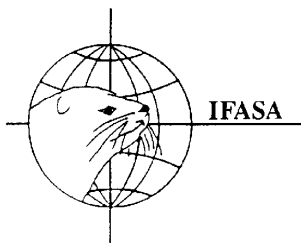
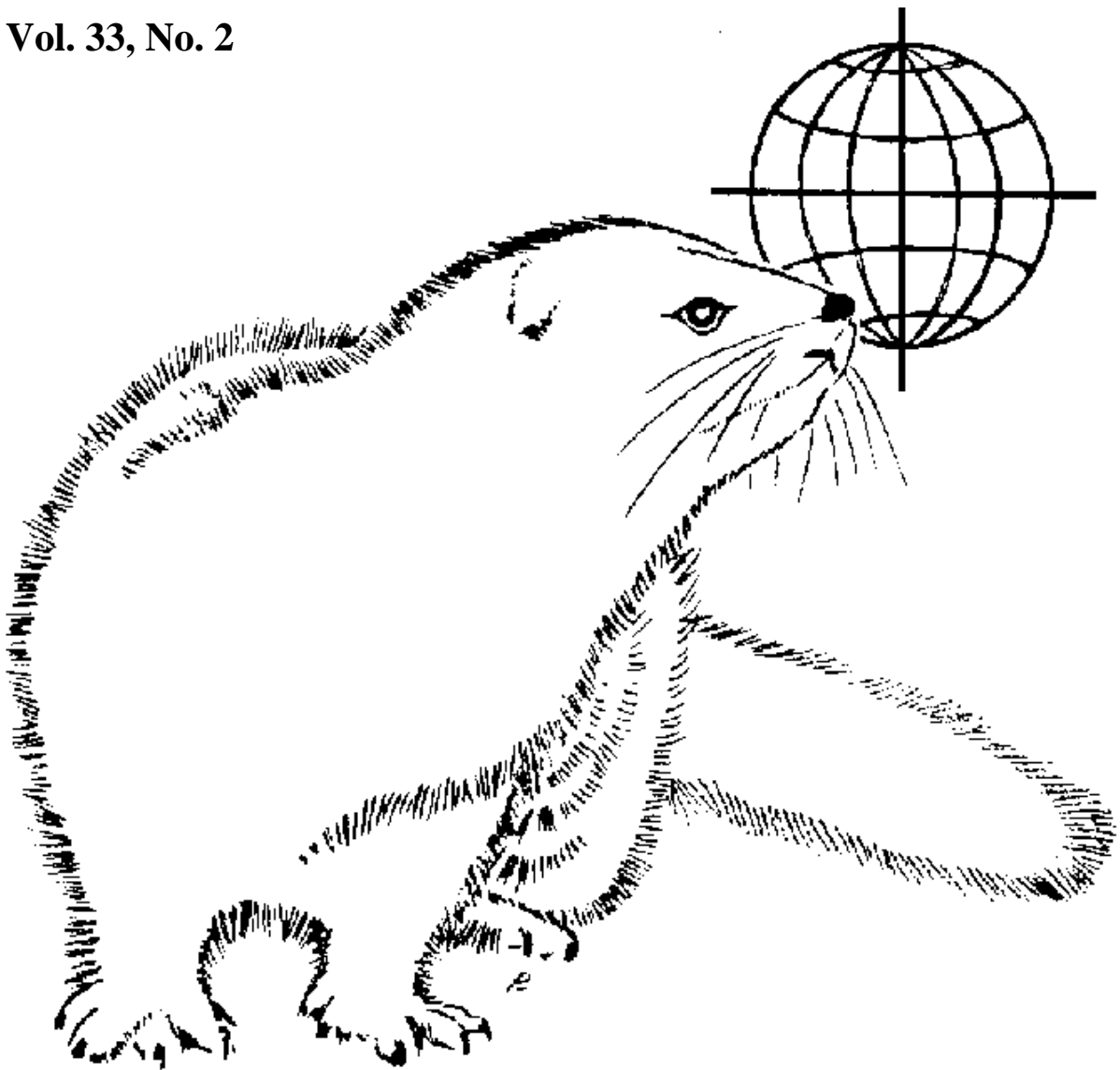


