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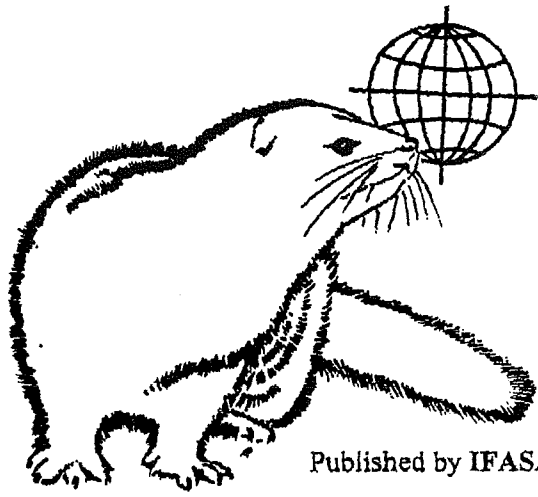

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5. Nutrition & nutritional physiology

6. Ethology, incl. Animal Welfare, Management & Production

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9. Pathology & Diseases

Notes

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Now the IFASA web site is a reality. We congratulate Bruce and Odette with this, in our opinion, good start of the future of IFASA and SCIENTIFUR.

You will from now on find a lot information on the IFASA web site which has the following address: http://www IFASA.ORG

It is the plan that approximately 25% of the contents of SCIENTIFUR vol. 24, 2000, shall appear also on the IFASA web site, as well as the entire proceedings from the VIIth INTERNATIONAL SCIENTIFIC CONGRESS IN FUR ANIMAL PRODUCTION to be held in Kastoria, Macedonia, Greece, 13-15 September, 2000.

The first announcement of the VII INTERNATIONAL SCIENTIFIC CONGRESS IN FUR ANIMAL PRODUCTION will be found on pages no. 186 and 245-246 in this issue of SCIENTIFUR and it will of course also be found on the IFASA web site.

As you will see there are many original as well as reviewed scientific reports in this issue of SCIENTIFUR. We hope that the authors can accept that it sometimes takes some months before their report occurs in SCIENTIFUR. We try very hard to do our best.

As you will see from the 1st announcement of the congress, IFASA also gives a discount to members of the organisation for participation in the congress. Together with the discount for SCIENTIFUR subscription you will earn the total payment for your membership.

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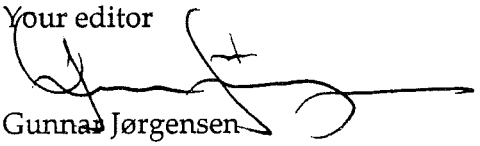

Gunnar Jørgensen

Reviewed scientific report

Bacteriological study on nutria meat

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Summary

Recognition of the contribution of the nutria (*Myocastor coypus*) industry to world food requirements has focused attention on the public health aspects of coypus meat. Operators engaged at the colonies in close contact with sick animals through management activities and slaughtering procedures are particular exposed targets. This survey investigated bacteriologically samples from the skin and tigh-muscle of the hind leg of 54 adult nutrias at pelting time. Mean values of minced beef expressed as $\log_{10}$ number of organisms per gram for Total Viable Count (TVC) at 37° C and 20° C, were 5.12 and 6.19, respectively; for *Enterobacteriacea*: 4.21; Streptococci: 1.61; *Staphylococcus aureus*: 1.13; *E.coli*: 4.10 and *Salmonella*: 2.46. Anaerobes (i.e. *Clostridium perfringens*) showed very low numbers. Mean value of TVC from surfaces was 9.19. Bacterial numbers found here are probably of a dangerous magnitude and seem to be of importance from a public health point of view.

Introduction

Besides valuable fur, nutria (*Myocastor coypus* Molina 1792) is a source of sizeable quantities of highly nutritive and tasty meat in several areas of South-America. Epidemiologic data indicate that meat and meat products account for between 50 and 90 % of the foods etiologically involved in outbreaks of classical food-transmitted disease (*Mossel,1988*). There are some 20 zoonoses or diseases that can be transmitted from nutria fresh meat to humans (*Scheuring, 1989; Wenzel, 1980*). According to our findings from carcasses routinely processed at our laboratory during the last years, infectious diseases accounted for almost 60 % of the casualties (*Martino and Stanchi, 1998*). Catastrophic bacterial infections by *Streptococcus equi* subs. *zooepidemicus, Salmonella Typhimuriun,* enteropathogenic *E.coli , Clostridium perfringens, Staphylococcus aureus* and *Yersinia pseudotuberculosis* are regularly diagnosed (*Stepanenko and Stepanenko, 1985; Cipolla et al,1987; Scheuring,*

1987; Martino and Stanchi,1991; Martino and Stanchi,1995). Most of these agents have been identified as potential human foodborne pathogens (*Acha and Szyfres, 1986; Mossel, 1988*). Concern that minced nutria meat might be a cause of food-borne illness has prompted interest in the numbers and types of bacteria commonly occurring in it.

Materials and methods

This survey investigated fifty-four, 7 months old animals killed at pelting time, coming from three farms. Fifty-four samples for bacteriological analysis were randomly taken with a bacteriological swab from the medial and lateral sides of the skin of the tigh before the carcasses were skinned (one sample, either lateral or medial, per each animal).

In addition, 54 samples from the tigh muscles (*gluteus medius*) of approximately 50 g of a minced meat were taken (one sample per each animal) and transported to the laboratory on ice packs in an insulated container. Twenty-five grams of each sample was added to 75 ml of maintenance medium comprising 0.85 % (w/v) NaCl and 0.1 % (w/v) bacteriological peptone (Oxoid L 37) in a polythene bag and homogenized on a stomacher for 1 min. Total viable counts (TVC) were done on standard plate count agar (Oxoid CM 463) incubated at 37° C for 48 h and 20° C for 96 h, and results were calculated using counts at two or more dilutions with weighing for dilution, as described by Farmiloe et al. (1954). The following counts were made by spreading duplicate 1/50 ml drops or 0.1 ml samples of the decimal dilutions on the media indicate: *Enterobacteriacea* (ENT): on violet red bile glucose agar (Oxoid CM 485), *E.coli* (EC) and *Staphylococcus aureus* (SA) on tryptose bile agar, Lancefield group D streptococci (STR) on kanamycin aesculin azide agar, *Salmonella* (SAL) on selenite broth base (Oxoid CM 395), and anaerobic blood agar medium (NBAPS) for anaerobes.

Results and discussion

Average population found from the skin-swabs was 10^4 to 10^5 aerobic bacteria per cm^2, meanwhile counts of 10^5 to 10^{12} aerobic bacteria per cm^2 are commonplace on other domestic animals' hides such as cattle, hogs or sheeps (*Ayres et al.,1980*).

Mean values of minced meat expressed as $\log_{10}$ number of organisms per gram for different type of bacteria are presented on the Table I.

Table 1. Mean values of minced meat expressed as $\log_{10}$ number of organisms per gram

Type of bacteria	at 37°C	at 20°C
TVC (total viable count)	5.12 ± 0.7	6.19 ± 1.2
Enterobacteriacea	4.21 ± 0.2	
Streptococcus (Lancefield group D)	1.61 ± 1.8	
Staphylococcus aureus	1.13 ± 2.1	
Escherichia coli	4.10 ± 1.1	
Salmonella spp	2.46 ± 1.3	
Anaerobes	0.09 ± 1.8	

Minced-meat-bacterial-numbers found here are probably of a dangerous magnitude, although assessment of risks by data on the total number of bacteria is of limited value, because there is no correlation between total counts and the numbers of *pathogens (Roberts and Hudson, 1987)*.

All meat animals carry large numbers of many different types of microorganisms in their intestines and on the skin, and most of the latter are encountered on the legs (*Mossel, 1988*). Nutrias are usually skinned before they are eviscerated. Huge numbers of organisms are associated with the animal's hide, hoof, and fur, with the gut, and with the faeces, and are an important source of contamination. Manual pelting of recently slaughtered coypus and subsequent evisceration contribute to extensive cross-contamination between carcasses. Bacteria may be introduced via the bloodstream by contaminated pithing rods or sticking knives (*Mackey and Derrick, 1979*). Such contamination can, of course, be limited markedly by adequate hygiene measures but residual levels of contamination with *Salmonella* spp, *E. coli* or streptococci represent a potential for enteric infection of consumers (*Gill,1995*) until rigid standards are imposed concerning freedom from foodborne pathogens. Slawon et al. (1995) reported the common presence of four serotypes of *Salmonella (Typhimuriun, Enteritidis, Dublin* and *Agona*) on faeces, soil and water of nutria farms, and pointed out that their presence in farm sewage is particularly dangerous from the point of view of environment protection.

Although consideration has not been given to unusual meats as nutria's, this meat must be processed in premises under supervision of the veterinary authorities and must be passed as sound and fit for human consumption, complying with the requirements of the standard health food's regulations and covered, at least, by the assessments made for diseases of other animals (livestocks, rabbits, etc.). Obviously, strict measures of hygiene should be taken to avoid post-mortem contamination by the use of inadequately cleaned and disinfected tools, tables and the staff's clothing. In addition, a dead or dying animal displaying symptoms of disease, having an abnormal temperature (> 38.5° C) or exhibiting other abnormal symptoms that would render its meat unfit for food, should be eliminated from the food chain. Therefore, there is good reason to monitor closely and constantly, numbers of bacteria in raw coypus meat offered for sale.

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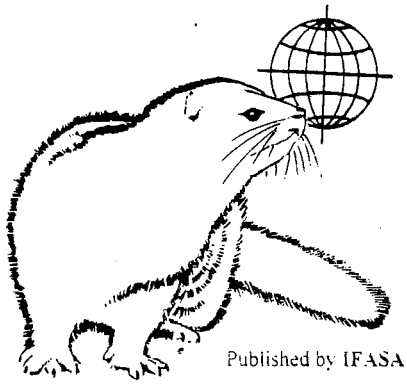

Original Report

Repeatability of mineral element content in the fur of female Greenland nutrias.

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Summary

The objective of this paper was to study repeatability of mineral composition of the fur in female Greenland nutrias in relation to their physiological condition. The trial involved 19 females of Greenland nutrias. The animals were housed in sheds, in one-storey cages with pools. They received the granular feed mixture KK, and alfalfa, and fodder beet as a saturation supplement. The concentrations of Ca, K, Na, Mg, Fe, Zn, Cu, Mn, Co in the fur of female nutrias were examined in selected body regions, i.e. in the middle of the dorsal and ventral regions, in relation to their physiological condition (periods): 1. primiparas - sexual maturity, age of 8 months – fur maturity stage, 2. females on the day of parturition, 3. females on the day of weaning. Concentrations of the mineral elements were determined by atomic absorption spectral photometry. The obtained results were evaluated mathematically and statistically. A conclusion can be drawn from our results that the mineral composition of fur in adult female nutrias varies in relation to age and physiological stage. Comparison of repeatability coefficients shows that repeatability of mineral element contents in the fur of Greenland nutrias is high, with only the repeatability coefficients of Co and Na being lower.

Introduction

The objective of this paper was to study repeatability of mineral composition of the fur in female Greenland nutrias in relation to their physiological condition.

Some of the main factors influencing the chemical composition of the animal's body are species and breed differences, effect of seasons, physiological state, age, lactation and pregnancy (*Georgievskij et al., 1982*). Marked alterations arise in nutritive conversion of the female's organism in relation to pregnancy and lactation. That is often shown in varied formation of fur and horn organs (*Kudlác and Elecko et al., 1987*). *Mertin et al.* (*1997, 1998*) studied the repeatability of mineral element content in the fur of female standard and silver nutrias. They found a high repeatability of the mineral element content within the genotype, studied body parts, age, and physiological stage.

Material and methods

The trial involved 19 females of Greenland nutrias. The animals were housed in sheds, in one-storey cages with pools. They received the granular feed mixture KK (produced by Cataj Cooperative Farm, Slovak Republic), and alfalfa (in the spring-summer period), and fodder beet (in the fall-winter period) as a saturation supplement. The concentrations of Ca, K, Na, Mg, Fe, Zn, Cu, Mn, Co in the fur of female nutrias were examined in selected body regions, i.e. in the middle of the dorsal and ventral regions, in relation to their physiological condition (periods): 1. primiparas – sexual maturity, age of 8 months – fur maturity stage, 2. females on the day of parturition, 3. females on the day of weaning. Fur samples (ca. 2g) were taken by clipping. Concentrations of mineral elements were determined by atomic absorption spectral photometry. Three measurements were performed from each sample. The obtained results were evaluated mathematically and statistically (*Winer, 1971; Grofík and Fl'ak, 1980*).

Results and discussion

Basic variations and statistic characteristics of mineral element contents in the fur of Greenland nutrias (mg/kg dry matter) according to periods and body regions are given in Table 1. In Table 2 are given the results of two- way variance analyses of the hierarchic classification with a firm effect of periods, random effect of animals with the periods and with error of experiment with content of mineral elements. Besides these items there are also F- tests of 1 – way analyses of variance of comparison of animals within the studied periods. When we compare the F – tests of periods of mineral element content in the fur of Greenland nutrias we find statistically significant differences in the concentrations of the elements Ca, K, Mg, Cu, Mn, and Co. It is a matter of course that the 2 – way analyses of variance pointed out the significant differences between the animals as these F – tests are in fact functions of 1 – way analysis of variance. Statistically significant differences between animals were not found in Na in the ventral region on the day of weaning, Ca, K, Na, Mg in the ventral region on the day of parturition, in Fe and Cu in the dorsal region on the day of parturition, Mn and Co in the ventral region on the day of parturition. The estimations of repeatability coefficients are given in Table 3 with their standard errors. The repeatability of individual elements in the fur of Greenland nutrias according to the individual regions of the body are high in Ca, K, Fe, Zn, Cu on the back (repeatability higher than 0.9), and in Ca, K, Mg, Fe, Zn, Cu, Mn on the abdomen. The repeatability was lower in Co and Na in the dorsal region. According to the repeatability coefficients we can state, in spite of the fact that there were only three repeated measurements in the animals, that there is a higher or high repeatability of the content of mineral elements in the fur of Greenland nutrias with the exception of Na in dorsal region (0.2730).

Our results document that the mineral composition of fur changes in dependence on age, genotype, and physiological state in adult females, and it is in line with studies of various animal species done by other authors (*Georgievskij et al., 1983, Kudlác and Elecko et al., 1987*), and in nutrias (*Mertin et al., 1997, 1998*). The changes in the content of mineral elements in dependence on age and physiological stages are similar with individual elements in standard, silver and Greenland nutrias.

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Table 1. Basic variation – statistical data on the contents of mineral elements (mg/ kg dry matter) in the fur of Greenland nutrias

			Calcium Ca				Potassium K				Natrium Na			
			back		abdomen		back		abdomen		back		abdomen	
P	b_i	n	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$
8m	3	9	377.60	13.37	510.17	20.94	62.21	11.45	75.55	9.38	65.97	6.69	50.51	5.60
Pa	3	9	1328.55	74.96	1801.39	11.52	425.08	3.91	189.94	6.19	355.24	7.73	126.93	4.72
W	3	9	1701.63	27.27	1441.72	95.92	661.11	91.63	336.40	46.56	227.22	74.61	45.23	5.61
T	9	27	1135.93	112.35	1251.10	111.30	382.80	56.65	200.63	25.95	216.15	33.47	74.22	7.90
			Magnesium Mg				Iron Fe				Zinc Zn			
			back		abdomen		back		abdomen		back		abdomen	
P	b_i	n	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$
8m	3	9	773.68	13.92	840.91	44.42	104.46	13.45	122.90	11.00	149.82	2.48	153.06	4.66
Pa	3	9	252.96	14.18	320.74	5.37	120.89	6.14	162.75	7.75	143.60	5.15	142.26	6.34
W	3	9	310.70	7.01	248.07	23.88	110.62	6.61	122.69	14.47	148.12	8.51	174.14	2.85
T	9	27	445.78	46.20	481.91	52.45	111.99	5,35	136.11	7,33	147.18	3.32	156.49	3.73
			Copper Cu				Manganesse Mn				Cobalt Co			
			back		abdomen		back		abdomen		back		abdomen	
P	b_i	n	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$
8m	3	9	5.8067	0.1408	5.9267	0.2869	1.1567	0.0773	1.6700	0.1213	0.7000	0.0495	0.7300	0.0337
Pa	3	9	5.2033	0.0941	8.5833	0.3012	2.8133	0.2796	5.4600	0.1020	0.7267	0.0597	0.7467	0.0464
W	3	9	9.9200	0.2691	10.6167	0.3126	3.7900	0.0896	4.4200	0.4093	0.6433	0.0287	0.7067	0.0480
T	9	27	6.9767	0.4234	8.3756	0.4118	2.5867	0.2343	3.8500	0.3436	0.6900	0.0274	0.7278	0.0242

P – periods, 8m – age of 8 months, Pa – parturition, W – weaning, T – total

Table 2. 2 – way analyses of variance of mineral elements content in the fur of Greenland nutrias.

	Calcium Ca			Potassium K		Natrium Na		Magnesium Mg	
	MS		F	MS	F	MS	F	MS	F
				Back					
P 2	4194813.49		54.71^{++}	819079.78	8.01^{+}	189101.39	6.70^{++}	733242.46	162.80^{++}
A 6	76681.15		127.02^{++}	102284.41	1399.03^{++}	282289.08	2.13	4504.10	16.38^{++}
e 18	603.70			73.11		13275.17		275.06	
P	f_{bi}	f_e							
8m	2	6	98.40^{++}		219.90^{++}		73.24^{++}		8.19^{+}
Pa	2	6	272.40^{++}		5.68^{+}		6.67^{+}		39.87^{++}
W	2	6	23.40^{++}		2241.62^{++}		2.06		50.65^{++}
				Abdomen					
P 2	3996589.78		34.68^{++}	153863.42	5.69^{+}	18815.76	25.06^{++}	872978.79	28.85^{++}
A 6	115232.06		170.98^{++}	27051.87	170.08^{++}	750.95	8.34^{++}	30262.20	151.21^{++}
e 18	673.97			159.05		90.06		200.13	
P	f_{bi}	f_e							
8m	2	6	307.62^{++}		157.65^{++}		67.46^{++}		283.26^{++}
Pa	2	6	0.67		2.15		1.09		0.18
W	2	6	492.23^{++}		407.40^{++}		16.38^{++}		789.15^{++}

Table 2. continued

	Iron Fe			Zinc Zn		Copper Cu		Manganese Mn		Cobalt Co	
	MS		F	MS	F	MS	F	MS	F	MS	F
Back											
P 2	620.35		0.21	93.09	0.08	59.2957	52.906^{++}	15.9493	16.396^{++}	0.0163	0.298
A 6	2956.46		46.37^{++}	1249.55	344.67^{++}	1.1208	36.089^{++}	0.9728	21.882^{++}	0.0548	
e 18	63.75			3.63		0.0310		0.0444		0.0091	6.004^{++}
P	f_{hi}	f_e									
8m	2	6	1555.85^{++}		39.55^{++}		29.940^{++}		33.475^{++}		7 .627^{+}
Pa	2	6	5.03		211.84^{++}		3.013		21.176^{++}		8.898^{+}
W	2	6	83.64^{++}		2107.04^{++}		138.783^{++}		23.123^{++}		0.593
Abdomen											
P 2	4790.51		1.05	2365.48	2.87	49.7826	15.907^{++}	34.5123	15.974^{++}	0.0036	0.072
A 6	4547.43		100.36^{++}	823.51	151.45^{++}	3.1296	78.240^{++}	2.1605	42.792^{++}	0.0506	9.120^{++}
e 18	45.31			5.44		0.0400		0.0505		0.0055	
P	f_{hi}	f_e									
8m	2	6	2392.41^{++}		227.91^{++}		99.439^{++}		27.017^{++}		12.974^{+}
Pa	2	6	16.97^{++}		277.09^{++}		54.954^{++}		0.545		4.990
W	2	6	289.70^{++}		34.54^{++}		98.389^{++}		210.904^{++}		16.031^{++}

$F_{0.05}$ (2, 6) = 5.143 $F_{0.05}$ (6, 18) = 2.661 P – period A – animals e - error
$F_{0.01}$ (2, 6) = 10.925 $F_{0.01}$ (2, 18) = 4.015 8m – age of 8 months, Pa - parturition
W - weaning

Table 3. Coefficients of repeatability of content of mineral elements in the fur of Greenland nutrias

	Calcium Ca		Potassium K		Natrium Na		Magnesium Mg		Iron Fe	
	ρ	S_ρ	ρ	S_ρ	ρ	S_ρ	ρ	S_ρ	ρ	S_ρ
Period	Back									
8m	0.970120	0.025361	0.986481	0.011603	0.960125	0.033614	0.705653	0.204890	0.998074	0.001666
Pa	0.989067	0.009399	0.609553	0.250121	0.653781	0.230629	0.928352	0.059085	0.573257	0.264429
W	0.882000	0.094152	0.998663	0.001157	0.261613	0.324682	0.943018	0.047473	0.964968	0.029630
T	0.976747	0.013543	0.997859	0.001265	0.272982	0.221645	0.836737	0.086075	0.937983	0.035173
	Abdomen									
8m	0.990311	0.008337	0.981208	0.016070	0.956810	0.036327	0.989483	0.009044	0.998747	0.001084
Pa	-0.124147	0.243938	0.277662	0.324318	0.029431	0.296671	-0.375100	0.099160	0.841821	0.122541
W	0.993930	0.005236	0.992672	0.006315	0.836773	0.125977	0.996208	0.003276	0.989716	0.008846
T	0.982656	0.010142	0.982566	0.010194	0.709827	0.138460	0.980419	0.011433	0.970693	0.017000

	Zinc Zn		Copper Cu		Manganese Mn		Cobalt Co	
	ρ	S_ρ	ρ	S_ρ	ρ	S_ρ	ρ	S_ρ
Back								
8m	0.927794	0.059522	0.906074	0.076249	0.915432	0.069109	0.688360	0.213816
Pa	0.985971	0.012036	0.401578	0.311494	0.870556	0.102428	0.724724	0.194646
W	0.998578	0.001231	0.978691	0.018192	0.880590	0.095180	-0.157076	0.229086
T	0.991346	0.005090	0.921237	0.044150	0.874381	0.068093	0.652171	0.166339
Abdomen								
8m	0.986951	0.011202	0.970426	0.025107	0.896612	0.083365	0.799653	0.150331
Pa	0.989251	0.009242	0.947326	0.044015	-0.178651	0.218676	0.570796	0.265344
W	0.917900	0.067209	0.970116	0.025364	0.985909	0.012088	0.833616	0.128110
T	0.980449	0.011416	0.962612	0.021568	0.933023	0.037855	0.730222	0.130898

8m – age of 8 months, Pa – parturition, W – weaning, T - total

Original Report

Effect of yucca feed additive on manure nitrogen content and production performance in mink and blue foxes

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Summary

Effects of a Yucca schidigera extract (Micro Aid) on manure nitrogen content as well as on weight gain, feed consumption, hemoglobin values and fur properties were studied in growing standard farm mink and blue foxes under traditional shed conditions. Dietary groups were comprised of control and yucca animals. N=42 and N=20 for mink and blue foxes of both sexes. Supplementation of the feed with yucca extract (120 ppm in daily feed ration) did not significantly affect growth or feed consumption in mink but did decrease weight gain in male blue foxes. Hemoglobin values in early September tended to be lower in male mink supplemented with yucca compared to controls. Skin length, fur quality, mass and cover were equal in all groups. Fur chewings were most common in female mink skins from the yucca group (14.6 % yucca vs. 7.3% control). Deteriorated belly furs were most common in male mink of the yucca-supplemented groups (33.3% yucca vs. 18.6 % control). Yucca extract decreased the total nitrogen content of mink manure but increased it in blue fox manure. Only decreases in the NO_3 N concentrations were parallel in both species. The effects of yucca extract on nitrogen in manure were small compared to the differences between the two manure collection periods (Sept vs. Oct).

Introduction

Ammonia is a volatile compound which easily escapes to the environment. In animal husbandry, high levels of ammonia are typically a by-product of large concentrations of manure. Ammonia can also get converted to nitrate and move to the soil. In addition to environmental pollution (*Pettigrew 1992*), excessive ammonia and nitrogen can cause damage to animal welfare and production. For example, ammonia concentrations of even below 100 ppm are known to reduce feed intake and average daily weight gain in pigs (*Stombaugh et al. 1969*).

Yucca saponin is a natural plant product made by drying and pulverizing the stems of Yucca schidigera (*Johnson et al. 1981*). The commercial product, Micro Aid, contains a combination of yucca saponin surfactants and a urease inhibitor. Micro Aid restricts ammonia production by stopping the urease enzyme from breaking down urea into ammonia in both manure and in the gut (*Micro Aid Information 1990, Asplund and Goodall 1991*). Commercial yucca extracts have been experimentally used as feed additives on swine and poultry farms to reduce ammonia malodors (*Johnson et al. 1981, Sutton et al. 1992, Walker 1993*). Some experiments with feed supplementation by yucca extracts have resulted in higher daily weight gain and/or feed utilization (*Goodall*

and Matsushima 1978, Johnson et al. 1981, Micro Aid Information 1990), although there are also results showing no observable or negative effects (*Dziuk et al. 1985, Johnson et al. 1982, Yen and Bond 1993, Balog et al. 1994, Kemme et al. 1995,Kiiskinen, 1995*).

Excessive ammonia and nitrogen can be problematic also on fur farms. Known examples are observed damages to neighbouring trees and extra load to waterways (*Latvala 1994*). In addition to manure gathered below cages, faecal and urine accumulation can occur also within nestboxes during the growing and whelping periods (*Harri et al. 1992, Pedersen and Jeppesen 1992*). Elevated ammonia levels, exceeding 40 ppm, have been measured in the nestboxes of farmed raccoon dogs and polecats (*Korhonen and Nurminen 1986, Korhonen and Harri 1986*). Thus, it is quite obvious that various means to diminish excessive ammonia and nitrogen release on fur farms should be encouraged. The present study sought to clarify how feed supplementation with the yucca saponin extract, Micro Aid, affects manure nitrogen content, weight gain, feed consumption, hemoglobin values and fur properties in growing blue foxes and mink.

Materials and methods

The experiments were carried out at Kannus research station. Subjects were 42 standard male and 42 female mink kits, and 20 male and 20 female blue fox kits. The mink were raised as male-female pairs in traditional wire-mesh cages measuring 40 cm wide x 60 cm long x 40 cm high. They had access to wooden nestboxes with dry oat straw bedding. The foxes were housed similarly, but without nestboxes, in cages measuring 103 cm wide x 110 cm long x 70 cm high. Experimental feeding of the mink and blue fox groups commenced on July 16th and August 6th, respectively. The litters were divided in half to form experimental dietary groups as follows: (1) a control group, given conventional standard feed, and (2) a yucca-supplemented dietary group (standard diet + 120 ppm yucca extract (Micro Aid, Distributors Processing, Inc., Porterville, CA, USA). The standard feed was a conventional fresh-mixed farm feed manufactured by the local feed kitchen. Dietary (Table 1) and chemical (Table 2) composition of the standard feed was based on the annual recommendations of the Finnish Fur Breeders' Association. Feed and water were available to the mink ad libitum throughout. Daily feed ration of the blue foxes was ad libitum until mid-September, but thereafter slightly restricted (700 g/animal/day) to prevent excessive obesity. The animals were fed twice a day until September and once daily thereafter.

Table 1. Composition of mink and fox diets.

