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Seasonal variations cause morphological changes and altered spermatogenesis in the testes of viscacha (*Lagostomus maximus*)

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ABSTRACT

This study complements the previous investigations of the reproductive biology of male viscachas, a rodent of a seasonal Hystricognathi that exhibits photoperiod-induced morphological variations in the reproductive system. In the present study, a quantitative analysis of spermatogenesis was performed during the summer and the spring. Spermatogonial cells were analyzed to determine by immunolabelling for STRA8 and DAZL, which are essential for spermatogenesis. Six free-living male viscachas were captured, three animals in the summer during the period of reproductive activity and three animals in the spring during the period of testicular regression. The testes of the viscachas were collected and processed for light microscopy, macroscopic and immunochemical analyses. The germ and Sertoli cells present in the seminiferous tubules were quantitatively analyzed in each animal. The efficiency coefficient for spermatogonial mitosis, meiotic yield, overall spermatogenesis yield and Sertoli cell index, revealed that the Sertoli cells in male viscachas captured during the summer had a reduced capacity to structurally and nutritionally support the developing round spermatids compared with the male viscachas captured during the spring. The animals produced less sperm during the spring than the summer, suggesting a seasonal impact on spermatogenesis. Immunolabelling for STRA8 and DAZL was detected during summer and spring seasons. These results suggest that in seasonal rodents, such as the male viscachas, the photoperiod promotes significant changes in the testis and in the germ cell yield.

1. Introduction

Many mammals that are seasonal breeders respond to annual changes in their natural habitat with several environmental factors, including food and water accessibility, temperature, and day length (photoperiod) regulating

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the reproductive cycle and spermatogenesis (Adam and Robinson, 1994). In rodents such as the Golden hamster (*Mesocricetus auratus*) and the Djungarian hamster (*Phodopus sungorus sungorus*), the photoperiod is the most important environmental signal that induces adaptive morphological changes (for example, diameter of the seminiferous tubule) and alters the physiology and behavior, including those associated with migration and hibernation (Gaston and Menaker, 1967; Stetson et al., 1975). In long-day sexually mature male hamster, short photoperiods cause testicular regression, whereas during a long photoperiod the fertile state is induced or maintained (Reiter, 1968; Wehr, 1997).

Viscachas have greater testosterone concentrations than many other species and increased reproductive activity in the summer and fall (Calvo et al., 1999). During the winter, testicular regression is characterized by a significant decrease in testosterone that corresponds with a marked reduction in the diameter of the seminiferous tubule. During this period, some animals undergo a substantial reduction in the number of spermatids and mature spermatozoa, and others almost completely stop spermatogenesis (Fuentes et al., 1993; Muñoz et al., 1997, 2001). This phenomenon is followed by the gradual recovery of the reproductive system during the spring (Mohamed et al., 2000; Muñoz et al., 2001; Fuentes et al., 2004).

Male germ cell apoptosis occurs via two major pathways, one involving mitochondria (intrinsic) and the other engaging cell surface death receptors extrinsic (Kerr, 2002; Shaha, 2007). Regulated cell death has several important functions in the testis, including maintaining the conversion of an appropriate number of germ cells into Sertoli cells, phagocytosis of degenerating germ cells and residual bodies and maintaining overall quality control in sperm production (Shukla et al., 2012).

STRA8 is a gene that encodes a cytoplasmic protein for which gene expression is induced by retinoic acid. In adult mice, the STRA8 gene is expressed in cells and the mitotic activity of spermatogonia and pre-leptotene spermatocytes is impacted (Baltus et al., 2006; Zhou et al., 2008). Most importantly, sperm production cannot occur without STRA8 gene expression (Ruggiu et al., 1997).

Another factor that may influence the continuity of spermatogenesis is the expression of the STRA8 and DAZL genes. These genes are expressed in cells during the early stages of spermatogenesis and meiosis and in pachytene spermatocytes. Without DAZL protein, mature sperm are defective because of anomalies in the acrosome and the tail (Lin et al., 1997; Dann et al., 2006).

The quantitative study of spermatogenesis permits an evaluation of basal spermatogenesis in different seasons and provides an enhanced understanding by correlating physiological and biochemical findings and degeneration of specific germ cell types (Hess et al., 1990; Johnson et al., 2000).

