B. leucurus* (from 6.1 to 182 nmols/mL ±53.7), *B. erythromelas* (from 3.6 to 66nmol/mL ± 21.6) and *B. moojeni* (from 6.1 to 428.4 nmols/mL ± 32.7). However, in comparisons of species, using a Kruskal-Wallis test, *B. moojeni* presented highest value of oxidative stress when compared to the other species (> 100 nmols/mL). This is the first lipoperoxidation assay with atretic snake eggs, showing a high content of MDA and of oxidative stress. This could induce apoptosis or control

cell proliferation and may induce the repair of this damage.
KEY WORDS: Lipoperoxidation, Apoptosis, Atresia, cell death, pit viper, lancehead

RESUMO: A atresia é um processo de morte celular ou apoptose da maioria dos folículos ovarianos, conhecido por uma variedade de condições morfológicas ou patológicas. Um grande número de ovos atrésicos foi liberado por diferentes grupos de serpentes, fêmeas cativas, no Laboratório de Ecologia e Museu Biológico, do Instituto Butantan. A lipoperoxidação induz ao dano tecidual, relacionado à patogênese de distintas doenças, que podem levar à morte celular. Nós investigamos por ensaio de lipoperoxidação, os ovos atrésicos liberados. A extração e purificação dos lipídeos foram feitas pelo método adaptado de Bligh & Dyer. Utilizou-se o método de reação do biomarcador malondialdeído (MDA), um produto secundário da peroxidação lipídica complexada com ácido tiobarbitúrico (TBA). A leitura foi feita a 532 nm. Analisando os resultados, houve uma grande variação na concentração de MDA em diferentes ovos atrésicos do mesmo espécime de viperídeo. Por exemplo: *Bothrops insularis* (de 7,4 a 58 nmol / mL ± 13,7 de lipídio), *B. jararaca* (de 9,4 a 100 nmol / mL ± 26), *B. leucurus* (de 6,1 a 182 nmol / mL ± 53,7), *B. erythromelas* (de 3,6 a 66 nmol / mL ± 21,6) e *B. moojeni* (de 6,1 a 428,4 nmols / mL ± 32,7). Entretanto, em comparações das espécies, utilizando o teste de Kruskal-Wallis, *B. moojeni* apresentou maior estresse oxidativo quando comparado com as outras espécies (> 100 nmols/mL). Este é o primeiro ensaio de lipoperoxidação com ovos atrésicos de serpentes, mostrando alta concentraação de MDA e elevado estresse oxidativo. Isso poderia induzir a apoptose ou controlar a proliferação celular e induzir o reparo desse dano.
PALAVRAS-CHAVE: Lipoperoxidação, apoptose, atresia, morte celular, viperídeo, jararaca.

1 I INTRODUCTION

1.1 Snakes

Latin America has a rich and diverse fauna of snakes, which has its distribution in different countries according to specific biotic and abiotic factors. They are distributed through a wide variety of habitats, from arid environments to forests and wetlands, except in polar caps (CAMPBELL and LAMAR, 2004). They belong to the order Squamata, which includes snakes, lizards and amphibians and is the main order of the Reptilia class with the largest number of reptiles described. Between the 3378 known snake species, 375 are Brazilian (UETZ, 2011).

Among the snakes of medical importance are those belonging to the families Viperidae and Elapidae, whose representatives are all venomous, endowed with venom producing glands and specialized dentition for their inoculation (CAMPBELL; LAMAR, 2004; MARQUES et al., 2001). In Brazil, viperids of public health importance basically comprise three genera: *Bothrops* (“jararacas”), *Crotalus* (“cascavéis”) e *Lachesis*

("surucucus").

1.2 Genus *Bothrops* wagler, 1824

The name of the genus is derived from the Greek *bothros*, meaning pit, and *ops*, meaning eye or face, alluding to the distinctive pit organ, located on the face, between the eye and the nostril (CAMPBELL; LAMAR, 2004).

According to the list of Brazilian reptile species (COSTA & BÉRNILS, 2015) Neotropical pit vipers of the subfamily Crotalinae ("pitvipers"), genus *Bothrops* ("lancehead"), consist of 29 species and are widely distributed throughout South America in a wide variety of habitats (MARTINS et al., 2001; CAMPBELL, LAMAR, 2004).

1.3 Reproductive aspects

Many manifestations or reproductive events are used to categorize the reproductive cycles of snakes. In females, the pattern and synchronization of follicular maturation (as primary and secondary vitellogenesis) and ovulation are among the most important events (ALDRIDGE, 1982). The secondary vitellogenesis and its duration vary with the species, period of the year, duration of the active season, availability of prey and amount of fat reserve (DILLER, WALLACE, 1984; NAULLEAU; BONNET, 1996).

