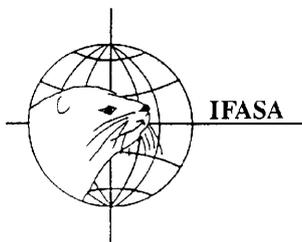
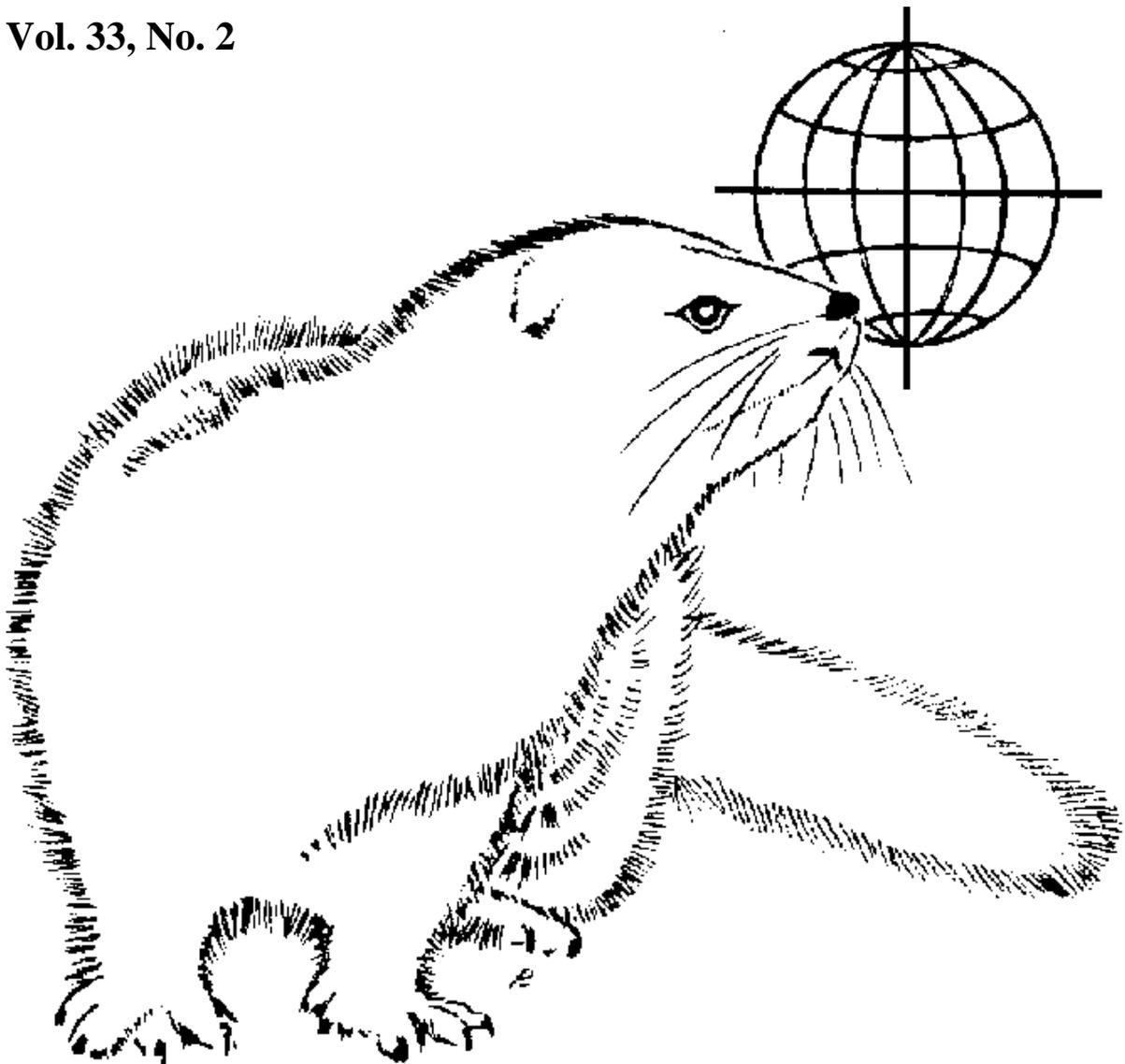


SCIENTIFUR

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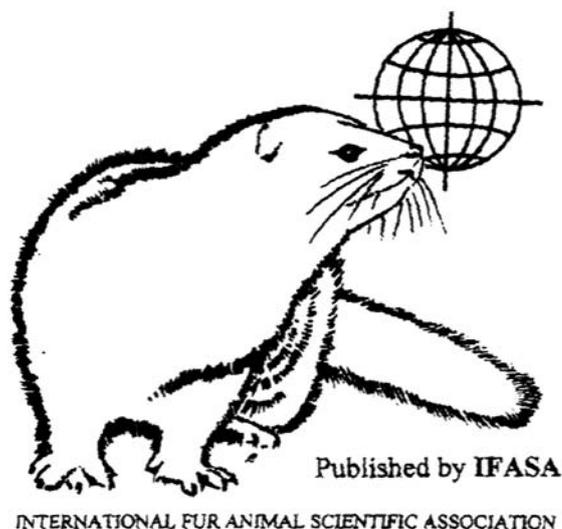
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Notes from the Editor

This issue of *Scientifur*, Volume 33, No 2, contains two articles and two short communications. A reviewed article shows different reactions to dietary sodium selenite on the antioxidant system in silver fox and blue fox. The second not reviewed article gives an overview of toxoplasmosis in mink based on a large number of observations in many years. This includes demonstration of hydrocephalus for the first time in mink. The two short communications present work in coypu. One of the

communications deals with histological observations of the cervix. The other presents a characterization of cellular types during the oestrous cycle.

It is a pleasure to publish original scientific work related to fur animal production. Submission to *Scientifur* of results from all scientific investigations regarding fur animal production is greatly encouraged.

Vivi Hunnicke Nielsen
Editor *Scientifur*

Effect of dietary sodium selenite on the antioxidant system in silver (*Vulpes vulpes* L.) and blue foxes (*Alopex lagopus* L.)

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Abstract

The influence of dietary sodium selenite (0.1 mg per kg body weight (BW)) on the activities of antioxidant enzymes – superoxide dismutase (SOD) and catalase (CAT) – and the level of glutathione (GSH) in six organs in fur-breeding Canids (silver foxes (*Vulpes vulpes* L.) and blue foxes (*Alopex lagopus* L.)) were evaluated. The treatment resulted in silver foxes with increased activities of SOD in liver and CAT in kidney and lung and also with increased level of GSH in spleen. There was no influence of selenite on the investigated indices in blue foxes.

Keywords: antioxidant system, blue fox, catalase, glutathione, silver fox, sodium selenite, superoxide dismutase.

Introduction

Cell defence against harmful effects of oxidative modification of bio molecules is known to be very important in adaptations to environmental factors. Therefore, species-related variations in tissue antioxidant status depend on the environmental conditions that species inhabit. In our research experimental animals, farmed for fur production, were the closely-related (family *Canidae*) blue and silver foxes. The species originated from different life conditions, *i.e.* arctic (blue fox) and temperate (silver fox) climate.

Selenium is an essential trace element in the diet of mammals. Antioxidant properties of selenium are mediated through the glutathione peroxidases

(GPx), helping to remove H₂O₂ and lipid peroxides generated in cells (Arthur *et al.*, 2003). Sodium selenite, one of chemical forms of selenium, is often used in animal breeding for the prevention of selenium deficiency in farm animals. So, the aim of the study was to determine the effects of dietary sodium selenite on the antioxidant system (AOS) in two *Canidae* species closely-related but differing in ecology.

Materials and Methods

Animals, diet and treatment groups

Twenty four foxes of each blue (*Alopex lagopus* L.) and silver fox (*Vulpes vulpes* L.) were used. The sex ratio was 1:1. All the animals were kept in standard farming conditions on a paste-like diet with two meals per day and water *ad libitum* as recommended for the species. The metabolizable energy (ME) is calculated on the basis of table values for feed ingredients. Diet composition (g/418 kJ of ME) was: meat soft by-products (5-10), meat-bone by-products (6-10), fish meal (15-20), minced fish (3-5), cooked grain (14.5-15.5), vegetables (8-10), dried yeast (2), tallow (1-2).

