Ultrastructural and Morphometric Study of the Sertoli Cell of the Viscacha (Lagostomus maximus maximus) During the Annual Reproductive Cycle

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ABSTRACT
Changes in the morphology of viscacha Sertoli cells were studied during the annual reproductive cycle. Sertoli cells exhibited marked nuclear and cytoplasmic changes. Seasonal variation in nuclear size and shape, chromatin texture, and nucleolus characteristics was observed. The seasonal patterns of the volume densities of the endoplasmic reticulum (ER), mitochondria, Golgi complex, dense bodies and lipid inclusions were distinct. Morphometric analysis revealed that the Golgi complex is the organelle most sensitive to seasonal change. It declined drastically in the regressed testes and its recovery was slow. The ER and mitochondria exhibited seasonal variations in their pattern and content, that was minimal during winter. In contrast, an accumulation of lipid and dense bodies, such as primary and secondary lysosomes, accompanied the spermatogenic arrest. The volume densities of both organelles were maximum during the restoration of spermatogenesis. The length and organization of the inter-Sertoli junctions also changed with the reproductive cycle. The Sertoli cell number per tubular cross section decreased significantly during the testicular regression, coincident with the presence of Sertoli cells with marked signs of involution. The degree of regression and recovery exhibited by the viscacha Sertoli cells was closely related to that shown by the associated germ cells. Therefore, seasonal endocrine fluctuations and local factors could be involved in the regulation of the morphological and functional characteristics of the viscacha Sertoli cells. These hormonal fluctuations are synchronized by the photoperiod through the pineal gland and its hormone, melatonin. Anat Rec 262:176–185, 2001. © 2001 Wiley-Liss, Inc.

Key words: Sertoli cell; Lagostomus maximus maximus; seasonal breeder; photoperiod
Sertoli cells were first described in 1865. They represent the only non-germinal elements within the seminiferous tubules, where they extend from the tubular base toward the lumen, surrounding the developing germ cells with numerous fine cytoplasmic processes. This topographical arrangement allows Sertoli cells to interact with all the other testicular cell types. The Sertoli cell represents the central point of convergence for both circulating hormones and local testicular factors that regulate or modulate spermatogenesis (Pescovitz et al., 1994). The Sertoli cell has follicle stimulating hormone (FSH) receptors on its basal membrane and nuclear androgen receptors (Means et al., 1976; Sanborn et al. 1977; Tindall et al., 1977; Orth and Christensen, 1978). FSH and testosterone synthesized by Leydig cells during luteinizing hormone (LH) stimulation interact with these specific binding sites and initiate spermatogenesis during puberty. Once the process is established, intratubular testosterone is critical for maintaining normal sperm production (Sharpe et al., 1988).

Conversely, the specialized junctional complexes between neighboring Sertoli cells constitute the principal component of the blood-testis barrier (BTB), that is responsible for the privileged physicochemical microenvironment required for completion of the meiotic process and spermiogenesis (Waites and Gladwell, 1982; de Kretser and Kerr, 1988).

Although the Sertoli cells from several mammalian species have been described, the majority of the work has been done in the rat. The morphology of this cell type is complex. Its cellular shape and size as well as the ultrastructural characteristics vary with species, age, season of the year and stage of the spermatogenic cycle (Sinha Hikim et al., 1989a; Ueno and Mori, 1990; Ghosh et al., 1992; Jégou, 1992). Furthermore, organelle distribution differs depending on the cytoplasmic region observed. Sertoli cells are mitotically active mainly in the sexually immature testis, whereas their number remains stable throughout most of the adult life of mammals (Clermont and Perey, 1957; Steinberger and Steinberger, 1971; Nagy, 1972). Sertoli cell mitosis is under gonadotropin control (Jégou, 1992). Data about Sertoli cell number in seasonal breeders, however, are contradictory (Bartke et al., 1994).

### TABLE 1. Seasonal morphometric data on viscacha Sertoli cells*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Component</th>
<th>Active</th>
<th>Regression</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertoli number/tubular cross section</td>
<td>Cell nucleus</td>
<td>15.24 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.53 ± 0.15</td>
<td>10.08 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vv%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ER</td>
<td>11.64 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>7.20 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vv%&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Mitochondria</td>
<td>8.20 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.49 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.11 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vv%&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Golgi complex</td>
<td>8.60 ± 0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>0.27 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vv%&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Dense bodies</td>
<td>3.39 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.22 ± 1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.20 ± 1.69&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vv%&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Lipid</td>
<td>0.15 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.36 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.72 ± 0.32&lt;sub&gt;&lt;sup&gt;d&lt;/sup&gt;&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

*Mean ± SEM. Vv%, volume density expressed as a percentage of Sertoli cell cytoplasmic volume. ER, endoplasmic reticulum.

