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REPRODUCTIVE CYCLE OF MALE GREEN IGUANAS, *IGUANA IGUANA* (REPTILIA: SAURIA: IGUANIDAE), IN THE PANTANAL REGION OF BRAZIL

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ABSTRACT

Adult male green iguana (*Iguana iguana*) (n=10) were collected in different seasons in the Brazilian Pantanal and their testes were examined using light and scanning electron microscopy. The annual reproductive cycle involved four stages which alternated between a maximum and a minimum of spermatogenesis. During the cycle, there was an inversely proportional relationship between the development of the seminiferous tubules and the interstitial tissue. In the first stage of the cycle (October-December, inundation season) there was minimum spermatogenesis, with only the initial divisions of spermatogonia, whereas the interstitial tissue and Leydig cells were well developed. This state continued during the second stage (January-March, flood season) with initial germ cell development and a reduction in interstitial tissue. In the third stage (April-June, drainage season) there was maximal germ cell activity with all phases of cell maturation present whereas the interstitial tissue was greatly reduced. Spermiation occurred in the last stage (July-September, dry season) and left large amounts of residual cytoplasm in the seminiferous tubule lumen. The possible social and environmental implications of this cycle are discussed.

Key words: annual cycle, Brazilian Pantanal, lizard, spermatogenesis

INTRODUCTION

The reproductive cycles of tropical lizards show great variability [27], with variation in the testicular cycle being affected by seasonality in many lizard species [7]. The reproductive cycles of temperate zone species have also been described by DeWolfe and Telford [5], Licht [17], Kasinathan and Basu [13] and Gavaud [8].

The morphological characteristics of lizard testes typically vary with the stage of the annual reproductive cycle. There are also changes in the developmental stages of germ cells, and in the quantity and metabolic activity of interstitial tissue [4,28,29]; changes in the latter tissues apparently vary inversely with the periods of germ cell production [9].

Several intrinsic and extrinsic factors influence the timing of the testicular cycle, with the main factors for *Iguana iguana* being climatic variations in photoperiod, temperature and precipitation.

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In this work, we describe the annual reproductive cycle of male green iguanas (*Iguana iguana*) based on structural and ultrastructural analyses of the testes, and correlate this with the seasonal variations in the Brazilian Pantanal.

MATERIAL AND METHODS

Study area

Adult male specimens of *I. iguana* (n = 10) were collected in the Pantanal region of the state of Mato Grosso do Sul, Central Brazil, from September 1998 to July 2000. The Pantanal located between 14º-22ºS and 53º-61ºW, is the largest regularly flooded area (approximately 168,000 km^2) in the center of South America [3]. The annual rainfall averages 1,500 mm, with a rainy season from October to March and a dry season from April to September. Based on the flood cycle, four seasons are recognized: flooding (“Cheia”), drainage (“Vazante”), drought (“Seca”) and inundation (“Enchente”) [1].

Light microscopy

The specimens were anesthetized by ethyl ether inhalation and were weighed and measured (Table 1). The testes were then removed and measured individually with a ruler. They were then fixed for 12 h in Bouin, Carnoy or Alfac (80% alcohol, 15% formol and 5% acetic acid) solutions, sectioned in half, and returned to the fixatives for another 6 h. After dehydration in a graded ethanol series, they were embedded in paraffin. Sections 5 µm thick were stained with hematoxylin-eosin.

Scanning electron microscopy

Testes fixed in Bouin solution were washed in 0.1 M cacodylate buffer for two days then infiltrated with increasingly concentrated sucrose solutions up to 3%. After fracturing in liquid nitrogen, the tissues were post-fixed in 1% osmium tetroxide and dehydrated in an ethanol series. They were then critical point dried, sputter-coated with gold, and observed with a Jeol JSM-5800LV scanning electron microscope.

RESULTS

Male reproductive system

The organs of the male reproductive system of *I. iguana* are located dorso-longitudinally in the abdominal cavity (Fig. 1A). The rounded testes are connected to the epididymis and vas deferens, which contact the kidneys posteriorly in the body cavity. The vas deferens ducts reach the copulating organ, which is inserted in the tail base and is everted during copulation.