Vitellogenesis is the process of hepatic synthesis of vitellogenin and its transport to the oocytes. It requires the mobilization of a significant maternal reserve and, consequently, depends on a threshold level of bodily condition (BONNET, NAULLEAU & MAUGET, 1994).

On the reproductive aspects, members of the genus *Bothrops* give birth to young alive, therefore they reproduce by viviparity. The females of the group have similarly conserved cycles (ALMEIDA-SANTOS; SALOMÃO, 2002), although the fecundity may vary according to the size of the animal body in the different species of the genus (MARTINS et al., 2001), being influenced by availability of resources in different regions or habitats (SHINE, 2005).

The larger snakes are more prolific, being able to generate more than 50 offspring at one time; however, those of moderate to small size - such as *Bothrops jararaca* - can generate 20 offspring or less in a litter (CAMPBELL; LAMAR, 2004; JANEIRO-CINQUINI, 2004).

1.4 Follicular atresia

Follicular atresia is a degenerative, hormonally controlled process by which the vertebrate ovarian follicles lose their integrity and are eliminated before ovulation. In primates, most ovarian follicles undergo atresia during development and are reabsorbed by the ovary before birth (HUGHES; GOROSPE, 1991).

Several studies suggest that apoptosis is the fundamental molecular mechanism

for the removal of germ and somatic cells in the ovary of mammals and birds (TILLY et al., 1991; HUSSEIN, 2005). In teleost fish, atresia occurs in natural conditions, as well as in captivity, especially after spawning.

In reptiles, morphological and histological studies have been performed on ovarian follicles in Squamates since the 1970s. Guraya, from the 1960s, studied and compared follicular atresia of mammals and non-mammals, and reported that vitellogenic follicles become atretic due to lack of appropriate gonadotropic stimulation or imperfect balance of various hormones (GURAYA, 1965, 1966, 1969, 1973). At this stage they are called atretic eggs, since they matured, but did not finish developing.

In captivity, females of Squamatas may have a higher incidence of infertile eggs, both in oviparous and viviparous species (MACHACHERN, 1991; RONNE, 1996).

1.5 Oxidative stress

Reactive oxygen species (ROS) are highly reactive oxygen groups that damage cellular components, including DNA, protein and lipid (ESSERS et al., 2004). Generally, ROS are classified into three categories: non-ERO (H_2O_2) hydrogen peroxide, oxygen free radicals (O_2^-) and hydroxyl radicals (OH) (STORZ, 2011). These radicals are generated by intracellular sources, such as normal aerobic metabolism, nutrient deprivation and ischemia, as well as environmental stress such as ionizing or UV radiation (STORZ, 2011; ESSERS et al., 2005).

It is believed that apoptosis induced by oxidative stress is one of the main causes of follicular atresia (MURDOCH, 1998). It has been reported that the accumulation of ROS resulting from mutations in different electron transport chain complexes leads to premature ovarian failure and follicular atresia in the human ovary (KUMAR et al., 2010).

The present work aims to evaluate the oxidative stress by Lipid peroxidation assay in lipids extracted from the yolk of the atretic eggs released in large quantities in captivity by the snakes of the *Bothrops* group. The snakes are kept and monitored in captivity at the Laboratory of Ecology and Evolution (LEEV) and Biological Museum, both of the Butantan Institute.

The species under study: *B. insularis* (an insular specie, endemic to the Queimada Grande Island, São Paulo-Brazil, critically endangered), *B. jararaca, B. erythromelas, B. leucurus* and *B. moojeni* have presented high rate of deposition of atretic eggs in captivity, at the same time of parturition. For long years of observation, several species of Squamata, viviparous and oviparous, has been through this phenomenon, but we have no data published up to now on the research to provide information on molecular aspects.

2 I MATERIAL AND METHODS

2.1 Maintenance of the snakes in captivity

We monitored the snakes behavior with emphasis on the reproductive cycle and oviposture (or release of atretic eggs) of 12 adult, captive viviparous female snakes of the *Bothrops insularis* species, kept in the Jararaca-ilhoa Conservation Laboratory, located in the Laboratory of Ecology and Evolution (LEEV) of the Butantan Institute (IB), under the responsibility of researcher PhD Selma Maria de Almeida Santos, under license from IBAMA 25650-1. A specimen of each captive female of the species: *B. moojeni* and *B. jararaca* are also part of the LEEV vivarium in the enclosure, and are monitored. Two species of female pitviper were included: *B. leucurus* and *B. erythromelas*, from the Biological Museum of the Butantan Institute, kept in captivity on the enclosure in exhibition to the public**.**

The animals of the Laboratory of Ecology and Evolution (LEEV) were kept individually in transparent plastic boxes (56.4 x 38.5 x 37.1cm), with lid and sides perforated and lined with corrugated cardboard (FIGURE 1). The natural conditions of photoperiod (latitude 23°S), temperature and humidity were maintained. The animals' diet was composed of mice (*Mus musculus*) from the Central Vivarium (IB) being offered at regular intervals of 30 days, respecting the proportion of 10 to 20% of the individual's weight. Fresh water was supplied *ad libitum.*

Biometric data were obtained twice a year. Data regarding behavior and sanity were daily passed on to the archives of the research projects.

