Seasonal spermatogenesis in the Mexican endemic oviparous lizard, *Sceloporus aeneus* (Squamata: Phrynosomatidae)

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Oviparous species of *Sceloporus* exhibit either seasonal or continuous spermatogenesis and populations from high-elevation show a seasonal pattern known as spring reproductive activity. We studied the spermatogenic cycle of a high-elevation (2700 m) population of endemic oviparous lizard, *Sceloporus aeneus*, that resided south of México, D.F. Histological analyses were performed on the testes and reproductive ducts from individual lizards collected monthly. This population of *S. aeneus* showed a seasonal pattern of spermatogenesis, with 4 successive phases common in other lizards. These include: 1) Quiescence in August, which contained solely spermatogonia and Sertoli cells; 2) Testicular recrudescence (September-January) when testes became active with mitotic spermatogonia, spermatocytes beginning meiosis, and the early stages of spermiogenesis with spermatids; 3) Maximum testicular activity occurred from March to May and is when the largest spermiation events ensued within the germinal epithelia, which were also dominated by spermatids and spermiogenic cells; 4) Testicular regression in June was marked with the number of all germs cells decreasing rapidly and spermatogonia dominated the seminiferous epithelium. February was a transitional month between recrudescence and maximum activity. The highest sperm abundance in the lumina of epididymides was during maximum testicular activity (March-May). Thus, before and after these months fewer spermatozoa were detected within the excurrent ducts as the testis transitions from recrudescence to maximum activity in February and from maximum activity to quiescence in June. Maximum spermatogenic activity corresponds with warmest temperatures at this study site. This pattern known as spring reproductive activity with a fall recrudescence was similar to other oviparous species of genus *Sceloporus*.

Introduction

According to previous studies,¹⁻³ spermatogenesis in lizards can be divided into 4 main phases: quiescence, recrudescence, maximum activity, and regression. The presence/absence of various germ cell stages, Sertoli cell, and Leydig cell sizes and morphologies can all be used concurrently to characterize each of the various testicular stages. Besides that, the presence and absence of mature sperm in the extra and intratesticular ducts may also help the characterization of the reproductive cycles, which are often different (i.e., dissociative) from the testicular cycle. These characterizations are made by the use of histological examinations, which strongly support the various spermatogenic stages in contrast to other types of evidence, such as testicular size (i.e., volume or mass) and/or opaqueness of the extratesticular ducts.¹⁻⁴

Although both seasonal and continuous spermatogenesis have been observed in lizards, seasonal spermatogenesis is by far the

most common type of male germ cell cycle. This pattern has been recorded in different genera within Phrynosomatid lizards including: *Phrynosoma*,⁵ *Uma*,⁶ *Cophosaurus*,⁷ *Uta*,⁸ *Petrosaurus*,⁹ *Urosaurus*,¹⁰ and *Sceloporus*.^{2,11} Within the *Sceloporus* genus, both seasonal and continuous spermatogenesis have been observed. Because this genus has a wide elevational range (0 m to > 4600 m¹²) and contains both oviparous and viviparous species, this genus makes an excellent model for reproductive studies. Previous studies have shown that oviparous species exhibit either seasonal^{2,3} or continuous spermatogenesis,¹³ and those from high-elevation show a maximum testicular activity during spring.^{2,3}

Both lizards *S. aeneus* (oviparous) and *S. bicanthalis* (viviparous) belong to the *scalaris* group, being considered as sister taxa.¹⁴⁻¹⁷ Although both of them are endemic to central México, their distribution is distinctive as *S. bicanthalis* takes the place of *S. aeneus* at elevations above 3000 m.¹⁶ *S. bicanthalis* was once

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considered a subspecies of *S. aeneus* but has now been elevated to the species level.^{15,18} Evolution of viviparity in lizards of the genus *Sceloporus* has promoted changes in different reproductive traits, especially in the female reproductive cycle.¹⁹

Independent of the elevation, *S. bicanthalis* shows continuous spermatogenesis at 3,200 m²⁰ and at 4,200 m²¹ in central México. Previous studies^{22,23} utilizing testis volume recorded that *S. aeneus* exhibited seasonal spermatogenesis with maximum testicular activity from February to June. Therefore, to test the hypothesis that male reproductive cycles of the oviparous *S. aeneus* differ from those of the closely related viviparous *S. bicanthalis* we examined the reproductive cycle histologically of a population of *S. aeneus* and compare it to the published data of *S. bicanthalis*.^{20,21}