Table 1. Body weight, snout-vent length (SVL) and testicular size of male *I. iguana* colleted in different seasons in the Brazilian Pantanal.

Period/Season	N	Body weight (g)	SVL (cm)	Testes (*) (cm)
September 1998 (Drought)	2	500	28	2.0x0.5
		1500	45	2.3x1.5
December 1998 (Inundation)	2	1000	33	2.0x0.5
		2500	41	2.4x1.0
March 1999 (Flood)	1	500	33	0.5x0.8
June 1999 (Drainage)	2	2000	40	3.0x2.0
		2000	39	2.5x2.0
October 1999 (Inundation)	2	2500	41	1.4x0.9
		1800	36	1.5x0.9
July 2000 (Drought)	1	2315	38	2.8x1.3

* length x width.

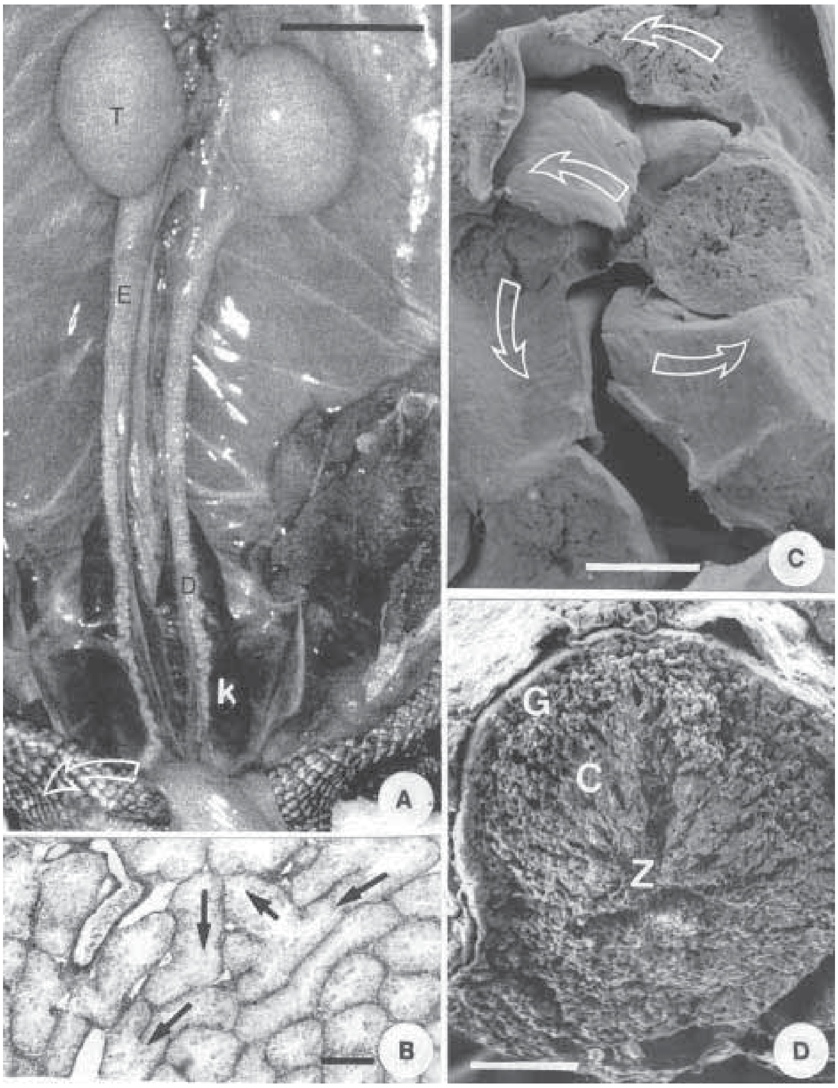

Figure 1 A. Male reproductive system of *I. iguana*. The testes (T) have reached their maximum size. (E), epididymis. The vas deferens (D) is intimately associated with the kidneys (k). The hemipenis is inserted in the tail (curved arrow). This specimen was collected in the drainage period (June 1999). Bar = 2 cm. **B.** Section of testis observed by light microscopy. Note the tangential sections of the seminiferous tubules, with their irregular arrangement (arrows). Bar = 100 µm. **C.** Seminiferous tubules observed by SEM are twisted in different directions (arrows). Bar = 50 µm. **D.** SEM view of a seminiferous tubule in which the germinal epithelium is organized in columns during the reproductive period. Note the region of spermatogonia (G), spermatocytes (C) and spermatozoa (Z). Bar = 20 µm.

