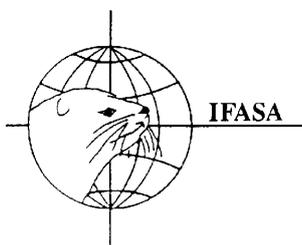
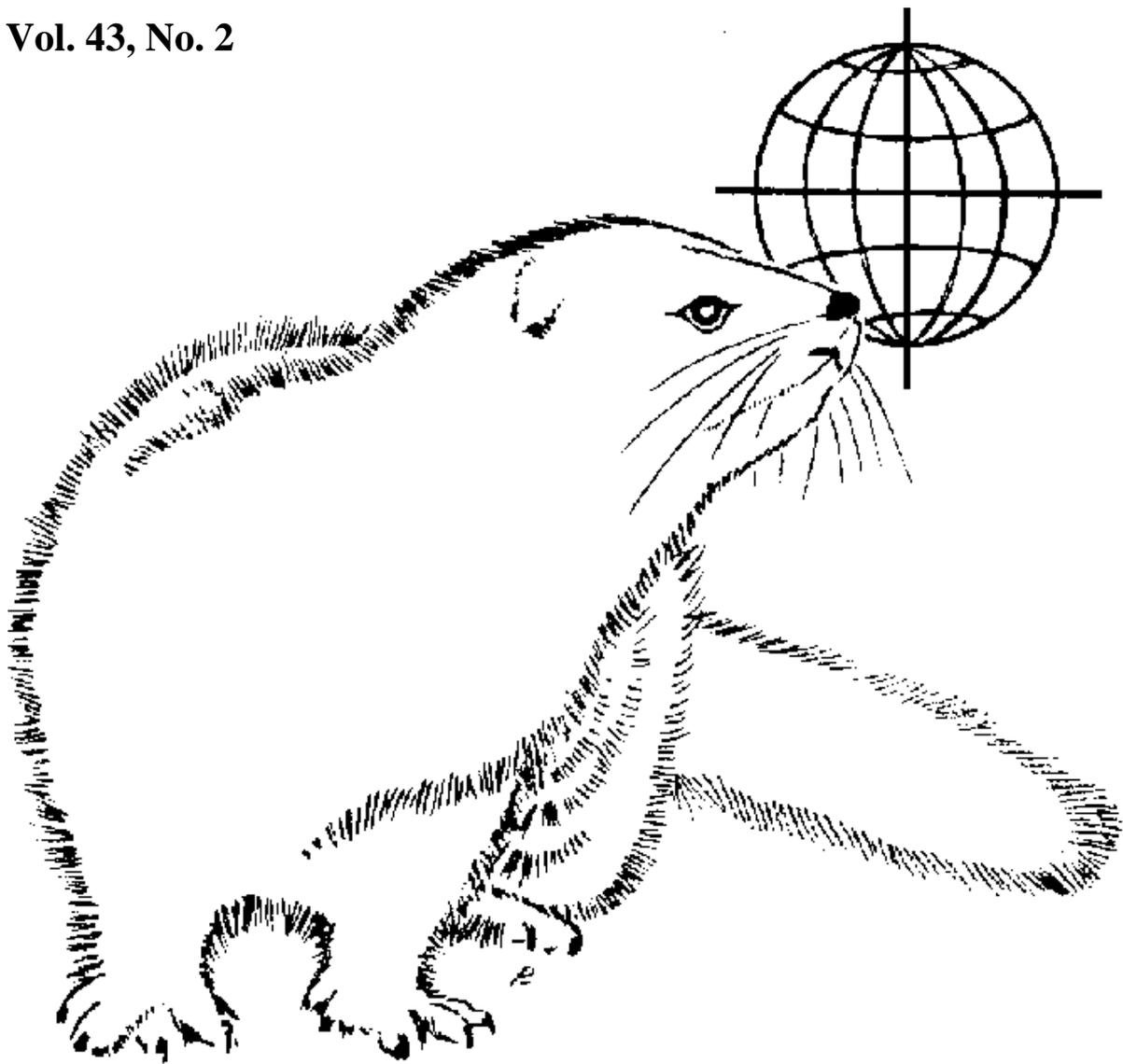


# SCIENTIFUR

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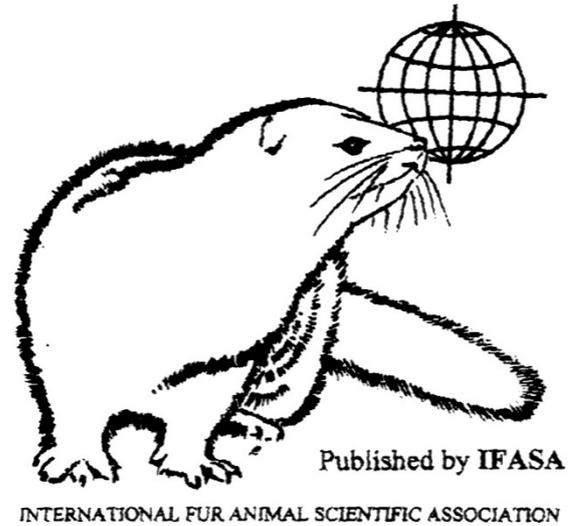
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## Notes from the Editor

Scientifur's main purpose has been to provide an overview of the efforts and results in fur animal research. From volume 40, it was decided to leave out the review procedure applied until then for manuscripts submitted for publication in Scientifur in line with less request on and submission of original work.

I am glad then to publish in this issue of Scientifur, original work in form of the article: "The evolution of the intraovular acrosomial reaction, a vital condition in gametes' fusion and zygotes formation" presenting central work in the author's career.

It is always a pleasure to inform about PhD-theses in Scientifur. The abstract from a Finnish PhD-thesis "Selection for welfare and feed efficiency in Finnish blue fox" defended at the Faculty of Agriculture and Forestry at the University of Helsinki in December

2018 is presented in this issue. The thesis points out the need to take into account production traits as well as animal health, welfare, conformation and feed efficiency in the Finnish blue fox breeding programme. From the results, it is concluded that health status and feed efficiency can be improved in blue foxes by including the traits in the breeding programme.

In the Finnish PhD-thesis, use of genomic selection is suggested as it allows selection of blue foxes at a young age, which reduces the need for fattening and slimming of the breeding animals. Other research presented in this issue of Scientifur deals with the opportunities for genomic selection in American mink and the results provide an initial framework for designing genomic selection in mink breeding programmes.

Vivi Hunnicke Nielsen

Editor Scientifur



**BREEDING, GENETICS AND REPRODUCTION****Opportunities for genomic selection in American mink: A simulation study**

Karimi K.<sup>1</sup>, Sargolzaei M.<sup>2,3</sup>, Plastow G.S.<sup>4</sup>, Wang Z.<sup>4</sup>, Miar Y.<sup>1</sup>

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Genomic selection can be considered as an effective tool for developing breeding programs in American mink. However, the genetic gains for economically important traits can be influenced by the accuracy of genomic predictions. The objective of this study was to investigate the prediction accuracies of traditional best linear unbiased prediction (BLUP), multi-step genomic BLUP (GBLUP) and single-step GBLUP (ssGBLUP) methods in American mink using simulated data with different levels of heritability, marker density, training set (TS) sizes and selection designs based on either phenotypic performance or estimated breeding values (EBVs). Under EBV selection design, the accuracy of BLUP predictions was increased by 38% and 44% for  $h^2 = 0.10$ , 27% and 29% for  $h^2 = 0.20$ , and 5.8% and 6% for  $h^2 = 0.50$  using GBLUP and ssGBLUP methods, respectively. Under phenotypic selection design, the accuracies of prediction by ssGBLUP method were 11.8% and 15.4% higher than those obtained by GBLUP for heritability of 0.10 and 0.20, respectively. However, the efficiency of ssGBLUP and GBLUP was not influenced by selection design at higher level of heritability ( $h^2 = 0.50$ ). Furthermore, higher selection intensity increased the bias of predictions in both pedigree-based and genomic evaluations. Regardless of selection design, TS sizes for GBLUP and ssGBLUP methods should be at least 3000 to achieve more accuracy than using BLUP for heritability of 0.50 and marker density of 10k and 50k. Overall, more accurate predictions were obtained using ssGBLUP method particularly for lowly heritable traits and low density of markers. Our results indicated that TS sizes should be optimized in accordance with heritability level, marker density, selection design and prediction method for

genomic selection in American mink. The results provided an initial framework for designing genomic selection in mink breeding programs.

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**Selection for temperament has no negative consequences on important production traits in farmed mink**

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**Genome analysis identifies the mutant genes for common industrial Silverblue and Hedlund white coat colours in American mink**

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The fur colour of American mink (*Neovison vison*) involves over 35 traits, but only three of these have been linked to specific genes. Despite being the most popular, coat colours Silverblue and Hedlund white remain uncharacterized genetically. The former is the first genetic mutant of fur colour identified in minks, while the latter is a commercially valuable phenotype that can be dyed easily. Here, we performed the whole genome sequencing for two American mink breeds with Silverblue and Hedlund white coats. We identified mutations in splice donor sites of genes coding melanophilin (MLPH) and microphthalmia-associated transcription factor (MITF) that regulate melanosome transport and neural-crest-derived melanocyte development, respectively. Both mutations cause mRNA splicing impairments that lead to a shift in open reading frames of MLPH and MITF. We conclude that our data should be useful for tracking economically valuable fur traits in mink breeding programs to contribute to global fur production.

*Sci Rep.* 2019 Mar 14; 9 (1): 4581.  
Doi: 10.1038/s41598-019-40918-7.

### **Molecular Cloning and Bioinformatics Analysis of DQA Gene from Mink (*Neovison vison*)**

Fan Z.<sup>1</sup>, Zhang H.<sup>2</sup>, Rong M.<sup>3</sup>, Meng D.<sup>4</sup>, Yu Z.<sup>5</sup>, Jiang L.<sup>6</sup>, Jiang P.<sup>7</sup>

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In the present study, we cloned, sequenced, and explored the structural and functional characteristics of the major histocompatibility complex (MHC)-*DQA* gene from mink (*Neovison vison*) for the first time. The full-length sequence of *DQA* gene was 1147-bp-long, contained a coding region of 768-bp, which was predicted to encoding 255 amino acid residues. The comparison between *DQA* from mink (*Neovison vison*) and other *MHC-DQA* molecules from different animal species showed that nucleotide and encoded amino acid sequences of the mink *DQA* gene exhibited high similarity with the ferret (*Mustela putorius furo*). Phylogenetic analysis revealed that mink (*Neovison vison*) *DQA* is grouped with that of ferret (*Mustela putorius furo*). The cloned sequence contained a 23-amino acid NH<sub>2</sub>-terminal signal sequence with the signal peptide cutting site located in amino acids 23-24, and had three Asn-Xaa-Ser/Thr sequons. Three cysteine residues were also identified (Cys-85, Cys-121, and Cys-138). The 218 to 240 amino acids were predicted to be the transmembrane domains. The prediction of the secondary structure revealed three  $\alpha$ -helixes and fourteen  $\beta$ -sheets in *Neovison vison DQA* protein, while random coil was a major pattern. In this study, the whole CDS sequence of *Neovison vison DQA* gene was successfully cloned, which was valuable for exploring the function and antiviral molecular mechanisms underlying the molecule. The findings of the present study have laid the foundation for the disease resistance and breeding of mink.

*Int J Mol Sci.* 2019 Feb 27; 20 (5). pii: E1037.  
Doi: 10.3390/ijms20051037.