Ingredient, %	Mink	Fox
Slaughterhouse offal[a)]	40	25
Fur animal carcasses	-	10
Fish[b)]	20	20
Fish silage[c)]	10	10
Fish meal	-	4
Cereals[d)]	12	18
Vitamins[e)]	2	2

a) beef offal, Pouttu Oy, Kannus
b) cod 56%, Baltic herring 35%, redfish 9%
c) Baltic herring
d) cooked wheat 50% and barley 50%
e) 1 kg mixture contains: vitamin A 500,000 IU; vitamin D_3, 50,000 IU; vitamin C, 6,000 mg; vitamin E, 4,000 mg; vitamin K, 10 mg; vitamin B_1, 1,500 mg; vitamin B_2, 600 mg; vitamin B_{12}, 1 mg; choline, 2500 mg; pantothenic acid, 500 mg; nicotinic acid, 1,000 mg; pyridoxin, 400 mg; folic acid, 50 mg; and biotin, 3 mg,

Table 2. Approximate chemical composition of the diets. ME=metabolizable energy

Analyzed	Mink	Fox
Dry matter, %	40.2	42.4
In dry matter, %		
Ash	7.5	9.4
Protein	33.1	28.3
Fat	27.9	33.6
Carbohydrates	31.8	29.2
ME, MJ/kg dry matter	19.2	19.9
% ME from		
Protein	29.2	24.1
Fat	51.9	59.2
Carbohydrates	18.9	16.7

The animals were weighed when grouped, and thereafter about once a month by a Lario balance. Feed intake was measured daily on a group basis. Each group received a weighed quantity of feed daily. Feed refusals were collected and weighed next day to feeding. Ten male mink were randomly selected for hemoglobin measurements. Blood samples for hemoglobin analyses were obtained by clipping the tip of a claw. Hemoglobin samples were collected twice during the experimental period: on Sept 3rd and Oct 14th. Total hemoglobin (g Hb/100 ml) was measured by a Spencer Optical B-hemoglobin Meter (American Optical Co., New York, USA). The experiment ended at pelting. After pelting, fur characteristics were evaluated by professionals. Quality, mass and cover were subjectively graded on a scale of 1 - 10, whereby 10 was the best and 1 the poorest. Fur chewing and deteriorated belly fur were considered to be fur defects. These were evaluated on a scale of 1 - 3, with 3 as the maximum and 1 the minimum degree of the defect.

Manure samples were obtained at two intervals: Sept. 9th - 11th, and Oct. 29th - 31st. Samples in all groups were collected from six randomly selected males and from the same individuals at each interval. A collection tray was placed below the cage of each animal sampled. Manure was collected individually every study day. Samples were frozen immediately after collection and stored at -20 °C until assay. The duplicate sample analysis included dry matter, pH, total N, soluble N, NH_4 N and NO_3 N and the analyses were performed by the Laboratory of Animal Nutrition, Agricultural Research Centre of Finland in Jokioinen. Feed samples were stored frozen (-20°C) until analyzed. Chemical composition of the diets was determined by the Feed Laboratory of the Finnish Fur Breeders' Association. The diets were analyzed for dry matter, ash and Kjeldahl nitrogen according to standard procedures. Dietary fat content was determined after HCL hydrolysis.Statistical analyses were performed by the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute, Inc. 1988) using the Tukey's Studentized Range (HSD) test for variables.

Results

In mink, feed consumption of control and yucca-supplemented groups was 30.6 kg/animal for the total study period (July 16th - Nov. 29th). The daily feed ration was excessive and more than that consumed by the animals each month. In the blue fox yucca- supplemented group, feed consumption between Aug. 6th and Nov. 25th was 68.6 kg/animal and 67.8 kg/animal in the control group. Only during August and September was some of the feed left uneaten by the foxes, while at other times the entire rations were consumed. No signs of deteriorated appetite or diarrhea were detected in either the mink or foxes. One female mink and one blue fox from the yucca-supplemented group died and therefore were omitted from the weight comparisons. Initial body weights in mink and blue foxes did not differ among the dietary groups (Fig. 1). Nor were any statistically significant differences encountered in the final body weights between the mink groups. Weight gain of blue fox females in both groups were similar. In males, however, control animals were significantly heavier ($p<0.05$) compared to yucca-supplemented ones (Fig. 1).

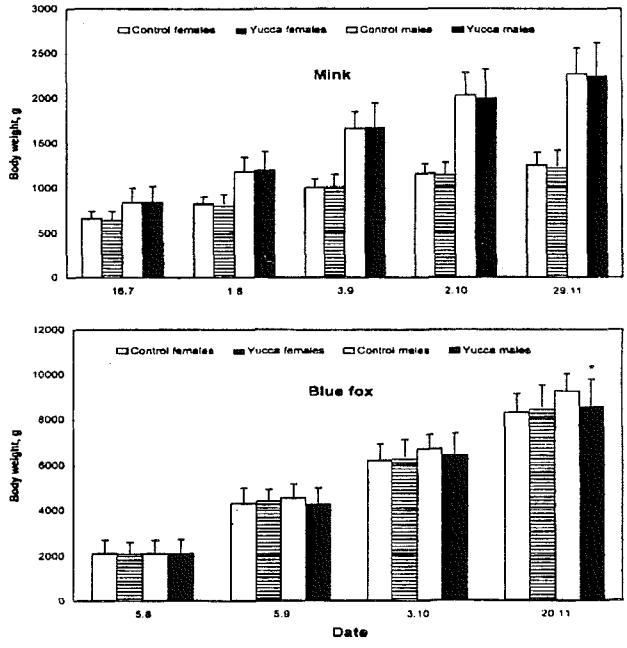

Fig.1. Body weights (mean ± SD) in dietary groups. Significantly different from the control: *$p<0.05$.

Hemoglobin was measured in 10 male mink from both groups twice during the test period. On Sept. 3rd the values for the yucca- supplemented and control groups were 17.8 ± 1.2 and 18.7 ± 0.9 g/100 ml, respectively. The difference was not statistically significant, but there was some tendency ($p<0.09$) for lower values in the yucca-supplemented groups. On Oct. 14th the hemoglobin concentrations measured for each group were identical, i.e. 18.3 ± 0.8 g/100 ml.

Skin length did not differ significantly between the mink and blue fox groups (Table 3). Fur quality, fur mass and fur cover were also similar among the groups in both species studied (Table 3).

Table 3. Skin length (cm) and fur properties. N=42 mink and N=20 blue foxes of each sex. The results are as mean ± SD. The groups within species did not differ significantly from eachother.

		MINK		BLUE FOX	
		Control	Yucca	Control	Yucca
Skin length	♂	72.9 ± 3.9	72.8 ± 4.8	105.3 ± 4.3	107.2 ± 2.9
	♀	60.8 ± 2.2	59.9 ± 2.9	101.2 ± 3.8	102.7 ± 2.8
Mass of fur	♂	7.3 ± 1.8	7.2 ± 1.6	7.2 ± 1.4	7.4 ± 1.3
	♀	9.0 ± 1.1	8.7 ± 1.0	6.5 ± 1.6	6.5 ± 1.7
Cover of fur	♂	8.8 ± 0.9	8.5 ± 1.6	7.7 ± 1.5	8.1 ± 1.1
	♀	9.3 ± 0.6	9.3 ± 0.5	7.8 ± 1.8	7.2 ± 1.7
Fur quality	♂	6.6 ± 2.5	6.8 ± 2.4	8.0 ± 1.7	8.2 ± 1.6
	♀	9.2 ± 0.9	9.0 ± 0.9	7.5 ± 2.0	7.2 ± 2.0

Table 4. Fur defects of the mink skins. Degree of defect is the sum of fur defect points per number of skins in the group. Note that data on fur chewing and deteriorated belly fur concerns different sexes.

		Control			Yucca		
		N	%	Degree	N	%	Degree
Fur chewing	♂	3	7.3	0.17	6	14.6	0.29
Deteriorated belly fur	♀	8	18.6	0.33	8	33.3	0.52

Fur defects are presented in Table 4. No fur defects were found in blue foxes. In mink, however, more fur chewings were detected in the yucca-supplemented females compared to control females. Furthermore, more males with deteriorated belly fur were in yucca than in control group.

Yucca feed additive decreased significantly the amounts of total ($p<0.05$) and soluble nitrogen ($p<0.01$) in the mink manure (Table 5). In blue foxes, on the other hand, the amount of total nitrogen was higher ($p<0.01$) in the yucca-supplemented animals but the amount of NO_3-nitrogen lower ($p<0.05$) than in the control animals. There were also a tendency that the amounts of soluble and NH_4-nitrogen were slightly higher in the yucca group in comparison to the control. Other nitrogen fractions, with the exception of NO_3 N, were higher in September than October in both species. Total nitrogen and NH_4 N concentrations were higher and, correspondingly, soluble nitrogen concentration lower, in blue fox manure compared to that of mink. The effects of

yucca extract on nitrogen in manure were small compared to the differences between the two manure collection periods.

Discussion

Previous results on the effects of yucca feed supplementation on animal performance have been ambiguous. The Swine Research Report by the manufacturer of Micro Aid (Micro Aid Information 1990) mentions that in 3 out of 4 trials yucca extract stimulated average daily gain in young pigs by approximately 6 %. Feed consumption and feed efficiency improved by over 4 %. However, Yen and Pond (1993) found that yucca extract had no effect on growth in young pigs. Johnson et al. (1981) reported a significant increase in the body weights of broilers supplemented with yucca, but no significant influence on feed efficiency. The other study of Johnson et al. of 1982 indicated a non-significant weight increase. When the concentration of yucca extract in feed was 125 - 150 ppm, the body weights of broilers decreased in the experiments of Balog et al. (1994) and Kiiskinen (1995). In the present study, no signs of increased weight gain or feed efficiency were observed in the mink or blue fox yucca- supplemented groups. On the contrary, the final body weights of blue fox male controls were significantly heavier than those of the yucca group. It appears to be obvious that the presently studied yucca extract neither promoted extra growth nor feed utilization in farmed juvenile mink and blue foxes. Obviously, excessive ammonia accumulations potentially detrimental to growing fur animals do not occur in the high ventilation conditions on traditional outdoor fur farms. In swine and poultry production, however, the situation is more pronounced because the animals are raised in closed facilities.

Table 5. Manure analyses. Nitrogen is presented as % in dry matter. Six samples were taken of mink and blue fox males in each month.

	CONTROL			YUCCA		
	Sept.	Oct.	Mean	Sept.	Oct.	Mean
MINK						
Dry matter %	29.1	28.3	28.7	28.7	28.2	28.4
pH	7.0	7.4	7.2	7.0	7.3	7.1
Total-N	5.57	4.22	4.89	5.27	3.96	4.62
Soluble-N	3.11	2.30	2.71	2.94	1.96	2.45
NH_4-N	0.36	0.24	0.30	0.36	0.23	0.29
NO_3-N	0.01	0.03	0.02	0.01	0.03	0.02
BLUE FOX						
Dry matter %	31.2	25.6	28.4	30.3	26.4	28.3
pH	7.2	7.0	7.1	7.5	6.7	7.1
Total-N	5.33	4.60	4.97	5.82	4.84	5.33
Soluble-N	2.35	1.91	2.13	2.49	2.01	2.25
NH_4-N	0.67	0.45	0.56	0.82	0.48	0.65
NO_3-N	0.02	0.03	0.03	0.01	0.03	0.02

Significance: *p<0.05, **p<0.01, ***p<0.001. NS=not significant.

	Between groups		Between months	
	MINK	BLUE FOX	MINK	BLUE FOX
Total-N	*	**	***	***
Soluble-N	**	NS	***	***
NH_4-N	NS	NS	*	***
NO_3-N	NS	*	***	***

In broilers, supplementation with yucca extract (125 ppm) has been found to decrease total hemoglobin values (*Balog et al. 1994*). This is obviously because saponins can reduce iron absorption (*Southon et al. 1988*). In the present study, the hemoglobin values of the experimental groups were within normal ranges. However, the values from early September showed some tendency toward decreased hemoglobin values in the yucca-supplemented dietary groups. Unfortunately, the hemoglobin values of the test animals were not measured in July - August when differences between the groups might have been more pronounced as a result of younger age. Possible adverse effects of Micro Aid on iron absorption in farmed fur animals requires further clarification. The yucca supplement did not significantly influence commercially important fur parameters, such as fur quality, mass or cover. Increased fur chewing on the skins of female mink, and, correspondingly, more deteriorated belly furs in male mink were found, however. Mink kits with low hemoglobin values in July - August have been found to develop a winter fur coat with defective pigmentation and hair structure of the under fur (*Näveri et al. 1984*). The present fur defects were not necessarily the most common symptoms of subnormal hemoglobin values and can, thus, indicatate behavioural or other nutritional disturbances (*Houbak and Hansen 1996*).

The effects of yucca extract on nitrogen in manure were small compared to the differences between the two manure collection periods. The clear difference in nitrogen content between September and October was due to the fact that feed conversion efficiency of mink and blue fox kits is increasing when the kits are growing (*Mink Production 1985, Einarsson and Skrede 1989*). Thus, still in September, the kits utilize feed nitrogen rather poorly which can be seen as high nitrogen content in the manure.

The present results showed that the Micro Aid supplementation of 120 ppm in the feed lowered the amount of total nitrogen in mink manure, but its effect on blue fox manure was the reverse. Only the decrease in NO_3 nitrogen levels were parallel in each species. Obviously, the differences observed reflect the fact that the digestive tract function and energy metabolism are somewhat different between the mink and blue fox. In the wild, the mink is known to have a more carnivorous diet in comparison to that of the blue fox.

According to the present results, the possibility of Yucca feed additive to prevent excessive ammonia and nitrogen releases from fur farm manure seems to be questionable. This conclusion agrees with previous findings: Rouvinen et al. (1996) carried out a nitrogen balance trial during a two-week period in autumn. Dietary supplementation contained 350 ppm and 500 ppm of Yucca shidigera extract in the mink feed. Their results showed that Yucca shidigera at the studied levels did not affect ammonia liberation from mink manure.

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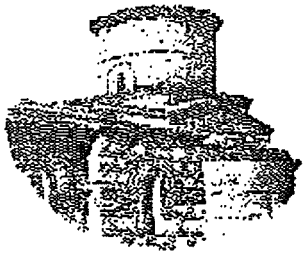
VII International Scientific Congress in Fur Animal Production

IFASA



13-15 September 2000
KASTORIA, MACEDONIA, GREECE

Organized by:

International Fur Animal Scientific Association
and
Prefecture of Kastoria
Prefecture of Kozani
Municipality of Kastoria
Chamber of Commerce & Industry of Kastoria
Greek Fur Trade Federation
Greek Fur Breeders Association
Greek Fur Center
Kastorian Development Agency
EDIKA S.A.

Dear friends,

On behalf of the International Fur Animal Scientific Association (IFASA), I am pleased to announce that the VII International Scientific Congress in Fur Animal Production will be held in Kastoria, Macedonia, Greece from September 13 - 15, 2000.

The purposes of the congress are to present scientific information on fur animal biology, production and welfare and to provide a forum for interaction among scientists from throughout the world.

As in previous successful congresses, the aim of IFASA is to maintain a high standard of scientific presentation at the VII Congress. The local Committee in Kastoria will also provide a similar high standard of organization of the Congress. Together we will succeed in putting together a gathering which will not only be very useful and productive for the future of fur animal science, but also will provide the attendees with an opportunity to sample the hospitality of the Kastorians and to have a very pleasant stay.

We look forward to seeing you in Kastoria.

Best regards,

Dr. Einar J. Einarsson
President of IFASA

More information at page 245-246

Original Report

Attempt to Use Unconventional Supplements in Growing Polar Fox Nutrition

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**Chair of Fur-bearing Animal Breeding*

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Abstract

The studies on using unconventional supplements in polar fox nutrition were conducted on 60 growing animals, in the period from weaning to slaughtering. The animals were divided into two equal groups. The experimental factor was a feed supplement, known on the market under a trade name DigDeo-Korector, which contains yeast that facilitates digestion, plant extracts from *Yucca schidigera*, probiotic bacteria and zinc bioplex. This preparation was added to the rations for the experimental group (II), in the amount of 5 g per animal. The other ration components were the same in both groups. The research included an analysis of daily gains, appearance and pelt evaluation. Additionally, digestibility-balance tests were conducted on five females from each group in September. The research results indicate that DigDeo–Korektor has a positive effect on the performance indices and digestibility of growing polar foxes.

Introduction

The feeds used in carnivorous fur-bearing animal nutrition are in most cases waste feeds. They contain different kinds of slaughter wastes, meat offal, meat from animal carcasses, wastes coming from the fishing, dairy, agricultural and food industry, etc. They are often characterized by lower sanitary quality and, compared with the initial product, changed chemical composition. Therefore, they require additional treatment and balancing of the deficiency of certain components in diet. Although products which cause diseases or other health problems should not be used on fur-bearing animal farms, such cases are noted from time to time, often leading to considerable economic losses.

A tendency towards maximum use of the genetic potential of animals, by means of securing them the optimum feeding conditions, is observed at present in carnivorous fur-bearing animal nutrition. Their maximum growth and pelt size, as well as the best parameters of fur cover quality (first of all fur cover thickness), are achieved through increasing the level of protein, fat and energy in rations (*Ahlstrom 1995; Skrede, Ahlstrom 1992*).

Rations are modified with the help of different supplements and protein-energy concentrates, often stabilized and treated with chemical substances. Although in this way the intended

goal is reached, such a solution ignores the effect of an excessive organism load and increased metabolism on the health state of animals. Due to a short production cycle in fox and mink breeding, the defects of a feeding system generally do not manifest themselves in the form of diseases of growing animals.

Another problem is connected with reproduction results which are worse than those noted a few dozen years ago, when on many farms animals received rations with an addition of whey or sour milk, containing larger amounts of complete feeds (e.g. animal carcasses) and feed supplements (e.g. cereal shoots, yeast). Research shows that fox and mink feeding, even if consistent with the relevant standards, may cause subclinical states of internal organs, mainly such as kidneys and liver, as well as inflammation of the alimentary tract (*Lorek et al. 1997; Lorek et al. 1998; Rotkiewicz et al. 1995*).

According to the recent trends observed in animal nutrition, the welfare of farm animals should be taken into consideration. Therefore, their productivity is increased through controlling metabolic processes, which allows for better use of nutrients and elimination of pathogenic factors. Subsequently, the health state of the animals improves.

Following the latest tendency concerning fur-bearing animal nutrition, a new research project was started at the Chair of Fur-bearing Animal Breeding, in co-operation with the Institute of Animal Nutrition and Feed Economy, University of Agriculture and Technology, Olsztyn. Its aim was to determine the usability of a preparation known on the market under a trade name DigDeo-Korector in polar fox nutrition. The preparation contains components which influence the processes taking place in the alimentary tract and improve the quality of fur cover. They increase the nutrient digestibility and reduce the putrefactive processes in the alimentary tract. The components in question are: yeast, plant extract from *Yucca schidigera*, probiotic bacteria and zinc bioplex.

Living organisms need zinc to perform different functions. Its deficiency leads to lack of appetite, growth inhibition, hair loss and disturbances in the process of reproduction (*Slawon 1987*). As for foxes, zinc plays an important role in the development of fur cover. In typical conditions, a zinc deficiency occurs relatively seldom, but some animal feeds, e.g. fish meal, may contain substances that decrease its assimilation. A high level of calcium and some microelements (e.g. copper and iron) also decreases zinc assimilation (*Baker, Ammerman 1995*). Generally, zinc from animal products is assimilated more easily than from plant ones. However, its availability from different kinds of mineral feeds varies. Scott and Zeigler (1963) noted that some feeds, e.g. liver extract, improve the assimilation of zinc from soybean oil meal. This is connected with the occurrence of zinc chelating bonds, most often with peptides and amino-acids. The results of numerous experiments indicate that organic compounds constitute a better source of trace elements (Bioavailability of ...1995). Such a correlation was observed in the course of research on both ruminants (*Clark et al. 1993; Boland et al. 1996*) and monogastric animals (*Du et al.. 1996*). Chelating agents and mineral proteinates belong to organic combinations of mineral compounds. The combinations of trace elements (Fe, Mn, Zn, Cu, Se) with amino-acids and peptides, known as bioplexes, are easily assimilated by animals. Such combinations are active in a lower concentration than non-organic combinations of those elements. The application of trace elements in the form of organic combinations (assimilated more easily) may also contribute to a decrease in their excretion to the environment (*Lyons 1992*).

Preparations containing extracts from *Yucca schidigera* are becoming more and more popular in animal nutrition. Saponins present in those preparations decrease the activity of urease, i.e. the enzyme which causes ammonia release from nitrogen compounds (Headon 1991). The mechanism of action of *Yucca schidigera* extracts consists in urease inhibition and fixation of ammonia and other harmful gases. The ex-

tracts in question affect quantitative and qualitative changes in the alimentary tract flora. The total number of microorganisms which use ammonia for bacterial protein synthesis increases, the development of urolytic bacteria (*Proteus sp.*) is inhibited, the development of lactic fermentation bacteria is stimulated, and cell walls are destroyed. Moreover, the above extracts have an effect on the activity of mitochondrial enzymes of some bacteria (*Jacques 1989; Killeen 1995*).

The results of using preparations from *Yucca schidigera* are as follows: a decrease in the ammonia level in the air, an improvement in the health state of animals and better production indices (*Al.-Bar et al. 1992; Goodall et al. 1982; Hussain et al. 1996*). It seems that in the case of fox nutrition, such preparations may be of special importance because rations for those animals contain large amounts of feeds of animal origin.

Intensive feeding methods are conducive to certain disturbances in the microbiological balance of the alimentary tract. Feed supplements which affect this balance are known as probiotics. They contain cultures of lactic fermentation bacteria or their mixtures. The following bacteria, among others, are used as probiotics: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Bacillus subtilis, Bifidobacterium bifidum, Pediococcus acidlactici, Streptococcus faecium.* Also some yeast species are used for the production of probiotics, e.g. *Saccharomyces cerevisiae,* or mildew mycelium *Aspergillus oryzae* (*Nousiainen, Setälä 1993; Vanbelle et al. 1990*).

Probiotics influence first of all the microbiological balance of the alimentary tract, which often results only in lower disease frequency in young animals and a decrease in the mortality level. An improvement in the production results may be a secondary effect. The action mechanism of probiotics is complex and multidirectional (*Ewing, Cole 1994; Lipinski 1997*). It is mainly connected with inhibition of the development of harmful bacteria, modification of the metabolism of microorganism in the alimentary tract and organism of animals, and stimulation of the immunological system.

Yeast is used in animal feeding in two ways: as a source of protein (fodder yeast) and, in small amounts, in the form of live bacterial cultures, in order to improve the functioning of the alimentary tract. It may also be a component of mixed probiotic preparations which contain, apart from yeast cultures, strains of lactic fermentation bacteria. Yeast is used in its pure form as well (*Lipinski 1998*).

Numerous positive results of yeast application are connected with its selective effect on the microorganisms present in the alimentary tract. *Saccharomyces cerevisiae* has a positive influence on the amount of lactic fermentation bacteria, at the same time decreasing the level of *E. Coli* bacteria.

The results of experiments indicate that yeast affects the detoxication of harmful metabolites (mycotoxins) produced by mildews. Research on broilers which received feed containing 500 ppm aflatoxin B_1 showed that an addition of 0.1% Yea-Sacc – preparation containing *Saccharomyces cerevisiae* - enabled them to improve production results. It was also found that yeast fixes fungal toxins.

Yeast increases the resistance to harmful bacteria and their toxins. Research on mice showed that *Saccharomyces boulardii* protected them against a harmful effect of *Clostridium difficile.* When the animals received yeast, the level of toxins in their intestines decreased. In those mice which did not receive yeast cultures, large amounts of cytotoxin and enterotoxin (produced by bacteria) were found, which led to their death. It may be concluded that yeast increases the resistance to infections in animals.

Material and Methods

The studies on DigDeo–Korektor were conducted on a commercial farm, in production conditions, on 60 young polar foxes born at a similar time. They included the period from weaning to slaughtering. The animals were di-

vided into two groups, with an equal number of females and males in each, taking into consideration their origin. The foxes were placed in typical cages, in the same pavilion. The experimental factor was a feed supplement, known on the market under the trade name DigDeo–Korektor. This preparation, as has already been mentioned, contains a composition of natural feed additives and is produced in a friable form.

The foxes of both groups were fed in the same way as the other animals kept on the farm. The differentiating factor was the above preparation, added to the rations for the animals from the experimental group (II), in the amount of 5 g/day per animal. It was carefully mixed with feed directly before giving to the foxes. The feed was prepared using typical components available on the domestic market. The rations were balanced according to the requirements of growing animals.

An analysis of body weight gains was performed on the basis of individual measurements, made every second week. In September, digestibility-balance tests were conducted on five females selected from each group. They included the determination of digestibility coefficients for individual nutrients and energy, as well as nitrogen balance. When the fur covers were fully developed, the animal appearance was evaluated by a group of specialists. After slaughter, the pelts were processed in a typical way and then evaluated and classified, taking into consideration their size and fur cover category. The results of the experiment were elaborated statistically, employing the method of a one-factor analysis of variance in an orthogonal design (*Ruszczyc 1981*).

Results and Discussion

Table 1 presents a list the components used for preparing the feed given to animals in the course of the research. The amount of meat from animal carcasses in the rations is relatively large, which is connected with the fact that the farm where the experiment was carried out is located in agricultural area and surrounded by numerous cattle farms. All the other components are characteristic of the Polish feed market.

Table 1. Composition of rations

Components	Percentage	
	Period (months) July – September	Period (months) September – to slaughtering
1. Medium-fat beef	30	25
2. Hard poultry offal	35	30
3. Steamed ground barley	20	20
4. Steamed potatoes	-	12
5. Green forage and vegetables:		
a) cow cabbage	6	6
b) carrot	6	5
6. Wheat bran	3	2
7. Polfamix L-N (as recommended by the producer)		

Animals of group II (experimental) received DigDeo-Korektor in the amount of 5g per animal/day

Table 2 shows the nutritive value of the rations. The amount of protein, fat, carbohydrates and metabolizable energy (in g) in 100 g of feed is given. This Table also presents the percentage of energy coming from individual nutrients and the amount of crude protein (in g) per MJ of energy. The values included in the Table are consistent with those quoted in 'Feeding Standards for Carnivorous and Herbivorous Furbearing Animals' (1995).

Table 2. Indices of the nutritive value of rations

Period (months)	Digestible nutrients in 100 g of feed			ME in MJ/100 g of feed	Percentage of energy from:			Digestible protein g/MJ ME
	protein	fat	carbohy-drates		protein	fat	carbohy-drates	
1 July-Sept	12,28	4,59	10,02	0,582	40	31	29	21,10
2 Sept-slaugh-tering	10,79	3,95	11,25	0,550	37	28	35	19,34

The results concerning the body weight gains of polar foxes are presented in Table 3. No statistical differences were found between the control and experimental groups, but the final body weight of foxes receiving rations with an addition of DigDeo-Korektor was 0.31 kg higher than that noted in the control group. It would be hard to draw conclusions concerning the effect of the preparation discussed on the body weight gains in growing foxes. However, the body weight of the animals from group II was a little higher during the whole experimental period. According to the professional literature available, the influence of such supplements on the production results achieved by fur-bearing animals has not been studied so far. Research on other animal species suggests a favorable effect of the components contained in DigDeo-Korektor on production results (*Al-Bar et al. 1992; Goodall et al. 1982; Hussain et al. 1996*). Very often a positive influence of such preparations may be a secondary effect of a general improvement in the health state of animals and better nutrient conversion (*Ewing, Cole 1994; Lipinski 1997*).