Testis morphology

The testes of *I. iguana* are enveloped by a mesothelium and a tunica albuginea, but the seminiferous tubules are not arranged in parallel within the testes (Fig. 1B,C). The seminiferous tubules are limited by a layer of myoid cells (Figs. 1D and 2A). Within the tubules, the germinal epithelium is made up of two types of cells, the nutritive or Sertoli cells and the germ cells (Figs. 1D and 2A). Cells in different stages of activity occur in the germinal epithelium, depending on the phase of testicular development. Within the seminiferous tubule, the germ cells are distributed from the periphery to the central lumen, according to their degree of maturity (Figs. 2A-D and 3A-D). The seminiferous tubules are surrounded by interstitial tissue made up of loose connective tissue rich in veins, nerves and Leydig cells (Figs. 2A and 3C, D). The Leydig cells are large, numerous, round or polygonal, and are usually arranged in groups.

Four stages of the spermatogenic cycle can be distinguished (Fig. 2A-D) and correspond to the four annual seasons. Two peaks are observed (Figs. 3A-D). In stage 1 (December), the initial division of germ cells begins. Spermatogonia are abundant and primary spermatocytes make up the intervening layers in the tubule lumen. The interstitial tissue and Leydig cells are quite evident (Figs. 2A and 3A-C), and indicate the peak of interstitial tissue activity. The initial division and development of germ cells occurs during March (stage 2). The testicular structure is similar to the previous period, although the number of Leydig cells in the interstitial tissue decrease, while spermatogenic cells begin to divide (Fig. 2B). Maximum spermatogenetic activity characterizes June (stage 3). Germ cells in all phases of maturation are present and numerous sperm occur in the lumen. The interstitial tissue is reduced and almost absent (Figs. 2C, 3B, D). In September (stage 4), possibly after copulation, mature germ cells are absent. Some primary spermatocytes and spermatogonia are still present. Large amounts of residual cytoplasm are observed in the lumen (Fig. 2D). Figure 4 shows the correlation of seasonal changes in the Pantanal (involving temperature and precipitation) with the testicular dimensions of *I. iguana*.