### Urban colonization through multiple genetic lenses: The city-fox phenomenon revisited

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Urbanization is driving environmental change on a global scale, creating novel environments for wildlife to colonize. Through a combination of stochastic and selective processes, urbanization is also driving evolutionary change. For instance, difficulty in traversing human-modified landscapes may isolate newly established populations from rural sources, while novel selective pressures, such as altered disease risk, toxicant exposure, and light pollution, may further diverge populations through local adaptation. Assessing the evolutionary consequences of urban colonization and the processes underlying them is a principle aim of urban evolutionary ecology. In the present study, we revisited the genetic effects of urbanization on red foxes (*Vulpes vulpes*) that colonized Zurich, Switzerland. Through use of genome-wide single nucleotide polymorphisms and microsatellite markers linked to the major histocompatibility complex

(MHC), we expanded upon a previous neutral microsatellite study to assess population structure, characterize patterns of genetic diversity, and detect outliers associated with urbanization. Our results indicated the presence of one large evolutionary cluster, with substructure evident between geographic sampling areas. In urban foxes, we observed patterns of neutral and functional diversity consistent with founder events and reported increased differentiation between populations separated by natural and anthropogenic barriers. We additionally reported evidence of selection acting on MHC-linked markers and identified outlier loci with putative gene functions related to energy metabolism, behavior, and immunity. We concluded that demographic processes primarily drove patterns of diversity, with outlier tests providing preliminary evidence of possible urban adaptation. This study contributes to our overall understanding of urban colonization ecology and emphasizes the value of combining datasets when examining evolutionary change in an increasingly urban world.



Fig. 1. Red foxes (*Vulpes vulpes*) have successfully colonized urban areas in Europe since the 1930s.

Photo credit: © L. Hamelbeck-Galle/stadtwildtiere.at

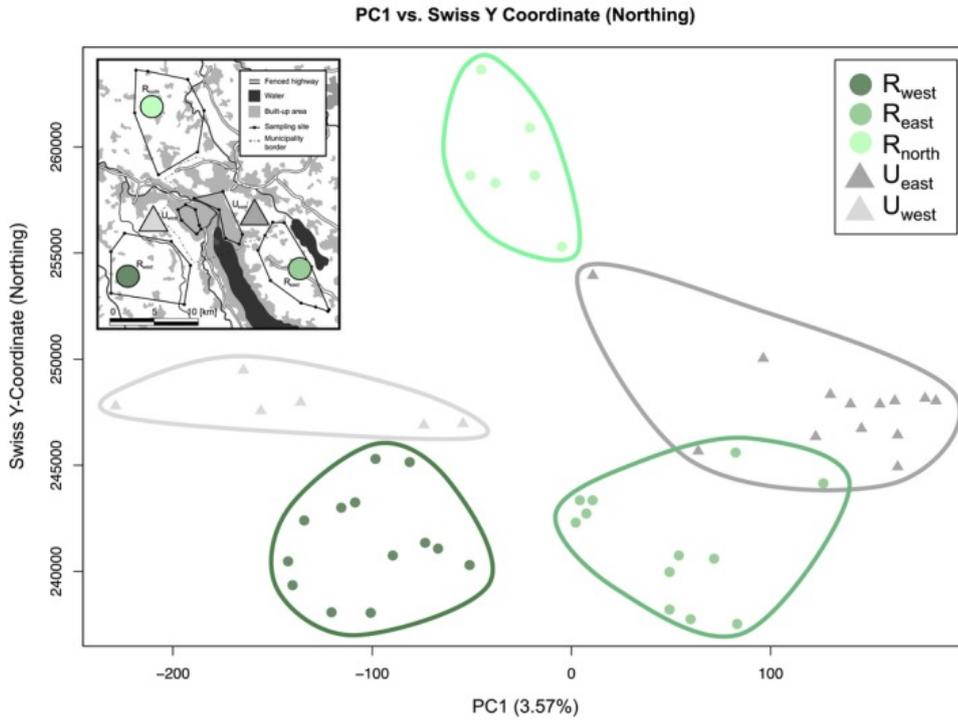
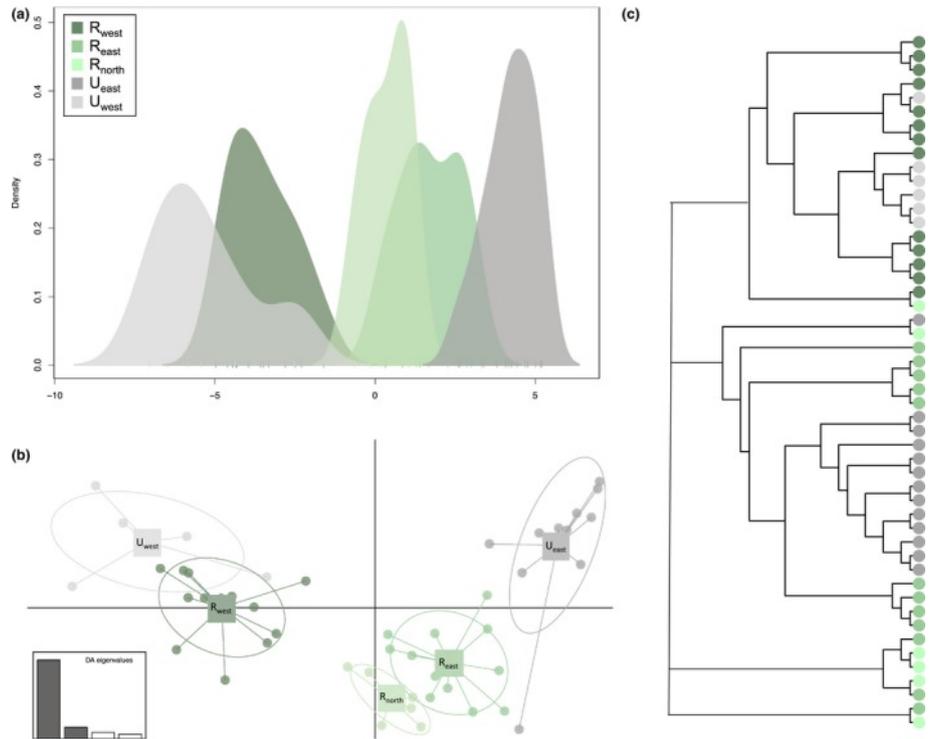


Fig. 2. Principal components calculated for 50 foxes across all 10,149 SNPs. When plotted against the Swiss Y-coordinate (northing), PC1 recapitulates the geographic sampling area (inset; adapted from Wandeler et al. ), thus mirroring the Swiss X-coordinate (easting).

Fig. 3. Discriminate analysis of principal components (DAPC) revealed (a) considerable overlap between the five sampling locations, with five distinct groups evident and (b) a divide between sampling locations east ( $R_{east}$  and  $U_{east}$ ) and west ( $R_{west}$  and  $U_{west}$ ) of Lake Zurich and the Limmat River, with  $R_{north}$  in the middle. (c) East-west subdivision also emerged in the major branches of the NJ tree, where each node represents an individual fox colored by sampling location.



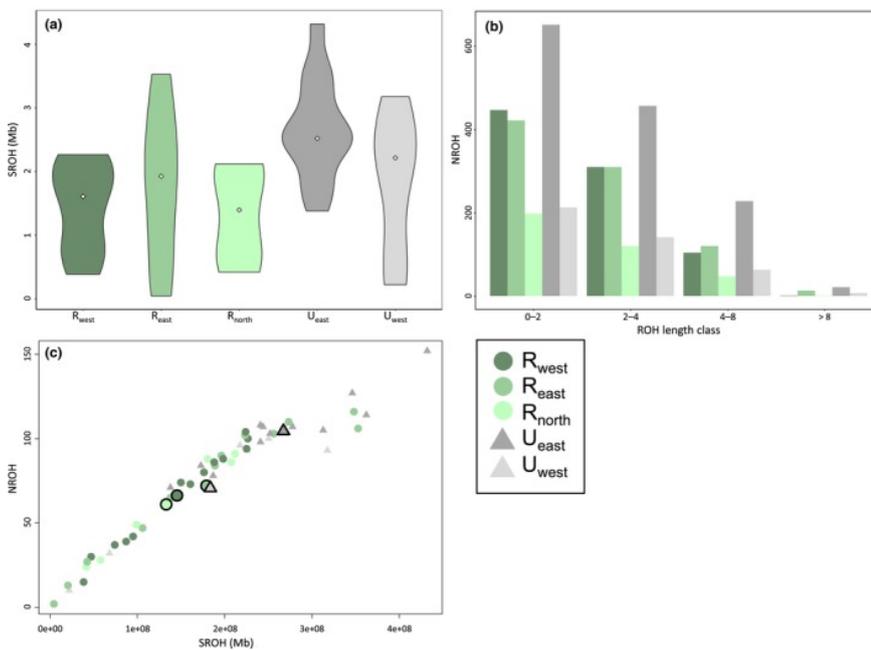


Fig. 4. Plots displaying (a) the sum of ROH (SROH) in Mb calculated per individual per subpopulation (median values indicated by white diamonds; nested ANOVA reported significant effect of urban vs. rural habitat on SROH;  $F = 9.843$ ,  $p = 0.003$ ), (b) the total number of ROHs (NROH) observed at each length class (0–2; 2–4; 4–8; >8 Mbs; nested ANOVA again reported significant effect of habitat on NROH;  $F = 7.91$ ,  $p = 0.007$ ) in each subpopulation (note sample size sensitivity of this metric), and (c) SROH vs. NROH per individual fox and averaged for each subpopulation (large points with black outlines;  $r^2 = 0.918$ ).

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Doi: 10.1002/ece3.4898. eCollection 2019 Feb.

### Comparative transcriptome analysis of embryo invasion in the mink uterus

Cao X.<sup>1</sup>, Xu C.<sup>2</sup>, Zhang Y.<sup>2</sup>, Wei H.<sup>2</sup>, Liu Y.<sup>3</sup>, Cao J.<sup>2</sup>, Zhao W.<sup>2</sup>, Bao K.<sup>2</sup>, Wu Q.<sup>2</sup>

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Epub 2018 Nov 16.

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### Glycogen metabolism in mink uterine epithelial cells and its regulation by estradiol, progesterone and insulin

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Epub ahead of print.

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**Comparative studies on testis, epididymis and serum hormone concentrations in foxes, and hybrids during the pre-breeding period**

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**BEHAVIOUR AND WELFARE**

**Juvenile Finnraccoons (*Nyctereutes procyonoides ussuriensis*) choose to allohuddle on the cage floor instead of resting on a platform**

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*Applied Animal Behaviour Science*, Volume 201, April 2018, Pages 102-110.

**Interaction with a bovine cortical bone in the Finnraccoon (*Nyctereutes procyonoides ussuriensis*)**

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*Applied Animal Behaviour Science*, Volume 196, November 2017, Pages 100-107.

**NUTRITION, FEEDING AND MANAGEMENT**

**Factors influencing exposure of North American river otter (*Lontra canadensis*) and American mink (*Neovison vison*) to mercury relative to a large-scale reservoir in northern British Columbia, Canada**

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Although reservoir creation increases fish mercury (Hg) concentrations, little information exists on its effects on Hg concentrations in aquatic mammals. River otters (*Lontra canadensis*) and American mink (*Neovison vison*) are two aquatic mammals that have been used as model species for assessing Hg bioaccumulation in aquatic systems. We assessed Hg and selenium (Se) concentrations in these two species within and outside of the Williston Reservoir (Peace-Williston (PW) watershed) in northern British Columbia (BC) and used these data to investigate potential explanatory factors (i.e., watershed, gender, trophic level ( $\delta^{15}\text{N}$ ), and regional geology) influencing Hg concentrations. Hg concentrations in otter and mink inhabiting the Mackenzie watershed (outside the PW) were significantly lower than other watersheds in Northern BC. The general trend was the same for both species; the Peace-Williston having the highest and Mackenzie having the lowest Hg concentrations. For mink, the Peace-Williston watershed, higher trophic levels, and higher proportions of igneous/metamorphic bedrock were all significant influences on higher Hg concentrations (logistic regression). Higher trophic levels or proportions of igneous/metamorphic bedrock, however, were not directly associated with the PW watershed suggesting there may be an impoundment effect. Baseline data on natural Hg inputs before planned anthropogenic

changes occur is a critical first step to aiding interpretations of Hg-related effects on wildlife populations and their related ecosystems.