The present study aims to complement previous studies on reproduction in viscacha (*Lagostomus maximus*) (Fuentes et al., 1991; Muñoz et al., 2001; Merlo et al., 2005a,b) with the primary objective being the quantification of spermatogenesis and protein localization in the testis of viscachas during the spring and the summer. In addition, the cellular localization of DAZL and STRA8 during both seasons was evaluated.

2. Materials and methods

Six animals were collected three in the summer and three in the spring. All the procedures involving animals were conducted in accordance with the Committee of Ethics of the Faculty of Animal Science and Food Engineering, University of Sao Paulo, Pirassununga, Brazil. The captured animals were handled and euthanized in accordance with the Guide for the Care and Use of Laboratory Animals. All the animals were anesthetized with 50 mg/kg ketamine chlorohydrate via intramuscular injection according to the method of Flamini et al. (2009).

2.1. Animal and tissue collection and processing

Six male, sexually mature viscachas (*L. maximus*) were obtained with free-living body weights of 3–5 kg in Estación de Cría de Animales Silvestres (ECAS), Buenos Aires (latitude 34°2′, longitude 58°3′ W). Testes were collected during the reproductive periods in summer (short night) and in spring (same length day and night) and the testicles underwent regression as described by Fuentes et al. (1991) and Mohamed et al. (2000) as assessed by macroscopic analyses. The testes from each animal were placed in Bouin's solution for 24 h. After dehydration in increasing concentrations of alcohol and then xylene, the testes were embedded in paraffin, Luna (1968). A microtome (Leica RM2145, Berlin, Germany) was used to cut 5 μm sections that were subsequently stained with hematoxylin–eosin (HE). To obtain microscopic descriptions of the organs, the slides were analyzed by light microscopy (Leica DM 2500, Berlin, Germany) (Assis-Neto et al., 2003). Tissues for transmission electron microscopy and scanning electronic microscopy were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4, postfixed in 1% osmium tetroxide (SEM®, Hatfield, Pennsylvania, PA). In transmission electronic microscopy the fragments were dehydrated in increasing concentration of acetone solution. The samples were pre-infiltrated in araldite (37 °C) for 2 h and examined under a transmission electron microscope (Morgagni 268D, FEI Company, the Netherlands; MegaView III camera; Soft Imaging System, Germany). In scanning electron microscopy, after fixing, the tissues were dehydrated in increasing concentrations of ethanol (70–100%) and dried in a critical point dryer (Balzer Union®, Critical point CPD 020, Liechtenstein, Germany). Samples were subsequently placed in stubs and covered with gold in a sputter coater (Emitech® K500, Ashford, Kent, England). These fragments were analyzed under scanning electronic microscopy (LEO 435 VP-Zeiss, Cambridge, England) (Flamini et al., 2011; Rezende et al., 2012; Fernandez et al., 2013).

2.2. Seminiferous tubule cell counts

The cell populations in the spermatogenic lineage were quantified by counting four cell types: Type A

spermatogonia (A), primary spermatocytes in pre-leptotene/leptotenes (PL/L) or pachytene (PQ), round spermatids (Ar) and Sertoli cells (CS). These counts were performed in 10 circular cross-sections of the seminiferous tubule that were in the same SEC stage in each testis. To count the different cell types, SEC Stage I was chosen according to the tubular morphology method (Courot et al., 1970). The counts were corrected for section thickness and the nucleolus diameter according to the Abercrombie and Johnson (1946) method as modified by Amann and Almquist (1962). The intrinsic efficiency of spermatogenesis was based on the report by Costa et al. (2004), that cells were counted in the first stage of the seminiferous epithelium cycle, and these cell numbers were used to calculate the coefficient of spermatogonial mitosis efficiency (PL/L:A), meiotic yield (Ar:PQ), overall yield of spermatogenesis (Ar:A) and prevalence of dead cells during meiotic prophase (PL/L:PQ).

