Ultrastructure of spermatogenesis in Spix’s yellow-toothed cavy (*Galea spixii*)

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Abstract

This was a pioneer study of the spermatogenic process from the onset of puberty in Spix’s yellow-toothed cavies (SYC, *Galea spixii*) bred in captivity. The study aimed to characterize fine structure of spermatogenesis. Twelve testes from pubertal and post-pubertal SYC males were studied using transmission electron microscopy. Spermatogenesis can be divided into three phases: proliferation, meiosis, and spermiogenesis. In proliferation phase, three types of spermatogonia were identified and characterized as A dark, A pale, and B. In the second phase, spermatocytes (2n) undergo meiotic divisions that generate spermatids (n); the process begins in spermatocytes in the preleptotene stage when they increase their nuclear size, differentiating into spermatocytes in the leptotene stage when cell division is initiated. In addition, we found chromatin condensation, and formation of a structure composed of proteins that formed a central shaft and two lateral bars associated with pairing of homologous chromosomes. During spermiogenesis, the following main events occurred: condensation of nuclear chromatin, formation of acrosome with perforatorium, elimination of residual cytoplasm, and development of the flagellum. The sperm head is different from that of other rodents. The endoplasmic reticulum and the Golgi complex are the two main organelles demonstrated during this process. These organelles collaborate through synthesis of proteins and hormones for the development of germ cells during spermatogenesis in SYC.

Introduction

Spix’s yellow-toothed cavy (SYC, *Galea spixii*) is found in the regions of northeastern Brazil (Eisenberg & Rerdford 1999). It is an herbivorous species with gray hair and a ring of white hairs around the eyes. When mature, cavies are 22.5–23.5 cm long, weigh between 375 and 405 g, and breed throughout the year, with a gestation period of ~48 days (Oliveira et al. 2008).

Although consumed as an alternative source of animal protein, Red List (IUCN 2013) has declared SYC as an endangered species. Nonetheless, the knowledge of reproductive biology and physiology is important for conservation and species management in captivity; such knowledge also aids efforts to ensure the propagation of endangered species (Busso et al. 2005, Wildt 2005).

However, due to risk of extinction of species and use of the same as an alternative source of animal protein, the search for information of captive breeding of these rodents has increased (Carvalho et al. 2003). Moreover, this serves as a model for biological discovery of therapies and prevention strategies for various diseases in humans (Domingues & Caldas-Bussiere 2007). For wild rodents, the cavy served as a model in studies of immunology (Von Ubisch & Amaral 1935) and chromatography (Cavalcanti et al. 1959) and in cases of leptospirosis (Castro et al. 1961). Aside from the work cited earlier, studies related to general biology of the species still lack. On this point, information of general biology, enhancement, preservation, and maintenance in captivity of any kind requires a basic knowledge of reproductive physiology.

Studies related to male SYC have described the establishment of puberty, the seminiferous epithelium cycle, and genital organs (Santos et al. 2011, 2012a, 2012b). For male animals, the study of spermatogenesis is particularly relevant to reproduction of the species, and in this regard, the ultrastructure view can promote morphological basic information necessary for reproduction biotechnologies. Thus, this study aimed to describe the process of germ cell differentiation in SYC ultrastructurally in order to determine the steps of spermatogenesis.

Materials and methods

**Animals, tissue collection, and processing**

Twelve pubertal and post-pubertal male specimens of *G. spixii* were studied. The animals were collected in northeastern...
Results

Testis

The testicular parenchyma consists of germ cells, Sertoli cells, Leydig cells, myoid cells, and vessels. During puberty, the seminiferous tubules with lumen and germ cells at different stages of division were noted and showed different stages of the seminiferous epithelium cycle (Fig. 1A). The myoid cells showed a cytoplasm with parallel filaments and a fusiform nucleus, with homogeneous chromatin. The single nucleolus was large, granular, and centrally located, and the cytoplasm had small mitochondria with few organelles and some extensions of the endoplasmic reticulum (smooth (sER) and rough (rER)).

Spermatogonia

At the ultrastructural level, only three types of spermatogonia can be identified accurately in SYC: spermatogonia type A ‘dark’, spermatogonia type A ‘pale’, and spermatogonia type B (Fig. 2). Spermatogonia type A ‘dark’ were elliptical in shape and adhered to the basal lamina, which had irregular projections and depressions. Sertoli cells were located close to the basal area of the germinal epithelium (Fig. 2A). The spermatogonia featured an oval center containing a fine, granular chromatin and ‘groups’ of condensed chromatin. The single nucleolus was large, granular, and irregular, usually centrally located. The cytoplasm contained larger mitochondria, extensions of the endoplasmic reticulum (smooth (sER) and rough (rER)).