*Ecotoxicology*. 2019 Apr; 28(3): 343-353.

Doi: 10.1007/s10646-019-02027-z.

Epub 2019 Mar 2.

### **Dental and Temporomandibular Joint Pathology of the Kit Fox (*Vulpes macrotis*)**

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### **Para-aminopropiophenone (PAPP) in canid pest ejectors (CPEs) kills wild dogs and European red foxes quickly and humanely**

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Lethal control remains an important approach to mitigating the impacts of predators on livestock and threatened fauna. This occurs in Australia, where wild dogs (*Canis familiaris*) and European red foxes (*Vulpes vulpes*) are commonly subjected to broad-scale poisoning programs. Ongoing refinement of lethal tools has led to the recent development of manufactured poison baits containing para-aminopropiophenone (PAPP). Canid pest ejectors (CPEs) have also been recently registered for use and are a target-specific poison delivery device; yet, there has been no confirmation that PAPP delivered via ejectors will provide similar efficacy to PAPP delivered via manufactured baits. We tested the efficacy of PAPP in

ejectors on wild dogs (1000-mg dose) and foxes (400-mg dose). Time-to-death, physical signs of poisoning and other related factors were assessed. Ten of 11 (91%) wild dogs used in controlled trials died within 3 h after PAPP administration; the mean time to unconsciousness was 65 min and the mean time to death was 84 min. Three of four (75%) foxes also died within 3 h after PAPP administration; their mean time to unconsciousness was 78 min, and their mean time to death was 121 min. Carcasses of eight deceased wild dogs and one fox were found during field trials, with distances between the nearest triggered ejector and the deceased animal ranging from 30 to 200 m. The presence of de-oxygenated blood in all necropsied carcasses and photographic evidence of triggered ejectors unequivocally demonstrated that using powdered PAPP in ejectors produces rapid anoxia and death in both wild dogs and foxes. Although anxiety and accompanying behaviours were observed in wild dogs (but not foxes), the use of PAPP offers a humane, additional option for the control of wild canids.

*Environ Sci Pollut Res Int*. 2019 Mar 14.

Doi: 10.1007/s11356-019-04818-7.

Epub ahead of print.

### **Innervation of the pineal gland in the Arctic fox (*Vulpes lagopus*) by nerve fibers immunoreactive to substance P and calcitonin gene-related peptide**

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The study demonstrates, for the first time, the presence of substance P (SP) and calcitonin gene-related peptide (CGRP) in the nerve fibers supplying the pineal gland in the Arctic fox. The expression and distribution pattern of the studied substances were examined by double-labeling immunofluorescence technique. The SP-positive fibers enter into the pineal gland through the capsule as the nervi conari. The

fibers formed thick bundles in the capsule and connective tissue septa, from where they penetrated into the pineal parenchyma. Inside the parenchyma, the nerve fibers created basket-like structures surrounding clusters of pinealocytes. The density of intrapineal SP positive fibers was slightly higher in the distal and middle parts of the gland than in the proximal one. Double immunostaining with antibodies against SP and CGRP revealed that the vast majority of SP positive fibers was also CGRP positive. The fibers showing a positive reaction to SP and negative to CGRP were scattered within the whole gland. The fibers immunopositive to CGRP and immunonegative to SP were not observed. In the habenular and posterior commissural areas adjoining to the pineal gland the immunoreactive nerve fibers were not found. Moreover, no immunopositive cell bodies were observed in both the pineal gland and the commissural areas. These results reveal that SP and CGRP are involved in the innervation of pineal gland in carnivores. In turn we suggest that these peptides can regulate/modulate melatonin secretion.

*Folia Morphol (Warsz)*. 2019 Mar 5.  
Doi: 10.5603/FM.a2019.0024. Epub ahead of print.

#### **Effects of Vitamin E and Selenium on Growth Performance, Antioxidant Capacity, and Metabolic Parameters in Growing Furring Blue Foxes (*Alopex lagopus*)**

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The objective of this study was to determine whether different dietary vitamin E (VE) and selenium (Se) levels affect the nutrient digestibility, production performance, and antioxidant abilities of growing furring blue foxes. A 4 × 2 factorial arrangement that included 4 levels of VE (0, 100, 200, or 400 mg/kg diet from  $\alpha$ -tocopherol acetate) and 2 levels of Se (0 or 0.2 mg/kg diet from glycine selenium) was performed from mid-September to pelting. A metabolism study was conducted for four days starting at the

30th day of the trial. Serum samples were collected at the last day of the study. The results showed that supplementation of growing furring blue fox diets with VE and Se significantly affected the average daily gain (ADG), average daily feed intake, and feed conversion ratio (F:G) ( $P < 0.05$ ). Dietary Se supplementation enhanced protein and fat digestibility of male blue foxes. There were significant effects of different VE and Se levels in diets on serum antioxidant parameters and metabolic parameters of blue foxes ( $P < 0.05$ ). In conclusion, this research indicated that dietary supplementation with VE improved ADG and F:G of blue foxes. Addition of VE and Se to blue fox diets increased the antioxidant capacity of blue foxes. The diet with high VE and Se supplementation reduced glucose and triglycerides concentrations in serum. The present study found that growing furring blue foxes had increased growth performance and antioxidant abilities when fed diets with 200 mg VE/kg and nearly 0.1 mg Se/kg.

*Biol Trace Elem Res*. 2019 Feb 20.  
Doi: 10.1007/s12011-019-1655-4.  
Epub ahead of print.

#### **Scent Chemicals of the Tail Gland of the Red Fox, *Vulpes vulpes***

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*Chem Senses*. 2019 Mar 11; 44(3): 215-224.  
Doi: 10.1093/chemse/bjz009.

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#### **Some segmental morphological and morphometric features of the intima and media of the aortic wall in *Chinchilla lanigera***

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The aim of this study is to describe the morphology, morphometry and ultrastructure of segments of the thoracic and abdominal aorta portions in *Chinchilla lanigera*. Thickness measurements of the tunica intima and media complex of the aorta were taken. In all observed specimens, the thickness values for the tunica intima and media complex of the cranial thoracic aorta were significantly higher (mean: 702.19µm) when compared to the values of other analyzed aortic segments (means: 354.18µm; 243.55µm). Complex statistical methods were used to assess the differences between various aortic segments. The components of the vessel walls show variations in structure and thickness, presumably due to an adaptation to functional demand.

*Folia Morphol (Warsz).* 2019 Mar 5.  
Doi: 10.5603/FM.a2019.0023. Epub ahead of print.

## HEALTH AND DISEASE

### Unique genetic features of canine adenovirus type 1 (CAV-1) infecting red foxes (*Vulpes vulpes*) in northern Norway and arctic foxes (*Vulpes lagopus*) in Svalbard

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Canine adenovirus type 1 (CAV-1) is the aetiological agent of infectious canine hepatitis (ICH) in domestic dogs (*Canis familiaris*). In spite of the widespread use of vaccination, CAV-1 continues to circulate in the dog population. Although a high number of serological screenings have indicated that CAV-1 is widespread in fox species, little is known about the potential role of foxes as reservoirs of CAV-1. Furthermore, very little data exist on the molecular features of this virus in foxes. To add to existing knowledge on CAV-1 circulating in wild carnivores, tissue samples from CAV-seropositive red foxes (*Vulpes vulpes*, n = 10) from the northern mainland of Norway and arctic foxes (*Vulpes lagopus*, n = 10) from the Svalbard archipelago, Norway, were investigated using a molecular approach to detect CAV-1 DNA and important structural and non-structural genes of the detected viruses were sequenced and analysed. Amplicons characteristic for CAV-1 were amplified from 14 out of 20 foxes (7 red foxes and 7 arctic foxes) and spleen and lymph node tissues resulted optimal targets for the viral DNA detection. The nucleotide sequences showed unique features that distinguished the viruses detected in this study from the CAV-1 to date identified in wild carnivores and dogs. Greater attention should be given to genetically different CAV-1 circulating in wild carnivores that may be transferred to dogs, potentially causing disease and reducing the effectiveness of available vaccines.

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Epub ahead of print.

### A comparison between intraperitoneal injection and intranasal and oral inoculation of mink with Aleutian mink disease virus

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**Identification of Arcanobacterium phocae isolated from fur animals by phenotypic properties, by MALDI-TOF MS analysis and by detection of phocaelysin encoding gene phl as probable novel target**

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**Infection and Propagation of Astrovirus VA1 in Cell Culture**

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*Curr Protoc Microbiol.* 2019 Feb; 52(1): e73.

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**Distribution and genetic diversity of Blastocystis subtypes in various mammal and bird species in northeastern China**

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**Background**

Blastocystis is one of the most common intestinal parasites in humans and animals worldwide. At least 17 subtypes have been identified in mammals and birds. In China, although some studies have reported the occurrence of Blastocystis in humans and animals, our understanding of the role of animals in the transmission of human blastocystosis is only superficial due to a paucity of available molecular data. The aim of the present study was to understand infection rates of Blastocystis and the distribution and genetic diversity of subtypes in various mammal and bird species in northeastern China, as well as to assess the zoonotic potential of Blastocystis isolates.

**Methods**

A total of 1265 fresh fecal specimens (1080 from ten mammal species and 185 from eight bird species) were collected in Heilongjiang, Liaoning and Jilin provinces of China. Each specimen was examined for the presence of Blastocystis by PCR amplification and sequence analysis of the partial SSU rRNA gene.

**Results**

Fifty-four specimens (4.3%) were positive for Blastocystis. Birds (7.0%) had a higher infection rate of

Blastocystis than mammals (3.8%). Blastocystis was found in seven mammal species, reindeer (6.7%), sika deer (14.6%), racoon dogs (7.5%), Arctic foxes (1.9%), dogs (2.9%), rats (3.7%) and rabbits (3.3%), as well as three bird species, pigeons (2.1%), chickens (13.0%) and red crowned cranes (14.0%). Eight subtypes were identified including ST1 (n = 5), ST3 (n = 3), ST4 (n = 13), ST6 (n = 8), ST7 (n = 6), ST10 (n = 13), ST13 (n = 4) and ST14 (n = 2). 64.8% (35/54) of Blastocystis isolates belonged to potentially zoonotic subtypes.

### Conclusions

To our knowledge, this is the first report of Blastocystis in reindeer (ST10 and ST13), rabbits (ST4), racoon dogs (ST3) and Arctic foxes (ST1, ST4 and ST7). The findings of potentially zoonotic subtypes suggest that the animals infected with Blastocystis might pose a threat to human health. These data will improve our understanding of the host range and genetic diversity of Blastocystis, and also help develop efficient control strategies to intervene with and prevent the occurrence of human blastocystosis in the investigated areas.

*Parasit Vectors. 2018 Sep 20; 11 (1): 522.*

*Doi: 10.1186/s13071-018-3106-z.*

### Outbreaks of canine distemper in Dutch and Belgian mink farms

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### Background

Vaccination of farmed minks against canine distemper virus (CDV) has proved to be very effective. In the Netherlands, vaccination of farmed minks against CDV was mandatory until the closure of the local agricultural product boards at the end of 2014.

### Objectives

To describe the first documented outbreaks of CD in Dutch mink farms since the closure of the agricultural product boards, as well as an outbreak in Belgium, with special attention to genotyping of the isolates.

### Methods

A full post-mortem was performed on three carcasses per submission from farms A-C and on two carcasses from farm D. Molecular detection with subsequent

typing was performed on eleven samples originating from four different farms. To assess genetic diversity partial sequences of the H gene of CDV were compared based on phylogenetic analysis.