SCIENTIFUR

SCIENTIFIC INFORMATION IN FUR ANIMAL PRODUCTION

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Characterization of cellular types during the estrous cycle of the *Myocastor Coypos* (COYPU)

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Abstract

The aim of the present study was to characterize qualitatively and quantitatively the cellular types observed in colpocytological samples and in the vaginal epithelia of coypu. Colpocytological samples were collected daily and stained with Harris's hematoxylin and Shorr's stain. In order to study the vaginal epithelial cells, samples from the vaginal wall were obtained. Later semi-serial cuts were made which were later stained with haematoxylin/eosin. Eight cellular types were differentiated in the smears: basal, parabasal, deep intermediate, superficial intermediate, superficial with vesicular nucleus, superficial with pyknotic nucleus, superficial without nucleus and polymorphonuclear lymphocytes. When the vaginal epithelium of the coypu was evaluated, three cellular layers were observed: deep layer, intermediate layer, and superficial layer.

Keywords: *coypu*, *colpocytology*, *Myocastor coypus*, *vaginal cells*.

Introduction

Coypu (*M. coypus*), mink and fox are the most important species in the international fur industry. In addition, coypu together with *Agouti paca* (*paca*), *Dasyprocta aguti* (*agouti*) and *Hydrochaeris hydrochaeris* (*capibara*) are considered one of the greatest species of economical relevance. Moreover, during the last decade, this group of rodents has become an important topic in international plans for

promoting sustainable development (Jori, 2001; Hardouin *et al.*, 2003).

The cyclic changes of the reproductive system during the estrous cycle have been reported in several fur species (Boue *et al.*, 2000; Finley, 1979; Guimaraes *et al.*, 1997; López Barbella, 1982; Mones & Ojasti, 1986; Stenson, 1988; Valdespino *et al.*, 2001; Yamaguchi *et al.*, 2006). Rodents of the Suborder *Miomorpha* show short estrous cycles (Snell, 1964) while animals belonging to the Suborder *Hystricomorpha*, as *M. coypus* present longer estrous cycles (Weir, 1974). Later studies have reported that the average duration of the estrous cycle in the coypu was 35.5 ± 10.8 days with a range of 20 to 60 days, nevertheless, a great variability in the duration of the estrous cycles among different animals as well as in the same animal was observed (Felipe *et al.*, 2001). Different cell types have been already recognised in the coypu vaginal smears (Felipe *et al.*, 2001) however, to our knowledge there are not a morphological description of them. Thus, the aim of the present study was to characterize qualitatively and quantitatively the cellular types observed in colpocytological samples and in the vaginal epithelia of coypu obtained during the estrous cycle.

Materials and Methods

Animals and housing conditions

18 virgin females of coypu aged 6.2 ± 0.5 months and weighting 5.7 ± 0.3 kg were used. Females were

kept under breeding conditions, in a partially roofed corral and males were housed individually in contiguous pens. Animals received food and water *ad-libitum*.

Collection of colpocytological samples

Colpocytological samples were collected daily between 11:00 and 12:00 a.m. during 6 months. Individual disposable Pasteur pipets of 151 mm with 2 mm of diameter, loaded with 0.2 ml of physiological solution (0.9 % sodium chloride) were used for each animal. Pipets were introduced 5 cm deep into the vaginal duct in order to take correctly the samples.

Analysis of the colpocytological samples

Samples were firstly analyzed fresh, registering the microscopic aspect of the vaginal cells, afterwards, staining was made with routine techniques (Harris's hematoxylin and Shorr's stain). Stages of the estrous cycle were defined according with Felipe *et al.* (2001). Smears were analyzed to determinate the stages of the estrous cycle and to make a cytometric evaluation of the different cell types.

