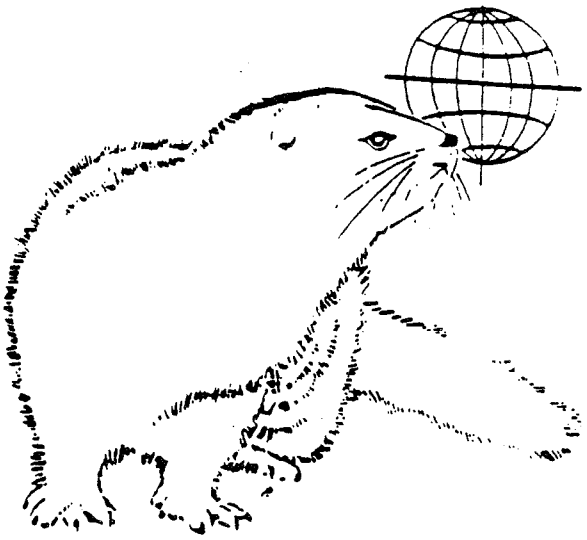

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INTERNATIONAL FUR ANIMAL SCIENTIFIC ASSOCIATION

SCIENTIFUR
ISSN 0105-2403
Vol. 22, No. 2
May 1998

List of other publications – not abstracted

Effective breeding increases productivity.
Michael Sønderup. Dansk Pelsdyravl, Vol. 58 (9), pp. 354-355, 1995. 2 tables. In DANH. Code 4-12-M.

5. Reproduction

List of other publications – not abstracted

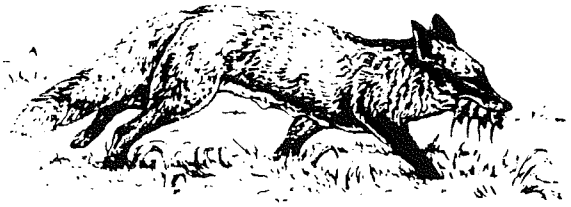
Some thoughts about the problem of greasy kits, 2.
Georg Hillemann, J. Skadborg. Dansk Pelsdyravl, Vol. 58 (12), pp. 491-492, 1995 4 ill. In DANH. Code 5-9-M.

6. Nutrition

List of other publications – not abstracted

Evaluation of the bioavailability of natural and synthetic forms of beta-carotenes in a ferret model. *W.S. White, K.M. Peck, E.A. Ulman, J.W. Erdman Jr. Annals of the New York Academy of Sciences, 691, pp. 229-231, 1993. Code 6-3-O.*

7. Veterinary



Notes
SCIENTIFUR
Vol. 22, No. 2, 1998

Now it looks as if SCIENTIFUR is going to manifest its status as the scientific link between all parties interested in fur animal research and production. Not only is the number of subscribers increasing - although a bit too slowly - but also the number of original reports for publication in SCIENTIFUR is increasing very fast. In connection with the present issue it has been necessary for us to keep some reports for the next issue because of the limited space, approx. 86 pages and totally 249.5 grams. This is only a luxury problem, which we shall be pleased to work on, if the "problem" is going in the right direction.

Many colleagues and some discipline groups within IFASA have asked us to establish a referee system for scientific reports presented in SCIENTIFUR. It is obvious that documentation of the scientific value of a report is important to give the author(s) scientific competence and to underline the scientific value of the information given to third parties for instance in the standing communication on fur animal welfare.

Therefore, we are now introducing this referee system so that authors, if they so choose, can have the scientific value of the actual report approved by senior scientists within the specific discipline. Already when you read these lines, we have a very well merited referee panel ready covering the following disciplines: NUTRITION & PHYSIOLOGY, ETHOLOGY INCL. ANIMAL WELFARE, MANAGEMENT & PRODUCTION, GENETICS & BREEDING, REPRODUCTION, VETERINARY SCIENCES AND HAIR & SKIN.

Until we know the development in the number of subscribers and reports for publication in SCIENTIFUR, we do not intend to change the form of production or the number of issues per volume. We would of course like to have the necessary finances to have the journal printed professionally with the possibility of presenting superfine illustrations of for instance photos included in the reports. So far we only have the possibility to collect the figures and photos on one sheet and print them professionally for later inclusion in SCIENTIFUR. Earlier we have done that on some occasions. Also in the present issue you will see this arrangement where the authors have together with the disc and text sent the desired number of ready printed figures for inclusion in the report when published. A good solution, costing a bit extra for the authors, but also presenting the material in a much more professional way. Other authors are welcome to do the same until we will be able to print the whole journal with the right techniques.

A web-side for IFASA/SCIENTIFUR is in preparation, and we hope that the SCIENTIFUR INDEX will be found on the Internet before the end of this year. Good luck with your work Bruce D. Murphy and collaborators.

Titles, names and addresses are not 100% correct in our files. Therefore, please send us a fax with the necessary corrections for our files. We like things to be correct - therefore please send us a short note.

Colleagues travelling around the world professionally often tell me that nobody or only very few in the audience know about SCIENTIFUR, the books published or IFASA. We therefore again ask you, dear readers - please tell your colleagues about all this and ask them to contact us to order a free introductory copy of SCIENTIFUR plus the basic information on IFASA. Hopefully, the Internet will give us a lot of help in this respect. Also here you can help by telling your colleagues about this possibility once it has been established.

Just these days we have sent a letter to all IFASA members who are not subscribing to SCIENTIFUR in connection with their membership because they have access to the journal at their library or some other place like that. We ask them to let us know whether they would prefer to pay their IFASA membership fee every year or for a 4-year period, in the latter case they would get a 25% discount. This would give us less work and saved costs on money

transactions would equal the 25% discount. We would all profit from such an arrangement and we therefore hope for a lot of positive answers.

For those of you who pay a combined membership and subscription fee, we are not going to make any changes, and the fact that a lot of you now pay your bills with credit cards helps us very much in the reduction of our banking costs.

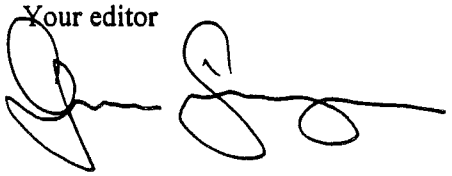
In 1997, the board meeting was held by correspondence. Above you have read about the new activities started here in 1998. The financial status of the organisation is satisfactory, especially thanks to the economic contribution we received from The European Fur Breeders Association (EFBA) also in 1997. In 1998, the board meeting will be held in Montreal, Canada, in the autumn.

It has earlier been pointed out that IFASA wishes to promote international scientific seminars or work groups in-between the international scientific congresses, of which the next will be arranged in Kastoria, Greece, in the year of 2000.

If any of our readers/members should have any suggestions for the board of directors to discuss, please send a message to the President, Prof. Einar J. Einarsson, or to the writer - your editor and secretary. Addresses are on the inside front cover of SCIENTIFUR.

Have a good summer!

Your editor



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Original Review

Status concerning the welfare of farm foxes

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Introduction

Rules and legislation on the housing of farm animals are often the result of a media-created debate between consumers, politicians and various "experts" (e.g. researchers, veterinarians, animal protection societies). At the moment the consumers are more conscious of the housing conditions of farm animals, and gradually they have also become willing to pay for goods which have been produced in a more ethically acceptable environment. It is therefore important that objective scientific results concerning the conditions of farm animals are available, the objectivity of which can guide the industry, politicians, and consumers in existing and future debates. Behavioural research can contribute with knowledge about the normal behaviour of farm animals, which is one of the basic preconditions of being able to assess welfare, and it can show possible welfare problems and the farm animals' state of welfare and suggest solutions to welfare improvements.

In Denmark, research in the behaviour of farm foxes began in 1986-87, and the recent 10 years' research has provided some answers to the behaviour and welfare of farm foxes. Some of these results have been implemented in the Regulations of the Veterinary Services concerning Breeding of Game and Foxes (1997). Welfare-wise the demand for a resting platform for all farm foxes is the most significant change compared with previous regulations. Finnish (a.o. *Harri et al. 1991*) and Danish (a.o. *Pedersen & Jeppesen, 1993*) studies have stressed that foxes use a resting platform to observe the surroundings both in quiet and disturbed situations, and having this possibility is important to the individual fox. Apart from this, the cage environment has been relatively unchanged for the past three decades, and there is no demand for any changes in the existing regulation. The Council of Europe has also in its recommendations concerning fox husbandry used recent years' Behavioural research. Among other things it recommends that breeding vixens have access to a whole-year shelter. Regarding the cage environment, the Ministry of Agriculture has financed a 5-year project where alternative cage systems are tested for both blue and silver foxes. Considerable differences between the alternative systems and the traditional system are cage size and improvements such as resting platforms, whole-year shelter, solid bottom, and sand tray. The results are expected to reveal whether the considerable changes of the existing cage environment in fact lead to a significantly improved welfare of the farm foxes.

This paper summarises some of the most recent results of the research in the behaviour of farm foxes and lists the areas which will be focused on in the nearest future.

Man-animal relationship

Behavioural research often focuses on breeding vixens when the effect of improving the cage environment is tested, while most of the studies of the production animals have concentrated on the man-animal relationship where attempts have been made to make the animals more trusting towards people. In a 3-year project, it has been shown that silver foxes'

fear of people is reduced by regular human contact during the early growth phase just before and after weaning. Besides reduction of fear, a reduced stress sensitivity and, later on, improved breeding results were seen (*Pedersen, 1993*). But what is the value of the man-animal relationship and the cage environment, respectively, for the welfare of the individual animal? Is one parameter more important to welfare than another? As far as blue foxes are concerned, a thesis project performed at the University of Copenhagen tried to provide the answer to that (*Bertelsen, 1996*). A group of blue fox cubs were treated tenderly 5 minutes daily from 7 to 10 weeks of age. The other group was a control group which was only subjected to normal routines. All the cubs were tested for behaviour at the age of 10 weeks and thereupon distributed to cages with or without a whole-year shelter (cage environment). The different behaviour tests showed that the handled cubs were less fearful towards people and less fearful generally compared with the control group. At the same time, cubs without a whole-year shelter were less fearful towards people and unknown objects compared with cubs with a whole-year shelter. At the age of 22 weeks, the cubs were subjected to a test in an unknown environment, a so-called "open field" test. Again it was shown that handled animals were more calm and curious during catching in their cages and in the open field test compared with the control group. No significant effect of cage environment was found on behaviour in the open field test, but a tendency opposite to that of the behaviour tests: namely that cubs with whole-year shelters were less fearful than cubs without whole-year shelters. Blood samples taken before the open field test were supposed to indicate the basic stress hormone level, and blood samples taken after the open field test should indicate the effect of acute stress on the cubs' stress hormone level. But results showed no effect of handling or cage environment on the stress hormone level. In the following breeding period, handled foxes showed a tendency to get more cubs, and more cubs survived the first 4 weeks compared with the control group.

The conclusion was that early handling of blue fox cubs significantly reduces their general fear as well as their fear towards people and thus increases their welfare. Physiological parameters could not confirm this relationship, but only that fearful foxes generally had a higher basic level of stress hormone in their blood. Whether a whole-year shelter could improve the welfare of blue foxes during the growth period could not be proven due to the opposing results. The results indicated, however, that human contact at an early age had a greater influence on the welfare of blue foxes than cage environment (access to a whole-year shelter during the growth period).

Cage environment during the growth period

As far as we know, tests describing how different rearing conditions affect the blue fox cubs with regard to growth, behaviour, welfare and later reproduction have never been performed. Perhaps there exists an optimal procedure which: 1) secures the blue foxes optimal conditions during the growth period, 2) secures the breeder larger furs at pelting, or if the foxes are going to be used for breeding, 3) secures the breeder optimal reproduction results. Two different rearing conditions were tested with regard to behaviour, welfare and weight development in the growth period as well as weight and reproduction success in the following breeding period. The rearing conditions tested were rearing in pairs or solitarily from weaning and separation at the age of 7 weeks. Other parameters such as e.g. age at weaning and weaning in litters a couple of weeks before separation will be tested at a later stage.

The blue foxes were weighed several times during the growth and breeding period to describe growth and weight loss. Furthermore, behaviour was evaluated in two different tests where the trust of the foxes towards people and their general level of fear were registered. Reproductive success was measured by number of born and weaned cubs of mated and pregnant vixens as well as loss of cubs from birth until weaning. The two groups of blue foxes did not diverge significantly in any of the behaviour tests as approx. half of both groups were curious towards people and accepted titbits out of peoples' hands. And the curiosity towards an unknown object was equally high in both groups. Growth until pelting as well as weight loss until mating were higher in the group reared solitarily. In the same group (solitary rearing) a higher percentage of the vixens were either not mated or were barren compared with the group reared in pairs (23% to 6%). Effect of rearing conditions on number of cubs at birth and weaning could not be proven.

Based on these results it could be concluded that it is advantageous to rear blue foxes solitarily in order to obtain bigger animals at pelting, but in the long run it is advantageous to keep blue foxes in pairs from weaning as more vixens in this group reproduce. Based on the behaviour tests and the number of cubs, it could not be concluded that the welfare was threatened in either group, but all things considered the prevalence of non-reproducing vixens indicates a welfare problem. The reasons for the lack of reproduction should, however, be ascertained. In this case, the reason is probably the lack of sexual imprinting on own species in the growth period and is not necessarily stress-related. Further studies of behaviour and physiology would affirm or confirm whether solitary rearing affects the welfare of blue foxes in the short and long term.

Selection

For a period of 30 years, Russian researchers have selected for trust in farm foxes and have now obtained a population which is very trusting towards people and does not need demanding handling during the growth period. Precisely the lack of trust in farm foxes has been considered a significant welfare problem. Therefore an initiative to a common Nordic project was taken in 1994, the objective of which was 1) to have more trusting foxes on private farms in Denmark, Finland, Norway and Sweden and 2) to examine the scientific and productional aspects of selection on behaviour. This project has been very well received by the participating breeders in Denmark (4 silver fox farms and 4 blue fox farms), and the first results have shown that trusting vixens of the selection group had more cubs than the other vixens on the farms (*Pedersen, 1995*). At the same time it became evident that more cubs born by trusting mothers are trusting at the age of 6 months compared with the cubs of the control group. Whether this trust is genetic or environmental cannot be determined for the time being. The most important thing, however, is that trust can be tested, trusting farm foxes exist, the trusting foxes have more cubs, and the trusting foxes have trusting cubs.

