SCIENTIFUR - scientific information in Fur Animal Production.

SCIENTIFUR scientific information for those involved in Fur Animal production, is published by the International Fur Animal Scientific Association (IFASA).

SCIENTIFUR is the contact link between fur animal researchers all over the world and serves as an outlet for scientific and other communication between researchers and others who are interested in the production of fur bearing animals. As such **SCIENTIFUR** will contain reports of scientific and applied nature as well as abstracts of information published elsewhere and information regarding congresses, scientific meetings etc.

SCIENTIFUR is published as four issues per year in the following way:

- Three issues containing short communications (max. 4 pages), abstracts, letters, book reviews etc.
- One issue containing only reviewed articles.

One year's issues (1 volume) are estimated to total 350 pages covering more than 500 titles of scientific reports.

SCIENTIFIC REVIEWED ARTICLES. Papers received for publication as Scientific Reviewed Article, will be sent to two referees for scientific approval, and will regardless of discipline appear in the issue entitled "Fur Animal Science" containing only reviewed articles.

SHORT COMMUNICATIONS. Other original papers can be published in SCIENTIFUR as short communications. In regard to such articles the author(s) alone is (are) responsible for the scientific validity of the article. Such papers must not exceed 4 printed pages.

EDITOR ADDRESS. All kinds of material suited for publication or abstracting in SCIENTIFUR have to be forwarded to the Editor:

Birthe M. Damgaard
SCIENTIFUR
P.O. Box 14
DK-8830 Tjele, Denmark

Tel: +45 89991512
Fax: +45 89991500

E-mail: Scientifur@agrsci.dk

SUBSCRIPTION 2001- : DKK 600.- per volume (year) including bank charges and postage.
Please note that members can subscribe, for personal use only, at a reduced rate.
Please apply for membership and further details at http://www.ifasanet.org or to the IFASA treasurer.

TRESURERS ADDRESS. All correspondence regarding subscription and payment should be addressed to the Treasurer:

Steen H. Møller
IFASA
P.O. Box 14,
DK-8830 Tjele, Denmark

Tel: +45 89991346
Fax: +45 89991500

E-mail: IFASA@agrsci.dk

INDEXING: Scientific Reports and Original Reports published in SCIENTIFUR are indexed in common international indexes covering data regarding animal science. All titles unless of origin which have been published in SCIENTIFUR from the very beginning, is covered in an electronic SCIENTIFUR INDEX, which is updated each year. This index can be downloaded from the Webb-site: http://www.ifasanet.org

SCIENTIFUR
ISSN 0105-2403
Vol. 25, No. 3
Reviewed Scientific Articles

Notes from the Group of Editors

This electronic version of Scientifur is the third issue of volume 25, and the first issue containing only reviewed articles. Thus, the Group of Editors has decided to issue all reviewed articles in a separate issue of Scientifur in order to make a clear distinction between reviewed articles and other scientific information. We plan to publish one issue per year with only reviewed articles, however, this number may increase as we receive more scientific articles. We hope that our readers will approve of this change.

The fourth issue of volume 25 will be published as an electronic version in the near future, and immediately after the third and the fourth issue will also be published as a paper version.

All the people involved in the publishing of the journal will make every effort to ensure that all the issues of volume 26 will be published in 2002.

On behalf of the
Group of Editors

Birthe Damgaard

Changes in the leucocyte alkaline phosphatase during the breeding period in female mink

L.B. Uzenbayeva, V.A. Ilukha

Institute of Biology, Karelian Research Centre, Russian Academy of Sciencies, Petrozavodsk, Pushkinskaya 11, Russia

E-mail : ILYUHA@MAZE.CENTRE.KARELIA.RU

Abstract
Activity of alkaline phosphatase (AP) in the leucocytes of standard mink females periferal blood during the reproduction seasons (mating period, pregnancy, lactation and post-lactation) was studied using cytochemical methods. The data about activity and localization of the enzyme in leucocytes and specificity of cytochemical reaction on AP in minks are submitted in comparison with other animal species. The changes of investigated parameters in dependence on phase of reproduction cycle are shown. The causes of changes and role of the hormonal status in regulation of cellular metabolism are discussed.

Keywords: blood leucocytes, leucocyte alkaline phosphatase, mink, reproduction

Introduction

Alkaline phosphatase (AP), classified according to the international enzyme as hydrolases (EC 3.1.3.1) plays a crucial role in cell metabolism. This is evidenced by the data on its presence in tissues with active transport – kidney canaliculus epithelium, intestine epithelium cells, osteocytes and osteoblasts (Metsler, 1980).

Leucocyte alcaline phosphatase (LAP), for some animals at least, is the cytochemical marker of secondary leucocyte granules, since the results of electronic-cytochemical and biochemical analyses showed it to be present in the secondary or the so-called specific granules.

Even compared to well-known hematological parameters (erythrocyte sedimentation rate, leucocytes, leucogram) LAP is the most sensitive test for changes in homeostasis (Shubich & Nagoev 1980). We found LAP activity to be a highly informative parameter for controlling the state of fur animal organism in healthy condition, with pathologies and under biologically active substancies effect. Thus, a marked reduction of phosphatase activity with simultaneous redistribution of cell elements in the leucogram was observed in mink kits showing nanism features (Uzenbaeva & Tyutyunik, 1994, 1995).

By present, extensive experimental and clinical material on LAP cytochemistry has been gathered, but still little is known of the role of the enzyme, except for its anti-microbial functions, and the mechanisms of controlling its activity in the organisms. The aim of the present paper was to study LAP activity in different phases of the breeding period in standard female mink.

Materials and methods

The standard female mink breading on the "Kondopogskii zverovod" Ltd. farm were used as the experimental animals. The investigations were made in the reproduction periods in 1988-1997. The parameters of leucopoesis (leucocytes, leucoformula) and leucocyte alkaline phosphatase were measured in 114 females (48 in the mating period, 31 pregnant female, 18 in the lactation and 17 in the post-lactation period).

Blood was sampled from the tail. A speciment for microscopic study prepared by spreading the blood across the glass slide without anticoagulant. Cytochemical analyses were made immediately after blood sampling. For leucocyte counting a standard routine light microscopic method was used (Berestov, 1971). For cytochemical determination of LAP the method of asocoupling reaction was used (Burstone, 1962). Naphthol AS phosphate as substrate and fast blue BB or fast garnet GBC as diazonium salt were used. The percentage content of phosphatase positive segmentonuclear leucocytes was estimated. The degree of intensity of coloring also was took into account. Leucocytes without enzymes were named as zero type. Coloured cells were separated into four types: with low (+), moderate (++), high (+++) and very high (++++) degree of activity.

Results and discussion.

Research demonstrated that cytochemical response to LAP in mink could only be found in part of leucocytes in peripheral blood samples. Comparison with data by other authors showed that mink, like polar fox, occupied a specific position, differing both from the animals with a very high level of phosphatase activity in leucocytes (rabbits, rats, guinea pigs), and from the species in which it had practically no cytochemical manifestation (dogs, cats, white mice). Because of considerable distinctions between species no model for the study of LAP modifications in humans could be found among the 18 animal species listed by F. G. J. Hayhoe et al. (1964).

LAP in mink peripheral blood is localized in neutrophilic segmented leucocytes (fig. 1 A, B). The used staining technique did not give a chance to find out which polynuclears - neutrophilic or eosinophilic - are phosphatase-positive. LAP activity in individual neutrophilic segmented leucocytes ranged from very low to very high. In some samples however leucocytes showing positive response could not be found in spite of an increased number of cells analysed.

As a rule mink lymphocytes do not contain LAP. It was only in preparations from some animals that single LAP-positive lymphocytes were observed. Some fragmentary data showed this phenomenon to be typical for some pathologies (Shubich & Nagoev, 1980).

Some interesting data were obtained for AP in thrombocytes (fig. 1 C, D). According to the literature, cytochemistry either does not reveal AP in thrombocytes or occasionally yields a weakly positive response (Butenko et al., 1974; Shubich & Nagoev, 1980). Female mink, though not all of them, show high activity of the enzyme in April, which is probably due to specific patterns of thrombocytopoiesis or thrombocyte functional activity during pregnancy. In other periods (mating, lactation and post-lactation periods) phosphatase-positive thrombocytes were not observed, at least in such high amounts and so intensively stained, in preparations from female mink blood. Surveys carried out for a comparison among polar fox females did not reveal highly active thrombocytes.

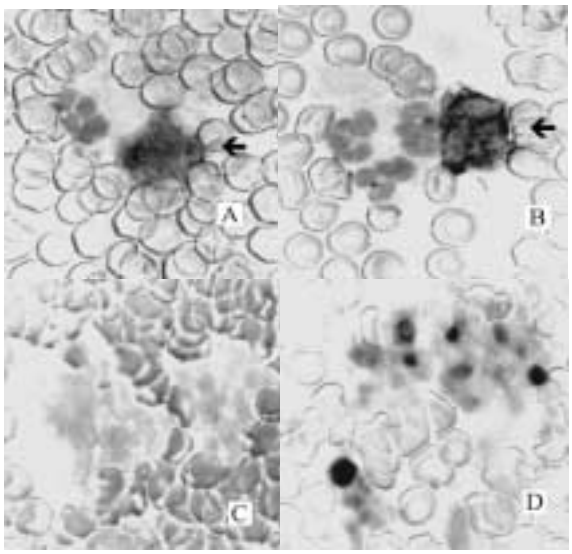

Figure 1. The alkaline phosphatase in various minks blood cell (is designated by a finger) according to Burstone (1965) method with modification. Leucocytes with weak (A) and moderate (B) activity. Thrombocytes with negative (C) and positive (D) reaction.

Shifts in the LAP activity were recorded in female mink during the breeding period (fig. 2 A). The greatest amount of phosphatase-positive leucocytes was observed during pregnancy and lactation. Detailed analysis indicated that the distribution into groups with different levels of LAP activity changed depending on the physiological status (fig. 2 B). A typical situation for the lactation period is the lack of animals with negative response to LAP. Furthermore, 30% of the mink examined had a relatively high (over 9%) content of phosphatase-positive leucocytes in the blood during pregnancy and lactation. During the mating period the number of such animals among females is negligible, and

practically equals zero after lactation, whereas the number of females showing no phosphatase response increases. These data have lead us to a conclusion that when evaluating the animal condition the distribution pattern of the relative frequencies of phosphatase activity levels should be taken into account in addition to the mean values.