Collection of vaginal samples

Samples from the vaginal wall were obtained from animals belonging to different breeders. Samples

were taken between the bladder and cervix and immediately fixed in Bouin's in order to study the vaginal epithelial cells. Routine techniques were used to process the samples until embedding in paraffin wax. Semi-serial 5 mm-thick cuts were made and stained with haematoxylin/eosin. Each cellular type in the epithelial layers was determined starting from the mean of two diameters at a right angle. A micrometric ocular incorporated to an Olympus CHL was used to determine the mean sizes of each cellular type and their nucleus. Measurements were obtained at a magnification of 1000x and were expressed as the mean \pm SD.

Results

a. - Cells in colpocytological samples

Eight cellular types were clearly differentiated in the smears: basal (Fig. 1 A), parabasal (Fig. 1 B), deep intermediate (Fig. 1 C), superficial intermediate (Fig. 1 D), superficial with vesicular nucleus (Fig. 1 E), superficial with pyknotic nucleus (Fig. 1 F), superficial without nucleus (Fig. 1 G) and polymorphonuclear lymphocytes (Fig. 1 H). Basal and parabasal cells could be observed isolated or in small groups. The characteristics of each cellular type are showed in Table 1.

Table 1: Characteristics of the cellular types in the vaginal smears of *M. coypus*.

Cellular types	Shape	Mean diameter (μm)	Harris/Shorr's stain	Nuclei	
				Shape	Mean diameter (μm)
Basal	Round	12.72 \pm 2.15	bluish red	Ovoid	6.06 \pm 2.82
Parabasal	Ovoid	14.94 \pm 3.76	bluish red	Spherical or ovoid	7.12 \pm 2.76
Deep intermediate	Ovoid	21.87 \pm 7.94	bluish or greenish	Vesicular	11.27 \pm 3.26
Superficial intermediate	Polygonal, flatened	23.93 \pm 5.65	red or bluish green (1).	Vesicular	9.36 \pm 2.17
Superficial with vesicular nucleus	Polygonal, squamous	32.55 \pm 8.16	red or sky-blue (1).	Vesicular, ovoid	5.95 \pm 2.64
Superficial with pyknotic nucleus	Polygonal, squamous	42.57 \pm 5.73	red	Round, dotted and hyperchromatic	4.17 \pm 1.17
Superficial without nucleus	Polygonal, squamous	39.14 \pm 11.24	red	-	-

(1) In some cases with both colours in the cytoplasm.

