Seasonal Ultrastructural Modifications of the Seminiferous Epithelium in Two *Eulemur* species: *E. fulvus* and *E. macaco*

B. Brun, R. Djelati, Y. Rumpler, C. Koehl, and M. Fabre

*Université Louis Pasteur*

**ABSTRACT.** A comparative ultrastructural study of the seminiferous epithelium was conducted during the mating and non-mating seasons of two *Eulemur* species: *E. fulvus* and *E. macaco*. The ultrastructure of the junctional complexes of the Sertoli cells, and the modifications in the spermatids during spermiogenesis are reported. Acridine orange staining of the sperm cells of these animals showed that the chromatin compaction was complete in all spermatozoa.

**Key Words:** *Eulemur; Reproductive activity; Spermatogenesis; Seasonal variations.*

**INTRODUCTION**

Seasonal breeders usually show histologic changes of the seminiferous epithelium and in testosterone steroidogenesis between mating and non-mating seasons, with significance variations depending upon species. In lemurs, *Microcebus murinus* presents a complete arrest of the spermatogenesis (Petter-Rousseaux, 1974), like the Vizcacha *Lagostomus maximus* (Fuentes et al., 1991) or the black bear *Ursus americanus* (Tsuiota et al., 1997). Only Sertoli cells and a few spermatogonia are visible during the non-mating season. Other lemurs such as *E. fulvus* and *E. macaco* present a continuous spermatogenetic activity throughout the year, with a reduced production (Rasamimanana et al., 1990) and teratospermia (Brun & Rumpler, 1990) during the non-mating season.

As, in human pathology reduced spermatogenesis and teratospermia are often associated with an abnormal blood testis barrier (Meyer et al., 1996) and an incomplete condensation of the chromatin of numerous spermatozoa revealed by the fluorochrome acridine orange (Tejada et al., 1984), in this study we have compared in two *Eulemur* species, *E. fulvus* and *E. macaco*: (1) the ultrastructural aspect of the seminiferous epithelium during the non-mating and the mating season in order to evaluate the blood testis barrier, and some particular aspect of the germ cells during spermiogenesis; and (2) the condensation of the spermatozoa chromatin with acridine orange.

**MATERIALS AND METHODS**

Two fertile adults *Eulemur fulvus* and two *Eulemur macaco* from our breeding colony were used for the ultrastructural study.

Testicular biopsies were carried out under general anaesthesia (10 mg/kg ketamine solution; Ketalar® Parke-Davis) at different periods of the reproductive cycle: in the breeding season (November or December) and in the non-breeding season (June or July). The samples of testicular parenchyma were fixed for 2 hr at 4°C in 0.1M cacodylate buffer containing 2% glutaralde-
hyde. The biopsies were rinsed three times during 20 min in the same buffer and post fixed for 1 hr in the same buffer containing 1% osmium tetroxide (OsO4). After dehydration, samples were embedded in epoxy resin. Semi-thin sections were cut and stained with toluidine blue and ultrathin section were contrasted with uranyl acetate and lead citrate, and examined with an electron microscope (Elmisco 102 SIEMENS).

STUDY OF THE SPERM WITH THE ACRIDINE ORANGE TEST

Semen samples from Eulemur fulvus were obtained by prostatic massage at different moments during the year.

No semen sample was obtained from Eutemur macaco. Indeed, as described by BRUN et al. (1987a, b) the use of an electrostimulating probe results in an irremediable blockage of the male genital duct of this rare species. The slides with spermatozoa were fixed in Carnoy solution (3 parts methanol/1 part glacial acetic acid) during 24 hr for coagulation of the seminal vesicle secretions. The slides were recovered from the fixative and allowed to dry for a few minutes before staining with acridine orange as recommended by TEJADA et al. (1984): 10 ml of the stock solution was added to 40 ml of 0.1M citric acid and 2.5 ml of 0.3M Na2HPO4; 7H2O. The final pH of the stain was 2.5 with a concentration of 0.19 mg/ml. The slides were stained for 5 min, gently rinsed in deionized water and observed the same day on a fluorescence microscope equipped with a 490-nm excitation filter and a 530-nm barrier filter.

PLASMA TESTOSTERONE CONCENTRATION

Blood from one animal of each species was drawn monthly through the femoral vein and testosterone concentrations determined with the radio immunoassay “kit testosterone direct immunotech” (cross-test with 5 α-dihydrotestosterone 10% and 11 β-hydroxytestosterone 2%) and the “T free HOECHST-Behring” as for human. Testosterone metabolism is accepted to be similar for all the primates.

RESULTS

OPTICAL MICROSCOPIC OBSERVATIONS

Using an optical microscope the organization of the seminiferous tubules of the two Eulemur species was similar to that of other primates, but presented important seasonal variations. During both the mating and non-mating seasons we had observed degenerating germ cells for all stages of the seminiferous epithelium cycle and exfoliated meiotic and spermatid cells for the two species. The thickness of the seminiferous epithelium and the number of germ cells were more reduced during the non-mating season (Figs. 1 & 2). We have noted apoptotic like cells characterized by coagulated chromatin at peripheral boundaries of their nuclei in all types of germ cells. These manifestations, as well as the presence of number of enlarged lipid droplets and lysosomes in Sertoli cells were more frequent during the non-mating phase (Figs. 1 & 2).

ELECTRON MICROSCOPY INVESTIGATIONS

THE SERTOLI CELL

The nucleus of Sertoli cells for the two species during the two seasons showed a similar