### Results

In 2017, there was a sudden series of CD outbreaks affecting four mink farms in the Netherlands (A-C) and Belgium (D). Gross, histologic and immunohistochemical findings were similar. There was a degree of genetic similarity between the viruses on farms A and D (98.5%) and between the viruses on farms B and C (97.3%), but the viruses from farms A and D belonged to a different clade than the viruses from farms B and C. Higher mortalities were reported in white and pastel minks.

### Conclusions

Findings indicated that the difference in severity of the outbreaks was partially related to the genetic composition of the farm populations. Vaccination against CDV on Dutch and Belgian mink farms seems warranted.

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### Genetic and spatial characterization of the red fox (*Vulpes vulpes*) population in the area stretching between the Eastern and Dinaric Alps and its relationship with rabies and canine distemper dynamics

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Information on the population dynamics of a reservoir species have been increasingly adopted to understand and eventually predict the dispersal patterns of infectious diseases throughout an area. Although potentially relevant, to date there are no studies which have investigated the genetic structure of the red fox population in relation to infectious disease dynamics. Therefore, we genetically and spatially characterised the red fox population in the area stretching between the Eastern and Dinaric Alps, which has been affected by both distemper and rabies at different time intervals. Red foxes collected from north-eastern Italy, Austria, Slovenia and Croatia between 2006-2012, were studied using a set of 21 microsatellite markers. We confirmed a weak genetic differentiation within the fox population using Bayesian clustering analyses, and we were able to differentiate the fox population into geographically segregated groups. Our finding might be due to the presence of geographical barriers that have likely influenced the distribution of the fox population, limiting in turn gene flow and spread of infectious diseases. Focusing on the Italian red fox population, we observed interesting variations in the prevalence of both diseases among distinct fox clusters, with the previously identified Italy 1 and Italy 2 rabies as well as distemper viruses preferentially affecting different sub-groups identified in the study. Knowledge of the regional-scale population structure can improve understanding of the epidemiology and spread of diseases. Our study paves the way for an integrated approach for disease control coupling pathogen, host and environmental data to inform targeted control programs in the future.

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### **Raccoons accumulate PrP<sup>Sc</sup> after intracranial inoculation of the agents of chronic wasting disease or transmissible mink encephalopathy but not atypical scrapie**

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Prion diseases are neurodegenerative diseases characterized by the accumulation of misfolded prion protein (PrP<sup>Sc</sup>) in the brain and other tissues. Animal prion diseases include scrapie in sheep, chronic wasting disease (CWD) in cervids, and transmissible mink encephalopathy (TME) in ranch-raised mink. We investigated the susceptibility of raccoons to various prion disease agents and compared the clinicopathologic features of the resulting disease. Raccoon kits were inoculated intracranially with the agents of raccoon-passaged TME (TME<sup>Rac</sup>), bovine-passaged TME (TME<sup>Bov</sup>), hamster-adapted drowsy (TME<sup>DY</sup>) or hyper TME (TME<sup>HY</sup>), CWD from white-tailed deer (CWD<sup>Wtd</sup>) or elk (CWD<sup>Elk</sup>), or atypical (Nor98) scrapie. Raccoons were euthanized when they developed clinical signs of prion disease or at study endpoint (<82 mo post-inoculation). Brain was examined for the presence of spongiform change, and disease-associated PrP<sup>Sc</sup> was detected using an enzyme immunoassay, western blot, and immunohistochemistry. All raccoons inoculated with the agents of TME<sup>Rac</sup> and TME<sup>Bov</sup> developed clinical disease at ~6.6 mo post-inoculation, with widespread PrP<sup>Sc</sup> accumulation in central nervous system tissues. PrP<sup>Sc</sup> was detected in the brain of 1 of 4 raccoons in each of the CWD<sup>Wtd</sup>-, CWD<sup>Elk</sup>-, and TME<sup>HY</sup>-inoculated groups. None of the raccoons inoculated with TME<sup>DY</sup> or atypical scrapie agents developed clinical disease or detectable PrP<sup>Sc</sup> accumulation. Our results indicate that raccoons are highly susceptible to infection with raccoon- and bovine-passaged TME agents, whereas CWD isolates from white-tailed deer or elk and hamster-adapted TME<sup>HY</sup> transmit poorly. Raccoons appear to be resistant to infection with hamster-adapted TME<sup>DY</sup> and atypical scrapie agents.

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**Endoparasites of Domesticated Animals That Originated in the Neo-Tropics (New World Tropics)**

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This review serves to summarize parasites found in Domesticated animals which were found in the Neo-Tropics. Indigenous domesticated Neo-tropical animals include South American camelids, (*Lama guanaco*, *Lama glama*, *Lama pacos*, *Vicuna vicuna*), guinea pigs (*Cavia porcellus*), chinchillas (*Chinchilla lanigera*), turkeys (*Meleagris gallopavo*) and ducks (*Cairina moschata*, *Anas platyrhynchos*, *Dendrocyga autumnalis*). These animals were chosen due to their origin of existence (Neo-tropics) and over time these animals became domesticated and were distributed throughout the world. Over eighty (80) references were collected for this review and the papers spanned over eighty (80) years from 1934 to 2018. The gastrointestinal parasites reported for each animal were tabulated and their effects in the animal noted. Parasites reported in domesticated Neo-tropical animals had little to no effect on wild and free ranging animals with a few cases of illness and decreased productivity. The majority of articles viewed these animals as reservoir host which can infect humans and other domesticated livestock. It must also be noted that research done in the past did not focus on the effect these parasites had on these animals but only observed their potential as reservoirs for parasitic diseases.

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## THE EVOLUTION OF THE INTRAOVULAR ACROSOMIAL REACTION, A VITAL CONDITION IN GAMETES' FUSION AND ZYGOTES' FORMATION

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### Abstract

The fecundity of some minks or other species of mammals (including humans) can, and not infrequently, be partially affected by the inefficacy of the acrosomal reaction in the generation of zygotes.

The acrosome is a structure derived from a modification of the Golgi apparatus. It is present in the anterior part of the spermatozoid head, which has the role of digesting the follicular columnar cells of the radial crown which surrounds the *zona pellucida* of the ovule for a certain time after the ovulation. Following the removal of these follicular cells, by enzymes, one of the spermatozoids punctually digests the membrane of the ovule, and penetrates inside the ovoplasma. These two stages constitute the first two segments described by current medical science in all existing embryology.

What we have discovered, in addition, by serendipity, is that segment represented by a temporary, intra-ovular traverse duct of the spermatozoid head, going towards the core of the ovule. This discovery has only been reported and published in the 1988 Scientificur edition. For documentation purposes, the original photos are published here. Our research shows the duct through which the spermatozoid head traverses by tropism the distance from the gateway through a slight curve from right to left towards the nucleus of the ovule, the amphimixis, and the mutual melting of the two haploid gametes.

**Keywords:** *zygote, acrosomal reaction, fecundity, ovule*

### Introduction

In the work are presented figures from the intracytoplasmic area. One shows in an extremely visible manner, that "road to life", respectively of the duct or of the tunnel created by endocytosis, the physiologic mechanism of penetration or capitulation in the ovule of external substances, which cannot arrive in the ovule directly of by passive or active transport.

Therefore, the endocytosis induces a reversible morphological modification (perforation or invagination) of the ovular membranes and, depending on the nature of the compounds, the receptor-mediated phagocytosis, pinocytosis and endocytosis are triggered. In Figure 3 can be noticed the entry gate in ovoplasma, the ovoplasma crossing tunnel and the junction of the tunnel with the ovule's nucleus, when the amphimixis of the two nuclei of the sexual gametes, as well as the mutual assimilation is performed, and implicitly the formation of the diploid zygote (zygoten), representing the second stage or meiotic prophase, where homologous chromosomes have conjugated and become bivalent.

In Figure 4 can be noticed the dissolution of the tubular walls of the sperm cell head's vehicular duct towards the ovule's nucleus before the finality of the chromosomes' conjugation.

### Material and method

In the present investigation, the fecundity of minks infected with Aleutian virus was compared with minks with negative reaction (CEP test). Culling was performed 20 days after mating, of an experimental batch of 32 female minks. The batch consisted of 16 positive females and 16 negative females. In both cases 8 adult females and 8 young females were investigated.

The sampled genitalia (in the period between 25<sup>th</sup> – 27<sup>th</sup> of March) was first examined and subsequently prepared for the perfusion of uterine horns for the full harvest of blastocysts. The ovaries, following a prior preparation, have been examined using a binocular magnifying glass (2,5 x 2,5 zoom power). The analysis of the perfusion fluid performed after the lavage of the uterine horns was performed using a stereomicroscope.

Using the same method, a number of 23 females coming from the March casualties were also analyzed in order to compare zygotes of various ages with those coming from the experimental batch.

The method used for the establishment of the fecundity diagnosis consists in examining the number and the evolution stage of the zygotes using a microscope, following the lavage of the uterine horns. To this end, in order to sample the material necessary for that examination, the full genitalia of the females (samples after culling or coming from casualties) were brought in the laboratory. Following the ablation by the surgical act of washing the oviducts and of the uterine horns, we went on to the examination, according to the principles described in the case of other mammal species (Paraipan, 1977, Paraipan si Bucur, 1981).

Following the detachment of the mesosalpinx, of the tubo-ovarian ligament and of the mesometrium from the oviduct's corpus and of the cornual extremity, two transversal sections were practiced, one superior, in the vicinity of the utero-tubar junction and a second, lower one, in front of the cervical ostium.

We consider that the perfusion of the uterine horns in minks, for the sampling of zygotes during their first stages of segmentation, is more efficient when performed from the cervical ostium towards the oviduct. The perfusion technique implies the introduction of a thin, blunt needle in the lumen of the uterine horn, a needle articulated to a 2 ml syringe, in which the perfusion fluid has been stored. Then the free extremity of the uterine horn is suspended over a pocket watch glass or in the lumen of a glass funnel which is introduced in a sampling test tube.

The sampling technique supposes the cautious actuation of the syringe's piston, to avoid the breakage of blastocysts and so that the perfusion fluid would slowly permeate the uterine horn. We would like to state here that after having inoculated 1 ml of the perfusion fluid, the syringe is disarticulated, is loaded with air, is rearticulated again to the needle left in the cornual lumen and the air is inoculated. The inoculated air pushes the remnant fluid column into the lumen, after which the operation is repeated, using the same quantity of fluid.

As perfusion fluids, in the present case we used saline solution and Hanks medium. In the situation where it is not imperative that the blastocysts are kept alive and viable, instead of aforementioned perfusion fluids, 2% formaldehyde solution can be used instead. The observations of the blastocysts, especially in the pink-reddish fluid of Hanks medium, can be per-

formed using a magnifying glass or even with the naked eye. They appear like minuscule, clear, pearly vesicles, which initially swim above, but subsequently lay at the bottom of the examination recipient and, depending on the washing fluid, may shrink.

Depending on the prospected goal, each test tube must contain all the blastocysts in a uterine horn, and be labelled with the specified record number of the mink, the horn from which the sample was taken, as well as the number of live and dead blastocysts.

Just like we had anticipated, the sampling of mink blastocysts must be performed on April 2<sup>nd</sup> at the latest, when most of the blastocysts become elongated and start nesting in the endometrium. This fact imposes that the sampling must be performed until March 27<sup>th</sup> – 28<sup>th</sup>.

For the microscopic examination of the components of the fluid resulting from the lavage of the uterine horns (gynogamets, zygotes in various stages of development, live or dead elements, etc.), several methods can be used: direct or identification exam, blastocysts exam, histologic sections exam, *in toto* exam, etc. In the present case, we made use of the first two methods.

The direct exam was performed using a stereomicroscope and a binocular magnifying glass. , A pocket watch glass with the Hanks perfusion fluid was laid on the microscope table. The zygotes, as they are heavier, after the settling of the fluid, lay on the bottom and in the center of the pocket watch glass.

In order to harvest all the elements in the analyzed fluid (primarily the zygotes), the drop exam can be performed by micropipetting , has the advantage of being more exact although it is difficult and needs more time.

