Animal Reproduction Science 136 (2012) 128–132

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

The seminiferous cycle of the rhinoceros

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ARTICLE INFO

Article history:
Received 13 April 2012
Received in revised form 21 October 2012
Accepted 22 October 2012
Available online 29 October 2012

Keywords:
Rhinoceros
Ceratotherium simum
Diceros bicornis
Seminiferous cycle
Spermatogenesis

ABSTRACT

The seminiferous cycle of the rhinoceros was investigated for the first time using testicular tissue from captive white (*Ceratotherium simum*, $n = 2$) and black (*Diceros bicornis*, $n = 1$) rhinoceroses. Stages of the seminiferous epithelial cycle were characterised using the tubular morphology method and relative frequencies of each stage determined. This method allowed for the identification of eight stages of cellular associations characteristic of the seminiferous cycle in white and black rhinoceroses. The eight stages of the seminiferous cycle observed closely approximated the stages previously described for the domestic horse (*Equus caballus*). Premeiotic (stages I–III), meiotic (stage IV) and postmeiotic (stages V–VIII) represented 44.8%, 5.3% and 49.9% of the seminiferous cycle respectively.

1. Introduction

Spermatogenesis is a cyclic, synchronous and well organised process in which diploid spermatogonia differentiate into mature haploid spermatozoa (Clermont, 1972). This highly coordinated process encompasses different associations of cells referred to as stages. The sequence of events that occurs from the disappearance of a given cellular association to its reappearance constitutes the cycle of seminiferous epithelium (Clermont, 1972; Berndtson, 1977). While the duration of the cycle is constant within a given species, cycle duration varies between 30 and 75 days in mammalian species examined to date (Franca et al., 2005).

In mammals the stages of the seminiferous cycle can be characterised by either tubular morphology (germ cell cellular associations, the presence of meiotic divisions, and spermatid nuclei morphology and orientation; Berndtson, 1977) or by the acrosomic system (acrosomic system development and spermatid morphology; Leblond and Clermont, 1952). Characterisation on the basis of tubular morphology typically yields eight stages of the seminiferous cycle. In contrast, characterisation on the basis of the acrosomic system yields a variable and typically higher number of stages due to species related differences in acrosome development (Leblond and Clermont, 1952). The stages of the seminiferous cycle can be further characterised into three distinct developmental phases: premeiotic (stages I–III), meiotic (stage IV) and postmeiotic (stages V–VIII) (Russell et al., 1990).

Despite concern regarding poor reproductive rates in captive rhinoceroses there has been limited investigation into potential male contributions to infertility and some basic aspects of reproductive anatomy and physiology remain undescribed in rhinoceros species. There are no published descriptions of the cycle of the seminiferous epithelium of any rhinoceros species. Accurate identification of the stages of the seminiferous cycle in rhinoceros species is a prerequisite for quantitative analysis of spermatogenesis and for understanding the various factors that may influence fertility. From a clinical perspective evaluation of tissue obtained by testicular biopsy, which has recently been used for the assessment of testicular function in rhinoceros species (Hermes et al., 2006), relies upon a sound understanding of normal testicular histology and

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http://dx.doi.org/10.1016/j.anireprosci.2012.10.013

the process of spermatogenesis for the species in question. The objective of this study was to describe and classify the cellular associations, and their frequencies, of the various stages of the seminiferous cycle in the black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros on the basis of tubular morphology.

2. Materials and methods

Testes ($n = 5$) were opportunistically obtained from one adult (age 35 years) and one sub-adult (age 5 years) male white rhinoceroses at post mortem examination and from one live adult male black rhinoceros (age 14 years) following hemicastration to remove a testicular seminoma. A seminoma was also present in the left testis of the adult male white rhinoceros. All rhinoceroses were captive animals. The two adult male rhinoceroses had sired offspring and the sub-adult male had not yet been given the opportunity to breed. Testes were trimmed out from the epididymides, washed in 0.9% NaCl solution, cut perpendicular to their long axis in 1 cm sections, and then fixed in Bouin's solution (Fronine Pty Ltd., Riverstone, New South Wales, Australia) until further processing for histologic examination. Sections of fixed testis were dehydrated, embedded in paraffin blocks, sectioned at 6 μm, mounted on glass slides and stained with haematoxylin and eosin. Sections were then examined under light microscopy at 400× magnification and images taken with a digital camera (DP70 Olympus Digital Camera, Olympus Imaging Australia Pty Ltd., Macquarie Park, New South Wales, Australia). The germinal epithelium of individual seminiferous tubules was staged on the basis of cellular associations of germ cells, the morphology and orientation of spermatid nuclei and by comparison with published data for the domestic stallion (*Equus caballus*) (Johnson et al., 1978; Russell et al., 1990; Heninger et al., 2004). Two hundred cross sectional seminiferous tubules from each of the five testes were examined and the relative frequency of each stage was determined as a percentage of the total number of seminiferous tubules examined.

