

Qualitative and quantitative variations of the vaginal epithelium in *Myocastor coypus bonariensis* (coypu) during the estrous cycle

Variaciones cualitativas y cuantitativas del epitelio vaginal en *Myocastor coypus bonariensis* (coipo) durante el ciclo estral

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ABSTRACT

The aim of the present study was to characterize the qualitative and quantitative changes in the vaginal epithelium in the coypu (*M. c. bonariensis*) during the estrous cycle. A daily colpocytological evaluation was performed in 36 females. Samples from three vaginal sections (cephalic, middle and caudal) were processed with routine histological techniques. The mean cycle duration was of 36.86 ± 10.52 days. The greater thickness of the epithelium was observed in the proestrous and the smallest in the metestrous. Variations in epithelial thickness in each cycle stage for the different sampled regions showed non-significant differences in the estrous and proestrous. Differences were observed during the metestrous between the cephalic and middle and middle and caudal regions, and also during the diestrous, being the most significant between cephalic and middle regions. At metestrous, a significant decrease in the number of epithelial layers was observed in all the regions. At diestrous, differences were observed between cephalic and middle, and cephalic and caudal regions.

Keywords: (coypu), (*Myocastor coypus*), (hystricognath rodents), (vagina), (estrous cycle)

RESUMEN

El objetivo del presente estudio fue caracterizar los cambios cualitativos y cuantitativos en el epitelio vaginal del coipo (*M. c. Bonariensis*) durante el ciclo estral. Se realizó una evaluación colpocitológica diaria en 36 hembras. Las muestras de tres zonas vaginales (cefálica, media y caudal) se procesaron con técnicas histológicas de rutina. La duración media del ciclo fue de $36,86 \pm 10,52$ días. El mayor grosor del epitelio se observó en el proestro y el menor en el metaestro. Las variaciones en el espesor epitelial en cada etapa del ciclo para las diferentes regiones muestreadas no mostraron diferencias significativas en estro y proestro. Se observaron diferencias durante el metaestro entre las regiones cefálica y media y media y caudal, y también durante el diestro, siendo las más significativas entre las regiones cefálica y media. En el metaestro, se observó una disminución significativa en el número de capas epiteliales en todas las regiones. En el diestro se observaron diferencias entre las regiones cefálica y media y cefálica y caudal.

Palabras clave: (coipo), (*Myocastor coypus*), (roedores histicognatos), (vagina), (ciclo estral)

INTRODUCTION

According to Ojasti's¹⁴ classification criteria of the wild fauna managing, the coypu is classified as a farming species which its native population is able to be used. Other South American hystricognath rodents of value for economical resources are *Hydrochoerus hydrochoeris* (capibara), *Dasyprocta aguti* (aguti), *Agouti paca* (pacas), *Cavia porcellus* (cuy or guinea pig), *Lagostomus maximus* (prairie viscacha), *Proechimys guairae* (casiragua or spinous rat or guaira spiny rat) and *Chinchilla laniger* (chinchilla). Most of the hystricognath, as the coypu, presents some differential characteristics from other groups of rodents, such as courtship activity, long gestation periods, delivery of precocious offspring and the presence of a vaginal occlusion membrane. In the coypu, the complete absence of this membrane once sexual maturity is reached has allowed colpocytological studies of the estrous cycle⁷. Researches on the reproductive biology of the coypu have been performed on wild natural populations⁴, under conditions of semi-captivity, at commercial farming or in experimental populations⁷. The reproductive system of the coypu has been anatomically and histologically characterized^{5,6}, however to our knowledge there are not information regarding its functional modifications under different physiological conditions. Among these modifications, morphofunctional variations during the estrous cycle are of great importance. Cyclic changes in the reproductive tract have been reported in many domestic and laboratory species¹⁵. The colpocytological studies performed on the

coypu have corroborated the existence of a typical estrous cycle, rendering it as an annual polyestrous rodent. The normal duration of its estrous cycle is 35.5 ± 10.8 days, ranking from 20 to 60 days⁷. The aim of the present study was to characterize the changes in the vaginal epithelium during the estrous cycle of *M. c. bonariensis* by using a qualitative and quantitative morphological analysis, taking into account macroscopic aspects of the mucosa, thickness of the vaginal epithelium, number of cellular layers and types of superficial cells.

