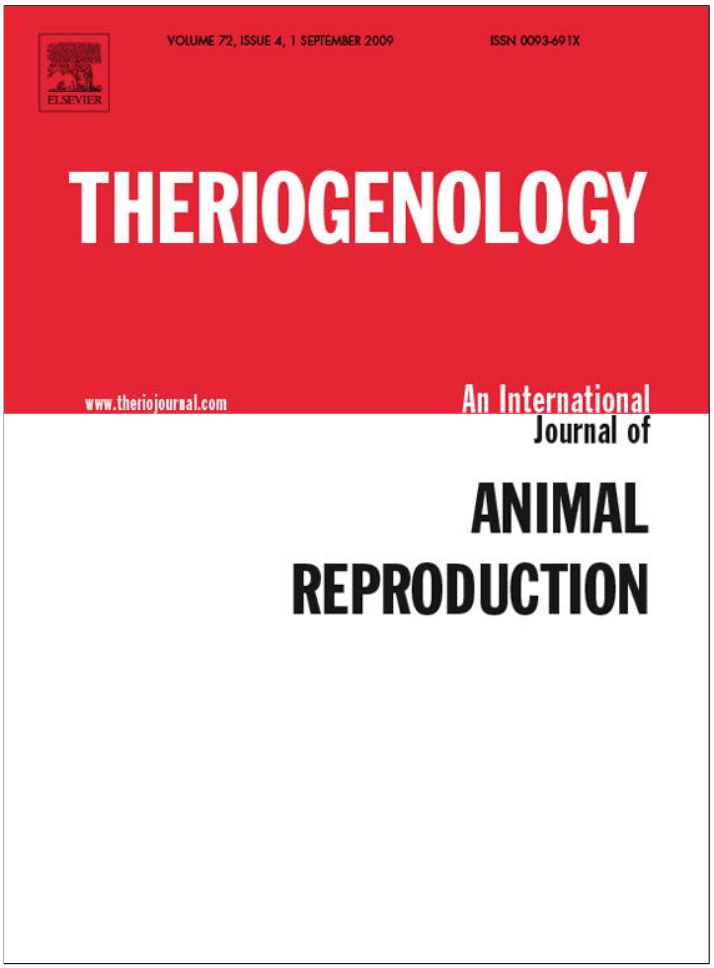

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Theriogenology 72 (2009) 484–492
Theriogenology
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Continuous spermatogenesis and the germ cell development strategy within the testis of the Jamaican Gray Anole, *Anolis lineatopus*

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Received 12 March 2009; received in revised form 7 April 2009; accepted 14 April 2009

Abstract

Testicular tissues from *Anolis lineatopus* were examined histologically to determine testicular structure, germ cell morphologies, and the germ cell development strategy employed during spermatogenesis. Anoles (N = 36) were collected from southern Jamaica from October 2004 to September 2005. Testes were extracted and fixed in Trump's fixative, dehydrated, embedded in Spurr's plastic, sectioned, and stained with basic fuchsin/toluidine blue. The testes of Jamaican Anoles were composed of seminiferous tubules lined with seminiferous epithelia, similar to birds and mammals, and were spermatogenically active during every month of the year. However, spermatogenic activity fluctuated based on morphometric data for February, May and June, and September–December. Sequential increases for these months and decreases in between months in tubular diameters and epithelial heights were due to fluctuations in number of elongating spermatids and spermiation events. Cellular associations were not observed during spermatogenesis in *A. lineatopus*, and three or more spermatids coincided with mitotic and meiotic cells within the seminiferous epithelium. Although the germ cell generations were layered within the seminiferous epithelium, similar to birds and mammals, the actual temporal development of germ cells and bursts of sperm release more closely resembled that reported recently for other reptilian taxa. All of these reptiles were temperate species that showed considerable seasonality in terms of testis morphology and spermatogenesis. The Jamaican Gray Anole has continuous spermatogenesis yet maintains this temporal germ cell development pattern. Thus, a lack of seasonal spermatogenesis in this anole seems to have no influence on the germ cell development strategy employed during sperm development.

Keywords: *Anolis*; Continuous spermatogenesis; Germ cells; Reproduction; Testes

1. Introduction

Three major germ cell development strategies have been described for vertebrates: anamniotes [1], amniotes; birds and mammals [2,3]; and reptiles [4]. Anamniotes have testes composed of tubules/lobules that are lined with cysts where germ cells develop together as a single population and are typically released into centralized lumina in a single spermiation event [1,5]. Classical literature involving only birds and mammals has shown that amniotic testes consist of seminiferous tubules that are lined with seminiferous epithelia in which germ cells develop and maintain consistent spatial relationships during spermatogenesis [2,6,7]. These consistent stages have two or three spermatids consistently associated with the same early

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0093-691X/$ – see front matter
doi:10.1016/j.theriogenology.2009.04.003

mitotic and meiotic germ cells within a single cross-sectional view of a seminiferous tubule. Cellular associations are also sequentially organized along the length of the seminiferous tubules, which leads to waves of sperm release from specific segments of the seminiferous epithelia [2,8].