Table 3. Body weight of foxes (kg)

Weighing	Statistical measures	Group	
		I	II
1	n	30	30
	x	1.56	1.61
	v	12.40	10.36
2	x	2.49	2.60
	v	10.02	10.06
3	x	3.28	3.42
	v	9.11	10.46
4	x	4.03	4.16
	v	9.36	10.75
5	x	4.95	5.22
	v	9.25	11.09
6	x	5.83	6.04
	v	8.71	13.06
7	x	6.44	6.69
	v	9.79	14.65
8	x	6.93	7.24
	v	11.16	16.38

No statistically significant differences

The chemical composition of the rations given to the animals during the digestibility-balance tests is presented in Table 4. An addition of the preparation in question caused only inconsiderable changes in the chemical compositions of the diets, which results from its small amount (5 g). The differences between the chemical composition of rations and their tabular nutritive value (Table 2) are connected with certain fluctuations in the content of basic nutrients in individual lots of feed components and lack of precision in feed rationing in production conditions.

Table 4. Chemical composition of rations in fresh matter (%)

Specification	Group	
	I	II
Dry matter	28.18	28.22
Crude ash	3.52	3.70
Organic matter	24.66	24.52
Crude protein	10.45	10.37
Crude fat	6.04	6.05
Crude fiber	0.49	0.53
N-free extract	7.68	7.57
Gross energy MJ/kg	6.148	6.164

The coefficients of digestibility, determined in the course of the studies (Table 5), indicate that the preparation evaluated improved the digestibility of organic and dry matter, N-free extractives and gross energy in a statistically highly significant way. The coefficients of digestibility of the other nutrients remained at a similar level in both groups. Research on different animal species confirmed a favorable effect of yeast live cells on digestibility processes in the alimentary tract. This is connected, among others, with an increase in the activity of some bacteria groups, observed after adding yeast cultures, and with the enzymatic activity of yeast. Subsequently, yeast increases the nutrient availability and, as a consequence, improves production results. Those processes probably also took place in the alimentary tract of the polar foxes examined.

Table 5. Coefficients of nutrient and energy digestibility (%)

Components	Statistical Measures	Group	
		I	II
Dry matter	x	63.79^{b}	67.56^{a}
	v	2.22	3.36
Organic matter	x	73.79^{b}	76.72^{a}
	v	1.32	2.02
Crude protein	x	74.18	75.13
	v	3.39	4.03
Crude fat	x	96.31	96.43
	v	0.38	0.34
Crude fiber	x	22.26	24.69
	v	6.65	14.03
N-free extract	x	61.85^{b}	70.03^{a}
	v	9.09	5.58
Gross energy	x	76.81^{b}	79.10^{a}
	v	1.61	1.93

a, b - $P< 0.05$

A, B - $P< 0.01$

Table 6 shows the nitrogen balance results. A higher level of nitrogen retention was noted in the experimental group, but the difference was statistically insignificant. Lower nitrogen losses in urea ($P<0.05$) were observed in the group receiving DigDeo-Korektor, which suggests better utilization of this element by the organism. It is beyond doubt related with the feed supplements used. The results obtained are consistent with those presented in Table 1. The animals of the experimental group retained more nitrogen and that is probably why they achieved higher final body weight.

Table 6. Nitrogen balance

Specification	Statistical Measures	Group I	Group II
Nitrogen (g/head)			
- taken	x	6.69	6.72
- excreted:			
with faeces	x	1.72	1.67
	v	9.78	12.10
with urine	x	1.86[a]	1.42[b]
	v	12.23	20.55
- digested	x	4.97	5.05
	v	3.40	3.99
- retained	x	3.11	3.63
	v	12.42	8.21
Retention in relation to nitrogen:			
- taken, %	x	46.49	54.02
	v	8.41	12.31
- digested, %	x	62.57	71.88
	v	7.23	9.38

a, b - $P < 0.05$

The data included in Table 7 show better growth indices of the animals from the experimental group. They got a higher score for size and body structure (the difference was statistically highly significant), additionally confirmed by measurements of the trunk length. The results obtained indicate that higher body weight of animals fed on rations containing DigDeo-Korektor results from the deposition of larger amounts of nitrogen (protein), and not fatty tissue, which affected the size of the animals. The fur cover quality was better in group II (the difference was statistically highly significant). The trait analyzed depends to a great extent on proper feeding, which found confirmation in numerous experiments (*Lorek, Gugolek 1993; Slawon 1987*). No effect of the preparation examined on the other traits (color type and color purity) taken into consideration during the appearance evaluation was noted. Both those traits are conditioned genetically, and as such are affected by environmental factors to a slight degree only.

Table 7. Evaluation of fox appearance

No.	Traits	Statistical measures	Group I	Group II
1	Size and body structure (points)	n	30	30
		x	3.00[B]	5.47[A]
		v	64.92	21.34
2	Color type (points)	x	3.00	3.00
		v	0.00	0.00
3	Color purity (points)	x	2.90	2.93
		v	10.52	8.65
4	Fur quality (points)	x	5.00[B]	6.37[A]
		v	19.65	12.70
5	Score (points)	x	13.83[B]	17.83[A]
		v	18.61	8.34
6	Trunk length (cm)	x	62.43[B]	64.57[A]
		v	3.68	4.14

a, b - $P < 0.05$
A,B - $P < 0.01$

The results of pelt evaluation are presented in Table 8. The pelts in the experimental group (DigDeo-Korektor) were almost 3 cm longer. The fur quality category was also better here, closer to category one (1.70). In the control group, the fur quality category was worse by 0.13 – 1.83. Those results are consistent with an intravital evaluation of the animals, presented in Table 2. An addition of zinc bioplex to in the rations for the foxes from the experimental group may have had a positive effect on the quality of their fur. It could also be affected by modification of digestibility processes in the alimentary tract, which allowed the foxes to develop fur characterized by better parameters.

Table 8. Pelt evaluation

Traits	Group	
	I	II
Size (cm):		
n	30	30
x	104.00	106.93
v	8.82	7.62
Fur quality category (head)		
1	8	12
2	19	15
3	3	3
n	30	30
x	1.83	1.70
v	32.30	38.31

No statistically significant differences

Conclusions

The research results suggest the usability of DigDeo-Korektor in growing polar fox nutrition. A full evaluation of this preparation will be possible after conducting additional anatomic and pathological tests, which will provide information on the animal health state, and analyzing the reproduction results.

1. The animals fed on rations containing DigDeo-Korektor achieved better body weight gains, i.e. their body weight was higher and their trunks longer.
2. The fur cover quality turned out to be better in the experimental group, both in the course of an intravital evaluation and pelt evaluation.
3. The preparation examined caused an increase in the digestibility of organic and dry matter, N-free extract and gross energy. Its application also resulted in the deposition of larger nitrogen amounts, expressed by the nitrogen retention index.

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Effect of *ad libitum* and restrictive feeding on seasonal weight changes in captive mink (*Mustela vison*)

Hannu Korhonen, P. Niemelä

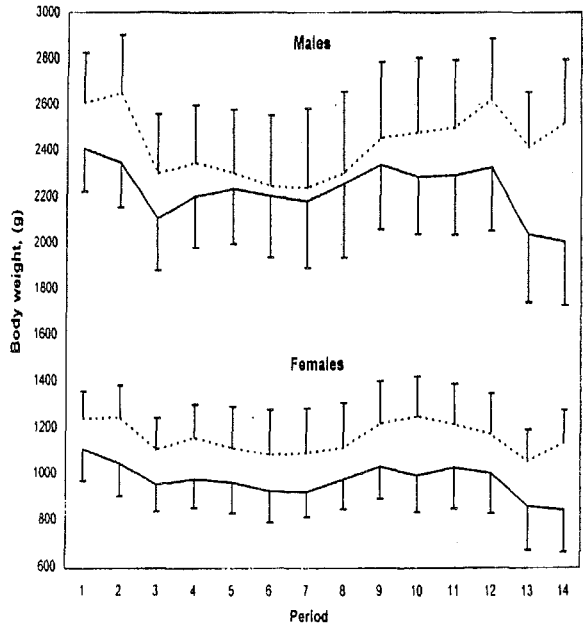

Fig.3. Seasonal changes in body weight (mean ± SD). Broken lines are for freely fed animals.

The present study aimed to clarify to what extent different feed availability during one year affects seasonal weight change and breeding body condition in male and female farm mink (*Mustela vison*). A comparison was made between animals fed *ad libitum* and those fed a restricted feed portion. The feed intake of freely fed animals was higher than that of restrictively fed ones. During March, however, the appetite of animals in each group dramatically declined due to breeding activities. Feed intake increased after the breeding season and levelled off during summer. Feed intake increased again towards autumn, peaking in October-November. The body weights in each dietary group showed distinct seasonal changes. The observed differences in feed intake were also reflected in body weights. Thus, the *ad libitum* group typically had higher body weights throughout the year than the restricted diet group. The same phenomenon was seen in both sexes, although the differences in the weights of the female groups were smaller. Just before and during the breeding season, the animals in all of the study groups had sharp weight losses. This was due to increased energy expenditure and decreased energy intake. This phenomenon obviously has an innate basis because it was found to occur despite the size of the feed ration. Thus, the mink voluntarily aims to set its body weight to an appropriate breeding condition. A very intensive weight decline in a short period should be avoided.

J. Anim. Physiol. a. Nutr. 79, pp. 269-280, 1998. 3 tables, 3 figs. 40 refs. Authors' summary.

Prevention of aflatoxicosis in farm animals by means of hydrated sodium calcium aluminosilicate addition to feedstuffs: a review

A.J. Ramos, E. Hernández

Mycotoxins are a wide group of fungal toxins that have been associated with severe toxic effects in man and animals. Aflatoxins are the most dangerous of these fungal secondary metabolites. Because there is no definitive way in which complete detoxification of feed and feed contaminated with mycotoxins can be achieved, new methods to eliminate mycotoxicosis are sought.

Hydrated sodium calcium aluminosilicate (HSCAS), a sorbent compound obtained from natural zeolite, has demonstrated an ability to sorb mycotoxins with a high affinity. Addition of this compound to feedstuffs contaminated with aflatoxins has shown a protective effect against the development of aflatoxicosis in farm animals.

Several authors have postulated that the main mechanism implicated in this process could be chemisorption of the toxins through the formation of a stable complex comprising HSCAS and the mycotoxins. This complex is not able to cross the luminal membrane of the gastrointestinal tract and therefore the bioavailability of aflatoxins is reduced in a dose-dependent manner.

This review comments on the in vitro and in vivo application of HSCAS to sorb aflatoxins and other mycotoxins. The effect on animal performance of dietary addition of HSCAS to feedstuffs contaminated with mycotoxins is discussed in a variety of farm animals.

Animal Feed Science Technology, 65, pp. 197-206, 1997. 46 refs. Authors' summary.

Different combinations of formic, propionic and benzoic acids in slaughter offal preservation for feeding to fur animals

Ilpo Pölönen, Vesa Toivonen, Jaakko Mäkelä

Washed rumens, intestines and lungs of beef cattle were ground, homogenized and acidified with formic acid, 6 g kg^{-1}. The acidified offal was divided into 30 plastic buckets, assigned to two storage temperatures (4°C, 20°C) and 5 treatments with propionic and benzoic acids, (in g kg^{-1}): 0 + 0, 2 + 0, 1 + 1, 0 + 2 and 3 + 1. At 4°C, the organoleptic quality of the silages containing additive acids was good for the whole storage time (35 d), while at 20°C all the silages containing 2 g kg^{-1} additive acids were spoiled. Yeasts predominated the deterioration process with all treatments. Benzoic acid inhibited yeast growth significantly better than formic acid alone, or added propionic acid. Biogenic amines increased only as a result of bacterial growth.

Animal Feed Science Technology, 71, pp. 197-202, 1998. 2 tables, 1 fig., 14 refs. Authors' abstract.

Effects of high amounts of dietary fish oil of different oxidative quality on performance and health of growing-furring male mink (*Mustela vison*) and of female mink during rearing, reproduction and nursing periods

Christian F. Børsting, R.M. Engberg, S.K. Jensen, B.M.Damgaard

In two experiments the effects of high levels of ethoxyquin-stabilized (300 ppm) fish oil, fed to four groups of mink, either fresh, or oxidized to peroxide values of 30, 70 and 100 meq O_2/kg, were investigated. During the growing-furring period 15 males per group and in the rearing period 15 females per group were fed diets providing 12% fish oil as the only fat source (58% of metabolizable energy) and supplemented with 90 mg α-tocopherol per kg. During the reproduction and nursing periods the diet for the females was modified to provide 3% fish oil as the only fat source (33% of metabolizable energy) and 50 mg α-tocopherol per kg. High dietary levels of ethoxyquin-stabilized fish oil of good quality in diets supplemented with high levels of vitamin E were tolerated quite well by males during the growing-furring period. However, the feed intake, body weight gain and skin length declined in response to decreased oil quality. The plasma content of α-tocopherol decreased with the decrease in fat quality, whereas there was no effect on other chemical, enzymatic or hematological indices in the males. In females the use of fish oil as the sole fat source led to small litter size, high incidence of diarrhoea among kits and high kit mortality irrespective of the quality, indicating that even fish oil of good oxidative quality cannot be used as the only fat source for breeding mink. Moderately oxidized fish oil caused depletion of vitamin E body stores, tissue degeneration and anemia in the females at the end of the nursing period.

J. Anim. Physiol. a. Anim. Nutr. 79, pp.210-223, 1998. 5 tables, 30 refs. Authors' summary.

Effect of folic acid supplementation on folate status and formate oxidation rate in mink (*Mustela vison*)

I.J. Pölönen, L.T. Vahteristo, E.J. Tanhuanpää

We investigated the folate-dependent toxicity of formate to mink to better understand the use of formic acid in fur animal feeds. Folic acid supplementation (0, 1, 5, 10, and 20 mg/kg of DM) in the feed of weanling mink for 4 wk resulted in hepatic tetrahydrofolate (H_4folate) concentrations of 3.94, 8.51, 9.15, 10.4, and 15.0

nmol/g, respectively (SE 1.03). Oxidation tests in metabolic chambers, preceeding a single injection of sodium [^{14}C]formate (500 mg/ kg BW), showed that the nonsupplemented mink oxidized formate into $C0_2$ at a rate 37% less than that of the supplemented mink. The oxidation rate increased with supplementation level and was maximal, 54.2 $mEq \cdot kg^{-1} \cdot h^{1}$ (SE 3.0), at 10 mg of folate/kg; at the highest level of supplementation (20 mg/kg), $C0_2$ production tended to be lower. Concentration of hepatic ^{14}C increased with the hepatic H_4folate, and its accumulation continued after the highest point of oxidation. These observations indicate that mink oxidize formate readily but at a slightly lower rate than do rats. However, if extra folate is not supplemented in the feed during the period of early intensive growth, hepatic H_4folate level may decline to the levels found in humans and monkeys, which are susceptible to formate accumulation. Average daily weight gain improved with each increase in supplementation of folic acid; however, only the differences between the nonsupplemented diet and the two highest levels of the vitamin reached statistical significance ($P < .05$).

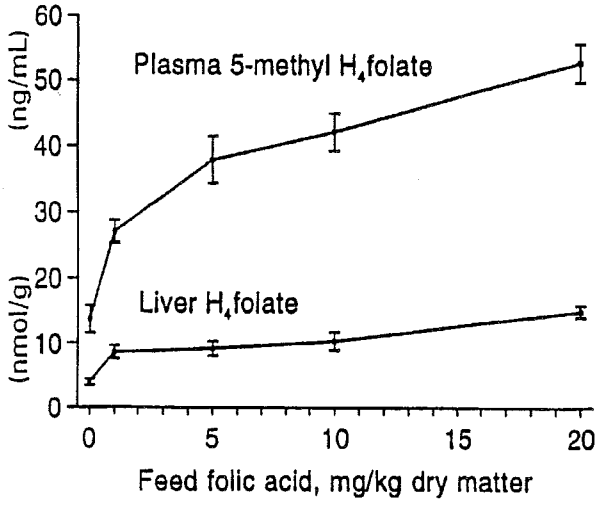

Figure 1. Effect of supplemented folic acid on plasma (5-methyl tetrahydrofolate [H_4folate]) and hepatic folate (H_4folate) concentrations in mink. Each value represents the mean of six animals ± SE.

Journal of Animal Science, 75, 6, pp. 1569-1574, 1997. 2 tables, 5 figs., 21 refs. Authors' summary.

Repeatability of mineral element content in the fur of female standard coypus

D. Mertin, K. Süvegová, P. Fl'ak, P. Sviatko

The objective of this paper was to study repeatability of mineral composition of the fur in female standard coypus in relation to their physiological condition. Experimentation took place at the Experimental Farm for Fur-bearing Animals of the Research Institute of Animal Production at Nitra. The trial involved 19 females of standard coypus. The animals were housed in halls, in one-storey cages with pools. They received granular feed mix KK (produced by Cataj Cooperative Farm), and alfalfa (in the spring-summer period) and fodder beet (in the fall-winter period) as a saturation supplement. The concentrations of Ca, K, Na, Mg, Fe, Zn, Cu, Mn, Co in the fur of female coypus were examined in selected body regions, i.e. in the middle of the dorsal and ventral regions, in relation to their physiological condition (periods): 1. primiparas - sexual maturity, age of 8 months - fur maturity stage, 2. females on the day of parturition, 3. females on the day of weaning. Fur samples (ca. 2 g) were taken by clipping. Concentrations of mineral elements were determined by atomic absorption spectrum photometry. Repeatability of the separate elements in the fur of standard coypus in the body regions as well as in the physiological periods was very high, and it was in fact higher than 0.95 and/or 0.99 in almost all elements. Average Ca content in the dorsal region was 1 754.56 ± 95.49 mg/kg dry matter, in the ventral region 1 756.79 ± 102.80 mg/kg dry matter, K content in the dorsal region amounted to 578.60 ± 50.01 mg/kg dry matter and in the ventral region to 257.99 ± 26.35 mg/kg dry matter, Na content in the dorsal region made 300.88 ± 19.95 mg/kg dry matter and in the ventral region 130.91 ± 11.23 mg/kg dry matter, Mg content in the dorsal region was 629.48 ± 41.77 and in the ventral region 656.66 ± 45.22 mg/kg dry matter, Fe content in the dorsal region amounted to 150.79 ± 13.95 and in the ventral region to 173.74 ± 13.10, respective Zn contents were 150.97 ± 2.20 and 160.41 ± 3.73 mg/kg dry matter, Cu content in the dorsal re-

gion made 5.9121 ± 0.2268 and in the ventral region 7.4095 ± 0.3092, respective Mn contents amounted to 2.4895 ± 1.732 and 5.3811 ± 0.3353 mg/kg dry matter, Co contents had the values of 0.7616 ± 0.0332 and 0.6758 ± 0.0375 mg/kg dry matter. Comparison of repeatability coefficients, although three repeated measurements of the animals were done, shows that repeatability of mineral element contents in the fur of standard coypus is high, for the body regions, the separate periods and in total. Hence it can be deduced that the contents of minerals are more or less constant taking into account only scarce differences between the periods. A conclusion can be drawn from our results that the mineral composition of fur in adult female coypus varies in relation to age, genotype and physiological stage. The experimental results suggest that the fur is a suitable subject for investigation within research on mineral metabolism and physiological and pathological changes in the animal organisms.

Zivocisna Vyroba, 42, 10, pp. 453-458, 1997. In SLOV, Su. ENGL. 3 tables, 17 refs. Authors' summary.

Flushing of mink (*Mustela vison*): effects on energy metabolism and some blood metabolites

R. Fink, A.-H. Tauson

Energy metabolism during flushing of mink (*Mustela vison*) in relation to a control group was studied by means of combining pooled data from balance and respiration experiments carried out in 1993 (experiment I) and 1994 (experiment II) and some blood metabolises from experiment II. The experiments, which were divided into six 1-week balance periods, started on 7 February and continued until 22 March. Flushing was performed by restricted feeding in periods 2 and 3 and refeeding in periods 4 and 5. Each balance period included a 24-h measurement of heat production by means of indirect calorimetry in open-air circuit respiration chambers. In experiment II blood samples were taken at weekly intervals and analysed for total triiodothyronine, total thyroxine, free thyroxine, glucose, insulin and fructosamine.

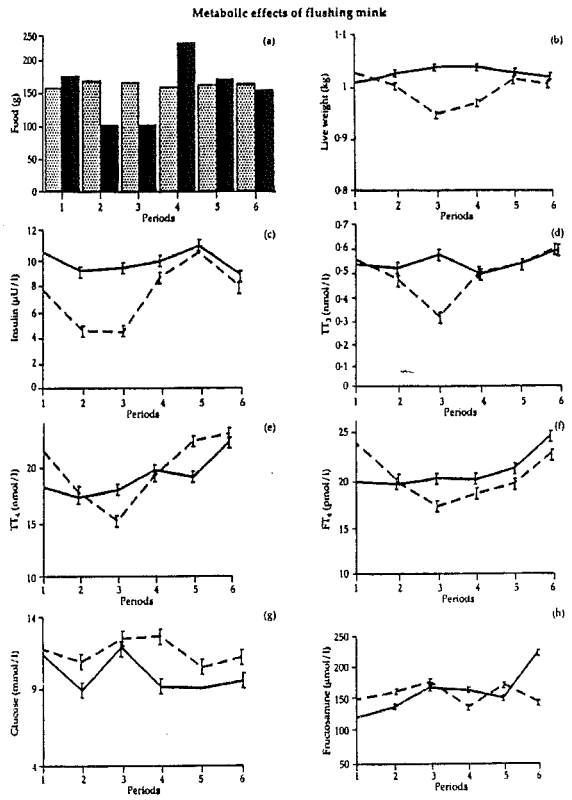

Fig. 1. (a) Feed consumption, (b) animal live weights, plasma concentrations of (c) insulin, (d) total triiodothyronine (TT_3), (e) total thyroxine (TT_4) (f) free thyroxine (FT_4), (g) glucese and (h) frucotosamine in the six experimental periods for mink females given feed *ad libitum* (▨; ------- control) and feed restricted in periods 2 and 3 and re-fed in periods 4 and 5 (■; - - - - - flushed).

Generally, intake of digestible nutrients and energy metabolism measurements within the flushed group were strongly influenced by period (energy supply), whereas they remained stable in the control group and differences between treatment groups over the total experiment were non-significant. The fluctuation in energy status induced by flushing did not involve major changes in body weight. Blood metabolites reflected the feed intake by being fairly stable in the control group and decreasing during restriction and increasing during re-

feeding in the flushed group ($P < 0.05$). As an effect of flushing the number of corpora lutea increased significantly ($P < 0.05$).

Animal Science, 66, pp. 277-284, 1998. 3tables, 1 fig., 34 refs. Authors' abstract.

Oxidation of substrates and lipogenesis in pigs (*Sus scrofa*), mink (*Mustela vison*) and rats (*Ratus norvegicus*)

A. Chwalibog, A-H. Tauson, R. Fink, G. Thorbek

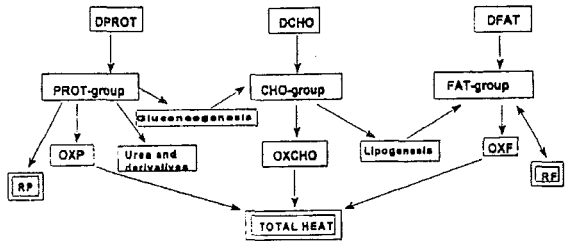

Fig. 1. Model of nutrient oxidation, lipogenesis and retention. Digested protein (DP), carbohydrate (DCHO) and fat (DFAT). Oxidized protein (OXP), carbohydrate (OXCHO) and fat (OXF). Retained protein (RP) and retained or mobilized fat (RF).

Data from experiments with 25 growing pigs at high feeding level, with 12 adult mink on a varied energy supply and with 36 rats on a maintenance level were used in a biological model of nutrient oxidation, lipogenesis and retention at the whole body level. Nutrient oxidation was calculated from gas-exchange measurements in respiration chambers working according to indirect calorimetry principles. Lipogenesis and nutrient retention were measured by means of carbon and nitrogen balances, in accordance with the demonstrated model. The results demonstrated that growing pigs had a high level of protein retention and low protein oxidation. Digested carbohydrates were oxidized or used for lipogenesis. Oxidation of carbohydrate was the main energy source, while lipogenesis was the main source of fat retention. Independent of dietary fat level, pigs did not oxidize fat but used all dietary fat for body fat retention. The mink, being fed with high protein and fat levels but only a small amount of carbohydrate, use protein and fat as their main energy sources. Rats fed near-maintenance level used dietary carbohydrate as a main substrate for oxidation. It was demonstrated that the present model of nutrient oxidation, lipogenesis and retention in different animal species and at different dietary composition can be quantified by means of indirect calorimetry and measurements of carbon and nitrogen balances.

Thermochimica Acta, 309, pp. 49-56, 1998. 3 tables, 3 figs., 20 refs. Authors' abstract.

Effect of lutein on beta-carotene absorption and cleavage

Henk van den Berg

Carotenoid interactions during absorption and in postabsorptive metabolism have been observed in both human and animal studies. We reviewed the mutual interactions reported between lutein and β-carotene and report new data on the postprandial β-carotene and retinyl ester response in the triglyceride-rich plasma lipoprotein (TRL) fraction in volunteers after a single test meal with β-carotene alone, or β-carotene combined with lutein or lycopene. Results indicate an inhibitory effect of lutein on β-carotene absorption, but apparently not on β-carotene cleavage. In a comparative study with two β-carotene/lutein ratios (2:1 and 1:2, respectively), this inhibitory effect of lutein was found to be most marked when lutein was the predominant carotenoid. In studies on plasma (serum) response also an inhibitory effect of β-carotene on lutein response was observed.

Internat. J. Vit. Nutr. Res. 68, pp. 360-365, 1998. 1 table, 1 fig., 23 refs. Author's summary.

Original Report

The proposal of a new behavioural test for the Polar fox. Empathic test

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Abstract

The author presents his original behavioural test designed for evaluating the temperament of the Arctic fox. The new method is termed empathic test as the investigator's ability to feel the animal's mentality, behaviour and feelings plays a crucial role.

The study was conducted in 181 young Polar foxes. The output was compared with the results of two other tests: the capture test and the hand test, which have been regarded as standard tests. In the empathic test similarly to the standard tests, the same classes (Aggressive, Normal, Timid) were used. The statistical analysis of the results obtained has not revealed any significant differences between compared tests, however the ease and simplicity of the new test give it a distinct advantage.

Introduction

Selection of breeding animals with high productivity is based on a number of independent factors. Selection based on behavioural tests is one of the methods employed in assessing appropriateness of animals for further breeding. Tests enable selection of animals with appropriate mental patterns which predispose them to passing on to their offspring positive behavioural forms. Behaviourally balanced animals, which are not afraid of man's presence and which do not display aggressive behaviour, should be selected for breeding. *(Hansen, 1998; Zon et al.,1998)*.

Docile animals are not only desirable from the point of view of the breeder, but should, according to the EU regulations, be promoted in breeding. Under the pressure of environmental organisations, breeding farms which do not meet conditions defined by the EU will soon lose their licence. Therefore, breeders are obliged to select animals in a manner compliant with the requirements.

These issues are inherently connected with application of relevant behavioural tests which verify whether an animal meets the breeding criteria. There exist a number of tests, more or less perfect, used to achieve the desired aim *(Hansen, 1998; Harri et al., 1995; Rekila et al.1997)*.