Animals of each species were divided into control and experimental groups (n=12 in each group). The experimental period lasted from July to October, during which animals were weighed monthly. The control animals were fed the basal diet and the experimental animals were fed the basal diet supplemented with 0.1 mg of sodium selenite per kg BW for 6 ten-day periods with ten-day intervals

between them, where animals were fed only the basal diet. This specific design was applied to prevent acquired tolerance of the organism to sodium selenite.

Sampling and assay methods

Samples of tissues (liver, kidney, spleen, lung, heart and skeletal muscles) were collected during the slaughter season in November. At pelting (killing of animals was performed according to European Convention [TA-P (96) 19] recommendations) samples of tissues were frozen and stored at -25°C.

To measure SOD and CAT activities, tissue samples were homogenized in 0.05 M phosphate buffer, pH 7.0, and then were centrifuged at 6000 g for 15 min. The total SOD activity was determined by the adrenochromic method based on the spontaneous autooxidation of epinephrine with the formation of end products which have an absorbance peak at 480 nm (Misra & Fridovich, 1972). This reaction depends on the presence of superoxide anions and is specifically inhibited by SOD. The amount of enzyme that caused 50% inhibition of epinephrine autooxidation is defined as 1 unit (U). Catalase activity was evaluated by measuring the decrease in H₂O₂ concentration at 240 nm (Bears & Sizes, 1952).

To measure GSH, tissue samples were homogenized in 0.02 M EDTA, and then centrifuged at 5000 g for 15 min. After that, the following mixture (supernatant, distilled water, 50% trichloroacetic acid) was centrifuged at 3000 g for 15 min. The supernatants were then assayed by the method of Ellman in the presence of 5,5'-dithiobis-(2-nitrobenzoic acid) (Sedlak & Lindsay, 1968). The results were expressed in mmol GSH per 100 g of raw tissue.

Data are presented as mean \pm standard error of mean (SEM). Statistical analysis was performed with Mann-Whitney's U-test. Differences between samples were considered to be significant when the *p* value was less than 0.05.

Results and discussion

The results on animal weights and weight gains are presented in Table 1. At the beginning of the study

the groups within species were identical ($p > 0.05$). Three- and 4-month-old (Aug and Sept) silver fox males and 4-month-old (Sept) blue fox males fed the selenite-supplemented diet had higher weight gain than controls. It indicates stimulating effect of sodium selenite on protein metabolism had a great role in the growth process of the animals. But lower weight gain was revealed in both 5-month-old (Oct) silver foxes and blue foxes (males and females) from selenite-supplemented group as compared with control group. Any indications of toxic effects of selenite were not detected. Rouvinen (1991) showed reduced appetite of blue foxes fed antioxidant supplemented diet before pelting.

Feeding foxes the sodium selenite resulted in both species- and tissue-related responses of the antioxidants to the treatment compared with feeding the basal diet. The investigated animals considerably differ in ecological conditions of their ancestors' life. Blue fox is a typical arctic animal while silver fox originates from temperate climate. It was shown that metabolic rate calculated from the level of oxygen consumption in blue fox throughout the year is almost twice lower than in silver fox (Casey *et al*, 1979). Various basal aerobic capacities of these species resulted in different reaction of their antioxidants to environmental factors.

Antioxidant enzymes' activities profiles in several organs of silver foxes fed the selenite-supplemented diet have been found to exhibit striking differences compared with control animals. The liver, kidney and lung retain the greatest amount of selenium of the internal organs and participate in selenium metabolism (Lopez *et al*, 1969). Increased activities of SOD in liver and CAT in kidney and lung of experimental silver fox were registered (Table 2). At the same time there were no differences in SOD and CAT activities between experimental and control blue foxes. Changes in the antioxidant enzymes' activities allow to understand the modifications of biochemical pathways, as the SOD synthesis is known to be regulated by oxygen level (Misra & Fridovich, 1972) and the CAT synthesis is activated by H₂O₂ (Aebi & Wyss, 1978) formed in both reaction of superoxide dismutation and oxidase reactions.

Table 1. Effect of sodium selenite on growth of silver and blue foxes

Variable measured	Species and groups							
	Silver fox				Blue fox			
	♂ control	♂ experimental	♀ control	♀ experimental	♂ control	♂ experimental	♀ control	♀ experimental
Initial body weight, kg	1.5±0.1	1.9±0.1	1.5±0.1	1.7±0.1	1.9±0.1	1.9±0.0	1.9±0.1	2.0±0.0
Body weight gain, kg								
– Jul 1-Aug 1	1.6±0.0	1.7±0.1*	1.4±0.1	1.4±0.1	2.7±0.1	2.3±0.2	2.5±0.2	2.3±0.0
– Aug 2-Sep 1	0.9±0.0	1.1±0.1*	0.9±0.0	1.1±0.2	2.4±0.1	2.7±0.1*	2.3±0.2	2.7±0.0
– Sep 2-Oct 1	2.0±0.2	0.6±0.1*	1.3±0.1	0.7±0.1*	1.1±0.1	0.8±0.1*	1.2±0.3	0.4±0.1*
Final body weight, kg	5.9±0.2	5.4±0.1*	5.1±0.1	4.8±0.4	8.0±0.0	7.8±0.1*	7.9±0.1	7.4±0.1

* – significantly different from control group.

Table 2. Effect of sodium selenite on activities of antioxidant enzymes and level of GSH in tissues of Canids.

Tissues	Species	SOD activity, U/g tissue		CAT activity, $\mu\text{mol H}_2\text{O}_2/\text{min}\cdot\text{g tissue}$		GSH, mmol/100 g tissue	
		control group	experimental group	control group	experimental group	control group	experimental group
Liver	Silver fox	105.4±8.3	300.5±83.6*	544.6±42.8	572.7±50.4	0.25±0.01	0.26±0.02
	Blue fox	233.1±36.4	434.8±79.1	445.0±59.6	561.8±121.6	0.37±0.01	0.33±0.01
Kidney	Silver fox	373.8±21.2	415.5±21.6	83.0±7.1	120.7±9.5*	0.20±0.02	0.21±0.01
	Blue fox	183.9±20.5	163.7±5.7	124.3±14.6	107.1±9.4	0.38±0.04	0.24±0.01
Lungs	Silver fox	76.1±3.6	68.4±6.9	7.1±0.8	12.6±1.8*	0.33±0.01	0.34±0.01
	Blue fox	33.8±8.0	45.6±8.4	34.4±4.8	27.1±3.6	0.26±0.00	0.26±0.01
Spleen	Silver fox	61.6±4.6	61.4±2.9	14.9±2.1	18.8±3.2	0.68±0.02	0.77±0.02*
	Blue fox	71.4±4.1	63.4±6.1	23.1±1.5	18.5±1.5	0.72±0.07	0.86±0.02
Heart	Silver fox	146.1±3.3	158.3±5.9	20.9±2.9	13.6±1.7	0.24±0.01	0.20±0.00
	Blue fox	112.6±7.4	146.1±25.9	13.1±1.4	20.9±6.0	0.17±0.04	0.14±0.02
Skeletal muscle	Silver fox	69.9±7.7	60.7±5.7	10.7±1.8	13.2±2.0	0.53±0.04	0.51±0.02
	Blue fox	70.9±9.6	77.0±15.8	13.5±2.0	16.8±3.1	0.34±0.05	0.35±0.08

* – significantly different from control group.

It is known that antioxidant effects of selenium were suggested to be mediated through the GPx with work of which nonenzymatic antioxidant – GSH – is connected (Arthur *et al*, 2003). It was revealed that influence of sodium selenite on the level of GSH in silver foxes was expressed by way of the increased content in spleen (Table 2). As spleen is an organ of the immune system, such effect of selenite could be explained by importance of selenium for an optimum immune response in mammals. At the same time no significant influence of sodium selenite on tissues levels of GSH in blue foxes was observed.

As sodium selenite is noneffective for Se retention in muscular tissues (Lopez *et al*, 1969), we haven't found any significant changes of antioxidants in heart and skeletal muscles of experimental animals ($p>0.05$).

The results indicated that sodium selenite caused more changes in the silver fox AOS as compared with the blue fox. It was revealed that silver foxes had lower selenium content in their livers than blue foxes (Rouvinen, 1991). The authors also showed nutritional effect in liver selenium level in silver foxes. As a result it may be presumed that the blue fox as compared with the silver fox possess a more resistant defense system against reactive oxygen species.