Infanticide

There is a theory in modern fox research that infanticide (cub killing) is based on a natural ability in individuals of the dog family (wolves, jackals, foxes etc.) to act as "helpers". "Helpers" abstain from reproduction but participate in the rearing of the litter of the dominant vixen. It has also been seen in the wild fox that a vixen eats her own cubs whereupon she helps the dominant vixen to rear her cubs *(MacDonald, 1979; 1980)*. Therefore, infanticide could be a natural occurrence of social behaviour and not necessarily an abnormal stress-related behaviour. This behaviour appears abnormal and extremely undesirable when otherwise healthy cubs have to be killed because of the higher social status of a neighbouring vixen, and because the vixen performing infanticide cannot perform the help function which caused the infanticidal act under normal farm conditions.

Bakken (1993) has examined the occurrence of cub killings in farm bred silver foxes of either very high or very low social status. This kind of basic research can be used to affirm or confirm the theory that cub killings in farm foxes are caused by social mechanisms. And the Norwegian results indicate that social status among neighbouring vixens affects reproduction success so that low-ranking vixens have a bad reproduction when the neighbouring vixen is high-ranking. On private farms, however, there is a far greater variation of social status among the vixens, e.g. because of differences in age, high replacement rate and frequent removal of the vixens. It would, therefore, be interesting to examine the stability of social status in an ordinary farm fox population and study the effect of social status on different welfare parameters. Three Danish projects aimed to examine this complex of problems, out of which two have been finished and the third will be terminated in 1998.

Project 1: The social status, behaviour and physiology were examined in silver fox vixens kept in pairs in a thesis project at the University of Copenhagen (*Bank, 1996*). Furthermore, the effect was examined of an acute (single) stressor and repeated stressors, respectively, on different behavioural and physiological parameters in the dominant and subordinate (inferior) vixens. Sixty unrelated silver fox vixens were paired off randomly. These vixens were tested for social status 8 times with a test procedure developed by Morten Bakken (Feed Competition Capacity Test, FCC-test, Bakken, 1993). In this test, the vixens compete for access to attractive feed after 24 hours without feed. The test was also performed with a ball as the competitive object (OCC-test). The vixen who was in possession of the feed for at least twice as long as the other vixen, was classified as dominant and the partner as subordinate. All the

vixens were exposed to an acute stressor (open field test) with blood sampling before and after the test. Half of the vixens (30) were exposed to repeated stressors, i.e. the open field test was repeated 8 times for each animal, without blood sampling, however. Finally, all vixens were exposed to an ACTH-stressor. ACTH is injected to make the adrenal glands produce stress hormones. The reaction of the adrenal glands to the ACTH-stressor provides information on the general stress level of the animal.

Results showed that the FCC and OCC-tests could not in all cases determine the vixens' social status, and when it could be determined, it was not stable in the course of a 6-month period. Furthermore, there was not agreement between the FCC and the OCC-test in the determination of social status as only 50% of the pairs had the same score in the two tests. There was no significant effect of social status on the measured behavioural and physiological parameters. Subordinate vixens, however, tended to be more passive in the tests than dominant vixens. Repeated stress affected the vixens but did not result in long-term stressed vixens, which was expected. Repeatedly stressed vixens showed signs of a passive tolerance to handling and the results of the physiological measurements indicated that they were less stressed on a daily basis compared with the acutely stressed vixens.

These results stressed that social status in randomly chosen fox vixens cannot be determined fairly on the basis of a single or a few tests as the dominance relationship among the vixens can change in a short time, and as there were no standardised results when the competition object was food and object. The non-significant results in behaviour tests and in physiological objectives seem to be due to the unstable dominance relationship. On the other hand, the results show that the daily farm routines were of greater importance to the welfare of the foxes than their mutual social status.

Project 2: In her thesis, Lise Overgaard continued to work with the above-mentioned dominance-tested vixens in the heat and breeding period (*Overgaard, 1997*). Each pair was placed in two adjacent cages, each with access to a shelter. The shelters were placed with the largest possible distance from eachother, and shields were put up to hide other neighbours so that only the two who were used to being together could see each other. During the heat measurements, the catching reaction of the vixens was observed. Furthermore, 3 behaviour tests and daily scanning observations to establish the use of shelter from birth and four weeks ahead were carried out during pregnancy. Cubs were counted often to register mortality, sex was determined and they were weighed at the age of 4 and 8 weeks.

The results indicated that the repeated stress to which half of the vixens had been exposed before the breeding period had caused a tolerance to farm routines for some vixens but also symptoms of a chronic state of stress in other vixens. Vixens who had been exposed to repeated stress bore and weaned fewer cubs compared with vixens exposed to only a single stressor (not significant), but the overall picture of the results did not show any difference in welfare between acutely stressed and repeatedly stressed vixens. Dominant vixens were generally calm (curious) in the various behaviour tests before and during pregnancy, while subordinate vixens often showed signs of fear or were passive. Dominance status did not affect reproductive success clearly, but on average dominant vixens bore and weaned a few more cubs than subordinate vixens (not significant). This result suggests that the dominant vixens experienced a slightly better welfare than the subordinate vixens.

The conclusion of the project was that there was only a small effect of dominance status and various earlier stress on the later reproduction success of a vixen. It seems possible, however, to improve the welfare of some vixens by accustoming them to farm routines (repeated stress), and to improve reproduction of foxes if status and behaviour are considered in the selection process.

Project 3: The third project is being carried out for the Faculty of Science, University of Copenhagen in 1997 and 1998. The objective of this project is to illustrate the occurrence of helpers and their social role in the wild population of foxes in Denmark, and to obtain a detailed knowledge of the social behaviour and reproduction of vixens held in groups through experimental tests. This article solely describes the preliminary results of the experimental part of the project.

Eleven groups were placed in separate aviaries of 28.8 m^2. The aviary was furnished with various resting platforms and several whole-year shelters where the foxes could spend their time. The first year the group consisted of three unrelated adults (1

male and 2 vixens), where the vixens were of different colour types for the sake of identification. The foxes were put together in January and observations of behaviour and interactions of the match were registered. Weekly tests and scannings of the use of aviary and equipment as well as of social behaviour have been performed concurrently with registration of births and possible infanticidal behaviour. To sum it up, the litters and the role of the adults in relation to the cubs were registered and followed until the cubs were 16 weeks old.

Only in two of the groups did both vixens have cubs that all survived. In both cases, the cubs were born with a few days' interval in different shelters, and after three days the vixens brought the cubs together in one shelter. Hereafter only one vixen suckled the cubs while the other vixen stopped producing milk. Later this vixen participated in the care of the cubs on equal terms with the other vixen. In these two groups, the dominant vixen adopted the litter of the subordinate vixen in one of the cases, and in the other case vice versa: the subordinate adopted the litter of the dominant vixen. Another group had two litters, but the subordinate vixen who had cubs first ate or killed her cubs 3 days before the dominant vixen whelped. All the cubs of the dominant vixen survived, and the subordinate vixen participated actively in the care of the cubs. In two groups only the dominant vixen had cubs, and the subordinate vixen was treated very aggressively and was kept away from the cubs and the shelter. In four of the groups, only the subordinate vixen had cubs and all the cubs were either killed or eaten, and in the last two groups there were no cubs. In all cases, the male played a minor role in relation to the cubs, but he was tolerant towards them and often brought them food when they left the shelter.

So it was a somewhat confusing picture emerging from the groups. It could not be established whether it was the lack of heat period or mating, sterile vixens, or absorbed foetuses causing the lack of litters in several of the vixens, but the high occurrence of infanticide and the fact that most often it was the subordinate vixen performing it, support Bakken's (1993) results: there is a social connection between status and infanticide in farmed silver foxes, also when they are housed together and physical contact is possible. It should be stressed, however, that it was possible for both vixens in a group to have cubs who all survived. If the reason could be established why exactly those vixens could live together and reproduce in harmony, we might have discovered a tool which could help to reduce socially conditioned infanticide on fox farms.

Summary

Recent years' behavioural research stresses the importance of a positive man-animal relationship both with blue foxes and silver foxes. Handling routines are, however, so time consuming that it could hardly be implemented on large fox farms. But if behaviour is taken into consideration in the selection of vixens, and the farmer keeps a trusting instead of a fearful vixen, the level of fear in the foxes will be reduced in the long run.

Infanticidal behaviour seems first of all to be caused by social mechanisms based on the natural ability of the species that individuals may act as helpers. This ability can probably not be eliminated by selection, but in some vixens it does not express itself even though they are housed together. Being unstable in the long run, it is difficult to work with social status on farm level. Curious and calm vixens probably have a higher status compared with fearful vixens, a fact that can be used in the selection process. But the effort of research should now be concentrated on the reason why vixens are able to reproduce successfully when housed together.

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This report is translated from DJF-Internal Report No. 94, 1997

Original Report

The Preference for Different Types of Floor in Silver Foxes *(Vulpes vulpes)* and Blue Foxes *(Alopex lagopus).*

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Abstract

The aim of the study was to test whether farmed foxes had a preference for a certain type of floor in the cage. A preference test was set up where 10 silver foxes and 10 blue foxes had a choice between solid floor and wire floor. Supplementary to this test, the effect of an early experience for either solid floor or wire floor was tested on 10 cubs of silver foxes and 10 cubs of blue foxes. Finally, it was tested whether 24 adult blue foxes had a preference for one of three types of wire mesh floor in the cage.

In the solid floor/wire floor test the silver foxes tended to rest on the solid floor while the blue foxes tended to rest on the wire floor. This difference is explained by the fact that blue foxes soil their solid plate with feces and urine, and warming up a cold and wet solid plate costs too much body heat. Therefore, choosing the wire floor is here more likely an avoidance of this soiled solid floor. The solid plate in the silver fox cages remained dry throughout the test, and therefore it was still the most protective against the weather. The cubs of both silver foxes and blue foxes chose the solid floor more often than the wire floor regardless of their early experience, probably due to small paws or not yet fully insulating fur. With age an effect of the early experience could be seen except for blue fox cubs born on solid floor. They chose the wire floor more often than expected, most likely due to the soiling of the solid plate as was seen for the adult blue foxes, and here it is also more correct to speak of an avoidance of the solid floor rather than a preference for the wire floor. Conclusively, it would seem to be an improvement of the barren environment, if silver foxes had access to a solid plate in half of the wire cage. Especially the silver fox cubs seemed to use this equipment very much, and the adult silver foxes tended to prefer to rest there, and they did not soil it with feces. Contrary to this, the improving effect of a solid plate in the cage could not be seen for the blue foxes, due to the soiling.

In the test with three different types of wire mesh no preference could be revealed. A significant individual preference was found, and it is suggested that the choice of resting place is more dependent on the resting place of a dominant neighbour than on the type of wire mesh. As a conclusion, the postulation that it is painful and unpleasant for the fox to walk on wire floor could not be verified.

Introduction

The increasing efficiency of today's husbandry has among other things caused more intensive housing conditions for our domestic animals. This intensification has often resulted in more barren cages. In today's welfare debate one of the main issues is therefore to what extent these barren environments reduce the welfare of the animal. If the welfare is threatened as a result of the barren cages, one way to improve it is to enrich the environment for the animal. But in order to avoid that useless "enrichments" are being forced on the animal merely to satisfy the human conscience rather than actually improving the welfare, it is of vital

importance that thorough research goes ahead of any decision on changing the housing conditions. Within fur farming such thorough research has been going on for years in order to improve the housing conditions for fur animals. In 1991 V. Pedersen & L. L. Jeppesen tested the effect of a whole year nest box in the cage. They showed that silver foxes with access to a whole year nest box were less fearful and less stressed than those without, and that enrichments in the cages such as a shelf or a whole year nest box are well used by the foxes, if present in the cage (Pedersen, V. & Jeppesen, L., L., 1993). Today, it is recommended in the legislation (*Danish Vet. Council, 1997*) that all cages are provided with an observational platform e.g. a shelf.

In the general public it is often heard that swimming is a behavioural need for mink (*Mustela vison*) and therefore ranch mink should have access to swimming water in the cages. If swimming is a behavioural need, deprivation of swimming would lead to an increased level of stress. And since reproductive success and stereotype behaviour can be used as indicators of stress, the effect of access to swimming water was tested on both the reproduction (*Skovgaard et al., 1997a)* and the behaviour (*Skovgaard et al., 1997b)* of ranch mink. But neither of these two parameters revealed a lower level of stress in those mink who had access to swimming water compared to those who did not, and in the light of these results it has not been possible to support the argument that mink have a behavioural need for swimming.

Another opinion often heard is that it must be both unpleasant and painful for farmed foxes to walk on the wire floor in their cages. The aim of this study was therefore to test whether or not farm foxes are averse to the wire floor and prefer a solid floor instead. To examine this two preference tests were arranged:

1) The preference for either solid floor or wire floor in the cage.
2) The preference for a certain type of wire mesh floor in the cage.

Material & methods

Solid floor/Wire floor
The material was 10 adult silver foxes and 10 adult blue foxes. All were born in traditional cages with wire floor. Each fox was placed in a double cage (measuring 2.0 x 1.2 x 0.75 m) where the floor in one half was ordinary hexagonal plastic covered wire while the floor in the other half was covered with a wooden plate. Feed and water was available in both halves of the double cage. The foxes were video recorded for 5 consecutive days and nights in 4 different recording periods, the first recording being immediately after they were put in the double cages and the fourth recording after 12 weeks. The video tapes were scanned every 15th minute and it was noted in what half of the cage the fox stayed and whether it was active or passive. This made a total of 4800 observations in each of the 4 recordings.

Thereafter the same test was carried out on 10 silver fox cubs and 10 blue fox cubs, five of each born in traditional cages with wire floor and the other five born in cages with only solid floor. These were video recorded for 5 consecutive days and nights in 7 different recording periods, the first recording when the cubs were 3 months old and the 7th recording when they were 7 months old. The video tapes were scanned every 15th minute and it was noted in what half the cub stayed and whether it was active or passive. This made a total of 2400 observations for every group of 5 cubs each of the 7 recordings.

Different types of wire floor
Three different types of wire mesh were tested:

1) plastic covered wire with hexagonal mesh,
2) stainless steel wire with 1 x 1 inch mesh,
3) electroplated wire with 1 x 1.5 inch mesh.