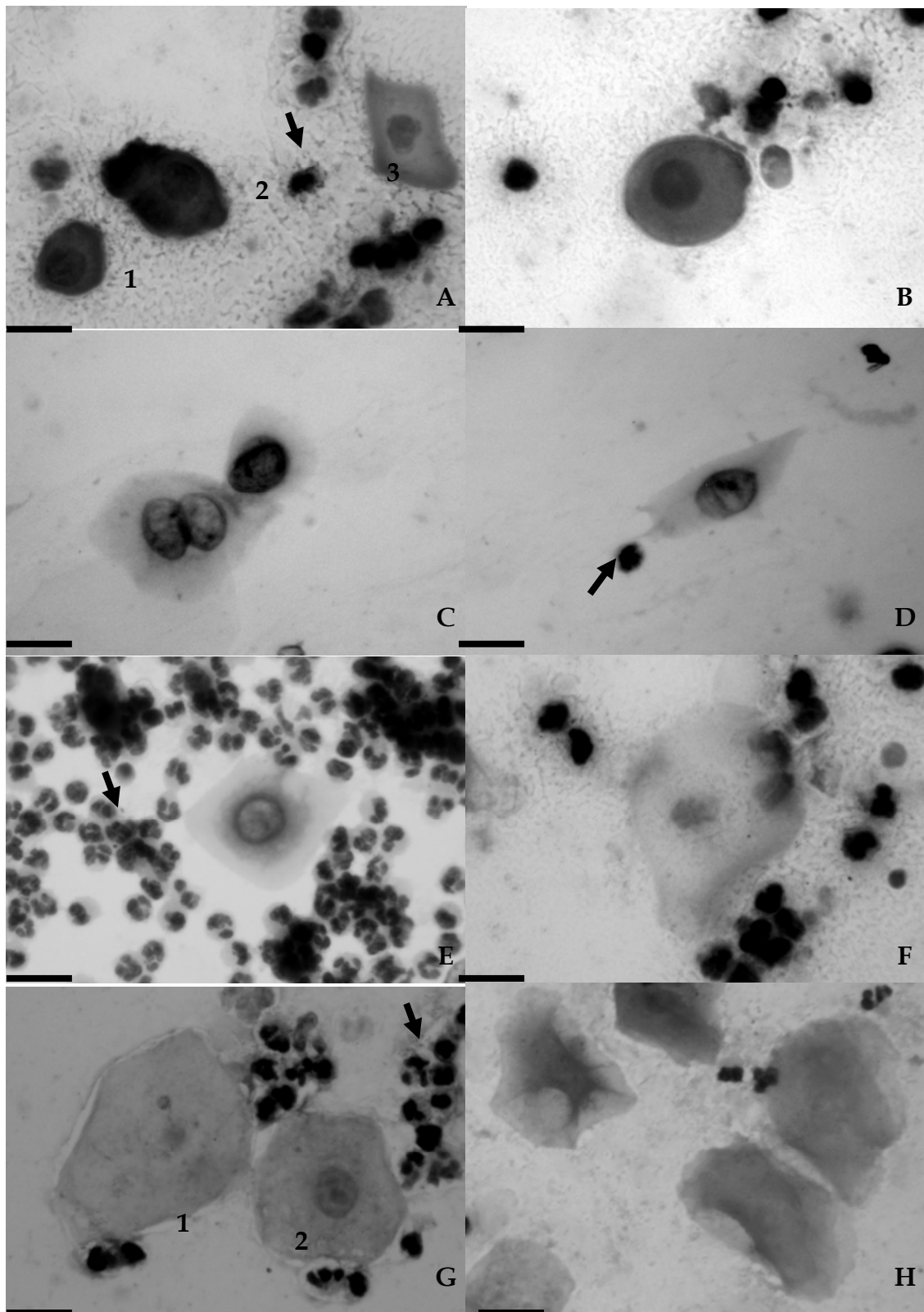


Figure 1: Cellular types observed in colposcycological samples: A.1- basal cell, A.2- parabasal cell, A.3- deep intermediate cell; B- parabasal cell; C- deep intermediate cells; D- superficial intermediate cell; E- superficial intermediate cell with keratinization around the nucleus; F- y G.2- superficial cells with vesicular nucleus, G.1- superficial cell with pyknotic nucleus, H- superficial cells without nucleus. Polymorphonuclear lymphocytes are indicated by arrows. 40X, Shorr's stain. Bar: 10 μ m.

b. - Cells in the vaginal epithelia

The following description of cellular layers was observed in the vaginal epithelium of the coypu:

1. Deep layer: it was possible to distinguish two cellular strata

a) The stratum of basal cells that was located along the basal membrane and was composed of a row of prismatic cells. These cells have a high nuclear to cytoplasmic ratio, a big ovoid or round nucleus (mean height = $7.6 \pm 1.4 \mu\text{m}$ and mean width = $3.8 \pm 1.1 \mu\text{m}$) perpendicular to the surface of the epithelium, and a small amount of basophilic cytoplasm. The mean height of the cells was $14.8 \pm 1.9 \mu\text{m}$ and the mean width was $4.8 \pm 0.6 \mu\text{m}$.

b) The stratum of parabasal cells was observed to be formed by two to three rows of ovoid cells, with round and vesicular nucleus (mean height = $8.2 \pm 0.5 \mu\text{m}$ and mean width = $5.9 \pm 0.7 \mu\text{m}$) and basophilic cytoplasm. Mean cells height was $14.3 \pm 1.8 \mu\text{m}$ and the mean width was $12.4 \pm 1.2 \mu\text{m}$.