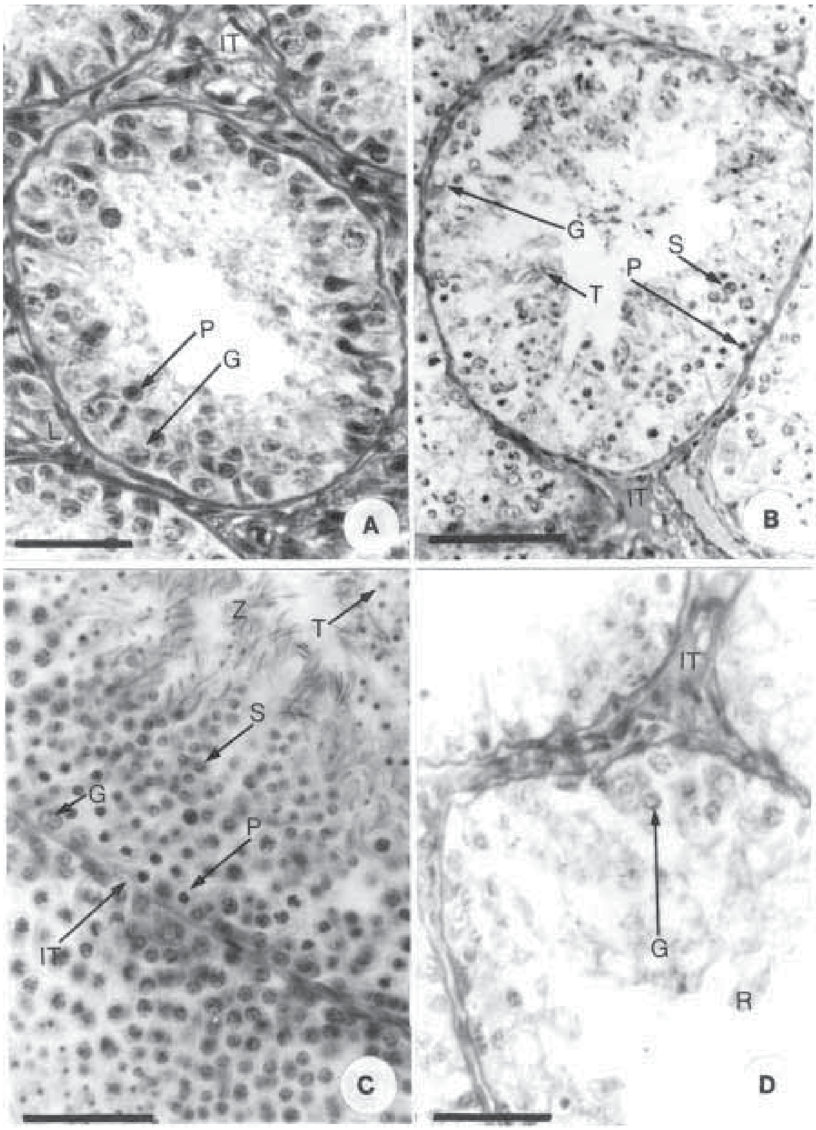

Figure 2. Transverse sections of seminiferous tubules in different stages of spermatogenesis. **A.** Stage 1, with spermatogonia (G) and primary spermatocytes (P) extending to the lumen; interstitial tissue (IT) is abundant and Leydig cells (L) are large. **B.** Stage 2, the germ cells initiate proliferation while the interstitial tissue (IT) begins to diminish. Note the spermatogonia (G), primary spermatocytes (P), secondary spermatocytes (S) and a few spermatids (T). **C.** Stage 3, with all stages of germ cells present. Spermatozoa (Z) are abundant near the lumen. Interstitial tissue (IT) is almost absent. (P), (S) and (T) as in fig. B. **D.** In stage 4, mature cells have been eliminated, leaving a large amount of residual cytoplasm (R). Only spermatogonia (G) are observed. Interstitial tissue (IT) is developing. Hematoxylin-eosin staining. Bar = 20 µm in all cases.

DISCUSSION

The annual testicular cycle in *I. iguana* includes the division, proliferation and maturation of germinal cells characterized by a short maximum peak and long periods of quiescence. This cycle is similar to that previously described for other lizard species, although most authors affirm that maximum spermatogenic activity occurs in the summer for lizards living in temperate zones [2,8-10]. In such lizards, the spermatogenic activity peaks when there are high temperatures and long photoperiods [9,13].

In some species, spermatogenesis and spermiation are triggered by exposure to long photoperiods that occur naturally, during long summer days [15], and are always associated with high temperatures, usually above 30°C [20,8]. These conditions are necessary to stimulate germ cell proliferation and spermiation. This has been demonstrated experimentally for the lizard *Anolis carolinensis* in tropical regions [16,17]. In the laboratory, this species has a long reproductive period, lasting from March to August [18].

Extrinsic factors, such as diet, hormonal factors [29], rainfall [5] and climatic variations at different altitudes [18], may affect the reproductive cycle. Some lizards, such as the Australian *Leiolopisma rhomboidalis* [28], have a slower process of spermatogenesis that extends throughout the year, with spermiation occurring only in spring.

Tropical lizards have a marked ability to adapt to different climatic conditions and can show markedly different requirements for reproduction and development [11,12,18,19,22,23]. In the genus *Sceloporus*, which inhabits tropical regions, the cycle is quite variable, with the reproductive period usually occurring in summer [9,19,21,22]. Similar patterns were found for *Liolaemus huacahuasicus* in Argentina [23] and *Barisia imbricata* in the mountains of Mexico [11]. However, *Sceloporus formosus,* an exception from Mexico, reproduced in spring [12]. If one considers a high temperature and a longer photoperiod as the most important environmental factors then the reproductive cycles of lizards in tropical countries are truly variable. Precipitation can also be an important factor [11].