Figure 1. Shelter of animals in captivity.

Transparent, perforated box with corrugated cardboard and water pot for the animal. Vivarium of Laboratory of Ecology and Evolution, Butantan Institute.

Source: Corrêa, P.G. (2018). Photo: Giuseppe Puorto.

In the Biological Museum, all the snakes were kept individualized in enclosures with an area of 1m^2, with a upper metal grille, and substrate of vegetal soil with cover of bark of trees (*Pinus* sp). In the room where the animals are exposed to the public were kept the natural conditions of photo-period of 10-14h, manual humidity control, to suit the conditions close to the ideal for the species. In all the enclosures there was a heated rock with temperatures ranging between 28 and 35ºC, branches for displacement and substrate (FIGURE 2). The diet of the snakes was composed of swiss mice (*Mus musculus*) and wistar rats (*Rattus norvegicus*) from the Central Vivarium (IB) that are offered at regular intervals of 30 days, respecting the proportion of 10 to 20% of the individual's weight. Fresh water was supplied *ad libitum*.

Figure 2. *Bothrops jararaca* on display at Biological Museum enclosure.

Enclosure with upper metal grille, branches for displacement and substrate.

Photo: Giuseppe Puorto.

2.2 Collection and preservation of atretic eggs

Once an atretic egg was expelled or released by the females snakes, it was collected under sterile conditions with gloves, avoiding the contact of the hands with it (FIGURE 3A). The eggs were measured and weighed and then quickly stored in a freezer (-20ºC or -80ºC) in zip plastic bags (10x7cm), in order to preserve the cellular content and humidity of the follicles.

The frozen atretic eggs were processed on an ice bath (Petri dish with dry ice). The germinal disc, when present (FIGURE 3B) was collected internally and the cells

processed for flow cytometer analysis - FACS (data not shown). The yolk or fatty tissue of the atretic egg was cut (in pieces of about 150 mg), and processed for the lipoperoxidation assay.

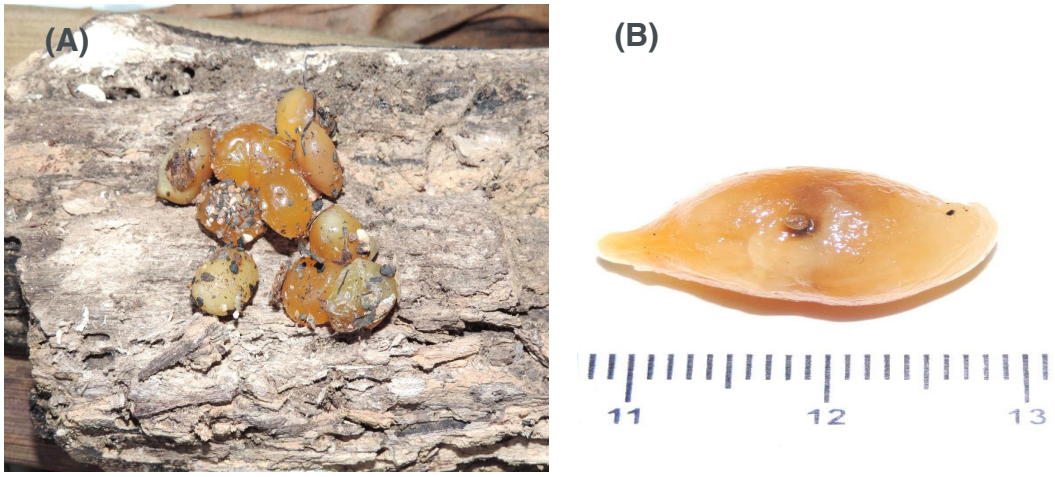

Figure 3. Atretic eggs from *Bothrops erythromelas.*

(A) Atretic eggs released at the parturition of offspring, on the substrate of wood gravel, enclosure of the Biological Museum, Butantan Institute. (B) Atretic egg of the same animal. The arrow indicates the presence of a germinal disc in the center. Photo: Giuseppe Puorto.