Recently, a new germ cell development strategy has been described in reptilian testes, which have a testicular structure similar to that of birds and mammals. However, reptiles do not have their germ cells organized into consistent cellular associations, and up to five spermatids are grouped with earlier mitotic and meiotic generations (similar to anamniotic amphibians) [4]. This plesiomorphic-like temporal germ cell development strategy has been recognized in all major taxa of reptiles to date (Chelonia [4]; Sauria [9]; Crocodylia [10]; and Serpentes [11]). These results demonstrate that this ancestral germ cell development strategy within a structurally amniotic testis may suggest decoupling of testicular organization and germ cell development strategy within the amniotic lineage.

However, recent histologic evaluations of testicular structure and germ cell development strategies within reptiles have been restricted to temperate species only. Spermatogenesis in these species is typically limited to warmer months, due to the lack of resources, which are presumably used to facilitate metabolically demanding processes such as spermatogenesis [12] in colder periods of the year. Several studies provided data on continuous spermatogenesis in tropical lizards [13–15]. Nevertheless, none of these studies described the germ cell development strategy employed during spermatogenesis within the testis. Thus, no information exists that provides evidence for whether continuous sperm production has an affect on the germ cell development strategy used by poikilothermic reptiles.

The purpose of the current study was to determine the germ cell development strategy within the Jamaican Gray Anole, *Anolis lineatopus*. This tropical anole is a medium-sized (50 to 65 mm) lizard commonly found in southern regions of Jamaica [16]. To date, information regarding life history characteristics, including reproductive characteristics of the Jamaican Gray Anole, is extremely limited, and to our knowledge this was the first study that explored spermatogenesis in this species.

Numerous reproductive studies involving anoles have been performed on both temperate and tropical species [14,17–20,21]; however, of the seven described species of *Anolis* found in Jamaica [22], published information regarding reproductive characteristics exists only for *A. opalinus* (native) and *A. sagrei* (introduced). *A. opalinus* testes are spermatogenically active every month of the year [14]. Conversely, *A. sagrei* has a more seasonal spermatogenic cycle [23]. Their testes are spermatogenically active from January to August, regressed September to November, and recrudescence begins in December. Data collected in this study were compared with the reproductive cycles of *A. opalinus* and *A. sagrei* and with known germ cell development strategy described in temperate reptiles to determine if continuous spermatogenesis had an effect on the reproductive strategy employed during sperm development in Reptilia.

2. Materials and methods

Adult male Jamaican Gray Anoles, *Anolis lineatopus*, were collected (N = 36) from southern Jamaica during every month of the year. Individuals were killed via decapitation (as this species is too small for proper intraperitoneal injection) and the testes were dissected, immediately cut into transverse sections, and stored in Trump's fixative (EMS, Hatfield, PA, USA) at 4 °C.

Testes were cut into small (3 mm) sections and dehydrated through a graded series of ethanol solutions: 70%, 85%, 95% × 2, and 100% × 2, for 30 min each. Tissues were then gradually introduced to Spurr's plastic (EMS) through a series of ethanol-plastic combinations (2 pt EtOH:1 pt plastic, 1 pt EtOH:1 pt plastic, 1 pt EtOH:2 pt plastic) before placing the tissue into pure Spurr's overnight on a rotary system. Tissues were then embedded in newly synthesized 100% plastic and allowed to cure for 2 d at 70 °C in a Fisher Isotemperature vacuum oven (Fisher Scientific, Pittsburgh, PA, USA).

The plastic blocks were sectioned (2- to 3-μm sections) using an LKB ultramicrotome (LKB Produkter AB, Bromma, Sweden) and dry glass knife. The sections were placed on glass slides and stained with a toluidine blue/basic fuchsin stain as described by Hayat [24]. Tissue samples were viewed using a Zeiss compound microscope (Carl Zeiss MicroImaging Inc., Thornwood, NY, USA) at various magnifications to determine testicular organization and germ cell morphologies present during each month. Photographs of the samples were taken using an attached SPOT digital camera (Diagnostic Systems Laboratories, Webster, TX, USA), and composite plates were constructed digitally using Adobe Photoshop CS (Adobe Systems, San Jose, CA, USA).

Twenty cross sections of seminiferous tubules representing each month were randomly chosen, and the tubule diameter and germinal epithelial heights of

each were measured using an ocular micrometer. Data analyses were performed using SigmaStat version 3.5 (Systat Software, Inc., San Jose, CA, USA) for Windows. Results were deemed significant if $\alpha \leq 0.05$. Tubule diameter and germinal epithelial height data were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov and Bartlett's tests, respectively, before statistical analyses were performed [25,26]. These data did not meet assumptions of normality or homogeneity of variance; thus, nonparametric Kruskal-Wallis analyses of variance were used to test for significant monthly variation in seminiferous tubule diameter and germinal epithelial height. Post hoc nonparametric multiple comparison tests using Dunn-Sidak procedures were then used to identify significant differences between pairs of monthly means [27].

3. Results

3.1. Testicular morphology and cell cycle

The testes of *A. lineatopus* contained seminiferous tubules, which had germinal epithelial linings that surrounded centrally located lumina. The epithelia contained developing germ cells and supporting Sertoli cells. The testes were spermatogenically active during the entire year, with multiple generations of spermatogenesis observed within a single cross-sectional view of a monthly seminiferous tubule. Spermatogonia A and B rested on the basal lamina of the seminiferous epithelium and continuously underwent mitotic divisions, ensuring new germ cell generation recruitment, which replenished cells that had completed spermatogenesis. Sertoli cell nuclei shared the basal compartment with spermatogonia and surrounded generations of developing germ cells with cellular processes as they migrated centrally toward the lumen during the maturation process.