2 - Intermediate layer: it was formed by 5 to 9 cellular rows and it was also possible to distinguish two cellular strata.

a) Stratum of deep intermediate cells or transitional intermediate cells: The cells of this stratum were ovoid and located close to the deep layer, containing rounded and smaller nuclei in central position (mean height = $10.7 \pm 1.1 \mu\text{m}$ and mean width = $5.2 \pm 0.5 \mu\text{m}$). The mean height of these cells was $7.1 \pm 0.7 \mu\text{m}$ and the mean width was $15.9 \pm 2.4 \mu\text{m}$.

b) Stratum of superficial intermediate cells that was conformed by rows of cells close to the superficial layer of the epithelium. The cells were slightly flattened, with a mean height of $6.1 \pm 0.8 \mu\text{m}$ and a mean width of $14.5 \pm 1.7 \mu\text{m}$. Cells nuclei had ovoid shape and prepyknotic aspect (mean height = $4.6 \pm 0.5 \mu\text{m}$ and mean width = $9.5 \pm 0.5 \mu\text{m}$).

3 - Superficial layer: five to eight rows of flattened cells were observed in this layer. Cells close to the intermediate layer had small, round, flattened or pyknotic nuclei, with punctuate and hyperchromatic aspect while nuclei could not be observed in the cells of the rows close to the surface. The mean cell height was $1.9 \pm 0.8 \mu\text{m}$ and the mean cell width was of $21.3 \pm 2.1 \mu\text{m}$.

Discussion

Vaginal cytology was successfully used to monitor the reproductive status of females in several species (Bekyurek *et al.*, 2002; Mayora *et al.*, 2003; Torres Rodrigues & Vieira Ferro, 1998). Cell types found in vaginal smears of coypu were classified as basal, parabasal, deep intermediate, superficial intermediate, superficial with vesicular nucleus, superficial with pyknotic nucleus, superficial without nucleus and polymorphonuclear lymphocytes according to their morphological characteristics. The types of exfoliated vaginal cells observed in the coypu were similar to other domestic (Ola *et al.*, 2006; Rodgers *et al.*, 1993; Zourgui *et al.*, 1976) and laboratory species (Barfield & Beeman, 1968). Intermediate cells were classified as small intermediate cells and large intermediate cells according with cells sizes reported in the chinchilla (*Chinchilla lanigera*) while superficial cells were divided in partly and completely cornified cells (Bekyurek *et al.* 2002). These latter cells resemble to the superficial cells with pyknotic nucleus cells and superficial without nucleus cells observed, in the present study, in coypu. Conversely to the chinchilla, smears globet cells, metoestrus cells and foam cells were not found in the coypu. Goblet cells were observed in the smears of chinchillas suggesting the presence of mucinous transformation of the superficial two or three layers, which is a known feature of the guinea pig (*Cavia porcellus*) (Deanesly, 1966), capivara (*Hydrochoerus hydrochaeris*) (Niño & García, 1999) and other rodents oestrous cycle (Vrcic *et al.*, 1991). Meanwhile, foam cells are in relation with the small intermediate cells or parabasal cells with multiple, clear cytoplasmic vacuoles. And metoestrus cells were small intermediate cells or parabasal cells with a neutrophil in the cytoplasm also recorded in chinchillas (Bekyurek *et al.*, 2002).

References

- Bekyurek, T.; Liman, N. & Bayram, G. 2002. Diagnosis of sexual cycle by means of vaginal smear method in the chinchilla (*Chinchilla lanigera*). Lab. Anim. 36: 51-60.
- Boue, F.; Delhomme A. & Chaffaux, S. 2000. Reproductive management of silver foxes (*Vulpes vulpes*) in captivity. Theriogenology 53: 1717-1728.
- Deanesly, R. 1966. Pro-oestrus in the Guinea-pig: hormonal stimulation of vaginal epithelium. J. Reprod. Fertil. 12: 205-212.