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Hydrocephalus and other morphopathologic aspects of toxoplasmosis in mink

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Abstract

Toxoplasmosis is a parasitic zoonosis (amphiheterosis), produced by the *Toxoplasma gondii* (*T. Gondii*) parasite, considered as pseudo-emergent or re-emergent, with not effective seizing upon and extension growing, but, due to the improvements in diagnosis techniques and to the more and more insistent control of preventing its baneful impact on the state of health both of human and animals, being more frequently rediscovered. Both, mink and human is infected by consuming not enough sterilized meat, by consuming other products and water, all contaminated with *T. gondii* oocytes resulted from cat excrements. The parasite has a strictly intracellular location, with intermediary host, represented by all hot blood species and definitive host is the cat, who also may be an intermediate host, presenting sometime clinical signs of illness. Mink younglings gravely affected by intrauterine infection with *T. gondii* present the same clinical tetrad as the human: microphthalmia and chorioretinitis, tonic and clonic convulsions, intracerebral calcifications and hydrocephalus. Regardless if it is primary or not, toxoplasmosis is compulsory seconded by an other morbid affection, macroglobulinemia, or, mono or polyclonal hypergammaglobulinemia, common disease and other secondary associated toxoplasmosis - in mink, the aleutine disease (A.D.), Sjögren's syndrome and other self-immune diseases and in human, the acquired immune-deficiency syndrome (AIDS). In both cases, the association to toxoplasmosis leads to a lethal finality of all affected subjects. Within their serum increased quantities of *IgM*, at least during the debuting stage are detected, accompanying inhibition of the other *Ig*, decreasing antibody synthesis and in detection of the persisting ones, the absence of antigenic

neutralizing capacity is ascertained. Serological tests are often irrelevant of falsely positive ones.

Keywords: hydrocephalus, hypergammaglobulinemia, infertility, toxoplasmosis.

Introduction

Toxoplasmosis affects both minks and humans, having negative influences on gestational females and on pregnant women. (Wallach & Boever, 1983; Dubey & Beattie, 1988; Antoniu, (2004); Palmer et al., 2005; Dubey, 2006). Losses by embryonic resorption, intrauterine fetal death, abortion, prematurely fetation, delays in intrauterine development, and appearance of congenital anomalies, may be such cases of maternal infections transmitted to the uterus during different gestation states.

Materials and methods

Research and observations have been carried out in a farm for animals bred for their furs (minks, arctic foxes, and polecats) during a period of several years, on the mink effective destined to be slaughtered, varying between 20,000 and 66,000 heads of 15 color varieties (homozygotes and heterozygotes). Research programmes have been diversified and have aimed at especial objectives being of epidemiologic and economic interest, respectively the furs' quality and quantity, according to the requirement. Research has been carried out on animals grouped in study lots (LS), reacting strongly positively to the not specific MALLÉN test (IAT-iodine agglutination test) used in mink for detecting hypergammaglobulinemia. Then, these animals have been submitted at the same time to the following tests: immunoenzymatic (ELISA), coprological, hematological and histological tests, followed by a strict clinical and paraclinical monitorization, compared to a witness lot (LM). The results have

been interpreted by comparison with the witness lots (LM). Lab investigations have had in view detection of *Ig M* and *Ig G* antibodies for toxoplasmosis and for the Aleutin Disease Virus (ADV) in minks.

Results and discussion

A. The 2860 gestational females of LS with plurifactorial determinism of risk have shown: 48.33% gestations losses; 19.6% embryonic fetal death; 6.33% premature fetation; 0.33% neonatal precocious death; 4.67% congenital malformations. In drawing up these results the following facts have been taken into account: a) deviations of maternal instinct (which naturally is exaggerated for this species), respectively by fetophagy of aborted pups or premature born ones and especially of those resulting from dystocic fetations, where some females forcedly intervened in the extraction of those blocked because of the fetopelvic disproportion and; b) difficult control on this discrete and prevailing nightly and extremely sensible animals to perturbations.

B. At the same time with these parameters, but separately, delayed intrauterine growths have been investigated, where positive variations at around 20.67% of females with positive deviations have been registered in an average of 4.05 days. The respective parameter is influenced by the physiological parameters of the gestation time. Normal gestations at these species are varying between 44 and 60 days, with an average rate of 49 days. Variations of time are influenced by the diapause and this, by the daylight length, age of females, number of former gestations, but especially, by the toxoplasmosis. The case of two infected females among the LS has been exemplified, where delayed intrauterine growths with a record duration of 85 days have been registered.

Subsequently, it has been confirmed that the infection of conception products caused delayed intrauterine growths, embryonic death, resorptions sometimes associated to hydrometer, fetal death, abortion, and perinatal morbidity. The appearance of visible modifications of the minks' state of health, with fast falling of reproduction indexes, imposed a diagnosis elucidation and preventive measures.

Out of the three cases met at the caretaker staff, where toxoplasmosis has been incriminated, one

deceased without a sure diagnosis, the second one, after a short crisis, has been diagnosed with a grave neuropsychic handicap, and the third one, after a dramatic state, with grave forecast, after a reevaluation of the toxoplasmosis diagnosis and re-directing the treatment, has been brought from the pre-coma state he reached, back to life. These episodes met at human have had a certain motivation of not observance hygienic rules and of inadequate behavior towards stealing confiscated meat products.

The most of the acquired infections with *Toxoplasma gondii* (*T.gondii*) have evaluated in an asymptotic manner, compared to the congenital ones, essential in evaluating this stage.

During the evolution of the congenital infection appeared during gestation, a temporary parasitemy has been found and the severity of the disease has depended on the infection's zygophase: 1) just after the service; 2) blastocytary; 3) diapause; 4) embryo – fetal; 5) fetal antepartum. Clinical effects of congenital toxoplasmosis in pups brought forth, respectively the probable period or moment of having been infected of serviced females and considered as made fecund, was difficult and sometimes, impossible to set up. Explanation of this fact consists in the high variety of the gestation time, determined by the physiological diapauses. (Dubey & Beattie, 1988; Constantin, 2005).

In precocious infection, by the generated parasitemy, changes in uterine trophoblast and, especially in the biochemistry of the embryotroph in formation, may occur. They are producing generally perturbations of the homeostasis (h), but especially on fecundated ovules' viability (immunologic h). Prolonged standing of blastocyst within the affected embryotroph makes it extremely vulnerable and induces a growth of its mortality rate.

The next stage of ovoimplantation starts after going out from diapause and staring nidation, when fecundated ovule penetrates trophoblaste and endometer, resuming the normal development cycle, similar to the other mammalians.

At the beginning of placenta formation, temporary parasitemy may initiate sometimes, the appearance of discrete focal lesions of placental coat, presuming that the parasite passed the placental barrier which holds the conception products. Subsequently, it has

been confirmed that the infection of conception products caused delayed intrauterine growths, embryonic death, resorptions sometimes followed by hydrometry, fetal death, abortion, and perinatal mortality (see Figure. 1 and 2).



Figure 1. Conception produces resorbtion and hydrometer.



Figure 2. Hydrocephalus in *T.gondii* infection.

Troubles on the uterine level by changes of the embryotrophic environment conditions induced

local biochemical changes, growths in diapause duration, disordering of uterine modulation mechanism and implicitly a blockage of genetic information to adequately respond to the modifications induced by the infection. It seems that disturber factors on a mono-zygote, or on a mono-zygote couple, constantly appear at the mono-zygote or the two ones of the mono-zygote couple and rarely on both bi- or polyzygoted members, supposing that a character is prevailing conditioned either genetic, or mezologic, as in this case.

After generally having affected conception products, the infection inclined to a step by step remission and apparently visceral tissues came back to their normal states. Not the same thing occurred out of the male genital organ and the nervous system. It may be considered that the mink is one of the extremely rare mammalian species, where congenital contaminated males too, remain with genital after-effects leading to a subsequent major sterility (Nesterov et al., 1981; Dubey, 2006).

In prenatal infections, infected fetuses presented various symptomatology. In light affections, discrete eye disturbances appeared and grave affections have been expressed by the complete tetrad of the symptomatic picture, as: a) eye diseases – microphthalmia and probably retinochoroiditis; b) nervous system affections - saltation or tetanic convulsion; c) intracerebral calcification; d) hydrocephalus. Hydrocephalus may be explained by not-suppurating encephalitis manifested by lymphoplasmocitary cell accumulation within the circum-vascular space, respectively within the Virchow-Robin space (Wallach et al., 1983).