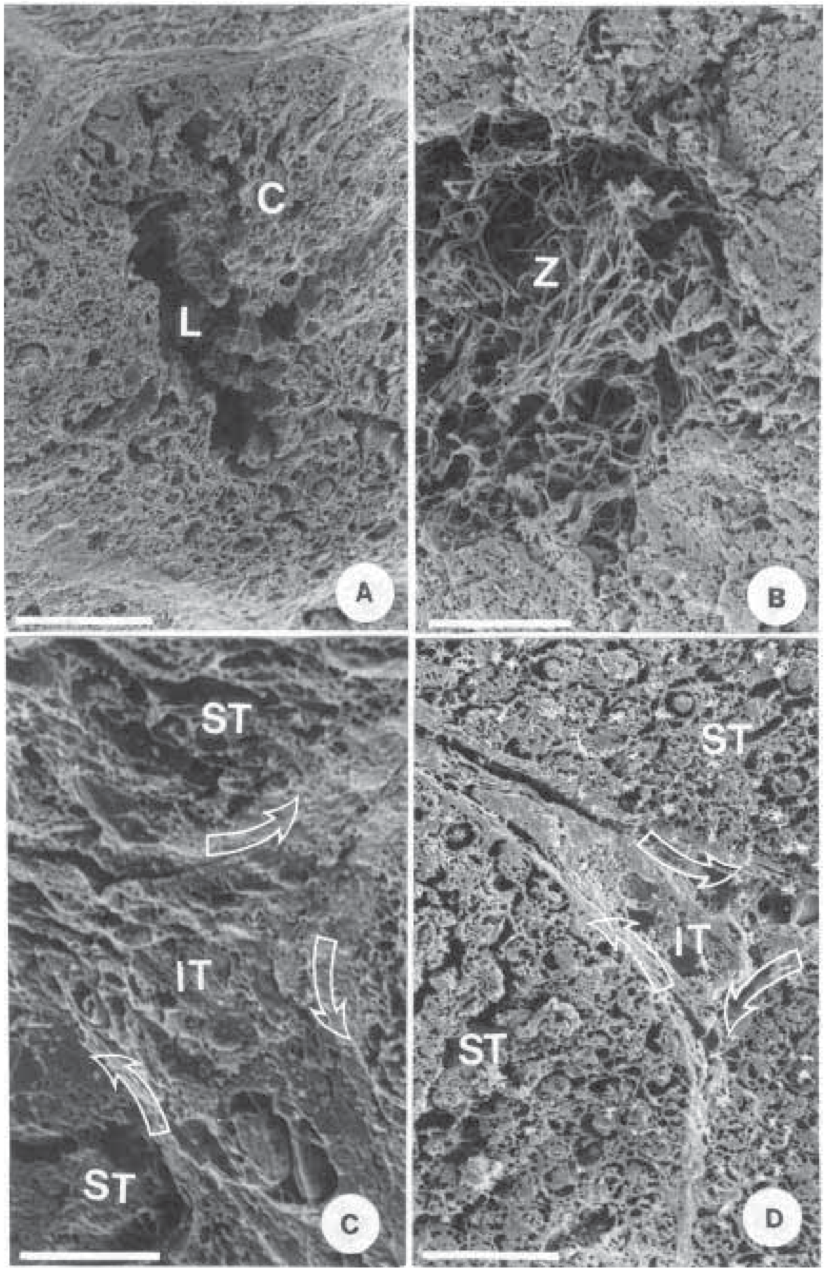

Figure 3. SEM micrographs. **A.** Lumen (L) of a seminiferous tubule in stage 1, with spermatogenic cells in reduced proliferation and some spermatocytes (C) reaching the lumen. **B.** Lumen of a seminiferous tubule in stage 3 with a well-developed germ cell layer. (Z, spermatozoa). **C.** Interstitial tissue (IT) of testis in stage 1. Fibrous tissue increases (arrow) as the seminiferous tubule diameter (ST) is reduced. **D.** Interstitial tissue (IT) of testis in stage 3, showing a reduction (arrows) around the developing seminiferous tubules (ST). Bar = 10 µm in all cases.

