Seasonal changes in testicular and epididymal histology of the tropical lizard, *Tropidurus itambere* (Rodrigues, 1987), during its reproductive cycle

Ferreira, A. a, Silva, DN. a, Van Sluys, M. b, and Dolder, H. c

aDepartamento de Biologia e Zoologia, Instituto de Biociências – IB, Universidade Federal de Mato Grosso – UFMT
Av. Fernando Correia, s/n, CEP 78060-900, Cuiabá, MT, Brazil

bDepartamento de Ecologia, Universidade do Estado do Rio de Janeiro – UERJ

Universidade Estadual de Campinas – UNICAMP

e-mail: adelina@ufmt.br

Received October 24, 2007 – Accepted January 17, 2008 – Distributed May 31, 2009

(With 4 figures)

Abstract

The reproductive cycles of lizards, including *Tropidurus* species, have been widely studied. However, few studies describe in detail the ultrastructure and the epithelial changes in the epididymis. Using histology and transmission electron microscopy, we show the seasonal changes in the testis and epididymis of the lizard *Tropidurus itambere*, during its annual reproductive cycle. The reproductive cycle of *T. itambere* was analysed from June 1988 to June 1989 and from June 2001 to June 2002. While the frequency of reproductive males in the population varied throughout the year, there were reproductive males in most months except for February through April. During this nonreproductive period, there is a reduction in the mean seminiferous tubule volume and few sperm were found in both the testis and the epididymis.

Keywords: spermatogenesis, reproduction, lizard, ultrastructure, Reptilia.

1. Introduction

In general, reproductive studies of tropical lizards have dealt with three distinctive aspects of reproduction. First, the reproductive period has been investigated to determine whether a species reproduces continuously throughout the year, and if so, whether the level of reproductive activity is constant. Second, which environmentally important selective pressures can be linked with successful reproduction in all period of the year. While some authors have focused on a single aspect of lizard reproduction, most studies have examined all three (Sherbrooke, 1975).

*Tropidurus itambere* (Squamata, Tropiduridae) is a medium-sized lizard (adult mean snout-vent length ±71.8 mm) which belongs to the *Torquatus* group. Their
bodies are brown with many white and black spots. *T. itambere* commonly occurs in open rocky areas in the Atlantic Forest domain, in central and southeastern Brazil (Rodrigues, 1987).

The reproductive cycle of *T. itambere* is highly seasonal (Van Sluys, 1993). Reproductive females occur from September to March and reproductive males are found throughout the year except March (Van Sluys, 1993). Herein we analyze morphological aspects of the gonads of *T. itambere* males in two time periods, from June 1988 to June 1989 and from June 2001 to June 2002. We describe the structure and ultrastructure of testes and epididymes.

2. Material and Methods

2.1. Study area

The study was carried out in Valinhos Municipality, São Paulo state, southeastern Brazil (22° 56' S and 46° 55' W). The vegetation of the area consists mainly of herbaceous species. For a detailed description of the study site see Van Sluys (1993). The ambient variable had been analyzed in the period of 1956 the 1991, mean air temperature is 20.6 °C (17.2 ± 23.1 °C), rainfall is seasonal: during the wet season (October to April), mean rainfall is 154.4 ± 45.8 mm, and during the dry season (May to September) mean rainfall is 55.1 ± 16.3 mm.

Adult males were collected monthly, from June 1988 to June 1989 (N = 176) and from June 2001 to June 2002 (N = 12). Right testes and epididymes were removed for histological analysis.

2.2. Light microscopy

We removed the right testes and epididymes for histological analysis. Testes and epididymes were sectioned at 5 µm and stained with toluidine blue 1% and observed with a light photomicroscope (Olympus®, BX60).