2.3. Immunohistochemistry

To conduct the immunohistochemistry for STRA8 and DAZL, paraffin sections (5 μm) of formalin-fixed testes were de-paraffinized and re-hydrated. For epitope retrieval the slices were boiled in citrate buffer (0.384 g of monohydrate citric acid and 2.352 g of sodium citrate tribasic dihydrate for 1 L of distilled water, pH 6.0) for 20 min. Endogenous peroxidase activity was blocked by incubation with 3% H_2O_2 in methanol for 20 min. Nonspecific protein binding sites were blocked with 10% goat serum (Vector Laboratories, Burlingame, CA) in PBS for 45 min; subsequently, the sections were incubated with the primary antibody (anti-STRA8 polyclonal rabbit antibody 1:100, ab49602, Abcam; or anti-DAZL polyclonal rabbit antibody 1:100, ab34139, Abcam) or a negative control (IgG, 1:100, ab27478, Abcam) at 4 °C overnight. The slides were washed in PBS, incubated for 30 min with biotinylated goat anti-rabbit IgG (1:100, Pk-4001, Vector Laboratories) at room temperature and then incubated with avidin–biotin complex (ABC; Vector Laboratories) for 1 h according to the manufacturer's instructions. 3,30-Diaminobenzidine tetrahydrochloride (DAB; Dako, Glostrup, Denmark) substrate development was performed for 30 s. The nuclei were counterstained for 30 s with 5% Mayer's hematoxylin.

2.4. Statistical analysis

All the data are presented as the mean ± SEM. The student's *t*-test and an analysis of correlation and variance (Newman–Keuls test) were performed using QuickCalcs (GraphPad Software, Inc.). The significance level was $P < 0.05$.

3. Results

3.1. Morphological analyses

Testes were located in the inguinal region, surrounded by the tunica albuginea rich in a local circulatory system, and consisting of dense connective tissue with collagen fibers, which emit septa into the parenchyma, dividing the organ into lobules. The testes have ovoid shape, regions of which are divided into *extremitas capitata*, have an epididymal margin and a free *extremitas caudata*. The blood supply in the testis resides in the spermatic cord that consists of adipose tissue surrounded by blood vessels and including a vas deferens, and a pampiniform plexus that is covered by visceral peritoneum. The testes are histologically composed of the testicular parenchyma, composed of numerous seminiferous tubules arranged in a tubular compartment system. The seminiferous tubules are composed on the tunic component and seminiferous tubule lumen. In tubules, the stratified epithelium is composed of spermatogenic cells, located between the tubular basement membrane and lumen. Scanning electron microscopy showed that around the seminiferous tubules, there are interstitial or Leydig cells, surrounded by the tunica albuginea. Transmission electron microscopy showed Sertoli cells with irregular shapes, located close to the basal membrane, having a nucleus with granular chromatin and a thin layer of heterochromatin, and an evident peripheral nucleolus and cytoplasm with vacuoles. The Sertoli cells also had organelles such as mitochondria and rough endoplasmic reticulum (Fig. 1).

The seminiferous tubules had morphologic changes in the epithelium during the two periods that the testes of these animals were analyzed. Microscopy data for animals that were collected in the spring indicate the seminiferous tubule had a reduced lumen of smaller diameter, a few late spermatids and no spermatozoa were observed in the lumen of tubules. For animals collected in the summer, testicles were larger, contained many late spermatids, and had a greater lumen diameter with the presence of sperm (Fig. 2).

3.2. Quantitative analysis of spermatogenesis

Quantification of the number of spermatogenic germ cells during summer and spring was conducted in the present study. The corrected number of Type A spermatogonia was greater in animals captured in spring (6.89 ± 0.71) than in those captured in summer (1.59 ± 0.55). Otherwise, smaller numbers of pre-leptotene/leptotene and pachytene spermatocytes and round spermatids were observed in animals captured during the spring compared with those captured during the summer (Table 1).

In viscachas, the mitosis efficiency coefficient (PL/L:A), that is the ratio of Type A spermatogonia and primary spermatocytes numbers, was 1:7.98 in summer and 1:1.23 in spring. In addition, the meiotic yield (PQ:Ar), was greater in the summer (1:3.41) than spring (1:1.92), showing that during the reproductive season the calculated value is closer to the theoretically expected ratio (1:4; Table 2). The yield of spermatogenesis generation (A:Ar), which accounts for the number of round spermatids and, therefore, number of sperm resulting from each Type A spermatogonia, was approximately 3.40 and 37.69 in the spring and summer, respectively. The Sertoli cell index was also determined; enabling an estimate of the efficiency and ability of Sertoli cells to mature and maintain the round spermatids. Each Sertoli cell could maintain and