2.3 Lipid peroxidation assay

Oxidative stress on unsaturated lipids in cell membranes was evaluated by determining the amount of malondialdehyde (MDA), a three-carbon aldehyde, which is the final product of fatty acid peroxidation (lipoperoxidized radical), which reacts with two molecules of acid thiobarbituric acid (TBA) to form a pink chromogen complex. The content of malondialdehyde was quantified using the TBA-reactive substances test (TBARS) (OHKAWA et al., 1979), formed as a by-product of fat degradation, unsaturated fatty acids and hydroxy peroxides.

In brief, from 10 to 20 released atretics eggs by specie were collected in captivity, measured and frozen (-80 ºC) to preserve the germinal disc region (GDR) when present, to further analysis on flow cytometer (FACS). The samples (about 150 mg/homogenate) were macerated, washed with PBS^{Na+} and the supernatant was collected and mixed with TCA solution (20%) and aqueous TBA solution (0.86%). The mixture was shaken and heated using a boiling-water bath for 30 min. After cooling, n-butanol was added, and the mixture was shaken. After separation of the butanol layer by centrifugation at 1500 *g* for 15 min, its optical density was determined using a spectrophotometer (ThermoPlate®), with wavelength adjusted to 532 nm (FIGURE 4).

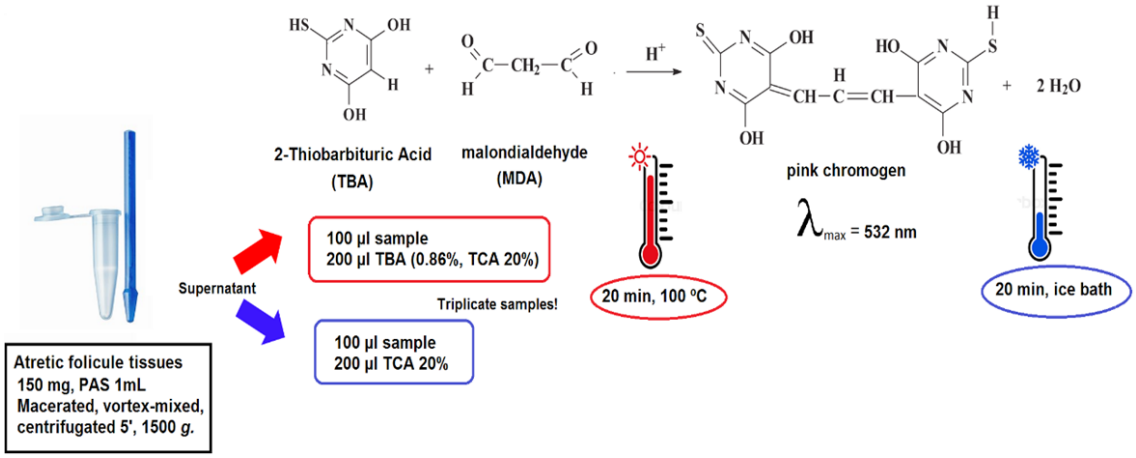

Figure 4. Simplified lipid peroxidation assay .

Extraction of yolk mass, maceration in conical microtube. Added solutions react in the lipid peroxidation and the chromogen product malonaldeído (MDA) is visualized in spectrophotometer, at 532 nm, in pink color.

Source: Corrêa, P.G. (2018).

The difference between results of the two optical density determinations was taken as the TBA value, and amounts of malondialdehyde (MDA) produced were calculated and compared to MDA standards and expressed as MDA nmoles/mL. Graphs and statistics were obtained using *GraphPad Prism* version 5.0 software and the Kruskal-Wallis test was used to compare MDA among species.

3 I RESULTS

3.1 Released eggs

Some wild snakes arrived pregnant from nature, such as *B. jararaca* and *B. insularis*. All the atretic eggs released by the females were collected in the enclosure or in the boxes at the time of parturition of the offsprings. The total of atretic egg samples were:

- *B. erythromelas* 12 eggs being four with germinative disk (GD); *B. jararaca* 11 eggs being five with GD; *B. insularis* 17 eggs of two females and two eggs with GD; *B. leucurus* 12 eggs and one with GD; and *B. moojeni*, 39 eggs, but we used only 12 eggs, of which, five had a GD. In figure five, we observed the different species of captive snakes, whose atretic eggs were used in this work, in the lipid peroxidation assay.

Figure 5. Snakes from *Bothrops* genus used to the assay.

(A) *Bothrops insularis*, (B) *B. jararaca*, (C) *B. alcatraz*, (D) *B.erythromelas*, (E) *B. leucurus*, Photos: Giuseppe Puorto. (F) *B. moojeni.* Photo: Bruno M. Costa.

Source: Corrêa, P. G. (2018).