These types of wire were distributed equally among 24 double cages (measuring 2.0 x 1.2 x 0.75 m) so that each half of a double cage had different types of wire floor. This made 3 combinations: 8 double cages with plastic covered wire/stainless steel wire, 8 with stainless wire/ electroplated wire and 8 with plastic covered wire/electroplated wire. For every combination the wire floor in half of the double cages was the reverse of the other half. Feed and water was available in each half of the double cage.

Twenty-four male blue foxes were tested - all born in cages with plastic covered wire floor. They were kept in cages in the two outer rows of a 4-rowed fox house and the manual observations were made from the path between the second and third row in order to minimize the human influence. The foxes were observed in 3 periods over a span of 5 months, the first period being right after the fox was put in the cage. In each period the foxes were observed for 10 days, in 3 x 12 rounds

per day, the first being in the morning before feeding, and the other two at midday and in the afternoon both after feeding. This made a total of 25632 manual observations. For each observation it was noted in what half of the double cage the fox was and whether it was active or passive.

Finally, each fox was video recorded continuously for 24 hours and the video tapes were scanned every 5th minute. This made a total of 6912 scannings. For each scanning it was noted in what half of the double cage the fox was and whether it was active or passive.

Results

Solid floor/Wire floor

Adult silver foxes and blue foxes distributed themselves more or less evenly on solid floor and wire floor and there was no significant difference in the frequency of stays on the two types of floor ($p>0.05$). The silver foxes distributed themselves equally on both types of floor when they were active, but tended to have a higher frequency on solid floor than on wire floor while being passive (fig. 1).

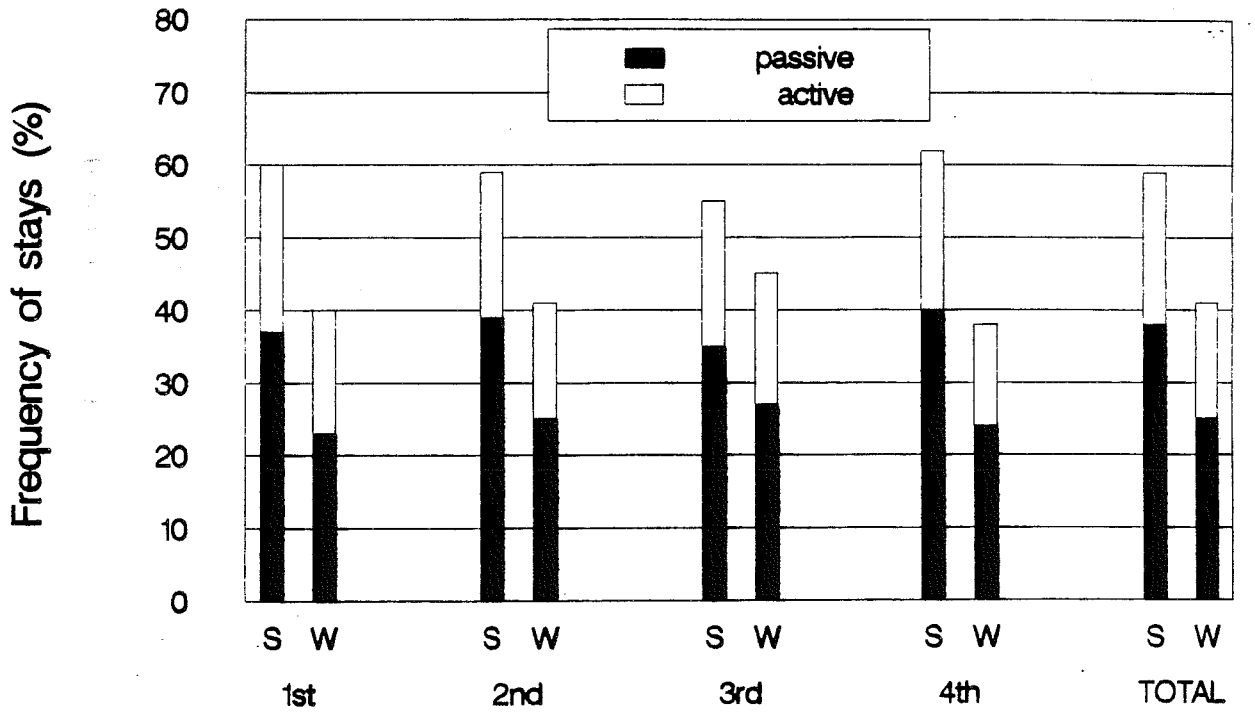
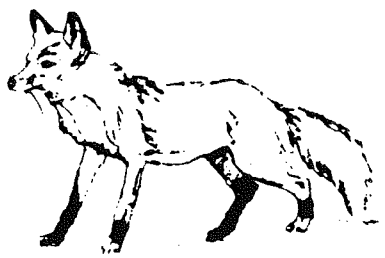


Figure 1. The relative distribution of the active and passive behaviour on solid floor (S) and wire floor (W) for adult silver foxes (N=10). The data are given in per cent for each of the four video-recordings (100 %=4800 observations), and as a total of the four (100 % = 19200 observations).



The blue foxes also distributed themselves equally on both types of floor when they were active, but contrary to the silver foxes they tended to have a higher frequency on wire floor than on solid floor while being passive (fig. 2). There was no significant difference in the frequency of stay between the 4 recordings ($p>0.05$).

Silver fox cubs were observed much more frequently on the solid floor than on the wire floor - regardless of whether they were born on solid floor or on wire floor (fig. 3a and b). For silver fox cubs born on wire floor the frequency of stays on the wire floor increased with age. Blue fox cubs born on solid floor were observed most frequently on the solid floor but with age they preferred the wire floor. Blue fox cubs born on wire floor had an equal distribution on the two types of floor while they were youngest but soon preferred the wire floor over the solid floor (fig. 4a and b).

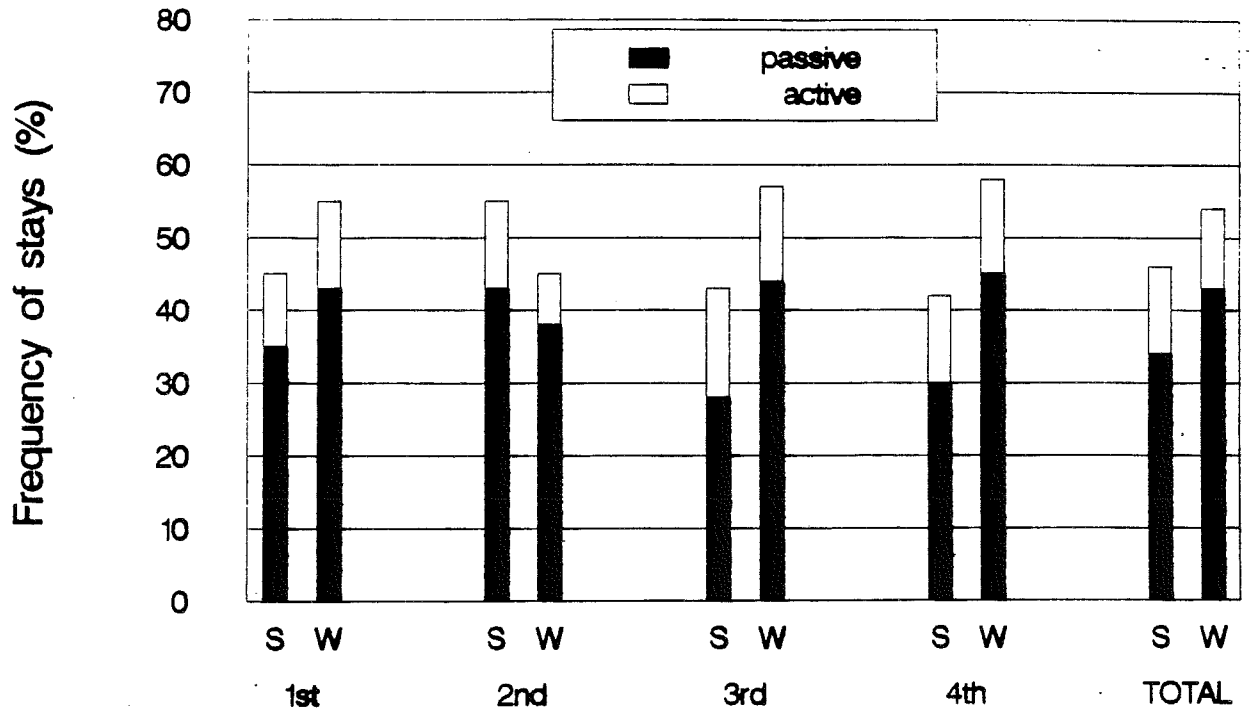
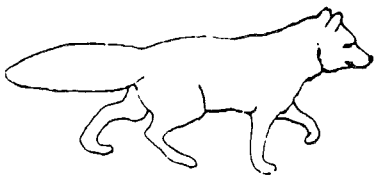


Figure 2. The relative distribution of the active and passive behaviour on solid floor (S) and on wire floor (W) for adult blue foxes (N=10). The data are given in per cent for each of the 4 video-recordings (100 % = 4800 observations) and as a total of the four (100 % = 19200 observations).



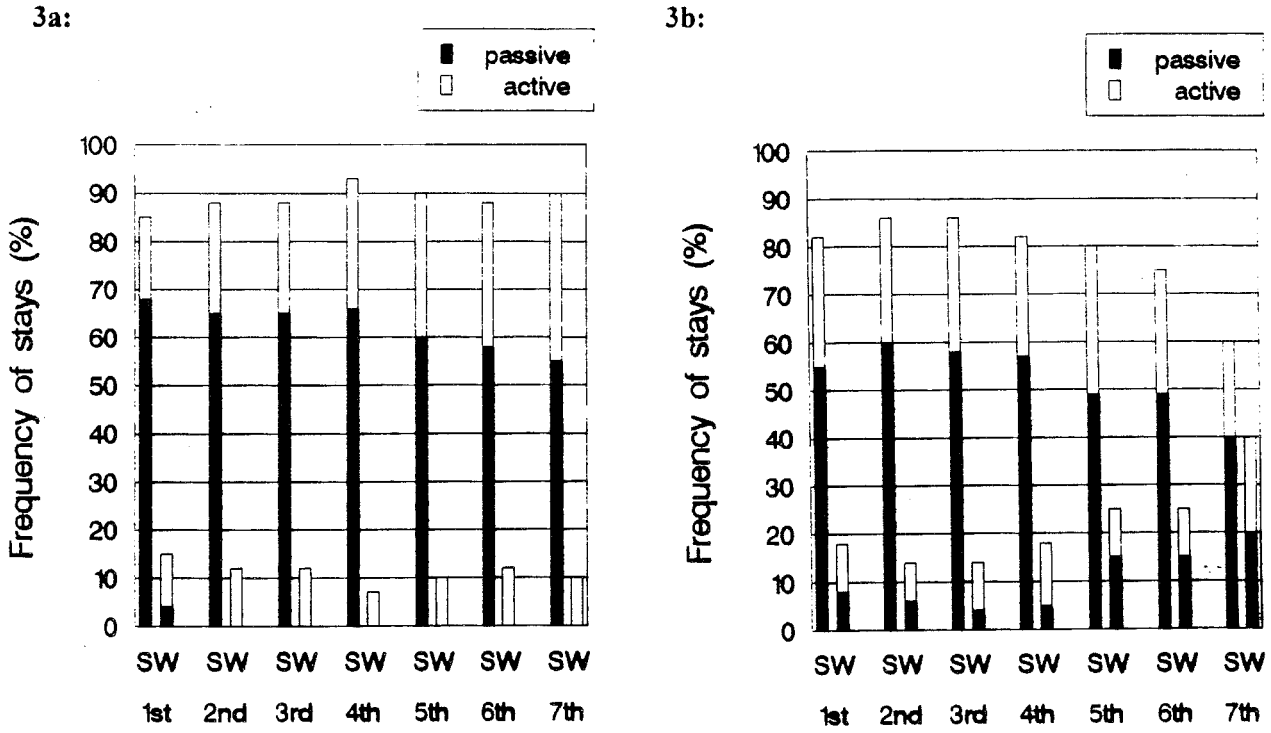


Figure 3a & b. The relative distribution of the active and passive behaviour on solid floor (S) and wire floor (W) for silver fox cubs born on solid floor (3a) and silver fox cubs born on wire floor (3b). The data are given in per cent for each of the seven times the cubs were video recorded. 100 % = 2400 observations for each recording and N=5 in each group.

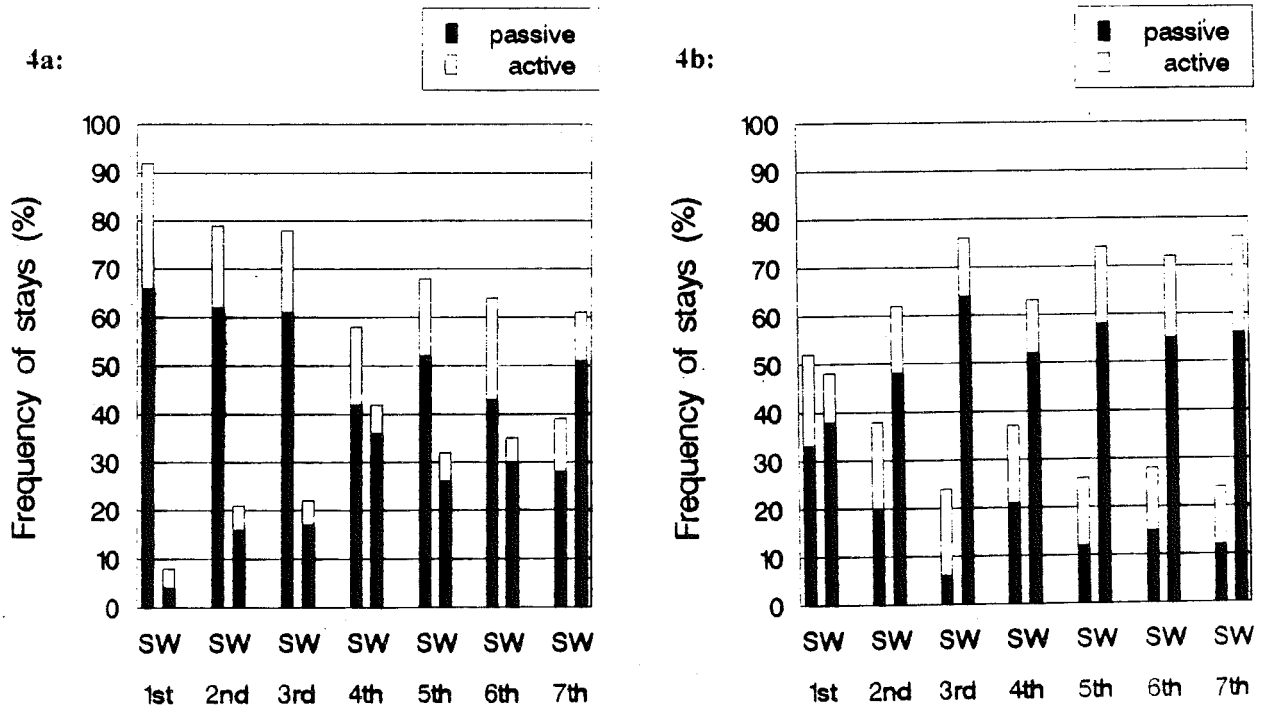


Figure 4a & b. The relative distribution of the active and passive behaviour solid floor (S) and wire floor (W) for blue fox cubs born on solid floor (4a) and blue fox cubs born on wire floor(4b). The data are given in per cent for each of the seven times the cubs were video recorded. 100 % = 2400 observations for each recording and N = 5 in each group.