The examination of the blastocysts has shown that they appear like clear vesicles, of uneven sizes, even at the same age. Except these vesiculous elements, smaller formations were observed, fully opaque, which represent blastocysts which died days before sampling and hence formed the "blastocyst sand" (Paraipan, 1971).

Depending on the observations' goal, the blastocysts, either whole, or crushed between the slides, can be colored with Methylene blue, and when the nuclei's features are observed, the crushed blastocysts are

fixed using a flame and colored for 45 minutes using Giemsa solution.

### Results and discussions

The approach of the fecundity process consists in proving the entire chain of events, which forms it. It starts with the sperm cells' contact with the ovule and it ends with the fusion of the pronuclei (of the paternal and of the maternal chromosomes), during the first metaphase of the zygote's mitotic division. A more concise characterization of the bivalents in this metaphase consists in that they are formed from the bichromatid chromosomes, which fix with the centromere on the filaments of the division furrow which travels towards the metaphasic plate, at the cell's equator, being normally oriented in a bipolar manner on the respective furrow's filaments. The homologous chromosomes' centromeres order randomly on one side and on the other side of the equatorial plan.

These events described above are the consequence of the ovule's fertilization by the sperm cell. We would like to demonstrate below that to arrive at this outcome of fusion between the two gametes, at least the male gamete must fulfill an impeccable physiological perfection.

Therefore, the fertilization strictly envisages the sperm cell's union phenomenon with the ovule. The contact between the two gametes is preceded by a series of events which happen quickly and which begin while the sperm cells approach the fertilization membrane which is developed from the sperm cell's union point with the ovule and which subsequently covers the egg, following the fecundation. This membrane also has the role to prevent the penetration of other sperm cells, being well known that the mink is a monospermic animal. The penetration process triggers the stimulation and the performance of amphimixis, together with the formation of the second polar body (polocyte). Once the female and male pronuclei have fused, the zygote can begin dividing by cleavage.

For the two gametes to fuse, we must mention, in the beginning of the stimulation and favoring of the fecundation process, the extremely important role of the modified structure of the Golgi apparatus (the acrosome), present in the anterior part of the sperm cell's head, similar in shape and role with a "spear tip". In the first stage, when the sperm cells come in contact with the multiparous species, their acrosome has the mission to digest the columnar follicular cells

area, which surrounds the pellucid area of the ovule, which persists a while after ovulation (Fig. 1, 2).

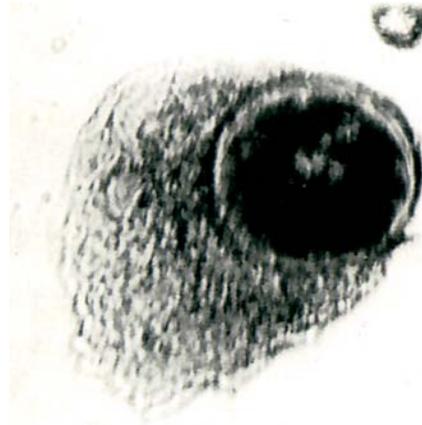


Fig. 1. The ovule resulting from the dehiscence of the Graaf follicle immediately following expulsion



Fig. 2. The ovule with the advanced denudation of abundant follicular cells presented in figure 1.

In the same chronological order, the sperm cell approaches the ovule's membrane, to penetrate it and to enter the ovoplasma.

These aspects can be characterized as the first two stages of the acrosomal reaction, perfectly visible and developed outside, as well as at the entering gate in the ovule's ovoplasma. They have been well described in the literature. What has not been discovered yet and is still only a supposition is the last stage of the acrosomal reaction (Figure 3 and 4).



Figure 1. The ovule following the dehiscence of the Graff follicle which, following expulsion, enters the uterine tube and going towards the uterus, massively surrounded by columnar follicular cells.

Figure 2. The ovule during full enzymatic assault process (the first stage of the acrosomal reaction), where can be noticed the gradual denudation of follicular cells surrounding the pellucid area, the thick membrane around the oocyte.

Figure 3. Complete peripheral denudation of the pellucid area, and including the opening of the entry gate of the sperm cell in the ovule's cytoplasm.

Fig. 3. The fecundation process (in vivo), performed by the fusion of the two gametes, which constitute the egg or the zygote, resulting in a new individual.

In the ovule's ovoplasma, it is visible the trajectory of the vehiculation by tropism of the sperm cell's head, representing the nucleus, towards the ovule's nucleus. The moment of junction between the two gametes leads to the reciprocal fusion and assimilation between them, representing the actual fecundation and the birth of the zygote, which will form a new individual.

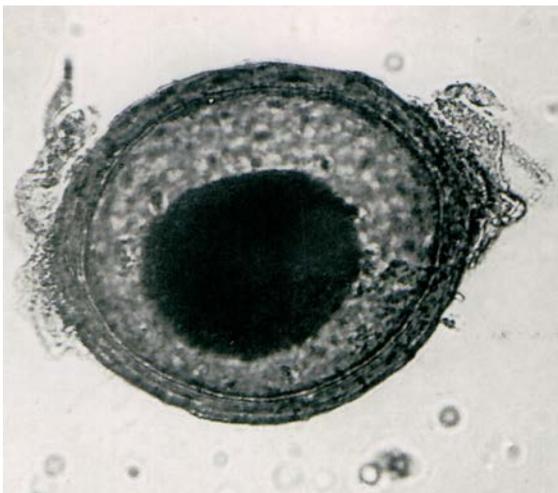


Figure 4. The increase in size of the nucleus to the complete volume of the zygote consecutively the fecundation and the beginning of the dissolution of the adduction tunnel in the same order as its formation.

Fig. 4. Increased (dilated) image of the ovular nucleus consecutively with the fecundation and with the beginning of duct dissolution.

Figure 5. In Mustelidae species (mink), gestation has a latency (inactivity) phase correlated with the light, day, weather conditions, etc. This makes that the blastomere produced by the cleavage of the zygote to include early stages of the embryo development, until the formation of the blastocyte and to wait for the optimal moment of implantation (nidation) in the endometrial mucosa.

The express mission of this work is to present and describe the third and last stage of the acrosomal reaction, which takes place in all mammals after the head of the sperm cell has passed through the entry gate and gets its way around the inside of the ovule, through cytoplasmic mass and towards the ovule's nucleus.

In the following figures we are presenting, for a careful examination, the four positions of the mink zygotes, carefully prepared and monitored chronologically, depending on the majority of the physio-reproductive parameters of the respective species.

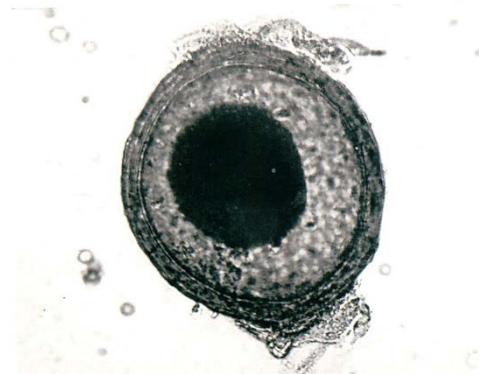


Fig. 5. Blastomere where can be noticed the existence of repeated divisions, without increase in volume

It is important to specify that the acrosome of the sperm cell contains a vesicle with hydrolytic enzymes (mostly hyaluronidase and acrosin) which allows it to penetrate the outer layer of the ovule and to enter the ovule's cytoplasm. The same receptors, which allow the entering of one single sperm cell in the ovoplasma, trigger in the ovule the lysosomal reaction by creating the crossing duct.

These lysosomes are cellular organelles in the ovular cytoplasm, with variable morphology and dimensions, of respectively 0.05 – 0.5  $\mu\text{m}$ , which are delimited by a glyco-proteic membrane. Inside about 40 hydrolytic enzymes (acid hydrolases, phosphatases, nucleases, proteases, polysaccharides, lipases) can be found. The lysosome represents the cellular organelle, which digests certain macromolecules, certain parts of the cell. The lysosomal enzymes act at an optimal acid pH (about 5, which is maintained by a membrane proton pump ( $\text{H}^+$ ), which uses the energy resulting from the ATP hydrolysis). The dependence of enzymes' action of an acid pH is extremely important, because it protects the components of the cytosol. In case the lysosomal membrane is injured, the discharged enzymes are widely and rapidly inactivated by the cytosolic pH, which is of 7.2. The synthesis of the lysosomal acid hydrolases happens in the endoplasmic reticulum. All the proteins destined for the lysosome contain the monoso-6-phosphate (M6P) as a marker. This group, present at the acrosome level (trans Golgi reticulum), a specific receptor, based on which the hydrolases are sorted and rapidly sent towards the lysosome. The kinetics of various products destined to be digested in the lysosome is performed via at least three ways:

a) Endocytosis, which consists in the transfer from endosomes to lysosomes. Generally, this consists in a physiologic act of penetration or caption in the cell of substances from the existing environment, which cannot arrive in the cell directly by diffusion (passive transport or active transport). During endocytosis, the plasmatic membrane undergoes a reversible morphologic modification (usually invagination). Depending on the nature of the compounds, like particles of ovular cytoplasm particles, the receptor-mediated phagocytosis, pinocytosis and endocytosis can be noticed. In this last reference, endocytosis happens after the fixation of a binder on the specific receptor. Following the formation of the binder-receptor complex, this enters the cell, without being accompanied by extracellular fluid.

b) The second pathway is autophagocytosis, by which cellular debris (cytoplasmic) are carried to lysosomes, a process during which autophagosomes are formed. This physiological process is extremely important for the aspects we are describing, because the head of the sperm cell which has penetrated in the ovule's cytoplasm cannot advance towards its nucleus only by removing cytoplasmic components which brake its advancement. The removal and the performance of the crossing duct is performed by digesting these residual cytoplasmic components, debried by the secondary lysosomes. This phenomenon is specific to mammals, especially during stages foregoing the embryo's development, when autophagosomes are formed.

c) The third and last pathway is the phagocytosis, which is in close connection to the first two pathways, and which is a natural addition to them. Phagocytosis only happens in specialized cells (macrophage, neutrofiles), where phagosomes which turn into phagolysosomes are formed.

From our findings and from what we have observed inside the mink ovules, it is clear that this phenomenon is indispensable in fecundation, plays a major role in mitosis and cannot only be specific to minks, but also to the entire animal kingdom, as well as to humans. Therefore, the occurrence of lysosome dysfunctions can lead to multiple modifications in the lysosomal pathology.

### Conclusions

1. Any hampering regarding the vehiculation of the sperm cells starting with the ovule's external membrane (the pellucid area) of the periviteline space and especially the crossing of these gears which have the role of covering and protecting the ovoplasma and of the nucleus, constitute serious causes of male sterility and the occurrence of a notable percentage of infertile females.

2. The hydrolysis of the endogenous proteins is influenced by numerous factors, between which are the distortion and activation of the lysosomes, which increase the process speed in all the tissues, like in the present case, the ovule's cellular structures.

3. The endocytosis is the intracellular caption process (micropinocytosis, pinocytosis, phagocytosis). The penetration inside the ovule is conditioned by the interaction between the ovoplasmatically captured ma-

terial and the non-specific or specific sites (Fe receptor, C receptor) present from the ovular membrane forward.

4. The involvement of the membrane leads to the gradual wrapping of the attached material penetrating the cytoplasm in a minuscule endocytic vacuole (pinosome, phagosome), but the coating of which is formed from the membrane portion which was interiorized and has become detached from the ovular surface.

5. The direct contact to a primary lysosome causes the lyophilisation of this minuscule organelle and the spilling of its contents into an endocytic vacuole.

6. The presented aspects of the endocytosis that have resulted from what was captured on photographic film, is that the ovoplasmatic material captured during the perforation and the performance of the duct destined for the crossing of the sperm cell, from its entry point in the ovoplasma towards the ovule's nucleus, by endocytosis, determines a distortion of the captured material (those small debris) by diverse mechanisms (pH, lactic acid, phagocytin, generation of H<sub>2</sub>O<sub>2</sub>, etc.).