2.3. Transmission electron microscopy

Testis and epididymis were fixed overnight at 4 °C in a 0.1 M sodium cacodylate buffer solution (pH = 7.2) containing 2.5% glutaraldehyde. After having been fixed following the above protocol, they were post fixed for 2 hours in 0.1 M sodium cacodylate buffer solution (pH = 7.2) containing 1% osmium tetroxide. Next, they were dehydrated in acetone and embedded in LR White resin. The ultrathin sections were stained with uranyl acetate and lead citrate and observed using a transmission electron microscope (Zeiss®, Leo 906).

3. Results

During the year, the testes presented seminiferous tubules with germ cells in various degrees of differentiation including spermatogonia, spermatocytes, and various spermatid stages. From February to April, a period of degeneration for the seminiferous tubules, spermatogenesis was interrupted. The seminiferous tubules were small from April (Figure 1h) but increased progressively from June to November (Figures 1a-1e). While the seminiferous tubules were completely occluded in February, March and April (Figures 1g, 1h and 1a), transmission electron microscopy was required to clearly ascertain the intense degeneration of germ cells (Figure 2a). From November, December and January, the majority of testes contained spermatozoa (Figures 1e, 1f and 2b). At the peak of the breeding season, there was tremendous testicular hypertrophy and very abundant sperm. This gradual increase in the amount of germ cells and sperm in the seminiferous tubules is associated with a reduction in the interstitial tissue (Figures 1d, 1e and 1f).

The epithelium of the epididymis is composed of two main cell types: secretory cells, which are the more numerous, and basal cells, which are probably important for cell replacement. This differentiation is particularly noticeable as the secretory cells have a columnar appearance compared to the basal cells which remain much smaller and wedge shaped (Figures 3a-3f and 4a-4f). From April to June, in synchrony with the testis cycle, there is a cycle in the secretory cells of the epididymis. During this period there is an absence of spermatozoa in the lumen and a gradual epithelial stratification which results in an increase in the cell cytoplasm height and the production of secretion vesicles (Figures 3a, 3b, 4a, 4b). Throughout the year, the epididymis contains varying amounts of spermatozoa; for example, from June to December (Figures 3c, 3d, 3e, 4c, 4d, 4e), the epithelial stratification is reduced to the point of total suppression of secretory activity (Figures 3f, 4f).

4. Discussion

In order to understand variation in reproductive activity of tropical lizards, Sherbrooke (1975) grouped the reproductive strategies into three possible types: (1) continuous, without variation in reproductive activity, (2) continuous, with variation in reproductive activity, noted by seasonal variation either in testis size and/or spermatogenetic activity or the percentage of fertile or ovigerous females, and (3) non-continuous, with periods when all individuals are reproductively inactive, males lack mature spermatozoa in their testes and epididymis and females lack large yoked ova and/or oviductal eggs.

A variety of reproductive patterns have been documented for the genus *Tropidurus* which is widespread in South America and the Galapagos Islands (Rodrigues, 1987). Continuous reproduction has been found in *T. hispidus* in both the uniformly hot Caatinga region of northeastern Brazil (Vitt, 1993) and under highly unpredictable environmental conditions. In contrast, a well defined seven month reproductive season has been found in *T. itambere*, a species which experiences regular seasons in the Atlantic Forest domain in southeastern Brazil (Van Sluys, 1993). This seasonal variation in reproduction is also found in *T. etheridgei* which has a six month repro-
Testicular and epidydimal histology of Tropidurus itambere


T. hispidus (Prieto et al., 1976), T. delanonis (Werner, 1978), T. quadrivittatus and T. theresioides (Goldberg and Rodrigues, 1986), and T. torquatus (Vieira et al., 2001; Wiederhecker et al., 2002) living in seasonal localities...