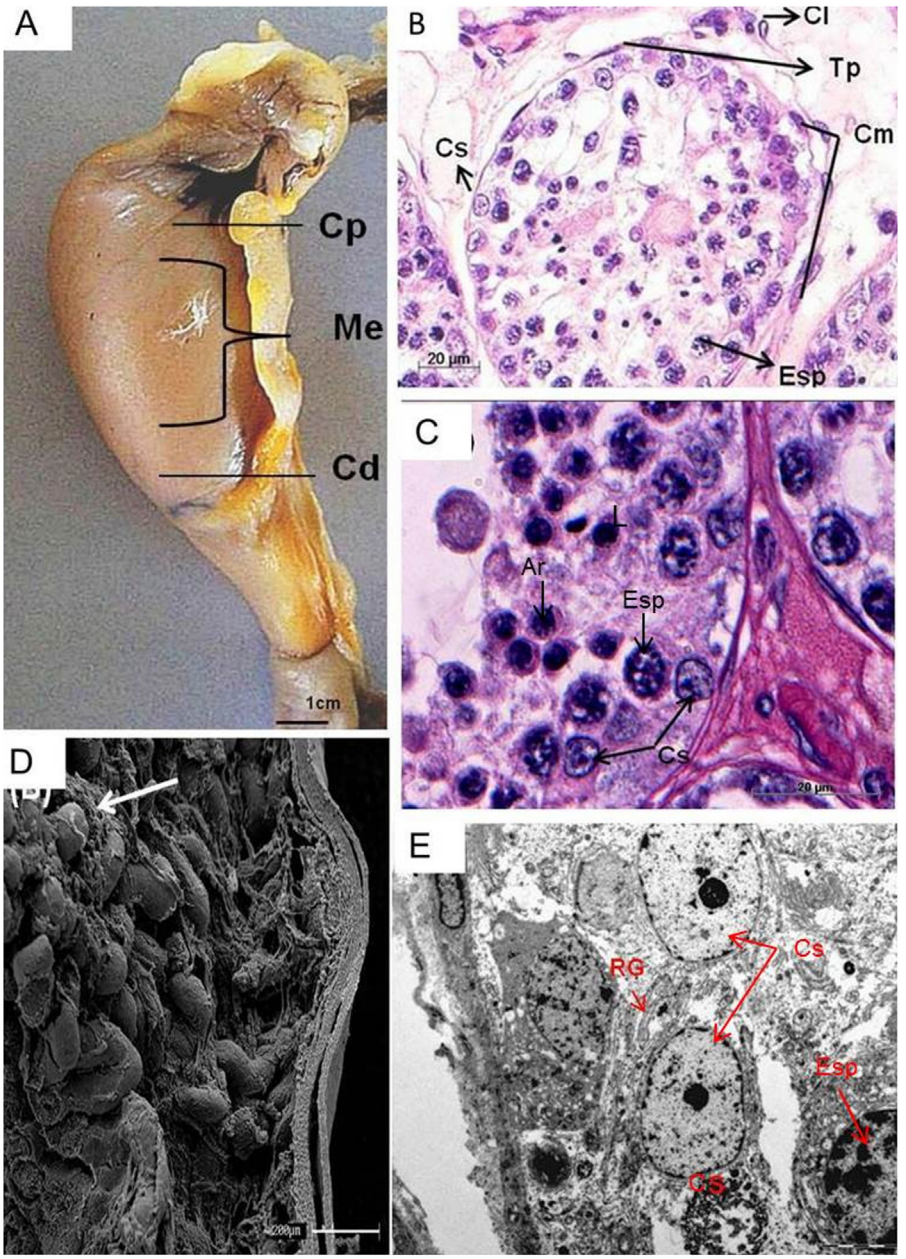

Fig. 1. Testis of viscacha. (A) Macroscopic anatomy. Divided into: capitada (Cp) epididymal border (Me) and caudata (Cd). (B) and (C) Hematoxylin and eosin. Seminiferous tubules with tunic (Tp), myoid cells (Cm) and basal membrane with Sertoli cells (Cs) and interstitial cells (CI), seminiferous epithelium and lumen (L) and the spermatogenic lineage cells: spermatogonia (Es), primary spermatocytes (Esp), round spermatids (Ar). (D) Scanning electron microscopy. Leydig cells between the seminiferous tubules. (E) Transmission electron microscopy. Sertoli cells (Cs) with irregular format, nucleus with granular chromatin and a thin layer of heterochromatin (H), peripheral evident nucleolus (Nc), cytoplasm with rough endoplasmic reticulum (RER), spermatocytes (Esp) with rounded nucleus and partially condensed chromatin and many organelles such as the Golgi complex and mitochondria (bar: 20 μm).

support 6.89 round spermatids in the spring and 13.40 in the summer.