3. Results

No significant differences between the stages of the seminiferous cycle were observed between black and white rhinoceros and testes from the two species were not considered separately. The sub-adult male white rhinoceros was considered to be physiologically sexually mature as mature spermatozoa were evident in the lumen of seminiferous tubules on histological evaluation. As with most other mammals, characterisation of the seminiferous cycle on the basis of tubular morphology produced eight stages. The eight stages of the seminiferous epithelial cycle are illustrated in Fig. 1 and are described below. The testicular cell types associated with each stage are detailed in Fig. 2. The relative frequency of each stage is detailed in Table 1. Premeiotic (stages I–III), meiotic (stage IV) and postmeiotic (stages V–VIII) represented 44.8%, 5.3% and 49.9% of the seminiferous cycle respectively.

Stage I: Characterised by the presence of type A spermatogonia adjacent the basal membrane; a single generation of spermatids with round nuclei, containing chromatin clumps, forming up to four layers within the germinal epithelium; and two generations of leptotene and pachytene spermatocytes located between the spermatogonia and the round spermatids. Sertoli cell nuclei were elongated in appearance and orientated perpendicular to the basal lamina in this and subsequent stages.

Stage II: Resembled stage I with two generations of leptotene and pachytene spermatocytes and type A spermatogonia. Spermatid nuclei have become slightly irregular in shape and have begun to elongate and orientate towards the nuclei of Sertoli cells.

Stage III: Two generations of zygotene and pachytene spermatocytes and type A spermatogonia are present. Spermatid nuclei have elongated forming bundles deeply embedded within the germinal epithelium.

Stage IV: Characterised by the presence of meiotic figures and one generation of bundled spermatids with elongated nuclei. Type B spermatogonia, with darker more rounded nuclei, are present adjacent the basement membrane, as are zygotene primary spermatocytes and secondary spermatocytes. Two populations of spermatids, both newly formed round and older elongated spermatids, are present.

Stage V: Characterised by the presence of type B spermatogonia and one generation of pachytene spermatocytes. Both round and elongated spermatids are present with the elongated spermatids remaining deeply embedded within Sertoli cell crypts and in conjunction with the Sertoli cell cytoplasm forming well demarcated columns. There is no migration of elongated spermatids towards the lumen.

Stage VI: Type B spermatagonia are still present, as are both round and elongated spermatids and one generation of pachytene primary spermatocytes. The cytoplasm of the Sertoli cells and the elongated spermatids bundled within their crypts continue to form well demarcated columns but some spermatids have begun to migrate towards the lumen.

Stage VII: Type B spermatagonia and one generation of pachytene spermatocytes are present. The elongated spermatids have migrated further towards the lumen with their flagella projecting into the seminiferous tubule lumen. Round spermatids are also present.

Stage VIII: Type A spermatogonia are present adjacent to the basement membrane; as are preleptotene primary spermatocytes. One generation of pachytene spermatocytes is present along with round spermatids. Spermatozoa are being released into the tubular lumen as spermiation occurs and residual bodies are evident immediately below the spermatozoa.

4. Discussion

The description of the seminiferous cycle in the present study is the first for any species within the family *Rhinocerotidae*. The stages of the seminiferous cycle in the rhinoceros were consistent with those described in other mammalian species and closely approximated those seen in the domestic stallion (Heninger et al., 2004) and to a

