

ESTROUS CYCLE COLPOCYTOLOGY IN CAPTIVE PACAS (*Agouti paca*, Linnaeus, 1766)

(COLPOCITOLOGIA DO CICLO ESTRAL DE PACAS *Agouti paca* Linnaeus,
1766 EM CATIVEIRO)

(COLPOCITOLOGÍA DEL CICLO ESTRAL DE PACAS *Agouti paca* Linnaeus,
1766 EN CAUTIVERIO)

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RESUMO

Com o objetivo de conhecer alguns aspectos reprodutivos da espécie *Agouti paca* em cativeiro, foram analisadas as células vaginais de seis fêmeas adultas, não-prenhes, submetidas à colpocitologia esfoliativa. Os animais foram mantidos no criatório do Centro de Estudos e Pesquisas com Animais Silvestres (CEPAS) da FCAV, Unesp, Campus de Jaboticabal. O estudo do ciclo estral baseou-se na identificação e contagem das células que ocorrem no epitélio da parede vaginal, desencadeadas por alterações hormonais. Foram considerados, ainda, os índices de taxa leucocitária, muco e restos celulares, sendo identificadas 4 fases do ciclo estral. A média e o desvio padrão para a duração do ciclo estral para as seis fêmeas foi de 33.4 ± 4.4 dias. As células superficiais predominaram na fase de estro, as células intermediárias e parabasais foram identificadas nas fases de proestro, metaestro e anestro. O metaestro foi caracterizado por grande número de células intermediárias e parabasais, assim como células de metaestro e *foam*, além de leucócitos. No anestro houve prevalência de células parabasais e um pequeno número de células intermediárias, de metaestro, e leucócitos. Pela colpocitologia esfoliativa foi possível detectar as alterações epiteliais e as células presentes durante as fases do ciclo estral, comprovando que este método permite a identificação do estro e a duração do ciclo estral da paca em cativeiro.

PALAVRAS-CHAVE: paca, colpocitologia, ciclo estral, cativeiro

SUMMARY

To study the reproductive aspects of captive *Agouti paca*, we analyzed the vaginal cells of six non-pregnant, mature pacas submitted to exfoliative colpocytology. The pacas were held in captivity at the Center for Studies and Research on Wild Animals (CEPAS) of the College of Agricultural and Veterinarian Sciences (FCAV), São Paulo State University (Unesp), Campus of Jaboticabal, Brazil. The study of estrous cycle was based on the identification and counting of the vaginal wall epithelial cells, as well as the leukocyte, mucus, and debris indexes. Four phases of the estrous cycle were identified. The mean and standard deviation for the estrous cycle duration was 33.4 ± 4.4 days. Superficial cells were predominant in the estrous phase, whereas intermediate cells and parabasal cells were identified in the proestrus, metestrus and anestrus. Metestrus was characterized by a great number of intermediate and parabasal cells, metestrus cells and foam cells, besides leucocytes. During anestrus, there was a prevalence of parabasal cells, debris and a small number of intermediate cells, metestrus cells and leucocytes. The vaginal exfoliative colpocytology allowed identifying the epithelial cells and detecting cellular changes that occur during the estrous cycle. It proved to be useful in the

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identification of estrous and the mean duration of the estrous cycle in captive pacas.

KEY - WORDS: Paca. Colpocytology. Estrous cycle. Captivity.