3.3. Immunohistochemistry

The cellular localization of STRA8 and DAZL proteins, which are essential in the initial phase of mitosis and meiosis, was determined. These proteins are responsible for the production of viable sperm in the testes during spermatogenesis. Localization of STRA8 and DAZL was determined by immunohistochemistry with a hematoxylin counterstain. STRA8 was detected in Type A spermatogonia and spermatocytes in the periphery of the seminiferous tubules. In pre-leptotene spermatocytes and Type A spermatogonia, the proteins appeared to be restricted to the cytoplasm during both seasons. The majority of STRA8-positive germ cells were adjacent to the basal lamina; very few were in the center of the tubule.

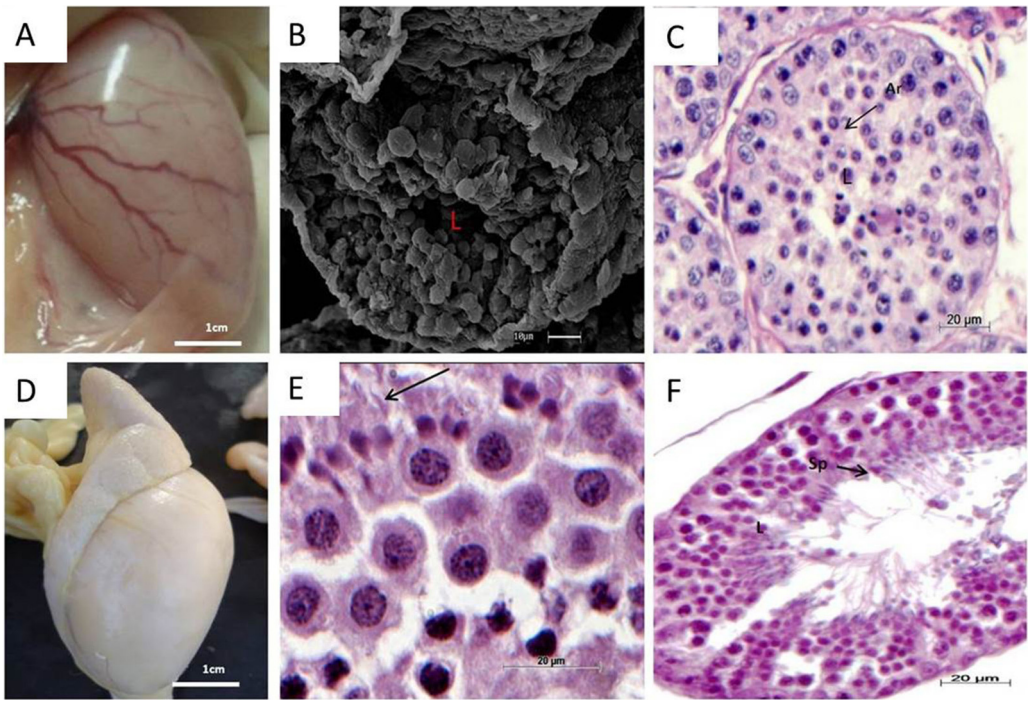

Fig. 2. Morphological variation in the testes of viscacha in summer and spring (A) and (B) scanning electron microscopy showing testis (spring), small seminiferous tubule, without the presence of sperm in the lumen (L), with round spermatids (Ar), and (C) hematoxylin and eosin showing spermatids around (Ar) in seminiferous tubules. (D–F). Hematoxylin and eosin. Testis (summer) with late spermatids (arrow), diameter lumen (L) and sperm (Sp) (bar: 20 μm).

Table 1
The corrected number of cells per cross-section of the seminiferous tubule in viscacha.

Population	Spring[a]	Summer[a]
Sertoli cells	3.42 ± 0.21	4.47 ± 0.19
Type A spermatogonia	6.89 ± 0.71	1.59 ± 0.55
Primary preleptotene spermatocytes	8.50 ± 2.36	12.69 ± 0.82
Primary pachytene spermatocytes	12.28 ± 4.67	17.54 ± 9.14
Round spermatids	23.49 ± 13.21	59.93 ± 1.35

[a] $P < 0.05$.

DAZL-positive staining was most evident in the cytoplasm of cells in early spermatogenesis and in meiosis, including pre-leptotene and pachytene spermatocytes, but not in Type A spermatogonia. DAZL staining was present in primary spermatocytes during the pre-leptotene through zygotene stages. Furthermore, in animals captured during the summer, positive DAZL staining was observed in the cytoplasm as well as in the round spermatids. There was no staining in Sertoli cells or in other somatic cells (Fig. 3).