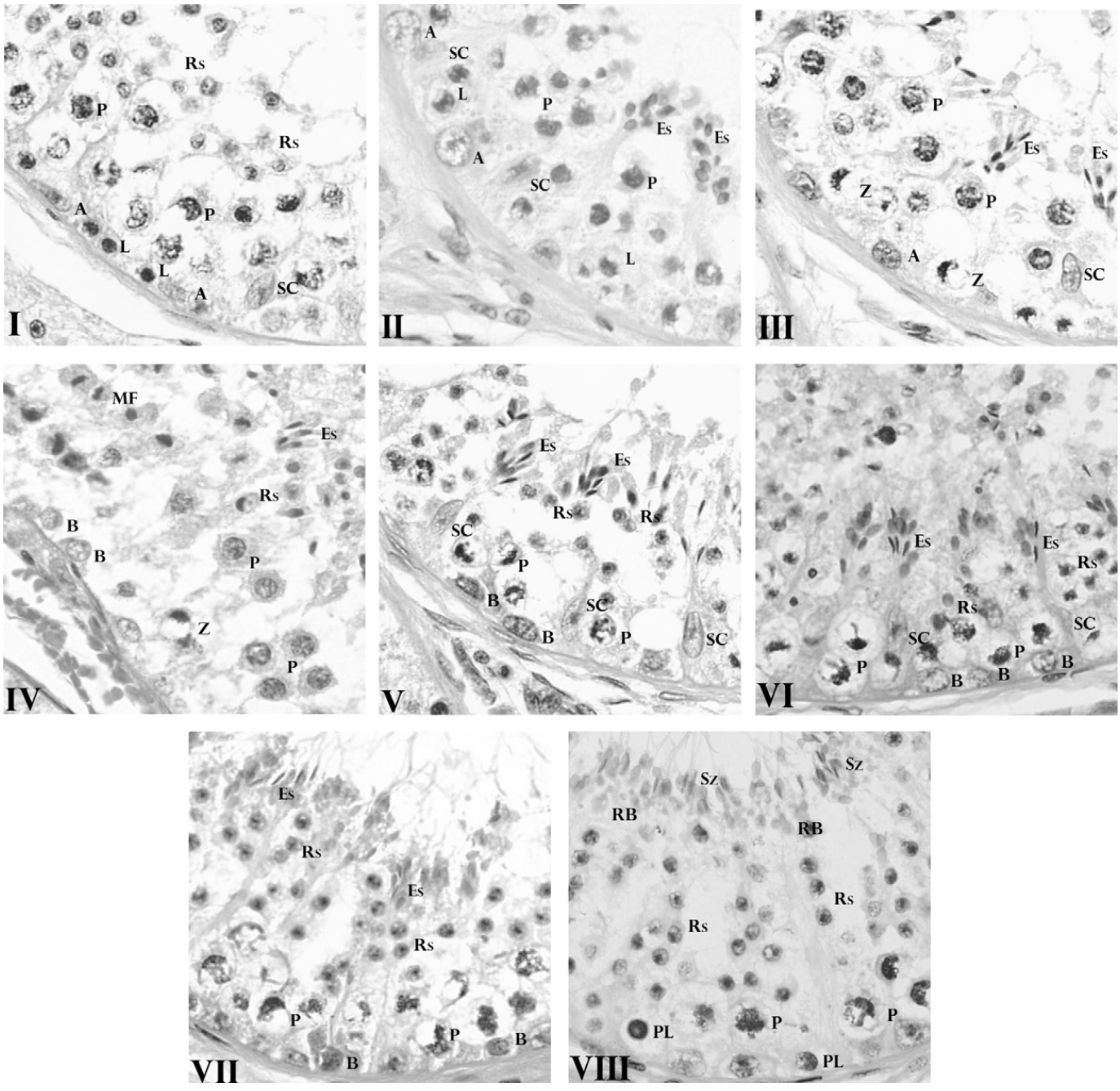

Fig. 1. Photomicrographs of the eight stages (I–VIII) of the seminiferous epithelium cycle of the rhinoceros in paraffin fixed sections stained with haematoxylin and eosin, 400×. A, A spermatogonia; B, B spermatogonia; SC, Sertoli cells; L, leptotene spermatocyte; P, pachytene spermatocyte; Rs, round spermatid; E, elongating spermatid; Z, zygotene spermatocyte; MF, meiotic figures; Sz, spermatozoa; RB, residual bodies; PL, pre-leptotene spermatocyte.

lesser extent the domestic donkey (*Equus asinus*) (Chiarini-Garcia et al., 2009). All stages were generally well defined with the exception of stages V and VI which were differentiated only by subtle changes in the positioning of some elongated spermatids as they began to migrate towards the tubular lumen.

The scope of this study was limited by small number of testes which were opportunistically available and the fact that two of the five (40%) testes available contained limited normal testicular tissue due to the presence of testicular neoplasia. However sufficient normal testicular tissue was available to accurately stage the seminiferous epithelial

Table 1
Relative frequencies ± standard deviation (%) of the stages of the cycle of the seminiferous epithelium in white ($n = 2$) and black ($n = 1$) rhinoceroses.

Stages of the cycle of the seminiferous epithelium							
I	II	III	IV	V	VI	VII	VIII
13.8 ± 1.2	16.3 ± 2.2	14.7 ± 1.2	5.3 ± 1.1	10.6 ± 1.4	15 ± 0.9	13.7 ± 0.8	10.6 ± 1.9

