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RESEARCH ARTICLE

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CHARACTERIZATION OF ESTROUS CYCLE PHASES IN RATS (*Rattus norvegicus*) BY VAGINAL SMEAR

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ABSTRACT

The determination of the estrous cycle in animals is important for studies of neuroendocrine function. The objective was to provide the researchers with the morphological characteristics of each phase of the estrous cycle in rats in. Proestrus is characterized by the predominance of a large number of round nucleated globular cells; in the estrus has identification of anucleated and conified epithelial cells; the next phase metaestrus has an equivalent proportion of nucleated and anucleated epithelial cells. The last phase is characterized by thin vaginal mucosa with predominance of leukocyte cells and presence of nucleated and round cells.

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INTRODUCTION

Determination of the estrous cycle in animals is important for studies of neuroendocrine function and can be monitored easily and non-invasively, observing the daily changes in vaginal cytology (Barril *et al.*, 2016). Rodents are animals that are easy to manipulate, have short cycles and short-term pregnancies, and are therefore more commonly used in reproductive experiments (Conceição *et al.*, 2005). The estrous cycle of rats has an average duration of four or five days, occurs at the onset of puberty and ends with a senility that is usually when the rats complete 12 months, being characterized by four phases, proestrus, estrus, metaestrus and diestrus. During the cycle, prolactin, luteinizing hormone (LH) and follicle stimulating hormone (FSH) remain low, increasing in the proestrus phase. Estradiol levels begin to increase in metaestrus, reaching extreme levels during proestrus and returning to the baseline in estrus. Progesterone secretion also increases during metaestrus and diestrus with a later

decrease. Then, the value of progesterone rises to reach its second peak at the end of proestrus (Vilela *et al.*, 2007). The knowledge of the estrous cycle allows accurate evaluation of the ovarian activity, since this changes abruptly due to fluctuations in estradiol levels, as well as any alteration that leads to the disruption of this pattern by environmental or toxic agents, besides avoiding a series of changes hormonal agents that occur during the cycle, enables to achieve more homogeneous important group in some types of assays (Long and Evans, 1922). The cytology of the vaginal smear is used to determine the phases of the estrous cycle (Hoar and Hickman, 1975; Marcondes *et al.*, 2002). The characterization of each phase is based on the ratio of three types of cells observed in the vaginal smear: epithelial cells, nucleated cells and leukocytes. The collection of vaginal secretion and the use of material usually takes from 1 to 2 hours. Thus, the objective of this study was to provide researchers with the characteristics of each phase of the estrous cycle in rats (*Rattus norvegicus*) in a fast and clear way. The present Study followed the ethical principles in animal experimentation, recommended by the

National Council for Animal Experimentation Control (CONCEA) and determined by Law 11,794 of 2008. The research project was submitted with protocol number 0158/2018 for prior appreciation by the Ethics Committee in Use of Animals of UESPI (CEUA-UESPI) having been the same deferred. Twelve rodents of the *Rattus norvegicus* species, females, maintained from birth at the Biotechnology and Biodiversity Research Center (NPBio / UESPI) vivarium were selected, with a mean age of three months and a mean weight of 300 g. They were kept in propylene cages (five animals per cage) with good hygienic conditions, fed with standard animal feed, feed (Labina TM) and water *ad libidum*. The determination of the phases of the estrous cycle was performed twice a day through the analysis of vaginal cytology for five consecutive days. The vaginal smear was collected using a pipette containing 0.9% saline. The vaginal fluid was analyzed after staining with crystal violet dye, under light microscopy, and the characteristics of the cells were made according to the estrous cycle (Brack *et al.*, 2006). The photographs were recorded for discussion. Rats with irregular cycles, characterized by the absence or permanence of one of the phases for an abnormal period, will be excluded from the study. The classification of the phases of the estrous cycle was determined by the ratio observed between the number of nucleated epithelial cells, keratinized cells and leukocytes (Marcondes *et al.*, 2002). The collection of the vaginal cytology of twelve female rats until observation of the material took 1-2 hours. During the estrus phase it was observed that the rats were able to be touched without any resistance, however when the animals were in another phase of the cycle they presented a certain aggressiveness when being manipulated. The material removed with the aid of a pipette was placed on a sheet of microscopy to make the coloration and subsequent photographic recording resulting in the figures that follow.