3.2. Premeiotic cells

Two distinct premeiotic cells were visualized within the testis of *A. lineatopus*; spermatogonia A and spermatogonia B (Fig. 1; SpA and SpB). Spermatogonia A (Fig. 1; SpA) were relatively ovoid in shape with centrally located nuclei and two nucleoli within their nucleoplasms. After a single mitotic division, spermatogonia A became spermatogonia B (Fig. 1; SpB), which had more spherical nuclei and a single nucleolus. These two premeiotic cells were found throughout the entire year and served the purpose of replenishing the germ cell population.

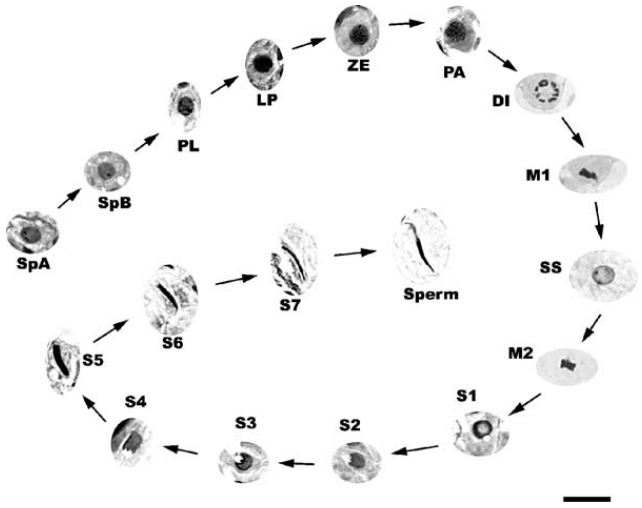

Fig. 1. Germ cell morphologies observed within the seminiferous epithelium of *Anolis lineatopus*. Scale bar = 25 μm. SpA, spermatogonia A; SpB, spermatogonia B; PL, pre-leptotene spermatocyte; LP, leptotene spermatocyte; ZE, zygotene spermatocyte; PA, pachytene spermatocytes; DI, diplotene spermatocytes; M1, meiosis I; SS, secondary spermatocytes; M2, meiosis II; S1, Step 1 spermatid; S2, Step 2 spermatid; S3, Step 3 spermatid; S4, Step 4 spermatid; S5, Step 5 spermatid; S6, Step 6 spermatid; S7, Step 7 spermatid; and sperm, mature spermatozoa.

3.3. Meiotic cells

During meiotic divisions, the nucleus began to increase in size, and the chromatin condensed into dark, easily distinguishable chromosomes. Spermatogonia B divided and entered prophase of meiosis I as pre-leptotene cells (Fig. 1; PL). These spermatocytes were approximately three-fourths the size of spermatogonia B and were the smallest of the developing germ cells. Leptotene spermatocytes (Fig. 1; LP) were easily distinguished from pre-leptotene generations because their nuclei were nearly filled with chromatin. Zygotene spermatocytes (Fig. 1; ZE) had a more discernible nucleoplasm and more intensely staining chromatin fibers. Pachytene cells (Fig. 1; PA) were the second largest spermatocytes next to diplotene cells. Their larger cell size and open nucleoplasm distinguished these meiocytes from zygotene spermatocytes. Diplotene cells (Fig. 1; DI) were the largest in volume of the spermatocytes and were found in close association with pachytene spermatocytes. These cells had a degenerating nuclear membrane, and the chromatin fibers formed a tight circle in juxtaposition to this membrane.

During metaphase of meiosis I (Fig. 1; M1), chromosomes aligned on the midplate of each cell. Secondary spermatocytes (Fig. 1; SS) marked the interphase before meiosis II, and two or three heterochromatic clumps could be seen within their

nucleoplasm. During this stage, nuclear membranes had reformed, and the entire cell remained relatively large. Metaphase of meiosis II (Fig. 1; M2) was similar to meiosis I, with the exception of being smaller in size and having approximately half the amount of chromatin.

3.4. Spermiogenic cells

Step 1 spermatids (Fig. 1; S1) were the outcome of the two meiotic divisions. The acrosome vesicle of this spermatid came in contact with the nuclear membrane and created a fossa on the apical surface of its nucleus. Step 2 spermatids (Fig. 1; S2) had slightly elongated acrosome vesicles and more pronounced fossae on their nuclei. The acrosome vesicle became deeply seated in the nuclear fossa of each Step 3 spermatid (Fig. 1; S3). Acrosome granules were often present against the inner acrosomal membranes of Step 2 and 3 spermatids. Step 4 (Fig. 1; S4) spermatids were the transition between the round spermatids (Steps 1 to 3) and the elongating spermatids (Steps 5 to 7). Step 4 spermatids had well-defined acrosome vesicles and granules, and their distal nuclei began the elongation process, which resulted in their slightly cylindrical nuclear shape. During Steps 5 to 7 (Fig. 1; S5 to S7), the nuclei continued to elongate and the chromatin within each nucleus underwent condensation, so that the final product of elongation was a thin, elongated, and slightly bent nuclear head. Upon completion of spermiogenesis, Step 7 spermatids were released as mature spermatozoa (Fig. 1; Sperm).