A test worth recommendation is the so-called "hand test" In farm conditions, sanitation and animal care frequently require staff to introduce their hand into the cage. The test assesses

the fox's reaction to the breeder's hand after it has been introduced into the cage (*Kenttamies, 1998*).

Preferred behaviour, typical of balanced and curious animals, consists of the animal quietly approaching and sniffing the hand. Aggressive behaviour, such as growling, charging with bared teeth and biting are symptomatic of aggressive animals which should be eliminated from further breeding. Animals trying to escape in a panic when the hand inserted into their cage should be also removed from breeding.

The capture test is based on similar principles. The animal is immobilised against the cage floor by means of fork. the fox's behaviour in such a situation shows its temperament and behavioural patterns. Animals which attempt to bite, growl and which focus on the investigator after they have been removed from the cage are classed as aggressive. Foxes sitting calmly on the floor, not trying to free themselves, are considered to be balanced. This type of temperament should be promoted during breeding selection. Similarly to aggression, timid behaviour is undesired. It manifests itself by the fox turning its head away from the keeper. The fox often scratches the floor and tries to run away from the perceived threat.

Presented behavioural tests provide a high level of probability in determining the temperament of the fox, which is crucial in making sure that only balanced animals are selected for breeding. However the hand test and capture test have some disadvantages. Both tests require opening the cage door and disturbing the animal's living space, which is threatening to the fox as it violates its territory. Creating such extreme conditions not normally occurring during everyday operations, may dramatically alter the animal's behaviour. Investigation under stress, caused undoubtedly by entering into the cage, equivalent to trespassing onto the animal's territory, may not reflect fully the temperament of the animal.

Studies on fox behaviour conducted in Chorzelów Experimental Breeding Station require precise determining of the temperament. Hand and capture tests, conducted to-date, were burdened with the aforementioned disadvantages, and so may not be treated as providing satisfactory results. A test without these drawbacks was developed to provide an optimum solution. Animals do not feel threatened, their territories are not invaded, and the test is not connected with the feeling of hunger and it does not create extreme conditions. The new method is termed empathic test as the ability of the investigator to feel the animal's mentality, its behaviour and feelings plays a crucial role.

This study was intended as a comparison between the results of the hand and capture tests with the empathic method.

Material and methods

The study was carried out in 181 young blue Arctic foxes which were kept two animals per cage. The cage was 90 centimetres long, 120 centimetres wide and 100 centimetres high. Capture, hand and empathic tests were conducted between August and November, and all behavioural forms recorded. The following symbols were used:

- Aggressive animals – **A**
- Curious, behaviourally balanced animals – **N**
- Anxious and timid animals – **B**

In total, 1,086 forms of behaviours were recorded using each test. Each animal was tested 6 times by the hand test, 6 times by capture, and 6 times by the empathic method.

Hand and capture tests were carried out in accordance with the presented procedure and they are referred to as standard tests, commonly applied in standard behavioural studies. In the empathic tests, proposed as a new method of behavioural studies, the same classes were applied as in standard methods (A,N,B).

The aim of the experiment was to test correlation between the results of the capture and hand tests on the one hand and the empathic method on the other. Similar results would be an indication of usability of the newly developed test.

During the empathic test, a flexible rod with a ribbon tied in a knot was inserted through the grid mesh into the cage; the door remaining closed. During the introduction of the rod, the investigator would remain 50 centimetres away from the cage, i.e. the same distance maintained by the staff feeding and nursing the animals. The tied knot was inserted by slow and steady movement, in the central part of the cage, at a height below the fox's head. Care was taken to avoid introducing the knot above the animal. Having inserted the rod, the knot was rotated at a distance of 30 centimetres from the animal. Trying not to distract the animal, the investigator carefully observed the fox and attempted to sense the fox's feelings when a new element entered the cage, which was its territory. The following forms of behaviour were observed during the first 15–20 seconds after the rod with the knot is inserted.

The foxes were classified as aggressive (A) if the following behavioural forms occurred:

1. growling and charging toward to bite the knot;
2. laying down ears,
3. eyes wide open, the animal stares at the knot all the time,
4. tail lifted up,
5. front paws spread widely, directed forward,
6. body down, ready to jump and attack,
7. upper lip lifted, the animal bares its teeth.

These behaviours occurred singly or in combination, with various intensity.

The foxes were classified as balanced and curious, with typical behavioural patterns (N) if the following behavioural forms occurred:
1. no interest in the knot,
2. the same position of the body, only looking at the knot,
3. approaching and sniffing the knot, with interest, no symptoms of aggressions,
4. jumping and displaying willingness to play.

These behavioural forms showed mainly curiosity or indifference.

Foxes were classified as anxious and timid (B) if the following behavioural forms occurred:

1. attempt to escape after introducing the rod with the knot into the cage;
2. searching for escape routes in cage corners or climbing cage walls,
3. turning the head away from the knot and closing eyes,
4. nervous pacing the cage,
5. ignoring „the problem" by turning back,
6. tail between the legs, ears low, and eyes closed.

If the stimulus exceeds a threshold value, anxious animals show aggressive behaviour as a form of self-defence. However, this should be treated as non-threatening direct desperate attack. It occurs some time after the knot is inserted to the cage and it is followed by symptoms of fear. Aggressive animals, in turn, after the first demonstration of power and attempt to threat the enemy may show the behaviour typical of an anxious animal. Therefore, the behaviour should be classified within the first 15 – 20 seconds.

Results

Each of 181 animals were tested six times using one test method, which means that in total each animal was tested 18 times, by all methods. In studies which employed the capture test, 62 animals (i.e. 34.5% of the test population) showed identical behaviour in all repetitions. In the hand test, this figure reached 50 animals (27.6%), and in the empathic test, 78 foxes (43.1%) behaved identically. These results indicate that the empathic test is a test method more accurate than the other methods tested.

Statistical difference of the results in specific tests was examined by the correlation method.

A high correlation of results was assumed to be in favour of interchangeable application of tests. The investigated behavioural forms, recorded in the specific tests, were analysed in order to attain the most representative data.

The results are presented in Table 1. As appears from the above there are no significant differences in application of the discussed tests, and the results obtained correlate to a large extent.

Table 1. Number of occurrences of specific forms of behaviour in particular tests

Temperament (type of behaviour)	Empathic test - E		Capture test - C		Hand test - H	
	Number	%	Number	%	Number	%
A (Aggressive)	228	20.99	188	17.31	206	18.97
N (Neutral)	786	72.38	826	76.06	748	68.88
B (Anxious)	72	6.63	72	6.63	132	12.15
Total	1 086	100	1 086	100	1 086	100

The correlation factor was calculated. The following symbols were assumed:

A_E, N_E, B_E – behaviour in in the empathic test,

A_C, N_C, B_C – behaviour in the capture test,

A_H, N_H, B_H, - behaviour in the hand test.

Aggressive behaviour:

	A_E	A_C	A_H
A_E	1.00000	0.83782**	0,73390**
A_C	0,83782**	1,00000	0,74076**
A_H	0,73390**	0,74076**	1,00000

Neutral (balanced) behaviour:

	N_E	N_C	N_H
N_E	1,00000	0,816881**	0,68115**
N_C	0,81681**	1,00000	0,65800**
N_H	0,68115**	0,65800**	1,00000

Anxious behaviour:

	B_E	B_C	B_H
B_E	1,00000	0,81537**	0,65358**
B_C	0,81537**	1,00000	0,57276**
B_H	0,65358**	0,57276**	1,00000

** - means high significance of the correlation factor.

Conclusion

The presented results of the comparison of the tests indicate that behavioural studies results are not statistically different. Applying the capture test, hand test and empathic test leads to similar results.

Introduction of the empathic test to breeding practice instead of capture and hand tests used so far seems a reasonable alternative which offsets the negative aspects of the standard tests. Undoubtedly, easy application of the test and elimination of stress to which animals are exposed in capture or hand tests are by no means beneficial. Each opening of the cage is an abnormal situation to the fox, thus investigation of the fox's temperament at that time may be burdened with an error. The empathic test requires of the observer, as the name would suggest, a more humane approach to the animal. Application of the test leads to the establishing an emotional tie between the breeder and the animal. Identifying of the breeder with the feelings of the animal requires some experience, but it should lead to creating better breeding conditions for foxes and better taking into account of their mental needs.

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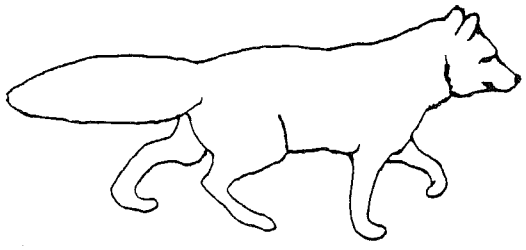

Do the stereotypies of pigs, chickens and mink reflect adaptive species differences in the control of foraging?

Georgia Mason, Michael Mendl

The food-related stereotypies of some captive species (e.g. mink) are performed most often prior to feeding, while those of others (e.g. pigs and chickens) occur at low levels before feeding and increase after food consumption. It has been suggested that these differences reflect adaptive species differences in how feeding behaviour is controlled. However, this hypothesis rests on several underlying assumptions for which there is incomplete support. One assumption is that there are indeed species differences in the design of motivational systems, and we suggest some specific predictions to test this idea. For example, the ingestion of small portions of food should lead to greater enhancement of local searching behaviour in species whose food supply is particulate and patchy. The basic premise underlying this evolutionary explanation for species differences in stereotypy is that such differences are genetically based, not an artefact of the way different animals are kept. However, we argue that variation in husbandry may also cause variation in stereotypies. For example, the autoshaping literature reveals factors likely to affect pre-feeding stereotypies: unreliable predictors of food delivery, or predictors that occur some time before food is presented, give rise to general locomotory search phases of appetitive behaviour rather than behaviour related to food handling. Farmed mink may therefore show high levels of pre-feeding locomotor behaviour principally because sounds predicting the delivery of their daily meal are quite unreliable and commence long before the food arrives. Lack of space may also inhibit locomotor forms of pre-feeding stereotypies in pigs and chickens. In addition, the high post-feeding appetitive behaviour of these two species may be caused by lack of satiation following food. Overall, evolutionary hypotheses make predictions about stereotypy based on feeding ecology, but there are also alternative causal hypotheses that make predictions based on aspects of husbandry. Together, these may help to explain the forms of existing stereotypies, and to anticipate the forms likely to arise in new husbandry systems or in newly captive species.

Applied Animal Behaviour Sciences 53, pp. 45-58, 1997. 2 tables, 68 refs. Authors' abstract.

Mating time and litter size in farm mink selected for confident or timid behaviour

J. Malmkvist, B. Houbak, S.W.. Hansen

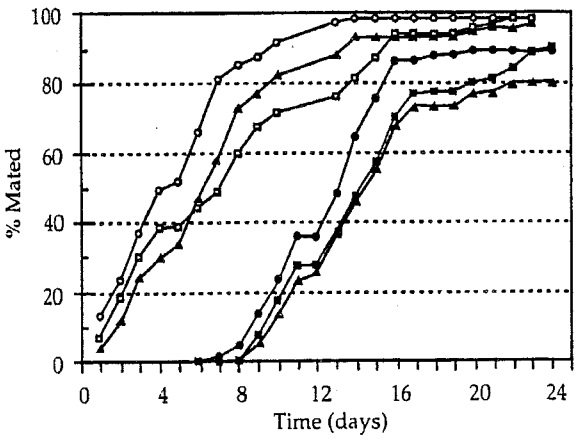

Figure 1 Percentage of mink females mated over time. Group A: curious (○,●), B: timid (△,▲), and C: control (□,■). First mating = open symbols; second mating = filled symbols. Day 1 is 28 February 1996.

Farm mink (*Mustela vison*) have been selected on the basis of their behaviour towards man since 1988 at the Danish Institute of Agricultural Sciences and in this study were offered the possibility to mate 1 week earlier than usual. The objective was to investigate if the behaviour-related selection has affected the reproduction of the farm mink, measured as mating willingness and reproductive success. The animals belonged to three breeding groups: A: selected for curious/confident reactions (17 males, 73 females), B: selected for timid reactions (17 males, 74 females), and C: selected without any demands on reactions towards humans (18 males, 73 females). The time when an average of 50% of the population were mated was 3-6 days for group A; thus the

confident were mated 1.7 to 2.1 days earlier than the timid (B) and the control (C) animals. The length of gestation was shorter in group A (46.0 (s.d. 3.0) days than in group C (47.6 (s.d. 3.6) days) but not different from group B (47.0 (s.d. 3.2) days). Group C had a lower kit mortality from birth to day 50 (11.3%) than groups A and B (20.4 to 21.2%). No significant differences were found between the groups regarding the frequency of successful matings, the ratio of remated females, number of interrupted matings, barren females or litter size. It is concluded that 8 years of selection had led to the development of reproductive differences primarily in the time of mating readiness, whereas the differences seen in kit loss may be related more to random effects.

Animal Science 65, pp. 521-525, 1997. 2 tables, 1 fig., 23 refs. Authors' summary.

The effect of an improved man-animal relationship on sex ratio in litters and on growth and behaviour in cubs among farmed silver fox (*Vulpes vulpes*)

Morten Bakken

Earlier experiments indicate that silver foxes' fear of humans can be reduced by humans offering them food titbits. Sex ratio, cub growth and behaviour were examined in litters produced by multiparous silver fox vixens that had been given a titbit twice a week during pregnancy (G1, N = 14) or received the same amount of human contact without any titbit (G2, N = 14). The cubs were tested in an open field (1. 15 X 1. 15 m square area divided into a 5 X 5 square grid) at 30 days of age, when they were in the early stages of primary socialisation. Cub activity was recorded (number of grid lines crossed [Lc] during 3 min). There was no treatment-related difference in number of cubs born or cubs weaned at 49 days (4.9 ± 0.3 and 4.1 ± 0.4 vs. 4.8 ± 0.5 and 3.8 ± 0.4 for GI and G2, respectively; NS). However, GI vixens delivered a significantly higher proportion of male cubs than G2 vixens (64% vs. 51 %, $p < 0.05$; G1: 3.1 ± 0.4 male cubs, 1.8 ± 0.3 female cubs; G2: 2.5 ± 0.4 male cubs, 2.3 ± 0.3 female cubs). Female cubs from GI vixens were more active in the open field (G1: 51.4 ± 4.8 Lc, G2: 34.2 ± 4.7 Lc, $p < 0.05$) and heavier (49 days old, GI: 1660 ± 42 g, G2: 1491 ± 40 g, $p < 0.01$) than the female G2 cubs. No significant differences in activity or live weight were found for male cubs (activity: GI: 46.4 ± 3.4 Lc, G2: 37.1 ± 4.3 Lc, NS; weight 49 days old: G1: 1716 ± 29 g, G2: 1729 ± 37 g, NS). Reduced fear of humans during pregnancy affected the sex ratio in the litter as well as the growth and activity of female cubs in the silver fox.

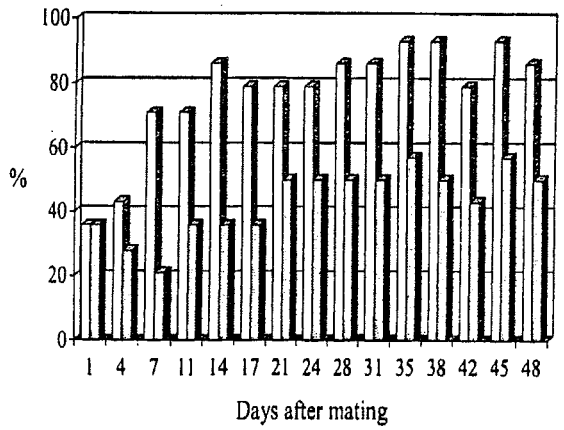

Fig. 1. Percent vixens observed in front of their cages during testing (open columns: vixens receiving a titbit, every day for one week after mating and then twice a week during the rest of pregnancy; hatched columns: vixens with the same amount of human contact, but without any titbit).

Applied Animal Behaviour Science, 56, pp. 309-317. 1998. 2 tables, 1 fig., 34 refs. Author's abstract.

Environmental enrichment for wild-born captive foxes

Anne Whiterow, Elaine Gill

Wild-born fox cubs aged three to four months were brought into captivity for use in feeding behaviour experiments. Individuals were initially housed separately in concrete and

mesh pens, where all foxes showed signs of stress, such as pacing, hiding and excessive chewing. This is unacceptable in terms of animal welfare and the validity of experimental work. Various methods of enriching the foxes' environment were therefore employed: the accommodation was varied by introducing and moving objects such as boxes, planks, vegetation, bowls of soil and toys, areas of cover were provided for security, the feeding regime was varied and made unpredictable by hiding food in different locations, and where possible, pens were linked and foxes housed in pairs. Whilst individuals responded to these techniques in different ways, companionship produced the most noticeable improvement in all foxes. Our observations provide information for the design of fox accommodation for future scientific study.

Animal Technology, Vol. 48, No. 2, pp. 37-43, 1997. 8 refs. Authors' summary.

Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals

Bjarne O. Braastad

This article is a review of research on effects of stress experienced by pregnant females on the sex-ratio, behaviour and reproductive success of their offspring. Implications of such effects for the behaviour and welfare of farm, zoo, and pet animals are discussed. Evidence mainly from studies of rodents and primates strongly indicates that prenatal stress can impair stress-coping ability, and is able to cause a disruption of behaviour in aversive or conflict-inducing situations in juvenile and adult offspring. In non-challenging situations, however, behavioural effects of prenatal stress are frequently not seen. Prenatally stressed animals are reported to show retarded motor development, reduced exploratory and play behaviour, and impairments of learning ability, social behaviour, and sexual and maternal behaviour. Prenatal stress may affect the sex-ratio at birth, and the reproductive success of these offspring in the first, and sometimes also in the second, generation. Individual variation in the susceptibility to prenatal stress may exist. Behavioural inhibition and anxiety when exposed to novelty are typical results which may underlie the effects of prenatal stress on learning and various behavioural responses. This seems to be related to increased or prolonged activity in the hypothalamic-pituitary-adrenal (HPA) axis produced by impaired negative feedback of glucocorticoids in the hippocampus, although other neuroendocrine pathways may be involved. Effects of prenatal stress may reflect evolutionarily adaptive mechanisms, favouring production of the sex which may serve as a helper-at-the-nest (usually females) and producing an increased HPA-axis dominance in these offspring which would favour defensive behavioural reactions in competitive or stressful situations. Since behavioural and neuroendocrine effects of prenatal stress in rodents are quite similar to those found in depressed humans, and since increased fearfulness and frustration is implicated, farm animals subjected to prenatal stress may be predicted to show a reduced ability to cope with a difficult environment and also have increased propensity for developing behavioural disturbances and reduced welfare. Recent results on farmed foxes, and indications in other farm species, show that prenatal stress may affect the behavioural development of farm animals. As knowledge in this area is scarce, more research is warranted. Effects of qualitative and quantitative aspects of handling, social relations and its disruption, and environmental conditions prior to mating and during gestation could be investigated. Effects should be sought on sexual maturation, sexual behaviour, maternal behaviour, fearfulness, behavioural responses to stress and novel stimuli, and behavioural effects of frustration. The interrelation between effects on offspring of necessary stressful treatment of pregnant mothers and effects of habituation to such treatment could also be studied.

Applied Animal Behaviour Science, 61, pp. 159-180, 1998. 110 refs. Author's abstract.

Effects of prenatal handling stress on adrenal weight and function and behaviour in novel situations in blue fox cubs (*Alopex lagopus*)

B.O. Braastad, L.V. Osadchuk, G. Lund, M. Bakken

Prenatal stress is known to affect the morphology, physiology, and behaviour of rodent offspring. This study investigated the effects of a 1-min daily handling stress given to farmed blue fox females in the last third of gestation on adrenal weight and function in male and female offspring and on behaviour in novel situations. In 10-day-old cubs (n = 68), records were made of scrum adrenocorticotropic hormone (ACTH), progesterone and cortisol, adrenal content of progesterone and cortisol, and in vitro production of progesterone and cortisol in adrenals, with or without synthetic ACTH in the incubate. At 35 days, three behavioural tests were made in succession (n = 72). In a Human test, the cub was held up by hand in a standardized way for 20 s. An Open-field test was followed by a Box test, where the cub was placed in a small box and was allowed 30 s to enter the open field. The adrenals of prenatally stressed (PS) cubs weighed only 60% of those in the control group (C), while body weight was equal in PS and C cubs. The serum level of progesterone and the in vitro adrenal production of progesterone were higher in PS than C cubs. In PS females, the in vitro adrenal production of cortisol was higher than in C females. No significant effect of ACTH stimulation was found. In general, the effects on progesterone parallelled the effects on cortisol. These results indicate that prenatal stress may enhance the postnatal adrenocortical function. PS cubs remained more active during the human test, crossed more lines and entered more squares in the open field, and more frequently entered the open field in the box test. These results may indicate a higher behavioural reactivity in novel situations in prenatally stressed cubs. Whether the increased adrenocortical function and behavioural reactivity is related to reduced welfare remains to be determined. Further studies on the effects of prenatal stress in farm animals are recommended.

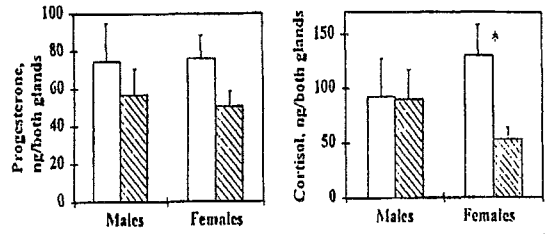

Fig. 2. Content of progesterone and cortisol in both adrenal glands of 10-day-old blue foxes subjected to prenatal handling stress (hatched columns) and without prenatal stress (open columns). $N = 5$ in each group. Mean and SEM are shown. *P<0.05, Student's *t*-test.

Applied Animal Behaviour Science, 57, pp. 157-169, 1998. 3 tables, 3 figs., 46 refs. Authors' summary.

Olfactory behaviour of blue foxes

H. Korhonen, S. Alasuutari, P. Niemelä

An account is given of 4 trials in Finland on the behavioural response of male and female blue foxes to various odours and their possible connection with production and social rank.

Finsk Pälstidskrift 31, 12, pp. 296-297; 312-313, 1997. In SWED. 4 tables, 1 fig., 2 refs. CAB-abstract.

Establishment of a preliminary genomic map of the American mink (*Mustela vison*)

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New Doctor in the family. We congratulate Dr. Klaus Brusgaard with the fine scientific work and the new title.

Summary
The purpose of the present work was to establish tools i.e. genetic markers for genetic and physical mapping with regard to production parameters in the American mink (*Mustela vison*). If feasible these tools or genetic markers would be employed in linkage analyses and population studies. Preliminary linkage analysis and mapping of candidate genes could provide a scaffold for a later development of a more comprehensive genetic map. The score of the population studies would be to correlate the genetic variance within and between different lines of mink with respect to their former ancestry.

The present thesis is a summary of 9 papers which are included in the appendix. The first paper address the development of a RFLP (Restriction Fragment Length Polymorphism) in mink. The three subsequent papers address the characterisation of a number of mink DNA microsatellites. Appendix 5 and 6 attend the chromosomal assignment of the characterised DNA microsatellites by *in situ* hybridisation of cosmids. Appendix 7 and 8, address the chromosomal assignment of a number of genes using Chinese hamster-mink hybrid cell lines. Appendix 9 deals with the utilisation of different polymorphic markers in the characterisation of different mink lines.

A RFLP marker have been developed. It is possibly related to potential production parameters. The *TYRP1* (Tyrosinase Related Protein 1) RFLP addresses a candidate gene for fur colour in mink, significantly different proportions in the allele frequency distributions (between 0.5 and 1.0) were found in the animal populations typed. Among the animals investigated the allele frequencies varied (from 0.5 to 1.0) between lines.

With the purpose of constructing a mink cosmid library, high molecular weight DNA was extracted from a male mink spleen. The DNA was partially digested with the restriction enzyme *Sau*3A, and size fractionated on a sucrose gradient, fractions containing DNA of approximately 50 kb were ligated into a cosmid vector. The library constituted 2 x 10^6 individual clones, equivalent to approximately 35 mink genomes. Screening of the cosmid library using a ^{32}P-labelled $(GT)_9$ oligonucleotide was performed to isolate clones harbouring DNA microsatellites. Ten new polymorphic DNA microsatellites habe been characterised. These DNA microsatellites showed varying degrees of polymorphism both between the markers and between the animal populations investigated. The PIC (Polymorphism Information Content) of the markers varied between 0.0 and 0.8, indicating that several of the markers might be useful in genomic mapping approaches, population analyses and QTL (Quantitative Trait Loci) studies. The distribution of mink DNA microsatellites was shown to be similar to that found in other mammals with approximately one $(dG\text{-}dT)_n$ DNA microsatellite every 63 kb. The presence of other types of DNA microsatellites in the mink genome is evaluated.

Seventy-seven DNA microsatellites have been mapped to chromosomal localisations using *in situ* hybridisation of cosmids harbouring mink

inserts. The distribution was at a 5% confidence interval shown to be even within the individual chromosomes. Thus, a scaffold for a nuance genetic map of the mink genome has been found. The cosmid SH376 was shown to hybridise to chromosome Y, being the first marker for the Y-chromosome in mink. The cosmid SH644 showed strong hybridisation to the nuclear organizer region of the mink.

Applying the DNA microsatellites characterised in the present study as well as a number of other mink DNA microsatellites an attempt has been made to establish linkage groups within the mink genome. Two presumed linkage groups have been established, one of these on chromosome 6.

Based on analyses employing Chinese hamster-mink hybrid cell lines the genes for mink growth hormone, prolactin, prion protein, immunoglubulin-kappa, homeo box B, immunoglobulin gamma and aldolase B, were mapped to mink chromosomes. *GH* and *HOXB* were mapped to chromosome 8, thus establishing a syntenic group in mink, *PRL* and *IGGC* were mapped to chromosome 10, *PRNP* and *IGKC* to chromosome 11, *ALDB* to mink chromosome 12.

Population studies of five different breeds representing three different mink colour types, employing calculations for deviations from Hardy-Weinberg equilibrium, assignment tests, based on the allele frequency distribution, phylogenetic relationships and analyses for shared alleles were performed. These studies clearly showed that it was possible to distinquish between related or unrelated individuals within and between different lines of mink. Thus, it is possible to assign an individual of a previously unknown origin to a particular breed.

Thesis, 53 pp. 13 tables, 13 figs., 158 refs. Author's summary.

