CHARACTERIZATION OF THE ESTROUS CYCLE OF THE *Myocastor coypus* (COYPU) BY MEANS OF EXFOLIATIVE COLPOCYTOLOGY

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ABSTRACT. The aim of this work was to contribute with new information about the estrous cycle of the *Myocastor coypus*. Firstly, making a characterization of the cellular types and, secondly, quantifying the changes in the cellular standards that occur at different stages of the cycle, applying the colpocytological indices used for other species. Vaginal smears were taken daily at the same hour in 36 females with an age of 8.3 ± 0.8 months and a weight of 4.6 ± 0.4 kg. Samples were taken with Pasteur pipets. Their analyses were made in fresh and after staining with Harris’s hematoxylin and Shorr’s stain. The colpocytological samples were analyzed to get qualitative and quantitative data. The duration of the estrous cycle was of 35.5 ± 10.8 days, with a range of 20 to 60 days. In the smears, 3 cellular types divided into 7 subtypes could be differentiated: 1- superficial cells, with vesicular nucleus, with pyknotic nucleus, or without nucleus; 2- intermediate cells, deep or superficial; 3- deep cells, parabasal or basal. In all the smears the abundance of polymorphonuclear leukocytes was outstanding. Cornification and eosinophilia indices proved to be good indicators of estrus. The cariopyknotic index was not an indicator of estrus, since it was higher in proestrus and metaestrus.

RESUMEN. Caracterización del ciclo estral de *Myocastor coypus* (coipo) mediante colpocitología exfoliativa. Se efectuó el análisis y la caracterización del ciclo estral en *Myocastor coypus* mediante colpocitología exfoliativa. El seguimiento colpocitológico se realizó a diario en 36 hembras mantenidas en condiciones de criadero a corral. Las muestras se tomaron con pipetas Pasteur individuales. Se realizó su análisis en fresco y postinción con hematoxilina de Harris y colorante de Shorr. Las muestras colpocitológicas se analizaron a fin de obtener datos cualitativos (tipos y formas celulares y su coloración) y cuantitativos (número y tamaño medio de cada tipo celular). La duración del ciclo estral fue de 35.5 ± 10.8 días, con un rango de 20 a 60 días. En los extendidos se diferenciaron 3 tipos celulares divididos en 7 subtipos: 1- células superficiales, con núcleo vesicular, con núcleo picnótico o sin núcleo; 2- células intermedias, profundas o superficiales; 3- células profundas, parabasales o basales. En todos los extendidos se destacó la abundancia de leucocitos polimorfonucleares. Los índices de cornificación y eosinofilia resultaron buenos indicadores del estado de estrus. El índice cariopyknotico no fue un indicador del estrus, dado que se mostró más alto en proestro y metaestro.

Key words: estrous cycle, exfoliative colpocytology, *Myocastor coypus*.

Palabras clave: ciclo estral, colpocitología exfoliativa, *Myocastor coypus*.
INTRODUCTION

The nature of all the aspects related with reproduction in rodents is extremely varied. Since the first works of Stockard and Papanicolau (1917) in the Guinea pig, Long and Evans (1920, 1921 and 1922) in the rat, and Allen (1922) in the mouse, it has been demonstrated that the estrous cycle in rodents is determined by a standard of changes in the cellular types observable in the vaginal smears. This standard of changes has allowed estimating the duration of the estrous cycle in different species. Thus, it was established that in rodents of the Suborder Myomorpha, the estrous cycles are relatively short (Bertalanffi and Lau, 1963; Smith and Kaltreider, 1963; Barfield and Beeman, 1968; Snell, 1969).

Most of the hystricomorph rodents show some characteristics that differentiate them from other rodents. These include the courtship not observed in the murids, long gestation periods, the birth of precocious litters, the lateral location of the mammas, and the presence of a membrane of vaginal occlusion and, in general, an easy reproduction in captivity (Rowlands and Weir, 1974).

The coypu (*Myocastor coypus*) is a semiaquatic hystricomorph rodent native of South America commonly called “nutria”. Its natural populations live in zones of lagoons, ponds, rivers, and other ecosystems of fresh water, in its zone of origin as well as in other countries where, due to its importation as a fur species, it could naturalize (Colantoni, 1993). The coypu, together with the mink and the fox, is one of the most important species in Argentina concerning the production and export of furs. Its use is also relevant in the international fur industry.