Different types of wire floor

In the manual observations the highest frequency of stays was observed on the plastic covered wire and the lowest frequency on the electroplated wire (fig. 5).

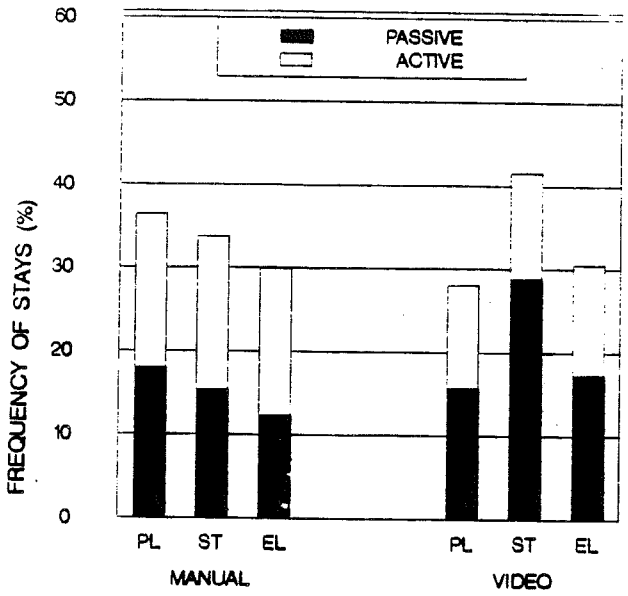


Figure 5. The relative distribution on the three types of wire mesh for both the manual observations and the video recordings. The data are given in per cent. 100 % = 25632 manual observations and 6912 scannings from the video recordings. 'PL' = plastic covered wire, 'ST' = stainless wire and 'EL' = electroplated wire.

There was no significant difference in the frequency of stays on the 3 types of wire mesh for the active behaviour ($p > 0.99$, Kruskal-Wallis). For the passive behaviour there was a significant preference for plastic covered wire over electroplated ($p<0.05$, Mann Whitney) but not for plastic covered wire over stainless wire nor between electroplated and stainless wire. However there was a significant difference in the individual preference for one of the three types of wire mesh for the passive behaviour ($p < 0.001$, X^2).

In the data from the video-recordings the highest frequency of stays was observed on the stainless steel wire and the lowest on the plastic covered wire, and while the foxes were equally active on the three types of wire ($p=0.87$, GLM), they showed a tendency to prefer the stainless steel wire while being passive ($p=0.08$, GLM). Within the passive behaviour there was a significant individual preference for a certain type of wire mesh ($p < 0.001$, X^2). And when comparing the significant individual preference for a certain type of wire in the manual observations and the video recordings, there was no general consequence in the animals' choice of wire type. Fifteen of the 24 animals preferred the same type of wire in the manual observations and in the video recordings.

Discussion

There was no clear preference for neither solid floor nor wire floor among both silver foxes and blue foxes. Silver foxes tended to prefer the solid floor while resting. The most immediate explanation to this is that the solid floor adds better protection against cold and wind than the wire floor, and since the test was carried out in the autumn this could explain why silver foxes seemed to use the solid floor more often than the wire floor when they were passive.

Contrary to the silver foxes, blue foxes seemed to choose the wire floor for resting. The explanation for this is most likely related to the defecation pattern of a blue fox. It differs from that of a silver fox in the way that blue foxes deposit a higher percent of their feces in nest boxes and on shelves than silver foxes do (*Pedersen & Jeppesen, 1992*). A likewise pattern was seen in this test, where the solid plate in most of the blue fox cages was covered with a thick layer of feces, despite a weekly cleaning. Korhonen (1987) suggests on the basis of a test with racoon dogs (*Nyctereutes procyonoides*) that warming up a cold and maybe wet/iced up plate costs body warmth, and therefore reduces the otherwise protective advantage of the solid floor. When analysing the results from preference tests it is worth bearing in mind that an animal's preference for a given choice may as well reflect an avoidance against the other choice represented to the animal (*Blom et al., 1992*). In the light of the more soiled and wet plates in the blue fox cages it would seem likely that they seeked the wire floor in order to avoid the solid floor rather than actually choosing the wire floor. The relatively long claws on a blue fox compared to a silver fox could influence on their choice of floor when walking. Pedersen (1990) has measured claw lengths on blue foxes of up to 4 cm.

The length of the claws could make it unpleasant for them to walk on the solid floor because the toes will be pressed upward when the claws rest on the solid plate. Vice versa the long claws could also make it unpleasant for them to walk on the wire floor because the claws might get stuck in the wire meshes. But the claws obviously did not seem to affect the blue foxes when walking since both silver foxes and blue foxes

distributed themselves evenly on the two types of floor while being active. In addition Pedersen (1990) also showed that access to a solid ground in the cage (nest box or shelf) did not have any wearing effect on the claws. So a solid plate do not seem to bother the blue fox when they are active, but neither does it prevent long claws.

It is well known that early experience affects the animals' relations to its environment later in life. It is important to bear that in mind when setting up a preference test, since the early experience that the animals in a test have had influences their choice in the test. Hughes (1976) showed with hens that early experience with a certain type of floor influenced the choice of floor given in a later preference test, so that hens reared on litter spent significantly more time on litter than those reared on wire floor. For silver fox cubs, the effect of an early experience did not appear in the results until they had grown up. Perhaps this delay was because the paws of the cubs are still so small that they step through the meshes in the wire, and therefore avoid the wire floor, despite an early experience. Another suggestion is that the fur of the cubs has not yet developed its fully insulating effect, and resting on the solid floor therefore was most profitable with respect to the heat loss of the body. An effect of an early experience was more clear in the blue fox cubs: cubs born on solid floor were hardly ever seen on the wire floor, while cubs born on wire floor chose the wire floor much more frequently. But all the blue fox cubs ended up choosing the wire floor over the solid floor - regardless of whether they were born on solid or wire floor. This might reflect the same pattern as with the adult blue foxes - namely that they soil the solid floor with feces and urine. In the light of this, it would be more accurate to say that the blue fox cubs avoid the solid floor rather than saying that they choose the wire floor, since it is expected that they too have small paws and therefore step through the meshes in the wire as the silver fox cubs do.

So, equipping the cages of the silver foxes with a solid plate on half of the wire floor could seem to be an improvement for silver fox cubs from weaning until pelting, since they seem to prefer the solid floor, and even avoid the wire floor. And because the adult silver foxes do not seem to bother, and because they do not soil the solid plate, it would be no problem to leave the solid plate in the cages even after pelting. On the other hand, it would not be considered an improvement to provide the blue foxes with a solid plate, since they obviously are averse to it due to the heavy soiling, and not even the weekly cleaning could keep the plate from getting more and more soiled with time.

The test with different types of wire floor revealed no significant preference for any of the three types, that were tested. The manual observations showed that the foxes chose the plastic covered wire a little more often than the other two types, while scannings from the video recordings showed that the stainless steel wire was chosen most frequently. This difference could indicate differences in the daily and nocturnal behaviour pattern since the manual observations took place during the day, while the video recordings were diurnal. But more likely the difference is due to the significant individual preference that was found in both the manual observations and in the video recordings. At first sight this could lead one to think that every single fox in the test had its own personal preference for a certain type of wire when resting. But with regard to the inconsequence in the individual preference between the two test sequences, it is more likely that the choice of resting place depends on other parameters than type of wire. For example, it may be purely fortutious - the fox picks its resting place where its latest activity had just ended. Dominance relations have been revealed for captive groups of blue foxes (*Korhonen et al., 1995*), and this fact may also be a parameter that affects the choice of resting place in this test. A subordinate animal may pick a resting place in the cage with respect to the resting place of its dominant neighbour, and if this neighbour has picked its resting place purely by chance this combination of the two parameters could explain the inconsequence in the significant individual preference in the manual observations and in the scannings.

Conclusion

For adult foxes there was no sign of any preference for a certain type of floor in the cage. In both tests the foxes distributed their active behaviour evenly on any given type of floor which indicates that it is not unpleasant for the fox to walk on wire floor - no matter which of the three types in question. Adult silver foxes tended to prefer to rest on solid floor while adult blue foxes tended to avoid the solid floor - most likely due to the soiling of the solid floor in the blue fox cage. Silver fox cubs preferred the solid floor over the wire floor - regardless of whether they were born on solid or wire floor, maybe because their paws were so small that they stepped through the wire mesh or because the

insulating effect of the fur was not yet fully developed. An effect of an early experience did not show until the silver cubs had grown up. An effect of an early experience in blue fox cubs was seen when the cubs were young but soon they all sought the wire floor - even those born on solid floor. This was interpreted as an avoidance of the solid floor rather than a preference for wire floor due to the soiling of the solid floor. As a conclusion, it is suggested that a solid plate in the cage could be an improvement for silver fox cubs, but not for blue fox cubs.

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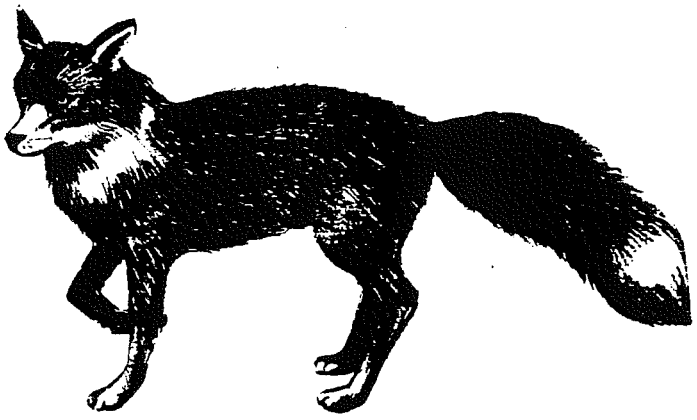

Original Report

Development of skin from mink (*Mustela vison*): From kits to adult animals

Bent Riis

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Summary

Skins from fourteen groups of minks, distributed in age groups spanning from two weeks to six months old adult animals, were tested. The following parameters were measured: weight, content of chemical extractable fat in non-dried skin, weight loss after drying and chemically extractable fat from dried skins. In short, the conclusion reached here is that the skin undergoes a remarkable developmental process. This could indicate that phase feeding small kits may be advantageous for obtaining a better skin quality in the adult animals.

Introduction

For the producers of mink and other fur-bearing animals, the skin is of immense importance because it is the sole income from the production. The skin is also the largest organ of any mammal and performs a large number of functions; it is the first defense against microorganisms, it prevents water loss and it contains the other body organs. The skin is a very complex structure, composed of many different molecules and atoms (*Riis, 1997*). Because the skin is of great value to the farmer, and because it is a complex biological structure, it is of importance to try to determine which parameters are important for the mink to develop a skin of high quality and large size. A better understanding of the developmental process may facilitate this goal.

This work was performed in order to characterize the major skin parameters (i.e. size, content of chemically extractable skin fat from non-dried skins, weight loss after drying and amount of chemically removable fat after drying of the skin). These parameters were measured in relation to development of the minks from two week old kits to mature pelting-ready animals at six months of age.

Materials and methods

The skin material was collected from Scanblack minks kept at the Experimental Fur Animal Farm situated at the Danish Institute of Agricultural Sciences, Research Centre Foulum, Denmark. The age groups were: 14, 21, 29, 40, and 45 days and 2, 2 ½, 3, 3 ½, 4, 4 ½, 5, 5 ½, and 6 months. Three animals from each group were tested. The animals were treated and fed according to the standard for the farm, until they were anaesthetized and killed. The minks were pelted shortly after the death of the animals, and the skins were stored frozen at -80^{0}C, until the described manipulations were performed.

Weight analyses of the skins were performed by weighing the raw, non-treated skins. Before performing further analyses all the skins were shaved in order to remove the under-fur and the guard hairs. The subcutaneous fat was removed using a scalpel to emulate the machine fleshing process. The control skins were fleshed using a fleshing machine. After

fleshing the skin was cut into smaller samples in order to be able to measure several parameters from the same skin. For drying samples of the skin the Experimental Fur Farm's drying facilities were used. All skin pieces were dried under production conditions for four days before performing the described experiments.

Two series of chemical fat removing experiments were performed: In one series non-dried skin samples were analyzed for "chemically removable skin embedded fat". The samples were transferred to a glass container, and at least 20 vol. of methanol (v/w) were added. This treatment was performed at a rocking table at room temperature for at least 60 min. The methanol was changed to a new batch and the treatment repeated. After this the samples were transferred to a new container, and at least 20 vol. of acetone (v/w) were added. The shaking was for more than 24 hours at room temperature. Before determining the weight loss, the samples were air dried over-night. In the other "drying and degreasing" experimental series, the skin samples were first dried as described above, and subsequently the weight loss was determined. Following the drying procedure the samples were chemically treated as described for removing the remaining fat using the above described procedure.