RESUMEN

Con el objetivo de conocer algunos aspectos reproductivos de la especie *Agouti paca* mantenida en cautiverio, fueron analizadas las células vaginales de seis hembras adultas, no gestantes, sometidas a colpocitología exfoliativa. Los animales fueron mantenidos en el Centro de Estudios e Investigaciones con Animales Silvestres (CEPAS) de la FCAV/Unesp-Jaboticabal, SP, Brasil. El estudio del ciclo estral se basó en la identificación y conteo de las células que ocurren en el epitelio de la pared vaginal, de acuerdo con las alteraciones hormonales. Fueron considerados los índices de la tasa leucocitaria, moco y restos celulares, siendo identificadas cuatro fases del ciclo estral. La media y el desvío estándar para la duración del ciclo estral para las seis hembras fue de $33,4 \pm 4,4$ días. Las células superficiales predominaron en la fase del estro, las células intermediarias y las parabasales fueron identificadas en las fases de proestro, metaestro y anestro. El metaestro fue caracterizado por el gran número de células intermediarias y parabasales, así como células de metaestro y *foam*, además de leucocitos. En el anestro hubo prevalencia de células parabasales y un pequeño número de células intermediarias, de metaestro y leucocitos. Por medio de la colpocitología exfoliativa fue posible detectar las alteraciones epiteliales y las células presentes durante las fases del ciclo estral, comprobando que este método permite la identificación del estro y la determinación de la duración del ciclo estral de la paca en cautiverio.

PALABRAS-CLAVE: Paca. Colpocitología. Ciclo estral. Cautiverio.

INTRODUCTION

Vaginal colpocytology in animal species, is used in the breeding handling, mainly for Carnivora, being specially useful in the identification of the estrous cycle phases and in diagnosing infertility problems such as abortion, vaginitis, vagina tumors, acute pyometra and metritis (FELDMAN e NELSON, 1996). For Stenson (1988), the use of vaginal smears is one of the simplest and most reliable methods for determining oestrus in live mammals. According to Simmons (1970), the phases of the estrous cycle can be determined based on the changes in the epithelial cells which occur in the vagina. Under estrogen influence, during the proestrus and estrus, the vaginal epithelium cells begin to proliferate, differentiate and exfoliate. As a result, the number of cell layers increases and the predominant cell type on the surface changes rapidly from basal and parabasal to intermediate and then to a superficial cell type (POST, 1985). The nomenclature of vaginal cells is based on cell morphology (FELDMAN e NELSON, 1996).

The *Agouti paca* belongs to the suborder of hystricomorphs (WOOD, 1955; EISENBERG, 1989) and to the family of Agoutidae (EISENBERG, 1989). Kleiman et al. (1979) and Fowler (1986) mention the caviomorphs as a group possessing reproductive characteristics, including long and variable estrous cycles, long gestations and the presence of a vaginal closure membrane. Due to its meat having excellent flavor, the paca is among the most hunted mammals in the neotropical America (Von IHERING, 2002,

GRZIMEK, 1975, KLEIMAN et al., 1979, SANTOS, 1984, DEUTSCH e PUGLIA, 1988, REDFORD, 1992, OCANA et al., 1997). The study of the biology and the development of handling techniques for the species in captivity contribute for the species preservation. The main purpose of this study was to determine the estrous cycle of the species in captivity based on the exfoliative cytology, considering that the reproduction studies are important aspects for the paca breeding either in captivity or in the wild.

MATERIAL AND METHODS

The material was collected from animals in captivity at the Center for Studies and Research on Wild Animals (CEPAS), and the staining and reading of the slides on exfoliative colpocytology was made at the Laboratory of the Animal Reproduction Department, both located at the Faculdade de Ciências Agrárias e Veterinárias (FCAV), Unesp, Jaboticabal *Campus*, São Paulo.

Six captive mature females, with age between three and six years, non-pregnant, kept isolated in captivity during the experimental period, were studied. The vaginal smears were collected along 48-hour intervals, totalizing 57 days. The vaginal smears were collected with sterile swabs (Steri-gamma-swab-I, IBRAS-CBO Indústrias Cirúrgicas e Ópticas S. A., Campinas, SP). After the females were held with a net, the swab was immersed in a physiological solution and after the perineal area cleaning,

it was carefully inserted in the vagina and moved from right to left. The collected material on the handle was smeared onto two slides for microscopy forming, each one of them, three parallel lines. The vaginal smears were fixed with a solution of alcohol-ether (1:1) until the collection was finished, and then stained by use of the Harris-Schorr technique, according to Mialot (1988). The material was examined under light microscopy, with 10x ocular and 40x lens (Olympus Inverted Research Microscope -Model IMT). From each slide examined 100 cells were counted and, later, the slides were kept for photographic documentation (Olympus Photomicrographic System - Model PM-10 AD). The vaginal epithelial cells were classified according to Feldman e Nelson (1996). The presence or absence of leucocytes and debris in the smears were observed as an aid in the recognition of the estrous cycle phases.