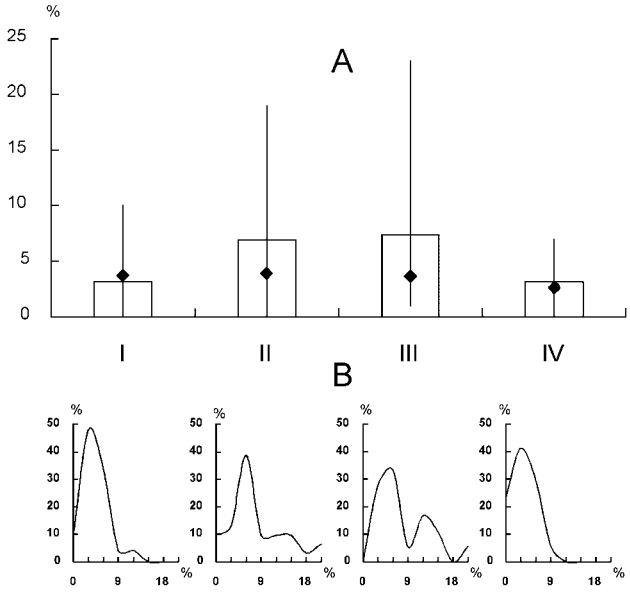

Figure 2. Changes of AP activity in minks leucocytes during reproduction cycle. Number of phosphatasepositive leucocytes (A) and distribution of relative frequencies of phosphatasepositive leucocytes levels (B).
The average value (columns), mode (rhombs) and limit of variation in mating period (I), gestation (II), lactation (III) and post-lactation periods (IV) are represented.
On ordinates axis (A) and on abscissa axis (B) – level of AP positive leucocytes in %. On ordinates axis (B) – frequency of cases in %.

Modifications in the phosphatase activity described above proceed against the background of changes in the leucocyte profile (Table 1). A certain relationship between the enzyme activity and the distribution of individual leucocyte types in the blood is observed. The relative content of basophils and eosinophiles was found to increase in the periods of higher phosphatase activity – pregnancy and lactation. The pattern of leucocyte distribution with respect to LAP activity apparently reflects the characteristics of leucocyte maturation and kinetics, and indicate to an exceptionally complicated mechanism of their functional activity control in seasonally breeding animals.

Table 1. Number of leucocytes and composition of the leucocytic formula of standard mink females. Average and min-max data represented.

	Periods			
Parameters	Mating period	Pregnancy	Lactation	Post-lactation period
Leucocytes, 10^9/L	8.72 5.25 – 13.80	8.16 3.75 – 17.60	7.97 4.35 – 16.55	4.96 2.15 – 9.00
Lymphocytes, %	37.20 17 - 55	44.58 26 - 64	46.58 22 - 66	47.80 32 - 63
Monocytes, %	7.60 3 - 18	7.09 3 - 20	4.42 1 - 10	4.00 0 - 9
Neutrophilic band, %	1.60 0 - 6	0.88 0 - 4	1.68 0 - 5	1.50 0 - 3
Neutrophilic segmented, %	52.15 37 - 75	43.39 21 - 62	43.84 17 - 70	45.60 33 - 58
Eosinophils, %	1.35 0 - 3	2.94 0 - 7	3.21 0 - 9	1.00 0 - 3
Basophils, %	0 0 - 0	0.18 0 - 1	0.26 0 - 2	0.10 0 - 1

The patterns of leucocyte functional activity cannot be considered in isolation from the hormone status, which in female mink undergoes notable changes in the period from February to June. Thus, low phosphatase activity with complete disappearance of phosphatase-positive leucocytes from the blood of some females observed in early February probably depends on the functional status of the adrenal gland. Simultaneously, increased phosphatase activity in mink in the periods related to pregnancy and lactation is apparently due to further dynamics of the sex hormones oestradiol and progesterone. Lower values of the studied parameters in the post-lactation period may be caused by extremely tense metabolism owing to the processes of recovery after lactation.

The effect of endocrine glands and physiological status of the organism on leucocyte metabolism is evidenced by a relationship between the level of phosphatase activity on the one hand and whelping dates and lactation duration on the other (Figure 3).

Investigations have demonstrated that, first, as whelping approaches females show a tendency towards a reduction in the content of phosphatase-positive leucocytes, which is in conformity with the known data on progesterone and oestradiol dynamics in this period (Savchenko et al., 1987). Second, the number of active leucocytes decreases during the lactation period. J.A. Pritchard (1957)

described similar tendention of LAP changes in pregnant women.

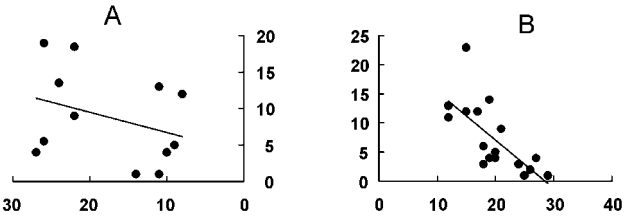

Figure 3. The influence of gestation and lactation on the number of phosphatasepositive leucocytes in standard mink females
On abscissa axis – days up to (A) and after parturition (B), on ordinates axis – level of AP positive leucocytes in %.

Thus, the functional activity of leucocytes in standard mink depends on the animal physiological status. A higher phosphatase activity in leucocytes is observed in the periods directly linked to gestation and lactation, which is related to an increased content of basophils and eosinophiles in the blood. A strongly positive response was recorded in thrombocytes of some mink during pregnancy, but further research is needed to find out the reasons for this phenomenon. Specific characteristics of the functional activity of leucocytes in female mink at different stages of the reproductive cycle appear to be due to high sensitivity of the blood system to hormone effects.

References

Berestov, V.A. 1971. Blood biochemistry and morphology of fur bearing animals, Petrozavodsk, 291 p. (In Russian)

Burstone, M.S. 1962. Enzyme histochemistry and its application in the study of neoplasms. Academic Press. NY & London. 464 p.

Butenko, Z.A., Gluzman, D.F., Zak, K.P. et al. 1974. Cytochemistry and electron microscopy of blood cells and hemopoietic organs. Kiev, 246 p. (In Russian)

Hayhoe F.G.J., Guoglino D., Doll R 1964. The cytology and cytochemistry of acute leukemias. London, 350 p.

Metsler D. 1980. Biological chemistry. Moskow, Vol. 2. 119 p. (In Russian)

Pritchard J.A. 1957. Leukocyte phosphatase activity in pregnancy // J. Lab. & Clin. Med., Vol. 50, N3. P. 432-436.

Savchenko O.N., Sirotkina L.N., Tyutyunik N. N. 1987. Hormonal function of sexual gland of some fur-bearing animals species // Sketches on physiology of the fur-bearing animals. Leningrad, P. 156-184. (In Russian)

Shubich M.G, Nagoev V.S. 1980. Alkaline phosphatase of leucocytes in norm and pathology. Moskow, 224 p. (In Russian)

Uzenbaeva L.B., Tyutyunik N.N. 1994. Some blood indices of mink nanism // Scientifur. V. 18. N 3. P. 157-160.

Uzenbaeva L.B., Tyutyunik N.N. 1995. Blood indices of mink nanism // Krolikovodstvo i zverovodstvo. N 3. P. 24. (In Russian).

Content of some mineral elements in the body of female standard coypu (*Myocastor coypus*)

D. Mertin [1], K. Süvegová [1], P. Sviatko [2], P. Fl'ak [1]
[1]Research Institute of Animal Production, Nitra, Slovakia
[2]Institute of Farm Animal Physiology SAS, Košice, Slovakia

Abstract
The objective of this study was to study the concentration of Ca, K, Na, Mg, Fe, Zn, Cu, Mn, Pb, Cd and Co in the body of female standard coypus in the period of fur maturity. The samples were analysed by the method of atomic absorption spectrophotometry. The highest concentrations were found with Ca (27.90±1.64 g/kg d.m.) and K (4.90±0.33 g/kg d.m.), and the lowest concentrations with Cd (0.44±0.03), Co (0.59±0.03) and Pb (1.57±0.04 mg/kg d.m.). Following significant correlations between minerals were found: Ca:Fe, K:Na, K:Zn, Na:Mg, Na:Fe, Na:Zn, Na:Cu, Na:Mn, Mg:Fe, Mg:Zn, Fe:Cu, Fe:Mn, Fe:Co, Zn:Cu, Zn:Pb, Zn:Cd, Cu:Mn.

Introduction
The content of biologically important mineral elements in body and tissues of animals is relatively stable under normal conditions. The normal values can thus be the starting material to judge the mineral status and value of nutrition. Bialkowski and Saba (1985), Työppönnen et al. (1988), Blus and Henny (1990), Kopczewski et al. (1990), Süvegová et al. (1993), Mertin et al. (1994), Niedbala et al. (1999) studied the mineral composition in the body and organs of carnivorous fur animals. There are only a few works dealing with the mineral profile in the organizm of coypus, and they are aimed mainly at determination of mineral elements in fur (Buleca and Sviatko, 1991b, Mertin et al., 1997, Hanusová et al., 2000a,b). Buleca and Sviatko (1991a) studied the content of microelements in blood and fur of coypu.

The objective of this study was to find out the concentration of Ca, K, Na, Mg, Fe, Zn, Cu, Mn, Pb, Cd and Co in the body of animals.

Material and methods
The experiment was performed at the Experimental Farm for Fur-bearing Animals of RIAP Nitra. Fifteen female standard coypus at the age of 8 months (during the period of fur maturity) were used. The animals were housed in a hall, in one-storey cages with pools. They were fed pellet feed mixture KK, and lucerne (during spring and summer period) and fodder beet (in autumn and winter period) as supplementary feed according to the feeding standard. Water from pools was used for drinking. The animals were clinically healthy and in normal condition.

The coypus were not fed for 24 hours before killing, which was performed in the period of fur maturity. After skinning the cadavers of animals were homogenised and an average sample of 200 g was taken from each body. The treated samples were frozen at -17oC. The samples were analysed by the method of atomic absorption spectral photometry using PERKIN-ELMER, model 5000 and graphite cell 500. Three measurements were done on each sample.

The gained results were processed mathematically and statistically (Grofik and Fl'ak, 1990) as follows:

- determination of arithmetic means and their standard errors,
- Pearson correlation coefficients and their significance.

Results and Discussion
The basic variation and statistical characteristics of the content of studied mineral elements in bodies of female standard coypus are given in Table 1. We found the highest concentrations of the studied macroelements expressed in dry matter (d.m.) in Ca (27.90±1.64 g/kg d.m.) and K (4.90±0.33 g/kg

d.m.). The content of Na was 1.69±0.06 and of Mg 1.13±0.15 g/kg d.m. The highest concentrations of microelements were found in Fe (211.55±16.53 mg/kg. d.m.) and Zn (69.59±4.80 mg/kg d.m.). The content of Mn was 10.87±1.21 and Cu 7.24±1.00 mg/kg d.m. The lowest concentrations were found in Cd (0.44±0.03), Co (0.59±0.03) and Pb (1.57±0.04 mg/kg d.m.).