7. In the end, follows the removal by digestion of dislocated materials, and the leftovers resulting from degradation can be used by the ovule or excreted to the outside, creating that dissolution of the printing created by the walls of the connection duct from the membrane entry and the junction point with the ovule's nucleus.

8. The aforementioned conclusions attempt to prove that a detailed knowledge of the acrosomal reaction, and respectively of the fecundation and generation process of zygotes essentially represents life and the procreation of all beings. This is why it is extremely opportune to add these small pieces and biological mechanisms to all that is already known in this field.

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KOPENHAGEN FUR FORSKNING

# FAGLIG ÅRSBERETNING 2018



KOPENHAGEN FUR RESEARCH

**ANNUAL REPORT 2018**



## BEHAVIOUR AND WELFARE

### Some measurements of mink welfare during the winter and growth periods depends on the date of observation, but the overall assessment does not

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The purpose was to test if the WelFur assessment changes with date within the assessment period. Repeated welfare assessments on the same mink farms in the winter and growth periods showed, that the result of some welfare measurements changes, while the overall categorization of the welfare at farm level rarely is affected. As it might change, however, it should be considered how the date of assessment can be addressed in WelFur-Mink.

*Annual Report 2018, 4-7. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

## BREEDING, GENETICS AND REPRODUCTION

### Use of auction data registrations in the genetic evaluation of mink

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The objective of the project was to investigate the perspectives of including auction data in the genetic evaluation of mink. We used registrations from individually culled mink from Aarhus University's Research farm in Foulum, to investigate heritability and correlations for size, quality and price, based on categorization from auction data, live skin grading and grading of the skins prepared for auction. We found a heritability for all traits, indication that genetic progress is possible for all traits. In general, heritabilities were lowest for auction data traits, where data were grouped in relative few categories, followed by traits graded on skin and live grading traits. We found a high positive genetic correlation between skin size

and price. We also found a positive genetic correlation between price and quality after correcting for skin size. We concluded, that is possible to increase genetic progress by including auction data records from relatives as a supplement to live grading of potential breeding animals, but more detailed registrations for size and quality are preferred over the present few categories for each auction trait.

*Annual Report 2018, 8-15. Copenhagen Research, Agro Park 15, DK-8200 Aarhus N, Danmark.*

## NUTRITION, FEEDING AND MANAGEMENT

### Foster kits are accepted quicker and obtain a higher growth following transfer day 2 than day 6 after birth

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Only few investigations have focussed on the optimal procedures and timing for the transfer of mink kits between litters. Therefore, a research project was initiated in 2018 – supported by the Danish Pelt Levy foundation – on transfer early after birth. In total 406 kits from large (9-14 kits) litters were transferred to 406 foster mother (4-7 kit) litters either day 2 or day 6 after birth. Besides the timing of transfer, also the dam experience was investigated, i.e. whether the dam was young (1st year) or older (2nd/3rd year). Mink dams fetched the unfamiliar kit quicker into the nest box day 2 than day 6 after birth. The offspring growth was higher following day 2 than day 6 transfer. The degree of maternal acceptance was equal between young and older dams; however, the growth of kits until 8 weeks was higher in the litters nursed by the older experienced foster dams. The early transfer (day 2 or 6) did not reduce the risk of kit mortality. In conclusion, it is recommended to move kits early (the first postnatal days) after birth, and select older rather than young dams as receiver of foster kits.

*Annual Report 2018, 16-21. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Effect of reducing litter size just after birth on the survival of kits raised by 1st and 2nd year females during the nursing period.**

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The purpose of the experiment was to investigate the survival from birth to day 28 of mink kits relocated to another female during the first days after birth. Kits from litter size 1-2 were relocated and litters with more than 10 kits were reduced to a maximum of 10. The kits were relocated within the first few days after birth. Kits were relocated to females with litter size 2-8 kits. 1-4 kits were relocated, but only up to 9 kits in the new litter were allowed.

Results showed that kits relocated from large litters have a greater chance of survival than kits relocated from small problem litters and there is the greatest chance of survival if kits are relocated to a second-year female compared to a first-year female. When we relocate the smallest kit from the big litters it has a lower survival rate than its new foster siblings, perhaps because it is the weakest kit from the big litter. In the case of litter size reduction after birth, it is recommended to relocate the kits within the first three days after birth, the largest kits are relocated, and preferably to a second year female with kits at approx. the same age, if possible, only one kit is relocated to each foster mother

*Annual Report 2017, 22-23. Kopenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Investigation of two different water systems for mink kits in the nursing period**

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The purpose of this study was to investigate whether installation of an extra water nipple in front of the nest box has a positive effect on growth and survival of mink kits and females. The study included white first year females divided into 3 groups consisting of 174 each. In the control group females and kits were housed in traditional cages with the water nipple at the end of the cage, in one of the experimental groups

a water system for kits were installed where the nipple was placed in the cage in front of the nest box. In the other experimental group a special water nipple device was placed over the nipple at the end of the cage.

The addition of additional water close to the nest box had in this study a positive effect on female kit body weight day 42, it reduced the number of kits who needed extra care in the nursing period and there were a tendency to better kit survival from day 28 to day 42.

*Annual Report 2018, 24-29. Kopenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Reduction of feed waste in the growing period**

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For this investigation we used 4 groups of 145 white male kits. The four groups were fed with the same amount of feed throughout the growing furring period. The control group (KON) was placed in standard cages and fed at the top of the cages, in one of the investigation groups the kits were fed on the top of the nest boxes until September 5 (REDE) after which they were fed at the top of the cages like the control group. In the remaining two investigation groups either a net (NET) or a plastic plate (PLA) were placed at the bottom of the cage under the feed wire.

Feeding on the nest box until September or plates or nets in the cage under the feed wire reduced feed waste at the start of the growing period from 4 to 2%. No problems with dirty nest boxes, nets or plates were observed in the investigation groups, and at the same time there was a tendency for better growth than in the control group at the same assigned feed amount.

*Annual Report 2018, 30-33. Kopenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

**The excretion of B-vitamins in the urine from female mink fed different levels of B-vitamins during the period January-May and from mink kits in June**

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The study elucidated the need of female mink for vitamin B during the winter and suckling period. Female mink excreted vitamin B2 (riboflavin), metabolites of vitamin B3 (niacin), vitamin B5 (pantothenic acid) and vitamin B6 (pyridoxal) in the urine. The excretion generally increased in response to increased content of vitamin B in the feed showing that the feed contained sufficient amounts of vitamin B. The number of kits at birth was not affected by vitamin B in the feed. The excretion of vitamin B in urine was very low from kits of mothers that were fed less than the recommended amount of vitamin B. This indicates a low transfer of vitamin B from mother to kit and/or low uptake of vitamin B from the feed. The weight of the mink kits did not differ on day 28 but on day 42 mink kits fed without addition of vitamin B weighed significantly less than mink kits fed the recommended amount. This suggests that the kits are getting enough vitamin B for growth when they suckle but when they start to eat the feed they have difficulties utilizing the vitamins in the feed ingredients and/or the feed ingredients in the actual feed contain too little vitamin B to cover the need of the kits. This investigation shows that when using the feed formulation of the present study the addition of some B vitamins may be reduced for the females, however the mink kits needs addition of vitamin B to the feed. The NRC specifications of requirements of vitamin B are of older date and primarily based on experiments with growing animals and the actual requirement of kits during the nursing period has not been established.

*Annual Report 2017. 34-42. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark*

**Iron supplementation to mink kits at day 3 after birth**

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The purpose of this experiment was to investigate the effect of injecting mink kits with iron day 3 after birth on the kits' hemoglobin level, counts of white blood cells, content of iron in the liver as well as growth. The experiment comprised 24 litters of which half of the mink kits in each litter was given an injection with 2 mg iron (Fe) day 3 after birth. Iron injected kits showed significantly higher hemoglobin levels day 18 after birth, but not day 39 and 58. Furthermore, iron injection resulted in higher counts of neutrocytes and monocytes day 58. Injection with iron also caused an increase in the iron content of the liver day 18 but had no effect on growth from day 3 to 103. Thereafter, interaction between sex and treatment (-/+ iron) on growth was observed, but whether this is a real interaction should be investigated in further experiments. The injected iron dose corresponded to the dose per gram of body weight used as a standard for suckling pigs day 3 after birth to prevent anemia. However, mink kits have a relatively higher growth rate compared with suckling pigs. Consequently, the used iron dose per gram of growth was considerably less for the mink kits compared with suckling pigs. This probably explains the lack of effect of iron on growth, hemoglobin day 39 and 58, as well as on white blood cells and lymphocytes. Therefore, further experiments with higher iron doses are needed to clarify that.

*Annual Report 2018, 43-50. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

**AlphaSoy Premium and Maltodextrin to 4 to 8 weeks old mink kits**

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<sup>1</sup>Kopenhagen Fur Forskning, Agro Food Park 15, DK-8200 Aarhus N, Denmark.

The purpose of the experiment was to investigate whether Maltodextrin and Premium SPC 68 (Triple A) has a positive influence on kit growth in the last

part of the lactation period. The experiment comprised three groups of 61 – 74 brown females with litters born in the period April 26 to May 2 and with 6 - 10 kits per litter. To one of the groups (PREM) AlfaSoy Premium was used to increase protein digestibility by replacing some of the high-pressure defatted poultry byproduct in the control group (KON). To the second group (MALTO) Maltodextrin was used to replace some of the barley-wheat mixture in KON to increase carbohydrate digestibility. In this experiment we observed no effects on the growth of kits in the period from day 28 to day 56 by using a high digestible soybean product (AlphaSoy Premium) instead of some of the high-pressure defatted poultry and highly digestible carbohydrate (Maltodextrin) instead of some of the barley and wheat.

*Annual Report 2018, 51-54. Copenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Impact of high proportion of fresh poultry, high-pressure boiled defatted poultry or fish offal on mink kit body growth in the last half of the lactation period**

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*<sup>1</sup>Kopenhagen Fur Forskning, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

The purpose of the experiment was to investigate the effect of feeding with highly digestible or low digestible poultry byproducts during the lactation period. The experiment comprised three groups of 130 brown females with litters born in the period April 27 to May 5. The three groups were fed three different diets from April 20 to day 42 after birth. The control group (KON) was fed a low level of high-pressure defatted poultry (8%) in combination with an increased level of fish offal, the second group (AFFJ) was feed a high level of high-pressure defatted poultry (20%), and the third group (ØLA) was feed a high level of raw poultry by-product (20% Øland). The experiment showed that feeding with raw poultry by-product (20%) resulted in a significant higher growth rate in male kits and a tendency to a higher growth rate in female kits from day 28 to day 42 compared to feeding with high-pressure defatted poultry (20%) or increased fish offal in combination with high-pressure defatted poultry (8%).

*Annual Report 2018, 55-59. Copenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **The effect of feeding high energy trout pellets as a supplement to standard lactation feed to mink females in the nursing period**

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The purpose of the experiment was to see whether trout pellets with high energy content given as a supplement to traditional lactation feed during the nursing period can increase the females milk production and thereby the growth of the kits. The experiment comprised two groups of 130 brown female mink with litters born in the period April 27th to May 2th. Both groups (KON and PIL) were housed in standard mink cages. All females were fed with feed from the local feed kitchen until April 20th and thereafter a standard lactation feed until the kits were 6 weeks of age. In the cages of the trial group (PIL), bowls were placed in the 4th mask from the top, right side of cage. In these bowls, high energy trout pellets were given as a supplement to the lactation feed from the day of birth until the kits were 6 weeks of age.

Feeding of trout pellets as a supplement to the mink females during the lactation period resulted in higher weight of male kit day 28, as well as fewer kits who needed additional water. The experiment needs to be repeated with females accustomed to pellets before birth.