RESULTS

Simply keeping the animal held by a net was satisfactory for the smear collection. The females were wild at first however and as the work progressed they became more and more docile and easy to handle.

The estrous cycle phases and the cell types occurring in the vaginal epithelial layers observed during the estrous cycle could be confirmed by exfoliative vaginal cytology. The epithelial cells observed in the smears were: superficial, intermediate, parabasal, foam cells and metestrus cells (Figure 1 to 4). It was observed individual variation about cells number in each phase of estrous cycle. During early proestrus, there was a large amount of intermediate and parabasal cells, a lower number of small superficial cells and the presence of leucocytes (Figure 1). As proestrus progressed, superficial cells became more cornified and increased in number, the number of intermediate and parabasal cells decreased as well as leucocytes. During estrus, nucleated superficial cells occurrence was almost total, normally at the end of this phase, changed into cell aggregates and non-nucleated superficial cells. Vaginal smears collected at this phase were clean and easy to visualize (Figure 2). Metaestrus is characterized by a sudden onset of a great number of intermediate and parabasal cells, besides leucocytes and a reduction in the number of superficial cells. The presence of great number of metestrus cells and foam cells were observed in this phase (Figure 3). At anestrus there was a prevalence of parabasal cells and debris and the presence of a small number of intermediate cells, metestrus cells and leucocytes. The background in the smear slides during this phase presented a lot of debris and cells in degenerative process (Figure 4). The presence of erythrocytes in the paca vaginal smears has not been noticed in any phase of the estrous cycle. The changes

from proestrus to estrus and then to metestrus were identified, respectively, by a typical increase in the superficial cells, as well as the sudden increase in leucocytes and the onset of a great number of intermediate, parabasal, metestrus and foam cells together with mucus. Determination of the duration of the estrous cycle was based on the cornification of the vaginal epithelial cells, that is, on the higher index of superficial cells. Therefore, the mean obtained for the duration of the estrous cycle, period beginning at the day of the estrus (high number of superficial cells) in a cycle until the day before the next estrus was of 33.4 ± 4.4 days for the six studied females. Estrus can be identified by the higher number of superficial cells which is a characteristic of this phase (Figure 2). The presence of vaginal smears dominated by cornified cells appears to be a good indicator of oestrus in the pacas. It has been observed, on the first vaginal smear collection day, that some of the females had the vaginal membrane perforated, combining with the results of the slides (estrous phase), and other females had the vaginal membrane enclosed.

DISCUSSION

This study has identified four phases in the paca's estrous cycle. Guimarães et al. (2003) observed four phases of the estrous cycle in pacas and have identified basal cells, but not leucocytes in the estrous smears. Matamoros e Pashov (1984) encountered three phases for the same species: proestrus, estrus and post-estrus, identifying the epithelial cells as parabasal, intermediate and superficial cells. These authors have identified the phase after estrus as post-estrus. The five estrous cycle patterns found by the authors have not been observed in this study. However, cell types encountered for the paca by Guimarães et al. (2003) in proestrus, estrus, metestrus e anestrus, were similar to the ones found in this study. Changes in the exfoliative cells in the vaginal epithelium during the estrous cycle found in this study are very close to the ones mentioned by Barbella (1982) for *Hydrochaeris hydrochaeris*, by Matamoros e Pashov (1984) and Guimarães et al. (2003) for *Agouti paca*, by Post (1985) and Feldman e Nelson (1996) for dogs, by Stenson (1988) for mustelids, by Guimarães (1993) for *Dasyprocta* spp. The high erythrocytic index observed during proestrus in canids by Feldman & Nelson (1996) was not observed in the pacas. According to POST (1985), erythrocytes may be present or not during estrus in dogs. The exact difference between the beginning and the end of metestrus, anestrus and proestrus could not be identified. Probably if the vaginal smears had been taken at shorter intervals with a greater number of animals, the passage from one phase to the other would have been clearly noticed. This would enable a more detailed observation in some aspects of the

Table 1 - Vaginal Exfoliated Cytology and blood cell indexes, stain and debris present at the vaginal smears during the estrous cycle phases of six pacas *Agouti paca* Linnaeus 1766. Jaboticabal, SP, Brazil.