Results

The smallest male with mature sperm in the seminiferous tubules and epididymal ducts was 45 mm SVL. The average SVL of the adult males was 52.5 ± 3.6 mm ($n = 30$), with a range of 45–60 mm. According to the Shapiro-Wilk test, the transformed $\log_{10}$ of the histological traits (Table 1), testes masses, and SVL were normally distributed ($P > 0.05$ for overall models), and met the assumption of homogeneity (Levene's test, $P > 0.05$ for overall models). The SVL of males did not vary monthly ($P > 0.05$). According to linear regression analyses, there was no relationship between SVL and diameters of seminiferous tubules, germinal epithelium heights, diameters of epididymal ducts, epididymal epithelium heights, or testes masses ($P > 0.05$ for overall models).

We recorded significant differences among months for diameters of seminiferous tubules, germinal epithelium heights, diameters of epididymal ducts, and epididymal epithelium heights (Table 1). Moreover, the testes masses exhibited significant differences among months (Table 1). Although no statistical analyses were performed, we also recorded variation on epididymal sperm concentration among months (Table 1).

Phases of spermatogenesis

Quiescence (August)

In August, the testis was in the quiescent phase and only spermatogonia (Fig. 1A, Sg) and Sertoli cells (Fig. 1A, Sc) were

observed within the germinal epithelium. The epididymal epithelium (Fig. 1B) was atrophied and no sperm were present within the lumina (Table 1). During this phase, the diameters of seminiferous tubules (118.7 μ m, letters c,d), germinal epithelium heights (20.0 μ m, letter d), diameters of epididymal ducts (93.4 μ m, letter b), epididymal epithelium heights (18.0 μ m, letter b), and testes masses (0.033 g, letter d) were at their lowest values (Table 1).

Recrudescence (September–January)

The onset of recrudescence occurred in September (Fig. 1C), when the diameters of seminiferous tubules (97.9 μ m, letter d), germinal epithelium heights (26.4 μ m, letters c,d), diameters of epididymal ducts (74.1 μ m, letters b, c), epididymal epithelium heights (11.3 μ m, letter c), and testes masses (0.016 g, letter e) were still at their lowest values (Table 1). Testicular activity began with spermatogonia (Fig. 1C, Sg) dividing mitotically and increasing in number within the germinal epithelium. Moreover, Sertoli cells (Fig. 1C, Sc) and a few primary spermatocytes (1°Sp) were observed in the germinal epithelium. However, a distinct lumen was not present and the epididymis (Fig. 1D) remained in an atrophied state and devoid of sperm (Table 1). In November and December testes, spermatogonial numbers were still high (Fig. 2A, C, Sg) and within the basal compartment early generations of germ cells have entered meiosis as primary, and a few secondary spermatocytes (Fig. 2A, C, 1°Sp and 2°Sp). The epididymis during this time remains atrophied and devoid of sperm (Fig. 2B, D; Table 1). In January, spermatogonial divisions slowed and fewer basal layers of gonial cells are observed (Fig. 3A, Sg) near the basal lamina and spermatocytes (Fig. 3A, 1°Sp and 2°Sp) dominated the germinal epithelium. During this stage, many of the germ cells have completed meiosis and have entered the early stages of spermiogenesis as round spermatids (Fig. 3A, Rpt). Small pockets of elongating spermatids are visible near the lumina of seminiferous tubules (Fig. 3A, Spt). The epithelium of the epididymis has enlarged and the lumina contained small amounts of sperm representing minor isolated spermiation events (Fig. 3B, Eps; Table 1). The diameters of seminiferous tubules, germinal epithelium heights, diameters of epididymal ducts, epididymal epithelium heights, and testes masses have increased significantly from November to January (Table 1).