*Annual Report 2018, 60-63. Copenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Feeding mink females and kits in the lactation period with Startex 100 – a carbohydrate source with high digestibility and water binding capacity**

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The purpose of the experiment was to investigate whether it is important for the growth of the kits in

the lactation period to be fed a highly digestible carbohydrate source with increased water binding capacity. The experiment comprised two groups of 130 brown females with kits in the period from birth to day 42. The control group received a barley-wheat mixture (1:1) as a carbohydrate source and the test group received a highly digestible pea starch product with a high-water binding capacity (Startex 100).

Use of Startex 100 had a negative effect on the growth of the kits from day 28 to day 42, most likely due to less water added to the feed and thereby a higher dry matter content of the feed than planned. Consequently, at the same calorie intake STAR kits needed to drink more water in addition to the amount of water that came from the feed in order to obtain the same total water intake as the KON kits. In addition, there were more females that had to be treated during the nursing period in the STAR group than in the KON group.

*Annual Report 2018, 64-69. Copenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

#### **Effect of feeding poultry by-products to mink kits during the growing and furring period**

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<sup>1</sup>*Kopenhagen Fur Forskning, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

The purpose of the experiment was to investigate the effect of feeding mink kits with different poultry by-products during the growing and furring period. The experiment comprised 5 groups of 138 brown male kits. Two groups were fed a raw poultry by-product (Øland) either 40% (Ø40) or 60% (Ø60). Two groups were fed a low temperature processed poultry by-product (Løgstør) either 40% (Ltemp40) or 60% (Ltemp60) % and one group were fed 5% of a poultry meal product (Sarval) (Fjmel5) in combination with industrial fish.

The experiment showed that feeding with the raw poultry by-product resulted in the highest growth, survival and feed efficiency, despite that some of the amino acids were below the current recommendations in the period.

*Annual Report 2018, 70-79. Copenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

#### **Effect of up to 5% fly larva meal in feed for mink during the growing and furring period**

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The purpose of the experiment was to investigate the effect of including up to 5% fly larva meal in feed for mink during the growing and furring period. The experiment comprised 3 groups of 138 brown male kits. The control group (KON) was fed without fly larva meal whereas the two experimental groups were fed with 2.5% (F12.5) or 5% (F15) fly larva meal (black soldier fly meal (*Hermetia Illucens*)).

Based on the available results, it appears that there is a negative effect on growth and skin quality, using 2.5 or 5% fly larva meal. A lower digestibility than expected of both protein (71%, against expected 82%) and fat (88%, against expected 90%) in the fly larva meal may be a contributing factor to this. Similarly, a lower content of certain amino acids in relation to some of the recommendations may have affected the growth, however the amino acid content in the KON group was also below some of the recommendations. Whether we could achieve better results using fly larva pulp, that has not been subjected to heat treatment and therefore has a somewhat higher protein digestibility, should be investigated in future studies.

*Annual Report 2018, 80-85. Copenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

#### **Feeding with a high proportion of vegetable proteins to female mink in the lactation period**

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The purpose of the experiment was to investigate whether a high proportion of vegetable protein sources, consisting of potato protein, corn gluten, soy protein, pea protein and wheat gluten can be used to female mink and kits in the lactation period. The experiment comprised two groups of 130 brown females with litter born in the period April 27th to May 2nd. From April 20th the groups were fed with a feed, where vegetable protein sources amounted 9.9% or

3.8% corresponding to that the proportion of digestible protein from the vegetable protein sources was 33,6 and 13,2%, respectively.

Feeding with 9.9% vegetable protein ingredients in the lactation period compared to 3.8% significantly reduced the growth of the mink kits from day 28 to day 42. There was no difference in weight of females and in kit survival during the period

*Annual Report 2018, 86-89. Copenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

## HEALTH AND DISEASE

### **Experimental inactivation of Aleutian mink disease virus (AMDV) in mink manure by increased pH and heat**

*Lise Kirstine Kvisgaard<sup>1</sup>, Mette Sif Hansen<sup>1</sup>, Mariann Chriél<sup>1</sup>, Lars Erik Larsen<sup>1</sup> & Charlotte Kristiane Hjulsager<sup>1</sup>*

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Sanitation of manure from mink farms is specified by Danish legislation, when more than three mink on a farm is tested antibody positive for Aleutian mink disease virus (AMDV). The sanitation is performed by increasing pH of the manure to pH 12. After sanitation, the manure can be used as fertilizer or transported to a biogas plant, where it will be heat-treated. The aim of this study was to test experimentally whether increase of pH to 12 or heat-treatment at 70 °C was sufficient to inactivate AMDV in manure and hence prevent spread of AMDV from infected herds. Four mink were inoculated with pH-inactivated AMDV in manure. One remained ADMV negative, but three mink became viremic. Another four mink were inoculated with heat-treated AMDV in manure. Of these, three mink were euthanized prematurely due to wounds. The surviving mink remained negative for AMDV. Under the conditions of this study, AMDV was not inactivated by elevating pH. However, the development of viremia was delayed compared to control mink inoculated with non-inactivated AMDV-manure, suggesting the elevating pH has an inactivating effect. Since only one mink inoculated with heat inactivated AMDV-manure survived, no conclusion regarding heat-treatment at 70 °C can be drawn.

*Annual Report 2018, 90-94, Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Experimental infection of mink with plasmacytosis virus in feed**

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Feed is often implicated in outbreaks of disease in mink, including outbreaks of Aleutian mink disease (AMD), also known as plasmacytosis. In this study we investigated the transmission of Aleutian mink disease virus (AMDV) via feed under experimental conditions. Experiments were performed with healthy Sapphire mink, fed with surplus feed left over by mink experimentally infected with AMDV Saebý strain virus. AMD virus was detected in samples of the surplus feed, but transmission to the recipient mink was not detected. Experiments were also performed with healthy sapphire mink, that were fed with different doses of Saebý-2016 or Holstebro-2016 virus spleen homogenates mixed into their feed, but transmission of the viruses was not detected. Finally, we fed the Saebý strain 2016 virus, mixed into feed, to sapphire mink, that were immunosuppressed by beforehand treatment with prednisolon. AMD virus was successfully transmitted to three out of six prednisolon treated mink. The remaining three animals were euthanized before transmission could be detected. The results from these experiments demonstrate that immunosuppressed mink are susceptible to AMDV virus in feed. We speculate that secondary transmission within the farm can proceed by the natural route of transmission, if single debilitated animals have been initially infected by feed.

*Annual Report 2018, 96-101. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Pathogenesis of mink plasmacytosis after aerosol and intraperitoneal inoculation**

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Though intraperitoneal (IP) inoculation is not the natural pathway of Aleutian Mink Disease Virus (AMDV) infection, this inoculation route is often used experimentally. Results from previous studies indicate that IP inoculation favors the liver in relation to development of histological lesions. For a better understanding of the development of AMDV infection, we compared the effect of IP and aerosol (AE) inoculation, and natural infection of sentinel mink. The sentinals were also used to investigate when the risk of natural infection between mink is highest. 46 sapphire mink puppies were divided into groups: negative controls; mink inoculated IP or AE (using a nebulizer and anesthesia mask) with AMDV; and sentinals exposed to natural infection from the IP-inoculated mink during 2 week periods. Mink from each group were killed after 2, 5 or 10 weeks. All AMDV IP-inoculated mink became virus positive. In the group of AE-inoculated mink, virus could not be detected after 2 weeks, while after 5 and 10 weeks 50% and 100% respectively tested positive for AMDV. Virus was detected in sentinals exposed to infection in weeks 2-6 of the study. As expected, IP inoculation resulted in more extensive lesions in the liver compared to AE inoculation. The aerosol model is considered to reflect natural AMDV infection better than IP inoculation. Results from the sentinals indicate that the risk of AMDV infection between mink is highest during early infection before antibody positive animals can be found on the farm.

*Annual Report 2018, 102-107. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Preliminary results of clinical and gross pathological studies of cystitis and urolithiasis in farm mink (*Neovison vison*)**

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The objective of this pilot study was to evaluate urine sample analysis as predictors of gross lesions in the urinary tract of farm mink kits with cystitis and urolithiasis. Urine samples (n=240) were collected by spontaneous micturition. The method is relevant for studies of the efficiency of prophylactic and treatment protocols on farms with urinary tract disease. Also the method could potentially provide information of the prevalence of subclinical (without symptoms) urinary infections and thereby provide information concerning the relevance of flock treatments. Based on urine analysis data two case groups (n=18/14) and one control group (n=12) of animals were selected. The case and control animals were euthanized and examined by necropsy. Lesions associated with cystitis and urolithiasis were recorded. Gross pathological findings were recorded in 7 animals. 6 of these were case animals identified by presence of struvite crystals in combination with erythrocytes, pH > 6.6, discolored urine and/or positive nitrite. No gross pathological findings were found in the control animals. In conclusion, urine analysis appeared to be a useful method for the identification of mink kits with gross lesions in the urinary tract, but also that urine color, nitrite, pH and the presence of struvite crystals should be evaluated in combination. The consequence of red bloodcells and protein in the urine of mink kits should be evaluated further.

*Annual Report 2018, 108-112. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Danmark.*

### **Immunological response against mink astrovirus**

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Mink astrovirus is a risk factor associated with pre-weaning diarrhea (PWD), a syndrome characterized by clinical symptoms such as diarrhea, greasy skin, and dehydration which might cause an increase in mortality on the mink farm. The purpose of the study was to investigate the specific immunological response in the form of virus-specific immunoglobulin G (IgG) in females and healthy kits and compare the response to PWD-affected mink kits. 100 females and their offspring were sampled from two farms (a control farm and a case farm with outbreaks of PWD) in Zealand. Blood samples were analyzed for the presence of mink astrovirus-specific IgG in both females and kits. Milk samples from 20 females, which were milked twice, were also analyzed for the presence of mink astrovirus-specific IgG. The results show that there is no immunological response towards mink astrovirus in neither females nor kits, irrespectively of their PWD status. This indicates that specific antibodies to mink astrovirus are not associated with the PWD syndrome, i.e. astrovirus is not involved, or the mink astrovirus strain found on the mink farm induces the production of IgG with another specificity that does not react with the astrovirus antigen used in this study.

*Annual Report 2018, 113-116. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

#### **Low concentration of immunoglobulin G is associated with pre-weaning diarrhea**

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Mink kits are born with a low concentration of immunoglobulin G (IgG) in the blood and the role of IgG in regards to protection against diseases, such as pre-weaning diarrhea has not been fully studied. In this study, we analyzed the concentration of IgG in blood

collected from kits from two mink farms, with one farm being a case farm with high prevalence of pre-weaning diarrhea (PWD), and the other being a control farm with no cases of PWD. Our results showed that PWD-affected kits had a significantly lower concentration of serum IgG compared to unaffected kits at 13-15 days of age. Our results suggest that a lower intake of IgG from the milk or a reduced absorption via the intestine could pre-dispose kits for PWD. PWD-affected kits had a significantly reduced weight gain compared to healthy control kits. Furthermore, litters born later in the breeding period were affected by PWD at an earlier age than litters born earlier.

*Annual Report 2018, 117-122. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

#### **Clinical trial comparing the effect of 3 alternative treatments and conventional amoxicillin therapy of mink kits with pre-weaning diarrhea**

*Julie Melsted Birch<sup>1</sup>, Jens Frederik Agger<sup>1</sup>, Mikael Leijon<sup>2</sup>, Karin Ullman<sup>2</sup>, Tina Struve<sup>3</sup> og Henrik Elvang Jensen<sup>1</sup>*

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Pre-weaning diarrhea (sticky kits) is a multifactorial syndrome in mink kits with an unclarified etiology. Evidence based treatment protocols are not available, hence the treatment is often empirical and based on the use of antimicrobials. The aim of this study was to compare the effect of three alternative treatments (probiotic, narrow-spectrum antimicrobial and fluid) with a conventional amoxicillin treatment on growth and mortality in mink kits with pre-weaning diarrhea. Secondly, the aim was to characterize the study population microbiologically. Staphylococci belonging to *S. intermedius* group, *E. coli*, *E. hirae*, astrovirus and calicivirus were identified from intestinal contents from litters included in the trial. Multivariable mixed model regression showed no significant difference in growth between the treatment groups. We did neither see a significant difference in mortality between the treatment groups.