3.5. Germ cell development strategy

Although spermiogenesis and spermiation occurred during every month of the year, there were distinct cycles between spermiogenesis/spermiation and the early events of spermatogenesis. Furthermore, the events of spermiogenesis seemed to be slower in development than the consistent mitotic and meiotic divisions, which resulted in layers of three to five spermatids found within the seminiferous epithelia during the months of the heaviest spermatid development

Even though histologically the Jamaican Gray Anole had continuous spermatogenesis throughout the year, there were monthly variations in seminiferous tubule diameter (Kruskal-Wallis: $H = 192.38$, $df = 11$, $P = 0.000$; Fig. 2, top) and seminiferous epithelial height (Kruskal-Wallis: $H = 56.83$, $df = 11$, $P = 0.000$; Fig. 2, bottom), with significant trends over all sampled months. Both the histologic and morphometric data closely paralleled one another; we inferred that three major spermiogenic and spermiation events occurred during a calendar year within the Jamaican Gray Anole. This cyclic pattern of spermiogenesis and sperm release was best seen in the histologic sections and in the seminiferous tubule diameters (TDs). Seminiferous epithelial (SE) morphometrics had the same trends, but not as dramatically as tubule diameter, which resulted in only two major superscript groups occurring within the seminiferous epithelial height data.

The first major wave of spermiogenesis occurred during the month of February. An increase in the spermatid population was apparent histologically (Fig. 3) and morphometrically (Fig. 2, top, subscript subset B) at this time, with elongation climaxing in the February seminiferous tubules (TD, 246 μm; SE, 62.5 μm). January testes (TD, 212 μm; SE, 54 μm) had fewer elongates and less spermiation than those of February, and the seminiferous tubules at this time were recovering from heavy late spermiogenesis and spermiation present in December testes (described shortly). March and April (Fig. 4) testes had few to no spermatozoa in their lumina; a rebound in the round spermatid population was occurring at this time to replace the spermatids lost during spermiation in the previous month. This loss of the spermatozoa population and a decrease in luminal size resulted in a significant drop in TD (March, 197 μm; April, 224 μm; Fig. 2, top, superscript subset C, A) compared with February.

The second major wave of spermiogenesis and sperm release occurred in a 2-mo increment. May and June (Fig. 5) had a considerable increase in both late spermiogenesis and spermiation. The enlargement of the SE in response to the increase in the elongate population and an increase in lumina size in response to a large burst of spermiation caused a significant boost in TD (May, 286.5 μm; June, 292.5 μm; Fig. 2, top, superscript subset D, E). Similar to the first wave, July and August seminiferous tubules (Fig. 6) showed a sizable decrease in the columns (Fig. 6B, black arrows) of the SE and a reduction in the luminal size, which led to a significant drop in TD (July, 194 μm; August, 220 μm; Fig. 2, top, superscript subset C, A).

The final wave of spermiogenesis and spermiation occurred over 4 mouths and led to a continuous increase in sperm release from September through December. There was very little difference between these months histologically (Figs. 7 and 8) except for an observable increase in size of the columns of seminiferous epithelium holding sequential generations of elongating spermatids, and large cohorts of spermatozoa were