Results and discussion

The first experiments show that the skin weight stays constant for the first two weeks (age groups: 14 days, 21 day and 29 days; fig. 1). The reason for this is unknown, but an explanation may be that the small kits are putting energy and effort into building and developing other vital organs, than skin (i.e. internal organs, muscles and bones). After the kits are more than one month old, the skin data show a linear increasing curve until the animals are five months of age. After five months, the growth rate starts to slow down (fig. 1). Assuming linear growth between one and six months allows linear regression analysis of the data. This gives the following equation: $Y = -131.03 + 5.15\ X$. The coefficient of correlation is: 0.99. This shows that the skins are gaining 5.15 g of weight per day in the period. This reflects the expansion of the skin to keep up with the gain in body weight. When the animals reach maturity the rapid skin growth ceases, because the expansion of the mink body is slowed down.

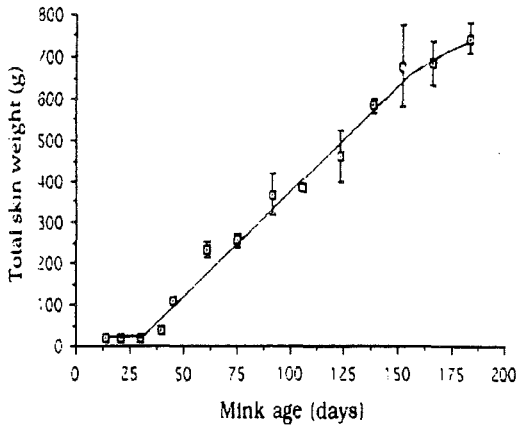

Figure 1. The figure shows the development in skin weight from mink kits two weeks of age till six month old adult animals. The standard deviation is shown.

After this analysis all hairs were removed from the skin samples for technical reasons. The hairs constituted on average 15.3% of the non-dried skin's weight with the coefficient of variation (CV%) being 11.4. Hairs from dried skins constitute 38.6% of the weight and CV% was found to be 4.3 (data not shown).

Another part of this work analyzed the weight loss in connection with chemical removal of the fat after drying of the skin. All skin samples were fleshed without the use of fleshing machines (apart from the control skins). This strategy was chosen because it was impossible to use a fleshing machine on the smallest of the mink kit skins. In order to compare the data directly, the older mink skins were also fleshed by hand, although this method is less efficient in removing fat (fig 4B).

The first part measured the content of chemically extractable fat from the fleshed mink skins without prior drying of the skin samples. The results showed that small kits contained a larger proportion of endogenously embedded fat compared to older animals (fig. 2). The reason is not known, but it is tempting to believe that small kits require more flexible skins than older nearly full grown minks do and therefore contain more fat in the skin. Fat storage as an energy reserve could also be part of the explanation.

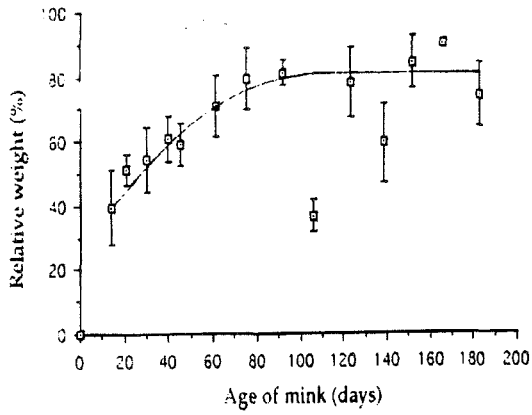

Figure 2. The figure shows the weight in percent of non-dried skins after the chemically extractable fat has been removed. The standard deviation is indicated.

The drying experiments showed that skin samples from the young kits lost relatively more weight compared to older animals indicating that young animals contain a relatively large proportion of volatile components (fig. 3.). Control samples of machine fleshed skin samples were dried under the same conditions and analyzed in order to estimate the relative effectiveness of hand fleshing versus machine fleshing.

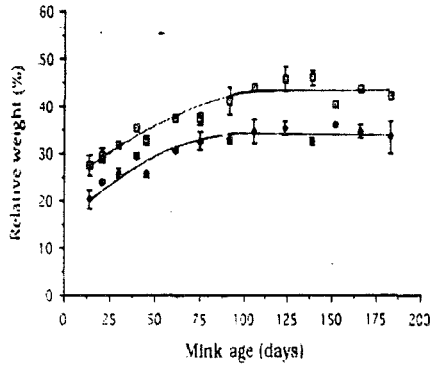

Figure 3. Relative development in skin weight after drying of the skin (open symbols) and after chemically degreasing the dried skin samples (closed symbols). The standard deviation is shown.

A statistically significant higher dried weight of machine fleshed adult skins was found when compared to the average of the dried weight of hand fleshed skins older than 75 days (fig. 4A). Not unexpectedly, this shows that machine fleshing is a more efficient way of removing subcutaneous fat compared to using a knife.

Finally, the dried skin samples were analyzed for chemically removable fat as described. The results showed that additional weight loss upon degreasing is approximately 7% for the small kits and 10% for adult animals (fig. 3). When comparing controls and hand fleshed samples from animals older than 75 days a statistically significant difference was found (fig. 4B). Again, this confirms that machine fleshing removes more subcutaneous fat compared to using a scalpel.

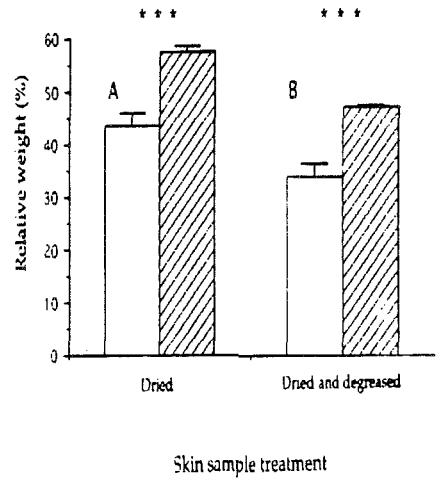

Figure 4. Relative values for average of all tested skin samples more than 75 days of age (open columns) and control machine fleshed skin samples (shaded columns). A is weight loss after drying of the skin samples and B is weight loss after degreasing of the dried skin samples. The standard deviation is shown.

These results shows that mink kit's skin physiology is different from adult animals and consequently, mink kits have special nutritional requirements. This may be part of the problems when the kits are re

moved from the dams. It is well known that the mink dam's milk contains an increasing proportion of fat compared to protein and hydrocarbons as the lactation period proceeds (*Glem-Hansen, 1985*). Part of this fat is probably embedded in the kit's skin. The reason for the relatively high amount of fat in the kit's skin is most likely to provide both a flexible skin and also to give the kit an energy reserve. Combined, this could indicate that phase feeding of mink kits may be beneficial for obtaining a better pelt quality. However, previous experiments have shown that phase feeding of minks may result in a lesser pelt quality (*Hejlesen et al., 1997*). It is also well known that using high amounts of fat as an energy source may create problems (*Børsting and Clausen, 1997; and references herein*).

Conclusions

The basic skin data clearly reveal that the mink kit's skin undergoes a developmental process which changes the composition of the skin from young to adult animals. This change was observed for all parameters investigated. Therefore, mink kit skin is physically and chemically different from adult skin. The results presented here indicate that phase feeding of small mink kits should be tested as a way of obtaining a better skin quality and size. However, this should be tested by practical experiments, before a firm conclusion is reached.

Acknowledgements

I thank Mr. Anders E. Østergaard of the Danish Institute of Agricultural Sciences, Research Centre Foulum, Denmark, for expert technical help and Dr. S. Michaelsen who collected the skins.

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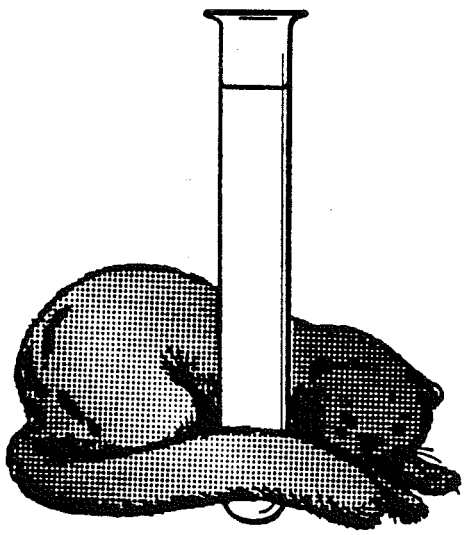


Development of the mammary glands in 1 year old mink females subjected to restricted or ad libitum feeding during the autumn, winter and gestation period

Steen H. Møller & Martin Tang Sørensen

Two groups of 200 standard female kits were fed to maintain the same weight from 1/9 to 25/2 or ad libitum from 1/9 to 1/12 followed by restricted feeding in order to reach the same weight by February 25th as they had on September 1st. From each group, 100 females were fed ad libitum and 100 females were fed restrictedly during the gestation period. From each of the four groups, 5 females with litters of 5-9 kits were killed on May 5th and June 10th, respectively. The number, size, height and weight of each active and passive gland were measured. The glands were fully active on May 5th, five days post partum in all four groups. The total gland area per female was 21.0 cm^2 and the total gland weight was 19.5 g. On June 10th, 42 days post partum most glands were still active. The total gland area per female was 24.3 cm^2 and the total gland weight was 20.5 g. There was no effect of feeding regime during autumn and winter on gland weight, neither on May 5th nor on June 10th. The feed allowance during gestation did not affect the gland weight on May 5th while the average gland weight on June 10th was reduced by 25% in the groups on restricted feed allowance during gestation (Table 1).

Table 1. Weight of mammary glands in mink females fed ad libitum or restrictedly from September 1st to December 1st or during gestation in April (lsmeans and standard deviation).

Sampling date	**Feeding regime 3/9 to 1/12**			
	ad lib.	restricted	sd	p
5/5	19.1	19.8	4.1	ns
10/6	21.5	19.5	9.9	ns
	Feeding regime during gestation			
5/5	19.8	19.0	4.4	ns
10/6	23.5	17.5	9.9	0.08

The reduction in gland tissue indicates that a feed restriction during gestation reduces the length of the lactation period. No difference was found in kit weight at 42 days post partum, indicating that the amount of gland tissue did not decline until shortly before the samples were taken. At this age, the kits have been eating for 2 weeks and are beginning to drink from the watering system and a decline in milk production may, therefore, not affect the weight significantly.

Technical Year Report 1997, pp. 89-92. 2 tables, 4 refs. In DANH. Author's summary.

Investigations on keratins extracted from mink hairs

Bent Riis

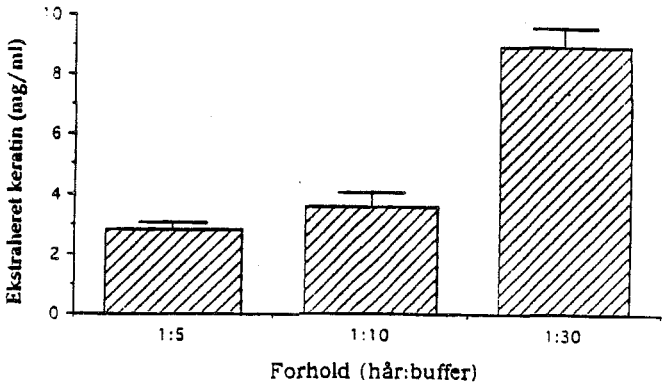


Fig. 2. The extracted amount of keratin depends on the buffer amount used to extract the keratins from the hair. It can be seen that the ratio 1 g mink hair to 30 ml buffer is optimal.

Introduction

Mink pelts are expensive quality products. The quality of the hairs are crucial for obtaining high sales prices during the auction. Therefore, mink producers have an interest in producing hairs of high quality and free from defects. Unfortunately, hair defects are quite common in some types of mink. 90-95% of the weight of a dry hair is accounted for by "hard" keratins. The "hard keratins" are structural proteins from the "intermediary filament" family and approximately 20 different species have been described. All "hard" keratins are very difficult to dissolve for biochemical analysis. This has created a lot of problems in analyzing both normal and defect mink hairs.

Materials and methods

The hairs were cut from dried mink skins obtained from the Experimental Fur Animal Farm situated at the research Centre Foulum. The hairs were washed in water and degreased in two batches of petroleum benzine (b.p. 40-60°C) followed by two batches of

acetone. The keratines were extracted in four different buffers: Buffer A (8 M Urea; 50 mM Tris/HCl pH: 8,0 og 0,1 M -merkaptoetanol), buffer B (8 M Urea; og 0,1 M -merkaptoetanol), buffer C (8 M Urea; pH: 10,0 adjusted with NaOH and 0,1 M -merkaptoetanol) og buffer D (8 M Urea; 50 mM Tris/HCl pH: 9,3; 1 mM EDTA and 20 mM -merkaptoetanol). The protein content was estimated using the Bradford method and S-carboxymethylation was performed as standard. Capillary zone electrophoresis (CZE) was performed using an Applied Biosystems 270-A HT system (Perkin Elmer, USA). The capillary was 50 cm long and the detection was at 280 mn.

Results and conclusions

The experiments described in this paper disclosed that mink hair keratins were extracted and dissolved using conditions similar to methods previously used for sheep hair keratins. Extraction methods efficient for human and horse hair were not found to be useful for extracting keratins from mink hairs. One single factor heavily influencing the solubility was the pH in the extraction buffer. Another result was that S-carboxymethylation of the mink hair keratins influences the behavior when analyzed using scanning spectrophotometric or Capillary Zone Electrophoretic methods. The main conclusion from these experiments is that it will be very difficult to develop good standard extraction methods which will allow direct comparison of different samples from different species. Therefore, caution must be exercised when comparing results from different laboratories especially when these results deal with different species.

Technical Year Report 1997, pp. 115-121. 5 figs, 7 refs. In DANH. Author's summary.

Colour measurement applied to understand visual judgment of colour shades in scanbrown mink pelts

Palle Vistisen Rasmussen

Abstract

Microspectrophotometric methods were used to provide objective correlates to the visually judged colour shade of the underfur in scanbrown mink pelts.