<sup>a</sup>Student’s T-test with Welch correction; P < 0.0001 vs. regression and recovery.

<sup>b</sup>Student’s T-test: P < 0.025 vs. regression.

<sup>c</sup>Student’s T-test: P < 0.0001.

<sup>d</sup>ANOVA, P < 0.0001.

<sup>e</sup>Student’s T-test, P < 0.0025 vs. active.

<sup>f</sup>Student’s T-test, P < 0.0001.

<sup>g</sup>Mann-Whitney test, P < 0.0001.

<sup>h</sup>Kruskal-Wallis test: P < 0.0001.

<sup>i</sup>Student’s T-test with Welch correction, P < 0.0001 vs. regression and recovery.

<sup>j</sup>Kruskal-Wallis test, P < 0.0001.

<sup>k</sup>Mann-Whitney test, P < 0.0001 vs. recovery.

<sup>l</sup>Mann-Whitney test, P < 0.001 vs. regression.

The viscacha (Lagostomus maximus maximus) is a seasonal rodent with nocturnal habits. The adult males exhibit an annual cycle of reproduction that is related to changes in the photoperiod (Fuentes et al., 1991, 1993). The testicular regression period during the short days of winter (July–August) ranges from a substantial reduction in the population of spermatids and mature spermatozoa in some animals to an almost complete arrest of spermatogenesis in others. The short gonadal atrophy is accompanied by a decrease in serum levels of testosterone, that is attributed to a fall in circulating LH, a testicular desensitization, and a reduced capacity for Leydig cells to synthesize and secrete the androgen (Fuentes et al., 1991, 1993; Muñoz et al., 1997). Specifically, testicular concentrations of LH, FSH, and prolactin (Prl) receptors are minimal in the regressed animals (Fuentes et al., 1993). Testicular restoration during spring is followed by a period of maximum activity during the long days of summer and autumn (Fuentes et al., 1991, 1993; Muñoz et al., 1997, 1998). Previous results have demonstrated that the photoperiod synchronizes the viscacha reproductive rhythmicity through the pineal gland and its hormone, melatonin (Domínguez et al., 1987; Fuentes et al., 1991; Pelzer et al., 1999).

It is well known that Leydig and Sertoli cells are key cells in testicular function, responding to photoperiod-induced endocrine changes (Sinha Hikim et al., 1989a,1989b; Muñoz et al., 1997). Therefore, the principal objective of the present work was to study the morphological changes exhibited by the viscacha Sertoli cell during the annual reproductive cycle, it being our wish to determine the structural parameters that are more sensitive to hormonal fluctuations.

### MATERIALS AND METHODS

Thirty adult male viscachas (Lagostomus maximus maximus), weighing 5–7 kg, were captured in their habitat near San Luis, Argentina (33° 20’ south latitude, 760 m altitude) between 1997 and 1999. They were anesthetized with Nembutal (pentobarbital) and quickly decapitated.

In San Luis, summer days have 14 hr of light and an average temperature of 25°C. In winter, the light phase
decreases to 10 hr and the average temperature is 10°C. The average rainfall is 206 mm in summer and 18 mm in winter.

The left testes were rapidly removed and sliced into 2–4 mm cross-sectional slabs from the middle region. They were fixed in Bouin’s fluid, dehydrated in a graded series of ethanols, and embedded in paraffin. Serial sections (5–6 µm thick) were obtained with a Reichert-Jung Hn-40 microtome, stained with hematoxylin-eosin, and examined in a Olympus CH2 microscope. Concomitantly, 2-mm blocks chosen randomly were fixed in glutaraldehyde-formaldehyde (Karnovsky, 1965), postfixed in cold 2% OsO4, dehydrated in acetone, and embedded in Spurr’s resin. One micrometer thick sections were cut with a Porter-Blum ultramicrotome and dyed with toluidine blue for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I electron microscope.

The seminiferous tubules examined in the present study were at Stages III and IV of the spermatogenic cycle, representing the spermiation stages in gonadally active viscachas. The identification of various germ cells and stages of the viscacha spermatogenic cycle was based on the description provided by us in active testes (Munoz et al., 1998). In the gonadally regressed animals, however, Stages III and IV were identified mainly by the appearance of preleptotene spermatocytes and B spermatogonia, which could be identified in active testes. Similar criteria were used by Sinha Hikim et al. (1988) to identify Stages VII and VIII in testes of hamsters exposed to short photoperiod.