Probably because of the cerebral hypertension, calcium dysmetaboly and of its chaotic distribution within the braincase, near to some less grave deformations, sometimes, discrete fractures on the maxilla's level, but also a light lability of the synarthrodial joints, have been observed.

Recent scientific information of high notoriety, attest that hydrocephalus (H) appears exclusively in human congenital toxoplasmosis and never has been referred to animals (Palmer et al., 2005). This study invalidates this assertion and presents for the first performance hydrocephalus also in other mammalians, excepting human being (see Figure 3 and 4).



Figure 3- Hydrocephalus and microphthalmia in congenital infection with *T.gondii*.



Figure 4. Toxoplasmosis in mink. Hydrometer by the persistence of corpus luteus.

Hydrocephalus (H), affection conditioned prevailing mesologically, represents an abnormal growth (stasis) of the cephalorachidian (spinal) liquid quantity (LCR) on the cerebral ventricular level (ensemble of *ependymar cavities*) (internal *H*) or/and within the pericerebral and perimedular subarachnoid spaces (external *H*). Congenital or acquired forms of H causes sometimes in children and mink pups an exaggerate growth of cranial volume. This is as such, determined by the LCR excess secreted by the choroids plexus, which may not be evacuated by the meningeal vessels and Pacchionian granulations, in the blood-vascular system.

In the actual acceptance the term of internal H more exactly, designates ventricular dilatations produced by a liquid excess, being secondary for some obstacles opposing to the LCR free circulation and resorption. They may be occlusive (smothering of the Sylviusduct – mesencephalic duct), but if LCR is accumulated in excess both within the ventricular

system and the perimedular subarachnoid spaces (brain tunic infection, hemorrhage, etc.), H are considered as communicant and manifest itself by intracranial hypertension (HI). Important for this case is that by the compression beard, it determines also ocular perturbations by the stasis choked disk, conducting sometimes to blindness by chorioretinitis. HI determines installation of brain edema with LCR accumulation in the Virchow-Robin spaces and of some intracranial expansive lesions (Wallach et al., 1983; Rusu, 2007).

Another observed symptom, inducted by the toxoplasmatic infection, was the discrete dermal form accompanied by fever and more or less circumscribed, or polymorphe macula-papillary eruptions and dermal lesion forms without being accompanied by fever.

If at a definitive host, such as the cat and other felines considered as auto-heteroxenes, the sexual cycle of the parasite *T. Gondii* occurs and represents the toxoplasme tank, in the mink only the second biological multiplication cycle, respectively the asexual cycle occurs (Wallach et al., 1983; Silva & Concearof, 2004).

During the asexual evolution of the parasite, ovocysts integrated by fodder, disseminate on lymphatic and hematogenic way, arriving in different tissues, where sporozoites are released, being transformed into tachizoites, which are multiplying and are forming cystoids within macrophages. If these are destroyed, the tachizoits released, are affecting other macrophages.

In suckling pups coming from infected mothers, who were re-infected, the affecting of mesenteric lymph nodes appeared, following then, on sanguine and lymphatic channel, the diffusion of the infection towards other organs. Some of the pups deceased consequently to the necrosis of the mesenteric lymph nodes and of the intestinal mucous membrane, former to the metastatic spreading of the infection.

Tissual cysts integrated by fodder, make bradyzoites to penetrate through the own intestinal lamina, where there multiply, transforming themselves into tachizoitess and, after a few hours, these are spreading hematogenous, developing a new extra-intestinal cycle with formation of cysts within the whole organism, but especially innervous system

(SNC), testicles, kidneys, liver, and muscular tissue. Compared to the immune defense of the host, cysts are meant to protect parasite for persisting for a long time within the organism, in an inactive form, during the whole lifetime of the animal. If oocysts are destroyed from the fodder only by freezing (-20°C during 30 days) or low pasteurization (+63°C during 30 minutes), in the presence of acid pH of the gastric juice, they are resting intact, representing a form of resistance and a permanent source of spreading.

The intervention of immune answer makes that tachyzoites of the most of affected organisms, to induce development of cysts by dormant

multiplication, i.e. bradyzoites. Beside the anatomic – clinical picture, the following lesions have been recorded: pneumonia, hepatitis, glomerulonephritis (Bright's disease), splenomegaly, myocarditis, epididymitis and during necropsic examination, often cachexy and sometimes, jaundice have been noticed. From histopathologic point of view, in the liver, kidneys, spleen and lymph nodes, necrotic micro-foci, determined by the toxoplasmatic cysts, presents within the cells of reticuloendothelial system and the macrophages, have been observed. (See Figure. 5, 6, 7, 8, 9, and 10). Sometimes, pneumonia showed dramatic forms, being accompanied by cuboidal metaplasia of the alveolar epithelium's cells (alveolar epithelization).

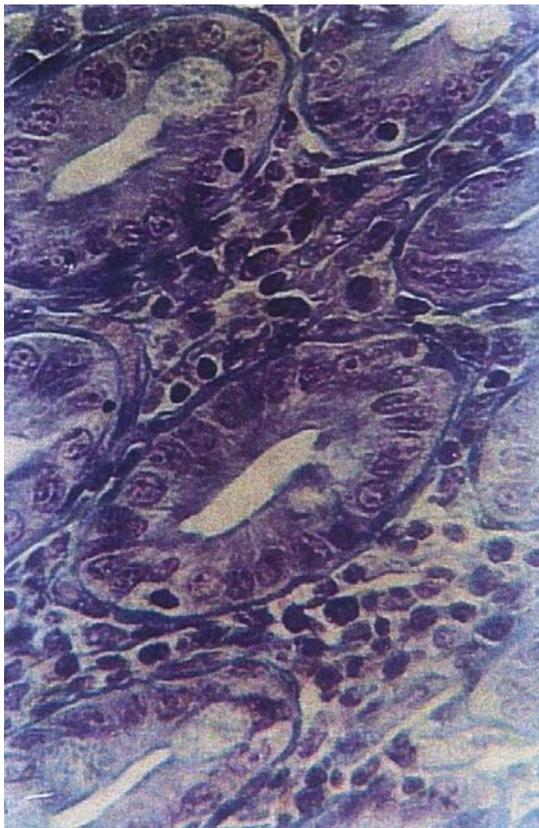


Figure 5. Kidney. *ADV* and *T. gondii* infection, (May Grunwald Giemsa x 1500).

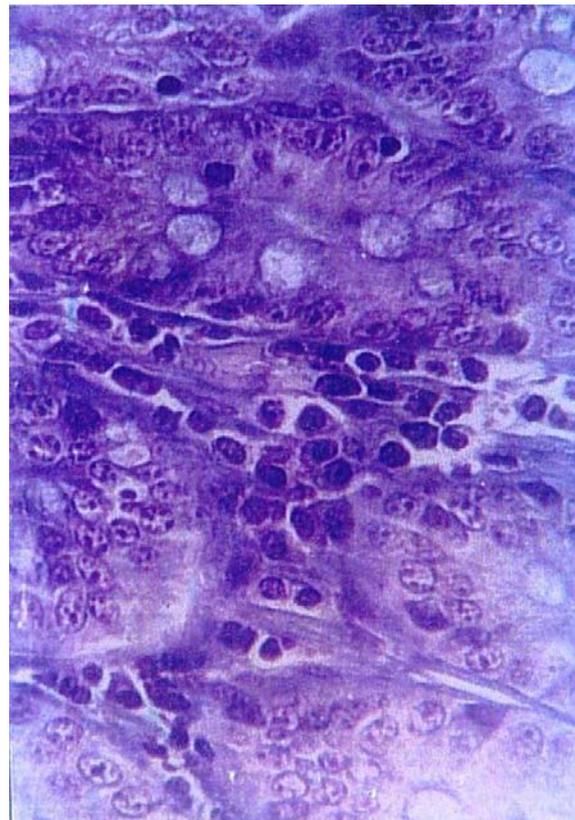


Figure 6. Peritubular lymphoplasmicitary infiltrations. Detail. (May Grunwald Giemsa x 1500)

