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# Cell proliferation in the seminiferous and epididymal epithelia of *Sus domesticus*

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ABSTRACT

It is important to understand the proliferative activity of the different structures of the male reproductive apparatus in livestock species, such as *Sus domesticus*, to ensure reproductive efficiency. The main aims of this study were (a) to evaluate the proliferative activity of the spermatogonia in the different stages of the seminiferous cycle and (b) to study the cell proliferation in the epididymal epithelium in each region, identifying the different cells involved. For this, the testes and epididymis of three healthy, sexually mature *Sus domesticus* boars were used. The organs were processed for light microscopy, and immunohistochemical techniques were used to detect proliferating cell nuclear antigen. The cells immunostaining positively and negatively for proliferating cell nuclear antigen were counted and several parameters and indexes were calculated to evaluate the proliferation in both epithelia, taking into account the stage of the seminiferous epithelium cycle, and, in the case of the epididymal epithelium, the different regions and cells are the same. Finally, a contrast analysis of equality between pairs of means was carried out followed by a least significant differences test, in which differences were considered significant at $P < 0.05$. In the seminiferous epithelium, the greatest total number of spermatogonia and proliferating spermatogonia was observed in the postmeiotic stages (mainly VII and VIII). The proliferation index of the spermatogonia increased from the meiotic to postmeiotic stages. As regards the epididymal epithelium, the total proliferation index was higher in the caput. In each region, the clear and principal cells showed the highest proliferation index with respect to the total number of cells counted, whereas the proliferation index of each cell with respect to the same type was higher in the clear cells, followed by the narrow and principal cells. In conclusion, the proliferative activity of spermatogonia in the seminiferous epithelium of *Sus domesticus* is stage-dependent, and mainly occurs in the postmeiotic stages. In the epididymal epithelium, proliferative activity takes place in several cell types and is dependent on the anatomical region of the epididymis. We think that these results may be of importance for understanding the pathologic or reproductive processes in which cell proliferation is involved in the male reproductive system.



## 1. Introduction

The male reproductive system is composed of different structures, including the testes and epididymis, which are responsible for the differentiation and transport of

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gametes. Meiosis takes place in the epithelium of the seminiferous tubules, and spermatozoids are formed and continuously released [1]. These spermatozoids mature and are transported in the epididymis, suggesting a constant and intense proliferative activity in the seminiferous epithelium, where a large number of spermatozoids are produced on a daily basis [2,3]. In both epithelia, stem cells are needed to make up for cell losses, maintaining the functionality of the tissues. However, it seems that such proliferative activity is not so intense in the epididymal epithelium.

In the case of the seminiferous epithelium, the continuous proliferation of stem cells (spermatogonia) ensures a continuous supply of cell precursors (spermatocytes) to undergo meiosis. This produces spermatids, which, through a differentiation process known as spermiogenesis, are transformed into spermatozoids [4]. The spermatogonia are essential for the maintenance of this epithelium and, in mammals, two main types have been distinguished: the so-called undifferentiated and differentiated types [5]. Moreover, spermatogonia are divided and differentiated according to the stages of the seminiferous cycle [6] and present proliferative activity and a distribution that depends on the stages of the cycle [7]. However, whether there is a relation between the total proliferative activity of spermatogonia and the different stages of the seminiferous cycle in mammals or whether their number varies as the cycle progresses has not been studied. Such information would be useful for understanding the cell kinetics of the spermatogenesis process. In rats, the existence of waves of proliferation with periods of greater activity, such as V, VIII, and IX stages, has been described [8]. Such processes are regulated by a variety of factors, provided mainly by Sertoli cells, which stimulate the proliferation or differentiation of specific types of spermatogonia [9] and support the continuous production of spermatozoid precursors [5].

Proliferative activity has been little studied in the epididymal epithelium. It seems that basal cells, which are considered to be responsible for cell renewal, show little or no proliferative activity [10] and any such activity in rodents has mainly been observed in the principal cells [1,11]. At present, it is not known whether other cell types in the epithelium show proliferative activity or whether such activity, if it does exist, differs according to the anatomical region of this duct (caput, corpus, and cauda).

Bearing the above in mind, the present study examines the proliferative activity in both seminiferous and epididymal epithelia because of their importance in gamete generation and maturation, respectively. The common boar (*Sus domesticus*) was used as model, and immunohistochemical staining against proliferating cell nuclear antigen (PCNA) was carried out to identify any proliferating cells. This animal model was chosen because of its importance for animal husbandry and because of our group's knowledge of the histology of both testis and epididymis in this animal [12–16].

The main aims were to study the proliferative activity of the seminiferous epithelium in *Sus domesticus* according to the stages of the seminiferous cycle and to evaluate the proliferative activity of the epididymal epithelium, according to the cell type and in the different regions of the epididymis (caput, corpus, and cauda).

## 2. Materials and methods

### 2.1. Animals

The testes and epididymis of three healthy postpubertal *Sus domesticus* boars were used in the study. The animals had been raised in a controlled environment at a mean temperature of 18 °C and fed a nutritive diet. The animals were sacrificed at 9 months and their reproductive apparatus was extracted and processed for light microscopy. The organs were fixed in methacarn (methanol:chloroform:acetic acid, in a proportion of 6:3:1), dehydrated in ethanol and immersed in paraffin at 56 °C to 58 °C. The study was carried out in accordance with the legal and ethical standards of Royal Decree 1201/2005 and law 32/2007 on animal welfare.

### 2.2. Qualitative analysis

#### 2.2.1. Testis of Sus domesticus

A morphologic study was made of the testicular parenchyma using hematoxylin–eosin staining. The stages of the seminiferous cycle were identified according to the morphologic characteristics described by García-Gil, et al. [15].

#### 2.2.2. Epididymis of Sus domesticus

Sections of the epididymis were studied morphologically using hematoxylin–eosin staining, differentiating the different regions of the same and the cell populations present, as described by Briz, et al. [12].

### 2.3. Semiquantitative analysis

#### 2.3.1. Percentage of tubular sections in each stage of the seminiferous cycle

All the tubular sections were classified, according to the stages of the cycle in four samples of each testicle. The percentage of tubular sections corresponding to each stage of the seminiferous cycle was then calculated.

#### 2.3.2. Spermatogonia in the seminiferous epithelium according to the stages of the cycle

The spermatogonia of all the tubular sections were calculated according to the stages of the cycle in the testicular sections stained with hematoxylin–eosin. The total number of spermatogonia was calculated by an indirect measurement, using a modified version of the method described by Woolveridge, et al. [17] and Bernal-Maña, et al. [16] with the equation (number of spermatogonia × number of tubules × 100)/(number of tubules). The results were expressed as the mean number of spermatogonia per section and per stage of the seminiferous cycle.

#### 2.3.3. Cell index of the epididymal epithelium in Sus domesticus

After differentiating the regions of the epididymal epithelium, the different cell types in each were counted: principal cells, clear cells, narrow cells, and basal cells. The mean index of each cell type was calculated with regard to the total number of cells and the different regions of the epididymis.