Studies on this species have been made on natural populations living wild (Crespo, 1975; Restelli et al., 1991), as well as in semicaptivity or captivity (Atwood, 1950; Norris, 1967; Gosling, 1981) and in production or experimental breeding farms (Bura et al., 1985; Bura, 1992 a and b). Such works comprise different aspects such as the populational ecology of the coypu, nutrition, epidemiology, genetic studies, and reproductive physiology. In the field of the Morphological Sciences some research referring to biostructure (Sincai et al., 1982; Felipe et al., 1998 a) and embryonal development (Felipe et al., 1997 a and b) has been made.

The coypu is a polyestrous rodent with estrous cycles that occur uninterruptedly during the year every 23 to 40 days, according to that determined in several studies in captivity or semicaptivity (Ehrlich, 1966; Newson, 1966). In the last years, several investigators have made general characterizations of the estrous cycle of the *Myocastor coypus* under breeding farm conditions (Callejas and Cabodevila, 1993; Felipe et al., 1998 b), establishing the existence of a typical estrous cycle for the species. Iudica and Alberio (1995) made a systematic study by means of exfoliative colpocytology.

Iudica and Alberio (1995) concluded that the average duration of the estrous cycle in the coypu was of 28.9 ± 12.6 days (range: 12-49 days). A tendency to keep conditions of greater reproductive activity was noticed in animals reared in corrals compared to those reared in cages. They observed four different cellular types in vaginal smears (squamous, intermediate, and parabasal cells, and polymorphonuclear leukocytes), a great variability in the duration of the cycles and in the estrous, among animals as well as in the same individual.

The purpose of this study was to contribute with new information about the estrous cycle of the *Myocastor coypus*. Firstly, making a detailed characterization of the cellular types and, secondly, quantifying the changes in the cellular standards that occur at different stages of the cycle, applying the colpocytological indices used for other species.

MATERIALS AND METHODS

Animals and housing

Thirty-six virgin females of *Myocastor coypus* of the Greenland variety were used. Their age was of 8.3 ± 0.8 months (range of 7 to 9.5 months) with a weight of 4.6 ± 0.4 kg. The females, organized from weaning in 7 groups (family nuclei), were kept under breeding conditions in a partially
roofed corral, with 2 m² for each animal. Each female was identified using ear-tags in the right ear and its number was registered in individual charts. Balanced food and water were administered ad-libitum. A unique male was placed in an adjacent corral.

**External examination**

During the colpocytological follow-up of the animals an external examination was made to detect variations that could be associated to the stages of the estrous cycle. The characteristics considered were changes in the tonality or color of the vulvar mucosa, presence of tumefaction, and presence of mucous secretion.

**Collection of vaginal samples**

The colpocytological follow-up was made during 3 months in each family nucleus. Samples were taken daily, between 10 and 11 a.m. Disposable Pasteur pipets of 151 mm with a diameter of 2 mm and loaded with 0.3 ml of physiological solution (0.9% sodium chloride) were used to take the samples. Ten animals were randomly selected to determine the ideal depth to take the samples and the possible existence of variations in the cellular composition of the smears. The procedure consisted in obtaining the sample of liquid by introducing the pipets 1, 3, or 6 cm deep into the vaginal duct. In all cases, pipets were introduced obliquely, upward and forward (toward dorsal) to avoid the incurvation angle of the vaginal vestibule. The total number of samples analyzed was 120.

**Analysis of the samples**

Samples were firstly analyzed in fresh, registering the macro and the microscopic aspect of the vaginal liquid (consistency and color). Afterwards, a staining was made with routine techniques (Harris’ hematoxylin and Shorr’s stain) (Lynch et al., 1972). Smears were analyzed to obtain a cytometric determination of the average sizes of each cellular type and of their nuclei, using a micrometric ocular incorporated to an Olympus CHL microscope with a magnification of 1000x. The determination of the number of each cellular type identified, using a lattice of known dimensions located in a microscope with screen was realized. A strategy of random sampling was used in the counting of cells. For each smear, a total of 30 fields were counted with a 10x ocular. All values were expressed as means ± the standard deviation of the mean.