3.2 Lipid peroxidation

Samples from 10 to 20 atretic eggs collected from the animals were used in the lipid peroxidation assay, but not all the samples were possible to obtain the results in triplicate of MDA reading in a spectrophotometer and were therefore dismissed. In Figure 6A we observed the pink lipid peroxidation product (MDA) observed in the presence of a strong acid, thiobarbituric acid (TBA), when heated. In Figure 6B we observed the samples applied in triplicate in a 96-well plate, flat-bottom, for reading the concentration of MDA by spectrometry, at the wavelength of 532 nm.

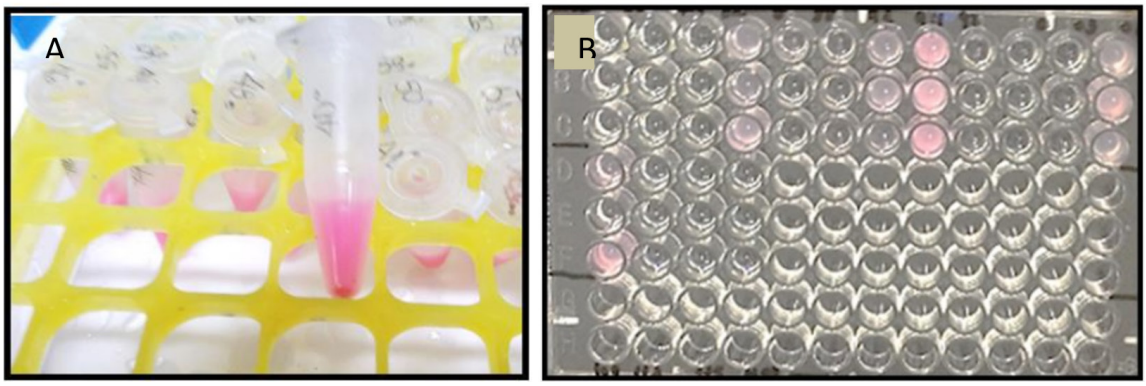

Figure 6. Lipid peroxidation assay (TBARS).

(A) Samples in the microtubes of 1.5 ml after heating with lipoperoxide chromogen (MDA) that was generated, pink. (B) 96-well plate with the samples in triplicate. Positive result for the production of MDA, for example, in wells A8, B8 and C8. Source: Corrêa, P.G. (2018).

The formation of lipoperoxidized radicals (MDA) from different samples of fertilized and unfertilized eggs of the snakes: *B. jararaca, B. insularis, B. moojeni, B. erythromelas* and *B. leucurus* showed significant differences in the levels of lipoperoxidized products. The analysis and calculations of these radicals, performed in *GraphPad Prism software* are present in graphs in figure 7. As we observed, there are significant differences in the concentration of malonaldehyde from one egg to another, even if they were released in the same parturition, of the same female. This indicates that one egg is under more ROS action than the others.