Table 1. Statistics of testicular and epididymal traits of *Sceloporus aeneus* from Milpa Alta, D. F. Different letters denote significant differences among months ($P < 0.05$). With the letter "a" representing the highest value, and + = low, ++ = medium, and +++ = high signifying relative abundance of sperm in the epididymis. The four phases within the spermatogenic cycle were: quiescence (August), recrudescence (September–January), maximum activity (March–May), and regression (June); February represents a transitional month between recrudescence and maximum activity

	F	DF	P-value	January	February	March	April	May	June	August	September	November	December
Diameters of seminiferous tubules	13.39	9,7	0.0012	a	a	a	a	a,b	b	c,d	d	a,b	b,c
Germinal epithelium heights	18.4	9,6	0.001	a	a	a	a	a	b,c	d	c,d	a,b	a
Diameters of epididymal ducts	14.85	9,6	0.0019	b	a	a	a	a	a	b	b,c	c	b,c
Epididymal epithelium heights	28.26	9,7	0.0001	b,c	a	a	a	a	a	b	c	c	b,c
Testes masses	20.83	9,20	<0.0001	a,b	a	a,b	a,b	b,c	c	d	e	c	d
Epididymal sperm presence				+	++	+++	+++	+++	++				

Transition from recrudescence to maximum spermiogenic activity (February)

In February, the diameters of seminiferous tubules (341.6 μm , letter a), germinal epithelium heights (110.7 μm , letter a), diameters of epididymal ducts (144.0 μm , letter a), epididymal epithelium heights (32.1 μm , letter a), and testes masses (0.317 g, letter a), were at their highest values (Table 1) compared to previous months. The testes in February still contained spermatogonia basally near the basement membrane (Fig. 3C, Sg); however, unlike January testes, there were fewer primary spermatocytes (Fig. 3C, 1°Sp). Cohorts of round spermatids (Fig. 3C, Rpt) and long columns of seminiferous epithelium containing elongating spermatids (Fig. 3C, Spt) dominated the February germinal epithelium. Furthermore, mature spermatozoa become evident in the lumen of the seminiferous tubules (Fig. 3C, Ms). The epithelium of the epididymis (Fig. 3D) at this time is greatly enlarged with the hypertrophied columnar epithelial cells that are filled with secretory material (*). Also, an increased amount of mature spermatozoa were seen within the lumina of the epididymal ducts (Fig. 3D, Eps; Table 1), which is evidence that spermiation is more common in February than in previous months. February was a preparation stage; the majority of the germ cell population were finishing meiosis and entering the early stages of spermiogenesis to prep the germinal epithelium for the climax of spermatid maturation.

Maximum testicular activity (March–May)

Spermiogenesis began in January and climaxed from March through May during which a massive spermiation event occurred. During March and April, the diameters of seminiferous tubules (319.1 μm , and 297.5 μm , respectively, letter a), germinal epithelium heights (110.3 μm , and 91.7 μm , respectively, letter a), diameters of epididymal ducts (191.3 μm , and 164.7 μm , respectively, letter a), epididymal epithelium heights (40.1 μm , and 39.7 μm , respectively, letter a), and testes masses

Figure 1. Testicular and epididymal histology of *Sceloporus aeneus* from Milpa Alta, D. F. (A) During August (quiescence), note in the seminiferous tubules the presence of Sertoli cell nuclei (Sc) and Spermatogonia (Sg). (B) During August (quiescence), epididymal epithelium is atrophied and no sperm are present. (C) During September (recrudescence), note in the seminiferous tubules the presence of Sertoli cell nuclei (Sc), a few primary spermatocytes (1°Sp), and Spermatogonia (Sg) dividing mitotically and increasing in number. (D) During September (recrudescence), the epididymis remains in an atrophied state and devoid of sperm.

(0.211 g, and 0.1806 g, respectively, letters a,b) were at their highest values (Table 1). During May, the diameters of seminiferous tubules (242.8 μm , letters a,b), germinal epithelium heights (83.5 μm , letter a), diameters of epididymal ducts (187.2 μm , letter a), epididymal epithelium heights (34.5 μm , letter a), remained at the peak values, except testes masses (0.1265 g, letters b,c see Table 1). The testis in March reached maximum activity and the germinal epithelium was dominated by large columns of elongating spermatids (Fig. 4A, Spt) and small cohorts of round spermatids are present below the columns (Fig. 4A, Rpt) with spermatogonia (Fig. 4A, Sg) and primary spermatocytes (Fig. 4A, 1°Sp) observed within the basal germinal epithelium. Additionally, mature spermatozoa (Fig. 4A, Ms) were evident in the lumen of the seminiferous tubules. During this time the epithelium of the epididymis cells was hypertrophied (Fig. 4B) and the lumina contained abundant spermatozoa (Fig. 4B, Eps; Table 1). This condition continued in April (Fig. 4C, D) and May (Fig. 5A, B) as large amounts of germ cells completed the stages of spermatogenesis and spermatozoa were transferred to the epididymis (Table 1).