**Determination of the stages of the cycle**

The stages of the estrous cycle and its duration were determined considering the cytological composition of the vaginal smears and taking into account the relative proportions of the desquamated cellular types, the infiltration of leukocytes, the presence of mucus, and the appearance of the vaginal fluid. In case of difficulties in the identification of the proestrus, a retrospective cytological analysis of the smears was made. Once the estrous was identified, the smears of the previous days were analyzed to confirm or not the identification of the proestrus.

**Elaboration of cytological indices**

Data obtained from the colpocytological analyses were used to elaborate the following cytological indices: a) cornification index (CI) (Evans and Savage, 1970): number of cornified cells / total number of epithelial cells x 100; b) eosinophilic index (EI): number of eosinophilic cells / total number of epithelial cells x 100; c) cariopyknotic index (KPI) (Schutte, 1967): number of superficial cells with pyknotic nucleus / number of superficial cells with vesicular nucleus x 100; d) superficial cell index (SCI) (Schutte, 1967): number of cells of the superficial layers / number of cells of deep layers x 100; e) maturation index (MI) (Lynch et al., 1972): obtained starting from the proportion of cells of the three principal layers of the vaginal epithelium (superficial, intermediate and deep). Indices were elaborated for different stages of the estrous cycle.

**RESULTS**

**External examination**

The vulvar surface was always of a grayish-pink color. None of the animals showed modifications in the external appearance of the vulvar region or the presence of mucous exudate.

**Sampling procedure during the colpocytological follow-up**

No differences were observed in the cellular components of the samples taken at 3 and 6 cm deep from the vaginal opening. In both cases the same macroscopic appearance of the liquid obtained and the same cellular types were observed. In the case of the samples obtained at 1 cm from the vaginal opening it was observed, in some cases, the presence of superficial cells (squamous), even at stages of the cycle where, at deeper levels of sampling, they were absent.
Characterization of cellular types

In the smears, three principal cellular types were differentiated: superficial cells, intermediate cells and deep cells.

Superficial cells were observed as squamous cells, polygonal in shape and generally with a clear, homogenous and transparent cytoplasm, red or sky-blue in color according to the stage of the cycle. Considering the nuclear characteristics, they were subdivided in: superficial cells with vesicular nucleus, with a size of $33.7 \pm 7.1 \mu m$ and an ovoid nucleus of $6.5 \pm 2.4 \mu m$; superficial cells with pyknotic nucleus, with a size of $41.8 \pm 6.7 \mu m$ and dotted and hyperchromatic nucleus, with a diameter of $3.9 \pm 1.4 \mu m$; and superficial cells without nucleus ($38.8 \pm 12.1 \mu m$).

Intermediate cells had an ovoid or polygonal shape, they were always flat, with a vesicular nucleus in central position. The ovoid cells were classified as deep intermediate cells (diameter of $22.5 \pm 7.9 \mu m$ and nuclear size of $10.4 \pm 4.4 \mu m$), bluish or greenish in color, and the polygonal cells were classified as superficial intermediate cells ($24.8 \pm 5.4 \mu m$ of diameter and nuclei of $9.1 \pm 3.3 \mu m$), red or bluish green, in some cases with both colors in the cytoplasm.

Deep cells were isolated or in small groups and were strongly stained bluish red. Their nuclei were well stained. They differentiated in parabasal cells (diameter of $15.4 \pm 3.5 \mu m$ with nuclei of $8 \pm 2.2 \mu m$), they were round with a big, spherical nucleus occupying most part of the cytoplasm; and basal cells (diameter of $12.3 \pm 2.4 \mu m$) ovoid in shape, with a big and eccentric ovoid nucleus (diameter of $7 \pm 2.9 \mu m$).

It was outstanding in all the smears the presence of polymorphonuclear leukocytes, predominantly at the diestrus.

Duration and stages of the estrous cycle

The duration of the estrous cycle was of $35.5 \pm 10.8$ days, with a range of 20 to 60 days. The duration of each stage of the estrous cycle is shown in Table 1. The percentages of the overall duration of the estrous cycle correspondig to each stage were as follows: proestrus 5%, estrus 4%, metaestrus 7% and diestrus 84%.

Characterization of the stages of the estrous cycle

The predominance of the polymorphonuclear leukocytes in the vaginal samples was evident not only at the diestrus and metaestrus but also at the proestrus (Table 2).