Publications included in this thesis:

1. Brusgaard, K., Malchenko, S.N., Shukri, N.M., Lohi, O. A *Eco*RI RFLP at the mink (*Mustela vison* L.) Tyrosinase Related Protein 1 (*TYRP1*) locus. Accepted for publicaiton in Animal Genetics.
2. Brusgaard, K., Shukri, S., Malchenko, S.N., Lohi, O., Christensen, K., Kruse, T. Three polymorphic mink (*Mustela vison*) dinucleotide repeats. Animal Genetics, 29: 150-160, 1998. *Abstracted in this issue of SCIENTIFUR.*
3. Brusgaard, K., Holm, L.-E., Lohi, O. Two mink (*Mustela vison*) DNA microsatellite loci. Accepted for publication in Animal Genetics.
4. Brusgaard, K., Lohi, O., Kruse, T. A polymorphic DNA microsatellite repeat in American mink (*Mustela vison*).Accepted for publication in Animal Genetics.
5. Brusgaard, K., Malchenko, S., Lohi, O., Christensen, K. 1996. A DNA microsatellite containing cosmid mapping to American mink (*Mustela vison*) chromosome Y by *in situ* hybridzation. Science Reports, 27: 41-44. *Abstracted in SCIENTIFUR, Vol. 20, No. 4, pp. 357, 1996.*
6. Christensen, K., Brusgaard, K., Malchenko, S., Lohi, O., Serov, O. 1996. Standardization of the American mink (*Mustela vison*) karyotype and some cosmid *in situ* hybridization results. Archivos de zootechnia, vol. 45, number 170-171, pp. 259-265. *Abstracted in this issue of SCIENTIFUR.*
7. Malchenko, S.N., Golovin, S.J., Koroleva, L.V., Mateeva, N.M., Beklemisheva, V.R., Grafodatsky, A.S., Brusgaard, K., Christensen, K., Serov, O. Chromosomal localization of growth hormone and prolactin in American mink (*Mustela vison*). In manus.
8. Khlebodarova, T.M., Malchenko, S.N., Matveeva, N.M., Pack, S.D., Sokolova, O.V., Alabiev, B.Y., Belousov, E.S., Permislov, V.V., Nayakhin, A.M., Brusgaard, K., Serov, O.L. 1995. Chromosomal and regional

localization of the loci for IGKC, IGGC, ALDB, HOXB, GPT, and PRNP in the American mink (*Mustela vison*): comparisons with human and mouse. Mammalian Genome, Number 6, pp. 705-709. *Abstracted in this issue of SCIENTIFUR.*

9. Brusgaard, K., Holm, L.-E., Lohi, O. Determination of genetic differences between lines of American mink (*Mustela vison*). Manuscript submitted to Acta Agriculturae Scandinavica, Section A, Animal Science.

Three polymorphic mink (*Mustela vison*) dinucleotide repeats

K. Brusgaard, N. Shukri, S.N. Malchenko, O. Lohi, K. Christensen, T. Kruse

Source description: A mink cosmid library was constructed from a Royal Pastel male. The cosmid clones SH201, SH585 and SH192 were isolated after screening with a γ-^{32}PdATP labelled $(GT)_9$ oligonucleotide. Sequencing of the microsatellite repeat was performed after subcloning of *Sau*3A digested cosmids in the *Bam*H1 site of pUC19 vector. The markers were named Mvi201, Mvi586 and Mvi192, respectively.

Chromosomal localization: The cosmids were localized to mink chromosomes by fluorescent *in situ* hybridization. SH201 was localized to chromosome 7q2,1, SH586 was localized to the chromosome 11-centromeric region, SH192 was localized to the chromosome 6-centromeric region.

PCR conditions: Approximately 40 ng of DNA was amplified in a total volume of 25 µl containing 15 pmol of each primer, 0.2 mM dNTP, 0.4 u Taq Polymerase (Pharmacia) and 2.5 µl 10 x PCR Buffer (100 mM Tris HCl, pH 9.0, 15 mM $MgCl_2$, 500 mM KCl, 0.1% v/w gelatine and 1% Triton X-100), Amplification was carried out in a OmniGene Temperature cycler (Hybaid). PCR cycling consisted of a denaturing step of 3 min at 93°C, followed by 30 cycles of 52°C for 20 seconds, 72°C 10 seconds followed by 30 seconds at 93°C, a final cycle at 72°C for 20 min.

Mendelin inheritance: Autosomal codominant segregation was demonstrated in a Danish full-sib mink pedigree.

Polymorphism: Studies for variation were performed in five populations of unrelated farm mink; two lines of Scanblack, two lines of Royal Pastel and one line of Standard mink "Wild". The size of the alleles was scored using a 50-500 bp ladder (Pharmacia) together with the amplified isolated clone as an external size marker.

1 table, 3 figs. Authors' summary.

Standardization of the American mink (*Mustela vison*) karyotype and some *in situ* hybridization results

K. Christensen, K. Brusgaard, S. Malchenko, O. Lohi, O. Serov

The mink karyotype consists of 14 autosomes and a par of sex chromosomes. A system is proposed where row 1 consists of five large metacentric chromosomes ordered by size, row 2 consists of five submetacentric chromosomes ordered by size, and row 3 consists of three acrocentric ordered by size, 1 small telocentric and the sex chromosomes. The identification of the individual chromosomes is based on R-, Q- and N-banding. A numbering system is proposed for the individual bands covering approximately a total of 100 bands.

Results are given for *in situ* hybridization for more than 50 cosmids selected for containing dinucleotide repeats. By these hybridizations a marker are located on all chromosomes. Five cosmids show strong centromere hybridizations results, including the nuclear organizer region and the Y chromosome. Some more detailed results can be seen on the www server http=//www.husdyr.kvl.dk/mink.htm.

2 tables, 3 figs., 10 refs. Authors' summary.

Chromosomal and regional localization of the loci for IGKC, IGGC, ALDB, HOXB, GPT, and PRNP in the American mink (*Mustela vison*): comparisons with human and mouse

T.M. Khlebodarova, S.N. Malchenko, N.M. Matveeva, S.D. Pack, O.V. Sokolova, B.Y. Alabiev, E.S. Belousov, V.V. Peremislov, A.M. Nayakshin, K. Brusgaard, O.L. Serov

Chromosomal localization of the genes for gamma- and kappa-immunoglobulins (*IGGC* and *IGKC*, respectively), aldolase B (*ALDB*), prion protein (*PRNP*), homeo box B (*HOXB*), and glutamate pyruvate transaminase (*GPT*) were determined with the use of mink-rodent hybrid cells. Analysis of segregation of the mink markers and chromosomes in these hybrid cells allowed us to assign the gene for *HOXB* to Chromosome (Chr) 8, *IGGC* to Chr 10, *PRNP* and *IGKC* to Chr 11, *ALDB* to Chr 12, and *GPT* to Chr 14 in mink. Furthermore, using a set of mink-mouse hybrid cells carrying fragments of mink Chr 8 of different sizes, we assigned the gene for *HOXB, PRNP, ALDB,* and *IGGC* are members of a conserved region shared by many mammalian species in common: the *IGKC* gene is a member of a conserved region common to carnivores and primates, not rodents; the *GPT* gene is a member of a syntenic gene group probably unique to the *Mustelidae* family or carnivores.

2 tables, 3 figs., 23 refs. Authors' abstract.

Biosynthesis of testosterone in fetal gonads of silver fox after long-term domestication

L.V. Osadchuk

Fetal gonad weight and testosterone content in serum and gonads were analyzed in silver fox every five days from the 35th day of pregnancy until delivery. Fetal testicles were also tested for testosterone production induced by chorionic gonadotropin (CG) in vitro. Pregnant females were sampled from an experimental population subjected to selection for domesticated behaviour and a commercial population (control). Fetal gonad weight was significantly lower in domesticated animals than in controls.

No differences were revealed in the testosterone contents in their serum and gonads and in the basal production of testosterone in fetal testicles. CG-induced production of testosterone was detectable from the 40th day of fetal development in domesticated animals and from the 50th day in controls. The results obtained suggest that domestication results in the heterochronic fetal development of the hypophysial-testicular complex in silver fox.

Genetika, 34 (7), pp. 941-946, 1998. In RUSS. Su. ENGL. 2 figs., 27 refs. Author's summary.

Phenogenetic analysis of prenatal development of the glucocorticoid function of adrenals in silver foxes after long-term selection for domestic behavior

L.V. Osadchuk

The level of cortisol in serum and adrenals and its production by adrenals in vitro was studied by the radioimmune method in male and female silver fox embryos, starting from day 30 of pregnancy every five days. Pregnant females from a commercial population and an experimental population, which had been selected for domestic behaviour, were used. It was shown that, at the end of the prenatal developmental stage, all investigated parameters of the glucocorticoid function of adrenals were significantly lower in embryos from selected mothers as compared to the unselected control group. The addition of adrenocorticotropic hormone into the incubation medium increased cortisol biosynthesis at all embryogenesis stages, but in the selected population the increase was less than that in the control group.

Genetika (Moskva), 33: 11, pp. 1534-1538, 1997. In RUSS, Su. ENGL. 5 figs., 17 refs. Author's summary.

Effects of long-term selection for behaviour on the level of progesterone in blood and its content in adrenals of silver fox embryos

L. V. Osadchuk

The level of progesterone in blood serum and its concentration in adrenals and gonads have been assayed in male and female silver fox embryos, starting from day 35 of pregnancy for every five days. Pregnant females from an experimental population, selected for the domestic type of behaviour and, as a control group, females from a commercial population, were used. At the end of prenatal development (days 45-50) the concentration of progesterone in adrenals was shown to be significantly lower in embryos from mothers of the selected population, than in the unselected control group. On the contrary, the domesticated and wild animals did not differ in the level of progesterone in blood. The results suggest that selection of animals for domestic behaviour decreases the synthesis of progesterone by embryonic adrenal glands.

Genetika (Moskva), 33: 12, pp. 1664-1668, 1997. In RUSS, Su. ENGL. 1 table, 2 figs., 22 refs. Author's summary.

Add colour to life with colour mutations

J. Hansen

An account is given of mating combinations required to produce Demi, Mahogany, Scan Black, Scan Brown, Scan Glow, Silverblue and Blue Iris mink.

Dansk Pelsdyravl, 61, 1, pp. 14-16, 1998. In DANH. 3 photos. CAB-abstract.

Breeding in 1997

K.R. Johannessen

For 57.694 mink, 99.358 blue fox and 20.071 silver fox females averaged 5.5, 5.0 and 3.2, respectively. Results are compared with those in 1996, and details are given of body measurements of breeding males and the freezing of semen.

Norsk Pelsdyrblad 71, 1, pp. 10-12, 1998. In NORG. CAB-abstract.

Original Report

Testosterone, estradiol and cortisol responses to sexual stimulation with reference to mating activity in domesticated silver fox males

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Summary

A weak sexual activity has frequently been observed among farmed fox males. However, there has been no good explanation for the observed variation. The aim of this study was to investigate plasma levels of testosterone, estradiol and cortisol with reference to breeding results in silver fox males, and to elucidate the role of the hormonal reactivity of testes to sexual stimulation in male reproductive performance. A group of adult silver fox males was studied for the pattern of seasonal variation in steroid hormone concentrations and hormonal responsiveness to sexual stimulation. Animals were tested during the different stages of the reproductive cycle, and hormonal levels were determined in males before and after a female introduction. Estimates of male reproductive performance were based on the number of mated females, litter size and the total number of cubs from a male per breeding season. This study demonstrated wide variations in plasma levels of steroid hormones between different stages of the reproductive cycle with the highest levels during the breeding period. It was observed that the highly sexually active males responded to the presence of a female by an increase in testosterone level. Their reproductive performance was best. The results suggest that there are differences in the testicular responsiveness to a sexual stimulation between males. These differences can be used as selection criteria of foxes for better sexual activity.

Introduction

The silver fox is a seasonal breeder like most mammals of temperate zones. The breeding season of the male silver fox is restricted, to a period of approximately 7-8 weeks, from the middle of January to the middle of March. Males are sexually active throughout the season, covering the season of females. In addition, it follows from breeding practice and studies that a proportion of males ignores mating (*Lanszki et al., 1996; Osadchuk, 1998a, 1999a*). The reasons for loss of sexual activity by some silver fox males are not, as yet, clear, and there is no way to predict the lower sexual activity.

Steroid hormone concentrations appear to be linked to the sexual behaviour in fox males (*Osadchuk, 1997, 1998b*). Disturbances in the hormonal system may be a possible reason for the reduction of the sexual activity in silver fox males. This aspect of fox reproduction has not been studied systematically. It has been recently shown that yearling fox males, which exhibit marked differences in sexual activity during their first breeding season, did not demonstrate any differences in the basal levels of sexual hormones before and throughout the

entire breeding season (*Osadchuk, 1999b*). These results suggest that baseline hormonal concentrations cannot be useful predictors of sexual activity in silver fox males. However, the testicular responsiveness to sexual stimulation was not evaluated as a hormonal test for the estimation of sexual behaviour problems.

There are no hormonal tests to predict sexual activity and other reproductive parameters and it is unknown how to select males for better reproductive performance in fur bearing animals. The idea that hormonal tests can be used to predict certain important reproductive traits is supported by investigations in other species (*Chubb, Nolan, 1985; Noguchi et al., 1993; Magistrini et al., 1996*).

The present study was conducted to investigate the role of the testicular reactivity to sexual stimulation in the breeding performance of silver fox males. Testosterone, estradiol and cortisol concentrations and breeding data were analysed in male silver foxes subjected to long-term selection for domestic behaviour. Domesticated silver fox was chosen as a model for research because of its obvious advantages which were discussed earlier (*Osadchuk, 1999a, b*).

Material and methods

Nineteen mature adult silver foxes (*Vulpes vulpes*) were used in this study. They have passed through at least one breeding season. Foxes were individually housed outdoors under natural conditions of daylight and temperature. They were kept within visual and olfactory contact of vixens. The studied silver foxes originated from a domestic population, which has been subjected to selection for domestic behaviour for more than 30 years. The behaviour of these animals and a method of selection has been described elsewhere (*Belyaev, 1979; Trut, 1995*). Shortly: domesticated silver foxes behave amicably towards humans and they are better adapted to farm conditions.

The silver fox is a multiparous mammal, seasonally breeding from the middle of January to the end of March. The silver fox female is monestrus and has only one estrus lasting two-three days. The time of onset of estrus and sexual receptivity varies markedly from one individual to another but is restricted by the reproductive season. The silver fox male is sexually active during the whole reproductive season. According to routine breeding practice in Russia, a female in estrus is introduced to the male's cage for 2-3 hr in the morning. If mating takes place, the female is allowed to mate again on the next day with the same male. If an estrus female is not receptive during the first exposure to male, it is placed every day with different males until mating occurs or estrus ends.

In this study, a female was placed in a male's cage for 1 h in September (during sexual quiescence), in January when they were in anestrus (the onset of breeding season), and in February when they were in estrus. In the latter case, the testes were in a state of maximum activity (*Osadchuk, 1993; Osadchuk, 1998b*). Blood samples were withdrawn from vein *saphena* of males before a female was placed into a male's cage and just after it. To avoid the effects of blood-sampling procedure on hormonal levels, the experimental males were sampled the first time each day before the introduction of a female (control) and the second time just after the treatment period (sexual stimulation). Each blood sampling including the time taken to catch the animal took approximately 3 min. Blood was taken from the males without anesthesia. Plasma samples were stored at -20°C after blood centrifugation.

The testosterone, estradiol and cortisol in plasma samples were measured by radioimmunoassay (RIA) using commercial kits (Sea-Ire-Sorin, France) with preliminary extraction with ethyl ether. The recovery from ether extraction (extraction yield of added tritiated steroids) was 0.9 to 1.0. The sensitivity of the assays expressed as the minimum detectable amount of hormone was 0.08 ng/ml for testosterone and estradiol and 4.0 ng/ml for cortisol. The intra- and interassay coefficients of variation were 6.0% and 10.0% for testosterone, respectively, 10.5% and 18.4% for estradiol and 5.4% and 9.6% for cortisol.

Estimates of male reproductive performance were based on the number of mated females per season, the number of pups born per mated female and the total number of pups from a male per breeding season.

Statistical analysis of the data was performed using one-way and two-way analysis of variance, and Student's t-test.

Results and Discussion

Considerable variations in the plasma levels of testosterone and estradiol between the different stages of the reproductive cycle were observed (Table 1). The maximum concentration of sexual hormones occurred in February (peak of mating activity), while the lowest values occurred in September during the deep seasonal quiescence of the reproductive system. Testosterone level in plasma considerably increased (10-20-fold) by the onset of the breeding season, reaching maximum values during this period. Plasma level of estradiol was virtually the same in September and January, but it also rose during the expected peak of sexual activity. The peculiar pattern of plasma estradiol may be due to a large extent to extragonadal sources, including the biotransformation of estradiol from testosterone in the brain (*Sodersten, Gustafsson, 1980*).

Table 1. Plasma hormone levels in silver fox males (n=19) during different periods of the reproductive cycle

Season	Hormonal level		
	Testosterone (ng/ml)	Estradiol (pg/ml)	Cortisol (ng/ml)
September	0.28 ± 0.11^{a}	12.7 ± 1.3^{a}	15.4 ± 2.0^{a}
January	3.09 ± 0.34^{b}	10.9 ± 0.8^{a}	30.5 ± 3.0^{b}
February	4.90 ± 0.50^{c}	20.4 ± 2.1^{b}	29.5 ± 2.4^{b}

The numbers in the columns with different superscripts were significantly different ($P<$ at least 0.05).

The hormonal activity of the adrenal cortex also increased towards the breeding season, as demonstrated by measurements of plasma cortisol level (Table 1). There were significant changes in the cortisol level in silver fox males related with the reproductive season. The highest levels of cortisol were observed during the breeding period (Table 1).

Reproductive performance and pattern of steroid concentrations were analysed in male silver foxes with reference to their sexual activity during the breeding season. The main criterion was the occurrence of coitus when a female in estrus was placed for the first time into a male's cage. The females were randomly selected from the farm population. A male from the breeding stock additionally tested the female for estrus (receptivity) a day before the female was introduced to the experimental male. According to this criterion the analysed males were tentatively divided into two groups. Group 1 (high sexual activity, n=11), males that mated with a first estrus female presented to it in the breeding season. Group 2 (low sexual activity, n=8), no coitus. Analysis of the two male groups demonstrated significant differences in their breeding results (Table 2). The number of mated females per breeding season was higher in Group 1 in comparison with Group 2. The number of cubs born from the males of Group 1 per breeding season was also higher than in males of Group 2.

It appeared expedient to set apart retrospectively a group with low sexual activity to analyse their endocrine parameters outside and in the breeding season. The results are given in the Figure 1. It was found that the endocrine status of the males of the two groups was different during the breeding season. This did not concern the basal level of the steroid hormones (the groups virtually were not different), rather the testicular endocrine response to the placement of a female (Figure 1). Testosterone plasma level in males of Group 1 significantly increased, when a female was introduced to a male, while in males of Group 2 there was no such increase. Thus, coitus occurrence in silver foxes coincides with the high reactivity of the testosterone production. The data obtained for estradiol are more difficult to explain. It was found that an estrus female had no effect on

estradiol plasma levels in Group 1, in contrast, estradiol level significantly decreased in Group 2 after the test. In males of both groups, there were no changes in testosterone and estradiol levels after exposure to a nonreceptive female outside the breeding season.

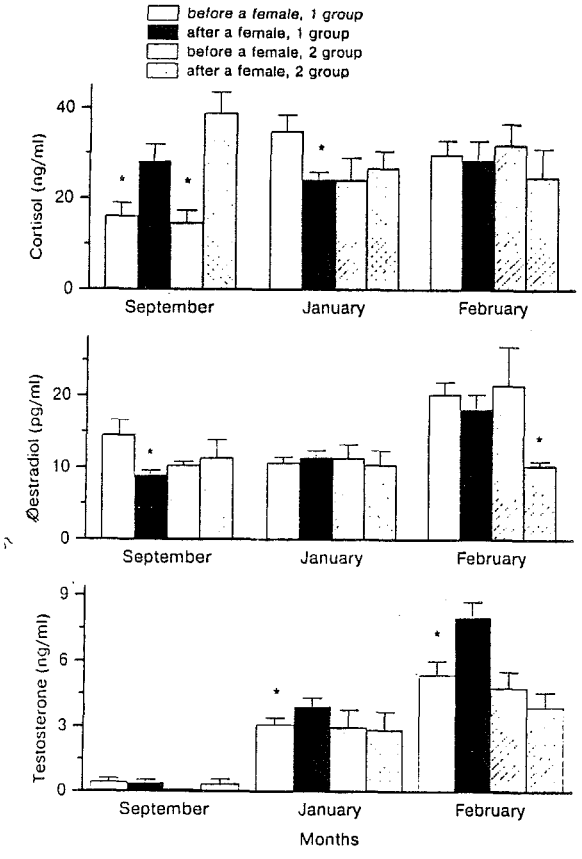

Fig. 1. Plasma testosterone, estradiol and cortisol levels in silver fox males before and after a female introduction during different stages of the reproductive cycle.

The presence of a receptive female stimulates the pituitary-gonadal axis in males of all the mammalian species that have been studied in this respect (*Macrides et al., 1975; Kamel, Frankel, 1978; Coquelin, Bronson, 1980; Gonzalez et al., 1988*). Stimulation was elicited without direct contacts with a female (*Osadchuk et al., 1985*). It was shown that the hormonal response evoked by the female pheromones is mediated through the vomeronasal system of the male. The response is accompanied by an increase in plasma level of luteinizing hormone (*Coquelin, Bronson, 1980; Johnston, Bronson, 1982; Wysocki et al., 1982; Wysocki et al., 1983*). There are also indications that in sexually inactive male rats the presentation of a female does not increase plasma testosterone (*Kamel, Frankel, 1978*). In the light of the above data, it seems possible that the non-occurrence of coitus accompanied by the absence of a hormonal response may be due to the irresponsivity of the males to pheromonal effects. If such is the case, sexual activity of males may correlate with gonadal endocrine activity.

In studies of the hormonal mechanisms of sexual behaviour, a regulatory role was assigned to corticosteroid hormones (*Liptrap, Raeside, 1978; Osadchuk et al., 1985*). An activation of the pituitary-adrenal axis was shown to be induced in male mice by the presence of a receptive female (*Osadchuk et al., 1985*). In this study, there was an increase in plasma cortisol by the onset of the breeding season (Table 1). However, there was no clear-cut evidence indicating that the increase might be relevant to the mechanisms of sexual behaviour (Figure 1). Nevertheless, the response of the pituitary-adrenal axis was noteworthy. It was the same type in males of the two groups: plasma level of cortisol increased during exposure to a non-receptive female out of the breeding season. The increase presumably reflects the stressful background of the social contacts between animals of the opposite sexes outside the mating period.

It is interesting to note that sexual activation affected the function of the pituitary-testicular axis in males of Group 1 presented with a non-receptive female in January (breeding season onset), once again, this was observed in males of Group I with a receptive female in February (Figure 1). This is of importance because a hormonal effect (increased plasma testosterone level) occurring at the onset of the breeding season might acquire predictive significance for reproductive performance. This assumption is partly supported by the data on the reproductive activity of the two fox groups (Table 2). Sexual activity, estimated as the number of mated females per breeding season and fertility as the number of pups born per breeding season, was higher in Group 1 than Group 2.

Table 2. Reproductive performance of silver fox males with different reaction to introduced female

Groups	No. of mated females per breeding season	No. of pups born per breeding season	No. of pups born per mated female
Group 1	4.9±0.7 (n=11)*	22.7±3.3 (n=11)*	4.8±0.4 (n=11)*
Group 2	0.7±0.5 (n=8)	3.0±2.7 (n=8)	2.5±0.7 (n=8)

Group 1: coitus during the first introduction of an estrus female
Group 2: males were unable to mate during the first introduction of an estrus female
* - $P<0.05$, Student's t-test between groups

Conclusive evidence indicates that the socio-sexual contacts are important regulatory mechanisms of male reproductive function. A feature of regulation of this kind is mediation through the endocrine activity of the gonads. More than that, from our data it may be inferred that the hormonal reactivity of the gonads to sexual stimulation may be crucial in determining reproductive performance in foxes. The work suggests that there are differences among fox males in the testicular responsiveness to sexual stimulation, which can be potentially used as criteria to select foxes for better sexual activity.

Acknowledgements

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Original Report

Development of the zygote and visualization of the pronuclei in mink (*Mustela vison*)

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Summary

The formation of the pronuclei, their fine structure and caryogamy in one-cell mink embryos were examined, using light and electron microscopy. The pronuclei expanded at higher rates in minks than rodents. The persistence of a single pronucleolus throughout the development of the pronuclei is probably a species-specific feature of mink. The one-cell stage is completed at 60 hours *post coitum*, and takes at least 13-16 hrs. It is advantageous to collect mink zygotes for injection of foreign DNA into the pronuclei 50-54 hrs *post coitum*. A brief centrifugation (15,000xg for 2 min) of the zygotes made possible the visualization of the pronuclei with a reliability of about 50%, using Nomarsky-optics. This centrifugation did not reduce considerably the viability of the zygotes during their *in vitro* culturing. Intact and centrifuged zygotes (38% and 24%, respectively) developed normally after transfer to recipient mothers. Pretreatment of the zygotes with colcemide reduced the egg volume, and the position of the pronuclei remained unaltered in the,center. Cytochalasin B had no effect on the volume of the zygotes, but altered their morphology. More than 70% of the centrifuged zygotes, pretreated with cytochalasin B or colcemide, failed to cleave normally.

Key words: mink, zygote, pronuclei, centrifugation, colcemide, cytochalasin B.

Introduction

Biotechnology has focused on farm animals rather than on questions pertaining to American mink (*Mustela vison*). However, mink have proven to be valuable research tools, particularly as information accumulated about *in vitro* culturing of mink embryos, their transfer *in utero* to foster mothers, and establishment of embryonic mink stem cells (*Zhelezova and Golubitsa 1978; Zhelezova and Sekirina 1982; Sukoyan et al., 1992, 1993; Moreau et al., 1995; Polejaeva et al., 1997*).

The rapid advances in modern animal biotechnology have come from *transgenesis (Rexroad 1992; Brower 1996*). The method relies on the correct timing of the one-cell stage events, which is species-specific (*Austin and Walton 1960; Hogan et al., 1986; Monk 1987*). The formation and the fine structure of the pronuclei

were extensively studied in laboratory and farm animals (*Austin and Walton 1960; Gondos et al., 1972; Zamboni et al., 1970, 1986; Chartrain et al., 1987; Wright et al., 1990; Farstad et al., 1993; Levron et al., 1995; van Wissen et al., 1995*). In descriptions of felids and canids, the presence of the pronuclei was recorded without reference to the time course of their changes *in vivo* (*Niwa et al., 1985; Farstad et al., 1993*). The pertinent data on mustelids are scanty (*Hamilton 1934; Hammond and Walton 1934; Chang 1968; Sundqvist et al., 1989; Maksimovskii et al., 1996*).

The efficiency of transgenesis undoubtedly depends on the correct microinjection of foreign DNA into the pronuclei. However, fertilized eggs of agricultural and fur animals are completely or totally opaque due to the presence of many different granules. Brief centrifugation has been used to visualize the pronuclei in cattle, sheep and pig zygotes (*Hammer et al., 1985; Bowen et al., 1996*).

The paper concerns a study of one-cell mink embryos performed to determine the period when mature pronuclei would be found. Also, brief centrifugation and chemical pretreatment were used to visualize the pronuclei in the opaque mink zygotes. Both procedures were estimated for their damaging effects on the zygotes. Also, we were the first to transfer mink one-cell embryos into the oviducts of recipient mothers.

Methods

Animals. Mink with different coat colors were used: Standard dark-brown (+/+ genotype); heterozygotes for the dominant mutation *shadow* (S^h/+ genotype); heterozygotes and homozygotes for the recessive mutation *white-hedlund* (*h*/+ and *h/h* genotypes). It should be noted that *h*/+ heterozygotes are dark brown with a white tail tip, whereas *h/h* homozygotes are all white. The dominant mutation *shadow* in homozygous state is lethal. For this reason, the progeny from crosses between S^h/+ and S^h/+ is dark-brown and *shadow* (*Ness et al., 1988*).