MATERIALS AND METHODS

Thirty six (36) virgin and sexually mature females of the subspecies *M. c. bonariensis* were used. Females, born in captivity, were kept under herding conditions located in parlors with the presence of a male of the same subspecies in an adjacent parlor. At the time of sacrifice, the animals were 23 months old and weighed 5.42 ± 0.31 kg. The colpocytological follow-up of the animals was carried out daily for 18 months from 5 month of age in order to obtain records of 7 complete estrous cycles per animal. Vaginal smears were observed as collected within 5 minutes of sampling and after its staining with hematoxylin and Shorr dye. To determine the stage of the cycle, the cytological composition of vaginal smears was considered as described by Felipe *et al.*⁷. Determination of the number of each cellular type identified was carried out by using a lattice of known dimensions located in a microscope with

screen. 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. The length of each estrous cycle was considered as the interval between the first day of estrous, determined by colpocytology, and the day before to the next estrous. To determine regional variations of the characteristics of the vaginal epithelium, the organ was divided into three sections: cephalic or of the vaginal fornix, middle (between the anal sac and the urinary bladder) and caudal. Samples were fixed in Bouin's liquid and processed with routine techniques and then embedded in paraffin. Serial cuts of 5 µm were performed and stained with Harris' hematoxylin and eosin. Ten sections of each region were examined to determine the thickness of the epithelium and the number of epithelial layers. In both cases, considering the ovoidal shape of the vaginal lumen, measures and counting were taken in four areas of the vaginal wall (dorsal, right and left laterals and ventral). Measures were done using a micrometric ocular of 100x attached to an Olympus CH2 microscope. Counting of the number of layers was done with a 400x.

Two serial cuts (one previous to and one after the other ten cuts used for the above mentioned determinations) were stained with Harris' hematoxylin and Shorr dye to facilitate the identification of the different types of superficial cells in each area for each stage of the estrous cycle. Classification of the cellular type was performed based on previous results⁷, considering cells as superficial or squamous, superficial intermediate, deep intermediate, parabasal and basal. Values

obtained were expressed as percentage of each cellular type per vaginal section. Statistical analysis of the data was done with GraphPad InStat software, version 3.0. Data are presented as mean ± standard deviation (S.D.) and the level of significance was always $P < 0.05$.

RESULTS

Cellular characterization of the stages of the estrous cycle

During the proestrous, smears were of different types of epithelial cells, which were alone or in groups, mixed with abundant leukocytes (Table 1). The observed basal and parabasal cells in the proestrous showed a marked acidophile in their cytoplasm. The intermediate cells presented a basophilous cytoplasm or lightly acidophilous and a vesicular nucleus. The superficial cells were translucent and basophilic. The samples collected during the estrous showed abundance of squamous cells, strongly eosinophilic and arranged as aggregates, with few leukocytes and other cellular types (Table 1). In the metestrous, the presence of basal, parabasal and intermediate cells mixed with cornified cells and a marked increment of leukocytes was observed. A predominance of leukocytes, either alone or in groups, was observed in the diestrous, as well as filaments of mucous aspect. The predominance of leukocytes in vaginal samples was evident not only at the diestrous and metestrous but also at the proestrous (Table 1).

Table 1. Distribution of the cellular types at the stages of the estrous cycle in *M. c. bonariensis*. Values expressed as percentages (%).

Cellular types	Proestrus	Estrus	Metestrus	Diestrus
Basal	0.61	-	0.50	1.87
Parabasal	4.22	-	3.30	7.85
Deep intermediate	5.55	-	8.42	5.63
Superficial intermediate	6.84	4.61	7.41	2.61
with	2.09	4.35	8.21	0.04
Superficial or				
vesicular nucleus				
with	2.11	7.03	5.08	0.05
squamous				
pyknotic nucleus				
without	4.17	55.80	5.26	0.83
nucleus				
Leukocytes	74.41	28.21	61.82	81.12

Thickness of the epithelium

No statistically significant differences were observed in the thickness of the vaginal epithelium between samples of the proestrous and estrous; however, there were differences between samples of these stages and those of

the metestrous and diestrous ($P < 0.001$) (Figure 1). The greatest thickness of the epithelium was observed in the proestrous and the smallest thickness in the metestrous.