Sertoli Cell Number Per Seminiferous Tubule Cross Section

Paraffin-embedded sections were used to determine the Sertoli cell number per seminiferous tubule cross section at the light microscopic level. The number of nuclei was counted using an objective of ×40. A total of 800, 300 and 400 sections were examined at the periods of maximum testicular activity, regression and recovery, respectively.

Sertoli Cell Volumetric Composition

To assess fine changes in Sertoli cell morphology, organelle morphometric quantification was performed at the electron microscopic level. Round seminiferous tubules were selected randomly to examine the basal cytoplasm from Sertoli cells cut approximately in their equatorial region. Five electron micrographs at an initial magnification ×10,000 were chosen per animal during the 3 periods of the reproductive cycle. They were taken at a final magnification of ×32,700 and used to determine the volume densities (Vv) of endoplasmic reticulum (ER), mitochondria, Golgi complex and associated vesicles, dense bodies and lipid inclusions. The proportion of rough endoplasmic reticulum (RER) present in the viscacha Sertoli cells is

Fig. 1. Seasonal volumetric composition of the basal cytoplasm of the viscachas Sertoli cells. ER: endoplasmic reticulum, M: mitochondria, G: Golgi complex, D: dense bodies, L: lipid. *ER and Golgi complex were not assessed in the regression period due to the marked dilatation of their components.

Fig. 2. Sertoli cell of the breeding viscacha. The nucleus (N) is irregular, euchromatic and contains a prominent nucleolus. The dominant organelles are the endoplasmic reticulum (ER), Golgi complex (G) and mitochondria (M). P, cytoplasmic processes. D, dense body. Magnification ×4,000.

Fig. 3. Basal cytoplasm from the active Sertoli cell. Multiple and well-developed Golgi complexes (G) associated with numerous vesicles (arrows) occur in the breeding period. ER, endoplasmic reticulum. M, mitochondria. D, dense bodies. Magnification ×10,000.

Fig. 4. Tight junctions (arrow) between two active Sertoli cells. Magnification ×12,500.

Fig. 5. Extensive desmosome like junctional complex (arrow) between two Sertoli cells from active testis. N, nucleus. ER, endoplasmic reticulum. M, mitochondria. R, ribosomes. Magnification ×12,500.
Figure 6–9.
low. Therefore, the ER data in the present study correspond to the sum of both rough and smooth endoplasmic reticulum (SER). The ER and Golgi complex were not assessed in the regressed testes due to the excessive dilatation of their components. The volume density of each organelle was obtained by the point-counting method, dividing the sum of points falling on each structure by the total number of points falling on the cytoplasm. A transparent grid with 15,552 points was used. The results were expressed as percentages (volume density ×100: Vv%).

Statistical Analysis

All results were expressed as the mean value ± SEM. The data were analyzed using the statistical software packages GraphPad Prism and GraphPad InStat (GraphPad Software, Inc.) (P < 0.05 was assumed to be significant).

RESULTS

Period of Maximum Testicular Activity

In the period of maximum testicular activity the viscacha Sertoli cells exhibited extensive cytoplasmic processes that enveloped the associated germ cells (Fig. 2). The nuclei were large, irregular, and localized to the basal portion of the cell. The nuclear membrane showed numerous deep infoldings. The homogeneous nucleoplasm contained euchromatin with a fine fibrillar granular texture and a prominent nucleolus, and heterochromatin was scarce. The perinuclear cytoplasm was organelle free, whereas the rest of the basal cytoplasm exhibited a high concentration of subcellular structures. The dominant organelles were the endoplasmic reticulum (Vv%: 11.64 ± 0.73), Golgi complex (Vv%: 8.60 ± 0.97), mitochondria (8.20 ± 0.50), polyosomes and single ribosomes (Table 1, Figs. 1–3, 5). The ER formed an extensive network of cisterns and interconnected tubules. The SER predominated over RER, usually occurring as short lengths of tubules or cistern associated with ribosomes. The SER was also present in the Sertoli cell cytoplasmic processes where it constituted large compacted masses of smooth membrane, and together with mitochondria, occurred in the apical cytoplasm that surrounded the differentiating spermatids. Multiple well-developed Golgi complexes characterized the active Sertoli cell (Fig. 3). They were associated with an appreciable number of vesicles, some of which appeared covered with dense material inside. Round, oval, rod-like, and irregular mitochondria with dense matrix and abundant tubular cristae occurred within the Sertoli cell cytoplasm. Few membrane-limited dense bodies in different stages (Vv%: 3.39 ± 0.52) and lipid inclusions (Vv%: 0.15 ± 0.12) were observed in the Sertoli cell base during the spermatogenic stages (Table 1, Figs. 1–3). The cytoskeleton, mainly microfilaments and microtubules, was elaborate in this period of the reproductive cycle. Similarly, extensive inter-Sertoli cell junctions were observed in the basal portion of the seminiferous epithelium (Figs. 4, 5). These junctional complexes included tight and gap junctions, and desmosomes. The Sertoli cell number per seminiferous tubule cross section was 15.24 ± 0.15 (Table 1).