Standard females (66 animals) mated twice during the breeding season with the same Standard males (+/+) served as embryo donors in this study of the timing of the one-cell stage. *Shadow* females (S^h/+, 26 animals) mated two times during the breeding season with *shadow* males (S^h/+) served as embryo donors in experiments with visualization of the pronuclei and transfer of the zygotes into the oviducts; recipient *h*/+ mothers (12 females) were first mated with *white-hedlund h/h* males and then with vasectomized males. With this experimental model, the transferred embryos developed into Standard or *shadow* pups and could be easily identified among the progeny (white and Standard with white tails).

Coitus was taken as zero point (0 hour) of the development timing. Timing was expressed as hours *post coitum* (hpc). The embryo donors were sacrificed 44, 46, 48, 50, 52, 54, 56, 58, 60 and 68 hpc by cervical dislocation. The animals were housed in the Experimental Farm of the Institute of Cytology and Genetics (SB RAS, Novosibirsk) in standard conditions. Females were not pretreated with hormones.

Embryo collection. Zygotes were recovered by flushing oviducts with Dulbecco buffer solution (*Zhelezova and Golubitsa 1978; Zhelezova and Sekirina 1982; Hogan et al., 1986; Monk 1987*). The embryos were placed in a small drop of Whitten medium supplemented with Hepes. The one-cell embryos were cultured in Whitten medium supplemented with 10% fetal calf serum at 37^0 C in a humidified 5% CO_2 atmosphere. The medium was renewed every day. An inverted microscope "Labovert" with Nomarsky-optics and phase-contrast (Leitz, Germany) was used to observe live zygotes. Some of the zygotes (85) were examined *in vitro* and the rest (76 zygotes) was transplanted into pregnant and pseudopregnant recipients (*Zhelezova and Golubitsa 1978; Zhelezova and Sekirina 1982*).

Embryo transfer. Transfer of one-cell embryos without *in vitro* culturing or after was carried out using a surgical method. The embryos were

transplanted into the oviducts of the recipient females anesthetised by a subcutaneous injection of 50 mg/ml Calypsovet (Gedeon-Richter, Hungary) solution at a dose of 160 mg per kg of body weight. A 2-3 cm long incision was made along the abdominal line, then the uterine horns were removed. The upper part of the oviduct was punctured with a syringe needle. Then, a glass micropipette was slipped into the puncture, and 5-6 mink embryos (in 5 µml Whitten medium solution supplemented with Hepes) were injected into the oviduct.

Embryo treatment. The zygotes were centrifuged in Whitten culture using Eppendorf microtubes at 15,000xg for 2 or 5 min. In another series of experiments, the zygotes were pretreated with cytochalasin B (5 mcg/ml CCB in Whitten medium, 30 min, 37° C) or colcemide (0.1 mcg/ml in Whitten medium, 30 min, 37° C) (*Hogan et al., 1986; Monk 1987*) and then centrifuged under the above conditions. After centrifugation, the zygotes were either transplanted or cultured for 24 or 72 hours as described above.

Optical and electron microscopy. The one-cell embryos were fixed in 2.5% glutaraldehyde and 2.5% formaldehyde in standard buffer phosphate solution (pH 7.6 - 7.8), washed three times in the same buffer, then postfixed in 1% OsO_4. Fixation was done on ice. Dehydration and embedding in Epon were standard for the carnivoran zygotes (*Farstad et al., 1993*). Two µm thick sections were cut with an ultramicrotome "Reichert". The sections were stained with fuchsin, pyronine, methylene blue, toluidine blue or safranin O. The zygotes were oriented before cutting then cut in a continuous series and reconstructed.

Results

1. Formation and migration of the pronuclei in mink

Three hundred seventy five (375) early mink embryos were examined with light and electron microscopy. The live mink zygotes, as seen with Nomarsky-optics, are shown in Fig.1 a. The zygotes are opaque due to the presence of many lipid granules. The same was observed up to the two-cell stage (Fig.1 e,f). Thus, neither interference, nor phase contrast microscopy provided visualization of the pronuclei at the one-cell stage.

In the examined sections, the male pronucleus (Fig.1 b) was already identified in about 40% of the zygotes at 44-46 hpc, whereas both pronuclei were observed approximately in 25% of the zygotes at that time (Fig.1 c; Table 1). At this stage, the male pronucleus appeared at the periphery of the cytoplasm usually irrespective of the position at which the polar bodies were extruded (Fig.1 b). In the fertilized mink egg, in contrast to the unfertilized, lipid granules were displaced away from the pericortical cytoplasm. In the fertilized mink egg this compartment contained a pool of mitochondria, microsomes, multivesicular bodies, cortical granules and other organelles (Fig.1 b and Fig.2). Many vesicles were observed in the perivitelline space (Fig.2 b). Sperm tails were never discerned in the cytoplasm at this stage.

Although the female pronucleus was revealed at 44-46 hpc, it was observed later at 48-50 and 52-54 hpc in most one-cell embryos (up to 60%) (Table 1). At 48-50 hpc, the pronuclei appeared as large, spherical (or ellipsoid) structures, showing a weak affinity for different stains (Fig. 1c). Chromatin was uniformly distributed in the pronuclear volume and no structures were visible within the pronuclei. However, single spherical pronucleoli were encountered in about 90% of the 52-54 hpc zygotes (Fig.4 d,e and Table 2). It appeared as though the pronuclei approached each other as they pass to the center of the zygotes at 52-54 hpc (Fig.1 c and Fig.3 a). It should be noted that the male pronuclei were consistently larger than the female (Table 2). Chromosomes completely condensed into the pronuclei were not observed. However, a breakup of the membranes at the contacting surfaces of the pronuclei occurred in the zygotes at 52-54 hpc (Fig.1 c and Fig.3 a). At same time, the zygotes with centrally located prometaphase chromosomes could be seen (Fig.1 d; Fig. 3 b).

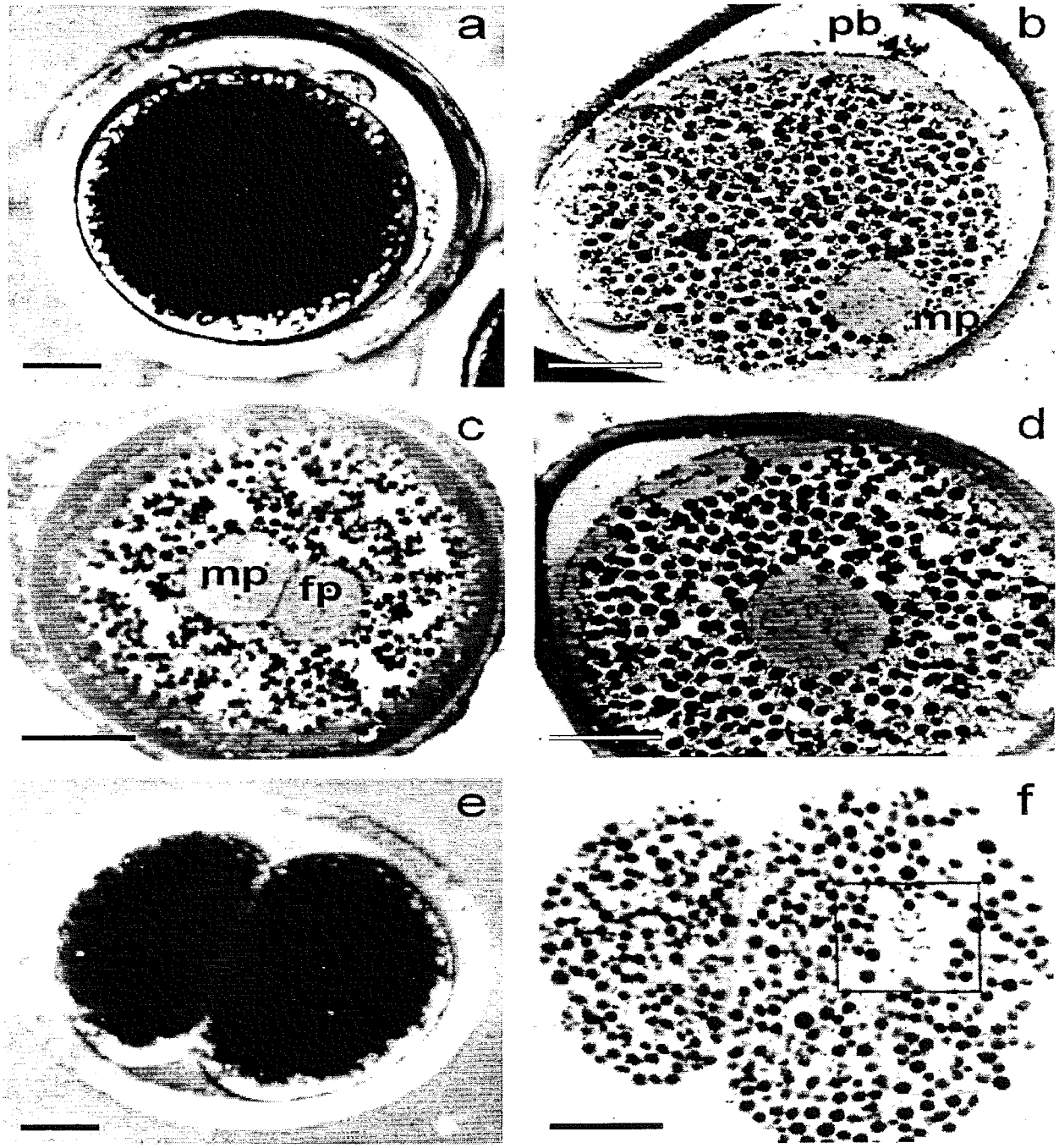

Fig.1. The one-cell and the two-cell stages in mink

a) A live mink zygote as seen under Nomarsky-optics.

b) A male pronucleus (**mp**) appears at the periphery of the cytoplasm far from the polar bodies (**pb**), 44-46 hpc.

c) Male and female (**fp**) pronuclei are closely approximated in the center of the zygote, 52-54 hpc.

d) A mink zygote at the end of the pronuclear stage, 54-56 hpc.

e, f) Two-cell mink embryos just before the second cleavage mitosis (a metaphase plate is shown inside the square), 66-68 hpc. Bar is 20 μm.

Table 1. Time course of the formation of the pronuclei in mink

Age of zygotes (hpc)	Total number of females	Total number of normal zygotes	Zygote without pronuclei (%)	Zygote with a male pronucleus (%)	Zygote with a male and a female pronuclei (%)	Zygote at the end of the pronuclear stage (%)
44-46	4	24	9 (38)	9 (38)	5 (24)	0 (0)
48-50	4	22	3 (13)	9 (41)	8 (36)	2 (9)
52-54	4	18*	0 (0)	0 (0)	11 (61)	7 (39)

* - five zygotes were not included in the pool due to evidence of degeneration

Table 2. Expansion rate of the pronuclei and the formation of the pronucleoli in mink

Age of zygotes (hpc)	Diameter of pronuclei, μm		Zygotes with pronucleoli (%)	Pronucleolar diameter, μm
	male (range)	female (range)		
44-46	19 (16-20)	15 (12-16)	33 %	3
48-50	20 (18-24)	17 (10-20)	50 %	3
52-54	22 (20-24)	17 (10-22)	90 %	3

Table 3. Timing of the one-cell and the two-cell stages in mink

Age of embryos hpc	Total number of females	Total number of embryos	Embryos		
			One-cell %	Two-cell %	Four-cell %
44-46	5	33	100	0	0
46-48	4	26	100	0	0
48-50	7	38	100	0	0
50-52	15	101	100	0	0
52-54	7	40	100	0	0
54-56	6	35	100	0	0
57	2	15	47	53	0
58	2	9	22	78	0
60	2	10	0	100	0
68	2	6	0	0	100

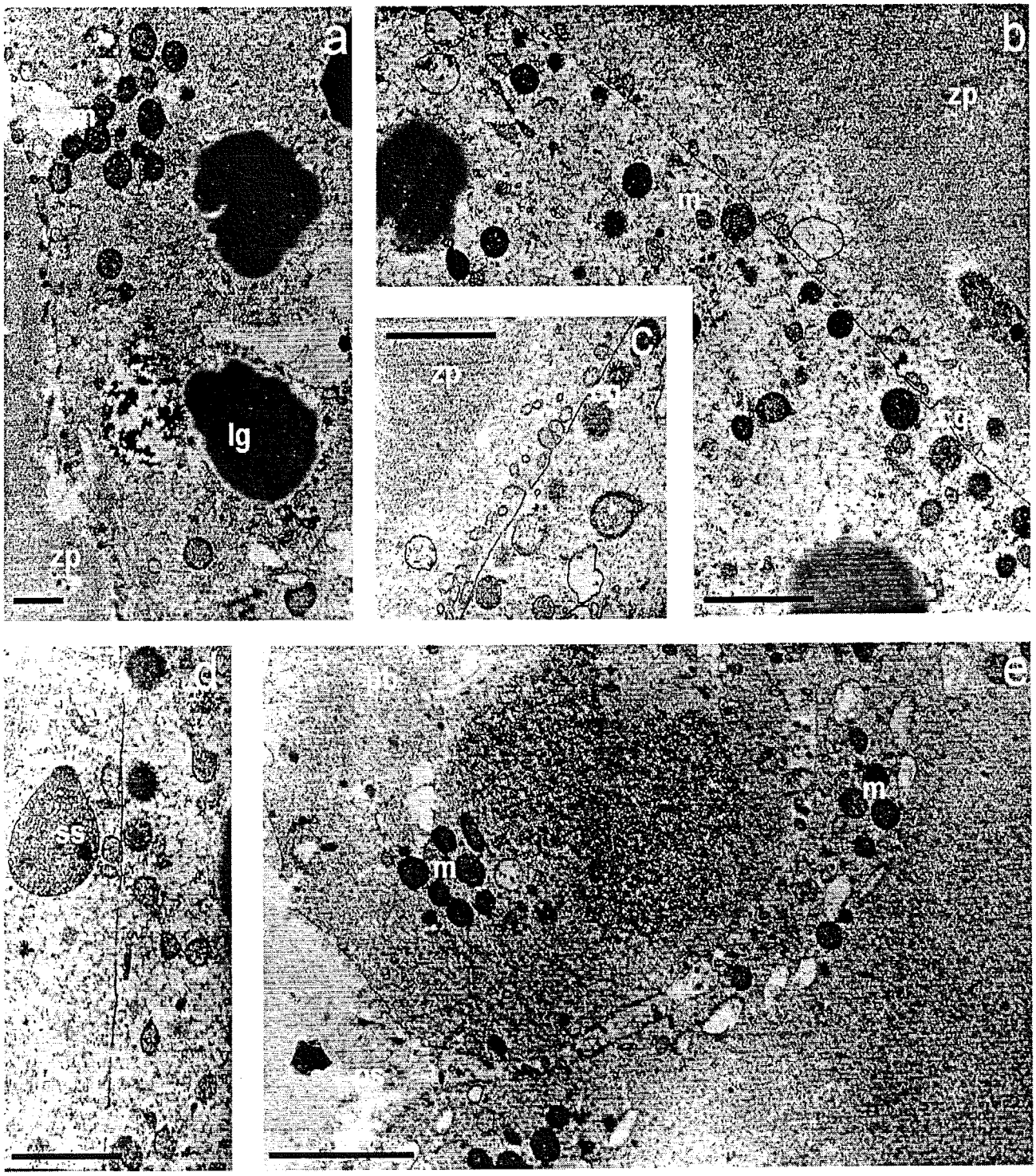

Fig. 2. Pericortical cytoplasm of unfertilized (b) and fertilized (a, c-e) mink oocytes

zp	- *zona pellucida*;	**cg**	- cortical granules;
ps	- perivitelline space;	**v**	- vesicles;
lg	- lipid granules;	**pb**	- polar body;
m	- mitochondria;	**ss**	- segrosome;

a, e) Bar is 2 μm; b, c, d) Bar is 0,5 μm.

At 52-54 hpc, some zygotes were deformed or partly fragmented (not included in Table 1). Probably, this was the pool of unfertilized eggs. If this were the case, the total number of unfertilized eggs would be estimated as about 25% (5 of the 23 embryos examined) at 52-54 hpc.

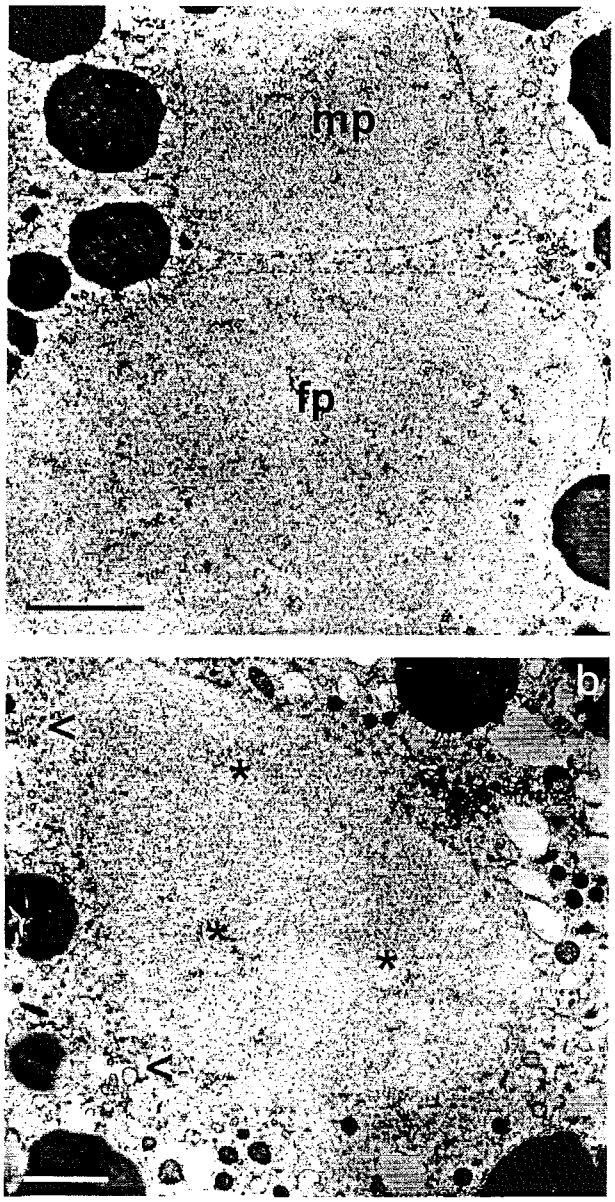

Fig. 3. Caryogamy in mink. The pronuclear membranes start to disrupt at 50-52 hpc (**a**). At 52-54 hpc (**b**) the pronuclear membranes disintegrate and deposit into the multivesicular aggregates (^). The chromosomes (*) are beginning to condense. Bar is 2 μm.

Table 3 demonstrates the timing of the one-cell and two-cell stages. The one-cell stage was completed by to hpc, when the zygotes were absent among the freshly flushed embryos. Thus, the one-cell stage takes at least 20-24 hrs in mink.

2. Effect of centrifugation on the morphology and the viability of the mink zygotes

The effect of brief centrifugation (at 15,000xg for 2 min) on the live zygotes is shown in Fig.4 a, c, d. Under Nomarsky-optics, cytoplasm polarity appeared after centrifugation; one third to half of the cytoplasm became pale and translucent, whereas the rest of the cytoplasm remained opaque (Fig. 4 a,d,f). It should be noted that centrifugation of the embryos did not provide visualization of the pronuclei under Nomarsky-optics. Histological studies of these centrifuged zygotes revealed that both pronuclei were always located within the dark portion of the cytoplasm (Fig. 4 b,c). However, at 52-54 hpc, the pronuclei were well seen in 50% of the zygotes centrifuged at 15,00xg for 2 min (Fig. 4 d,e, f, g). Probably, the pronuclei have distinctive physical properties at the stages of maturation and, hence, they can co-sediment with either the light lipid granules (the opaque portion of the cytoplasm) or with the heavy organelles (the translucent portion of the cytoplasm). Also, it should be taken into account that the orientation of the zygotes during centrifugation is established randomly with respect to the gravitation field and, consequently, the pronuclei can be localized with equal probability either in the opaque (Fig. 4 d, e) or in the translucent portion of the cytoplasm (Fig. 4 f, g).

The results of electron microscopy of the zygotes after their brief centrifugation are shown in Fig. 5b, c, d, e. The heavier translucent portion of the cytoplasm contained mitochondria, multivesicular bodies, lysosomes, and fragments of reticulum. Lipid granules entered the pericortical cytoplasm, which was devoid of granules in the intact zygotes (Fig 1 a, b; Fig.2 b,e).

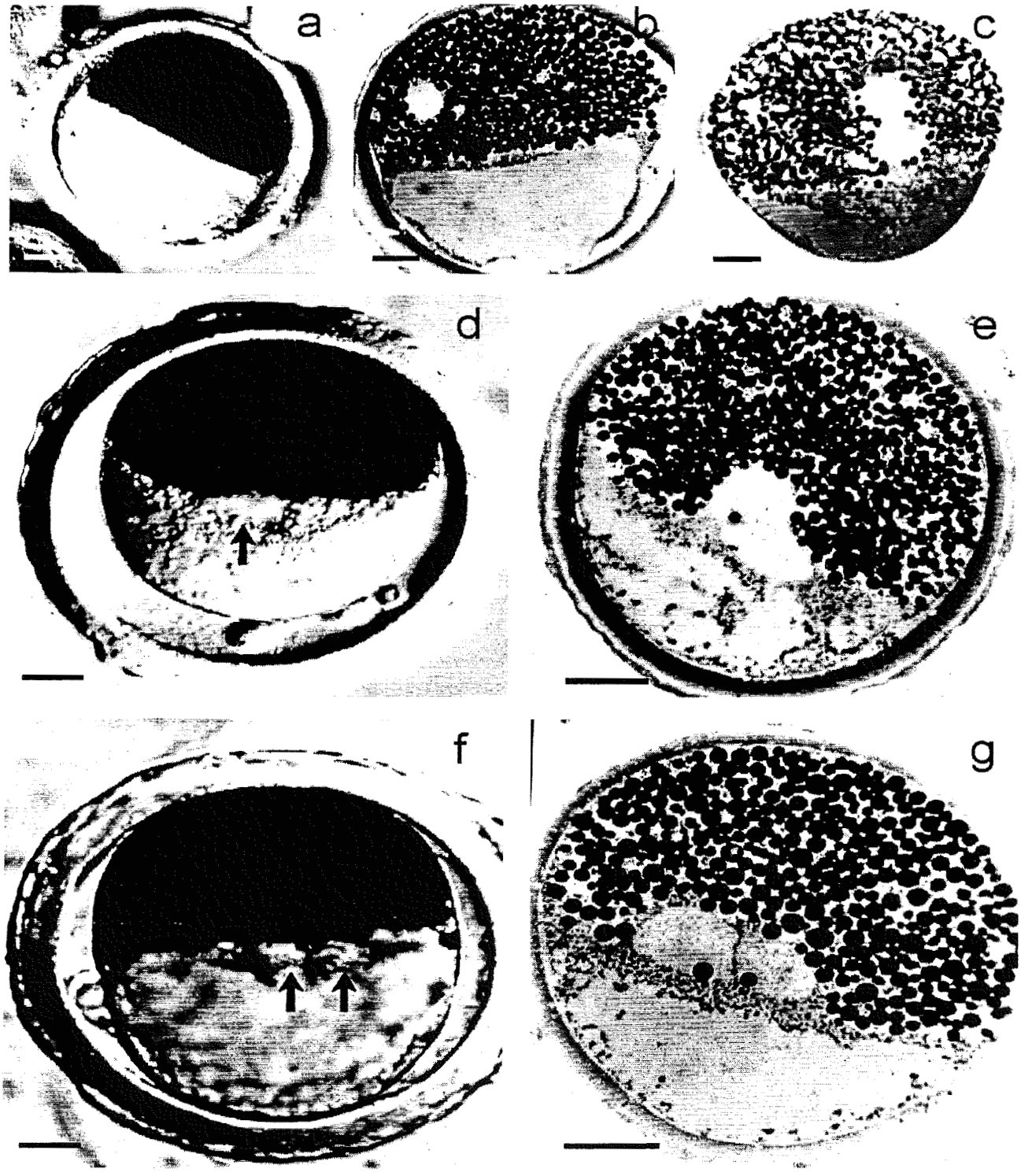

Fig. 4. The pronuclei visualized after brief centrifugation (15,000xg for 2 min). The pronuclei at 44-48 hpc are barely, if it all, visible (**a, b, c**). The pronuclei (d, **f**) are partly visible because the zygotes occupy random positions with respect to gravitation fields (e, **g**). Arrows (↑) point to the pronuclei. Bar is 20 μm.

Table 4. The combined effects of mechanical and chemical treatment on the viability of mink zygotes *in vitro*

Centrifugation (min)	Chemical treatment	Total number of zygotes	Severely deformed zygotes (%)	Zygotes with normal morphology after 7 h culturing (%)	Normally cleaved zygotes (%)
--	--	8	0(0)	8(100)	4(50)
2	--	8	0 (0)	8 (100)	4 (50)
5	--	8	8 (100)	0 (0)	0 (0)
5	CCB	10	10 (100)	0 (0)	0 (0)
2	CCB	6	0 (0)	3 (60)	2 (30)
2	colcemide	6	6 (100)	3 (60)	2 (30)
2	glycerol (0.1%; 1%; 5%; 10% in DPBS)	10	10 (100)	not cultured	

The brief centrifugation had no appreciable effect on the definitive position of the pronuclei in the 50-54 hpc zygotes (Fig.4 d, e, f, g). Prolonged centrifugation (at 15,000xg for 5 min), however, severely deformed the zygotes (Fig.6 a, b).

Clearly, the *in vivo* or *in vitro* viability of the zygotes is a reliable criterion for estimation of the effects of brief centrifugation. For this reason, we used both *in vitro* and *in vivo* tests. According to the *in vitro* observations made using Nomarsky-optics, the effect of brief centrifugation (15,000xg for 2 min) on the mink zygotes is quite reversible. The lipid granules and the other organelles are normally redistributed by 7 hrs of *in vitro* culturing of the centrifuged zygotes. (Fig.5 f). Moreover, the centrifuged zygotes cleaved normally *in vitro* (Fig.5 g and Table 4). The prolonged centrifugation (15,000xg for 5 min) detrimentally affected the zygotes (Fig. 6 a). As a rule, these drastically deformed zygotes broke up into fragments during *in vitro* culturing (Fig. 6 b and Table 4).

From the data in Table 5, it follows that 40% of the freshly flushed zygotes developed normally after transfer into the oviducts of the recipients. Bearing in mind that 25% of the S^h/S^h embryos die *in utero* due to the lethal effect of the S^h mutation (see Methods), the percentage of the live-born pups should be higher, when wild mink are used. When transferred into the oviducts, 25% of the zygote centrifuged at 50-54 hpc developed normally (Table 5), i.e. the total efficiency of manipulation was about 60% of the control.

Unlike the brief centrifugation, the detrimental effect of the prolonged centrifugation (15,000xg for 5 min) was stronger: all the treated zygotes were unable to further develop *in vitro* (Fig.6 a, b and Table 4).