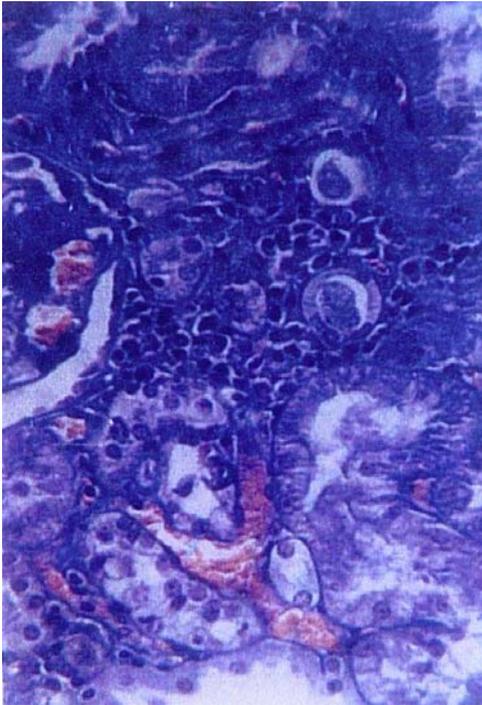


Figure 7. Kidney. Peritubular and perivascular Infiltrations.
(Tricrom Masson x 700)

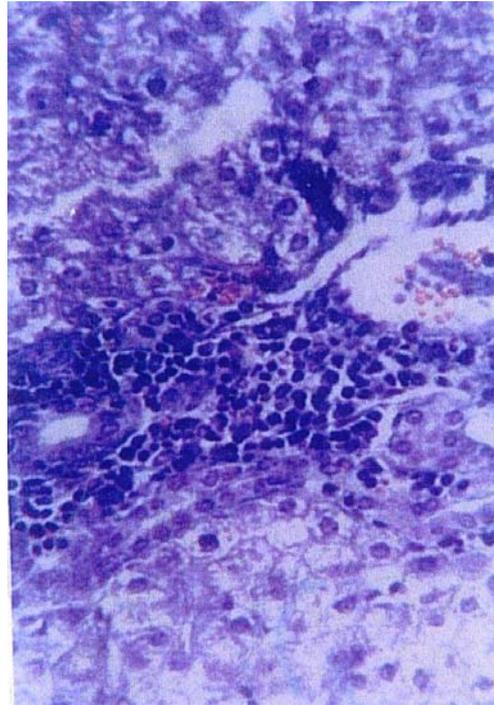


Figure 8. Liver. Plasmocytary infiltrations in the interlobular Space.
(Tricrom Masson x 800)

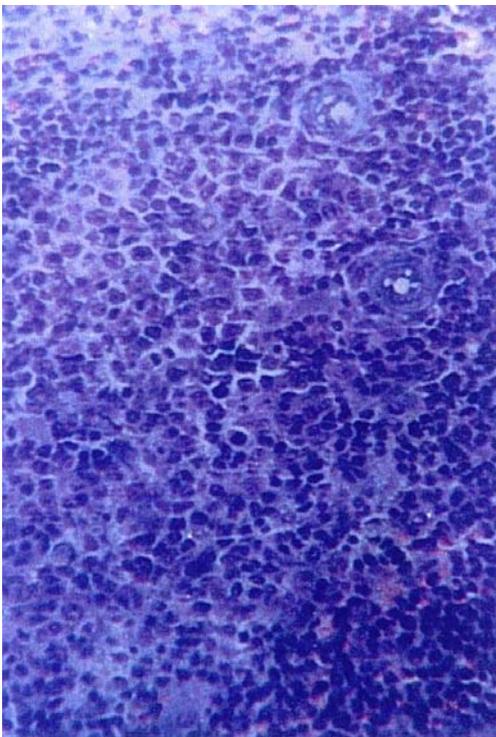


Figure 9. Lymph node. Abundant plasmocytary infiltrate in the lymph node's medullary.
(Tricrom Masson x 700)

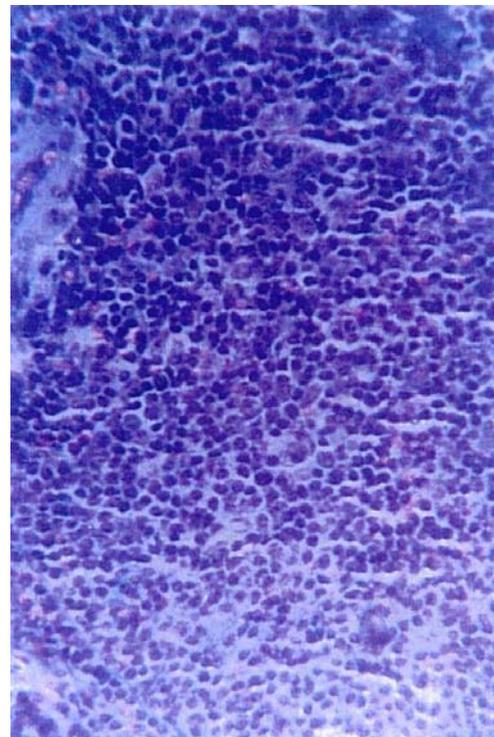


Figure 10. Spleen. Intense plasmocytary infiltrate in the spleen's structure.
(Tricrom Mosson x 700)

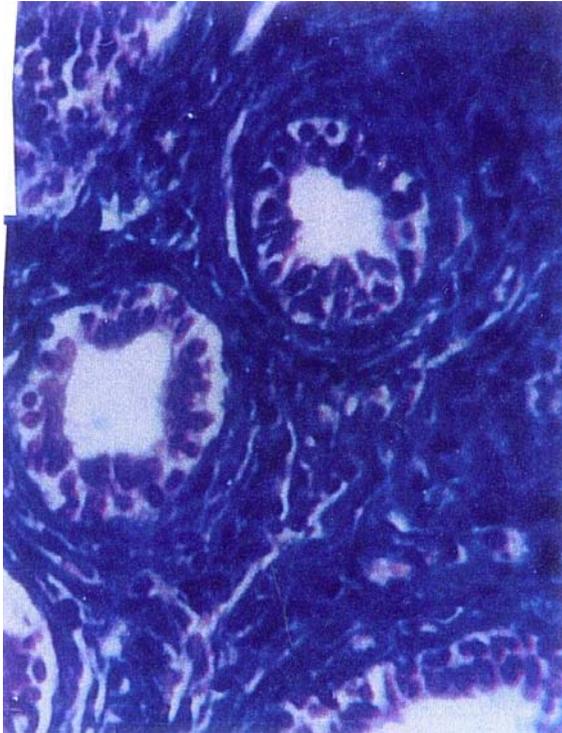


Figure 11. Mink epididymia. *ADV* and *T. gondii* infections Peritubular plasmocytary infiltrate. (Tricrom Mosson x 700)