Table 2
Ratio of the number of spermatogenic cells per seminiferous tubule cross-section in viscacha.

	Spring	Summer
Efficiency of spermatogenesis (PL/L:A)	1:1.23[a]	1:7.98[a]
Meiotic yield (PQ:Ar)	1:1.92[a]	1:3.41[a]
Spermatogenesis yield (Ar:A)	1:3.40[a]	1:37.69[a]
Sertoli cells index/round spermatids (Ar:Cs)	1:6.86[a]	1:13.40[a]

Note: Ar: round spermatids; Cs: Sertoli cells; A: spermatogonial cells; PL/L: preleptotene/leptotene spermatocytes; PQ: pachytene spermatocytes.
[a] $P < 0.05$.

4. Discussion

The current study presents new findings on the male reproductive system of viscachas with an emphasis on spermatogenesis and the proteins that are essential for this process. Previous studies on *L. maximus* have evaluated female reproductive biology, which is characterized by the poly-ovulation of hundreds of oocytes, the loss of implantation and the development of 1–3 offspring and unrestricted proliferation of germ cells (Flamini et al., 2011; Leopardo et al., 2011), and the relationship between seasonality and photoperiod. The present study complements previous studies on the reproductive biology of viscachas (Morales and Cavicchia, 1993; Muñoz et al., 1997; Merlo et al., 2005a,b; Aguilera-Merlo et al., 2009; Chaves et al., 2012).

In viscachas the testicles were in the inguinal region, as described by Weir (1974) for histricomorfs. This organ had similar morphological and anatomical characteristics to those described by Menezes et al. (2003) for the Agouti; however, the anatomical shape differed from other rodents (Cooper and Schiller, 1975; Hebel and Stromberg, 1986). Morphologically testes of viscacha were formed by the assembling of seminiferous tubules, *rete testis* and, tunica albuginea. Tubules of the testicular parenchyma divided into two compartments, with seminiferous tubules,