Regression Period

During gonadal quiescence, the viscacha Sertoli cells exhibited a marked reduction in their size and organelle content (Figs. 6, 7). The nuclei were round, oval and irregular, whereas the chromatin showed a dense granular texture. The nuclei remained in the basal region of the cell during testicular involution. Nucleoli were not often observed. The Sertoli cell cytoplasm usually contained many clear vacuoles, that seemed limited by membrane in the electron microscope (Figs. 6, 8). The degree of vacuolization, however, was related to seminiferous tubule damage (Figs. 6, 7). Vacuoles of variable configuration were normally dispersed within the cell. This morphological detail correlated with a pronounced dilatation of the ER and Golgi complex. The ER was constituted mainly of short tubules and cisterns, and a few vesicles of smooth membrane. The Golgi complex was rudimentary and the number of associated vesicles was low (Figs. 7, 8). The ribosome, RER and mitochondria content also decreased. Annular and tunescent mitochondria were often observed (Vv%: 4.49 ± 0.60, Table 1, Fig. 1, 6–8). The spermatogenic arrest was accompanied by a drastic increase, in the basal cytoplasm, of lipid inclusions (Vv%: 1.36 ± 0.66) and membrane-limited dense bodies (Vv%: 10.22 ± 1.23, Table 1, Fig. 1). The lysosomal system observed in regressed Sertoli cells was constituted principally of dense bodies, such as primary and secondary lysosomes (Figs. 6–8). The cytoplasmic matrix was clear and the cytoskeleton was disorganized. The junctional complexes between neighboring Sertoli cells appeared short, tortuous and frequently interrupted (Figs. 7, 8). The Sertoli cell number per seminiferous tubule cross section recorded in the involution period (9.53 ± 0.15) was significantly lower than in the breeding period (Table 1). Sertoli cells with marked signs of involution were frequently observed in the regressed seminiferous tubules (Fig. 9).

Period of Testicular Recovery

During spring, the viscacha Sertoli cells progressively recovered their nuclear and cytoplasmic characteristics (Figs. 10–12). Irregular nuclei with numerous infoldings and prominent nucleoli were localized to the basal region of the cell, whereas the nucleoplasm still exhibited a granular texture. The organelle content varied according to the different proportions and patterns of subcellular struc-
tures (Figs. 10, 11). In this period, the dominant organelles were dense bodies (Vv%: 12.20 ± 1.69), ER (Vv%: 7.20 ± 0.63) and mitochondria (Vv%: 6.11 ± 0.71, Table 1, Fig. 1). Specifically, the volume densities of dense bodies and lipid inclusions (Vv%: 1.72 ± 0.32) were maximum during the gonadal recovery (Table 1, Fig. 1). The dense bodies, such as secondary lysosomes, predominated in this period of the reproductive cycle (Figs. 10–12). An important ultrastructural characteristic was the presence of a poorly-developed Golgi complex (Vv%: 0.27 ± 0.17, Table 1, Fig. 1). The increase in the Sertoli cell number per seminiferous tubule cross section (10.08 ± 0.17) compared to the regression period was statistically significant (Table 1). Furthermore, Sertoli cells in the process of involution were frequently observed (Fig. 13).