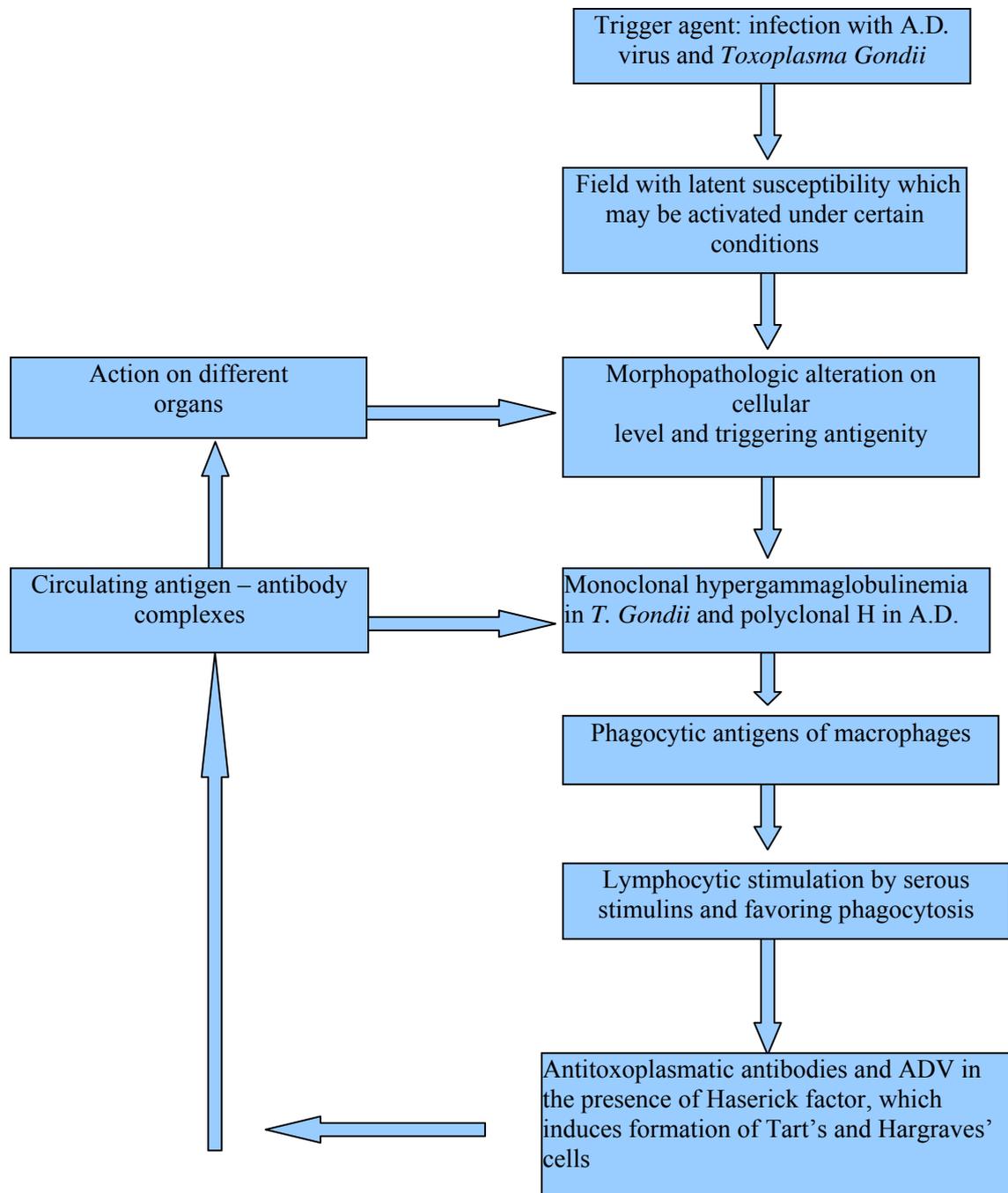
Generally, lesions of the male genital apparatus and especially of the epididymis, were expressed by lymphoplasmocytar hyperplasia (see Figure 11). Aspermatogenous orchiepididymitis manifested by degenerations of seminal epithelium, eosinophile, neutrofile and lymphoplasmocytary infiltrate, frequently having an aspect of necrotic orchiepididymitis. Self-immune phenomena which appeared concurred to the formation of anti-spermatozoid self-antibodies, with appearance of self-immune aspermatogenous orchitis, with azoospermia. Finally, these modifications led to the obturation of testicular ways, determining spermatostasis. The sperm retained within excreting channels of epididym, in the seminiferous tubs have affected in a first stage their function and those of the epididymar epithelia. Subsequently, aggravating histopathologic processes appeared, finally compromising in a rate of 65% of male, the spermatic and endocrine function of testicle.

A part of the infected minks, presenting clinical signs of toxoplasmosis, have healed due to the installed immunity.

Tachizoites localized especially in the brain and testicles, have been protected against specific anti-toxoplasmosic antibodies, which are efficient in destroying extra-cellular localized parasite forms of the rest of organism. For the immune process, compared to the humoral ones, cellular effectors seemed to be more efficient. Cytolytic effect of lymphocytes T, which stimulates lymphocytes B in producing antibodies, seems to be extremely important for secondary toxoplasmosis A.D.

Reactivity of latent cysts in the case of decreasing the organism's immunity (in A.D., AIDS, etc.) explains the appearance of toxoplasmic infection. In mink, cysts with reactivating potential are those located on cerebral, orchiepididymar and lymphonodeic level, which during serologic screening before reactivation, are resting without reaction. Disturbance by excessive stimulation of the lymphocyte B's proliferation, which in terminal differentiation stage became plasmocytar with exclusive role in producing and secreting antibodies, but also co-participation of lymphocytes T to this phenomenon, lead to abundant infiltrations of plasmocytar type in the most of the organs.

As a result of the intensive antigenic stimulation, consequently to the plasmocytar disfunction and to the concomitant synthesis of exaggerated quantities of monoclonal gamma-globulin in toxoplasmosis and, polyclonal in A.D., grave perturbations of antibody-genesis have appeared. On the ground of the active antibody decrease, persistent antibodies, without antigenic neutralizing and coordinating capacity and tropism towards specific receivers of target cells, appeared. Some of the A.D. virus' features are similar to those of the acquired immunodeficiency (AIDS) virus in man, such as: persistence of the virus, presence of antibodies unable to neutralize the virus, increased number of lymphocyte T4, bearer of CD4 membranar marker, as well as hypergammaglobulinemia, reflecting a mono- and polyclonal hyperactivity of lymphocyte B, favoring the brutal intervention of opportunistic infections on organisms without immunologic protection (7, 8). On the other hand, mature lymphocyte T8 is characterized by the CD8+ membranar marker, who's activity leads to the multiplication (under the effect of intraleukine 2 secreted by the adjuvant lymphocyte T4) and the differentiation of this cytotoxic lymphocyte, effector cell on the *T. gondii* target cells.



Scheme 1 - Pathogenic and self-maintaining circle in Toxoplasmosis and A.D.

Table 1. Classification of macroglobulinemias*

Waldenström's macroglobulinemias	Secondary macroglobulinemias
1) Malignant lymphoplasmocitary diseases - Waldenström's macroglobulinemia - Multiple myeloma with IgM - Extra-medullary plasmocytoma	1) Parasitary diseases - Toxoplasmosis - Trypanosomiasis
2) Malignant proliferations of the β series - lymphocytic - lymphocytic chronic leukemia	2) Catching diseases - infectious hepatitis (Botkin's disease)
3) Dysglobulinemia: - cold agglutinin syndrome - benign macroglobulinemia	3) Different neoplasias - lung - gallbladder, etc.
	4) Self-immune diseases - Aleutic disease - Lupus erythematosus - Rheumatic polyarteritis nodosa (Kussmaul's disease) - Sjögren's syndrome, etc.

*) adapting after Bucur Ghe., 1987

The installed immunity doesn't blocks totally the infection, because the intracellular toxoplasmic oocysts, partly are resting unaffected and are persisting for different laps of time and, under certain conditions, bradyzoites may generate new cysts, without transformation into tachyzoites. The released bradyzoites may be newly destroyed by the immune effectors and the infection seems to be in remission, but under the reserve of a recurrence with resuming cycles of oocysts (See Scheme 1).

Following the observations it results that both the self-immune disease (A.D.) of the mink and the acquired immunodeficiency syndrome (AIDS), which represents the final stage of HIV infection in human being, characterized by a grave immunosuppression and production of some opportunistic infections, have a special affinity and susceptibility of toxoplasmosis, probably related to neurotropism and incapacity of the organism to protect itself. If AIDS is declared in the presence of suppression where the lymphocyte number decreases to less than 200 CD4 lymphocytes / ml, in the situation of the AIDS – cerebral toxoplasmosis association, its declaration is done regardless of the CD4 level / ml (8).

On the analogy of AIDS also A.D. may be declared in the case of its association to toxoplasmosis, regardless to the number of CD8+ lymphocytes / ml.

Both in mink and in human being, referring to the pathogenicity resulted from the collision between A.D. and toxoplasmosis, similarly also in AIDS, the pathogenic power increases with each disease's joint effect of hypergammaglobulinemia and toxoplasmosis. One of the appearances of A.D. associated to toxoplasmosis was hypergammaglobulinemia, morbid disease within serous dysglobulinemia group of high molecular weight (see Table 1).

This state has been characterized by the presence of increased quantities of *Ig M* and the inhibition tendencies of the other *Ig*'s synthesis (Constantin, 2005). If concerning clinical appearances, between Waldenström's macroglobulinemia and secondary macroglobulinemia, no difference compared to normal exists the differences consisted only in their plasmatic concentration. At the same time, *IgM* growths induced both ESR acceleration and irrelevant and false positive serologic reactions and decreases of the organism's defending capacity by reducing the antibody synthesis.