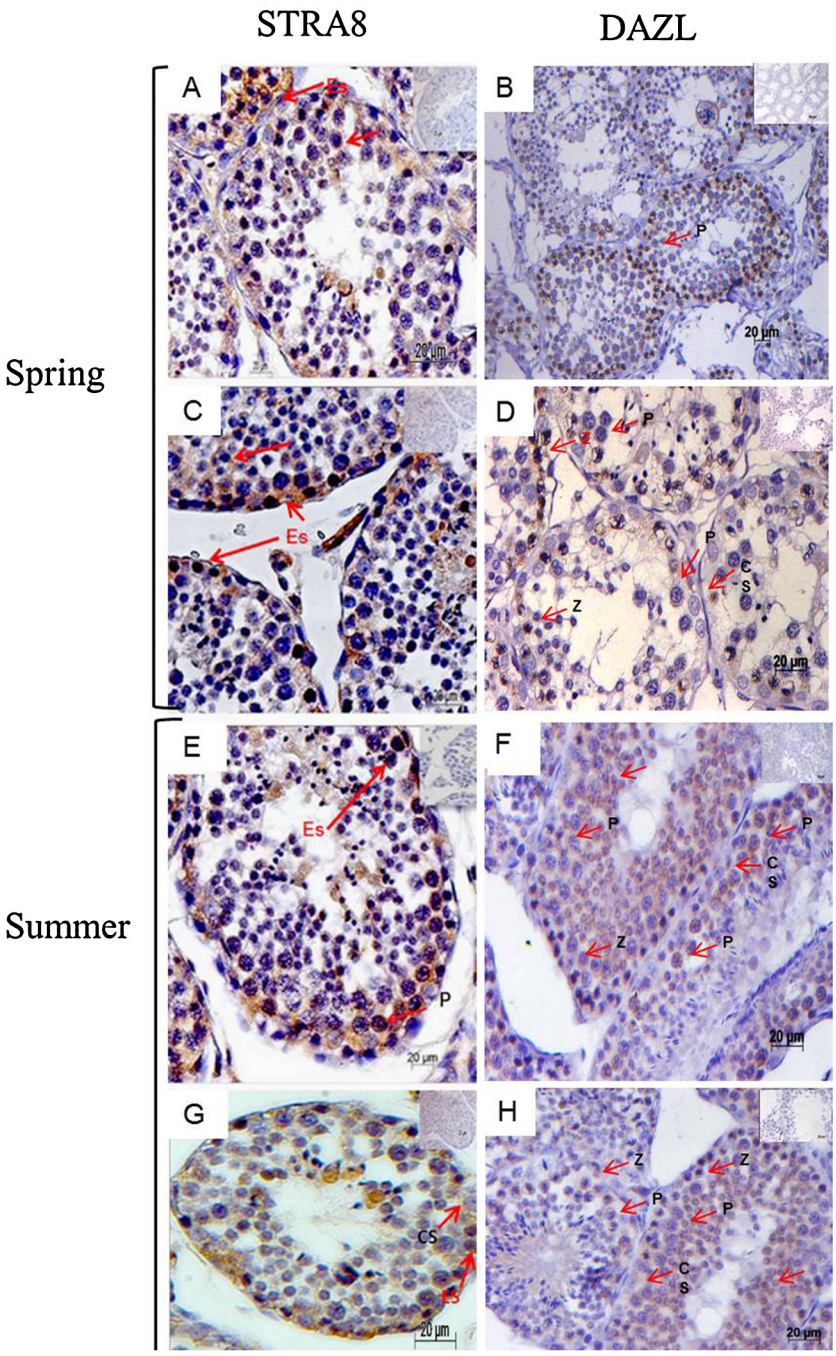

Fig. 3. Photomicrographs identify the cells that are immunohistochemically positive for STRA8 and DAZL in the testes of viscachas in summer and spring. In (A), (C), (E) and (G) the arrows denote the STRA8-positive seminiferous tubules that are STRA8 in the pre-leptotene spermatocytes (P) and spermatogonia (Es) of animals captured during the spring and summer. In (B), (D), (F) and (H) DAZL-positive staining was observed in the cytoplasm of pre-leptotene spermatocytes (P), round spermatids (arrow) and zygotene spermatocytes (Z) in animals that were captured during the same season. The staining was negative in Sertoli cells (Cs) (bar: 20 μm).

consisting of a tunic component and lumen as well as an intertubular compartment composed of interstitial cells as occurs in other mammals (König and Liebich, 2002). According to França and Russell (1998) and Hess and França (2007), the tubular linings make up most of the testicular tissue in mammals occupying 70%–90% of testicular parenchyma there are similar findings noted by Menezes et al. (2003) for the aguti.

According to Fuentes et al. (1991, 2004) the seminiferous tubules of viscacha had morphologic changes in the epithelium, tubular diameter and height of the epithelium in different reproductive periods with this

previous analysis being made in two periods (summer and winter). These were similar to changes in the period analyzed in the present study in the summer and spring. Spermatogenesis is not a continuous process in viscachas. During the winter, testosterone concentrations decrease and Leydig cells hypertrophy (Fuentes et al., 1991; Muñoz et al., 1998). Consequently, there is less Type A spermatogonia in animals captured during the spring than in those captured during the summer. This difference may be related to the function of these cells in the spermatogenic cycle. Type A spermatogonia are capable of reproducing 1000-fold and are essential for the initiation of spermatogenesis. However, the corrected number of Type A spermatogonia observed in the present study was similar to that reported by Assis-Neto et al. (2003) in the agouti and by Segatelli et al. (2004) in the gerbil.

Cell death and degeneration during the spermatogonial phase can be quantified using the methods reported by De Rooij and Lock (1987) and Sharpe (1994). In viscacha, the mitotic yields were greater in the summer compared with spring; these data differ from the results described by Segatelli et al. (2004) in the gerbil. During mitosis, a greater apoptotic rate (60%–90%) occurs in mammalian germ cells (Roosen-Runge, 1973), which is similar to the findings for cell death rate in viscachas during the spring in the present study.

According to the efficiency of meiotic divisions reported by Roosen-Runge (1973) and França and Russell (1998), round spermatids can theoretically be generated from each pachytene spermatocyte. The rate of meiotic yield of round spermatids in the viscacha was similar with other phylogenetically related rodents, such as capybara, that is reproductively similar to viscacha even though these animals do not inhabit the same locations (Lee et al., 1997).