The study included 21 scanbrown mink pelts (winter coat), representing a larger group of 87 mink pelts. Based on visual evaluations, the 87 pelts were subjectively graded by integer values from "1" (blue-greyish) to "5" (reddish) primarily in respect of the colour shade (CS) of the underfur seen on the edge of the pelt. The evaluation was done across the visual impression of lightness of the fur surface (the belly side). Prepared hair samples of underfur fibres visually became darker in proximal-distal direction and were consequently examined at three levels above the leather surface. Reflection curves were obtained from bundles of underfur fibres, and the hue (dominant wavelength) of the underfur was determined. Based on reflection curves the X Y Z tristimulus values and subsequently the CIE Lab values of L* (lightness), and the chromaticity coordinates a* (red colour direction), and b* (yellow colour direction) were calculated.

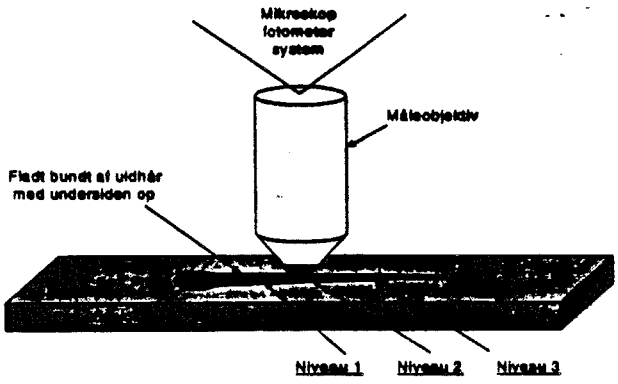


Fig. 1. The colours of medicated wool can be measured precisely by means of a microscope-photometer.

The visual blue-greyish colour shade was negatively correlated to the visual lightness of the fur in both groups ($r = -0.68$; $P = 0.0001$; $N = 87$ and $r = -0.75$; $P = 0.0001$; $N = 21$, respectively). The means of the hues were 589.40 nm, 588.00 nm and 586.70 nm, respectively, indicating that the variation between levels was small. Based on mean values of L* it was documented that the underfur fibres became darker in proximal – distal direction. The ranges of L*, a* and b* across colour shades and levels were 27.7 – 56.3, 2.2 – 5.4 and 4.0 – 10.7, respectively. Based on mean values of L*, a* and b* the predicted value of the colour shade (CSp) was estimated by the following polynomial regression: $CSp = -33.16 + 1.56L^* - 0.018L^{*2} + 0.12a^* + 0.56b^*$, ($r^2 = 0.58$; $P = 0.005$). The partial regression coefficient of a* was not significant.

The model for the scanbrown type demonstrated that the visual colour shade of the underfur was correlated

primarily to the lightness (L*) and to the yellow chromaticity coordinate (b*) of the underfur samples. The investigation showed that underfur fibres with a (visually) blue-greyish colour shade were relatively dark and relatively less yellow, and that (visually) very reddish underfur fibres were not the lightest coloured. So the model improves the colourimetrical understanding of visual colour shade and may eventually standardize and thus improve the subjective grading of this property. Further, the photometric methods and the model may be used to establish discriminating threshold values in grading and selection of (reference) pelts and probably also of animals.

Technical Year Report 1997, pp. 123-129. 1 table, 2 figs, 10 refs. In DANH. Author's summary.

Fur chewing behaviour and reaction to handling and straw in mink belonging to breeding lines with different occurrence of fur chewing

Jens Malmkvist

This study focuses on fur chewing (removal of hairs without penetration of skin) in farm mink, by examining in detail the behaviour of chewers during day and night (part 1), and by investigating the effects of repeated stress and of periods with alternating straw availability (part 2). The animal material consisted of mink from breeding lines successfully selected for and against fur chewing (2 x 12 males) and a control group of unselected mink (12 males). Males from all groups were housed with an unselected female in pairs.

Part 1: 24-hour video recordings of each individual in cage and nest box.
The behavioural analysis included all kinds of fur chewing (self, female), other kinds of oral manipulations, interactions with the female, scratching, drinking behaviour and passivity. Recordings of fur chewing mink revealed a marked diurnal rhythm in the behavioural categories (chewing of own fur, interaction with female) primarily responsible for the observed fur chewing, with most occurrences around sunrise and sunset.

Part 2: Blood sampling after repeated stress and periods of alternating straw availability.
Handling (3 x 15 minutes daily in a trap for 6 days) resulted in a significant increase in the total number of plasma leukocytes in comparison with the non-handled control group. Evaluated on the basis of changes in eosinofiles and total number of leukocytes, mink selected for fur chewing did not show increased sensitivity to the stress of handling compared with mink selected against fur chewing. Furthermore, cell populations of the blood did not change in connection with 7-day periods with and without straw, indicating that fur chewing males did not have a reduced tolerance to straw. That is, no sign of hypersensitivity towards straw (incl. mites within) were found. Besides, the typical occurrence of fur chewing is done systematically on smaller parts of the body, and given that the second-most frequent type of fur chewing is done on another individual (neck chewing), makes allergy unlikely as the main cause of fur chewing in mink.

This study, therefore, cannot confirm the hypotheses that stress sensitivity or allergy to straw are primary causal factors in fur chewing. On the basis of behavioural observations, fur chewing of the cage mate was not a result of aggression in connection with fights for (obvious) resources (e.g. access to feed, water, sleeping place). The concurrence of the act of fur chewing and periods of high activity would suggest that fur chewing occurs as a part of a naturally governed circadian rhythm when the animal cannot use other possibilities of activity. It can therefore be speculated that understimulation could play a role in the execution of systematic chewing.

Technical Year Report 1997, pp. 139-147. 2 tables, 2 figs., 17 refs. In DANH. Author's summary.

Housing juvenile blue foxes in pairs or singly: Effects on behaviour, growth and later reproduction

Vivi Pedersen

Two groups were formed at weaning with 66 female blue fox cubs in each group. One group was housed singly (solitary rearing, SR) in standard cages (1m x 1.2 m x 0.75 m) (w x d x h) with a wooden platform. The other group was housed in sibling pairs (pairwise rearing, PR) in similar cages with one wooden platform. Siblings in the PR group had two siblings in the SR group to diminish genetic factors. At the normal pelting time in December, 32 individuals (litterwise, 2 from SR, 2 from PR) were eliminated from the experiment and thus 50 in each group continued in the experiment for breeding purposes.

From January all foxes were kept singly. When mating were completed each female was given access to a traditional nest box in the adjoining cage.

The PR- and SR-foxes were exposed to 6 titbit tests during autumn. It was registered if the fox took the titbit within 15 seconds, the latency to the titbit and the fox's position in the cage in the 15th second. In the SR-group 45% of the foxes took the titbit and 50% of the PR-foxes did so ($p>0.5$). No significant differences were found between the SR- and PR-group in any of the other measured parameters related to the titbit test ($p>0.5$) either. An object test was performed 14 days after the last titbit test. The object was a red plastic ball with a diameter of 20 cm. It was placed just within the cage door, and the observer withdrew 1 m. The latency to have tactile contact with the object within 30 seconds was registered. After each trial the object was desinfected in a 10% dilution of Rodalon. In the SR-group 68% of the foxes had tactile contact with the object. This difference was not significant ($p>0.5$). No significant differences were found as regards the other measured parameters in the object test either.

Table 1. Growth and weight loss in 100 female blue foxes kept either singly (SR) or pairwise (PR) during rearing. Means of each group in grams ± the standard deviation is shown

	GROWTH	WEIGHT LOSS
PR-group	5950.4±1209.1	2068.7±1009.8
SR-group	6586.5±1310.7	2815.2±1344.9

All foxes were weighed individually at 10 weeks of age, at 6 months and just prior to the first mating. Initially (at 10 weeks of age), no difference in weight was found between SR- and PR-foxes ($p>0.5$), but at 6 months of age, the SR-group showed a higher body weight than the PR-group ($p<0.01$). Prior to the first mating this difference in body weights between SR and PR-foxes was eliminated ($p>0.5$). The growth of SR-foxes from weaning to the pelting season was higher than in PR-foxes ($p<0.01$), and the weight loss from the pelting season to mating time was higher in SR-foxes than in PR-foxes ($p<0.005$, see table 1).

Eight of the blue foxes were not mated due to lack of or vague heat signs (3=6% in the PR-group, 5=10% in the SR-group). The PR-group had no barren females whereas the SR-group had 6 (13%) barren females. This difference in number of barren females was significant ($p<0.05$). In total, there were more non-reproducing females in the SR-group than in the PR-group ($p<0.05$). The reproductive success expressed as number of cubs born and weaned to mated and pregnant females did not differ significantly between the SR- and PR-group ($p>0.5$).

The study concluded that since no differences between solitary reared and pairwise reared female blue foxes were found in the behavioural parameters and in reproductive success it is premature to state whether welfare was better or worse in either of the groups. More detailed behavioural studies including physiology would give us more information regarding this aspect. However, since a higher proportion of females in the solitary reared group did not reproduce, it seems to be a benefit on long term to keep blue foxes in pairs during rearing, especially if the feeding schedule is controlled carefully.

Technical Year Report 1997, pp. 149-158. 5 tables, 4 figs, 5 refs. In DANH. Author's summary.

Use of IsoPrime for characterisation of protein composition in mink milk

Tove N. Clausen, Kirsten Mortensen, Hilmer Sørensen, Jens Christian Sørensen, Susanne Sørensen

In the present study, focus has been placed on separation and characterisation of mink milk proteins in order to provide information about the importance of the mink milk composition in relation to optimal development of mink kits and for studies of the problems concerning "sticky" kits. Milk samples were collected in connection with feeding trials comprising two groups (5 mink per group) of standard mink fed a diet high in protein and a diet high in carbohydrate, respectively, and one group of wild mink (7 mink) fed a diet high in protein content. Of the wild mink four had litters with "sticky" kits. The milk samples were extracted by supercritical fluid extraction (SFE). SFE has been shown to be an effective method for separation of relatively small sample volumes of mink milk into groups of lipids and protein+carbohydrates including other hydrophilic compounds. The residual after lipid extraction was separated using a new IsoPrime (Pharmacia)

technique which involves quantitative isoelectric focusing using compartments separated by membranes containing immobilised ampholines for generation of different pH area compartments. The fractions obtained from IsoPrime were further analysed in micellar capillary electrophoresis, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing thereby offering the possibility for determination of individual compounds in the mink milk. The dry matter content and composition with respect to lipid content and protein+carbohydrate content did not seem to be influenced by the differences in diets given to the two groups of standard mink. Three different milk samples were fractionated using IsoPrime, distributed with one sample from the group of mink fed high protein diet, and two samples from the group of wild mink, with the samples taken from mink with and without "sticky" kits, respectively.

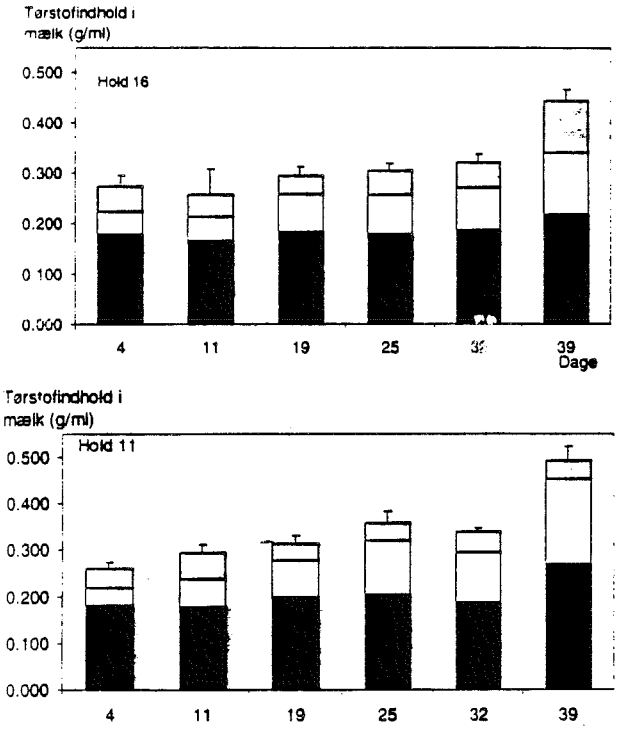


Fig. 2. The dry matter content of mink milk in two groups of standard mink (group 16 (top) and group 11 (bottom)) having received different feed in the lactation period. Mink milk from 5 females from each group was collected on the days stated during the lactation period. The total dry matter content in the milk is indicated with standard deviations. The fat content (white marking) and protein+carbohydrates content (black marking) have been found gravimetrically according to SFE, while the residual fraction (light grey marking) has been stated as the difference between the total dry matter content and the two SFE fractions.

The three analysed samples showed similar protein compositions, where the major part of the proteins was distributed with pI values between 4.7 and 5.9. SDS-PAGE showed that the quantitatively dominating proteins had molecular weights of 17-25 kD. However, SDS-PAGE also seemed to reveal small differences in the protein composition for the high pI value proteins between the different milk samples, but this has to be further investigated. In conclusion, IsoPrime seems to be a promising technique for group separation of both proteins and other milk constituents in order to allow possible identification of specific compounds present in mink milk.

Technical Year Report 1997, pp. 213-220. 4 tables, 4 figs, 10 refs. In DANH. Author's summary.

Variations of fat content in mink milk analysed by Supercritical Fluid Extraction (SFE)

Charlotte Bjergegaard, Tove Clausen, Kirsten Mortensen, Hilmer Sørensen

The energy content of milk increases from birth to weaning, with mink milk having a higher content of fat than e.g. cow milk and human milk. The composition of the fat fraction in mink milk is related to certain feed-related, hereditary, and environmental factors and may be of importance in relation to the problem concerning "sticky" kits. In the present study, supercritical fluid extraction (SFE) (Buskov *et al.*, 1997) has been utilised in order to get a closer look of the fat fraction in milk samples from 2 groups of Standard mink (group 11 and 16; 5 mink/group) and 1 group of wild mink (group 5; 7 mink/group). The milk samples for group 11 and 16 was obtained at day 4, 11, 19, 25, 32; and 39 from birth, and at day 24-27 for group 5. Group 16 was given a diet high in carbohydrate and low in protein, whereas feed for group 11 and 5 had a high content of protein and a low content of carbohydrate. The results showed considerably higher fat content in milk from Standard mink compared to Wild mink and a slightly higher fat content in milk from group 11 compared to group 16. The fat content increased from birth to weaning, independent of the group. The fat included in the different diets varied only by a few percent and cannot explain the variation in milk composition obtained. No systematic variation was observed for fat content in milk samples from mink with and without "sticky" kits. The level of fat-soluble vitamins in the milk, investigated by UV-spectroscopy of the SFE extracts, showed considerable variation, however more sensitive methods

may change this result. In conclusion, SFE provides a promising starting point for a characterisation of the fat fraction and fat-soluble vitamins as well as other amphiphilic components in milk. The SFE technique is fast, effective, and applicable to even small amounts of starting material (50-100 mg), and a selective extraction of specific compounds are easily performed by the addition of small amounts of modifier (e.g. MeOH) to the extraction media (CO_2). Combined with group separation techniques this procedure thus provides an efficient tool for specific analyses of the extracted compounds.