In the proestrus and metaestrus, the percentages of deep and intermediate cells were higher than in estrus and diestrus. Superficial cells without nucleus presented an increase in proestrus and metaestrus, being predominant in the estrus (Table 2).

It was difficult during the work to determine the proestrus accurately in the animals without the certainty of the observation of the estrus. By means of the retrospective analysis of the definitive smears of all the females, the presence of basal and parabasal cells was determined in 61.1% of the females in the day previous to the estrus in more quantity than in the preceding days, and in 22.2% of the females since two days before the estrus, but in all cases the presence of polymorphonuclear leukocytes was outstanding (Table 2).

The other animals (16.7%) did not show peculiarities in the colpocytological smears in the days previous to the estrus, the image being that of the diestrus type.

The macroscopic observation during the proestrus showed a flushing liquid translucent, with a fluid consistency and small-scattered clots. The smears were formed by different types of epithelial cells of various sizes, which were isolated or in groups, intermín-

Table 1: Length of each stage of the estrous cycle in M. coypus, values expressed in days ($x \pm s.d.m.$).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Length $^1$</th>
<th>Range $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proestrus</td>
<td>$1.7 \pm 0.9$</td>
<td>0 - 3</td>
</tr>
<tr>
<td>Estrous</td>
<td>$1.5 \pm 0.5$</td>
<td>1 - 2</td>
</tr>
<tr>
<td>Metaestrus</td>
<td>$2.3 \pm 1.1$</td>
<td>1 - 5</td>
</tr>
<tr>
<td>Diestrus</td>
<td>$29.6 \pm 10.4$</td>
<td>14 - 54</td>
</tr>
</tbody>
</table>

$^1$ Values expressed in days ($x \pm s.d.m.$).
Distribution of the cellular types at the stages of the estrous cycle in *M. coypus*. Values expressed as percentages (%).

<table>
<thead>
<tr>
<th>Cellular Types</th>
<th>Cellular Subtypes</th>
<th>Estrual Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Proestrus</td>
</tr>
<tr>
<td>Deep cells</td>
<td>Basal</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Parabasal</td>
<td>1.5</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Deep intermediate</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Superficial Intermediate</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Superficial with Vesicular nucleus</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Pyknotic nucleus</td>
<td>0.9</td>
</tr>
<tr>
<td>Superficial</td>
<td>Superficial without Nucleus</td>
<td>6.4</td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td>75.7</td>
</tr>
</tbody>
</table>

Gled with abundant scattered polymorphonuclear leukocytes (Table 2). The basal and parabasal cells observed at the proestrus stood out for the acidophile of their cytoplasms. The intermediate cells showed a basophilic or slightly acidophilic cytoplasm, translucent and with vesicular nuclei. The superficial cells were basophilic and translucent.

In the samples obtained during the estrus the vaginal fluid was yellowish white, with abundance of cellular elements and a tendency to adhere slightly to the walls of the pipet (flowing at the minimum pressure). At the microscopic observations in fresh, the abundance of big, squamous, transparent and in cumulus cellular elements was outstanding and without the apparent presence of other cellular elements. At the post-staining observation, it was confirmed the predominance of squamous cells strongly eosinophilic and opaque, determining the presence of polymorphonuclear leukocytes and a very scarce number of other epithelial types (Table 2).

At the metaestrus, the macroscopic aspect of the liquid was similar to that of the proestrus. Microscopically, it was determined the presence of intermediate, basal, and parabasal cells among the cornified cells and a marked and sudden increase in the number of polymorphonuclear leukocytes, parallel to the decrease of the squamous cells.

At the diestrus, it was possible to observe the mucous and lumpy appearance of the liquid, with a whitish and filamentous aspect, tendency to strongly adhere to the walls of the pipet and flowing with difficulty. The microscopic observations showed the polymorphonuclear leukocytes, isolated as well as in groups (clots), and trapped in mucous filaments. In 75.3% of the females, during the diestrus, a sporadic increase of superficial cells with and without nucleus was registered. This increase was observed in only one sample per animal and the percentage of superficial cells increased from 1.03% (Table 2) to 9.7%.

**Cytological indices**

The cytological indices obtained from the
The cornification, eosinophilic and superficial cell indices were higher in the estrus than in the proestrus and metaestrus. However, the cariopyknotic index in the estrus was lower than in the stages of proestrus and metaestrus (Table 3).