Figure 1. a-h) Light Microscopy of the seminiferous tubule in the testis. 400x. a) June: seminiferous tubule with recognizable to initial germ cells, spermatogonia (g) and spermatocytes (c). The lumen is evident. b) July: kept the characteristic of the month before, with a sensitive increase of spermatocytes (c). c) August: initiated spermiogenesis, with an appearance of round spermatids (et). d) September and October: elongated spermatids (lt) present in the lumen. The interstitial tissue (it) has undergone initiated reduction. e) November and December: germ cells possess a layered organization in the interior of the seminiferous tubule, where spermatocytes (c) are basal, followed by spermatids (t) and in the lumen there are numerous spermatozoa (z). The interstitial tissue (it) is extremely reduced. f) January: period of maximum spermatogenesis, with all types of germ cells. g) February and March: lumen is occlusion, occurring all types of germ cells. and h) April: lumen occlusion, with a few types of germ cells.

ductive cycle and lives in the Argentinian Chaco (Cruz, 1997), a region with predictable seasonality.

Additional studies of other Tropidurus species, for example, T. albermalensis (Stebbins et al., 1967),
have found that females reproduce only during the wet season while males are potentially reproductive throughout the year. *T. torquatus* and *Platynotus semitaeniatus* are an exception to this seasonal rule reproducing mainly during the dry season, in northeastern Brazil (Vitt and Goldberg, 1983). The small number of females reproducing during the wet season suggests that this season is unfavorable for reproduction (Vitt and Goldberg, 1983).
This finding demonstrates that, while reproduction has been closely related to wet-dry seasons, there is no single reproductive strategy.

There is an apparent tendency to concentrate reproductive activities during the regular rainy seasons (Wiederhecker et al., 2002). However, the lack of information for a large number of localities hampers a better understanding of the association between reproductive activity and environmental factors for Tropidurus lizards. In T. itambere, the reduction of mean testis volume was associated with a drop in the frequency of males containing sperm in either the testes or epididymis (Van Sluys, 1993). A similar reduction in testis size has been associated with diminished sperm production in various tropical lizards which have continuous, year-round spermatogenesis (Daniel, 1960; Licht and Gorman, 1970; Sexton et al., 1971). In T. itambere, the reproductive period coincides with the rainy season which occurs from January to April (Van Sluys, 1993). Van Sluys’ (1993) findings were confirmed in the present study by documenting testis and epididymis morphology. In addition, the epithelial epididymis cells were found to show variation in the secretion production, a factor which is considered important for sperm maturation.

The pattern of reproductive activities in Squamata has often been associated with limiting environmental factors. In temperate regions, reproduction is seasonal and dictated by temperature and daylight hours (Fitch, 1970; Duvall et al., 1982). In tropical areas, however, Squamata exhibit a broad variety of reproductive patterns, ranging from continuous to strongly seasonal reproduction, making it difficult to identify the limiting environmental factors (Vitt, 1992; Clerke and Alford, 1993). Two main hypotheses have been advanced for these areas: 1) the lack of microhabitats with adequate moisture for egg development (Sexton et al., 1971; Andrews, 1988), and 2) the lack of food resources for reproduction and/or development of the young (Rocha, 1992; Van Sluys, 1993; Vrcibradic and Rocha, 1998). An association of all these factors could happen (Ferreira et al., 2002) during an unfavourable season. In those species with broad or wide-ranging geographical distributions, phenotypic plasticity in reproduction has been demonstrated (Seigel and Ford, 1991).

Of the few studies demonstrating epididymal variations during the annual reproductive cycle in lizards, most are experimental and investigate the effect of castration (Haider, 1985), various drugs (Haider and Rai, 1986) or hormones (Gigon-Depeiges and Dufaure, 1977; Haider and Rai, 1987). The present research, which examined the natural reproductive cycle of T. itambere, determined that there was synchronous development of the testes and epididymis and that maximum testes volume and epididymis diameter (especially secretory cell development) occurred from June to December. Dufaure et al. (1986) studied the annual weight variations of testes during the sexual cycle, confirming in vivo experimental evidence.
previously obtained that associated testosterone control of the secretory activity.