Table 1. Contents of some mineral elements in the body of female nutria (M±s.e.)

Element	M	s.e.
Ca (g/kg dry matter)	27.90	1.64
K (g/kg dry matter)	4.90	0.33
Na (g/kg dry matter)	1.69	0.06
Mg (g/kg dry matter)	1.13	0.15
Fe (mg/kg dry matter)	211.55	16.53
Zn (mg/kg dry matter)	69.59	4.80
Cu (mg/kg dry matter)	7.24	1.00
Mn (mg/kg dry matter)	10.87	1.21
Pb (mg/kg dry matter)	1.57	0.04
Cd (mg/kg dry matter)	0.44	0.03
Co (mg/kg dry matter)	0.59	0.03

M = mean, s.e. = standard error of the mean

Comparing the concentration of mineral elements in the body of female mink, which are given in Table 2 (Mertin et al., 1994) with the results achieved in coypus we can state that the content of K, Na, Zn, Cu, Cd and Co are almost the same. The concentration of Mn, Ca, Pb was higher, and the content of Fe lower in the body of coypus.

Table 2. Contents of some mineral elements in the body of female mink (M±s.d.), (Mertin et al., 1994)

Element	M	s.d.
Ca (g/kg dry matter)	13.98	8.43
K (g/kg dry matter)	4.56	0.93
Na (g/kg dry matter)	1.58	0.12
Mg (g/kg dry matter)	0.74	0.30
Fe (mg/kg dry matter)	247.49	34.60
Zn (mg/kg dry matter)	70.43	6.33
Cu (mg/kg dry matter)	6.49	1.12
Mn (mg/kg dry matter)	3.36	0.93
Pb (mg/kg dry matter)	0.82	0.36
Cd (mg/kg dry matter)	0.47	0.15
Co (mg/kg dry matter)	0.59	0.14

M = mean, s.d. = standard deviation

The average concentration of mineral elements in fur of standard coypus at the age of 8 months in the region of back were measured by Mertin et al. (1997) to be: Ca 1.71, K 0.51, Na 0.24, Mg 0.68, all in g/kg d.m., Fe 168.69 mg/kg d.m., Zn 139.94, Cu 5.98, Mn 2.51, Co 0.73, all in g/kg d.m. If we compare the content of studied elements in fur and in the body of coypus, higher content of Ca, K, Na, Mg, Fe, Cu and Mn was measured in the body of animals, whereas the content of Zn in the body was much lower. The Co concentration was at approximately the same level or only slightly higher in the fur.

The concentration of a certain element in organism depends not only on feed, genotype, age, region of the body but also on mutual relation between the elements (Lohi and Jensen, 1991; Mertin et al., 1997; Hanusová et al., 2000a,b). We therefore studied also the correlation relations between the elements.

The average correlation coefficients (r) of mineral elements in the body of coypus are given in Table 3.

Table 3. Correlation coefficients (r) between the contents of mineral elements in the body of standard nutria

Mineral elements	K	Na	Mg	Fe	Zn	Cu	Mn	Pb	Cd	Co
Ca	0.33	0.38	0.45	0.58*	0.20	0.33	0.28	0.10	-0.03	0.15
K		0.56*	0.16	0.13	0.61*	0.50	0.28	-0.28	0.33	-0.04
Na			0.79**	0.75**	0.77**	0.66**	0.59*	-0.29	0.34	0.44
Mg				0.74**	0.66**	0.30	0.39	0.19	0.20	0.36
Fe					0.42	0.61*	0.74**	0.07	0.16	0.57*
Zn						0.55*	0.46	0.54*	0.64*	-0.13
Cu							0.85**	0.06	0.28	0.16
Mn								0.19	0.20	0.36
Pb									0.33	0.23
Cd										-0.24
Co										

* $P<0.05$ for $r(13) \geq 0.5139$
** $P<0.01$ for $r(13) \geq 0.6411$

We found the following significant correlations:
sodium : with Mg (r=0.79**), Fe (r=0.75**), Zn (r=0.77**), Cu (r=0.66**), Mn (r=0.59*), K (r=0.56 *);
iron : with Ca (r=0.58*), Na (r=0.75**), Mg (r=0.74**), Cu (r=0.61*), Mn (r=0.74**), Co (r=0.57*);,
zinc : with K (r=0.61*), Na (r=0.77**), Mg (r=0.66**), Cu (r=0.55*), Pb (r=0.54*), Cd (r=0.64*),
potassium : with Na (r=0.56*), Zn (r=0.61*);,
calcium : with Fe (r=0.58*) and
copper : with Na (r=0.66**), Fe (r=0.61*), Zn (r=0.55*), Mn (r=0.85**).

Lohi and Jensen (1991) studied the interactions between elements in mink fur and found significant correlations only between Ca, Mg and P on one hand and between Mg, Na and K on the other hand. Hanusová et al. (2000a) studied the correlation relations of mineral concentrations in fur of nutria in the period of fur maturity and found significant correlation between several minerals. Corresponding to our results the correlations were significant between K and Zn, Fe and Cu, Zn and Cu, Zn and Pb, and Cu and Mn with the exception that the correlation Fe:Cu on fur was negative.

References

Bialkowski, Z., Saba, L. 1985. Investigations over relationship between occurrence of mineral elements in blood serum and hair of black-silver foxes. Scientifur 9, 1, p. 21-23.

Blus, L.J., Henny C.J. 1990. Lead and cadmium concentrations in mink from Northem Idaho. Northw. Sci., 64, p. 219-223.

Buleca, J., Sviatko, P. 1991a. Minerálny profil makroelementov v krvi a srsti nutrií. Proc. Scientific Symposium on Fur Animal Production, ZSVTS Košice, p. 34-35.

Buleca, J., Sviatko, P. 1991b. Analýza mikroelementov v srsti nutrií. Proc. Scientific Symposium on Fur Animal Production, ZSVTS Košice, p. 93-94.

Grofik, R., Fl'ak, P. 1990. Štatistické metódy v pol'nohospodárstve. Bratislava, Príroda, 344 p.

Hanusová, E., Mertin, D., Lohi, O., Süvegová, K., Stepanok, V. 2000a. Relationship between the content of various elements in fur of nutria (Myocastor coypus). Scientifur, 24, 3, p. 193-196.

Hanusová, E., Lohi, O., Süvegová, K., Mertin, D., Stepanok, V. 2000b. Factors affecting the mineral composition of nutria fur (Myocastor coypus). Scientifur, 24, 3, p. 197-202.

Kopczewski, A., Wroblewska, M., Zdunkiewicz, T. 1990. Okreslenie zawartosci pestycydow polichlorowych, polichlorowanych dwufenily, oraz metali: olowiu, kadmu, miedzi i cynku w tkankach norek, lisow i tchorzofretek. Przeglad naukowej literatury zootechnicznej, Vol. 35, p. 218-223.

Lohi, O., Jensen, L.V. 1991. Mineral composition of mink feed and hair. National Institute of Animal Science, Denmark, Report no. 688, p. 99-114.

Mertin, D., Süvegová, K., Oravcová, E., Sviatko, P. 1994. Concentrations of some mineral elements in the mink body in the period of fur maturity. Živocíšná výroba, 39, 2, p. 121-127.

Mertin, D., Süvegová, K., Fl'ak, P., Sviatko, P., Točka, I. 1997. The effect of genotype and physiolgical condition on the content of some minerals in the fur of coypu females. Živoc. výroba, 42, 4, p. 149-157.

Niedbala, P., Szeleszczuk, O., Mertin, D., Süvegová, K., Sviatko, P. 1999. Preliminary investigation on heavy metal content in kidneys, liver and spleen of Polar foxes (Alopex lagopus). Appl. sci. rep. 42 Fur animals production and breeding, p. 69-79.

Süvegová, K., Mertin, D., Sviatko, P., Oravcová, E. 1993. Content of some mineral elements in chosen organ of silver foxes (Vulpes vulpes). Scientifur 17, 4, p. 257-262.

Työppönnen, J.T., Smeds, E., Pölönen, J. 1988. Antioxidant trace elements and enzymes in mink. Proc. IV. Int. Congr. in Fur Anim. Prod. Biology, Pathology and Genetics of Fur Bearing Animals, Ontario, p. 358-368.

Morphometric parameters of body and skin of nutria (*Myocastor coypus*)

J. Hanusová [1], D. Mertin 2, I. Točka [1], K. Süvegová 2 , P. Fl'ak [2]
[1]Slovak Agricultural University, Nitra, Slovakia
[2]Research Institute of Animal Production, Nitra, Slovakia

Abstract
In this work we studied the morphometric parameters of body (length and width of head, index of head, length of body on the belly and back, live weight, girth of chest) and of skins (length of raw and dry skin, girth of dry skin and weight of raw skin) in standard coypus at the age of eight months (males n = 68, females n = 45). On the basis of our results no marked differences between the sexes were found in the studied parameters. Significant differences ($p<0.01$) were found only in the length of head (males 122 mm, females 117 mm), width of head (78 mm, 74 mm, resp.) and girth of chest (393 mm and 370 mm, resp.). The mentioned differences are due to the sexual dimorphism, as the higher values of morphometric parameters of head and girth of chest are the typical male signs in nutria. In the evaluation of live weight we found a tendency to higher body weight in males (males 4637 g, females 4496 g). The average length of raw skins was in males 641 mm and in females 634 mm. Highly significant correlations between different morphometric measures were found in almost all cases with the exception of length of head to weight of raw skin, length of body on back to index of head, index of head to girth of dry skin and length of raw skin, and length of raw skin to weight of raw skin. The live weight, length of body on back and belly, which from the practical point of view are important parameters, correlated strongly with other studied parameters.

Introduction
Dixon et al. (1980) and Willner et al. (1980) studied the live weight in free living nutrias and in semi-free herds in regard to age and season. Their results show that the live weight in free living nutrias at the age of one year reaches to 3000 - 3500 g. Kladovščikov et al. (1979) were engaged in the growth of live weight in standard nutrias kept on farms with respect to the level of nutrition. They reported live weight of males 4700 g and females 4000 g. The live weight of females is 10-15 % lower than in males. Barta et al. (1989) reported the average live weight of nutrias 4244 g. Ocetkiewicz et al. (1972) studied the relation of body weight to length and surface of the skin. They found a direct dependence between these parameters. Kukla and Pitrun (1982) also found highly significant correlation between live weight and length of body, and live weight and length of skin. They stated that it is possible to gain skins 65 cm and longer from animals with body weight over 3600 g if the body length is 51 cm. According to Kukla (1977) the length of body ranges between 44-65 cm. Holdas (1982) and Barta et al. (1989) found also a close relation between live weight and size of skin. Gerberová (1977) concluded that males fed full-value feed achieve the required constitution and body size at the age of seven months. Cholewa (1997) found differences between sexes in the length of skins - 78.1 cm in males and 77.4 cm in females – and he concluded that females of the age of seven months only in few cases produce skins large enough. Barta et al. (1984) reported the weight of raw skins to be 556 g in males and 506 g in females.