Figure 2. Testicular and epididymal histology of *Sceloporus aeneus* from Milpa Alta, D. F. (A, C) During November and December (recrudescence). Note in the seminiferous tubules the presence of Sertoli cell nuclei (Sc) and Spermatogonia (Sg) that are located in the basal compartment and germ cells entering/progressing through meiosis as both primary spermatocytes (1°Sp) and secondary spermatocytes (2°Sp). (B and D) Show the epididymis remains atrophied and devoid of sperm in this recrudescence period.

Testicular regression (June)

Testicular regression began in June when a significant decrease was recorded in diameters of seminiferous tubules (178.1 µm, letter b), germinal epithelium heights (43.05 µm, letters b,c), and testes masses (0.0813 g, letter c). Diameters of epididymal ducts (172.9 µm, letter a), and epididymal epithelium heights (33.5 µm, letter a) did not decrease during testicular regression (Table 1). In this phase, no new germ cells entered the spermatogenic cycle within the germinal epithelium and the number of spermatids (Fig. 5C, Rpt, Spt) observed within the epithelium had decreased. The epithelium of the epididymis (Fig. 5D) remained hypertrophied. However, sperm decreased in the lumina of the seminiferous tubules (Fig. 5C, Ms) and epididymis (Fig. 5D, Eps) in comparison to earlier maximum testicular active months (Table 1).

Discussion

In this endemic population of oviparous lizard, *Sceloporus aeneus*, spermatogenic activity was seasonal. The histological examination enabled us to divide the spermatogenic activity into 4 main phases: quiescence, recrudescence, maximum activity,

and regression. These phases have been recorded in other species within the *Sceloporus* genus including: *S. mucronatus*,^{4,11} *S. spinosus*,² *S. consobrinus*.³

The spermatogenic activity of *S. aeneus* with peaks of spermiogenesis and spermiation occurring during late winter and spring (i. e., highest sperm abundance in epididymis during March-May), is similar to that found in other oviparous species of *Sceloporus*.^{2,3,24,25} According to the classification scheme provided by Rheubert et al.³ this population of *S. aeneus* represents a fall recrudescence pattern, which is common in oviparous lizards in temperate habitats. Although this population occurs at a tropical latitude it is located at high elevation where climatic patterns are similar to those found at temperate latitudes.

A number of studies have shown that temperature is an important environmental cue in male reproductive

cycles,^{20,26,27,28} although other factors such as rainfall and day length have also been indicated elsewhere.²⁸ The warmest temperatures during March (17.0°C), April (17.9°C), May (18.3°C), and June (17.2°C) at the study site²⁹ correspond with maximum testicular activity for this population of *S. aeneus* (March-May), and the highest concentration of spermatozoa in the lumina of the epididymis. These findings are consistent with previous studies in which warm temperatures appear to favor the maximum spermatogenic activity.^{1,30} Experimental studies through manipulation of reproductive cues for gonadal development³¹ represent one important step in the understanding of male reproductive cycle control in lizards and other reptiles.

The maximum testicular activity (March-May) is also associated with the reproductive cycle of female in which receptive females (i.e., vitellogenic females within the population) are present in abundant numbers from March to June.³² However during June, we found a male in a testicular regression phase copulating with a female. As suggested previously in viperid snakes from temperate zones,³³ females determine mating season and males adapt to the female reproductive phenology. During June, it is also true for the *S. aeneus*. Elevated plasma androgens during testicular regression have been reported in previous studies.³⁴ In lizards, the epididymis is an androgen-dependent

structure that secretes proteins required for sperm maturation.³⁵ In *S. aeneus*, epididymal epithelium was highest from February to June, which may be related to elevated androgen levels³⁴ (before and after maximum testicular activity). This dissociated pattern has already been observed for other squamates, where the mating during testicular regression may be linked to the hypertrophy of the sexual segment of the kidney.³⁶ Behavioral, hormonal, and histological (i.e., sexual segment of the kidney) studies in *S. aeneus* will clarify why mating may take place during testicular regression and thus warrants further investigation.