Technical Year Report 1997, pp. 221-227. 1 table, 2 figs, 6 refs. In DANH. Author's summary.

Can mink mothers distinguish between own offspring and foster offspring in the litter?

Kith Skovgaard

It is a well known practice on mink farms to average out litters by moving cubs from big litters to mink mothers with small litters. If such mixed litters are to be used for breeding later it is important for the farmer to know which cubs originally came from what mothers. The question then is, whether not only the farmer, but also the mink mother can distinguish between her own biological offspring and her foster offspring. On the Research Farm North experience with pearl and pastel mink showed that they might, to some extent.

Ninety-three pastel cubs were distributed among 91 pearl litters and 94 pearl cubs among 89 pastel litters, so that 187 foster cubs were given to 180 mink mothers and so that each foster cub differed in colour from the biological offspring. All foster cubs were female and they were on average 5.8 days old when they were given to the mink mother. Weaning took place when the cubs were 8 weeks old.

Of the 187 foster cubs 17 were killed by the mink mother and of the 1197 biological cubs 12 were killed by the mother. This means that significantly more foster cubs were killed by the mother than biological cubs ($p << 0.0001$; X^2 two-sample test). Ninety percent of all killings took place when the cubs were 40-50 days old which means primarily in the 7th week. Of the 180 mink mothers, 18 killed one or several cubs, 7 were pearl mink and 11 were pastel mink. Of these 18 mothers, 10 killed only foster cubs, 4 only biological cubs while the remaining 4 killed both foster cubs and biological cubs. Of the 12 biological cubs that were killed 5 were males, 5 were females and for 2 cubs the sex was unknown. The litter size varied from 6-9 cubs for those mothers that killed one or several cubs.
The conclusion is that more foster cubs than biological cubs are killed by their mother. But whether it is due to a true recognition of the biological offspring based on imprinting or whether it is caused by some of the factors mentioned above (age, type, colour, litter size or sex) is difficult to conclude at the moment. In order to conclude anything specific about which parameters affect the mink mother it is necessary to test each of these parameters more thoroughly.

Technical Year Report 1997, pp. 229-232. 2 tables, 3 refs. In DANH. Author's summary.

Standard figures for fur animal manure. A model for calculation of feed consumption and N-input and output on a mink farm

Steen H. Møller

Increasing public concern about pollution has brought attention to the utilisation of wastes from animal production. The need to know the exact amount of plant nutrients in all steps from feed delivered at the farm to land application increases accordingly. In this paper the amount of nutrients in a typical Danish feed ration was calculated for a 'model' mink farm during a normal production year. The calculations were based on the analysis of weekly feed samples from all feed kitchens in the feed control programme, which covers almost all mink feed produced in Denmark. The average feed allowance per animal was calculated for each month on the basis of registrations from 59 farms with a total of 64,000 females. All data was from 1995.

The model farm had 1000 females, 5.22 kits per mated female and 8% barren females. The amount of feed delivered to the farm was 185 kg with 309 Mcal per female equal to 35.5 kg and 59.2 Mcal per skin produced. The nitrogen content in the feed is either excreted in urine or faeces or retained in the body as shown in Table 1.

Table 1. Nitrogen content in mink feed, feed waste, body, manure and urine from a Danish model farm with 1000 females and 5220 kits on July 1st. The amount of N excreted pr. female and pr. skin produced is also given.

N delivered in the feed	4898 kg
N in feed waste	392 kg
N ingested	4506 kg
N in body, skin, hair	310 kg
N excreted in urine and faeces	4196 kg
N in faeces	676 kg
N in urine	3520 kg
N in urine and faeces pr. female	4196 g
N in feed waste pr. female	392 g
N total pr. female	4588 g
N in urine and faeces pr. skin	804 g
N in feed waste pr. skin	75 g
N total pr. skin produced	879 g

Technical Year Report 1997, pp. 233-236. 3 tables, 5 refs. In DANH. Author's summary.

Stress physiological status and fur properties in farm mink placed in pairs or singly

Birthe M. Damgaard, Steffen W. Hansen

The effects of keeping farm mink in pairs or singly from weaning to pelting on haematological, clinical-chemical and stress physiological variables, on body and organ weight, and on fur properties were examined in 96 mink kits in order to assess the consequences on welfare and productivity. Mink kept in pairs had better pelt quality than mink kept singly. No significant difference was found in frequency of hair chewing between mink in pairs and single mink. Bite marks on the leatherside of the pelt occurred only in mink kept in pairs. No significant difference was found between the groups in body weight, and organ weight corrected for body weight. Based on the physiological variables applied, no differences in welfare were shown between mink in pairs and single mink. Sexual differences were found for the enzymes ASAT and ALAT, for number of leucocytes, and for adrenal glands relative to body weight.

Acta Agric. Scand., Sect. A, Animal Sci. 46, pp. 253-259, 1996. 5 tables, 24 refs. Authors' summary.

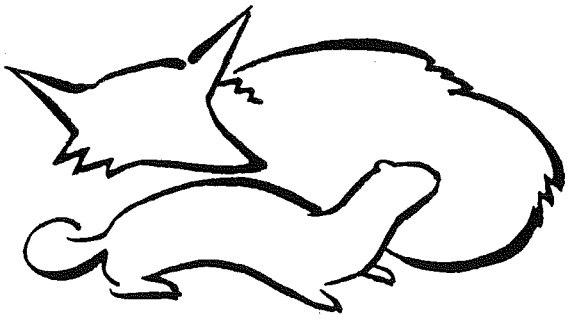
Effect of melatonine treatment in the development of fur in furbearing animal. A review.

Jozsef Lanszki, Krisztina Lengyel

Result of melatonine treatments and regulation of light have been summarised for carnivorous fur animals. As a result of the experiments carried out in the investigated animal species (Canadian mink, silver foxes, polar foxes and fox hybrids), the fur became mature 1 to 6 weeks earlier due to the treatment. The fur quality did not decrease in the adult animals, - however, the treatment of growing animals caused differences in the quality of fur (Scanblack mink, fox hybrids).

Longer than natural daylength delayed fur maturation while the shorter daylength accelerated it. Decrease in the intensity of lighting also accelerated the development of winter fur. In the case of simultaneous lighting and melatonine treatment, the role of melatonine treatment was the determinant.

Magyar Allatorvosok Lapja 51, 5, pp. 307-309, 1996. In HUNG, Su. ENGL. 1 fig., 18 refs. Authors' summary.



Selection for maternal traits. – Results of the first year of selection

Bente Krogh Hansen, Peer Berg, Niels Therkildsen, Ulla Lund Rasmussen

Preliminary results from the present selection experiment confirm that there are two ways to affect the early growth of kits by selection: to select for the kits own growth capacity or to select for maternal ability to induce growth on kits. In both cases the phenotypic effect on kit body weight at 4 weeks is similar.

The future plans include optimizing the model and description of genetic correlations. Furthermore the relation between different traits must be well defined before new traits are included in the breeding program.

Technical Year Report 1997, pp. 7-9. 2 tables, 2 refs. In DANH. Authors' conclusion.

Observation of heredity of fur colour in coloured mink

Manfred O. Lorek, Andrezej Gugolek, Bozena Gawarecka

The investigation was carried out on 80 female mink in the second year of reproduction. Half of them consisted of the standard variety mink (S), while the second half were white Hedlund variety (B). The females were divided into 4 sub-groups of 20 each and mated 3 times in two estrus periods alternatively with males of a different fur colour. The results were calculated on the basis of the number of litters obtained and distributed with regards to the fur colour of the kits as well as the litter size on the basis of the number of kits delivered. It has been shown that, irrespective of the mink mating performed, the majority of litters consisted of inter-variety crossbreds. The mean number of progeny in these litters was also higher. Moreover, the results obtained confirmed the fact that mating mink in two estrus periods is appropriate since it improves the effectiveness of fertilization expressed in the litter size.

Anim. Prod. Rev. (Poland). Appl. Science Reports 15, pp. 217-218 (poster), 1994. In POLH, Su. ENGL. 3 tables. Author's summary.

Selection for litter size, body weight, and pelt quality in mink (*Mustela vison*): Correlated responses

Gabrielle Lagerkvist, K. Johannson, N. Lundeheim

In a five-generation selection experiment, separate lines of standard mink (*Mustela vison*) were subjected to selection for litter size at 3 wk (F line), body weight in September (BS line), underfur density (P line), or combined selection for litter size and body weight (I line). One unselected line served as a control (C line). The present paper focuses on correlated responses to selection regarding fertility and fitness traits, fur quality, and body size traits. Genetic and environmental parameters were estimated with REML (Restricted Maximum Likelihood) techniques using a multi-trait, reduced animal model in a derivative-free way. Genetic and phenotypic correlations were estimated from four subsets of data consisting of 1) female September weight, litter size, and kit mortality; 2) body size traits; 3) September weight and fur traits graded on live mink; and 4) fur traits graded on live mink and skins. September weight was found to be negatively correlated with fertility and fitness traits as well as with fur traits. Selection for underfur density resulted in an improvement in guard hair quality and in general impression of the fur and almost eliminated the fur defect metallic sheen.

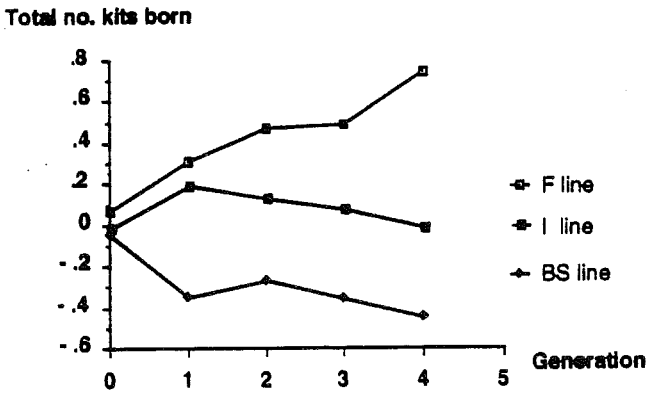


Figure 3. Genetic trends in litter size (total born) in F BS, and I lines (Data set 1).

Journal of Animal Science, Vol. 72 (5), pp. 1126-1137, 1994. 9 tables, 6 figs., 28 refs. Authors' abstract.

Economic profit from increased litter size, body weight and pelt quality in mink (*Mustela vison*)

Gabrielle Lagerkvist

Economic benefits of improving litter size, body weight (i.e. pelt length) and pelt quality were estimated in mink. Effects of the size of the litter in which the kit was born on pelt length and pelt quality were also assessed. The traits were recorded during a selection experiment involving the traits litter size, body weight and underfur density. Skins originating from litters of ≥10 kits tended to be smaller and sold at a lower price, compared with pelts from smaller litters. Litter size did not seem to affect fur quality. Pelt quality score decreased with increasing September weight. Pelts of animals with September weights of <2000 g and pelting weights of <2300 g had a lower sales price than pelts of heavier animals. Pelt quality had only a small effect on the price. The highest economic gain was achieved by increasing litter size. The net revenue from each extra kit per litter was, in Swedish Kronor, SEK 70, 122 and 170 at an average sales price of SEK 150, 200 and 250, respectively.

Acta Agric. Scand., Sect. A., Animal Sci. 47, pp. 57-63, 1997. 1 table, 6 figs., 13 refs. Author's summary.

Partial sequence of an expressed major histocompatibility complex gene (*DQA*) from Arctic fox (*Alopex lagopus*)

D.I. Våge, I. Olsaker, K. Rønningen, Ø. Lie

A 508 bp cDNA sequence of a major histocompatibility complex gene (*DQA*) from Arctic fox (*Alopex lagopus*) was isolated and sequenced.

The oligonucleotide primers used for specific amplification of the *DQA* locus were based on sequences found in conserved regions of *DQA* genes in man, mouse, and rabbit. The PCR product included a part of the alpha-1 domain (residues 12-87) and the alpha-2 domain (residues 88-180). Cysteine residues were identified at positions 110 and 166, and two glycosylation sites were identified at positions 81-83 and 121-123. The analysed sequence showed 96% nucleotide similarity to *DQA* in dog, 86% to that in man, 77% to that in the mouse, and 84% to that in the rabbit.

The sequence was also compared to the human alpha genes *DNA, DPA* and *DRA*, showing a nucleotide similarity below 70% in all comparisons. The results suggest that the amplified product is the *DQA* equivalent in Arctic fox.

Animal Biotechnology 5(1), pp. 65-68, 1994. 1 fig., 15 refs. Authors' abstract.

Colour variation of the fur of Japanese marten (*Martes melampus melampus* Wagner) in Japan

Tetsuji Hosoda, Kazuo Oshima

The nominotypical subspecies of Japanese marten, *Martes melampus melampus* Wagner, 1841 was once divided by Thomas (1905) into two subspecies, Kiten (*M. m. melampus*: type of yellow fur) and Susuten (*M. m. bedfordi*: type of brown fur) by the difference in the colour of the fur. In order to fully understand the distribution of the variations, an on-the-spot investigation and a survey through questionnaires were conducted in their distribution area – Honshu, Shikoku, and Kyushu. The results were entered in the blank map of Japan by prefecture (fig. 1).

The relation between these two subspecies was examined from the aspect of molecular genetics (rDNA-RFLP). Genetic differentiation in neither case was recognized. The variations in phenotypes of Japanese marten were the only difference before the detection limits on the nuclear DNA level.

Reprinted from NANKISEIBUTU: The Nanki Biological Society, Vol. 35, No. 1, pp. 19-23, 1993. In JAPN, Su. ENGL. 2 figs. + 2 colour photos of the 2 types. 10 refs. Authors' summary.