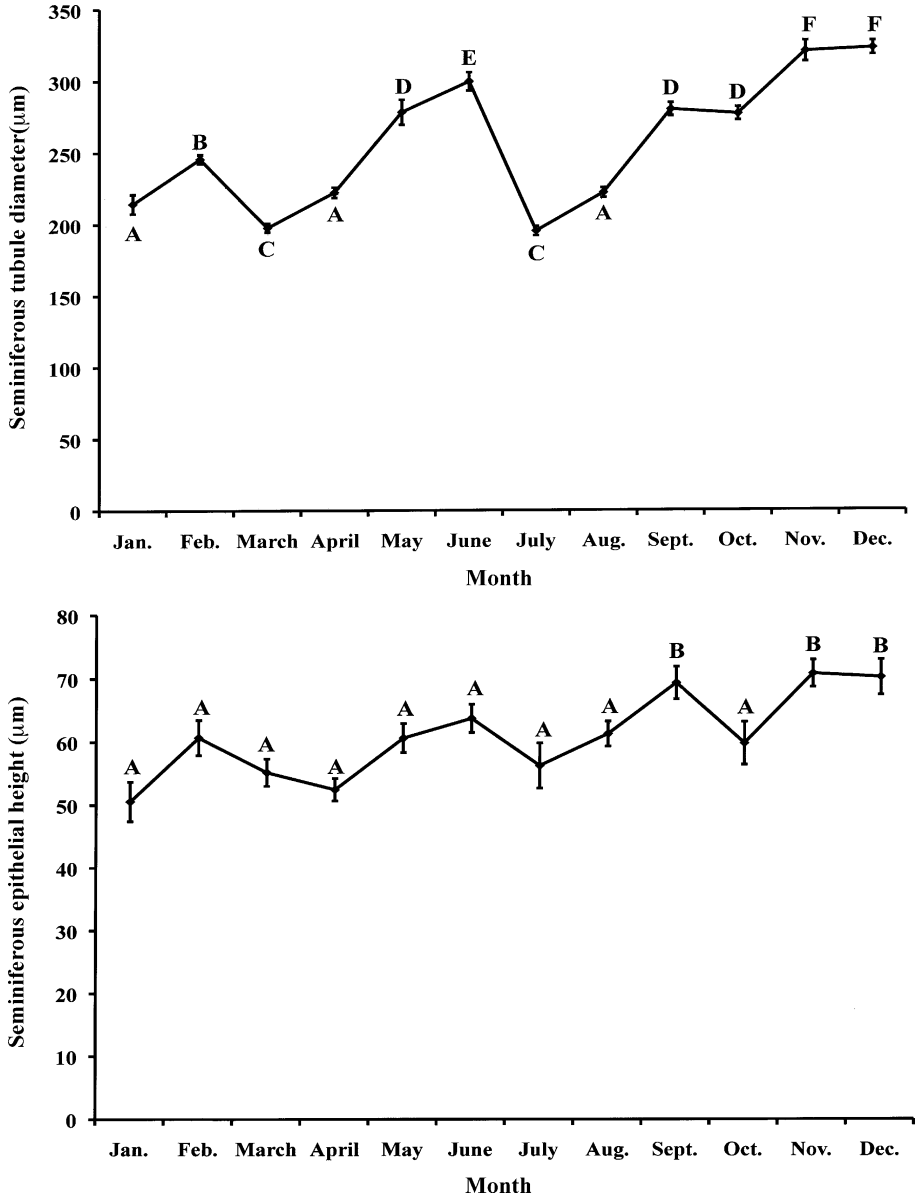

Fig. 2. Top: Variation in seminiferous tubule diameter. Bottom: Variation in germinal epithelial height during the months of January–December within the testis of *Anolis lineatopus*. Values represented on this graph are means ± 1 SEM. $^{A–F}$Values without a common superscript differed significantly ($P \leq 0.05$; Dunn-Sidak multiple range test).

being released to the lumina of seminiferous tubules over these months. The morphometrics (Fig. 2, top, superscript subsets D, F) followed the histologic observations robustly; the TD and the SE increased significantly over these months, leading to the largest TD and second highest SE measurements (TD, 318 μm; SE, 68 μm) within December testes. Throughout all months, spermatid generations (≤5) were not consistently associated with any of the earlier generations of mitotic and meiotic cells. Thus, consistent cellular associations were not present, and the germ cell development strategy was temporal in nature within each of the three cycles of spermiogenesis and spermiation.

4. Discussion

The testes of *A. lineatopus* consisted of seminiferous tubules lined with seminiferous epithelia where germ cells matured into spermatozoa. Spermatogonia A and B were present throughout the entire year in close association with basally located Sertoli cell nuclei. This testicular structure was similar to that reported in all other major reptilian taxa (Chelonia [4]; Sauria [9]; Crocodylia [10]; Serpentes [11]) and similar to the testicular structure exhibited by birds and mammals [8].

Although spermatogenesis occurred in every month of the year, similar to that of other tropical lizards [14,15,28], there were activity differences between each

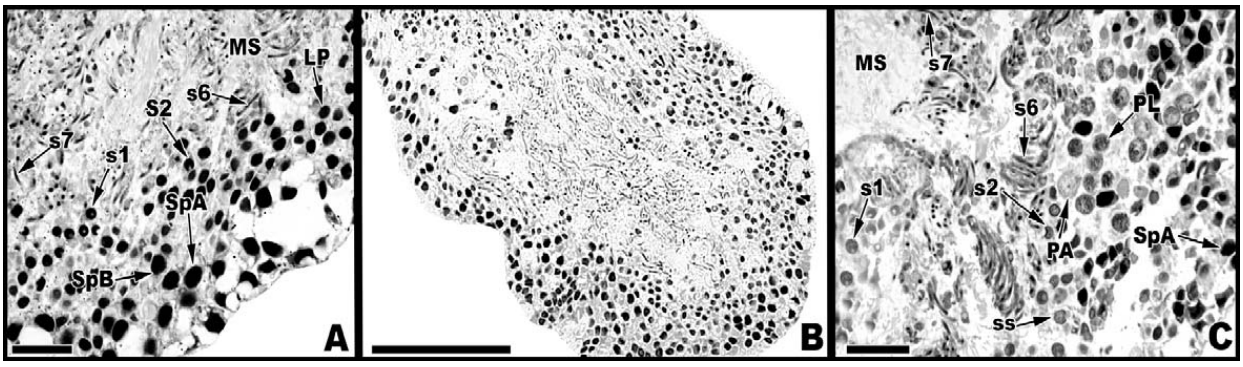

Fig. 3. (A) January and (C) February seminiferous epithelia with represented cell types. Scale bar = 30 μm. The seminiferous tubules of these two months are similar in morphology, thus (B) (January) is a representative micrograph of a low-power seminiferous tubule for January and February. Scale bar = 100 μm. Represented cell types within the germinal epithelia of both months: SpA, spermatogonia A; SpB, spermatogonia B; PL, pre-leptotene spermatocyte; LP, leptotene spermatocyte; PA, pachytene spermatocyte; ss, secondary spermatocyte; s1, Step 1 spermatid; s2, Step 2 spermatid; s6, Step 6 spermatid; s7, Step 7 spermatid; and MS, mature spermatozoa.