Material and methods
The experiments were performed at the Experimental Farm for Fur-bearing Animals of RIAP Nitra with standard nutrias. The animals were kept in one-storey cages with pools in a hall. They were fed pellet feed mixture KK (AC Čataj), and green lucerne (in spring-summer period) and fodder beet (autumn-winter period) as moisture supplement. The animals drank the water from the pools. For the experimental observations 68 males and 45 females were chosen at the age of 8 months, during the period of fur maturity. The main criterion in selection was the live weight of animals with respect to balanced degree of body development.

After euthanising the animals by mechanical break of the cervical vertebra and skinning the following morphometric parameters of body and skin were measured: length and width of head (mm), length of body on belly and back (mm), live weight during the period of fur maturity (g), girth of chest (mm), length of raw and dry skin (mm), girth of dry skin (mm) and weight of raw skin (g). We used the digital scales with the accuracy of 1 g to weigh the animals and raw skins. Linear measures were taken using a tape measure with the accuracy of 5 mm. The length of body was measured from the tip of nose to the root of tail on both the dorsal and ventral side of the body. The length of head was measured with a slide-rule from the tip of nose to occipital eminence, and the width of head at the broadest facial part. The index of head was calculated as proportion of head length to head width. The girth of raw skin was measured with a tape-measure at the middle of the skin, and the girth of dry skin at the same point after the skin was stretched on wooden standardised board.

The gained results were processed mathematically and statistically (Grofik and Fl'ak, 1990) as follows:

- determination of arithmetical means and their standard errors,
- Pearson correlation coefficients and their significance.

Results and Discussion

Arithmetic means and standard errors of mean of morphometric parameters of body and skin of nutrias are given in Table 1 for the total material and for each sex. Our results show no significant differences between sexes in most of the studied parameters.

Table 1. Arithmetic means and standard errors of mean in morphometric parameters of coypu (M ± s.e.)

Parameters	Males (n = 68)		Females (n = 45)		Total (n = 113)	
	M	s.e.	M	s.e.	M	s.e.
Length of head ** (mm)	122	1	117	1	120	1
Length of body ventral (mm)	506	3	508	5	507	3
Length of body dorsal (mm)	510	3	504	4	508	2
Live weight (g)	4637	72	4496	62	4522	63
Index of head	1.6	0.01	1.6	0.02	1.6	0.01
Girth of chest (mm)**	393	4	370	5	384	3
Width of head (mm) **	78	1	74	1	76	1
Length of dry skin (mm)	761	6	766	7	763	4
Girth of dry skin (mm)	1721	11	1701	17	1713	9
Length of raw skin (mm)	641	5	634	9	638	5
Weight of raw skin (g)	472	8	470	15	471	8
Girth of raw skin (mm)	1515	10	1513	19	1514	10

Significant differences between sexes:
*p<0.05 for t (111) ≥1.982; **p<0.01 for t (111) ≥ 2.621

The only significant differences, at the level of significance p <0.01, were found in the length of head (males 122 mm, females 117 mm), width of head (78 mm or 74 mm, resp.), and girth of chest (393 mm or 370 mm, resp.). In all parameters higher values were found in males. Significant differences are due to sexual dimorphism, as higher values of morphometric parameters of head and girth of chest are typical traits for the male nutria, as reported also by Točka (1984). We found a tendency to higher live weight in males compared to females. However, the difference was not statistically significant (males

4637 g, females 4496 g). The mentioned live weight values of nutrias correspond with the results of Kladovščikov et al. (1979), Kukla and Pitrun (1982) and Barta et al. (1989). The length of raw skin represents the biological skin length. The average length of raw skin was 641 mm in males and 634 mm in females. The average lengths of dry skins were in males and females 761 mm and 766 mm, respectively. – The reverse sex difference in dried skin can be due to human influence during the pelting procedure as the female skins are thinner and thus are easier to stretch when blocking on the drying boards.

As no significant differences between sexes were found in most of the parameters the total material including both males and females was used to calculate the correlations between the studied morphometric parameters of body and skin in nutrias. (Table 2). Highly significant correlations were found between almost all parameters, with the exception of length of head to weight of raw skin, length of body on back to index of head, index of head to girth of dry skin and length of raw skin, and the length of raw skin to weight of raw skin. In the practical production it is important to have parameters measurable on live animals, which have strong correlation to important skin measures. In our study live weight, length of body measured both on back and belly showed high positive correlation to skin weight and skin length. These relations are also confirmed in the works of the other authors cited here.

Table 2. Correlations of morphometric parameters in coypu

Parameters	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
1. length of head	0.24*	0.44**	0.42**	0.37**	0.43**	0.53**	0.32**	0.33**	0.37**	0.11	0.39**
2. length of body ventral		0.82**	0.70**	-0.29**	0.46**	0.46**	0.68**	0.67**	0.50**	0.60**	0.67**
3. length of body dorsal			0.76**	-0.14	0.49**	0.50**	0.71**	0.70**	0.59**	0.54**	0.69**
4. live weight				-0.25**	0.57**	0.60**	0.70**	0.62**	0.53**	0.61**	0.70**
5. index of head					-0.37**	-0.59**	-0.45**	-0.13	-0.15	-0.21*	-0.21*
6. girth of chest						0.71**	0.21*	0.35**	0.44**	0.36**	0.49**
7. width of head							0.32**	0.41**	0.46**	0.29**	0.54**
8. length of dry skin								0.86**	0.50**	0.58**	0.64**
9. girth of dry skin									0.56**	0.51**	0.66**
10. length of raw skin										0.15	0.79**
11. weight of raw skin											0.31**
12. girth of raw skin											

*P<0.05 for r(111) ≥ 0.19; **P<0.01 for r(111) ≥ 0.24

Conclusion

1. In the period of fur maturity (age 8 months) there was no sex difference in the studied morphometric parameters of body and skin of standard nutrias, with the exception of length and width of head, and girth of chest which are characteristic of the sex dimorphism in nutria.
2. The correlations between the studied parameters show that the length and width of head, length of body on belly and back, and length of dry skin were in correlation with all studied traits. On the basis of the studied correlations we recommend the dorsal body length to be used as information for size in the selection of breeding animals.

References

Barta, M., Palanská, O., Točka, I. 1984. Tissue composition of the body and carcass yield of standard nutrias. Agriculture 30, 11, 1036-1042.

Barta, M., Jakubicka, I., Fl'ak, P. 1989. Female fertility, increase of live weight and area of hides in hybrids from reciprocal crossing of standard nutrias with black recessive nutrias. Agriculture 35, 6, 545-554.

Cholewa, R., 1997. Growth of nutria youngs and peculiarity their skins i.e. furs. Proc. The 2nd International Conference on animal-protection, trapping, farming, skin processing and fur marketing in Budapest City from 25th till 27th February, 1997, 17.00-17.08.

Dixon, K.R., Willner, G.R., Chapman, J.A., Lane, W.C., Pusley, J., 1980. Effect of trapping and weather on body weights of feral nutria in Maryland. J. Appl. Ecol. 16, 69-76.

Gerberová, H., 1977. Vývoj nutrií od narození do kožešinové zralosti. Československý kožešinář 11, 4, 17-19.

Grofik, Fl'ak, 1990. Štatistické metódy v pol'nohospodárstve. Bratislava, Príroda, 344 p.

Holdas, S., 1982. A nutria tenyésztése. Budapest, Mezogazdasági Kiadó, 240.

Kladovščikov, V.F., Kuznecov, G.A., Jakovenko, J.A., 1979. Kletocnoe rozvedenie nutrij. Rossl'chozizdat Moskva, 208 p.

Kukla, F., 1977. Chov kožešinových zvírat. VŠZ Brno, 98-100.

Kukla, F. and Pitrun, J., 1982. Vzt'ah hmotnosti nutrií k délce kožek. Československý kožešinář 17, 3, 21-24.

Ocetkiewicz, J., Rychlicky, Z., Kawinska, J., Niedwiadek, S., Wrona, J., 1972. Wyniki badan laboratornyjnych futerek nutrii pochodzacych ze zwierzat ubijanych w wieku 10, 12 i 14 miesiecy zycia. Rocz. nauk. roln. Ser. B, 94, N 1, 67-79.

Točka, I., 1984. Chováme nutrie. Príroda, Bratislava, 144p.

Willner, G.R., Dixon, K.R., Chapman, J.A., Stauffer, S.R., 1980. Model for predicting age-specific body weights of nutria without age determination. J. appl. Ecol. 7, 343-347.

Tracheo-bronchial microflora of nutrias (Myocastor coypus) from two hygienically contrasting farms

P. Martino, N. Stanchi, R. Rule, D. Arias, M. Gatti

Cátedra de Microbiologia-CIC-Facultad de Ciencias Veterinarias
Universidad Nacional de La Plata
60 y 118. CC 296 (1900) La Plata. Argentina

E-mail: pemartino@yahoo.com

Abstract
Bacteria and fungi were cultured from non-surgical-tracheo-bronchial aspirates (TBA) from clinically healthy nutrias in two different, hygienically contrasting colonies. Bacteria were isolated from 53.4 % of the samples on the farm with the best sanitary conditions (group A), and from 61 % on the other farm, with poor conditions (group B). The difference between these isolation rates was not significant ($p > 0.05$). Coagulase-negative *Staphylococcus sp., Escherichia coli* and α-haemolytic *Streptococcus* sp. were the most common organisms, and were isolated from 16 %, 13.7 % and 13.1 % of the TBA samples respectively. Anaerobic bacteria and fungi were detected in 4 and 3 samples, respectively. Most of the isolated genera showed no significant differences in their distribution in both groups. The number of bacterial species isolated from each TBA culture ranged from 1 to 8 (mean, 3 ± 1.6 in group A and 3 ± 1.3 in group B). The number of colony forming units tended to vary inversely with the number of bacterial species detected in each sample. Sensitivity of isolates to antibiotics was highest for gentamicin and polymyxin B. The bacterial microflora of the caudal respiratory tract of these nutria appeared to be comprised of common opportunistic pathogens or potentially transient contaminants, and was not particularly influenced by the immediate surroundings.