Spermatocytes

Spermatocytes developed from the differentiation of spermatogonia during the first meiotic division. Chromatin condensation occurs at this stage, and the formation of the synaptonemal complex, a structure composed of proteins that form a central shaft and two lateral bars associated with chromosome pairing of homologous chromosomes. The preleptotene spermatocyte had a circular shape with a circular core containing homogeneous chromatin. The cytoplasm was accompanied by a few small organelles (Fig. 3A).
The core of the leptotene spermatocyte was larger, demonstrating the beginning of chromatin condensation, which occurred gradually during the transition of zygotene spermatocytes to diakinesis spermatocytes (Fig. 3B). The organization of the nucleolus was apparent even among preleptotene and zygotene spermatocytes (Fig. 3C). The disorganization and break-up of the nucleolus began in pachytene spermatocytes, and the nucleolus lost its morphology and became more fragmented (Fig. 3D).

Diplotene spermatocytes and diakinesis spermatocytes had nucleoli fragments that may have been associated with specific chromosomal regions (Fig. 3E). In diakinesis, there was a reduction in the number of nucleoli fragments. In some areas, it is found associated with a single chromosomal region (Fig. 3F). At this stage, chromosome pairing was observed (Fig. 3F).

**Spermatids**

Morphological changes, such as the formation of the flagellum and the acrosome, chromatin condensation,
nuclear elongation, and removal of the cytoplasm, occurred during spermatid differentiation. Our results showed that this process is divided into four phases: the Golgi phase, the cap phase, the acrosomal phase, and the maturation phase (Figs 4 and 5).

In Golgi phase, spermatids originated from the second meiotic division. They had a spherical nucleus with condensed chromatin points, and a region of concentrated chromatin was seen near the nuclear envelope. The Golgi complex was developing, increasing in size, and blisters began to fuse to form the proacrosomal vesicles. The centrioles tended to be located close to pole opposite the formation of the axoneme (Fig. 4A, B and C).

In cap phase, spermatids had a developed Golgi apparatus with highest production of proacrosomal vesicles joining the nuclear envelope to make the cell nucleus concave. Note the formation of the acrosome. The acrosome began to cover the core, surrounding almost the entire surface (Figs 4D and 5A).

In acrosomal phase, the acrosome condensed in a strong, dense, and granular arrangement. It can be seen at the beginning of the disorganization of the nucleolus. The acrosome covered much of the nuclear surface in association with microtubules in the distal region, and the nuclear elongation of the spermatid occurred in this phase (Fig. 5B, C and D).

The acrosome covered the entire core and the nucleolus could not be seen in the maturation phase (Fig. 5E, F and G). Spermatids were elongated, and the heads had prominent perforatorium. In this phase, the spermatids become similar to spermatozoa. The mitochondrial sheath was organized in the middle part, and a fibrous sheath formed the main part. There was great change in the cytoplasm when compared with spermatids in the other steps. It was the last phase before the release of sperm into the tubular lumen.

**Spermatozoa**

The spermatozoa consisted of a head, neck, intermediate part, main part, and an end part. The head with a fusiform shape was covered externally by a plasma membrane on top of the core, with a space separating the outer acrosomal membrane and the equatorial segment connecting to the end portion in the terminal region of the acrosome.
The proliferation phase had three spermatogonia types: meiosis, and spermatogenesis (Clermont 1972). The mammals can be divided into three phases: proliferation, contributing to the knowledge of reproductive biology. Onset of puberty in SYC bred in captivity, thus the description of the spermatogenic process from the genital organs (Santos et al. 2011). Previous studies in SYC have been carried out on sexual and testicular development (Santos et al. 2012a), frequency and stages of the seminiferous epithelium cycle (Santos et al. 2011), and development of male genital organs (Santos et al. 2012b). This study permitted the description of the spermatogenic process from the onset of puberty in SYC bred in captivity, thus contributing to the knowledge of reproductive biology.

The spermatogenic process of SYC, as well as other mammals, can be divided into three phases: proliferation, meiosis, and spermatogenesis (Clermont 1972). The proliferation phase had three spermatogonia types: Ad (dark), Ap (pale), and B, identified by the nuclear morphology of chromatin condensation and position within the seminiferous epithelium (Smithwick et al. 1996). At this phase, there was an increase in the number of mitochondria, and this contributes to the increase in ATP nuclear promoting cell proliferation (Warburg 1966).