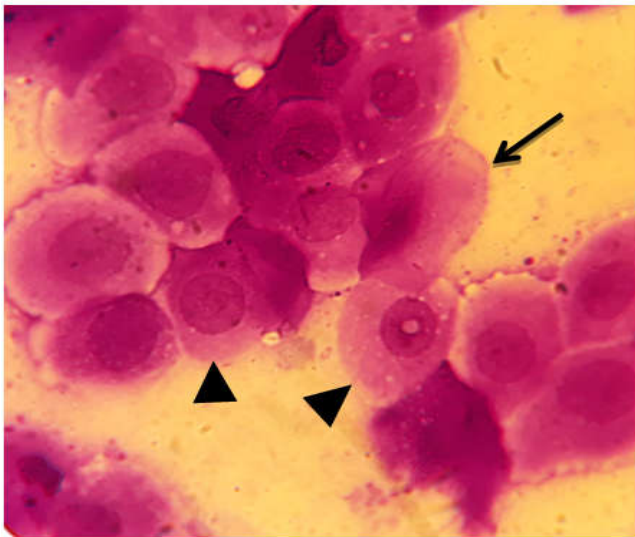


Figure 1. Violet smear photomicrographs stained with violet crystal of rats observed under light microscopy, without the use of a condenser lens, with 1000x increase photos taken with the camera itself, indicating anucleated rounded epithelial cells (arrow) and rounded nucleated cells (arrowhead) during the prophase phase

Figure 1 indicates proestrus, is characterized by the predominance of a large number of globular cells with well evident and rounded nuclei and there is also evidence of anucleated epithelial cells. The proestrus phase lasts from 12 to 14 hours, occurring to the maturation of one or more follicles (Brack *et al.*, 2006; De Carvalho, 2017). According to Marcondes *et al.* (2002) the levels of estradiol (main estrogen) and progesterone levels are high, and at this stage maturation of the ovarian follicle begins. In the next phase called estrus, figure 2, is the period in which the female is receptive to the male culminating with intercourse, lasts in average 25 to 27 hours, being identified by the presence of anucleated epithelial cells and cornified in large quantity and overlapping (Brack *et al.*, 2006).

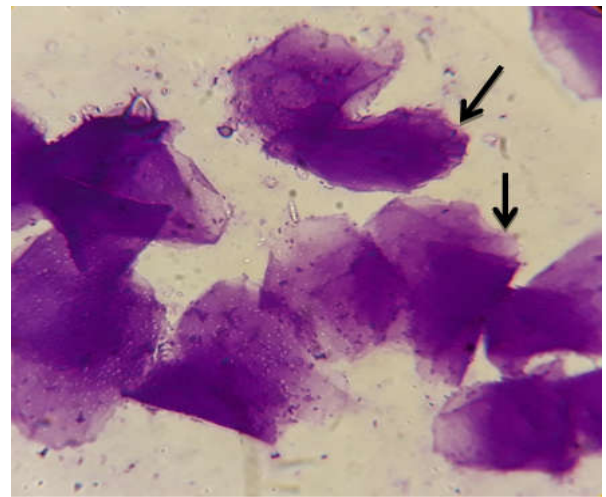


Figure 2. Vaginal smear photomicrographs stained with violet crystal from rats observed under light microscopy, without the use of a condenser lens, with 1000x increase photos taken with the camera itself, indicating anucleated cornified epithelial cells (down arrow) and rounded nucleated cells (up arrow) during the estrus phase