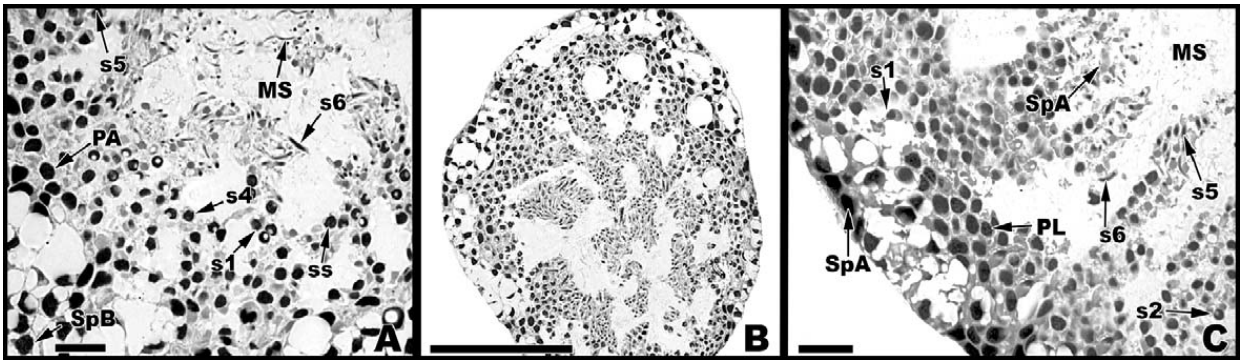

Fig. 4. (A) March and (C) April seminiferous epithelia with represented cell types. Scale bar = 30 μm. The seminiferous tubules of these two months are similar in morphology, thus (B) (March) is a representative micrograph of a low-power seminiferous tubule for March and April. Scale bar = 100 μm. Represented cell types within the germinal epithelia of both months: SpA, spermatogonia A; SpB, spermatogonia B; PL, pre-leptotene spermatocyte; PA, pachytene spermatocyte; ss, secondary spermatocyte; s1, Step 1 spermatid; s2, Step 2 spermatid; s4, Step 4 spermatid; s5, Step 5 spermatid; s6, Step 6 spermatid; and MS, mature spermatozoa.

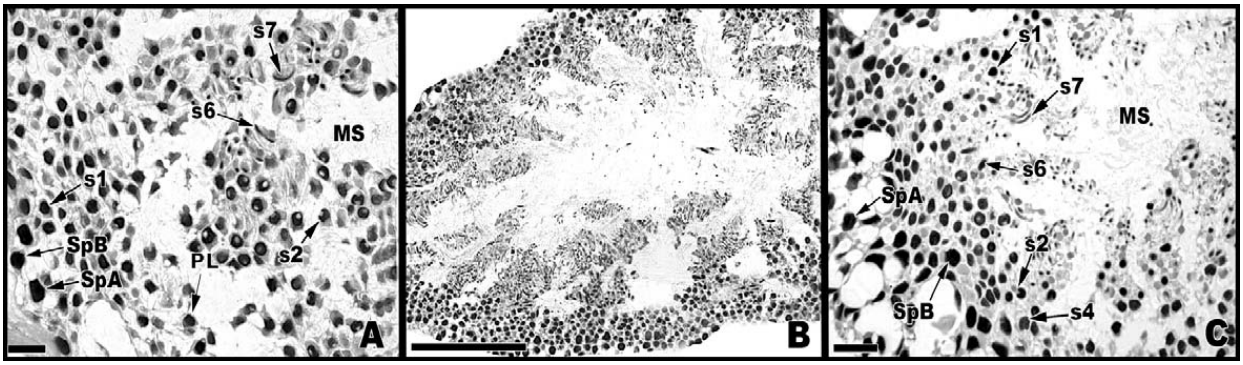

Fig. 5. (A) May and (C) June seminiferous epithelia with represented cell types. Scale bar = 30 μm. The seminiferous tubules of these two months are similar in morphology, thus (B) (May) is a representative micrograph of a low-power seminiferous tubule for May and June. Scale bar = 100 μm. Represented cell types within the germinal epithelia of both months: SpA, spermatogonia A; SpB, spermatogonia B; PL, pre-leptotene spermatocyte; s1, Step 1 spermatid; s2, Step 2 spermatid; s4, Step 4 spermatid; s6, Step 6 spermatid; s7, Step 7 spermatid; and MS, mature spermatozoa.

phase of spermatogenesis during certain time periods within the annual spermatogenic cycle of the Jamaican Gray Anole. These inconsistencies seemed to be between spermiogenesis and mitosis and meiosis. Mitotic and meiotic activity was similar for every month; however, spermiogenesis occurred in three increasing waves of activity, with three rebound periods between these waves within the annual cycle. Spermio-