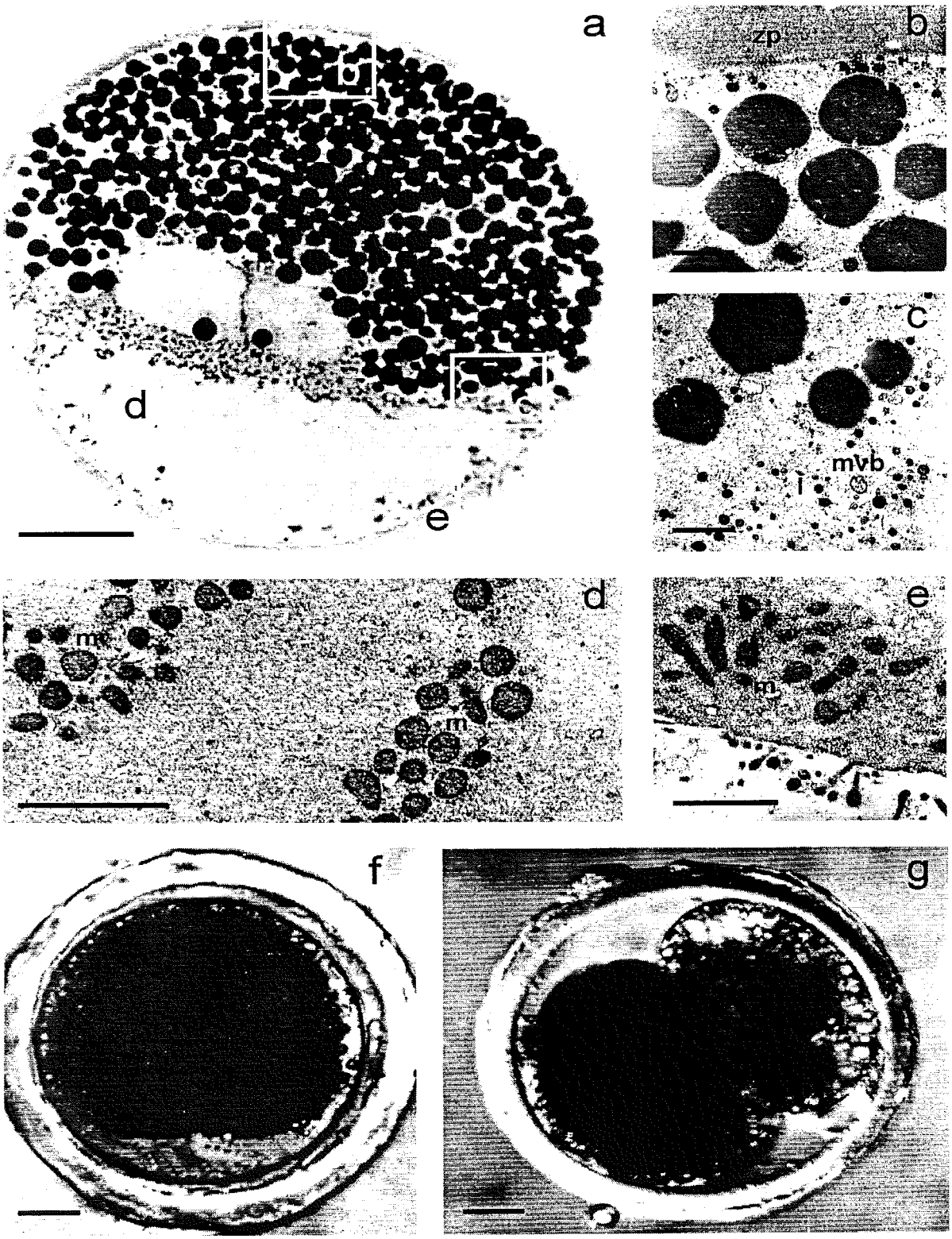

Fig. 5. Effect of brief centrifugation on the mink zygotes
b-d) Segregation of the organelles in a mink zygote centrifuged at 52-54 hpc (**a**).
e, f) Native morphology of the zygote is recovered after 7 hours of culturing and then the zygotes cleave normally *in vitro*.
m - mitochondria; **mvb-** multivesicular bodies; l - lysosomes
a, f, g) Bar is 20 μm b, c) Bar is 2 μm d, e) Bar is 4 μm.

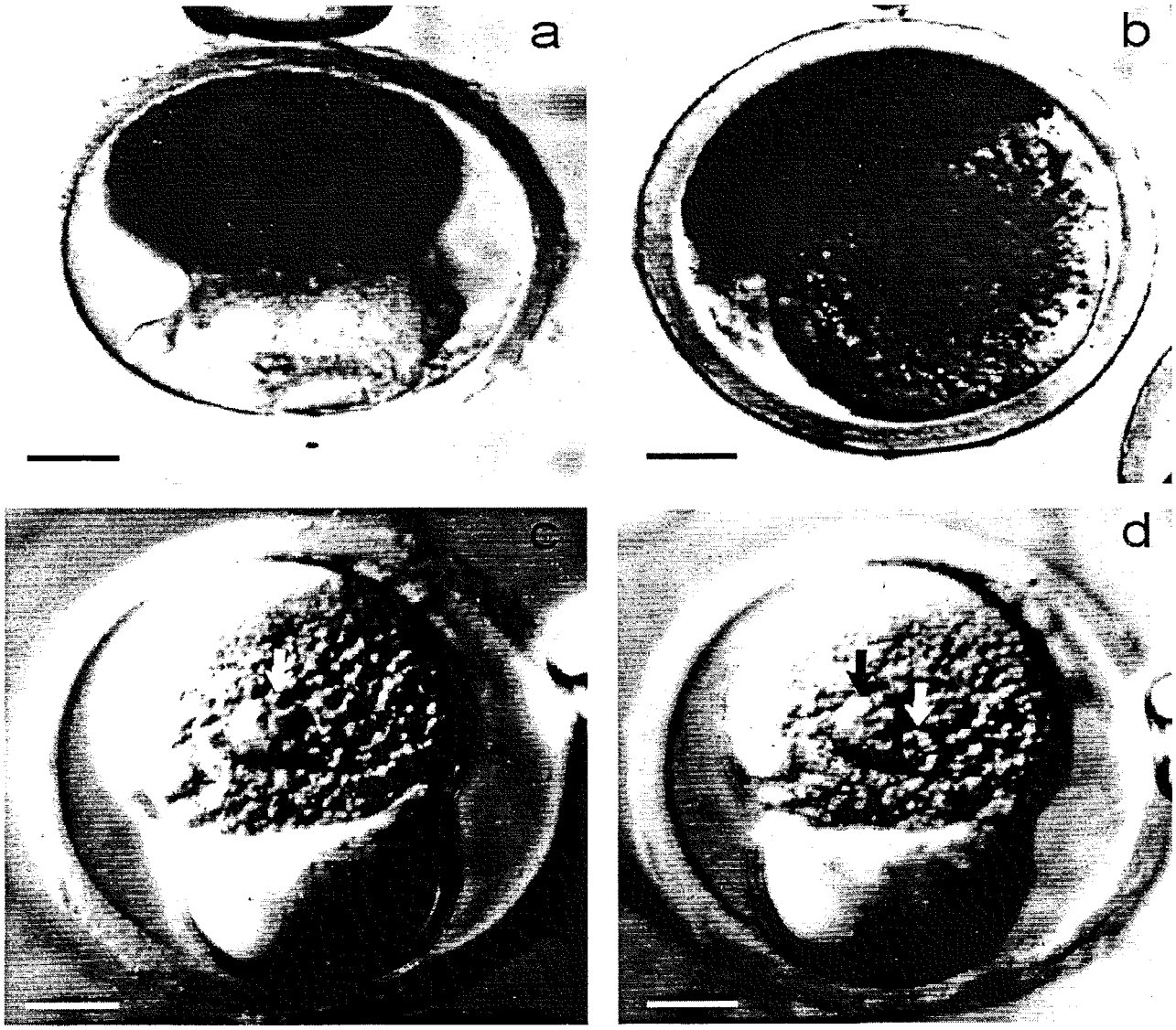

Fig.6. Damaging effect of prolonged centrifugation (a, b) or incubation of zygotes in a glycerol solution (c, d) **a, c)** at 46-48 hpc; **b, d)** at 52-54 hpc. Bar is 20 μm.

Table 5. Effect of brief centrifugation on the viability of mink zygotes after transplantation to recipient females

Treatment	Total number of transplanted zygotes	Number of host females	Number of live-born pups (%)
centrifugation (15,000xg for 2 min)	45	7	11 (24)
control (without centrifugation)	31	5	12 (38)

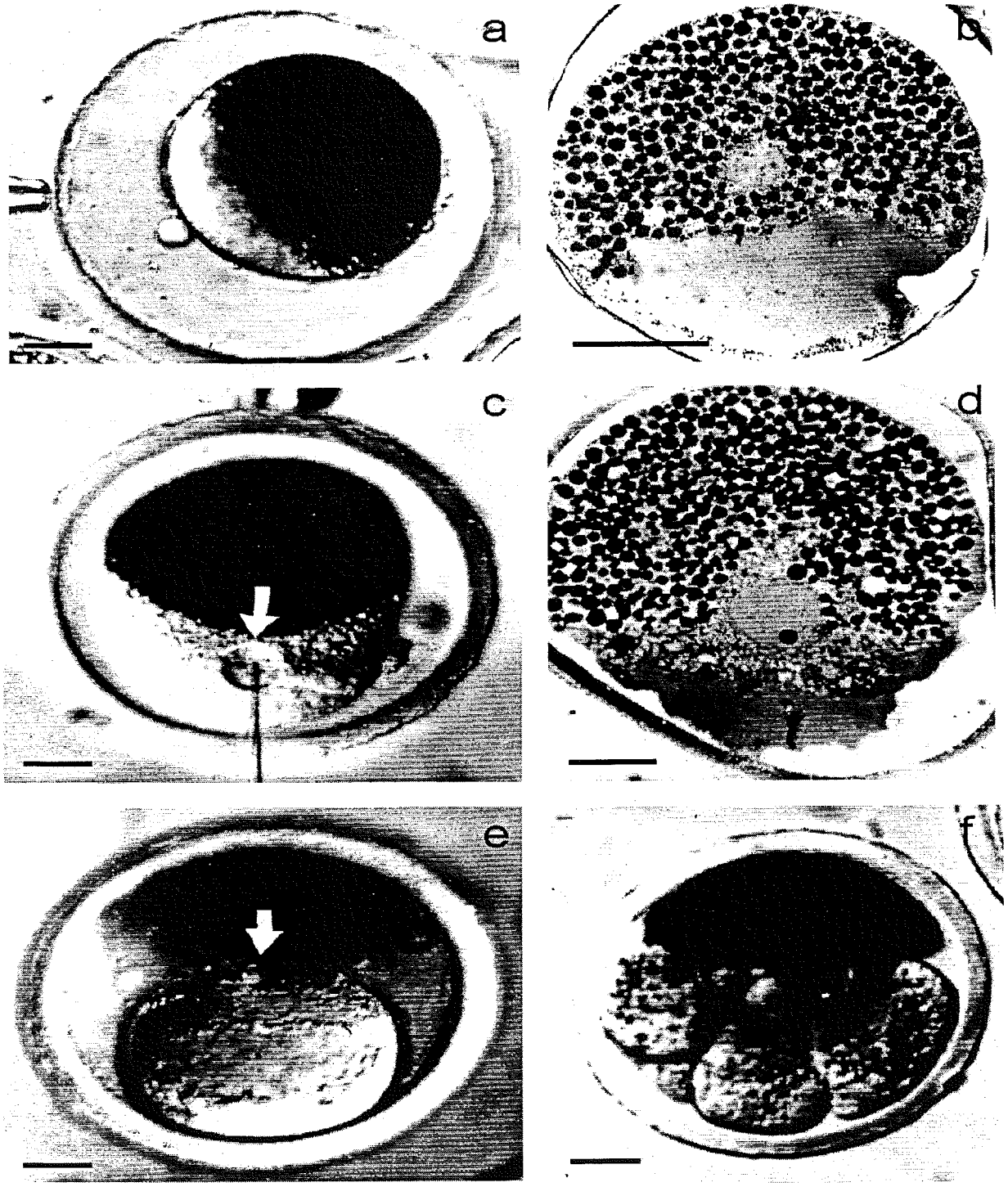

Fig.7. Combined effect of chemical pretreatment and centrifugation on mink zygotes
a, b) The effect of brief centrifugation on the colcemide pretreated zygotes at 52-54 hpc;
c, d) The effect of brief centrifugation on the 52-54 hpc CCB-pretreated zygotes;
e) Prolonged centrifugation of the CCB-pretreated zygotes at 52-54 hpc provokes the entry of lipid granules into the perivitelline space.
f) The majority of the chemically pretreated zygotes fragmented during 7 hrs of culturing *in vitro*.
Pronuclei are shown by arrows (↑). Bar is 20 μm.

After brief centrifugation, the embryos were also incubated in a glycerol solution (0.1%, 1% 5% and 10% glycerol in DPBS) to visualize the pronuclei in mink. Using the Nomarsky-optics, it was shown that in these zygotes the pronuclei can be visualized at the highest frequency (70-80%). However, the zygotes were not viable and died, as a rule, during manipulation (Fig.6 d, e and Table 4).

3. Combined effects of CCB, colcemide and centrifugation on the mink zygotes

It is known that in some cases pretreatment of one-cell embryos with cytochalasin B (CCB) or colcemide, followed by gentle centrifugation, improves visualization of the pronuclei (Hogan et al., 1986; Monk 1987). These agents affect polymerization of actin and tubulin (Maro et al., 1984; Schatten et al 1985).

We applied this approach to the mink zygotes. Pretreatment of the zygotes with colcemide reduced the egg diameter by 1.4 times (Fig.7 a). It is seen that in these zygotes the pronuclei occupy the same central position and they are always immersed in the opaque portion of the cytoplasm (Fig.7 a, b). Thus, this procedure of colcemide pretreatment did not improve the visualization of the pronuclei when Nomarsky-optics was used.

Pretreatment of the zygotes with CCB had no appreciable effect on the volume of the zygote. The pronuclei in the 44-48 hpc CCB-pretreated zygotes were invisible and not seen after centrifugation. In the CCB-pretreated 50-54 hpc zygotes, a single pronucleus was prominent in the translucent portion of the cytoplasm, the other pronucleus remained within the lipids (Fig.7 b, c). After centrifugation for 5 min, the lipid granules were seen outside the cytoplasm (Fig.7 e). The majority of the centrifuged zygotes, which had been pretreated with CCB or colcemide, became fragmented when cultured *in vitro* (Fig.7 f; Table 4). All the zygotes, whose lipids were extruded into the perivitelline space, failed to cleave normally.

Discussion

In mustelids, the passage of sperm into the reproductive tract precedes ovulation and fertilization proceeds quite rapidly. The time course of events is such that the male pronucleus was already formed by 44-46 hpc. At 50-52 hpc, both pronuclei reached the center of the egg and lie in close approximation. The two pronuclei come close together in the periphery of the cytoplasm in less than 3% of the zygotes we examined. Caryogamy in the zygotes fertilized *in vivo* takes place mostly at 52-54 hpc. The first cleavage occurs at 57-58 hpc; at least 50% of the embryos reached the two-cell stage at that time. These data are quite comparable those with reported *earlier (Gustafsson et al 1987)*.

In mink, as in most other studied eutherian mammals, the pronuclei are not fused during caryogamy, but rather remain as separate juxtaposed entities until the nuclear membrane break down during the prophase of the first mitotic division (*Gondos et al 1972; Longo 1973; Ashley and Pocock 1981; Sathananthan and Trounson 1985; Zambony 1970; 1986*). Caryogamy in mink and *rabbit (Gondos et al 1972)* is similar, but interconnections or invaginations of the pronuclear envelope were never observed in the present study in mink.

The development of pronuclei in mink follows mainly the course described for laboratory and farm-bred animals. However, the formation of pronuclei in mink is somewhat species-specific. Pronuclear expansion is slower in mink than in rodents (*Austin and Walton 1960*) The persistence of a single pronucleolus (*Dyban 1988*) throughout the period of pronuclear development is also a distinctive feature of mink. The pronuclei move toward the center without the pronucleoli. The pronucleoli were first seen after the pronuclei had occupied their definitive central position.

The mink is an induced ovulator with ovulation occurring in about 32-36 hpc (*Hansson 1947; Sundqvist et al 1989*). Undoubtedly, there are individual differences in the rate of follicle maturation and oocyte ovulation between and within mothers (*Belyaev and Zhelezova 1968*). The variations in the timing of the development of the zygotes may be caused by this phenomenon.

Mink zygotes are opaque and remain opaque to the two-cell stage. The pronuclei are invisible under the interference and phase-contrast optics. We used three approaches to visualize the pronuclei:

1) centrifugation of the zygotes (15,000xg for 2 or 5 min);
2) treatment of the centrifuged zygotes with glycerol at different concentrations;
3) pretreatment of the zygotes with colcemide or CCB followed by centrifugation.

From the experimental results it is clear that brief centrifugation (15,000xg for 2 min) allows us to visualize the pronuclei in 52-54 hpc zygotes with a reliability of about 50%. However, the pronuclei remain invisible in 44-46 hpc zygotes. The morphology of the centrifuged zygotes is distinguishable by the formation of translucent and lipid granules containing opaque portions. These changes are reversible: the native morphology is recovered by 7 hrs of culturing, and the zygotes cleave normally *in vitro*. In contrast, prolonged centrifugation (15,000xg for 5 min) causes irreversible changes and the zygotes develop abnormally *in vitro*.

We used a direct test to estimate the viability of the zygotes by transferring them into the oviducts of recipient mothers. The test demonstrated that intact and centrifuged eggs developed to birth with an efficiency of about 40% and 25%, respectively. It may be suggested that the percentage of live-born pups would be higher, if the genotype of the transplanted embryos were Standard (+/+), not mutant S^h. Thus, the efficiency is quite high and the test can be recommended to breeders.

Summarizing, recommendations can be given for improving the procedure of microinjection of foreign DNA into the mink zygotes:

i) it is best to remove the mink zygotes at 50-54 hpc;
ii) gentle centrifugation (15,000xg for 2 min) allows one to visualize the pronuclei at least in 50% of the mink zygotes;
iii) a transfer of the zygotes into the oviducts (without culturing *in vitro*) provides at least 35% live-born pups.

The recommendations may encourage microinjections of foreign DNA into zygotes to produce transgenic minks.

Acknowledgments

We especially thank Denis M. Larkin and Alexander V. Dubynin for computer assistance. We also thank Dr. Victor G. Kolpakov and Dr. Oleg V. Trapezov for commenting on a very early draft of the manuscript. We are also grateful to A. Fadeeva for translating the paper from Russian into English.

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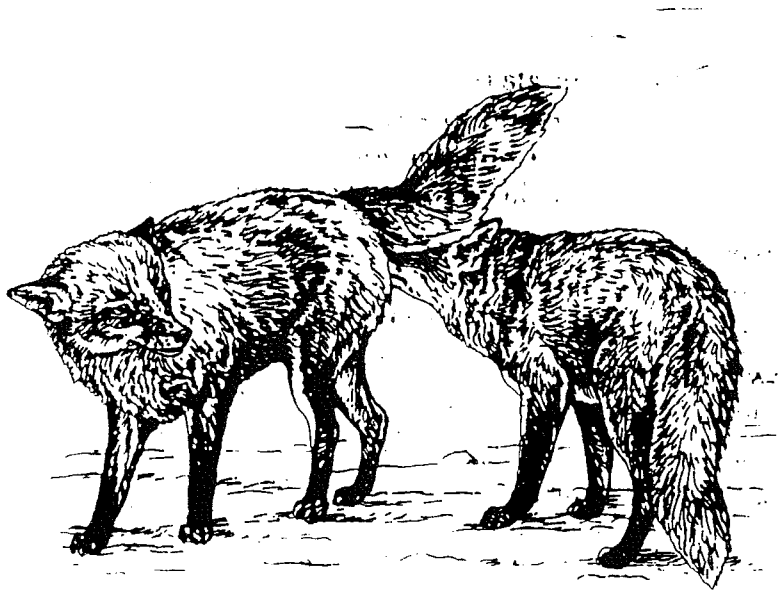


Prolactin profiles of pregnant, lactating and non-mated female mink (*Mustela vison*)

A-H. Tauson

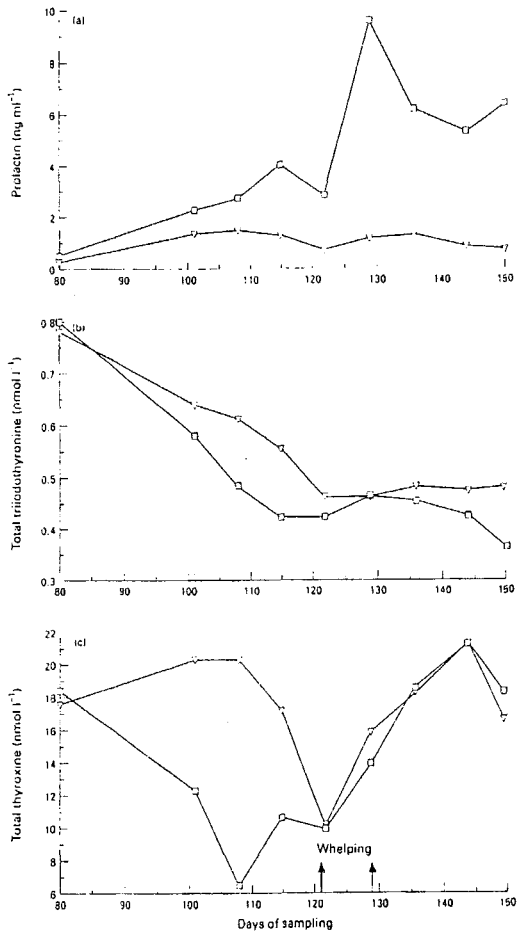


Fig. 2. Profiles of plasma concentrations of (a) prolactin, (b) total thyroxine and (c) total triiodothyronine from late March (day 80) until late May (day 150) in female mink that were not mated (n = 3; ∇) and in pregnant and lactating females (n =10;). SE of the LS-means for comparions between females that were not mated and pregnant or lactating females were 3.1 and 1.7 for prolactin, 0.03 and 0.05 for total triiodothyronine, and 2.8 and 5.0 for total thyroxine, respectively.

This study was part of an experiment on energy metabolism in pregnant and lactating female mink (*Mustela vison*). Ten mated and three non-mated female mink were kept in metabolic cages in the laboratory from immediately after mating until the kits were three to four weeks old. Consecutive energy balance experiments with periods each of one week duration, including a 22 h respiration experiment (only three with females that were not mated) were performed, and weekly blood samples were collected for determination of plasma concentrations of thyroid hormones, prolactin, insulin and glucose. Prolactin profiles of pregnant and lactating females had a biphasic pattern: there was an increase in April, a decline immediately before parturition, and peak values were recorded in early May, usually when the females were in the first week of lactation. In females that were not mated plasma concentrations of prolactin did not rise above basal concentration. These females also exhibited a delayed spring moult, and had a lower feed intake. In addition, plasma profiles of thyroid hormones of mated and non-mated animals were different; concentrations of thyroid hormones decreased in April to early May, but the decrease started earlier and was most pronounced in pregnant female mink. These data indicate that prolactin secretion in female mink is regulated by photoperiod, but that other endocrinological events during pregnancy may also be involved.

Journal of Reproduction and Fertility Supplement 51, pp. 195-201, 1997. 1 table 2 figs., 14 refs. Author's summary.

Etiology of reproductive disorders in female farmed foxes

E. Smielewska-Los, S. Klimentowski, K. Rypula, R. Karczmarczyk

The aim of the studies was to estimate the range of bacterial and viral infections in etiology of reproductive disorders in polar and silver fox females.

The examinations were carried out in vixens, in which early fetal death, abortions, stillbirths and neonatal losses were observed.

In serological examinations for listeriosis, low titres of antibodies were found in 13 females

(from 30 examined), but Listeria sp. was not isolated from fetuses and dead new-born foxes. All females were free from brucellosis and leptospirosis. Toxoplasma gondii infections were noted in all the examined vixens derived from one polar fox farm (5 farms were examined). The blue fox parvovirus infection was observed in 3 polar fox farms. The high antibody levels were noted only in vixens with early fetal death.

The bacteriological examinations of vaginal swabs confirmed the important influence of Salmonella sp. and 0-hemolytic strains of Escherichia coli infections on the occurrence of abortions and stillbirths. All the negative results were obtained during the examination for Chlamydia psittaci and Campylobacer sp. infection.

Zycie Weterynaryjne, 73: 3, pp. 93-97, 1998. In POLH. 4 tables, 32 refs. Authors' summary.

Use of products of Mutilus Mariculture Processing (*Mutilus Hydrolyzate*) in fur breeding

N.N. Tyutyunnik, L.B. Uzenbaeva, V.A. Ilukha, H.I. Meldo

A study of the rational use of the bioresources of the White sea for medical-biological purposes is relevant. At the Laboratory of Animal Ecological Physiology of the Institute of Biology evaluation on the influence of mutilus hydrolyzate on the reproductive function and physiological condition of mink was carried out. The action of hydrolyzate depends on dose, duration of application and condition of the animals. A significant part of the research is devoted to the study of the hydrolyzate efficiency with viral plasmocytosis or aleutian disease of mink (AD). Use of mutilus hydrolyzate as a food additive results in changes in the ratio of the fractional composition of blood serum proteins, structure leucoformula and weight increase. Normalization of the spectrum protein in mink with aleutian disease apparently reduces the development of disturbances connected with hypergammaglobulinaemia.

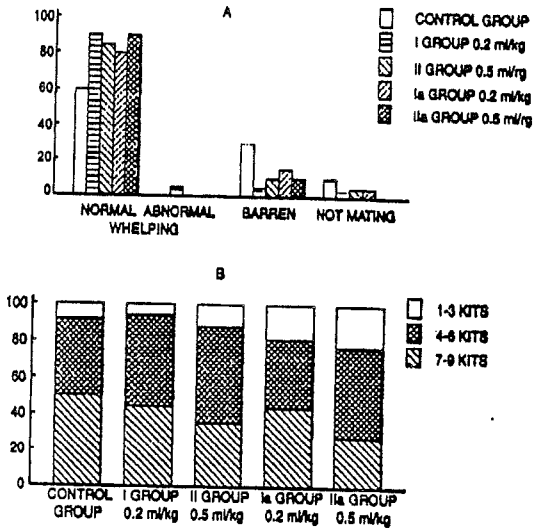


Fig. 1. Effect of mutilus hydrolysate on reproduction parameters of standard mink. A – number of mating and whelping females; B – litter size.

Karelia and Norway: the main trends and prospects of scientific cooperation. Proceedings of the Scientific Conference held in Karelian Research Centre RAS within the framwork of the Days of Norway in Republic of Karelia (Petrozavodsk, 28-31 May, 1997). 5 figs., 8 refs. Authors' abstract.

Artificial insemination of foxes in 1997

E. Smeds

In 1997, in Finland, 229.000 blue, silver and crossbred foxes were inseminated resulting in a CR of 89, 87 and 89%, respectively, and a litter size per inseminated female of 612, 2.86 and 5.4 cubs. In the Articop scheme, in which inseminations are carried out by farmers, 3630 females of the 3 breed types were inseminated, resulting in a CR of 80, 81 and 79% and a litter size at birth of 4.8, 2.17 and 4.56.

Finsk Pälstidskrift, 31,1 2, pp. 305, 1997. In SWED. 2 tables. CAB-abstract.

Vaccination with Aleutian mink disease parvovirus (AMDV) capsid proteins enhances disease, while vaccination with the major non-structural AMDV protein causes partial protection from disease

Bent Aasted, Søren Alexandersen, Jesper Christensen

Vaccination studies were performed with partially purified recombinant AMDV VP1/2 capsids as well as with the major AMDV non-structural protein (NS1). All vaccine constructs induced an antibody response, but did not prevent infection upon challenge with AMDV. The severity of Aleutian disease (AD) was judged by the serum gammaglobulin level, the quantity of peripheral blood CD8 lymphocytes, antibody titers to VP1/2 and NS1 proteins and mink death rates. The VP1/2 vaccine constructs enhanced the disease process with drastic death rates for the vaccinated mink. On the contrary, the NS1 vaccine constructs resulted in milder AD than seen in the non-vaccinated mink.