Conclusion

Maternal infections with tachyzoites produced by *Toxoplasma gondii* have a major role in gestation losses of minks and their presence in females with gestation risks present a determining factor, which associated to grave reproduction troubles of young contaminated males, manifesting infertility, partly or totally, in a percentage of 55% - 75%.

Congenital toxoplasmosis in mink represents the consequence of the gestational female infection and of those immuno-compromised by the Aleutine disease and induction of abortus or some grave malformations.

In gestational females the highest infection risk appeared during the first third period of gestation, but the parasite's transplacental transmission occurs during the last third period of gestation, after the diapause.

Congenital contamination occurred in the acute phase of the disease, observation proved by the significant presence of *IgM* in the blood serum of females.

Following this study, hydrocephalus was noted for the first time in mink pups infected with *T. gondii*. Up to the present, hydrocephalus has been described exclusively in congenital toxoplasmosis of human being.

The management of primary prevention of toxoplasmosis in mink follows avoiding the consumption of not enough processed fodder and of the contact with potential infection vectors.

The strategy of secondary prevention has in view to decrease the fetal transmission rate by precocious detection of the disease, by a serologic screening and clinical detection of abnormal gestations, reforming proposals and clearance of these females from the reproduction effective group.

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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.

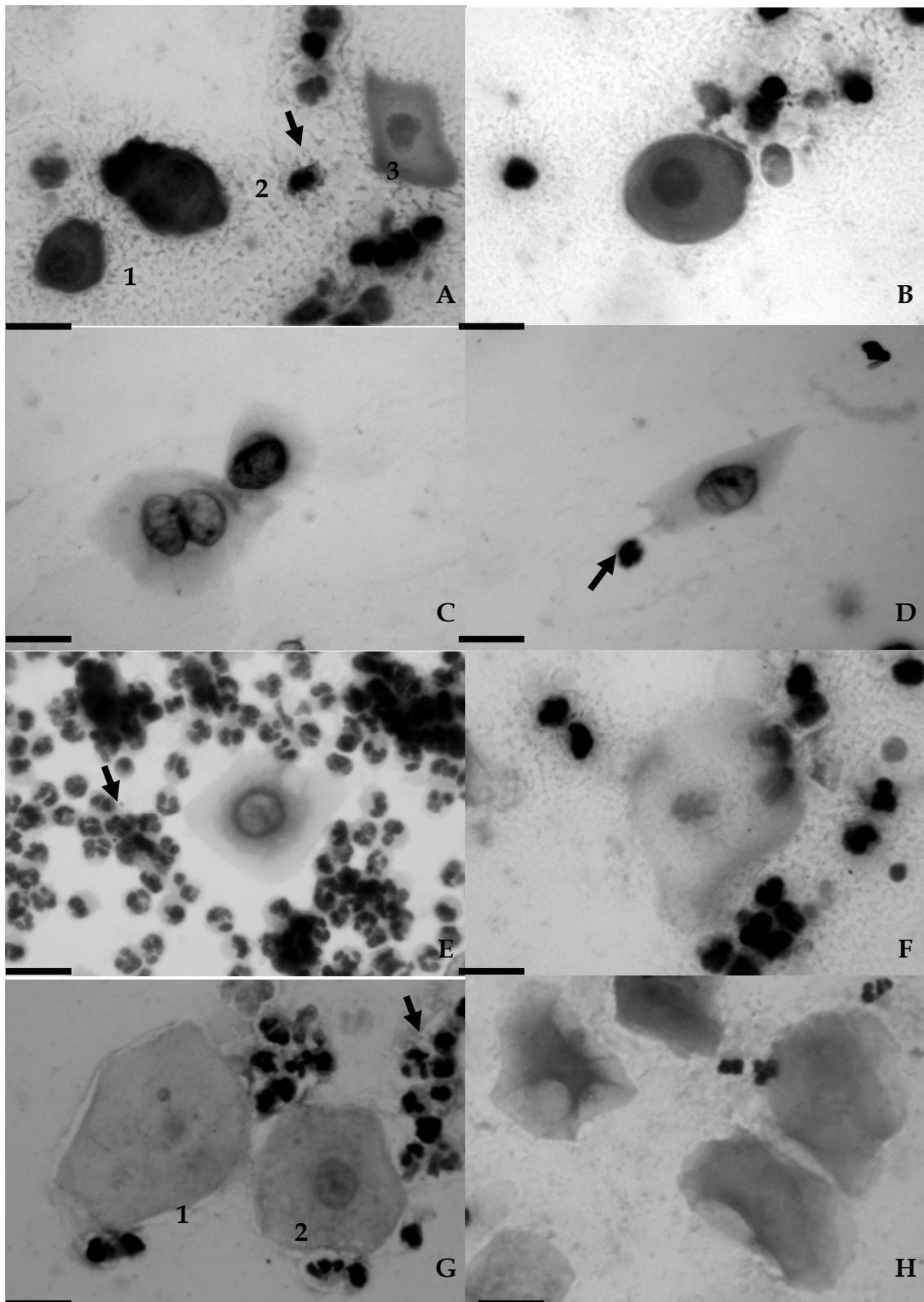


Figure 1: Cellular types observed in colposcycological samples: A.1- basal cell, A.2- parabasal cell, A.3- deep intermediate cell; B- parabasal cell; C- deep intermediate cells; D- superficial intermediate cell; E- superficial intermediate cell with keratinization around the nucleus; F- y G.2- superficial 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).

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Histological observation of the cervix of coypu (*Myocastor Coypus Bonariensis*)

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Abstract

The aim of the present study was to examine the histology of the cervix of coypu. Samples from mature sexually animals were used. Tissue was processed by routine histological techniques. The histological stains used were Mayer's hematoxylin and eosin, Masson's trichrome stain, PAS and picosirius-red. The exocervical mucosa showed squamous stratified epithelium and the lamina propria consisted of dense irregular connective tissue. The zone of transformation was found to be covered by squamous stratified epithelium. Surface epithelium of the endocervical mucosa was stratified with a superficial layer of cylindrical cells with secretory aspect. The upper limit of the endocervical channels showed small folds. Positive PAS staining was observed in the luminal edge of the cylindrical cells of the surface epithelium. Additionally, Picosirius-red staining was seen inside of the folds of the endocervical mucosa, showing the presence of collagen type I and II in the deep stroma.

Keywords: coypu, cervix, *Myocastor coypus*.

Introduction

General morphology of the reproductive organs of coypu has been studied in sexually mature animals by Rowlands & Heap (1974) and Felipe *et al.* (1998 and 2000). These studies reported that the reproductive system of coypu was similar to that of other hystricomorph rodents, like viscacha (*Lagidium sp.* and *Lagostomus maximus*) (Weir, 1974), chinchilla (*Chinchilla laniger*) (Weir, 1974), African porcupine (*Hystrix africaeaustralis*) (Van Aarde &

Skinner, 1986) and brush-tailed porcupine (*Atherurus africanus*) (Mayor *et al.*, 2003).

The uterine cervix is not only an extension of the uterus. Instead, the cervix presents its own histological and functional properties (Ludmir & Sehdev, 2000). The uterine cervix is a dynamic structure with a high capacity to adapt to different physiological events (estrous cycle and gestation) and has differential biological responses to modifications to the hormonal milieu (Challis *et al.*, 2000). These different responses involve many cellular and extracellular events. However, the structure of the cervix of the coypu is still not fully known. This information results of importance to establish the normal anatomical and histological characteristics and to allow a better understanding of the physiology of the cervix in coypu.

Material and methods

Samples were obtained from 35 animals belonging to different breeding farms. After collection, samples were fixed in buffered formol at 10 %, processed with routine techniques and then embedded in paraffin. Serial cuts of 5 µm were performed and later stained with Mayer's hematoxylin and eosin, Masson's trichrome stain, Periodic-acid-Schiff (PAS) and Picosirius-red, specifically to detect collagen (Noorlander *et al.*, 2002). Slides stained with Picosirius-red were analyzed with epipolarization microscope. Sections were placed between two crossed polarization filters (polarizer slider U-POT UP110 and analyzer slider CH3-AN CO500; Olympus, Tokyo, Japan).

Collagen type I was observed with a bright red color and collagen type II with a bright yellow-green color.

To characterize the cervix, the particular features of the mucosa of the exocervix and endocervix or endocervical channel, of the external and internal openings, of the zone of transformation and the cervical stroma were considered. A micrometric ocular incorporated to an Olympus CH2 microscope was used to determine the morphometric characteristics of the tissues. Measurements were expressed as the mean \pm S.D. Nomenclatures used for the different structures are according with *Nomina Histologica* (1994).