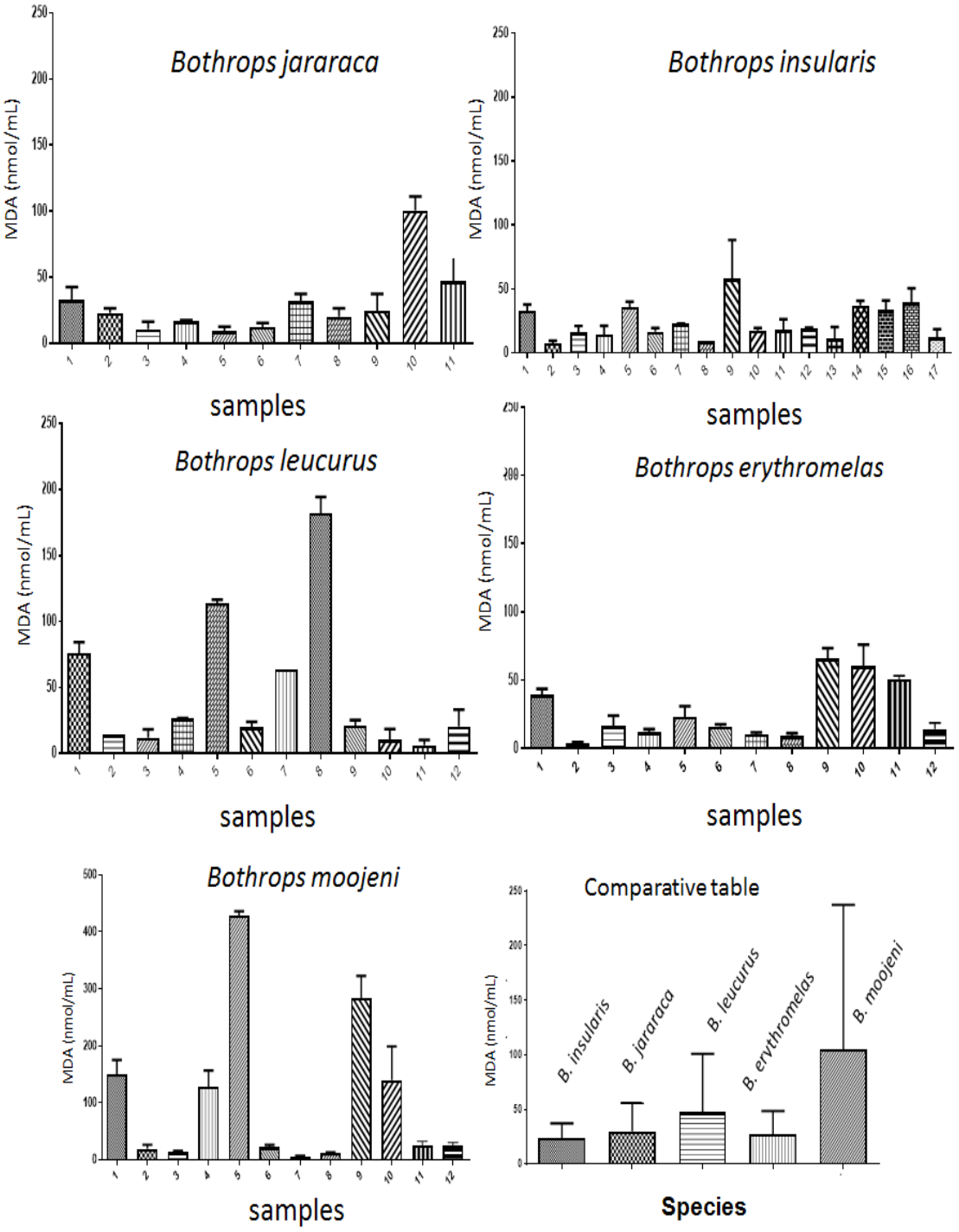

Figure 7 Statistics on GraphPad Prism - TBARS Assay.

Concentrations of MDA expressed in nmol/mL of sample. It's shown the concentration and standard deviation per individual atretic egg. Graphics represented by animal species. Comparative table of species mean by Kruskal-Wallis test.

Analyzing the results, there was a great variation in the concentration of MDA in different atretic eggs of the same viperid specimen. For example: *Bothrops insularis* (from 7.4 to 58 nmol/mL of lipid ± 13.7), *B. jararaca* (from 9.4 to 100 nmol/mL ± 26), *B. leucurus* (from 6.1 to 182 nmol / mL ± 53.7), *B. erythromelas* (from 3.6 to 66 nmol / mL ± 21.6) and *B. moojeni* (from 6.1 to 428.4 nmols / mL ± 32.7). However, in comparisons of the species, using the Kruskal-Wallis test, *B. moojeni* presented higher concentration of peroxidized radicals (MDA) when compared to the other species (> 100 nmol/mL).

4 I DISCUSSION

4.1 Atretic eggs and reproductive cycle

All the snakes released atretic eggs of medium size (~ 25 mm), yellowish color with hardened yolk, coincident with the parturition season (late spring and early summer). The snakes that showed the highest release of atretic eggs coincided with females that had never copulated since they came from nature.

We observed that the number of atretic eggs increased after the females were kept in captivity in relation to those females that came from the nature pregnant (or vitellogenic).

4.2 Lipid peroxidation

Reactive oxygen species (ROS) affect multiple physiological processes in reproduction and fertility, from oocyte maturation to fertilization, embryonic development and pregnancy.

In lipid peroxidation, there is a loss of selectivity in the ion exchange and release of the organelles content, as lysosomal hydrolytic enzymes and formation of cytotoxic products, as in the formation of malondialdehyde (MDA), capable of inducing cell death. Malondialdehyde is a reactive aldehyde with low stability and widely used as a biomarker in the evaluation of oxidative stress (PILZ et al., 2000). The imbalance between antioxidant and pro-oxidant systems, with predominance of oxidizing action and consequent damages, results in oxidative stress. It promotes changes such as lipid peroxidation, DNA fragmentation and oxidation of different molecules, leading to cell death (apoptosis).

Some snakes in the study group came pregnant from nature, while others were born in the enclosure (captivity) and were never placed to mate. It was observed that although the reproductive cycle of *Bothrops* has been biennial, the snakes when captive undergo to secondary vitellogenesis and release atretic eggs annually at the time coincident with parturition. The percentage of germinal disc between atretic eggs is around 19%, and was higher in *B. jararaca* specie (45%).

The production of free radicals is a continuous and physiological process, necessary for functions such as cell signaling, differentiation and cell death to occur properly. During the metabolic processes, these radicals act as mediators for the transfer of electrons in the diverse biochemical reactions. Nevertheless, overproduction can cause oxidative damage. When ROS generation exceeds the capacity of antioxidant defense systems, oxidative stress occurs in the cell.