Contrary to *T. itambere*, the epididymis of *T. torquatus* presents a clear-cut annual cycle in which the epididymis lumen remains completely filled with spermatozoa. In addition, there was little difference in the quantity of spermatozoa and epithelial cell development between seasons (wet-dry) (Vieira et al., 2001). For *T. itambere* the occurrence of spermatozoa in the epididymis lumen was most prevalent from August to January.

The investigation by Znari and El Mouden (1997) on the annual reproductive and fat body cycles of *Agama impalearis* in the central Jbilet Mountains (Morocco) had similar findings to those of the current study with *T. itambere*. First, the development of the testes and epididymis was synchronous. Secondly, testes volume and epididymis diameter were at maximum from April to July with post-reproductive regression from late summer through autumn.

While Mesure et al. (1991) affirmed that degeneration of the epididymis epithelial cells occurs in *Lacerta vivipara* during its reproductive cycle, this was not observed, even with transmission electron microscopy, in *T. itambere*.

With the identification of different proteins and glycoproteins that influence sperm maturation, Dufaure and Saint-Girons (1984), Depeiges et al. (1985) and Ravet et al. (1987), studied the biochemical and histochemical characteristics of epididymal secretions in lizards. They observed an increase of epididymal secretory activity during the period of reduced reproductive activity which was thought to contribute to the maturation of the future germ cells. Based on variations in epididymal maturation, Dufaure et al. (1986) correlated cyclic development of the testes with that of the epididymis and its secretions.

Mesure et al. (1991) postulated a degenerative process leading to the destruction of the epithelium. However, the routine fixation methodology he used made confirmation of this impossible.

Gigon-Depeiges and Dufaure (1977) suggested that this epididymis in lizards, which secretes large granules, could be used as a model for the role played by epididymal secretions in the maturation of spermatozoa.

Seasonal variation in reproduction is common among lizards of the temperate zones (Fitch, 1970). Temperature and photoperiod are determinative factors for the reproductive activity of lizards in temperate zones and extreme conditions can determine the hibernation period for many species (Bartholomeu, 1953; Mayhew, 1964; Licht, 1973). In contrast, temperature and photoperiod under go less variation in the tropics and the dry period is comparable to the cold of temperate zones (Tinkle, 1969; Pianka, 1970). Precipitation has been demonstrated as the main factor influencing the reproductive cycle of some lizard species (Licht and Gorman, 1970; Sexton et al., 1971; Ruibal et al., 1972). The rains are important as a regulating factor of the reproductive cycle with the higher humidity preventing egg desiccation and guaranteeing the availability of animal and plant resources for the hatchings (Vallejo and Vallejo, 1981; Rocha, 1992).

The observed reproductive season for male of *T. itambere* was determined to be very similar to that described by Van Sluys (1993), to remain intact with the same characteristics after a period of one decade in relation to the reproductive cycle. In fact, the well defined climatic changes in Brazil and other regions of the world did dictate the male’s reproductive cycle. Van Sluys (1993) found that the *T. itambere* males in Atlantic Forest domain appear to have the potential of reproducing during the first reproductive season and electron microscopy in this study found spermatozoa were degenerated from February to April.

Acknowledgements — The research was supported by FAPEMAT (Fundação de Amparo a Pesquisa do Estado de Mato Grosso, process # 0769/2006) and FAPESP (Fundação de Apoio à Pesquisa do Estado de São Paulo, processes # 01/06744-4 and 89-0031/2). We thank MC Kiefer for help during the 2001-2002 field work and M. Petreire Jr., who made facilities available for the 1988-1989 study. MVS holds a research grant from CNPq (# 301401/2004-7). The animals were collected with authorization of the MMA/IBAMA (Ministério do Meio Ambiente / Instituto Brasileiro do Meio Ambiente, process # 02027.003521/02-17).

References


DUVALL, D., GUILLETTE JR., L.J. and JONES, RE., 1982. Enviromental control of reptilian reproductive cycles. In GANS,
Testicular and epidydimal histology of *Tropidurus itambere*