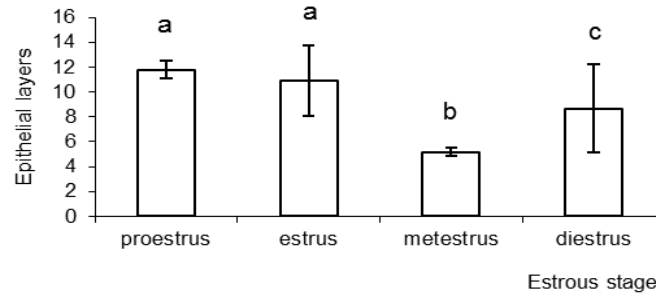


Figure 1. Variation in the thickness of the vaginal epithelium of *M. c. bonariensis* at different stages of the estrous cycle. Bars with different letters are significantly different ($P < 0.001$).

The analysis of the epithelial thickness in the different regions during each stage showed no significant differences among them when comparing proestrous and estrous (Figure 2). However, differences were observed during metestrous between the cephalic and middle regions ($P < 0.05$) and between the middle and caudal ($P < 0.001$) regions. Also, there were differences during diestrous among the three

regions, being more marked that between the cephalic and middle regions ($P < 0.001$) (Figure 2). The comparison among regions of the vagina at different stages of the estrous cycle showed no significant differences in the proestrous and estrous among the cephalic, middle and caudal regions, but there were differences between regions in these two stages and the regions in the metestrous and diestrous ($P < 0.001$).

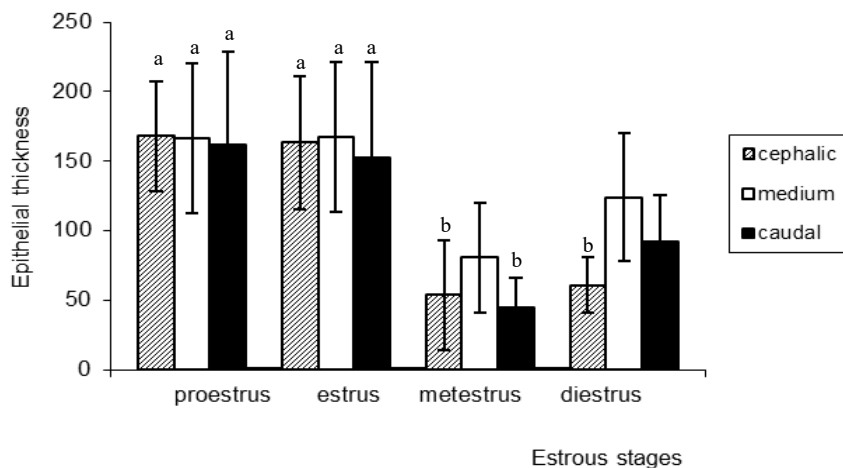


Figure 2. Variations in the epithelial thickness of *M. c. bonariensis* within each stage of the cycle considering the different vaginal regions analyzed. Bars with different letters are significantly different ($P < 0.001$, except b vs. c, $P < 0.05$).

Number of epithelial layers

The greatest number of layers was observed in the proestrous (Figure 3). The analysis of regional variations in number of layers within each stage of the cycle showed that in the proestrous there were no differences among regions. The decrease in the number of layers in the estrous was more marked in the caudal region (Figure 3), differences were significant when compared to the cephalic and middle regions ($P < 0.001$). In the metestrus, a decrease in the number of epithelial layers was

observed in all the regions (Figure 3), and the middle region presented significant differences compared to the cephalic and caudal regions ($P < 0.01$). In the diestrus, differences were observed between cephalic and middle and cephalic and caudal regions ($P < 0.001$). In the metestrus and estrous, the middle region showed a greater number of layers compared to the other two regions (cephalic and caudal regions) (Figure 3).