### DISCUSSION

The results show that viscacha Sertoli cells undergo marked nuclear and cytoplasmic changes during the annual reproductive cycle. The seasonal patterns of the volume densities (Vv%) of the principal organelles, however, are quite distinct. If we compare these results with those obtained in viscacha Leydig cells (Muñoz et al., 1997), we may conclude that the Sertoli cells are less sensitive to photoperiod-induced endocrine fluctuations. The regressive effect of a short photoperiod is more severe on the viscacha Leydig cells, whereas the recovery rate of this cellular type is higher (Muñoz et al., 1997). The results of the morphological study of the interstitial compartment in *Lagostomus maximus maximus* are supported by the circulating testosterone profile throughout the reproductive cycle (Fuentes et al., 1993). The Sertoli cell contains FSH and androgen receptors, and it is thought to be the mediator of FSH and testosterone action on the spermatogenic process (Means et al., 1976; Sanborn et al. 1977; Tindall et al., 1977; Orth and Christensen, 1978; Sharpe et al., 1988; Pescovitz et al., 1994). Despite this evidence, the Sertoli cells from short-term hypophysectomized hamsters showed few morphological changes at a time when the levels of both hormones were drastically decreased, whereas the long-term hypophysectomy resulted in similar, but slightly more severe regressive changes than those occurring during seasonal regression (Sinha Hikim et al., 1989a; Ghosh et al., 1992a).

Sertoli cells are known to change functionally and morphologically during the seminiferous epithelial cycle (Mo-
Bergmann, 1987; Ghosh et al., 1992a, 1992b). In contrast, a morphometric study in the golden hamster showed that the volumes of lipid and lysosomes were not significantly different between active and inactive Sertoli cells (Sinha Hikim et al., 1989a).

The SER and mitochondria are two of the dominant organelles present in the Sertoli cell cytoplasm of breeding viscachas. This ultrastructural feature is common in other species studied and consistent with the marked capacity of Sertoli cells for synthesizing and metabolizing many steroids, some of which are not produced by Leydig cells (Wiebe et al., 1987). The tubular steroids have been implicated in the autocrine, paracrine and endocrine regulation of spermatogenesis (Jégou, 1992; Pescovitz et al., 1994). The content and pattern of both organelles change during the reproductive cycle of Lagostomus maximus maximus. The presence of many clear vacuoles limited by membrane within the Sertoli cell cytoplasm from inactive viscachas coincides with a marked dilatation of the SER, that occurs as short tubules and cisterns, and vesicles of smooth membrane. This pattern of the SER is accompanied by a significant decrease in the mitochondria volume density, and mitochondrial damage. The percentage of both organelles increases during the spermatogenesis regression, and mitochondrial damage. The percentage of both organelles varies during the reproductive cycle.

The length and organization of the inter-Sertoli cell junctions vary seasonally in Lagostomus maximus maximus. Extensive and organized junctional complexes are characteristic of the Sertoli cells from breeding viscachas. This feature is consistent with the presence of a functional blood-testis barrier (BTB) to an electron-opaque tracer, such as lanthanum (Morales and Cavicchia, 1993). The capacity of the viscacha seminiferous epithelium to exclude lanthanum disappears in completely regressed testes (Morales and Cavicchia, 1993), coincident with the presence of short, tortuous, and disorganized junctions. Contradictory results on the cyclical formation and disassembly of the BTB have been reported in other seasonal breeders (Gravis et al., 1977; Budney and Fawcett, 1985; Pelletier, 1986, 1988; Bergmann, 1987; Sinha Hikim et al., 1988).

The decrease in the viscacha Sertoli cell number per seminiferous tubule cross section recorded in the regression period could be interpreted as a consequence of the tubular retraction (Fuentes et al., 1991). The presence of Sertoli cells with marked signs of involution in regressed and recovery testes, however, suggests that the cellular number fluctuates with the season. New studies are necessary to test this hypothesis, as it has been established that Sertoli cells do not divide in the adult or hypophysectomized animals (Clermont and Perey, 1957; Steinberger and Steinberger, 1971; Nagy, 1972). Information about Sertoli cell numbers in other seasonal breeders is scarce and contradictory. Apparently, significant seasonal variations in Sertoli cell numbers are only seen in the stallion, but not other species (Johnson and Thompson, 1983; Johnson and Nguyen, 1986; Sinha Hikim et al., 1988; Johnson, 1991).

The present work also reveals that the degree of regression and recovery exhibited by the viscacha Sertoli is closely related to that shown by the associated germ cells. These observations allow us to postulate that the seasonal changes noted in the Sertoli cells of Lagostomus maximus maximus are not just a response to photoperiod-induced endocrine fluctuations. Rather, it is likely that several local factors are involved in this process (Pescovitz et al., 1994; Muñoz et al., 1999).

In summary, the viscacha Sertoli cells exhibit marked nuclear and cytoplasmic changes, and probably variations in their number, throughout of the annual reproductive cycle. These changes are synchronized by the photoperiod through the pineal gland and its principal hormone, melatonin. Regional and stage-dependent morphological changes, however, remain to be investigated.

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LITERATURE CITED