Results

The cervix of the coypu was observed as a thick fibromuscular projection in the vaginal fornix (*portio vaginalis cervicis*), with a length of 1.59 ± 0.7 cm and a diameter of 0.98 ± 0.56 cm. The endocervical channels were completely independent one from each other, ending in two external openings.

Exocervical mucosa

The exocervical mucosa (Fig. 1 A) was conformed by squamous stratified epithelium (5.4 ± 0.7 cellular layers) and the lamina propria consisted of dense irregular connective tissue. The height of the mucosa was 54.35 ± 8.84 μ m. Different areas of cornification were observed in relation to the points of enlargement of the epithelium or to the interpapillar sectors (Figure. 1 A).

Zone of transformation or squamous columnar junction

The area of the external openings was found to be covered by squamous stratified epithelium. The transition between this latter epithelium and the secretory columnar epithelium was extended, being characterized by stratified cylindrical epithelium adjacent to a dense connective tissue.

Endocervical mucosa

Endocervical mucosa presented a stratified surface epithelium (3.21 ± 0.71 cellular layers and a width of 34.32 ± 6.8 μ m) with a superficial layer of high cubic cells or cylindrical cells. These cells showed basal, small and oval nuclei with a supranuclear

cytoplasmatic area with light staining (Fig. 1 B). The adjacent connective tissue was irregular and dense. The mucosa of the endocervical channel showed great ramified folds with different sizes (length 40.92 ± 4.95 μ m) and crypts (Fig. 1 B and C).

Cervical stroma

The connective tissue of the cervical stroma was irregular dense and vascularized. It was observed a predominance of collagen and elastic fibers (Fig. 1 B and D).

Muscular tissue

The smooth muscular tissue changed throughout the organ. It presented fascicules that surround a vascular central area in the extremity of the vagina. In the middle zone of the vaginal area, it was forming a circular layer surrounding the endocervical channels. Throughout the supravaginal portion of the cervix, it was observed the constitution of two layers of muscular tissue: an internal layer with muscular fasces in a circular disposition and an external layer with an oblique disposition (Fig. 1 C).

Definition of the internal openings

The upper limit of the endocervical channels was conformed by a transitional zone between the endocervical epithelium and the endometrium, characterized by the presence of short folds (Fig. 1 D, E and F).

PAS positive reaction

Positive reaction to PAS was observed in the luminal edge of the cylindrical cells of the surface epithelium (Fig. 1 D, E and F).

Distribution of collagen fibers

The staining with Picrosirius red allowed observing a great amount of collagen fibers with a different disposition depending on the area. In the internal side of the folds of the endocervical mucosa it was possible to detect the presence of collagen type I in the superficial stroma parallel to the epithelium. Additionally, it was also recorded collagen type II in the deep stroma perpendicular to the epithelium. In the basis of the folds, there was collagen fiber type II and in the middle zone between both channels, the fibers were disposed parallel and horizontal between themselves.

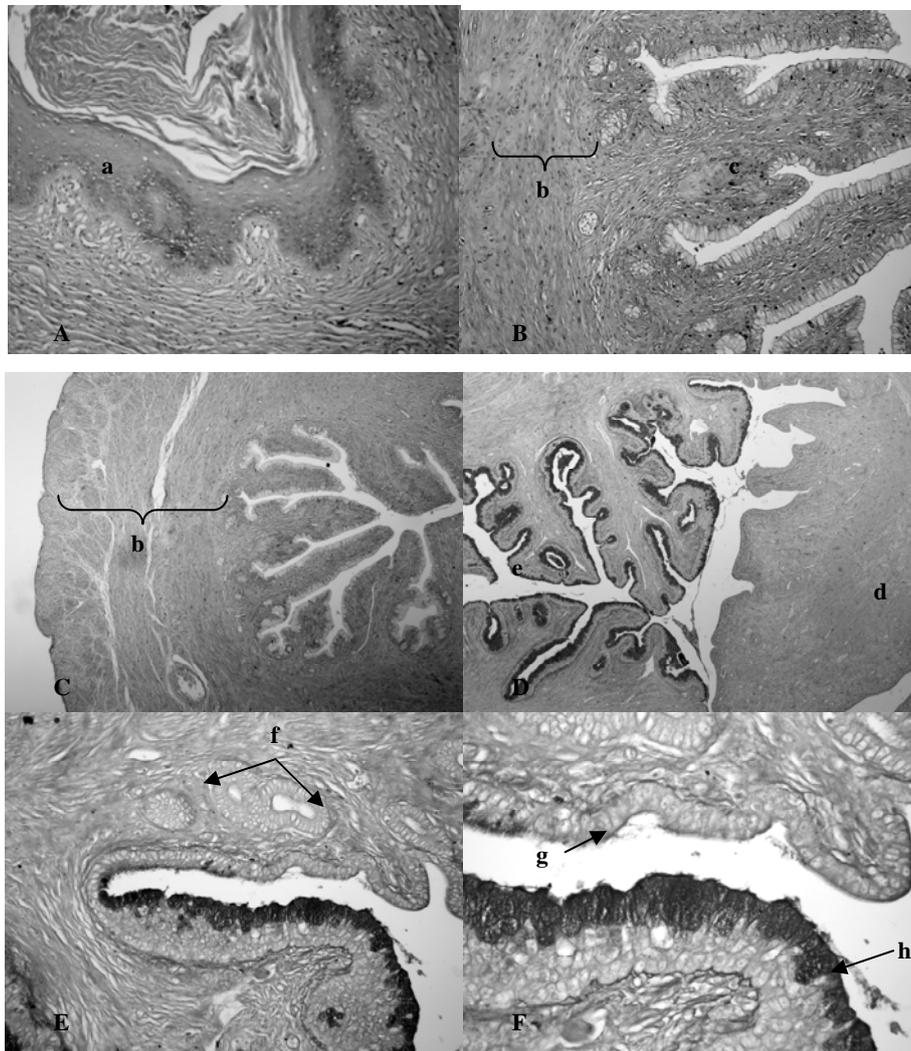


Figure 1: Pictures of the cervix of an adult *M. c. bonariensis*. A- Exocervix, 40x; B- Cervical wall, 10x; C- Supravaginal segment of the cervical wall, 10x; D- Transitional zone between uterus and cervix, 4x; E- Transitional zone between uterus and cervix, 20 x; F- Endocervical epithelium containing cells with secretory aspect (dark staining), 40 x. References: a- Exocervical epithelium, b- Miometrium, c- Stroma, d- Area of uterine horn, e- Area of the endocervix, f- Endometrial glands, g- Epithelium of the uterine horn, h- Secretory cells of the endocervical epithelium. A, B y C: Mayer's hematoxylin and eosin stain. D, E y F: Periodic-acid-Schiff (PAS) stain.

Discussion

Similarly to other hystricomorph rodents like *Myoprocta pratti* (acuchi) (Weir, 1971 c), *Chinchilla laniger* (chinchilla), *Lagostomus maximus* (vizcacha de montaña) (Weir, 1971 a) and *Dasyprocta aguti* (aguti) (Weir, 1971 b), coypu presented two endocervical channels with independent external openings. The same feature has been recorded in other non hystricomorph species like castor (*Castor fiber*) (Doboszynska, 1978), rabbits and rats (Hafez, 1993).

The presence in the cervix of a vaginal portion with solid and compact wall is comparable to that observed in other domestic species (Barone, 1996; Dyce *et al.*, 1987).

The description of the exocervical mucosa was identical to that previously recorded for the vaginal mucosa (Felipe *et al.*, 1998). The endocervical mucosa with the complex system of primary and second folds has been also reported in other domestic animals by Barone (1996). Conversely to other species in which the epithelium of the cervix has been described to be columnar and composed of ciliated with intercalated mucosal cells (Hafez, 1993; Barone, 1996), coypu presented stratified epithelium throughout the entire organ.

Crypt formation as observed in the present study has been previously reported in small ruminants, gilts and queens (Barone, 1996).

Other hystricomorph rodents as acuchi (Weir, 1971 c) and mountain viscacha lack of muscular component in the cervix, however it was possible hereby demonstrate the presence of abundant smooth muscular tissue in the cervix of coypu.

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