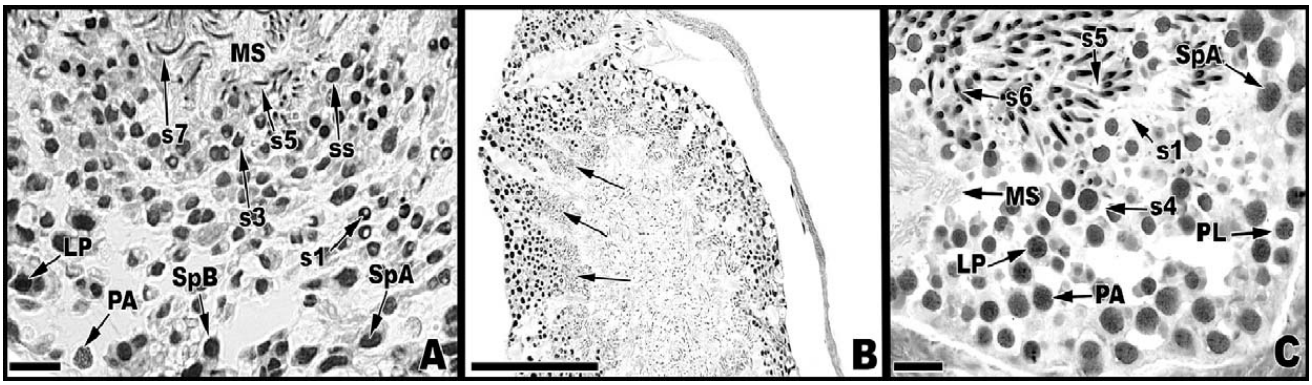

Fig. 6. (A) July and (C) August seminiferous epithelia with represented cell types. Scale bar = 30 μm. The seminiferous tubules of these two months are similar in morphology, thus (B) (June) is a representative micrograph of a low-power seminiferous tubule for July and August. Scale bar = 100 μm. Note black arrows in (B) point to columns of seminiferous epithelia that hold developing stages of spermatids. Represented cell types within the germinal epithelia of both months: SpA, spermatogonia A; SpB, spermatogonia B; PL, pre-leptotene spermatocyte; LP, leptotene spermatocyte; PA, pachytene spermatocyte; ss, secondary spermatocyte; s1, Step 1 spermatid; s3, Step 3 spermatid; s4, Step 4 spermatid; s5, Step 5 spermatid; s6, Step 6 spermatid; s7, Step 7 spermatid; and MS, mature spermatozoa.

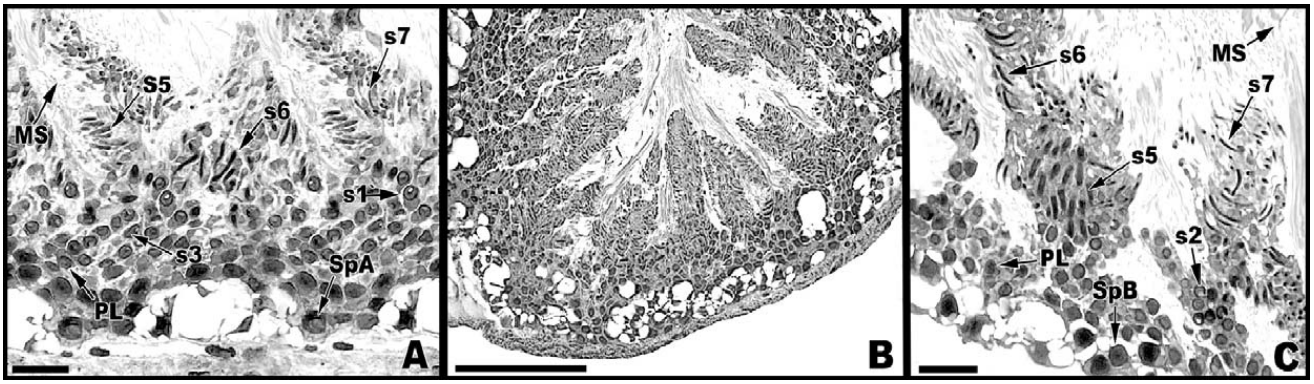

Fig. 7. (A) September and (C) October seminiferous epithelia with represented cell types. Scale bar = 30 μm. The seminiferous tubules of these two months are similar in morphology, thus (B) (September) is a representative micrograph of a low-power seminiferous tubule for September and October. Scale bar = 100 μm. Represented cell types within the germinal epithelia of both months: SpA, spermatogonia A; SpB, spermatogonia B; PL, pre-leptotene spermatocyte; s1, Step 1 spermatid; s2, Step 2 spermatid; s5, Step 5 spermatid; s6, Step 6 spermatid; s7, Step 7 spermatid; and MS, mature spermatozoa.