**DISCUSSION**

The members of the Suborder Hystricomorpha, like the coypu, show estrous cycles of very variable duration. Among the hystricomorph rodents that have been studied in captivity and for those whose reproductive characteristics have been reported (Weir, 1974), the cavies show relatively short periods. However, these are longer than those of the myomorph rodents. Thus, in *Cavia porcellus* (Guinea pig) the cycle lasts 16.5 days (Bland, 1980) and in *Cavia aperea*, 20.6 days (Rood and Weir, 1970). The estrous cycle is long, as in the coypu, in most cases. In *Galea musteloides* is of 22.3 days (Rood and Weir, 1970), in *Chinchilla laniger*, 38.1 days (Weir, 1970), and in *Lagostomus maximus*, 45 days (Weir, 1971 a). In the dasyprocta (*Dasyprocta aguti* and *Myoprocta pratti*), the cycle is of 35 days, with a range of 12 to 59 days for the first (Weir, 1971 b and c) and of 16 to 68 days for the second (Kleiman, 1970). Also *Hystrix cristata* has a cycle of 35 days, but with less variations (range of 30 to 37 days) (Weir, 1967).

The variations in the length of the cycle indicated for the dasyprocta is present also in any species of the hystricomorph rodents. These can be so wide as those observed in *Myocastor coypus* in this work and by other authors (Judica and Alberio, 1995). For example, a variation of 11 to 48 days was determined in *Galea musteloides* (Rood and Weir, 1970), of 16 to 69 days in *Chinchilla laniger*, of 16 to 94 days in *Lagostomus maximus* (Weir, 1971 a), and of 13 to 25 days in *Cavia porcellus* (Asdell, 1964). The estrous cycles registered for *Cavia aperea* by Rood and Weir (1970) also showed great variability in their duration, in wild animals as well as in animals reared under laboratory conditions, being such duration of 12 to 41 days. These authors concluded that the variation observed in the length of the estrous cycle would be normal for the hystricomorph rodents as it is present in all the species studied and under different breeding conditions.

Despite the variations registered, Weir (1974) has estimated that the average length in most of the species is within the range of 30 to 40 days. For Weir and Rowlands (1974) this would indicate a long luteal phase. These authors says that, in general, the length of the

<table>
<thead>
<tr>
<th>Cytological indices</th>
<th>Stage of the estrous cycle</th>
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<tbody>
<tr>
<td></td>
<td>Proestrus</td>
</tr>
<tr>
<td>Cornification (1)</td>
<td>34.6</td>
</tr>
<tr>
<td>Eosinophilia (1)</td>
<td>67.5</td>
</tr>
<tr>
<td>Cariopyknotic (1)</td>
<td>12.6</td>
</tr>
<tr>
<td>Superficial Cells (1)</td>
<td>2.1</td>
</tr>
<tr>
<td>Maturation (2)</td>
<td>34.6 / 51.8 / 13.6</td>
</tr>
</tbody>
</table>

(1) Values expressed in percentages.
(2) Proportions.
ESTROUS CYCLE IN *Myocastor coypus*

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Cycle is determined by an extended duration of the secretory activity of the corpus luteum and that the maximum lifespan of the corpus luteum of the different hystricomorph species is very variable, but within the same species it is constant.

Like in the coypu, in most of the hystricomorph rodents, the estrus state is not easily determined by the external appearance of the vulva. Many rodents of the suborder secrete mucus during the estrus, but only in the plains viscacha (*Lagostomus maximus*), the tucu-tucu (*Ctenomys* spp.), the casiragua (*Proechymis guairae*), and the degu (*Octodon degu*) can be seen sometimes the concomitant vulvar tumefaction (Weir, 1974). Kleiman (1970) observed the external vulvar appearance in *Myoprocta pratti* during its estrous cycle and determined that at the beginning of the opening of the vaginal occlusion membrane, during the estrus, the vaginal wall is dry and wrinkled and then it turns smooth and wet, and finally a slight whitish discharge is detected, assuming this moment as the metaestrus stage.