Key words: nutria, tracheo-bronchial microflora

Introduction
Nutria (*Myocastor coypus*) is a large histricoid rodent of South-american origin which has been introduced to many countries through fur-farming and meat production. Regretably, the nutria has not been studied enough in many aspects, therefore we lack extensive knowledge on their biological characteristics as potential laboratory animals. On the basis of the laboratory explorations done on fur bearing animals, we are only at the beginning in this field in comparison to the other domestic animals, due to two main problems: insufficiently studied fixing procedures and blood techniques which would both provide such quantities and quality of samples that could meet the contemporary requests of any specific analysis. Nutria's respiratory flora has never been determined. Knowledge of normal caudal respiratory tract microflora would enable the clinician to initiate rational preventive and therapeutic measures. Use of the nutria for experimental bacteriological studies requires a thorough understanding of the normal microflora of these animals. Assuming that the qualitative and quantitative nature of the bacterial indigenous flora in mammals and humans could be affected by the environment (Sonnenwirth et al., 1980), the survey reported here was conducted on adult nutria kept in hygienically contrasting environments on two commercial farms. Therefore, we describe the bacterial and fungal flora demonstrated in a non-surgical- tracheo-bronchial aspirate (TBA) from clinically healthy nutrias providing information on the frequency of isolation of each microorganism.

Materials and methods

Animals. Forty-three groenland mutation nutria from farm A (28 females and 15 males with a mean age of 8.2 ± 1.7 months), and 47 animals from farm B, mostly black and amber golden mutations (19 females and 28 males, mean age: 7.9 ± 1.2 months), were selected randomly among the surplus cull animals. Both farms had a population of between 400 and 500 adult nutrias which were reared on concrete premises with the same kind of water facilities. Farm A was apparently free of pneumonic cases and had very good hygiene, whereas farm B had a history of sanitary problems and animals which were kept overcrowded resulting in poor hygienic conditions (Martino and Stanchi, 1998). A careful assessment of the nutria clinical state was necessary to ascertain whether they would withstand the procedure. An estimation of total plasma protein was obtained by a protein refractometer (normal values range from 5.2 to 7.9 g/dl plasma) and the blood sugar was quickly assessed (normal range: 200 to 350 mg/100 ml) by using a glucose test strip (BM Test Glycemil, Boehringer). Packed cell volume was measured and should have been in the range of 40 to 55 per cent. Respiratory abnormalities such as dyspnoea or discharge from the nostrils were unnoticed. Females were anaestrus (prior to puberty) or inter-estrus. All animals were housed individually on their own farm 40 days before the study in outdoor concrete cages (107 cm x 120 cm x 70 cm) with indoor nests equipped with straw. The animals were fed once a day (200 - 250 g for each animal) a mixed ration of green alfalfa silage, hay and concentrated feed with 1050 kcal/kg of metabolizable energy, and had free access to tap water by means of an automatic watering system. All procedures involving handling and management of the animals were carried out according to the guidelines of the Code of Practice for the Care and Handling of fur-bearing animals (1994).

Parasitology. All animals were screened for the presence of *Giardia* sp and *Cryptosporidium* sp on faecal samples (Verdes,1986; Howerth et al, 1994) by direct microscopy and latex agglutination test (Biocientifica Lab., Argentina), and for other endoparasites by the routine faecal examinations using a zinc sulfate centrifugal flotation technique.

Sample collection and bacterial culture procedure. Nutria were fasted from the evening before the day of sampling. Medication with atropine (0.04 mg/kg BW, S.C.) and acepromazine (0.01 mg/kg, I.V.) preceded induction of total anaesthesia with an intramuscular injection of thiopental: 30 mg/kg (Scheuring, 1989). Anaesthesia was maintained by further 1 to 2 ml injections whenever necessary. The nutria were placed in dorsal recumbency and a heat pad was provided to prevent heat loss during anaesthesia. Oxygen supplementation was available in case of substantial respiratory compromise, but was not deemed necessary in any of the procedures. To obtain the TBA, a commercially available 8-gauge through-the-needle catheter with a 15.88-cm tubing was used according to Martino and Stanchi, 1995.

The dimension of the equipment necessary for catheterization and the depth of penetration of the were based on previous studies with necropsied nutrias. The site over the midcervical part of the trachea was shaved and surgically prepared. For the aspirate, after inserting the tubing into the incision, approximately 1.3 ml of a sterile saline solution (without a bacteriostatic agent) was delivered endobronchially and then recolected by a sterile 5 cc syringe appropriately adapted to the needle. The samples were placed in sterilized tubes in an ice bath for transport to the laboratory. All specimens were processed by the laboratory within 1.5 to 2 hours of acquisition. They were cultured on two tripticase soy agar (with 0.1 % agar) (Difco laboratories, Detroit, Michigan) plates containing 7 % (v/v) horse blood. One plate was incubated in a 5 % CO2 atmosphere at 37ºC. Both plates were incubated at 37ºC and examined after 48 hours for evidence of bacterial growth. Gram stains were prepared for all colonies, and representatives colonies were subcultured onto blood agar and incubated aerobically at 37ºC. Isolates were identified according to Lennette (1995) and Murray (1999). The number of different types of organisms in each culture was determined and the number of colonies of each type were counted. The amount of growth was scored as 1+ for 1 to 10 colony forming units (CFU), 2+ from 10 to 50 CFU, 3+ for 50 to 100 CFU, and 4+ for > 100 CFU for each type. For total counts of aerobes and anaerobes samples were plated on Plate Count Agar (Difco Laboratories, Detroit, Michigan, USA, CM325) and incubated aerobically and anaerobically at 37 C for 2 days, 20 C for 3 days or at 5 C for 5 days. β-haemolytic streptococci were serologically grouped according to the method of Lancefield (1933) (Streptex, Wellcome Diagnostics, Dartford, England), and biochemical reactions were determined using a

commercial test system (API20 Strep, Mérieux, France). Specimens for mycoplasmal culturing were placed in vials of sterile modified Hayflick broth containing penicillin and thallium acetate inhibitors (Holt, 1984) and cultures were incubated at 37 C in a 5 % CO2 atmosphere. Plates were examined daily under 40x magnification and reduced light for signs of the typical fried egg appearence of mycoplasmal colonies. For anaerobic bacteria, the specimens were inoculated in pre-reduced, anaerobically sterilized (PRAS) media prepared according to Holdeman and Moore (1977). In addition, anaerobic chopped meat carbohydrate broth (Carr-Scarborough Microbiologicals, Atlanta, GA, USA) was inoculated as a backup broth. The Sabouraud agar plates inoculated at 20 C were examined perodically for evidence of fungal growth. Fungi were identified according to Carter (1984) and Roberts et al. (1985). Investigation on *Chlamydia* sp was performed in McCoy cells inoculated with samples according to Schachter, 1991.

Antimicrobial susceptibility studies. All bacterial isolates were tested for antimicrobial susceptibility, using disk-diffusion methods. The testing was performed for the following antimicrobials: penicillin, ampicillin, tetracycline, nitrofurantoin, neomycin, gentamicin, streptomycin, erythromycin, kanamycin, sulfamethoxazole + trimethropim, chloramphenicol and polymyxin B. Diffusion methods were also used for testing antifungal drug susceptibilities. Commercial manufactured tablets (Media Kitchen, University of California,Davis) that contained either 20 IU of amphotericin B, 50 µg of natamycin, 50 µg of nystatin, 25 µg of griseofulvin, 15 µg of ketaconazole, 10 µg of miconazole and 10 µg of 5-fluorocytosine were applied to buffered YMA agar (Difco) inoculated with a single isolate and incubated at 30ºC for 24 to 48 hours. Susceptibilities were determined by measuring zones of inhibition and applying interpretative criteria (NCCLS 1997).

Statistical analysis. The data were analyzed using the Chi square test for association (Snedecor and Cochran, 1978) using $p < 0.05$ as the cut off for significance.

Results

Parasitology. All animals were free for the presence of *Giardia* sp and *Cryptosporidium* sp meanwhile helminth ova and coccidia were absent or in low numbers in the faecal examinations.

Bacteroiology. All animals recovered successfully from the anaethesia and sampling procedure. Bacteria were isolated from 23 of the 43 TBA samples of farm-A-nutria (53.4 %), and 29 of the 47 TBA from farm-B-nutria (61%). The difference between these isolation rates from both groups was not significant ($p > 0.5$). The total number of aerobic isolates was 175, and were representative of 18 recognized genera (Table I). The greatest variety of organisms was isolated from farm-B-nutria, although not significantly ($p > 0.5$). The most common isolates (coagulase-negative-*Staphylococcus*: 16 %, *E.coli*: 13.7 % and α-haemolytic *Streptococcus* sp: 13.1 %) were observed in both groups. Only four genera were significantly higher in their isolation rate among both groups: *Actinomyces pyogenes, Haemophilus paracuniculis*, unidentified *Streptococcus* and coagulase-negative *Staphylococcus* in group B, and α-haemolytic *Streptococcus* in group A. The number of bacterial species per nutria ranged from 2 to 8 (mean, 3 ± 1.6) in farm-A-nutrias, and from 1 to 6 (mean, 3 ± 1.3) in farm-B animals. Cultures with several species had relatively low numbers of CFU per isolate. When only one or two species were present in a sample, high numbers of CFU were generally observed. Anaerobic bacteria were isolated from four samples, all present in fairly low numbers (1 to 2+), meanwhile fungi were isolated from three samples and yielded moderate growth. An organism was isolated for which a positive identification was not possible. Two *Haemophilus*-like organisms were isolated from 1.1 % in each group. It was found in pure culture and had 4+ growth from one nutria. Identification was not possible because the test results were different from those of all known species of *Haemophilus*. No *Mycoplasma* and chlamydial organisms were recovered.

Antimicrobial susceptibility tests. Sensitivity to antibiotics were: gentamicin: 91 %, polymyxin B: 83 %, chloramphenicol: 54 %, sulfamethoxazole + trimethropim: 32 %, kanamycin: 29 %, erythromycin: 26 %, ampicillin: 9 %, streptomycin: 5 %, neomycin: 3 %, nitrofurantoin: 3 %, tetracycline: 2 %, penicillin: 5 %. The antifungal activity was highest for nystatin, miconazole and natamycin (≥ 90 %) and lowest for the other five drugs (≤ 80%).