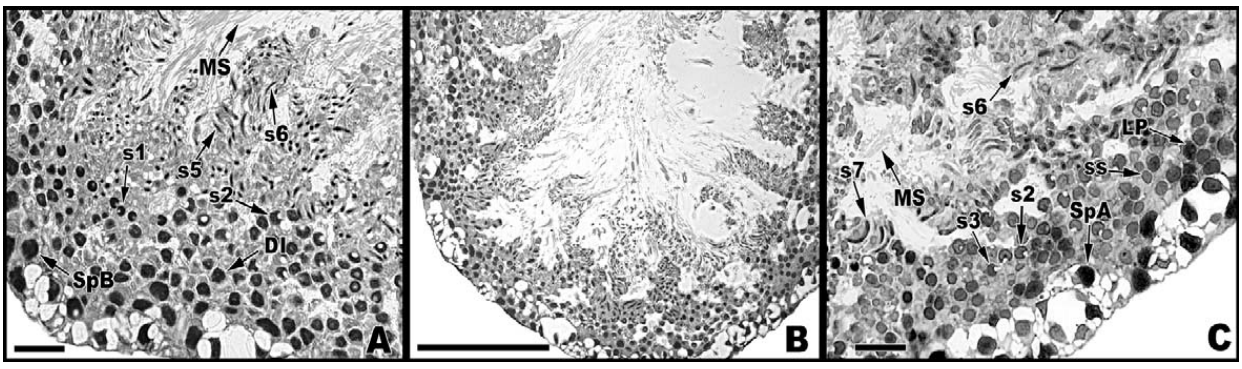

Fig. 8. (A) November and (C) December seminiferous epithelia with represented cell types. Scale bar = 30 μm. The seminiferous tubules of these two months are similar in morphology, thus (B) (November) is a representative micrograph of a low-power seminiferous tubule for November and December. Scale bar = 100 μm. Represented cell types within the germinal epithelia of both months: SpA, spermatogonia A; SpB, spermatogonia B; LP, leptotene spermatocyte; DI, diakinesis; ss, secondary spermatocyte; s1, Step 1 spermatid; s2, Step 2 spermatid; s3, Step 3 spermatid; s5, Step 5 spermatid; s6, Step 6 spermatid; s7, Step 7 spermatid; and MS, mature spermatozoa.

genesis climaxed in February, May and June, and September–December, and major spermiation events occurred at the end of these spermiogenic peaks, leading to burst of spermatozoa release during these months. Between spermiogenic peaks, the seminiferous epithelium was in spermiogenic rebound where new round spermatids were recruited from spermatocytes that had just completed meiosis. These recovery events lead to drops in TD and SE over the months of January, March/April, and July/August. These waves of spermiogenic activity may explain the inconsistencies reported in other anoles regarding spermatogenic activity reported and in other studies within lizards that use testicular mass as a measure of testis activity [14,28,29].

Typically in more northern lizards with temporal germ cell development, spermiogenesis ensues in one extended event [4]. These lizards frequently have seasonal spermatogenesis and mating due to the long, cold winters, which hinder the energy intake required to maintain such demanding metabolic processes as spermatogenesis [12]. *Anolis lineatopus* occupies a tropical niche where resources are available year-round and can maintain the three large spermiogenic events seen during spermatogenesis. Because spermatozoa are released over these three increased periods of spermiogenesis, which span the entire annual cycle, *A. lineatopus* potentially can mate throughout the year. Unfortunately, to the best of our knowledge, there is no published information that specifically details when the Jamaican Gray Anole reproduces. Our data, however, closely resembled that reported for *A. opalinus* in Jamaica [14]. Though fluctuations are observed in testis mass in this species of anole, histologically their testes were always active in spermiogenesis and spermiation, and the epididymis was packed with sperm year-round. Furthermore, this study also reported that male *A. opalinus* were observed copulating with females in every month of the year. Thus, *A. lineatopus* most likely practices the same type of reproductive behavior as *A. opalinus* and would potentially reproduce with fertile females at any time during the year.

Although the Jamaican Gray Anole has continuous spermatogenesis like many birds and mammals, its germ cell development strategy was more reminiscent of that in anamniotes. This temporal germ cell development was much different than the typical spatial amniotic germ cell development strategy found in mammals and birds [6,30–34]. The temporal development of cohorts of germ cells within the seminiferous epithelium of *A. lineatopus* was similar to the germ cell development strategy in more temperate squamates [4,11,35]. The waves of spermiogenesis were also consistent with the *Agkistrodon piscivorus* [11] and the introduced *Hemidactylus turcicus* within Louisiana (Gribbins, unpublished data). These two reptile populations inhabited more moderate temperate regions (Louisiana) within their northern ranges. Thus, they have more warm months in which to maintain spermatogenesis and consequently can support multiple waves of spermiogenesis unlike, for example, *Podarcis muralis* (Ohio) [9] or the *Seminatrix pygaea* (Georgia) [36], which support only one major wave of spermiogenesis in the summer months. Overall, based on our data, whether a reptile practices continuous or seasonal spermatogenesis has no impact on the germ cell development strategy employed by the reptiles studied to date. There are simply modifications to the number of spermiogenic waves seen during germ cell development within temperate versus tropical species.

Anolis lineatopus adhered strictly to the temporal development strategy previously described for other reptiles and for anamniotes such as anurans. However, the testis of *A. lineatopus* differed from anamniotes in that the seminiferous tubules, like other amniotes, are not lined with cysts. Thus, this anole supported a plesiomorphic-like germ cell development strategy within the typical tubular testis of amniotes, which was considered a synapomorphy for this clade. As birds are direct descendants of the reptilian group Archosauria, then the spatial germ cell development within birds should be considered a synapomorphy for Aves. Linking the spatial germ cell development strategy shared by Aves and Mammalia evolutionarily is more difficult. Mammals are considered a sister taxon to modern reptiles and do not share a common ancestor with birds [37,38]. Thus, the most parsimonious explanation is convergence of the spatial pattern of germ cell development when considering mammals and birds, which may be linked to the practice of homeothermy in these two amniotic groups. Our understanding of germ cell development strategy and the evolution of the process of spermatogenesis in reptiles have increased over the last 6 yr. However, other tropical, semitropical, and temperate reptiles should be studied along with basal Monotremata mammals so that our understanding of spermatogenesis and the evolution of the testis within the amniotic clade becomes clearer.

Acknowledgment

This research was funded in part by competitive research grants from Wittenberg University.

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