- Felipe, A.; Callejas, S. & Cabodevila, J. 2001. Characterization of the estrous cycle of *Myocastor coypus* (coypu) by means of exfoliative colpocytology. *J. Neotrop. Mammal.* 8: 129-137.
- Finley, G.G. 1979. Basic facts on reproduction of foxes in North America. *Fur Rancher*; 59: 10.
- Guimaraes, D.A.; Moreira, D. & Vale, W.G., 1997. Determinação do ciclo reproductivo da cutia (*Dasyprocta prymnolopha*) a través do diagnóstico colpocitológico. *Acta Amazónica* 27: 55-64.
- Hardouin, J.; Thys, É.; Joiris, V. & Fielding, D. 2003. Mini-livestock breeding with indigenous species in the tropics. *Livestock Research for Rural Development* 15 (4). Retrieved May 2, 2004, <http://www.cipav.org.co/lrrd/lrrd15/4/hard154.htm>
- López Barbella, S. 1982. Determination of the estrous cycle of the capybara (*Hydrochoerus hydrochaeris*). *Acta Cient. Venez.* 33: 497-501.
- Mayora, P.; López-Béjar, M.; Jori, F.; Fenech, M. & López-Gatius, F. 2003. Reproductive functional anatomy and oestrous cycle pattern of the female brush-tailed porcupine (*Atherurus africanus*, Gray 1842) from Gabon. *Animal Reprod. Sci.* 77: 247-259.
- Mones, A. & Ojasti, J. 1986. *Hydrochoerus hydrochaeris*. *Mammalian Species* 264: 1-7.
- Niño, M.I. & García, G. 1999. Histochemical Characterization of the Mucous Secretion of the Vaginal Epithelium of the Chigüire (*Hydrochoerus hydrochaeris*). *Rev. Fac. Cs. Vets. UCV* 40(2): 111-117.
- Ola, S.I.; Sanni, W.A. & Egbunike, G. 2006. Exfoliative vaginal cytology during the oestrous cycle of West African dwarf goats. *Reprod. Nutr. Dev.* 46 (1): 87-95.
- Rodgers, J.B.; Sherwood, L.C.; Fink, B.F. & Sadove, R.C. 1993. Estrus detection by using vaginal cytologic examination in miniature swine. *Lab. Anim. Sci.* 43: 597-602.
- Snell, G.D. 1969. The early embryology of the laboratory mouse. In: *Biology of the laboratory mouse* (Snell G.D., ed.), Blakiston, Philadelphia, pp. 1-54.
- Stenson, G. B. 1988. Oestrus and the vaginal smear cycle of the river otter, *Lutra Canadensis*. *J. Reprod. Fertil.* 83: 605-610
- Torres Rodrigues, J.F. & Vieira Ferro, E.A. 1998. Morphological changes in the vaginal epithelium during the oestrous cycle of *Calomys callosus* (Rodentia, Cricetidae). *Rev. Brasil. Biol.*, 58: 527-539
- Valdespino, C.; Asa, Ch. S. & Bauman, J. E. 2001. Estrous cycles, copulation, and pregnancy in the fennec fox (*Vulpes zerda*). *J. Mammalogy* 25: 99-109
- Vrcic, H.; Horvat, B. & Damjanov, I. 1991. Lectin histochemistry of mouse vagina during the oestrous cycle. *J. Histochem. Cytochem.* 39: 1685-1692.
- Weir, B. J. 1974. Reproductive characteristics of hystricomorph rodents. *Symp. Zool. Soc. Lond.*, 34, 265-301.
- Yamaguchi, N.; Dugdale, H. L. & Macdonald, D. W. 2006. Female receptivity, embryonic diapause, and superfetation in the European badger (*Meles meles*): implications for the reproductive tactics of males and females. *Q. Rev. Biol.* 81:33-48.
- Zourgui, A.; Neophyton, G. & Elize, K. 1976. Results of vaginal cytology studies and examination of the arborisation phenomenon of the vaginal mucus for the determination of estrous cycle activity in sheep. *Arch. Exp. Veterinarmed.* 30(6):889-96.