Cell types (epithelial cells) and indexes	Estrous Cycle Phases			
	ANESTRUS	PROESTRUS	ESTRUS	METESTRUS
SUPERFICIAL	---	+	+++	+
INTERMEDIATE	++	+++	+	+++
PARABASAL	+++	++	---	++
METESTRUM	+	---	---	++
FOAM CELLS	---	---	---	++
Leucocytes	++	+	---	+++
Debris	++	+	---	++
Stain	Basophilic	Acidophilic and basophilic	Acidophilic	Basophilic and Acidophilic

--- Insignificant or absent, + low (up to 20%), ++ moderate (up to 50%), +++ abundant (> 50%)

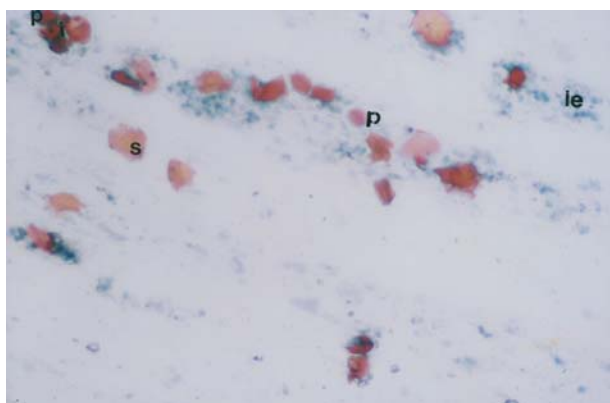


Figure 1 - Photomicrograph of the paca exfoliative cytology characterizing proestrus (137 X). Harris-Schorr stain. Intermediate cell (i), parabasal cell (p), superficial cell (s), leucocyte (le).

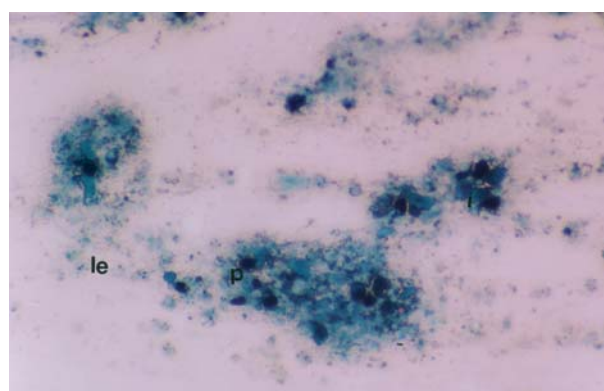


Figure 3 - Photomicrograph of the paca exfoliative cytology characterizing metestrus (275 X). Harris-Schorr stain. Intermediate cell (i), parabasal cell (p), superficial cell (s), leucocyte (le), metestrum cell (me), foam cell (f).

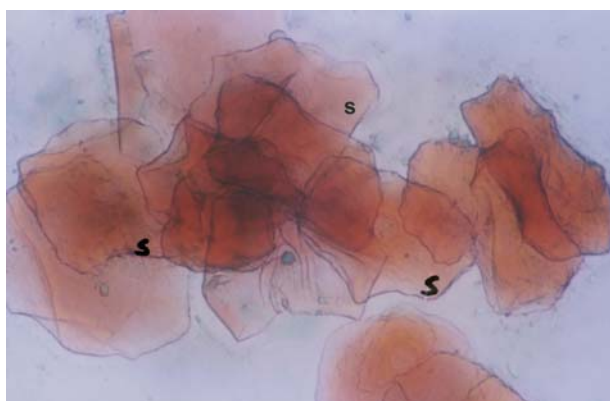


Figure 2 - Photomicrograph of the paca exfoliative cytology characterizing estrus (275 X). Superficial cell (s).