Our findings regarding the spermatogenic activity in *S. aeneus*, especially the length of the different phases, differs from previous studies.^{22,23} For example, according to histological examination (this study) the maximum testicular activity occurs from March to May; however based on the testis volume,^{22,23} the maximum activity of *S. aeneus* was from February to June. Several studies within lizards have shown that the testis mass or testis volume may not accurately represent the spermatogenic events of the testis.^{1-3,11,20} We agree with the efforts in using multiple indicators to describe the spermatogenic cycle of lizards, especially designs that include histological examination of reproductive organs.^{1-11,20-21} However, the use of a single indicator may be misleading. For example, some of the data collected for *S. aeneus* (testis mass) suggest that February should be included in the months classified as maximum testicular activity (Table 1). However, although no statistical analyses were performed, February showed intermediate quantity of spermatozoa in the epididymis with respect to the massive spermatozoa release recorded during maximum testicular activity (March–May). The present data, along with numerous other studies,¹⁻⁴ suggest that histological data is the most accurate technique compared to size of testis (volume or mass) alone in determining the seasonality of male reproductive/germ cell activity and length of different phases of the spermatogenesis. Therefore, studies involving exclusively

Figure 3. Testicular and epididymal histology of *Sceloporus aeneus* from Milpa Alta, D. F. (A) During January (recrudescence), there are spermatogonia (Sg) near the basal lamina, primary spermatocytes (1°Sp), secondary spermatocytes (2°Sp), and germ cells have completed meiosis and entered the early stages of spermiogenesis as spermatids (Rpt, Spt) apically. (B) January recrudescence shows that the epithelium of the epididymis also has enlarged and the lumina contain very few sperm (Eps). (C) During February (transition of recrudescence to maximum testicular activity), note the presence of spermatogonia (Sg) at the base of the germinal epithelium, both primary (1°Sp) and round spermatids (Rpt) dominate the epithelium with a few of the germ cells have entering spermiogenesis as elongating spermatids (Spt). A few mature sperm (Ms) are seen in the lumen of seminiferous tubules. (D) February (transition of recrudescence to maximum testicular activity) epithelium of the epididymis has greatly enlarged and the cytoplasm of the epididymal epithelial cells is filled with secretory material (*), and a few mature sperm (Eps) are seen in the lumen.

macroscopic data to evaluate the male reproductive cycle should be interpreted with caution.^{37,38}

The SVL (45 mm SVL) at sexual maturity of males of *S. aeneus* was equal to that of females.³² The ratio (104.5%) male SVL (52.5 mm) to female SVL (50.2 mm) were higher than other intersexual SVL ratios recorded previously in *Sceloporus* (101.3%³⁹). The SVL at sexual maturity of males *S. aeneus* within this population was the smallest recorded in this species. In fact the SVL at sexual maturity of the high elevation lizard, *Sceloporus bicanthalis* is the smallest within the entire *Sceloporus* genus (34 mm and 35 mm,^{20,21} respectively; see Fitch³⁹). Apparently early sexual maturity could be an inherent trait within the *scalaris* group and linked to high elevation within these Mexican spiny lizards (see Fitch³⁹ and Méndez-de la Cruz et al.¹⁹). A comparative analyses, including different life history traits, will clarify the evolution of early sexual maturity within *scalaris* group thus warrants further research.

Figure 4. Testicular and epididymal histology of *Sceloporus aeneus* from Milpa Alta, D. F. (**A, C**) During March and April (maximum activity), note the presence of spermatogonia (Sg) and primary spermatocytes (1°Sp) at the base of germinal epithelium; the germinal epithelium is dominated by round and elongating spermatids (Rpt, Spt) with mature sperm (Ms) abundant within the lumina of seminiferous tubules. (**B, D**) The March and April (maximum activity) epithelia of epididymal cells are hypertrophied, and the lumen of epididymis is filled with mature spermatozoa (Eps).

Although many hypotheses and explanations have been provided for the evolution of viviparity in lizards, especially within the *Sceloporus* genus,¹⁹ many of these focus on female reproduction without connecting the possible correlation between changes in male reproductive patterns. Rheubert et al.³ investigated the link between parity mode and male reproductive patterns and effectively found no link. However, their study focused on the entire *Sceloporus* genus and looked at large scale differences (i.e., fall vs. spring recrudescence). It may be plausible that male reproductive variations are correlated to variations in female reproductive patterns, as viviparous and oviparous species vary in their timing of reproduction, which may cause or stimulate deviations in male cycles as well.¹⁹ *S. bicanthalis* and *S. aeneus* are sister taxa and thus represent a good model system for studying such disparities and links between parity mode and spermatogenic cycle. The presence of sperm in February and June in *S. aeneus* may signify a prolonged maturational phase to spermatogenesis, which may represent an intermediary step toward the continuous spermatogenesis observed in *S. bicanthalis*.^{20,21} Continuous spermatogenesis in *S. bicanthalis* (which probably is related to the extended presence of the receptive females in the population⁴⁰) is a complete prolongation of the reproductive season and may represent