*Annual Report 2018, 123-127. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Transfer of amoxicillin from dams to suckling mink kits**

*Julie Melsted Birch<sup>1</sup>, Henrik Lauritz Frandsen<sup>2</sup>, Tina Struve<sup>3</sup>, Jens Frederik Agger<sup>1</sup> og Henrik Elvang Jensen<sup>1</sup>*

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Antimicrobial treatment of pre-weaning diarrhea in mink kits is empirically and “off label” based and often by the use of amoxicillin. In larger outbreaks of diarrhea, single animal treatment is very time consuming. Therefore, in some farms treatment of the dam, either through the feed or as injection, is used instead. The aim of the study was to examine the transfer of amoxicillin from the dam to the suckling mink kits during the first 8 hours after orally or intramuscular treatment, respectively. Serum samples from mink kits showed the highest concentration of amoxicillin 8 hours after treatment of the dam, independently of the administration route. The concentrations were judged too low to exert antibacterial effects on relevant gut bacteria.

*Annual Report 2018, 128-131. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **The stability of amoxicillin in feed**

*Desiree C. K. Lassen<sup>1</sup>, Nanett K. Nikolaisen<sup>1,2</sup>, Amir A. Ronaghinia<sup>3</sup>, Katja A. Kristensen<sup>1</sup>, Mariann Chriél<sup>4</sup>, Tina Struve<sup>2</sup>, Henrik L. Frandsen<sup>1</sup>, Lars Bogø Jensen<sup>1</sup>, Karl Pedersen<sup>5</sup>*

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Bacterial infections in mink are usually treated with antibiotics. Amoxicillin can be prescribed as a powder for oral administration and mixed into the feed. The aim of this study was 1) to investigate the stability of amoxicillin in mink feed within 24 hours, and 2) to investigate if it influences the bacterial composition and the occurrence of antibiotic resistant bacteria in the feed. Both aims were tested at two temperatures; 4°C and 20°C, representing a cold winter day and a warm summer day. The study showed that the concentration of amoxicillin is temperature dependent. After 24h the concentration of amoxicillin decreased to app. 30% of the initial concentration at 20°C, whereas the concentration decreased to 75% of the initial concentration at 4°C. *Escherichia coli* and *Staphylococcus* spp. were identified in the feed mixtures and further tested for antibiotic sensitivity. Half of the *Escherichia coli* were tetracycline resistant and a few *Staphylococcus* were resistant against tiamulin and erythromycin. This study demonstrated 1) the importance of sick animals eating their ration of feed mix within few hours, to obtain the intended therapeutic antibiotic dosage and 2) the occurrence of antibiotic resistance in the tested bacteria was low, compared to previous studies on pathogenic bacteria from mink.

*Annual Report 2018, 132-135. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Experimental exposure of mink with MRSA supplemented feed**

*Mette Fertner<sup>1</sup>, Karl Pedersen<sup>2</sup>, Mariann Chriél<sup>1</sup>*

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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been found in approximately 40 % of Danish mink farms, hypothesized to be a spill-over from the pig production where the bacterium is widely distributed. Contaminated

slaughter offal used in the production of mink feed is considered the most likely route of transmission.

We performed a longitudinal experimental study where 24 naïve mink were exposed to LA-MRSA-contaminated feed ( $5.1 \times 10^8$  cfu/mink) for five days. Four mink were retained as negative controls receiving LA-MRSA-negative feed. Twenty-four hours after initiation of spike, all 28 mink, including the four negative control mink, tested LA-MRSA-positive by paw swabs. At day 31 in the study period, 26 days after LA-MRSA-positive feed was ceased, the bacterium could be re-isolated from paws (11/28) and pharynx (9/28) of mink.

Results from the present study demonstrate that LA-MRSA in feed can be re-isolated from paws and pharynx of exposed mink and may persist on both anatomical sites for more than 26 days. In addition, the results indicate the potential of the bacterium to spread directly between neighboring mink or through contaminated surfaces.

*Annual Report 2018, 136-138. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Prevalence of MRSA in mink and environmental sites on Danish mink farms**

*Mette Fertner<sup>1</sup>, Karl Pedersen<sup>2</sup>, Vibeke Frøkjær Jensen<sup>1</sup>, Gitte Larsen<sup>1</sup>, Mikkel Lindegaard<sup>1</sup>, Julie Elvekjær Hansen<sup>1</sup>, Mariann Chriél<sup>1</sup>*

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Livestock-associated MRSA (LA-MRSA) is a bacterium resistant towards commonly used antimicrobials, and it is able to cause infections in humans and animals. In the present study, the aim was to identify the animal prevalence and environmental reservoir of LA-MRSA on-farm, based on carcasses and environmental swabs from five Danish mink farms, collected during May-September 2017.

On one farm, LA-MRSA could not be isolated from the animals, while the bacterium was isolated from mink on four farms, found in 20% [13;29] CI95% to 29% [22;38]CI95% of the animals. The bacterium was isolated from non-weaned whelps, indicating a

direct transmission from the dam or from a contaminated environment. On the four farms with LA-MRSA-positive mink, the bacterium was further isolated from all tested environmental sites (feed, glove, cages, nest boxes), except air. Negative air samples are in contrast to swine production, where LA-MRSA is found in high concentrations. Due to the anatomical location of the bacterium on mink (paws and pharynx), farmers should be aware of the risk of LA-MRSA through bites and scratches in the handling of mink.

*Annual Report 2018, 139-143. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Vaccination of females during the winter period to enhance their overall immunity**

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The purpose of the investigation was to see whether a general enhancement of the immunity of females through vaccination (adjuvant) can reduce disease problems in the nursing period with fewer greasy kits and mastitis. To the investigation we used two groups with each 79 second year and 250 first year brown mink females. One group was vaccinated in January against Distemper and Virus enteritis in order to strengthen their overall immunity.

There were significantly higher numbers of kits at birth and day 28 in second year females in the vaccinated group, compared to the control group. Concurrently, a slightly lower body weight increase in male kits from these females compared to male kits from second year females in the control group. In this study, no significant difference in the number of treatments during the period and the number of dead females was found by vaccinating the females in January.

*Annual Report 2018, 144-147. Copenhagen Fur Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*



RIITTA KEMPE | SELECTION FOR WELFARE AND FEED EFFICIENCY IN FINNISH BLUE FOX

## SELECTION FOR WELFARE AND FEED EFFICIENCY IN FINNISH BLUE FOX

RIITTA KEMPE



# Selection for Welfare and Feed Efficiency in Finnish Blue Fox

Doctoral thesis by Riitta Kempe

Department of Agricultural Sciences

Faculty of Agriculture and Forestry

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2018



Finland is the world's largest producer of blue fox pelts, and fur animals form the second largest group of production animals in Finland (Profur 2016; SVT 2017). The fur industry has a national breeding Value evaluation of blue fox production traits and it is used by one third of fox producers. The primary goals in blue fox breeding are to improve the fertility traits and fur quality of foxes and to produce large pelts, whereas animal health, welfare, conformation traits or feed efficiency are not included in the current breeding value evaluation. The emphasis on production traits has resulted in unfavourable changes in blue fox conformation and health traits. There is an obvious need for revision, if any of the current breeding goals weakens the animals' welfare. In addition to welfare traits, an important new breeding goal would be feed efficiency, given that feeds are a major production cost and their inefficient utilization may lead to poor growth and to nutrients being wasted into the environment.

Improvement of any economically or ethically important trait through animal breeding requires that the trait is heritable and is recorded into a breeding software database. The main objectives of this thesis were to estimate genetic parameters for new conformation, health and production traits for potential introduction into the national blue fox breeding programme, and to determine their correlations to the production traits in the current breeding value evaluation. Phenotypic and genetic evaluation systems for the proposed traits were created in this research project, namely for feed efficiency, body condition score, body length, leg conformation, ability to move about and susceptibility to eye infection.

The study data, consisting of altogether 2076 foxes, are from a two-year experiment carried out during the growth period in 2005 and 2006 at the fur animal research station of MTT Agrifood Research Finland in Kannus (now Kannus Research Farm Luova Ltd). Multiple-trait restricted maximum likelihood (REML) estimation was used, since it enables taking several traits into account at the same time, to calculate the genetic parameters and to determine any antagonistic genetic or phenotypic correlations between conformation, health and production traits.

The heritability estimates for feed efficiency, daily gain and daily intake were moderate (0.23-0.29) (II-IV). The studied conformation and welfare traits were shown to have a genetic background. Moderate heritabilities were found for leg conformation, ability to move about, body condition score (BCS) and susceptibility to eye infection (0.21-0.30) (IV, V). Animal body weight had large genetic variation and moderate to high heritability

(0.37-0.50) (II, IV, V). High heritability estimates were obtained for pelt size (0.47-0.50), while the highest estimates were for fox body length (0.51-0.57) (II-V).

Grading size and pelt size, the two size traits in the current breeding value evaluation, had moderately high to high positive genetic correlations with body weight, daily gain, body length and BCS (fatness) (0.42-0.74) (III). Pelt size and daily gain had moderate to rather high positive genetic correlations with feed efficiency (0.36 and 0.51-0.56, respectively), but all studied size traits had unfavourable positive genetic correlations with feed intake (0.49-0.95) (II-IV). Grading size, October body weight, daily gain and BCS had moderately high unfavourable genetic correlations with leg conformation (-0.40 to -0.53) and high unfavourable genetic correlations with ability to move about (-0.58 to -0.65) (IV). The genetic correlations between the size traits (grading size, BCS, body length and body weight in November) and susceptibility to eye infection did not differ from zero, as the standard errors of these genetic correlations were high (V). However, grading density of fur had an unfavourable genetic correlation with susceptibility to eye infection (-0.49). Body length showed a high positive genetic correlation with grading size and pelt size (0.63-0.87), but its genetic correlations with BCS and susceptibility to eye infection were low and hardly differed from zero (0.04 and -0.18, respectively) (II-V). Genetic correlations between body length and foreleg conformation, and between body length and the animal's ability to move about were negative, although their standard errors were high (-0.38±0.21 and -0.42±0.19) (IV).

While the current, relatively strong emphasis on selection for larger animal and pelt size in blue fox breeding does improve feed efficiency indirectly, it is unlikely to reduce feed intake. Selection for longer pelts tends to favour fast-growing and fat individuals, simultaneously increasing their feed intake and, hence, feeding costs. Fast growth rate and extreme fatness also pose a risk to animal welfare. The results reported in this thesis show that fast growth rate, high body weight, large grading size and BCS (fatness) have unfavourable genetic correlations with leg weakness and impair the ability to move about in the cage in less than six-month-old blue foxes.

Although the current emphasis on size traits in the breeding value evaluation does not significantly weaken the foxes' eye health, the focus on thicker fur density can expose them to eye infection due to the antagonistic genetic correlation between the two traits. High BCS (fatness) is also associated with an undesirable reddish fur colour and a lighter pelt colour. The

use of animal body length as a selection criterion can open up the possibility to breed wellstructured, long, slim foxes instead of fat ones. Selection for longer animal body does not increase the risk of fatness or susceptibility to eye infection nor does it have unfavourable effects on pelt quality traits.

The genetic parameters estimated for conformation, health and feed efficiency traits indicate that these traits are heritable and that genetic improvement through selection has potential to improve the health status and feed efficiency of Finnish blue foxes. The results of this research project can be implemented into the national blue fox breeding scheme taking into account the genetic connections between health and production traits.



Riitta Kempe

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