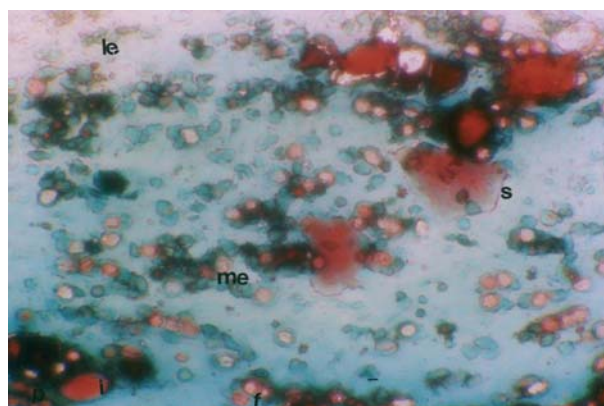


Figure 4 - Photomicrograph of the paca exfoliative cytology characterizing anestrus (137 X). Harris-Schorr Stain. Intermediate cell (i), parabasal cell (p), leucocyte (le).

paca's estrous cycle such as the duration of estrus, of the other cycle phases, estrus and postpartum estrus. This information is important to reproductive knowledge. For Nelson & Feldman (1996), vaginal cytology is usually used in helping an owner to determine the proper time to breed a bitch and is an excellent aid in distinguishing between proestrus, estrus and diestrus, but vaginal cytology cannot be used for pregnancy diagnosis or identify the day of ovulation or fertilization.

The result obtained for the mean estrous cycle is similar to the one mentioned by Guimarães et al. (2003) in Belém, Brazil (32.5 ± 3.69 days) and Matamoros e Pashov (1984) for pacas in Costa Rica (31.16 days) and it matches the figures obtained for the caviomorphs mentioned by Kleiman et al. (1980), except that in order to obtain the mean for the estrous cycle, Matamoros e Pashov (1984) considered the perforation of the vaginal membrane from one cycle to the next and not based on the vaginal exfoliative. These authors considered the duration of the estrus cycle as the period between the first day of a cycle and the day before the following cycle.

According to Kleiman et al. (1979) and Rowlands & Weir (1984) there is a clear indication that all caviomorphs, except *Myocastor coypus*, have a vaginal closure membrane. For Rowlands e Weir (1984) and Fowler (1986) this membrane is formed due to the hyperplasia of epithelial cells, which becomes perforated only at estrus and parturition. Matamoros (1981) refers to the absence of the vaginal membrane during inspection of the external genitals of hystricomorphs, as one of the means to determine estrus in these animals. Smythe (1991) and Smythe e Guanti (1995) reported that the vaginal membrane opening is not necessarily related to the female's reproductive state, since it varies from one female to another. The authors have observed a slight opening in the vagina of pregnant pacas in some occasions. In this work, the vaginal membrane remained open during the entire collection period, probably because the vaginal smears were taken every 48 hours. It was noticed, however, that after three days without taking vaginal smears, the membrane reappeared. Therefore, the absence of vaginal membrane may be observed during estrous and birthing, but it should not be the only tool for diagnosing estrous in pacas. For the campesino, it may be an ally along with the behavioral observation of animals, mainly the male that search restlessly for the female during estrous.

This study has confirmed by observations of exfoliative vaginal cytology that the paca has spontaneous ovulation, as reported by Matamoros (1981) and Collet (1981), and presents a postpartum estrus and anestrus by lactation as observed by Matamoros (1982); Nogueira (1997) and Guimarães et al. (2003).

CONCLUSIONS

The results obtained lead to the following conclusions:

1. By colpocytological changes analysis during estrous cycle and the cell types present can be detected, proving that the exfoliated colpocytology technique is efficient and enables identification of estrus in the species.
2. The staining method used was adequate to distinguish the epithelial cell morphology and was easy to handle at low cost.
3. The 33.4 ± 4.4 day-mean obtained for the estrous cycle duration for pacas is similar to the one mentioned by other authors and is among the average figure reported for the caviomorphs.
4. More detailed studies on the paca's estrous cycle and reproductive behavior will be essential for the understanding of the species biology and will contribute to the success in preserving and breeding to this animal.

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