Table 1. Bacterial isolates from respiratory tracts of two groups of nutrias

Bacteria isolated	No. of isolates and percentage (%) Farm A (n = 43)	Farm B (n = 47)
Aerobes		
Escherichia coli	14 (8)	10 (5.7)
Staphylococcus aureus	1 (0.5)	1 (0.5)
Staphylococcus intermedius	1 (0.5)	0
Staphylococcus(coagulase -)	8 (4.7)	20 (11.4)
α-haem.*Streptococcus* spp	17 (9.7)	6 (3.4)
Streptococcus zooepidemicus	0	4 (2.2)
beta-haem.*Streptococcus* spp*	0	3 (1.7)
non-haem.*Streptococcus* spp	2 (1.1)	1 (0.5)
Streptococcus unidentified	1 (0.5)	8 (4.7)
Aerococcus sp	2 (1.1)	1 (0.5)
Micrococcus sp	1 (0.5)	0
Bacillus sp	1 (0.5)	0
Corynebacterium sp.(non-haem.)	8 (4.7)	7 (4.0)
Actynomyces pyogenes	0	4 (2.2)
Enterobacter cloacae	1 (0.5)	0
Klebsiella pneumoniae	2 (1.1)	5 (2.8)
Flavobacterium sp	1 (0.5)	2 (1.1)
Moraxella sp	4 (2.2)	3 (1.7)
Acinetobacter sp	3 (1.7)	5 (2.8)
Pasteurella haemolytica	3 (1.7)	2 (1.1)
Pasteurella multocida	2 (1.1)	0
Pseudomonas aeruginosa	0	2 (1.1)
Haemophilus paracuniculus	0	4 (2.2)
Haemophilus sp	2 (1.1)	0
Haemophilus sp-like	2 (1.1)	2 (1.1)
Simonsiella	0	1 (0.5)
Anaerobes		
Bacteroides sp	2 (1.1)	0
Bifidobacterium	0	1 (0.5)
Lactobacillus sp	0	2 (1.1)
Fungus		
Fusarium	0	1 (0.5)
Epidermophyton	1 (0.5)	0
Absidia	0	1 (0.5)
Total	**79 (45.2)**	**96 (54.8)**
	175	

*Not *Streptococcus zooepidemicus*

Discussion

To our knowledge, this was the first comprehensive study conducted in fur-farming nutrias to evaluate the normal tracheo-bronchial flora on live nutrias. There have been a number of reports in the literature concerning the study of the respiratory tract microflora by surgical or non-surgical TBA-procedures on different animal species (Chauhan et al, 1987; Raphel & Beech, 1981). In our survey the TBA procedure proved to be safe for the life of the animals and did not cause unneccessary disturb to them. Culturing at post-mortem is often conducted on lung samples of nutria in an attempt to identify the aetiological agents associated with pleuropneumonia, considered to be one of the most common diseases of captive nutrias (Gadzhiev, 1977, Martino & Stanchi, 1995). Many different bacteria have been isolated from clinical cases and even from sacrificed animals during pelting time, mainly belonging to the following genera: *Staphylococcus, Pasteurella, Streptococcus*, and *Klebsiella* (Scheuring, 1989, Dousek, 1987). Data presented here showed how frequently potential pathogens such as *Streptococcus equi* subsp. zooepidemicus, coagulase-positive *Staphylococcus*, *E.coli* or *Pasteurella* spp can be isolated from the TBA from clinically healthy nutrias. Many of them are also commonly found in nutria cadavers from main organs (Zwierchowsky, 1987). *Streptococcus zooepidemicus* is the most common bacterial species causing epizootic pneumonia in nutrias (Martino & Stanchi, 1995), but the role of the rest of the species involved in this pathology has not been specifically established (Scheuring, personal communication). Most of the isolated genera in this survey, showed no significant differences in their distribution among both groups, and the *Streptococcus* genera outnumbered the rest (24 %). Coagulase-negative *Staphylococcus*, *E.coli*, α-hemolytic *Streptococcus* spp. and non-haemolytic *Corynebacterium* spp were the species most commonly found here. Ivascu et al. (1982) reported that *Streptococcus* sp and various gram-negative bacteria predominated among the organisms isolated from infected skin wounds in nutria. Four different strains of β-haemolytic *Streptococcus* were isolated from female coypus suffering from mastitis (Jidong et al. 1992), whereas β-haemolytic *Streptococcus* species are found as pure or miscellaneous culture from the skin and ear infections of domestic and fur-bearing animals (Niskanen, 1996). Despite the very common presence of *Salmonella* in faeces, water and soil of nutria farms (Slawon et al. 1995), this species was not isolated in our survey. Anaerobes and fungus were isolated in few cases, and this is an area that deserves more attention in the study of nutria respiratory disorders. Our failure to isolate *Mycoplasma* and *Chlamydia* suggests that they would not be part of the caudal respiratory tract of the nutria. Bacteriologically negative cultures can be the result of the bacteria dying during transport to the laboratory, a failure in collecting specimens or that the nutria are on treatment with antibiotics. There was no statistically significant difference ($p > 0.05$) between the proportions of TBA that proved to be bacteriologically sterile on aerobic and anaerobic culture from both groups, and the striking differences in hygienic standards between farms A and B would not have obvious effects, either quantitative or qualitative, on the tracheo-bronchial flora of the investigated animals. In our survey, caudal respiratory tract microfloras were not significantly influenced by the nutria's immediate surroundings. Nutria are in close contact with their environment, which act as a constant source of bacteria for them. That can give an impression of a persistent flora, either autochthonous or allochthonous, even when the bacteria do not colonize. On the basis of our findings, bacterial microflora of the caudal respiratory tract of nutrias consists of common opportunistic pathogens or potentially transient contaminants.

Bacterial isolates from our survey had the highest susceptibilities to commonly antibiotics in fur-bearing animals economy, e.g. gentamicin and polymyxin B. These are raw data, and their clinical importance remains to be seen. Mass broad spectrum antibiotic chemoprophylaxis is often empirically used to prevent acute respiratory disease and control epidemics among nutria colonies, particularly those caused by *Streptococcus zooepidemicus* infections. In addition, amiglycosydes and fluoroquinolones are the most frequently used and misused antibiotics in *Enterobacteriacea* infections by the owners. However, the efficacy of these interventions and their impact on residual antibiotics have not been fully evaluated. Many factors, including local epidemiologic factors, antibiotic policies, over-the-counter use (which often leads to inadequate use), lower standard of farming in developing countries, lack of information on the prudent use of antibiotics in animal husbandry may contribute, not only to the emergence of resistant organisms, but also to become the nutria meat unappropriated as an animal

food. This stresses the need for a multidisciplinary approach to determine the levels permitted for residues of antibiotics, antihelmintics or banned growth promoters in nutria.

References

Carter G.R. 1984. Diagnostic procedures in Veterinary bacteriology and mycology. 4th ed. Springfield,III: Charles C. Thomas, Publisher, USA.

Chauhan R.S., Gupta S.G., Satija KC. 1987. Bacterial flora of upper respiratory tract in apparently healthy camels. Indian J. Anim Sci 57, 424-426

Code of Practice for the care and handling of farmed mink, fitch and fox in Europe. 1994. Council of European Fur Breeder's Associations, Annual meeting, The Netherlands

Dousek J. 1987. The health situation in coypus farms in the Czech socialist Republic. Proceedings of the International Scientific Conference Coypu'87, Novi Sad, Yugoslavia, 121-124

Gadzhiev A.T. 1977. Some diseases of young coypus reared on farms in Azerbaijan. Proceedings of the 2nd All-Union Scientific Conference on Fur-Bearing Animals, Iyulya, USSR, 296-297

Holdeman L.V., Moore W.C.C. 1977. Anaerobe laboratory Manual. 4th edition, Virginia Polytechnic Institute and State University, Blacksburg, USA

Holt J.G. 1984. Mycoplasmas. In: Bergey's Manual of Systematic Bacteriology. Volume 1. Williams & Wilkins (Ed), Baltimore.

Ivascu I., Steopan E., Sahleanu C., Matyas L. 1982. Untersuchungen uber lokale infektionen beim nutria. Deutsche Tierärztliche Wochenschrift 89, 155-157

Jidong Z., Minshan F., Peiguo, L. 1992. Susceptibility of Streptococcus haemolyticus causing mastitis in coypus. Heilongjiang J Ani Sci Vet Med (China) 9, 34-35

Lancefield R.C. 1933. A serological differentiation of human and other groups of hemolytic streptococci. J Exp Med 57, 571- 595

Lennette E.H. 1995. Manual of clinical microbiology (Lennette E.H. et al, eds) American Society of Microbiology, Washington DC, USA

Martino P.E., Stanchi N.O. 1995. Epizootic pneumonia in nutria. J Vet Med B 41, 561-566.

Martino P.E., Stanchi N.O. 1998. Causes of death in captive nutria (Myocastor coypus) in Argentina, Isr J. Vet Med 3, 83-88

Murray P.R., Baron E.J., Pfaller M.A., Tenover F.C., Yolken R.H. (eds). 1999. Manual of Clinical Microbiology. 7th ed. ASM Press, Washington, D.C.

National Commitee for Clinical Laboratory Standards. 1997. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. NCCLS, Wayne, Pa. USA.

Niskanen M. 1996. Basic features of skin bacteria. Post-Graduate Course on Hair and Skin Research Methods. Research Centre Foulum Danish Fur Breeders Association, Tjele, Denmark.

Raphel C.F., Beech J. 1981. Pleuritis and pleural effusion in the horse. Proc Am Asoc Equine Pract 27: 17-25

Roberts G.D., Goodman N.L., Land G.A. 1985. Detection and recovery of fungi from clinical specimens. In: manual of Clinical Microbiology (EH Lenette et al, ed) American Society of Microbiology, Washington DC, 500-513

Schachter J. 1991. Chlamydia. In: Ballows A.;Hausler WJ; Hermann KL et al (ed) Manual of Clinical Microbiology.5th edition Washington DC,American Society of Microbiology.1045-1053.

Scheuring W. 1989. Choroby Nutrii (Diseases of nutria). Panstowowe Wydawnictwo Rolcnize i Lesne (ed), Warszawa, Poland

Scheuring W. 1994. Personal communication

Slawon J., Bis-Wencel H., Saba L. 1995. Salmonella bacilli on nutria farm. Scientifur 19, 47-50

Snedecor G., Cochran W.G. 1978. Statistical methods. Iowa State University Press, Ames, Iowa, USA.

Sonnenwirth A.C., Gibbons R., Socransky S. 1980. Indigenous bacteria. In: Davis BD, Dulbecco R, Eisen HN & Ginsberg HS (ed). Microbiology. Harper & Row, Philadelphia. pp: 807-816

Verdes N., Pop T. Observatii asupra unor enzootii de enterocolita la nutrii. Rev.de Cresterea Animalelor (Romania) 36,4: 41-44.