the penultimate stage of prolonged reproductive activity in male *Sceloporus*^{19,41} and may represent a step in the progression of male reproductive cycles in response to the evolution of viviparity. Future studies, including populations of *S. aeneus* and *S. bicanthalis*, that are similar in geographic location, may provide insight into this hypothesis.

Materials and Methods

Study site and specimen collection

Monthly samples, from April 1993 to March 1994, of *S. aeneus* at Milpa Alta (to the south of México, D. F.) were collected (19°13'46" N, 99°03'10" W; 2700 m above sea level); with the exception of July and October in which no adult males were seen. This study site has a relatively cool climate (range 13.6–18.3°C), with the heaviest rainfall occurring in summer and warmest temperatures from March to June.²⁹ Vegetation at this high elevation is

dominated by pine forest and alpine bunchgrass (that lizards utilize for perches, and for shelter). However at this study site there are extensive areas of different types of agricultural crops and only small patches of native vegetation remaining.⁴²

Captured males were euthanized via an intraperitoneal injection of sodium pentobarbital. Specimens of *S. aeneus* were collected under the scientific collector permit SEMARNAT-FAUT 0074. The whole study was conducted in accordance to the ethical principles for the laboratory use of animals; all work met the relevant legal regulations as well as institutional procedures for animal research of the Facultad de Ciencias, UNAM. Snout-vent length (SVL) was measured to the nearest 0.1 mm, the testes and extratesticular ducts were removed via gross dissection, and the testes was weight to the nearest 0.0001 g. Specimens were fixed in 10% neutral buffered formalin, preserved in ethanol (70%), and deposited in the Colección Nacional de Anfibios y Reptiles, Instituto de Biología, UNAM (CNAR, UNAM).

Histological examination

Standard histological techniques were performed on the left testis of mature males. Histological procedures included:

dehydration in ascending concentrations of ethanol, cleared in xylene, and embedded in paraplast (J. T. Baker 9490–03, 9014–03; Leica Ref 39601006). Embedded tissues were sectioned at 5 μm with a rotary microtome and slides were stained with hematoxylin-eosin and Mallory's trichrome (Sigma H-9627, E-4009, O3756, A5268, F8129, 42755). Measurements were made using an ocular micrometer and included: diameter of seminiferous tubules, germinal epithelium height, diameter of epididymal ducts, and epididymal epithelium height (in 30 representative microscopic fields per individual). Germinal and Sertoli cells were classified according to Villagrán-Santa Cruz et al.⁴³. Moreover, to characterize the spermatogenic phases, we recorded the presence of spermatozoa within the lumina in both seminiferous tubules and epididymal ducts.

Figure 5. Testicular and epididymal histology of *Sceloporus aeneus* from Milpa Alta, D. F. (A) During May (maximum activity), note the presence of spermatogonia (Sg) and primary spermatocytes (1°Sp) basally, spermatids (Rpt, Spt) apically, and mature sperm (Ms) were most abundant within the lumina of seminiferous tubules. (B) In May (maximum activity), epididymal sperm (Eps) were most abundant in the lumina. (C) During June (regression), note the presence of spermatogonia (Sg) and the absence of early generations of germ cells entering spermatogenesis within the germinal epithelium. Also, spermatids (Rpt, Spt) have greatly decreased; mature sperm (Ms) additionally have decreased within the lumina of the seminiferous tubules. (D) During June (regression), the epithelium of the epididymis remained hypertrophied, and mature sperm concentration (Eps) decreased within the lumina respective to the maximum activity.

Statistical procedures

Diameters and epithelial heights of both seminiferous tubules and epididymal ducts, testis mass, and SVL were log₁₀ transformed to meet the assumptions of normality.⁴⁴ We used ANOVA (analyses of variance) on histological data and testes masses, with the month as factor; followed by a multiple comparison test (Duncan's test) in order to determine the differences among averages for each of the above measurements. All analyses were performed with α set at 0.05.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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