Vaccine, Vol. 16, No. 11/12, pp.1158-1165, 1998. 4 figs., 30 refs. Authors' abstract.

Production and characterization of monoclonal antibodies against mink leukocytes

Wensheng Chen, Michael Pedersen, Sanne Gram-Nielsen, Bent Aasted

Three monoclonal antibodies (mAbs) were generated against mink leukocytes. One antibody reacted with all T lymphocytes, one with all monocytes and one had platelet reactivity. Under reducing conditions, the T lymphocyte reactive antibody immunoprecipitated 18 kDa, 23 kDa, 25 kDa and 32-40 kDa polypeptides and the platelet reactive antibody 17 kDa, 22 kDa plus two high molecular weight (> 100 kDa) polypeptides. Immunohistological studies of the mAbs were performed in order to localize the cellular distribution of the detected antigens in various organs. The T lymphocyte reactive antibody detected an antigen, which was widely distributed in the T cell area of lymph nodes and spleen and in the thymic medulla. We conclude that this antibody is an anti-CD3 mAb and suggest that the platelet reactive antibody reacts to the CD41/CD61 integrin molecule. In addition to our own mAbs, more than 100 mAbs against leukocytes of human and various animal species have been analysed for cross-reactivity to mink leukocytes. We found eight to cross-react with mink. Of particular importance was an anticanine CD11a mAb, an antihuman CD79a mAb and an antihuman bcl-2 mAb.

Veterinary Immunology and Immunopathology 60, pp. 161-170, 1997. 1 table, 5 figs., 15 refs. Authors' abstract.

Tuberculosis of polecat-ferrets caused by Mycobacterium tuberculosis

M. Holub, T. Kubinski, I. Barcz, W. Jurkowski

A disease case was described in a large pack of polecat-ferrets. Post-mortem, histopathological and bacteriological examinations showed that a fast-acid bacillus was the cause of the infection; it was classified as Mycobacterium tuberculosis. A man suffering from tuberculosis of the lungs, who looked after the animals, was probably the source of the infection.

Medycyna Weterynaryjna 47, 2, pp. 61-62, 1991. 11 refs. In POLH, Su. ENGL. Authors' summary.

Septic infection of a companion chinchilla with *Salmonella* Enteritidis

Satomi Yamagishi, Yoshimasa Watanabe, Hidenori Tomura, Takashi Sekine,Munehito Mimura, Yuji Iijima and Mitsuyuki FujiI

Fron a companion chinchilla that collapsed and died, *Salmonella Enteritidis* was isolated from the major organs and intestinal contents.

Pathologically, necrotic enteritis, focal necrosis in the liver, in the renal glomeruli and spleen microvascular thrombosis, meningitis and panophthalmitis were observed, and immunohistochemically, salmonella antigens were detectable in the lesions and bacterial clumps in the kidneys and heart.

J. Jpn. Vet. Med. Assoc., 50, pp. 345-348, 1997. In JAPN, Su. ENGL. 3 figs., 19 refs. Authors' summary.

Analyses of leucocytes in blood and lymphoid tissues from mink infected with Aleutian Mink Disease Parvovirus (AMDV)

Wensheng Chen, Bent Aasted

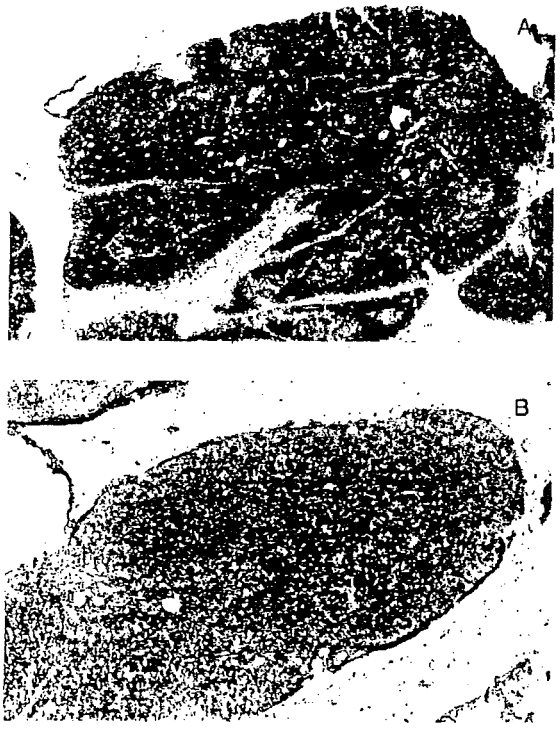


Fig. 5. Immunostaining with a monoclonal antibody to mink CD3 of formaldehyde fixed thymic sections from an uninfected mink (A) and from a mink infected 10 months earlier (B) with AMDV. Technically, the APAAP immunostaining protocol of DAKO (Glostrup, Denmark) was used.

Mink were infected with Aleutian Mink Disease Parvovirus (AMDV) and sacrificed at monthly intervals after infection. During this time humoral immune responses and leucocyte numbers in blood, mesenteric lymph node, spleen and thymus were monitored. Serum hypergammaglobulinaemia was observed together with elevated antibody responses to AMDV NS 1 and VP 112 proteins. In blood, a highly significant increase in $CD8^+$ lymphocytes was observed. However, (presumed)$CD4^+$ cells defined as $CD3^+CD8^-$ cells, and B lymphocytes remained relatively constant throughout the study. The (presumed)$CD4^+/CD8^+$ ratio decreased significantly from greater than 2 to less than 0.5 and MHC-11^+ blood leucocytes increased significantly during infection, a large proportion of these being $CD8^+$. Similar changes were observed in the mesenteric lymph node and spleen. Immunohistology of lymph nodes showed a massive expansion of the paracortical area due to increased numbers of $CD8^+$ cells. The staining intensity of B lymphocytes in lymph nodes with a CD79a reactive monoclonal antibody was decreased in the late infection, indicating a possible greater number of plasma cells. Thymic involution was observed during the AMDV infection, although relative increases in $CD3^{high}$(presumed)$CD4^+$ and $CD3^{high}CD8^+$ single positive cells were observed. These increases were countered by a corresponding reduction in the $CD3^{low}$(presumed)$CD4^+CD8^+$ double positive cell population. Immunohistology of the thymus in normal mink showed that most of the matured $CD3^+$ T cells were present in the inner medulla, while only few $CD3^+$ cells could be found in the outer cortex. In severely infected mink the thymic structural organisation vanished, and $CD3^+$ cells were found throughout the organ.

Veterinary Immunology and Immunopathology 63, pp. 317-334, 1998. 4 tables, 5 figs., 29 refs. Authors' abstract.

Examinations of new-born foxes towards CHV and Mycoplasma infections as well as Toxocara canis infestation

E. Smielewska-Los, S. Klimentowski, C. Kaszubkiewicz, J. Pacon

The purpose of the study was to establish the influence of canine herpes virus (CHV), Mycoplasma sp. and Toxocara canis on new-born fox mortality. The examinations were carried out in 25 new-born foxes which had died at the age of up to 5 days. They were derived from 5 blue and silver fox farms. Virological investigations were done using direct IF method and virus isolation attempts. Hepatic, splenic and renal tissue suspensions were inoculated into monolayers of cell lines: A-72, @YMCK, Vero, RK-13. PPLO (Difco) medium was used to isolate mycoplasmas. Parasitological examinations were performed using larvoscopy technique. At autopsy lung congestion was observed in all examined pups. Renal hemorrhages in the subcabsular region of the kidney were found in some pups. No microorganisms were isolated in basic bacteriological examinations of the pups. Low levels of antibodies against CHV were found in some vixens. Histologically interstitial pneumonia was found in all examined foxes. Canine herpesvirus was not isolated from any cases, nor Toxocara canis larvae in lungs and livers. Mycoplasmas were isolated from lungs of 5 pups derived from 2 farms. This suggests that mycoplasmas may cause interstitial pneumonia in new-born foxes.

Medycyna Wet. 54 (4), pp.253-257, 1998. In POLH, Su. ENGL. 1 table, 28 refs. Authors' abstract.

Potential agents of reproductive failures in vixens

B. Mizak, A. Rzezutka, J. Matras.

The studies on the influence of bacteria and parvovirus infection on reproductive failure in silver and blue foxes were carried out on 21 Polish farms. Parvoviral antibodies were detected in 35 out of 229 sera tested. Parvovirus infection could be the main cause of small litter size in only 6 farms An abundant growth of E. coil, Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgaris and Clostridium perfringens was demonstrated in vaginal and prepucal swabs collected from animals tested. Isolated bacteria were susceptible to norfloxacin and amoxicillin.

Antibiotic treatment, according to results of in vitro tests, of all animals before reproduction and all vixens before parturition, resulted in a decreased number of "empty vixens", abortions and neonatal deaths. Application of a proper antibiotic or elaboration of an empiric scheme of infections for foxes would eliminate reproductive losses on fox farms.

Medycyna-Weterynaryjna 54, 4, pp. 271-275, 1998. In POLH, Su. ENGL. 3 tables, 24 refs. Authors' summary.

Helicobacter mustelae-associated gastric MALT lymphoma in ferrets

Susan E. Erdman, Pelayo Correa, Leslie A. Coleman, Mark D. Schrenzel, Xiantang Li, James G. Fox

Gastric lymphoma resembling gastric mucosa-associated lymphoid tissue (MALT) lymphoma linked with Helicobacter pylori infection in humans was observed in ferrets infected with *H. niustelae* Four ferrets with ante- or postmortem evidence of primary gastric lymphoma were described. Lymphoma was diagnosed in the wall of the lesser curvature of the pyloric antrum, corresponding to the predominant focus of *H. mustelae*-induced gastritis in ferrets. Two ferrets had low-grade small-cell lymphoma and two ferrets had high-grade large-cell lymphoma. Gastric lymphomas demonstrated characteristic lymphoepithelial lesions, and the lymphoid cells were IgG^+ in all ferrets. Lymphoma was confirmed by light chain restriction, which contrasted with the 1.2:1 κ:λ ratio observed in

H. mustelae-associated chronic gastritis *H. mustelae* infection in ferrets has been used as a model for gastritis, ulcerogenesis, and carcinogenesis. The ferret may provide an attractive model to study pathogenesis and treatment of gastric MALT lymphoma in humans.

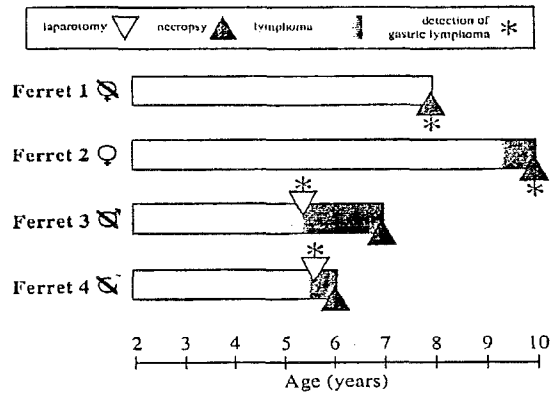


Fig. 1. Progression of primary gastric lymphoma in four ferrets.

Am J Pathol 151, pp. 273-280, 1997. 1 table, 3 figs., 37 refs. Authors' summary.

Epidemiological studies on Mycobacterium avium infections in carnivores

Karel Hejlicek, Frantisek Treml

Susceptibility of dogs, cats and mink to experimental infection with M. avium was tested by subcutaneous inoculation of a M. avium serotype 2 suspension, oral administration of liver tissue of M. avium-infected chickens, and long-term contact with infected chickens. Neither clinical signs, nor post-mortem microscopic lesions were found in any of the experimental animals. Mycobacteria were isolated from muscles, mesenteric lymph nodes and the intestinal walls and lung tissue one and one cat infected orally, respectively. M. avium was demonstrated in feces of the infected dogs, cats and mink 2-4 days after the oral infection.

Veterinarstvi 47, 7, pp. 293-295, 1997. In CZECH, Su. ENGL. 35 refs. Authors' summary.

Analysis of the immunological cross reactivities of 213 well characterized monoclonal antibodies with specificities against various leucocyte surface antigens of human and 11 animal species

R. Brodersen, F. Bijlsma, K. Gori, K.T. Jensen, W. Chen, J. Dominguez, K. Haverson, P.F. Moore , A. Saalmüller, D. Sachs, W.J. Slierendrecht, C. Stokes, 0. Vainio, F. Zuckermann, B. Aasted

213 Monoclonal antibodies (mAbs) raised against leucocyte surface antigens from human and 11 animal species were analyzed for reactivities against leucocytes from human and 15 different animal species. We found 77 mAbs (36%) to cross-react. Altogether, 217 cross reactions were registered out of 3195 possible combinations (7%). Most of the cross reacting mAbs had integrin or MHC class II specificities. This study defined cross reactions on the following markers: CD1a, lc, 2,4, 5, 8, 9, 1la, 1lb, 14, 18, 20, 21, 23, 29, 31, 41, 43, 44, 45, 45R, 46,49, 61, 62L, TCR γ/δ, BCR, Thy-1, MHC class 1 and MHC class 11, Swine-WC7 and Cattle-WC1. In order to characterize the molecular weight (MW) of the corresponding cross reacting antigens, selected mAbs were used to immunoprecipitate the antigens. The MW's of the analyzed precipitated antigens were in good agreement with the MWs of the homologous antigens. The followed strategy was found to be efficient and economical in defining new leucocyte antigen reactive mAbs.

Veterinary Immunology and Immunopathology 64, pp. 1-13, 1998. 2 tables, 43 refs. Authors' summary.

Pneumonyssoides caninum, the canine nasal mite, reported for the first time in a fox (*Vulpes vulpes*)

William P. Bredal, Bjørn K. Gjerde, Hege Kippenes

This is the first report describing the finding of the canine nasal mite, *Pneumonyssoides caninum*, in a silver fox (*Vulpes vulpes*). It is also the first time *P. caninum* has been found in a species

other than the dog (*Canis familaris*). A severely debilitated 10-month-old, male silver fox was euthanised due to suspected renal failure. During autopsy, a female mite matching the description of *P. caninum* was found in the nasal cavity of the fox. The finding of *P. caninum* in the fox suggests the existence of a new host, or at the very least a transient host. The fox's role in maintaining and/or propagating canine nasal mite infection needs to be explored.

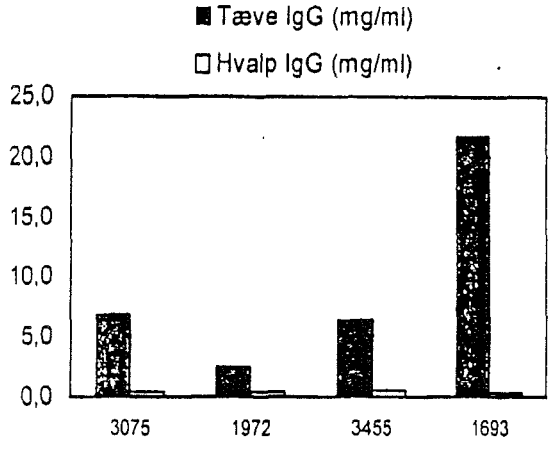
Fig. 1. A female nasal mite, *P. caninum*, 1.14 mm long and 0.63 mm wide, recovered from the nasal cavity of a young captive silver fox (*Vulpes vulpes*). Note four pairs of legs and the single granulated ovum in the gravid uterus. The abdomen was damaged duirng preparation for photography.

Veterinary Parasitology 73, pp. 291-297, 1997. 2 figs.,19 refs. Authors' summary.

Investigations of immunoglobulins in mink

Åse Uttenthal, Per Henriksen, Jørgen Østergaard, Tove Clausen, Fred Costello

Diseases in newborn mink are widely studied, but the work is hampered by the lack of basal knowledge of the immunology in mink kits. We have studied the development of antibodies in mink kits and the transfer of immunoglobulins from the female to the mink kit. In all experiments their own mothers nursed the mink kits. The investigations were based on total measurement of immunoglobulins based on a DELFIA system and analysis of passive immunity using antibodies to Mink enteritis virus (MEV) as a marker for the antibodies transferred from the female.



The investigations have reached the following conclusions:

1. Mink kits are born with very low levels of antibodies.
2. The access to and uptake of colostrum is crucial for the mink kit.
3. If the female is revaccinated to MEV the increase in antibodies to this agent is also measurable in the serum of the mink kits of this female.
4. Antibodies ingested by the mink kits are transferred to the serum of the mink kits; thus within a few days the total concentrations of immunoglobulins in the kit is at the same level as their mothers.
5. The concentration of immunoglobulin is not dependent on the number of kits in the litter.
6. The passively transferred antibodies starts to decay about 2 weeks of age of the mink kit.

7. Mink kits born from vaccinated females are capable to produce active antibodies as a response to vaccination (MEV) at 55 days of age in spite of a measurable passive antibody response.

Further investigations are needed to elucidate the impact of these findings.

Tecnical Year Report 1998, pp. 189-196, PFR, Febr. 1999. In DANH, Su. ENGL. Authors' summary.

Morphologic and hematologic characteristics of storage pool deficiency in beige rats (Chédiak-Higashi syndrome of rats)

Kiyokazy Ozaki, Hiroyuki Fujimori, Syohsaku Nomura, Tetsu Nishikawa, Masahiko Nishimura, Hidemitsu Pan-Hou, Isao Narama

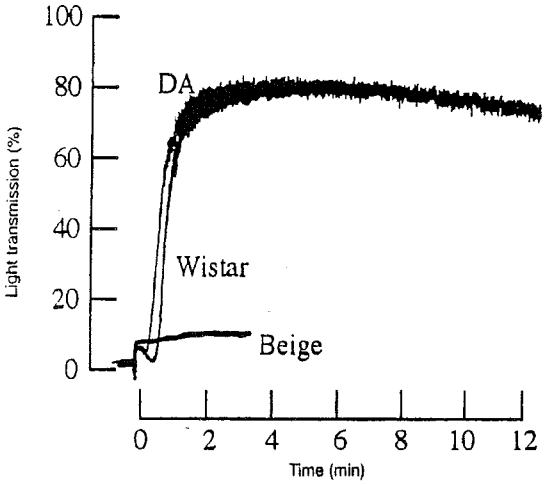


Fig. 1. Collagen (50 µg/ml)-induced platelet aggregation in beige, DA, and Wistar rats. Notice lack of aggregation in beige rats.

Characterization of beige rats as having a platelet storage pool deficiency (SPD) was undertaken. Platelets from beige rats, an animal model of Chédiak-Higashi syndrome (CHS), completely lacked the ability to aggregate when stimulated with high dosages of collagen (50 µg/ml), and lacked secondary aggregation induced by adenosine diphosphate (ADP). Concentrations of ADP, ATP, and serotonin in the platelets of beige rats were significantly lower than those of the control rats. However, platelet count remained within normal values. Electron microscopy revealed that platelets had fewer dense granules, whereas other organelles had normal structure. This morphologic and functional evidence confirms that platelets of beige rats have the typical characteristics of SPD.

Laboratory Animal Science, Vol. 48, No. 5, pp. 502-506, 1998. 1 table, 3 figs. Authors' abstract.

The host range of chronic wasting disease is altered on passage in ferrets

Jason C. Bartz, Richard F. Marsh, Debbie I. McKenzie, Judd M. Aiken

Chronic wasting disease (CWD), a member of the transmissible spongiform encephalopathies (TSEs), was first identified in captive mule and black-tail deer in 1967. Due to the failure to transmit CWD to rodents, we investigated the use of ferrets (*Mustela putorius furo*) as a small animal model of CWD. The inoculation of CWD into ferrets resulted in an incubation period of 17-21 months on primary passage that shortened to 5 months by the third ferret passage. The brain tissue of animals inoculated with ferret-passaged CWD exhibited spongiform degeneration and reactive astrocytosis. Western blot analysis of feret-passaged CWD demonstrated the presence of PrP-res. Unlike mule deer CWD, ferret-passaged CWD was transmissible to Syrian golden hamsters (*Mesocricetus auratus*). Increasing the passage number of CWD in ferrets increased the pathogenicity of the agent for hamsters. This increase in host range of a field isolate on interspecies transmission emphasized the need for caution when assessing the potential risk of transmission of TSEs, such as bovine spongiform encephalopathy, to new host species.

Virology 251, pp. 297-301, 1998. 5 figs., 13 refs. Authors' summary.

IFASA

VIIth INTERNATIONAL SCIENTIFIC CONGRESS IN FUR ANIMAL PRODUCTION

Kastoria, Macedonia, Greece
13 - 15 September 2000

REGISTRATION FEE

IFASA members	$200
Non members	$250
Accompanying persons	$100

Registration fee includes:	Members and non members	Accomp. persons
Participation to all scientific program	*	
Congress bag	*	
Proceedings	*	
Three lunches (Wed.-Th.-Fr.)	*	
Coffee breaks (during congress)	*	
Opening reception	*	*
City tour	*	*
Social Events	*	*
Accompanying persons program		*

INFORMATION

For all information you may contact
SYMVOLI - Congress Organizers Ltd.
Patmou 8, Kalamaria, 551 33 Thessaloniki, Greece.
Tel: ++3031 425 159; Fax: ++3031 425 169
e-mail: symvoli@yahoo.com
attention. VII IFASA Congress

Further information you may visit our web site in the following address:
http://www.IFASA.ORG.

GENERAL INFORMATION

- The official language of the congress will be English.
- Kastoria may be reached by airplane: from most cities in Europe either by the airport of Argos Orestiko (11km from Kastoria) through Athens, or by the airport of Thessaloniki (200 km from Kastoria) and then by route via EGNATIA Road.
- Transportation will be provided to attendees from the airports of Kastoria (Argos Orestiko) and Thessaloniki, on September 12 and September 16 and 17 to coincide with the schedule of major arrivals and departures.
- Hotel rooms at a range of special prices will be available; prices will be announced in 2nd announcement.
- Transportation to and from hotels to congress hall will be provided.
- In September the average temperature in Kastoria is 17 - 25°C.
- Passports are required by all foreign delegates at any point of entry. Visas are requested from some countries which will be provided by the Greek Embassy of your country.
- Electrical equipment 220 V.

TOURS

Pre-congress as well as **post-congress** tours in the region of Macedonia will be organized.

Pre-congress tours, *Friday, September 8 - Tuesday, September 12*

Those who would like to extend their summer vacation, may enjoy the beautiful sea of Chalkidiki at one of the resorts or stay in Thessaloniki (second largest city of Greece) - a city of great culture, archeological and entertainment interest and visit the nearby areas of Pella - Vergina (birthplace of Alexander the Great), Dion, sacred city founded in the 5th century BC at the root of Olympos, homeland of the Gods, or take a cruise to the holy mountain Athos, which has been inhabited solely by monks for over a thousand years.

Average temperature at Chalkidiki and Thessaloniki during September is 22 - 27°C.

Post-congress tour *Saturday, September 16*

There will be a visit to the lakes of Prespa, Greece's largest wildlife sanctuary and a very interesting site for observation of wild birds. Details of further 3-4 day tours in other regions of Greece will be included in future announcement.

VENUE

The congress will be held in Kastoria, which is located in West Macedonia, Greece. Kastoria is a Byzantine city, rich in prehistorical and archeo-

logical findings and built on a peninsula of Orestiada lake with 25,000 permanent residents. It is an international center of fur production and trade and approximately 3,000 fur manufacturing artisans operate in the city.

CONGRESS STRUCTURE

The scientific program will consist of plenary sessions, oral presentations and posters.
The papers will cover topics according to the 5 working groups of IFASA.

1. Breeding, reproduction and genetics.
2. Nutrition.
3. Pathology and diseases.
4. Behavior and welfare.
5. Fur properties.

The IFASA Council and Board of IFASA meetings will be held during the Congress.

SUBMISSION OF PAPERS

Titles and abstracts must be submitted by January 2000 and manuscripts by March 2000.
Instructions to authors will be provided in the 2nd announcement which will be circulated during September 1999.
All papers will be reviewed by the scientific committee and authors will be notified promptly.

SOCIAL PROGRAM

A reception, fur fashion show and a tour of the city will be provided to all delegates and accompanying persons. Other social events, as well as an excursion, will be organized in the city, visits to Byzantine and folklore museum, Byzantine Churches (72) - from the 11th century.

Visits to the areas of Dispilio (Neolithic lake settlement 7.000 years old) and Nostimo (petrified forest 20 million years old) are planned.

ORGANIZING COMMITTEE

Georgios Kapahtsis, *Prefect of the County of Kastoria*
Pashalis Mitliagas, *Prefect of the County of Kozani*
Dimitrios Papoulidis, *Mayor of the City of Kastoria*
Georgios Nasiopoulos, *Chairman of the Chamber of Commerce and Industry*
Lazaros Fotiadis, *President of the Greek Fur Trade Federation*
Lazaros Chionos, *President of the Greek Fur Breeders Association*
Konstantinos Bousios, *President of the Greek Fur Center*
Nikolaos Zouloumis, *President of EDIKA S.A*
Demetrios Mirtsios, *Managing Director of the Kastorian Development Agency, ANKAS*

SCIENTIFIC COMMITTEE

Prof. Bruce Murphy, *Univ. of Montreal, Vice President of IFASA*
Prof. Maija Valtonin, *Kuopio University, Finland*
Prof. Marian Brzozowski, *Warsaw Agricultural University*
Prof. Anastasios Kovatsis, *Dept. of Biochemistry & Toxicology, School of Veterinary Medicine, Aristotle Univ. of Thessaloniki, Greece, Member of the Scientific Committee of Animal Nutrition (SCAN) of the European Commission*
Prof. Alexandros Spais, *Dept. of Animal Production, Ichthyology, Ecology and Protection of Environment, Director of Laboratory of Animal Nutrition, School of Veterinary Medicine, Aristotle Univ. of Thessaloniki, Greece*
Dr. Spyros Tsitsamis, *Director of Research in Veterinary Nutrition*
Dr. Gerasimos Kussunis, *Agronomist, Councilor on Fur Farming to Hellenic Fur Trade Federation*

TECHNICAL COMMITTEE

Paschalis Ikonomidis, *Dr. of Veterinary Medicine, Director of Projects and Development of the Prefecture of Kastoria, Prefecture Councilor*
Ioannis Tsamisis, *Furrier, Prefecture Councilor*
Iordanis Michailidis, *Furrier, Prefecture Councilor*
Naoum Ditsios, *Fur Dresser, Prefecture Councilor*
Dimitrios Emmanouilidis, *Furrier, Prefecture Councilor*
Georgios Nikitidis, *Special advisor to the Mayor of the City of Kastoria*
Gerasimos Kussunis, *Agronomist Councilor on Fur Farming to Hellenic Fur Trade Federation*
Konstantinos Arvanitakis, *Business Consultant, Ass. General Secretary to the Hellenic Fur Trade Federation*
Vassilios Tsaparas, *Director of the Kastorian Development Agency*
Christos Karantinos, *Director of the Greek Fur Center*
Leonidas Pouliopoulos, *Director of EDIKA S.A*
Pantelis Samarinis, *President of the Hotel owners Association of the city of Kastoria*

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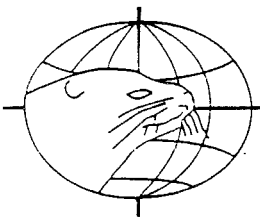

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First Announcement