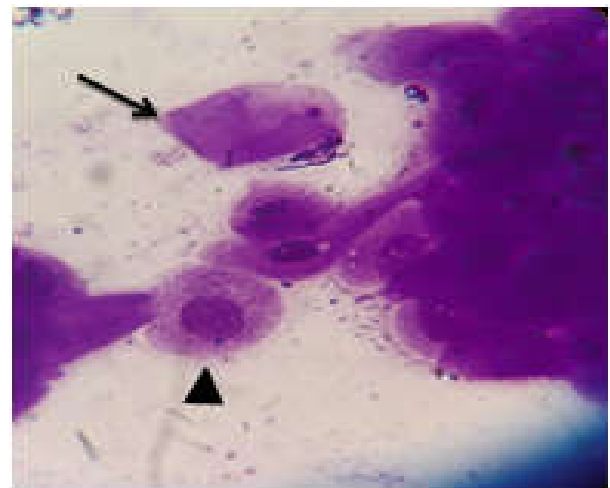


Figure 3. Vaginal smear photomicrographs stained with violet crystal of rats observed under light microscopy, without the use of a condenser lens, with 1000x increase photographs photographed with the camera itself, indicating anucleated cornified epithelial cells (arrow) and rounded nucleated cells (arrowhead) during the metaestrus phase

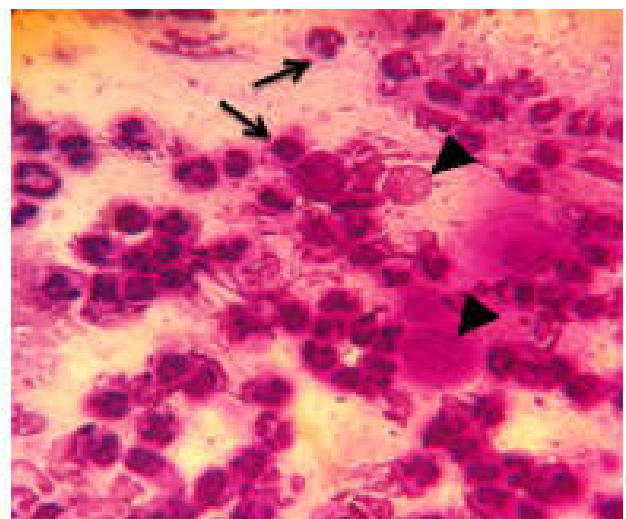


Figure 4. Violet smear photomicrographs stained with violet crystal of rats observed under light microscopy, without the use of a condenser lens, with 1000x increase photographed with the camera itself, indicating leukocyte cells (arrows) and anucleated epithelial cells (arrowhead) during the diestrus phase

According to Bracket *et al.* (2006) the ovarian follicle is already mature and ovulation occurs. If there is no conception, after estrus there is a recovery period called metaestrus, figure 3, whose duration was 24 to 48 hours. The metaestrus has an equivalent proportion of conical anucleated epithelial cells, remnants of stratum cells, as well as the presence of rounded nucleated epithelial cells with a well-defined nucleus and globose (Barril *et al.*, 2016; Brack *et al.*, 2006; De Carvalho, 2017). The levels of estradiol and progesterone begin to rise again (Barril *et al.*, 2016). The figure 4 is characterized by a thin vaginal mucosa with a predominance of leukocyte cells, there are nucleated and rounded cells lasts about 24 hours (Brack *et al.*, 2006; De Carvalho, 2017). At this stage, estradiol and progesterone levels are increased, reaching one of their highest concentrations and then reduced before they rise again in the proestrus stage (Brack *et al.*, 2006; De Carvalho, 2017). At the end of this experiment it is quite clear that after the violet crystal stained vaginal smear its characterization of the different phases of the estrous cycle in rats (*Rattus norvegicus*) is made easily, since the current literature is only supplying schemas and drawings of books and with this there is a difficulty to characterization of the different phases of the estrous cycle in rats, reducing the errors that occur during their classification for later studies.

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