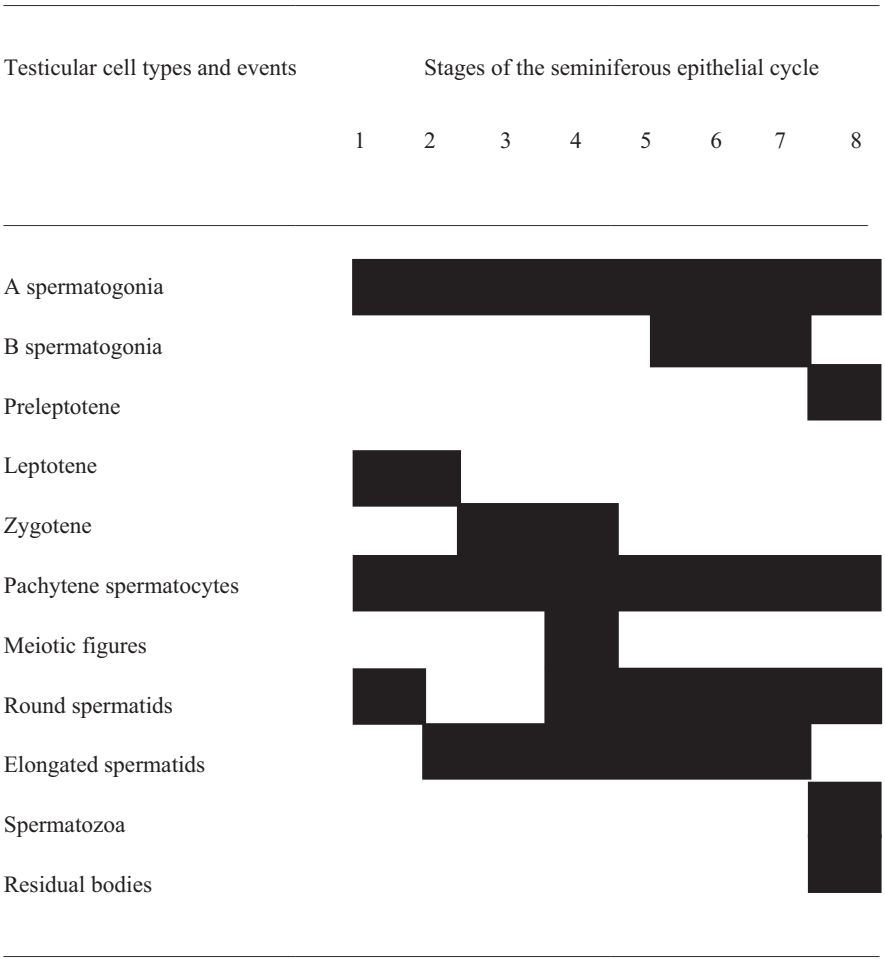

Fig. 2. Summary of the association of testicular cell types and the eight stages of the seminiferous epithelium cycle in the black and white rhinoceros.

cycle. Determination of the duration of the seminiferous epithelium cycle using ante mortem intratesticular administration of tritiated thymidine, a well-established technique in both domestic and non-domestic species, was not possible in this study utilising opportunistically available tissues. Application of this methodology in the future would allow additional quantitative information of the rhinoceros seminiferous cycle to be determined and for species specific differences in seminiferous cycle duration between rhinoceros species to be determined.

The relative frequencies of each stage of the seminiferous epithelial cycle were determined. However potential limitations in determining the frequencies of these stages include the presence of neoplastic tissue (seminomas) in two of the five testes available for examination. While all stages of the cycle were present in these two testes it is possible that the frequency with which the various stages were present may have been influenced by potential endocrine perturbations or the space occupying effect of the seminomas. While detailed endocrine evaluation of the affected rhinoceroses was not conducted seminomas in domestic stallions, a taxonomically related species, are rarely hormonally active (Brinsko, 1998). The influence of the seminomas on spermatogenesis in rhinoceroses remains unknown. Additionally the testes examined were from animals of varying ages and two separate rhinoceros species although no obvious differences between the stages in the two species were noted. Relative histological frequencies of the stages of the seminiferous epithelial cycle were broadly comparable with those reported for domestic stallions. However the observed frequency of stage IV was considerably less for rhinoceroses in this study ($5.3 \pm 1.1\%$) than reported for reproductively normal stallions (14.6%) (Heninger et al., 2004).

Despite the limited scope of this study characterisation of the stages of the seminiferous cycle of the black and white rhinoceros provides basic anatomical and physiological reproductive data previously undescribed in any rhinoceros species. This information is a prerequisite for elucidating the normal reproductive biology of rhinoceroses and for understanding causes of infertility in captive males. The recent addition of testicular biopsy to the suite of tools available for assessing reproductive soundness in male rhinoceroses relies upon accurate descriptions of the normal seminiferous cycle epithelium for the interpretation of biopsy results (Hermes et al., 2006). Examination of larger numbers of testes, the examination and comparison of testes from additional rhinoceros

species and the application of techniques for determining the duration of seminiferous cycle would provide useful baseline data for the investigation of fertility and infertility in captive male rhinoceroses.

Acknowledgement

The authors would like to acknowledge Phillip Ladds for guidance with interpretation of the histological images in this work.

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