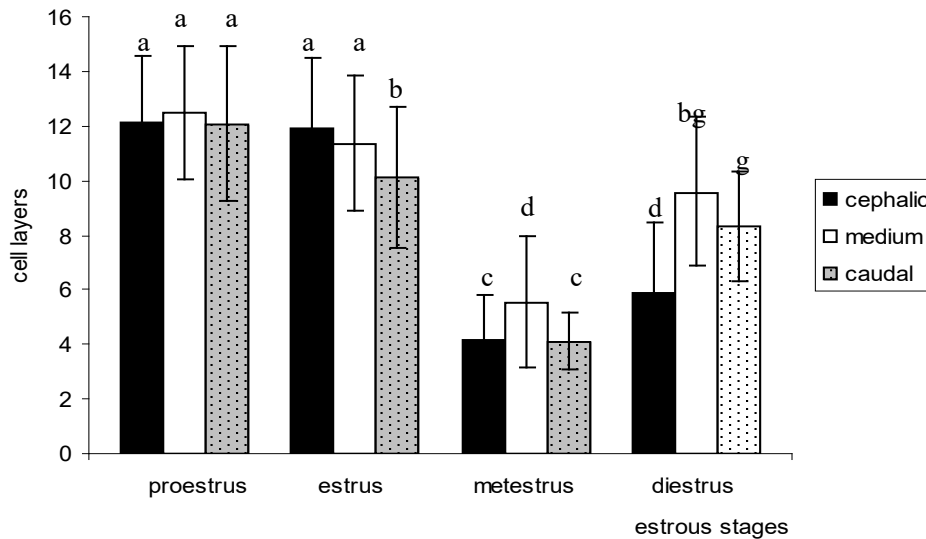


Figure 3. Number of epithelial cell layers in different stages of the estrous cycle and regions of the vagina of *M. c. bonariensis*. Bars with different letters are significantly different ($P < 0.001$, except c vs. d, $P < 0.01$).

Types of superficial cells during each stage of the estrous cycle

During the proestrous and estrous, a predominance of superficial cells of the squamous type in all the regions was observed (Table 2). In the vaginal epithelium, during the proestrous, a tendency to detachedness of the more superficial layers of the epithelium was observed. In the metestrus, the predominant cells on the surface were of the intermediate

deep type in the middle and caudal regions and intermediate superficial type in the cephalic region. Both cellular types showed a cuboidal shape with round or slightly ovoidal nuclei. In the diestrus, samples presented a predominance of intermediate superficial cells (Table 2).

Figure 4 shows microphotographs of the cell types observed in colpocytological samples.

Table 2. Cell types in the different regions of the vagina and in different stages of the estrous cycle in *M. c. bonariensis*. Values expressed as a percentage.

Estrous cycle stage	Cellular type	Vaginal regions		
		Cephalic	Medium	Caudal
Proestrus	Superficial or squamous	99	98	96
	Superficial intermediate	1	2	4
	Deep intermediate	-	-	-
	Parabasal	-	-	-
	Basal	-	-	-
Estrus	Superficial or squamous	98	99	99
	Superficial intermediate	2	1	1
	Deep intermediate	-	-	-
	Parabasal	-	-	-
	Basal	-	-	-
Metestrus	Superficial or squamous	23,2	7	-
	Superficial intermediate	39,1	32	33,1
	Deep intermediate	37,7	40,6	45,7
	Parabasal	-	20,4	21,2
	Basal	-	-	-
Diestrus	Superficial or squamous	12	-	-
	Superficial intermediate	45	47,8	52,4
	Deep intermediate	20,5	23,7	17,4
	Parabasal	22,5	28,5	30,2
	Basal	-	-	-