4.3 Oxidative damages

The main biological molecules, notably DNA, proteins and lipids, can be adversely affected by ROS. In addition, the reaction with these macromolecules generates

additional ROS, starting a cascade of damage if not controlled. It is estimated that ROS are responsible for 10,000 modifications of the DNA nitrogen bases per cell per day (AMES et al., 1991). Oxidation or methylation of the bases may have the most severe phenotypic consequences (FALNES et al., 2007).

We can say that the vitellogenic eggs of the snakes of the *Bothrops* group, even when fertilized, either with mating or by parthenogenesis (females born in the enclosure that never copulated) suffer the action of ROS and that ends up making the development of the embryos unfeasible.

Oxidative damage to oocyte lipids has been implicated in the cause of loss of quality of the oocytes. It is believed that the ability to resist oxidative stress may be a key factor for a specimen, which in turn may have a greater chance of survival and reproductive success.

A complementary study to elucidate if this is actually cell death by apoptosis would be the study of these germinative disc with different cell differentiation markers.

5 I CONCLUSION

Changes in the levels of peroxidized lipids obtained in this study indicate the involvement of lipid metabolism in the phenomenon of atresia in snakes, especially the genus *Bothrops*, which is an unpublished data.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the Post-Graduation Program in Wild and Domestic Animals Anatomy (Anatomia dos Animais Domésticos e Silvestres - PPGAADS) from Faculty of Veterinary Medicine and Zootechny at the State University of São Paulo (FMVZ-USP), and CNPQ.

REFERENCES

ALDRIDGE, R. D. The ovarian cycle of the watersnake *Nerodia sipedon* and effects of hypohysectomy and gonadotropin administration. **Herpetologica, Chicago**, (38):71-79. 1982.

ALMEIDA-SANTOS, S. M., SALOMÃO, M. G. Reproduction in Neotropical pitvipers, with emphasis on species of the genus *Bothrops*. In G. Schuett, M. Höggren, and H. W. Greene (eds.), **Biology of the Vipers**. Biological, Sciences Press, Carmel, IN. p. 445–462, 2002.

AMES, B.N. Oxygen radicals and 8-hydroxyguanine in DNA. **Jpn. J. Cancer Res**. 82(12):1460-1461.1991.

BONNET,X., NAULLEAU, G., MAUGET, R. The influence of body condition on 17-β estradiol levels in relation to vitellogenesis in female *Vivera aspis* (Reptilia, Viperidae) **General and Comparative Endocrinology**, 93, 424-437. 1994.

CAMPBELL, J. A.; LAMAR, W. W. (Eds) In: **The venomous reptiles of the western hemisphere**. Ithaca, New York: Cornell University Press., v. 2, p.687-689, 2004.

CORRÊA, P.G. **Monitoramento e estudos moleculares de folículos atrésicos de serpentes do grupo *Bothrops*.** 2018**.** 57 f. [Tese de Qualificação (Doutorado em Ciência, PPGAADS, FMVZ-USP)]. São Paulo, SP. 2018.

COSTA, H.C., BÉRNILS, R.S. (org.). **Répteis brasileiros: Lista de espécies 2015**. Herpetologia Brasileira - 4(3):75-93. Disponível em <http://www. sbherpetologia.org.br/images/LISTAS/2014.03-07-Mudancas Taxonomicas.pdf>. Acesso em 01/12/2017.

DILLER, L.V., WALLACE, R.L. Reproductive biology of the northern Pacific rattlesnake (*Crotalus viridis oreganus*) in northern Idaho **Herpetologica**, 40, 182-193.1984.

ESSERS, M. A., WEIJZEN, S., DE VRIES-SMITS, A. M., SAARLOOS, I., DE RUITER, N. D., BOS, J. L., AND BURGERING, B. M. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. **EMBO J**. 23, 4802–12. 2004.

ESSERS, M. A., DE VRIES-SMITS, L. M., BARKER, N., POLDERMAN, P. E., BURGERING, B. M., AND KORSWAGEN, H. C. Functional interaction between -catenin and FOXOin oxidative stress signaling. **Science**. 308, 1181–1184. 2005.

FALNES, P.O., KLUNGLAND, A., ALSETH, I. Repair of methyl lesions in DNA and RNA by oxidative demethylation. **Neuroscience,** 145: 1222–1232. 2007.

GURAYA, S. S. A histochemical study of follicular atresia in the snake ovary. **J. Morphol.**, 117: 151–169. 1965.

GURAYA, S.S. A histochemical study of the rhesus monkey ovary. **Acta Morphol Neerl Scand.** 6(4):395-406. 1966.