The hystricomorph rodents, unlike the *Myocastor coypus*, are characterized by the presence of a membrane of vaginal occlusion that only opens during the estrus and for a short time near labor (Weir, 1967, 1974). The presence of this membrane has provided an easy way to determine the periodicity of the reproductive cycle in some members of the suborder (Hunter et al., 1969; Kleiman, 1970; Weir, 1974), but has complicated or has made impossible the follow-up of the estrous cycle in almost all the species of such group of animals. But the coypu has allowed the possibility of doing numerous works about its estrous cycle in a similar way to that made with the myomorph rodents (Iudica and Alberio, 1995; Felipe et al., 1998 a).

The detection of the estrus starting from the interpretation of vaginal smears is not possible in most of the hystricomorph rodents as can be the guinea pig, in which the predominance of superficial cells first and the reappearance of leukocytes in the smear later, are considered as indicators of estrus and ovulation, respectively (Bland, 1980), or like in the coypu, where a clear predominance of squamous cells was observed.

The sequence of cellular types found in the vaginal smears of the coypu is the same that has been reported for other species. The successive dominance of leukocytes, nucleated epithelial cells and cornified cells has been described in other murid rodents, like the rat (Allen, 1922), the hamster (Peczenik, 1942), the mouse (Bronson et al., 1969; Evans et al., 1990) and the gerbil (Barfield and Beeman, 1968).

In laboratory rodents such as the rat and the mouse, the detection of the proestrus allows to predict the beginning of the estrus (Long and Evans, 1922; Evans et al., 1990). However, the difficulty observed for detecting the proestrus in the coypu would indicate that, under conditions of colpocytological follow-up, it is very difficult to foresee the estrus with more than 24 hours of anticipation. The cytological indicators of the proestrus state are masked by the marked predominance of polymorphonuclear leukocytes in the colpocytological samples.

In a similar way to that observed in the mare (Witherspoon, 1971), the sheep (Ghannam et al., 1972), and the hamster (Peczenik, 1942), the stages of proestrus and metaestrus in the coypu do not show a differential characterization in the colpocytological samples allowing to identify them unmistakably.

As for the morphological characteristics of the exfoliated cells, there are no differences with the descriptions made by Iudica and Alberio (1995).

About the colpocytological indices, the principal objection to their use in the coypu is that they only consider epithelial cells, unknowing the presence of lymphocytes which, in the case of this species, are the majority during almost all the cycle. The increase in the cornification index observed between the proestrus and the estrus coincides with that expected according to Schutte (1967). For this author, while the estrus progresses, the epithelial cells go on with their keratinization process. Similar observations can be made about the eosinophilic index that is based exclusively on the eosinophilic color of the cytoplasms, independently
of the appearance of the nucleus. The maximum value of this index is reached, according to Schutte (1967), at the ovulation moment, at least in the bitch. The cornification of the vaginal epithelium has been used as an indicator of the biological activity of sexual steroids in many mammal species (Fowler et al., 1970) and to determine the appropriate times for mating.

The cariopyknotic index, according to Schutte (1967), would be indicative of the action of estrogens on the vaginal epithelium during the estrus, causing that the nuclei of the superficial cells show pyknotic changes. But, as it was indicated, this nuclear appearance in the squamous cells of the coypu is more characteristic of the proestrus and of the metaestrus.

The index of superficial cells, also suggested by Schutte (1967), is applicable to the coypu, as there is a notable variation in the proportion of the different cellular types. As it was observed when analysing the percentile distribution of the cellular types, there is an increase in the number of nucleated (from 2% in the proestrus to 4.1% in the estrus) and without nucleus (from 6.4% in the proestrus to 53.8% in the estrus) superficial cells near the estrus and a decrease in the number of cells of the deep layers. The increases in the cornification and eosinophilic indices are good indicators of the estrus state in *M. coypus*.

The finding of superficial cells with pyknotic nuclei is more frequent during proestrus and metaestrus than during estrus, and this determines higher cariopyknotic indices at these stages. In its turn, the united finding of superficial cells with vesicular and pyknotic nuclei would be an indicator of very early or late stages of estrus. The cariopyknotic index does not seem to be applicable as such in the coypu to define the estrus state, due to the absolute predominance of cells without nucleus over the vesicular and pyknotic cells at this stage. A low cariopyknotic index was obtained at the estrus, as a result of the peculiarities of the species.

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**LITERATURE CITED**