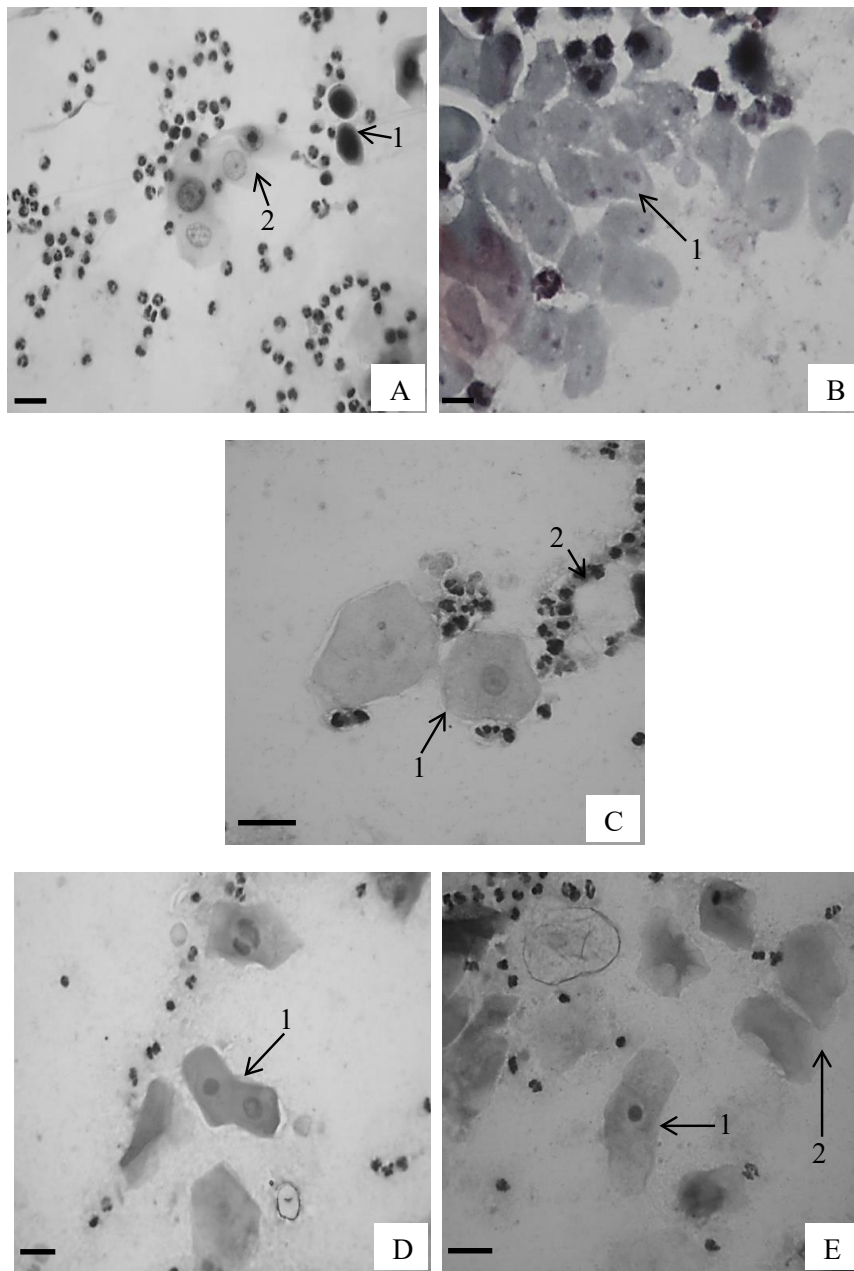


Figure 4. Cellular types observed in colposcycological samples. A.1- basal cell, A.2- parabasal cell; B.3- deep intermediate cell; C.1- superficial intermediate cell, C.2- polymorphonuclear lymphocytes; D.1- superficial cells with vesicular nucleus; E.1- superficial cell with pyknotic nucleus, E.2- superficial cells without nucleus. Shorr's stain. Bar: 10 μ m.