GURAYA, S.S. Histochemical observations on the corpora atretica of amphibian ovary. **Gen. Comp. Endocr.**12(1): 165-167. 1969.

GURAYA, S.S. Follicular atresia. Proceedings of the Golden Jubilee Symposium on Perspectives in Reproductive Physiology of the Female, **Proc. Indian Natl. Sci. Acad., Delhi University.** 3 (39b): 311-332.1973.

HUGHES, F.M., GOROSPE, W.C. Biochemical identification of apoptosis (programmed cell death) in granulosa cells: evidence for a potential mechanism underlying follicular atresia. **Endocrinology.** 129: 2415–22. 1991.

HUSSEIN, M.R. Apoptosis in the ovary: molecular mechanisms. **Hum Reprod Update** 11,162–177. 2005.

JANEIRO-CINQUINI, T.R.F. Capacidade reprodutiva de *Bothrops jararaca* (Serpentes, Viperidae). **Iheringia, Série Zoologia** 94(4):429-431, 2004.

KUMAR, M., PATHAK, D., KRIPLANI, A., AMMINI, A. C., TALWAR, P., AND DADA, R. Nucleotide variations in mitochondrial DNA and supraphysiological ROS levels in cytogenetically normal cases of premature ovarian insufficiency. **Arch. Gynecol. Obstet.** 282, 695–705. 2010.

MARQUES, O. A. V.; ETEROVIC, A.; SAZIMA, I. **Serpentes da Mata Atlântica: Guia Ilustrado para Serra do Mar**. Ribeirão Preto: Holos, v. 1, p.184, 2001.

MARTINS, M.; ARAUJO, M. S.; SAWAYA, R. J. & NUNES, R. Diversity and evolution of macrohabitat use, body size and morphology in a monophyletic group of Neotropical pitvipers (*Bothrops).* **Journal of Zoology**, v. 254, p. 529-538, 2001.

MARTINS, M.; MARQUES, O. A. V. & SAZIMA, I. Ecological and Phylogenetics Correlates of Feeding Habits in Neotropical Pitvipers of the Genus *Bothrops*. In: **Biology of the vipers.** Schuett, G., Höggren, M., Douglas, M. E., Greene, H. W. (Org.). Eagle Mountain Publishing, L.C., p. 307-328, 2002.

MCEACHERN, M. J. **Keeping and breeding corn snakes. Advanced Vivarium Systems,** Lakeside. pp. 60. 1991.

MURDOCH, W. J. Inhibition by oestradiol of oxidative stress-induced apoptosis in pig ovarian tissues. **J. Reprod. Fertil.** 114, 127–130. 1998.

NAULLEAU, G., BONNET, X. Body condition thresholdfor breeding in a viviparous snake. **Oecologia** 107:301–306. 1996.

OHKAWA H, OHISHI N, YAGI K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Anal Biochem.** 95:351–8. 1979.

PILZ, J., MEINEKE, I., GLEITER, C.H. Measurement of free and bound malondialdehyde in plasma by high-performance liquid chromatography as the 2,4-dinitrophenylhydrazine derivative. **Journal of Chromatography B: Biomedical Sciences and Applications.** 742, 315–325. 2000.

RONNE, J. Revelations of a boa breeder. Reptiles 4:24–43. 1996.

SHINE, R. Life-history evolution in reptiles. **Annual Review of Ecology and Systematics**, 36:23–46. 2005.

STORZ, P. Forkhead homeobox type O transcription factors in the responses to oxidative stress. **Antioxid. Redox Signal**. 14, 593–605. 2011.

TILLY, J.L., KOWALSKI, K.I., JOHNSON, A.L., HSUEH, A.J. Involvement of apoptosis in ovarian follicular atresia and postovulatory regression. **Endocrinology.** 129,2799-801. 1991.

UETZ, P. et al. (eds.) **The Reptile Database.** Disponível em < http://www.reptile-database.org/db-info/taxa.html#Ser>. Acesso em 04/11/2015.

SOBRE A ORGANIZADORA

DAIANE PATRICIA OLDIGES Possui graduação em Biomedicina pela Fundação Universidade Federal de Ciências da Saúde de Porto Alegre (2010), com habilitação em Análises Clínicas e Bioquímica. Mestre (2011) e doutora (2016) pelo programa de Biologia Celular e Molecular da Universidade Federal do Rio Grande do Sul, com período de estágio sanduíche na Washington State University (Pullman - WA). Seu foco de pesquisa é a caracterização de proteínas com potencial uso no desenvolvimento de vacinas contra o carrapato bovino *Rhipicephalus microplus*, bem como na manipulação gênica do protozoário *Babesia bovis* no intuito de utilizá-lo como plataforma vacinal.

Agência Brasileira do ISBN
ISBN 978-85-85107-61-1
9 788585 107611