The second phase of spermatogenesis, the meiotic phase, is the process wherein spermatocytes (2n) undergo meiotic divisions that generate spermatids (n) (Russell et al. 1990). The process begins in spermatocytes in the preleptotene stage when they increase their nuclear size, differentiating into spermatocytes in the leptotene stage when cell division initiates. The nuclear characteristics of spermatocytes were similar to those of rodents and other mammals (Schleirmacher & Schmidt 1973, Solari & Moses 1973).

In the last stage of spermiogenesis, a round spermatid differentiates into a spermatozoon capable of fertilization (Clermont 1972). The spermatid differentiation occurred in four phases: the Golgi phase, the acrosomal phase, and the maturation phase (Gunawardana & Scott 1977, Singwi & Lall 1983, Lin & Jones 1993, Góes & Dolder 2002, Segatelli et al. 2002). Chromatoid bodies observed in spermatids have been reported in guinea pigs, other mammals, birds, and reptiles. Moreover, the presence of chromatoid bodies promote the part connection in mature spermatozoa (Sud 1961, Fawcett et al. 1970).

During this process, the following events occurred: condensation of the nuclear chromatin, formation of the acrosome, elimination of residual cytoplasm, and development of the flagellum procedure. The process is similar to that of the rodents (Lalli & Clermont 1981).

The spermatozoa is the final process of spermatogenesis, which occurs through the meiotic and mitotic divisions in the seminiferous epithelium of the testis (Eddy 2006). The sperm head of SYC is fusiform shaped, differing from other rodents such as rats, mice, and hamsters (Pesch & Bergmann 2006), which have

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**Sertoli and Leydig cells**

At the pubertal stage, the Sertoli cells remained attached to the basal membrane, where they had cytoplasmic extensions involving the germ cells. The core was large and triangular in shape, with a homogeneous euchromatin nucleoplasm. The nucleolus appeared scattered, occupying part of the nuclear area. The cytoplasm contained subcellular structures such as the ER, Golgi apparatus, mitochondria, and ribosomes (Fig. 7A).

Leydig cells were paired and associated with blood vessels. The Leydig cells show round and oval nuclei. In the cytoplasmic compartment, the Golgi complex, an extensive sER, and few lipids were observed. The Leydig cells filled most of the interstitial space into a smaller intercellular space between the seminiferous tubules (Fig. 7B).

**Discussion**

Previous studies in SYC have been carried out on sexual and testicular development (Santos et al. 2012a), frequency and stages of the seminiferous epithelium cycle (Santos et al. 2011), and development of male genital organs (Santos et al. 2012b). This study permitted the description of the spermatogenic process from the onset of puberty in SYC bred in captivity, thus contributing to the knowledge of reproductive biology.

The spermatogenic process of SYC, as well as other mammals, can be divided into three phases: proliferation, meiosis, and spermatogenesis (Clermont 1972). The proliferation phase had three spermatogonia types:

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**Figure 6** Ultrastructure of spermatozoa, Spix’s yellow-toothed cavy (SYC, Galea spixii). Longitudinal section of the spermatozoon (a, acrosome; Af, axial filament; An, ring; Bp, basal plate; Es, equatorial segment; Fs, fibrous sheath; m, mitochondria; N, nucleus; Od, outer dense fibers; P, perforatorium; Pc, proximal centriole; Pm, plasma membrane). Transmission electron microscopy; scale bars: 2 μm.

**Figure 7** Sertoli and Leydig cells in Spix’s yellow-toothed cavy (SYC, Galea spixii). (A) There is a triangular nucleus, nucleolus with condensed chromatin, and the presence of many mitochondria in the cytoplasm. Adherence to the basal lamina and a greater cytoplasmic area. (B) Many groups of mitochondria are associated with the ER. Few lipid droplets were seen. BL, basal lamina; m, mitochondria; N nucleus; No, nucleolus; rER, rough endoplasmic reticulum; sER, smooth ER; LC, Leydig cells; L, lipid droplets; v, vesicles. Transmission electron microscopy; scale bars: 2 μm.
different from those of other rodents. In addition, to allow for a usual spermatogenesis, an interaction between the germ cells and Sertoli cells, Leydig cells, and myoid cells is necessary. These cells demonstrated activity in support of spermatogenesis, and followed that of other mammals (Fawcett et al. 1970, Hoffer 1982).

Declaraton of interest
The authors declare that they have no conflict of interest with relation to the development and publication of the study.

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References


Figure 8 The sperm head of rodents. The spatulate-shaped heads of guinea pig; the falciform-shaped heads of mouse; and the fusiform-shaped heads of Spix’s yellow-toothed cavy (SYC, Galea spixii). The spermatozoon head size and shape is specific to each species.


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