DISCUSSION

Results of the present study indicate that the estrous cycle of *M. c. bonariensis* is characterized by its long duration. The prolonged duration of the estrous cycles of the coypu is common in the members of the Suborder *Hystriognathi*^{9,11,13}. In the cavies, such as *Cavia porcellus*, the cycle lasts 16.5 days¹ and in *Cavia aperea* 20.6 days. In *Galea musteloides*, it lasts 22.3 days²³, in *Chinchilla laniger*, 38.1 days^{18, 20} and in *Lagostomus maximus*, 45 days^{21, 22}. Conversely, in the rodents of the Suborder *Miomorpha*, estrous cycles are relatively short. For example, it lasts 5 days in the mouse, 4 to 6 days in the Mongolian gerbil (*Meriones unguiculatus*)², 5 to 6 days in the rat²² and from 8 to 9 days in *Oryzomys y Sigmodon*. In rodents of the Suborder *Sciurognathi*, such as *Cricetomys gambianus*, the mean duration of the cycle is 7.9 days, ranging from 3 to 15 days¹⁰, being more prolonged in *Pectinator spekei* (22.7 days) and in *Ctenodactylus gundi* (23 to 25 days)¹¹.

The composition of the vaginal smears from the coypu showed a typical sequence of rodents. The successive dominance of leukocytes, nucleated epithelial cells and cornified cells has been described in murine rodents such as the hamster¹⁵, mouse¹⁵, gerbil², rat¹⁹ and *Calomys callosus*¹⁶, and in a hystriognath such as the guinea pig^{9,23}, *Cavia aperea*, *Galea musteloides*¹⁷, and *Atherurus africanus*¹¹. These changes are directly associated with the modifications in the vaginal epithelium, for example the variations in the type of superficial cells, mitosis and apoptosis indexes, the keratinization, the increase of the thickness and the number of cellular layers and the leukocytic infiltration¹⁶.

Besides, the qualitative and quantitative changes observed during the estrous cycle in the coypu are similar to those described for other rodent species. Weir^{20, 21} reported that, in *Dasyprocta agouti* and *Myoprocta pratti*, morphological variations in the thickness of the vaginal epithelium were coincident with the reproductive status, taking place a great desquamation during the estrous. Mayor *et al.*¹¹ reported that in *Atherurus africanus* the vaginal content and the epithelium vary in accordance with the reproductive status. In diestrous, females of this species showed no cornification and less

than 5 cellular layers. During the follicular phase, there was an increase of the stratification and cornification of the epithelium and the vaginal content presented abundant eosinophilic cells. Similar observations were realized in the wild black agouti (*Dasyprocta fuliginosa*)¹².

The vaginal epithelium is sensitive to the effect of the sexual steroids, mainly estrogens, and presents predictable changes throughout the estrous cycle in response to the changes in plasma concentrations of ovarian hormones. The increase in the estrogen levels determines the keratinization of the vaginal epithelium. This keratinization of the vaginal epithelium has been used as an indicator of the biological activity of circulating sexual steroids in many mammalian species and to determine the appropriate time for mating. Estrogens, both natural and exogenous, possess a direct effect on the vaginal epithelium²². When an increase of the circulating estrogen levels takes place, the growth of the number of cellular strata and cornification of the uppermost superficial layers are stimulated. Hence, when the estrogen levels decrease, an extensive desquamation of the vaginal epithelium occurs. These variations determine different cellular scenes during the estrous cycle.

CONCLUSION

Results of the present study demonstrate that the estrous cycle of the *M. c. bonariensis* is characterized by its long duration. The composition of the vaginal smears showed a sequence that is very similar to other rodents. Differences in the epithelial thickness observed between the different vaginal regions suggest that not all the vaginal areas respond in a similar way to the ovarian hormones. The lesser epithelial thickness in the metestrous, compared to that in the diestrous, may be interpreted as that during this last phase the follicular growth would be taking place. This could suggest that an increase of plasma estrogens is occurring in the female.

The information presented in this study show the necessity of more research studies, suggesting that more samples during the intermediate stages of the diestrous and a correlation between macroscopical observations and ovarian status could allow a better comprehension of the changes that occur during the estrous cycle in coypu.

Ethics: Females were sacrificed according to the methods established by the Animal Welfare Act of the Facultad de Ciencias Veterinarias de la Universidad Nacional del Centro de la Provincia de Buenos Aires (2002)³.

Conflict of interest: The authors declare that they have no conflict of interest.

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