Zwierchowsky J. 1987. Papilonaceae in coypu feeding and their influence on health and general condition of coypus. Proceedings of the

International Scientific ConferenceCoypu'87,
Novi-Sad, Yugoslavia, 140-144

Controlled mating in Myocastor Coypus (COYPU)

A.E. Felipe [1], M.T. Teruel [1-2], J.A. Cabodevila[2], S.S. Callejas[2]
[1]Area de Embriología. [2] Area de Reproducción. Facultad de Ciencias Veterinarias. Universidad Nacional del Centro de la Provincia de Buenos Aires
(7000) Tandil, Argentina

Abstract
The aim of the present work was to determine the viability of a controlled mating technique in the Myocastor coypus. Twelve sexually mature virgin females and one male of proven reproductive performance from the same breeding farm were used. Animals were maintained in a yard breeding system. A daily colpocytological examination was performed and when oestrus was detected, mating was allowed. Copulation with ejaculation was confirmed by direct observation and by the presence of spermatozoids in colpocytological samples taken 1 hour post-coitus. Copulation took place on the oestrus day for all mated females. The presence of embryos was determined. Embryos were collected by flushing of the oviducts and hemi-uteri at 48-hour intervals between days 1 and 7 post-coitus. A 100% pregnancy rate was achieved, which demonstrate the viability of the technique.

Keywords: colpocytology, controlled mating, Myocastor coypus.

Introduction

Myocastor coypus (coypu) is reared under zootechnical conditions mainly for producing fine furs and, secondly, meat as human food (Actis et al., 1989). Hence, it becomes of interest to prove the usefulness of procedures used in other domestic animals to improve production, remarking those to achieve a high reproductive efficiency. The achievement of high pregnancy rates in species of zootechnical interest, as it is the coypu, is an essential condition for a profitable production. Among those factors that contribute to this, the knowledge about the reproductive characteristics of the species is relevant (Heyman, 1988; Hafez, 1993). There are many studies on the reproductive morpho-physiology of the coypu. We can cite works done by Rowland and Heap (1966), Afasaniev et al. (1970), Crespo (1981), Bura et al. (1985), Cotea et al. (1986), Jakubicka et al. (1989), Bura (1992a, 1992b) and Felipe et al. (1998). Different authors have established the minimum age for the beginning of reproductive activities at 6 month, for both sexes, with a body weigh between 3 and 4 kg (Actis et al., 1989). Wilson and Dewees (1962) determined that the coypu is an annual polyoestrus animal, with oestrus cycle being characterised by a great variability. On this matter, Iudica and Alberio (1995) determined a range from 12 to 49 days, with a mean of 28.9 ± 12.6 day and an oestrus of 2-5 days.

In the present study, some previous data on the oestrus cycle of the coypu and breeding management in yard system conditions were applied to determine the applicability and efficiency of rearing techniques for controlled mating.

Materials and methods

Animals

The animals used in this study came from four family nuclei of *Myocastor coypus*, all of the Groenland variety in order to avoid variations due to differential fecundity (Afasaniev et al., 1970; Bura 1992a, 1992b). Each of the family nuclei was composed of young, virgin and sexually mature females destined to zafra. Twelve females with a mean weight of 4.6 ± 0.6 kg and a mean age of 8.9 ± 0.9 months were used. A male of proven reproductive performance, weighing 5.8 kg and 8 month-old was used.

The females were kept in a half-roofed pen with a built floor (half-roofed floored pen). The male was kept in a pen adjacent to the females to avoid undesirable mating. Each female had a surface of two m2. Once the family nucleus was constituted, no new female was introduced. At the beginning of the study, a 15-day period for adaptation was allowed to the females. The animals were fed 300

g/day/animal of a commercial concentrate for this species and water ad libitum.

Mating programme
A daily colpocytological follow-up and observation of the external appearance of the vulva all along the experimental period were done. The colpocytological analyses were done using standardised routine techniques (Callejas and Cabodevila, 1993; Iudica and Alberio, 1995). The observation of the unfixed vaginal samples was done within 5 minutes of collection and after staining with Harris' hematoxylin and Shorr dye. In both cases, a qualitative evaluation of both cellular and prevailing type was done to establish the oestrus cycle phase present. The information was recorded, indicating oestrus cycle phase, time of mating, and presence of spermatozoids in the vagina and established time of sacrifice from mating for corroborating gestation.

The controlled mating methodology was used, therefore, once the oestrus was determined by colpocytology, the female was immediately placed with the male in the same pen. The copula with ejaculation was corroborated by direct observation of the animals' behaviour and colpocytological sampling for detection of spermatozoids one-hour post-coitus (h.p.c.). In all the cases, a second sampling was done 5 h.p.c. for determining the possibility of new mating.

To determine gestation, animals were sacrificed at 48-hour intervals from day 1 to 7 post-coitus, the oviducts and hemi-uteri were taken out by laparatomy incising on the white line (Barros, 1992). These organs were washed with saline solution. Embryos were identified in the flushing liquid in dishes divided into squares, using a stereoscopic microscope.

Results
During handling, animals got used quickly to working conditions. After a week of handling, no one of the females showed signs of disturbance when the operator entered the pen.

The daily colpocytological analysis allowed oestrus detection, observing that scuamous cells prevailed in non-fixed smears. The number of such cells decreased between 24 h.p.c. (83.3% of the females) and 48 h.p.c. (16.7% of the females).

The 1-hour interval between observation of copulation and the colpocytological sampling was considered as the more acceptable. The reason for this is that in the immediate time post-coitus, the females were more nervous than ordinary with the seeing of the operator and ran away from him, looking for safe with the male. One hour later, the normal behaviour was back. In any case, changes in the external appearance of the vulva were observed.

The 83.3% of the mating took place within 15 minutes after the female was placed in the pen. In 91.6% of the cases, the presence of spermatozoids was confirmed 1 hour later of the observation of the mating. Only in one animal, spermatozoids were found in the vaginal sample 5 hours after the female was placed with the male. Double mating were not observed. Changes observed in the post-mating colpocytology are shown in Figure 1.

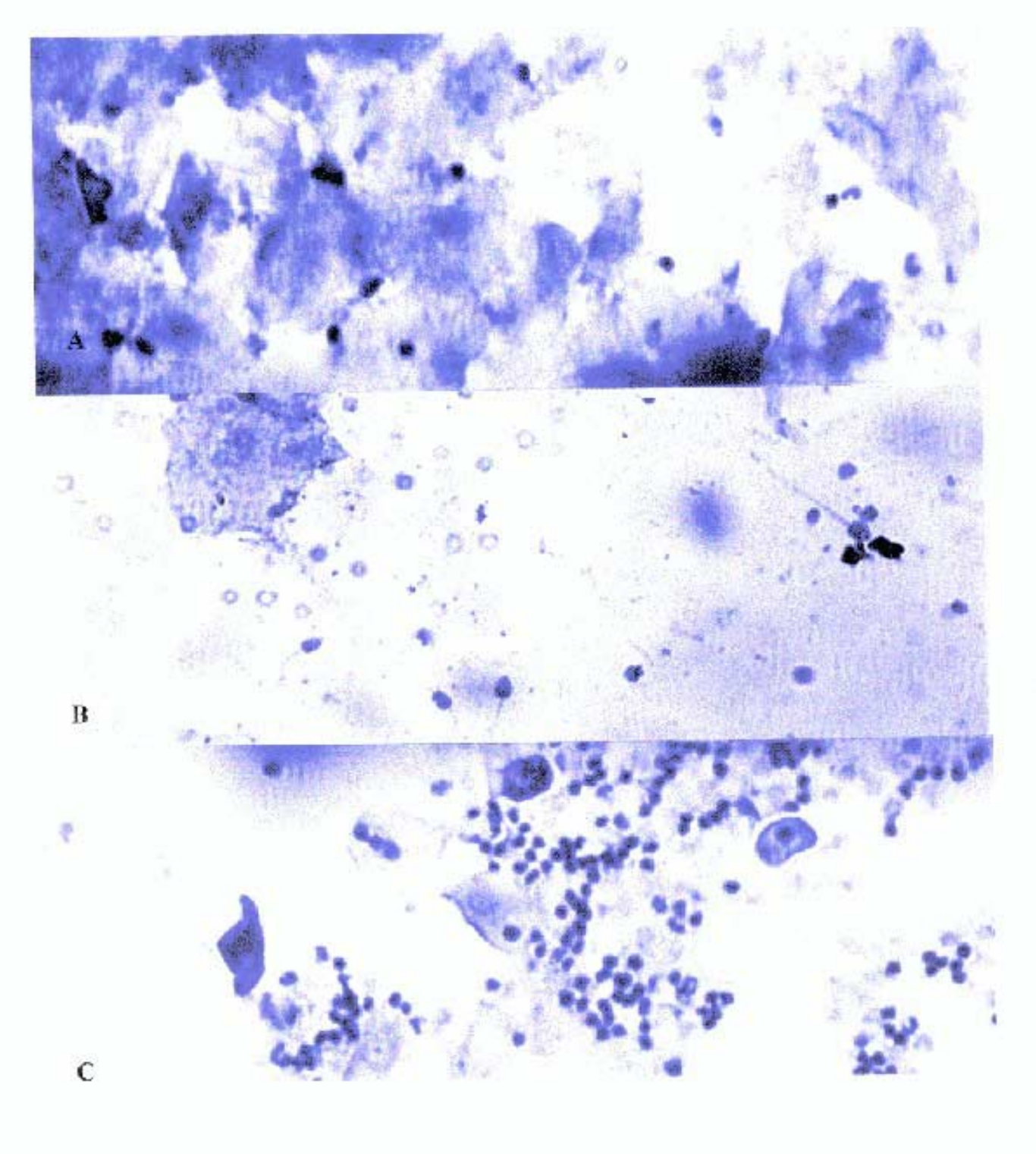

Figure 1. Pre- and post-mating changes in the vaginal cytology. A: oestrus; B: 1 hour post-coitus; C: 24 hours post-coitus. Harris' hematoxylin and Shorr dye, 40X.

In all the cases, post-mating colpocytology showed the presence of a vaginal plug, making difficult the introduction of the pipette farther than 1.5 cm to the vaginal canal (Figure 2). Macroscopic observation of the vagina allowed to corroborate the presence of the vaginal plug. It was composed of central core of semen surrounded by scamous cells derived from the superficial layer of the vaginal epithelium. All the females lost the vaginal plug 24 h.p.c.