Significant cell death occurs at the pachytene spermatocyte stage of meiotic divisions, but the end of meiosis (metaphase) is the most important for sperm production. During each of these spermatogenesis phases, there are several significant changes in gene expression that regulates differentiation, and unique genetic recombination occurs in the spermatocyte stage (Allan et al., 1992; Lee et al., 1997). The spontaneous death of male germ cells is a widespread phenomenon in numerous animal species in which mitosis and meiosis have an important role in maintaining appropriate cell numbers (Billig et al., 1995). The reason for this cell death is unknown; however, for example, mutation of exonuclease-1 induces checkpoint activity during meiosis in MRL mice. The detailed apoptotic structure indicated that cellular arrest occurred immediately after the beginning of metaphase (De Rooij and Russell, 2000). In the rat, maximal cell death was observed during Stages XII and XIV as well as, to a lesser degree, in Stage I primary metaphase spermatocytes that persisted from the immediately preceding stage XIV. In domestic animals, the apoptosis rate is 5%–30% at the end of meiosis (França and Russell, 1998); however, when analyzed in the present study in the viscachas captured in spring, cell death rate was greater than had been previously observed in adult animals (Muñoz et al., 1998; Costa et al., 2004).

Muñoz et al. (1997) reported that viscachas have lesser testosterone concentrations during the spring season, which coincides with Leydig cell atrophy and significant changes in Sertoli cells, suggesting a mechanism for the greater apoptosis rate in this species.

In mammals, significant apoptosis occurs during the spermatogonial phase. Therefore, 70%–80% of sperm undergo apoptosis during spermatogenesis, starting with Type A spermatogonia (França and Russell, 1998; Paula et al., 1999; Weir, 1971). Regarding the yield of spermatogenesis in viscachas, the average production of Type A spermatogonia is four sperm in the spring and 38 in the summer. This confirms that the number of viable sperm in the summer is greater than that during other seasons (Russell and Peterson, 1984; Neves et al., 2002; França and Godinho, 2003). This rodent exhibits seasonal spermatogenesis, with limited sperm production during the spring and a much greater production rate during the summer. Similar results have been observed in the Syrian hamster (Sato et al., 2005), a rodent with seasonal reproductive characteristics similar to those of the viscacha (Leal and França, 2006).

In viscacha, altered Sertoli cell morphology is synchronized with the photoperiod, the pineal gland and melatonin release (Muñoz et al., 2001). Sertoli cells provide a support network for a limited number of stem cells (Sinha-Hikim et al., 1988; Almeida et al., 2006). In viscachas, Sertoli cells exhibit reduced supportive capacity during the spring, as evidenced by the lesser rate of spermiogenesis, and consequently sperm production is less in the spring (Muñoz et al., 2001). This ability to nutritionally and structurally support round spermatids in the summer was compared with that of other rodents, such as the hamster (Sinha-Hikim et al., 1988; Almeida et al., 2006), mouse (Leal and França, 2008; Richardson et al., 2009) and capybara (Aguilera-Merlo et al., 2009); this analysis revealed that the viscacha have greater Sertoli cell indices values, which are similar to those reported for the guinea pig (Rocchietti-March et al., 2000).

Rodent research has confirmed that DAZL is essential for spermatogenesis and quality sperm in domestic animals (Koubova et al., 2006; Ma et al., 2013). DAZL protein was detected in pachytene and pre-leptotene spermatocytes, Type B spermatogonia, and round and elongated spermatids in the testes of viscachas during both seasons. DAZL has been evaluated in adult rats (Rocchietti-March et al., 2000) and mice (Ruggiu et al., 1997). In viscachas, meiotically active germ cells located at the periphery of the seminiferous tubules contained STRA8 primarily in the spermatogonia and the pre-leptotene spermatocytes, similar to what was observed by Zhou et al. (2008) in mice. STRA8 is associated with meiotic prophase and is restricted to the peripheral layer of the seminiferous tubules in mice Zhou et al. (2008). The inactivation of STRA8 in mice arrests spermatogenesis because it blocks DNA replication and the subsequent entry into meiotic prophase. Therefore, these animals are infertile (Koubova et al., 2006). STRA8 was present during both seasons, suggesting that the expression of the STRA8 gene is not involved in the changes in spermatogenesis. Results of the present study demonstrated that the changes in spermatogenesis with

significantly altered sperm production are related to the external environment and the reproductive cycle of this rodent, confirming the observations of Fuentes et al. (1991). STRA8 and DAZL were detected during spring and summer.

5. Conclusion

In the present study, it was demonstrated that during the spring, cell death was increased during spermatogenesis, leading to a lesser yield and reduced sperm production. These data suggest that in seasonal rodents, the spermatogenic cycle does not completely stop during the period of reproductive quiescence in this species and the photoperiod promotes statistically significant changes in the population and yield of male germ cells.

Conflict of interest

None declared.

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