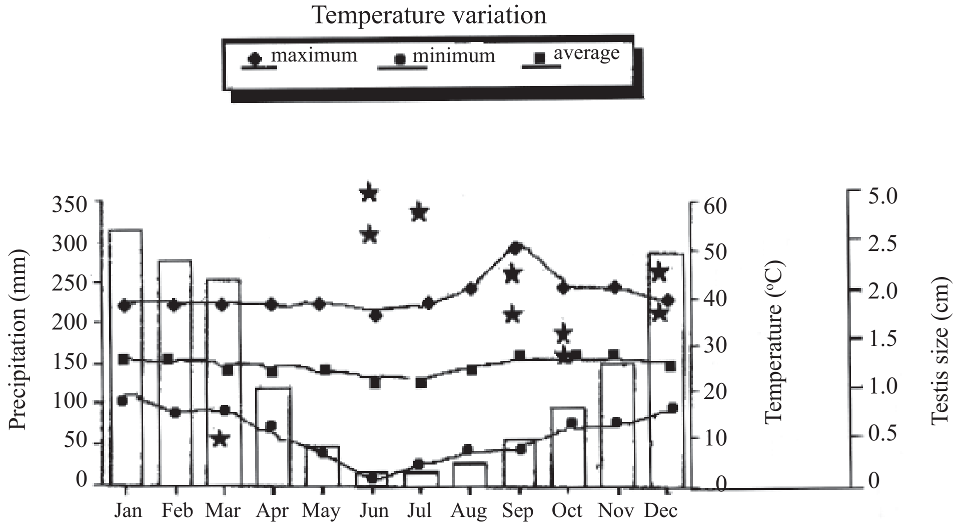

Figure 4. Seasonal variation in testicular dimensions (★) versus the climatic conditions of the Pantanal. The lines indicate temperature variations (minimum, average, and maximum). Precipitation is shown by vertical bars. (Climatic data from C. Strussmann, Master's thesis, 1992, State University of Campinas, Campinas, Brazil).

Morphological adaptations of *I. iguana* to environmental conditions have been described [7,26]. In contrast to these reports, we found no relationship between body length or weight and the testicular variations during the cycle. However, a relationship between testicular size and climatic variations was observed (Table 1). Microscopical analyses of testes obtained in different seasons (Fig. 2) demonstrated the modifications in germ cell production even more clearly.

The reproductive cycle of male green iguanas from the Brazilian Pantanal reflects a series of physiological adaptations to this unusual environment. Mating occurs in the dry season and oviposition in the summer, thus providing appropriate conditions for embryonic development [7,14]. There is, however, little information on the histological changes in the testes of *I. iguana,* in relation to environmental conditions.

Since only a few geographic locations show similar environmental conditions, including periodic flooding, to those of the Brazilian Pantanal, comparisons with other studies are difficult to make. Nevertheless, an annual cycle in the interstitial tissue of other lizards has been reported [29,28] with Leydig cells increasing cyclically in volume and activity prior to spermatogenesis [4,9].

Thus, the iguana's reproductive activity is influenced by external factors, also noted by Rand and Bock [24]. This conclusion supports the suggestion that *I. iguana* is strongly adaptable to environmental conditions [7]. Hormonal variations can obviously influence the annual reproductive cycle of lizards [25]. However, these variations can in turn be influenced by ambient conditions, such as temperature and photoperiod [6,15,16]. In tropical regions, an increase or decrease in precipitation is particularly important [11,12,21].

The reproductive cycle of *I. iguana* is annual, and the period of maximum testicular development is relatively short, occurring from July to September. Lizards, including tropical species, typically reproduce in the summer [12,21,27], when high temperatures stimulate spermatogenesis. In the case of *I. iguana*, the alternating drought and flood seasons provide ideal conditions for the formation of couples followed by a warmer period appropriate for egg and embryonic development. This periodicity also favors the survival of the young, which are bright green and lack the ability of the adults to modify their color. In the abundant green foliage of summertime, the young can easily hide.

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