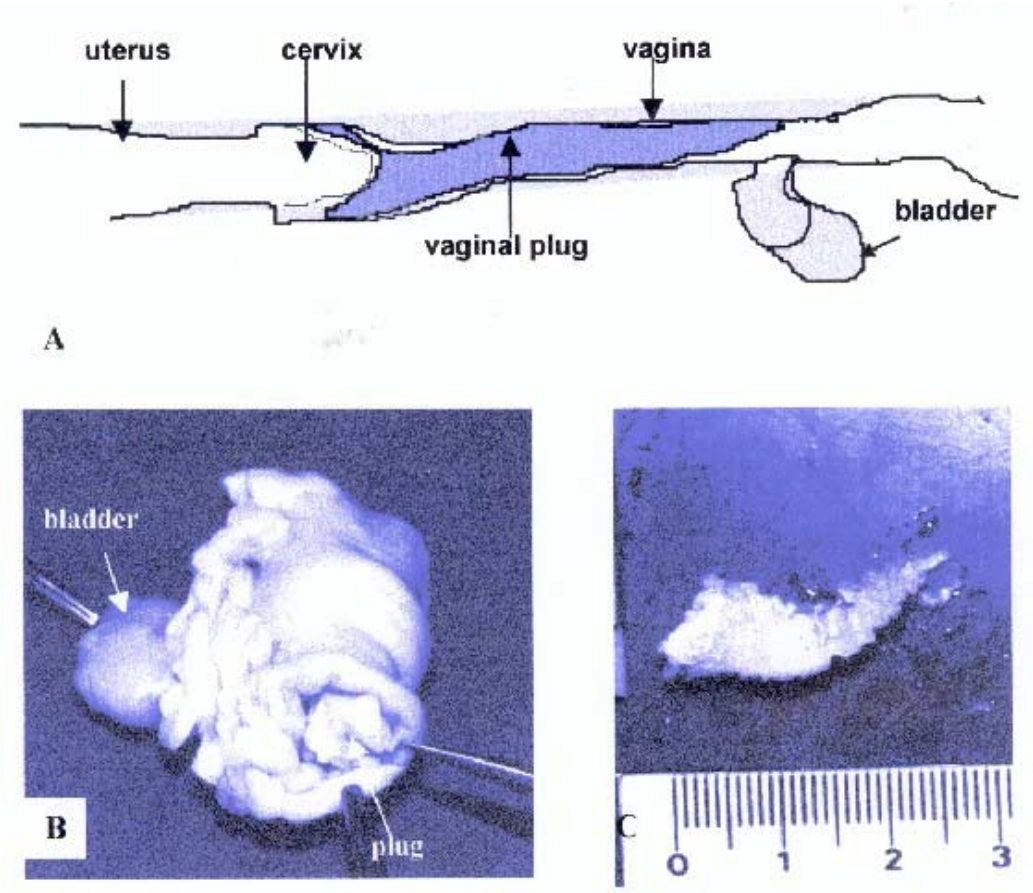

Figure 2. Vaginal plug in *Myocastor coypus*. A: Diagram depicting the position of the vaginal plug 1 hour post-coitus in a hypothetical longitudinal section; B: Photograph of a transverse section of the vagina showing the vaginal plug; C: Photograph showing the vaginal plug obtained 24 hours post-coitus.

Most of the observed matings was characterised by a receptive female, being absent signs of avoidance. Only in two cases, matings of females with aggressive behaviour were observed. These behaviours consisted in resistance to be mounted, characterised by continuous walking in the pen, loud grunts and bites.

The oocytes and embryos were successfully collected from all the females. In general terms, by flushing of the oviducts and of the uterotubal junctions, the collected embryos were in the stage of zygote up to 18-cell morula and by flushing of the uteri, the collected embryos were from 18-cell morula up to developing blastocysts.

Discussion

Myocastor coypus differs from the other hystricomorph rodents in the absent of a vaginal occlusion membrane (Weir, 1967a; 1974). This membrane makes difficult or impossible the follow-up of the oestrus cycle in almost all the members of this animal group. Many works have been done on the oestrus cycle of the coypu (Iudica and Alberio, 1995; Felipe et al., 1998).

As in the coypu, for most of the hystricomorphs, the oestrus is not easy to detect by external appearance of the vulva. Many rodents of this suborder secrete mucus during oestrus, but only in the plains viscacha (Lagostomus maximus), the tuco-tuco (Ctenomys sp.), the casiragua (Proechymis guairae) and the degu (Octodon degu) sometimes can be observed the swelling of the vulva during oestrus (Weir, 1974). The oestrus detection from the interpretation of vaginal smears is not feasible in most of these rodents as it is in the guinea pig (Bland, 1980). In this species, the prevailing of superficial cells, first, and the reappearance of leukocytes in the smears, later, are considered as indicative of oestrus and ovulation, respectively (Rood and Weir, 1970).

In domestic animals, mating takes place only during oestrus, which is coincident, in most of the cases, with the ovulation. To date, no studies have been done on the implementation of the controlled mating in other hystricomorph species except for the guinea pig due to its use as laboratory animal. However, observations at the time of mating in hystricomorphs kept o bred in captivity have been done. For example, in the chinchilla (Chinchilla laniger), the mating can take place at any time when the vagina is open (Weir, 1967b). The plains viscacha normally mates at the end of the open vagina period (Weir, 1971a; 1971b), and the cuis (Galea musteloides) (Rood and Weir, 1970), degu (Weir, 1974) and casiragua (Weir, 1973) mate at the beginning. For all these species, the colpocytological stage at mating showed scuamous cells prevailing, as for the coypu.

One-hour post-coitus, the vaginal plug was complete. This plug would retain spermatozoids in the vagina, keeping them in close contact with the cervix, allowing a maximum access of the spermatozoids to the cervical mucus, as was suggested by Harper (1988). The vaginal plug has been considered as indication of mating in hystricomorphs like the guinea pig and plains viscacha (Weir, 1971a), mountain viscacha (Lagidium sp.) (Pearson, 1949), chinchilla (Weir, 1967b), degu (Ctenomys sp.), casiragua and cuis (Weir, 1974). In the acuchi (Myoprocta pratti), Kleiman (1970) reported the lack of formation of the vaginal plug. The maximum persistence of the vaginal plug in the coypu, estimated in 24 h.p.c., was similar to that reported for the mouse by Snell (1969).

The results obtained under the described conditions in the present work suggest that the controlled mating technique is viable in Myocastor coypus. However, further experiments using a greater number of animals and under different breeding conditions are needed to determine the efficiency of the technique.

References

Actis, J.J., Cacciamani, M.A. and Vuegen, C. 1989. Informe sobre la nutria de criadero en la Argentina, Bol. Div. Téc. Nro. 78, Buenos Aires Norte, INTA-CR.

Afasaniev, V.A., Pereldik, N.S., Kladovscikov, V.F., Kuznetov, G.A. and Hronopulo, N.P. 1970. Cresterea animalelar de Blana, Bucuresti, Ed. Ceres.

Barros, C. 1992. Biología del desarrollo de mamíferos y criopreservación de embriones. Barros, C. (ed.), Santiago, Chile, pp. 6-9 and 19-21.

Bland, K. P. 1980. Biphasic follicular growth in the guinea pig oestrous cycle. J. Reprod. Fert., 60, 73-76.

Bura, M. 1992 a. Studiu asupra indicelui de fecunditate la nutrie (Myocastor coypus Molina), Lucrari Stiin Zootechnique, 26,141-145.

Bura, M. 1992 b. Studiu asupra ratei conceptiei la femele primipare si lutipare de nutrie (Myocastor coypus Molina), Lucrari Stiin Zootechnique, 26, 147-151.

Bura, M., Gluhovschi, N. and Radulescu, V. 1985. Incercári de combatere a infecundit tii determinate de anestru prelungit la femelele de nutria, Rev. Cresterea Animalelor, 1, 52-55.

Callejas, S.S. and Cabodevila, J.A. 1993. Características colpocitológicas del ciclo estral de la nutria (Myocastor coypus), Rev. Arg. Prod. Anim., Suppl.1, 83.

Cotea, C., Cotofan, V. and Hritcu, V. 1986. Histology and histochemistry of accesory sex glands of the male coypu, Myocastor coypus. Repr. Patol. Anim., 10, 527-530.

Crespo, J.A. 1981. Observaciones sobre la reproducción de la nutria en estado silvestre. In: La experiencia argentina en la cria de la nutria coypus. Aporte para el inicio de la producción en México, Griva E.E. (ed). México: Pider-S.A.R.H., pp 27-41.

Felipe, A., Cabodevila, J. and Callejas, S. 1998. Anatomicohistological characteristics of female genital tubular organs in the south american nutria (Myocastor coypus). Anat. Histol. Embryol., 27 (4), 245-250.

Hafez, E.S.E. 1993. Foliculogénesis, maduración del óvulo y ovulación. pp. 142-181. In Reproducción e inseminación artificial en animales. E.S.E. Hafez, 5ta. ed., Interamericana, México.

Harper, M.J.K. 1988. Gamete and Zigote Transport. In: The Physiology of Reproduction, E. Knobil and J. Neill (ed.). Raven Press, Ltd., New York.

Heyman, Y. 1988. Moment de la transplantation et succes de la gestation chez les mammiferes. Reprod. Nutr. Develop., 28 (6B), l773-l780.

Iudica, C.E. and Alberio, R.H. 1995. Prelimnary study of the sexual cycle of the South-American nutria (Myocastor coypus) by the method of

exfoliative colpocytology, Scientifur, 19 (1), 33-42.

Jakubicka, I., Barta, M., Nitray, J. and Szeleszezukova, O. 1989. Fluctuations in the progesterone concentration of the peripheral blood of nutria during pregnancy. Veter. Med., 34 (4), 251-256.

Kleiman, D. G. 1970. Reproduction in the female green acouchi, Myoprocta pratti Pocock. J. Reprod. Fertil., 23, 55-65.

Pearson, O. P. 1949. Reproduction of a South American rodent, the mountain viscacha. Am. J. Anat., 84, 143-174.

Rood, J.P. and Weir, B.J. 1970. Reproduction in female wild guinea pig. J. Reprod. Fert., 23, 393-409.

Rowland, I.W. and Heap, R.B. 1966. Histological observations on the ovary and progesterone levels in the coypu (Myocastor coypus). Symp. Zool. Soc. Lond., 15, 335- 340.

Snell, G.D. 1969. The early embryology of the laboratory mouse. In Biology of the laboratory mouse, G.D.Snell (ed.), Blakiston, Philadelphia, p.1-54.

Weir, B.J. 1967a. The care and management of laboratory hystricomorph rodents. Lab. Anim., 1, 95-104.

Weir, B.J. 1967b. Aspects of reproduction in some hystricomorph rodents. Ph.D. Thesis, Univ. of Cambridge Press, Cambridge.

Weir, B.J. 1971a. The reproductive physiology of the plains viscacha, Lagostomus maximus. J. Reprod. Fert., 25, 355-365.

Weir, B.J. 1971b. The reproductive organs of the plains viscacha, Lagostomus maximus. J. Reprod. Fert., 25, 365-373.

Weir, B.J. 1973. Another hystricomorph rodent: keeping casiragua (Proechimys guairae) in captivity. Lab. Anim., 7, 125-134.

Weir, B.J. 1974. Reproductive characteristics of hystricomorph rodents. Symp. zool. Soc. Lond., 34, 265-30l.

Wilson, E.D. and Dewees, A.A. 1962. Body weights, adrenal weights and oestrous cycles of nutria. J. Mammal., 43 (3), 362-364.