Comparison of microstructure of white winter fur and brown summer fur of some arctic mammals

John E. Russell, Renn Tumlison

Several species of Arctic mammals have brown hair in the summer and moult into a white pelage in the winter. It is unknown whether characteristics other than colour of the hair also change during the colour transition between seasons. We borrowed guard hair

samples from museums to represent summer and winter pelages of five species: *Alopex lagopus* (Arctic fox), *Lepus americanus* (snowshoe hare), *Lepus Arcticus* (Arctic hare), *Mustela erminea* (ermine) and *Mustela nivalis* (least weasel). Micro-structural differences exist between the brown and white hairs. In general, white winter hairs had larger upper shaft medullas comprising more air-filled cells and smaller lower shafts. These structural changes may function in conservation of heat or in increasing light reflection to whiten the fur and aid as camouflage.

Acta Zoologica (Stockholm), Vol. 77, No. 4, pp. 279-282, 1996. 1 table, 15 refs. 1 appendix. Authors' summary.

Phylogenetic relationships within *Martes* based on nuclear ribosomal DNA and mitochondrial DNA

Tetsuji Hosoda, Hitoshi Suzuki, Kimiyuki Tsuchiya, Hong Lan, Liming Shi, Alexei P. Kryukov

Restriciton fragment length polymorphism (RFLP) in the spacer regions of the nuclear ribosomal DNA (rDNA) and sequence variations in mitochondrial DNA were examined in 4 species in the genus *Martes*; the Japanese marten (*M. melampus*), the sable (*M. zibellina*) from Japan and from Russia, the American marten (*M. americana*) from North America, and the yellow-throated marten (*M. flavigula*) from China. The RFLPs of rDNA showed that interspecies diversity among *M. melampus, M. zibellina,* and *M. amēricana* was small with 0.1%-0.4% estimated sequence divergence. This divergence indicates that speciation occurred during the late Pleistocene. Sequence divergence between these three species and *M. flavigula* based on rDNA-RFLP was 2.5%-2.9%, suggesting that divergence occurred during the early Pliocene. We sequenced the 402-bp portion of the mitochondrial cytochrome *b* gene for the 4 species of martens. Sequence divergences between *M. melampus* and *M. zibellian, M. americana,* and *M. flavigula* were 2.8%, 3.6%, and 14.4%, respectively. Assuming that the divergence rate of the cytochrome *b* gene is 2.5% per million years per pair of lineages, divergence of the mtDNA of *M. flavigula* and those of the other three species was approximately 6 million years ago, which corroborates the data from rDNA-RFLP. However, the amount of divergence among the mtDNAs of these three species was slightly greater than that of rDNA-RFLPs. These data suggest that genetic exchange continued intermittently during the Pleistocene after the geographic isolation of Japan nearly 2 million years ago.

Martes: taxonomy, ecology, techniques, and management, pages 3-14. G. Proulx, H.N. Bryant, and P.M. Woodard, editors. 1997. Provincial Museum of Alberta, Edmonton, Alberta, Canada. 4 tables 4 figs., 29 refs. Authors' abstract.

Restriction site polymorphism in the ribosomal DNA of eight species of canidae and mustelidae

Tetsuji Hosoda, Hitoshi Suzuki, Takuzo Yamada, Kimiyuki Tsuchiya

Heterogeneity in the external spacers of the ribosomal DNA (rDNA) from eight species of terrestrial carnivores (Canidae, Mustelidae) was analysed using twelve different restriction enzymes and cloned mouse rDNA probes. We constructed species-specific maps of restriction site on rDNA. Based on differences in the arrangements of the restriction sites, sequence divergence among the repeating units of rDNA was estimated in order to construct a molecular-phylogenetic tree and to estimate the timing of the divergence of species. The divergence between *Nyctereutes procyonoides viverrinus* and *vulpes vulpes japonica* was 8.4%. The sequence divergence between *Mustela putorius furo* versus *Mustela sibirica itatsi, Mustela nivalis nivalis* and *Martes melampus melampus* was 0.65%, 1.59% and 7.27%, respectively.

Cytologia 58, pp. 223-230, 1993. 2 tables, 3 figs., 26 refs. Authors' summary.

Original Report

Changes of testosterone level and sperm production and morphology in male silver foxes *Vulpes vulpes* throughout the breeding season

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Summary

The number and morphology of spermatozoa in the ejaculate, as well as plasma levels of testosterone in the basal condition and after sexual stimulation, were studied throughout the breeding season in yearling males of the farmed silver fox. The plasma testosterone level was considerably increased in the middle of the breeding season compared to its onset and end. Sexual stimulation (the introduction of a female to the male) increased testosterone level in males only at the onset of the breeding season. The percentage of sperm abnormalities and the number of spermatozoa in the ejaculate did not change during the breeding season, being 15.7% and 145 million, respectively.

Introduction

The species-specific features of the reproductive processes in mammals subjected to domestication quite recently remain poorly studied. The reports concerning spermatogenesis and endocrine control of reproduction in some fur animals, such as foxes, which have been bred in captivity from the turn of this century, are scant. However, studies of reproductive physiology in captive-bred animals are needed to gain better insight into the general patterns of mammalian reproduction, as well as to promote the development of new methods for breeding commercially valuable species.

The silver fox is renowned for its fur of good quality. Its reproduction is strictly seasonal, monoestrous and multiparous. The seasonal changes in testosterone and estradiol levels in male silver foxes have been studied (*Osadchuk, 1993*). Sex hormone levels were highest during the breeding season and were low out of it with the lowest levels in summer. Spermatogenesis in male silver foxes also changes seasonally: it starts in the middle of December and mature spermatozoa appear in the epididymus in the beginning of January *(Osadchuk, Zelezova, 1994)*. Certain morphological features of the sperm have been considered with reference to selection of males for artificial insemination (*Fougner, 1989; Jalkanen, 1993*). Subtle changes in sperm count and morphology and in sex hormone level

have been scarcely, if at all, studied throughout the breeding season in male silver foxes. Our aim was twofold: (i) to analyse the course of changes in plasma testosterone level during the entire breeding season and the effect of sexual stimulation on testosterone concentration and (ii) to observe the course of changes in the number of spermatozoa in the ejaculate and determine the percentage of abnormal spermatozoa during different periods of the breeding season.

Material and methods

Yearling male foxes (*Vulpes vulpes*) were used in the experiments. The foxes were maintained at the Experimental farm of the Institute of Cytology and Genetics (Novosibirsk) and the commercial farm Magistralny (Altai). As noted above, the silver fox is a seasonal breeder. The breeding season is from late January to the end of March. Males remain sexually active throughout the entire breeding season.

Ejaculate was obtained by masturbating males during different periods of the breeding season: 22nd-28th January (12 males), 14th-21st February (14 males) and 13th March (6 males). The volumes of the ejaculates were measured. After the measurement, 0.9 ml distilled water was added to 0.1 ml sperm, and an aliquot was placed in a sperm counter. The spermatozoa were counted visually in a light microscope at a magnification of x120.

To analyse the abnormal forms of spermatozoa, sperm was fixed in Hancock's solution (4.3% buffered formalin) and stored at 4^0C. Subsequently, these samples were used to stain the spermatozoa with hematoxylin. The stained preparations were examined in a light microscope (x 600). The first 500 spermatozoa were estimated. Seven forms of abnormalities of the spermatozoa were distinguished: 1) abnormalities of the head including defects of the acrosomes, abnormal size and shape of the head, double head; 2) detached head (without the tail); 3) abnormalities of the middle piece, thickened and bent middle piece; 4) abnormalities of the tail including looped, double, hairpin, broken tail: 5) cytoplasmic droplets; 6) agglutinated spermatozoa; 7) multiple defects including some of the above listed occurring in a spermatozoon.

Blood was withdrawn from *vena saphena* on 3rd-5th January (18 males), 20th-28th January (8 males), and 18th-19th March (8 males). A model of sexual activation was used in the experiments. A female in oestrus or anestrous was placed in a male's cage for 40 min. Blood was collected from the male a day before the exposure of a female to avoid stress connected with the procedure of taking blood, and promptly after it. The collected blood was centrifuged and plasma separated and stored at -20^0C until analysed. Testosterone concentrations were determined by RIA after extraction of plasma by ethyl ether. Commercial kits were used, products of the Institute of Bioorganic Chemistry (Minsk, Belorussia).

The sensitivity of the testosterone RIA has been determined as being 0.07 ng/tube. The antiserum used in testosterone tests showed cross-reactivity as follows: testosterone 100%; dihydrotestosterone 20%; androstanediol 2%; androstenedione 2%; androsterone 0.6%; other steroids <0.1%. The inter-assay coefficient of variation was <15% and intraassay coefficient of variation was less than 10%.

A computer package of programs Statgraphics ANOVA (1988) was used for statistical treatment of the data. All values are means ±SEM. One-factor analysis of variance was applied to analyse the temporal changes in the parameters of the reproductive function. To compare plasma levels of testosterone in males before and after the introduction of a female, Student's t-test was used.

Results

One-factor analysis of variance demonstrated no significant differences ($F_{2, 29}$=1.168, p=0.216) in the number of abnormal forms of spermatozoa throughout the breeding season. There were also no significant variations in each type of the structurally abnormal spermatozoa at each time point of the breeding season (see Table).

Table. The percentage of abnormal forms of spermatozoa throughout the breeding season.

Abnormal forms of spermatozoa	Onset of breeding season (n=12)	Middle of breeding season (n=14)	End of breeding season (n=6)
Cytoplasmic droplets	1.1 ± 0.4	1.4 ± 0.4	0.2 ± 0.1
Abnormalities of the head	2.6 ± 0.8	1.8 ± 0.5	0.9 ± 0.3
Detached head	2.1 ± 1.1	1.1 ± 0.5	2.1 ± 0.9
Defective middle piece	4.3 ± 2.1	0.7 ± 0.2	0.7 ± 0.1
Defective tail	7.6 ± 2.8	7.5 ± 1.7	5.8 ± 1.8
Agglutinated spermatozoa	1.2 ± 0.2	0.6 ± 0.2	1.0 ± 0.2
Multiple defects	1.3 ± 0.5	0.4 ± 0.2	0.5 ± 0.2
Total	20.4 ± 4.6	13.4 ± 2.5	11.5 ± 1.8

Values are means ±SME. The number of animals in the group is given in parenthesis.

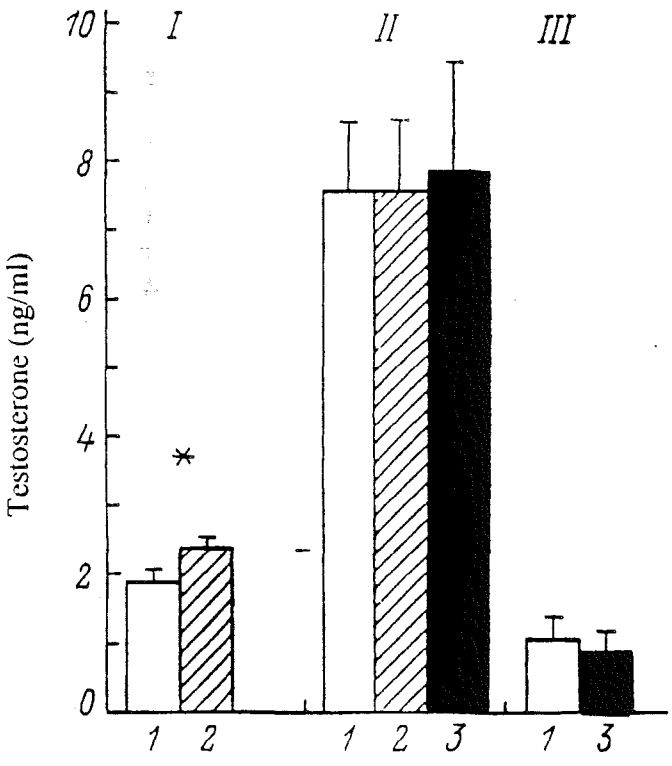

Figure. Plasma level of testosterone in male silver foxes throughout the breeding season. I - onset, II - middle, III - end of the breeding season. Vertical bars - testosterone concentrations (ng/ml). 1 - basal level; 2 - level after introduction of anestrous female; 3 - level after introduction of estrous female. Asterisk designates the significant difference between the basal and treatment levels.

The occurrence frequencies of the abnormalities were arranged in decreased order. Abnormalities of the tail ranked first (7.2±1.4%), defects of the middle piece (2.1±0.8%) and of the head (1.9±0.4%) followed, then spermatozoa without the tail (decapitated head) (1.7±0.5%). The percentages of the following defects were low: cytoplasmic droplets, usually occurring in the middle piece 1.0±0.2%), agglutinated spermatozoa (0.9±0.1%), and multiple defects (0.8±0.2%). The total percentage of abnormal forms of spermatozoa was, on the average, 15,7±2.1%.

The numbers of spermatozoa in the ejaculate of male silver foxes ranges from 17-605 x 10^6. No significant variations in the number of spermatozoa were found during the different periods of the breeding season ($F_{2,29}$=1.896, p=0.168). However, the number tended to decrease by the end of the season. Thus, the number of spermatozoa in the ejaculate was 195.7±48.1 x 10^6 (n=12) at the onset of the season, it was 123±19.8 x 10^6 (n=14) in the middle and 97.6±20.5 x 10^6 (n=6) at the end of the season. The average number of spermatozoa in the ejaculate was 145.6±20.5 x 10^6 (n=32).

Plasma level of testosterone varied widely during the breeding season ($F_{2,31}$=45.4, p<0.001). The level was maximal in the middle of the breeding season and minimal at the end (figure). Exposure of a nonreceptive female to the male at the onset of the breeding season produced a significant increase in plasma testosterone level (p<0.05). Exposure of a female in anestrous or estrous in the middle of the

breeding season did not elicit an increase in plasma testosterone level in males. No significant changes in plasma testosterone were found in males when a female was exposed to the male at the end of the breeding season (see figure).

Discussion

The numbers of spermatozoa in the ejaculate showed wide variations, being 145.6 $x10^6$, on average. The results shown in this study are in general agreement with other reports on this species. Jochle and Lamond (1980) have estimated the numbers of spermatozoa in the ejaculate of the red fox as 80 $x10^6$. Barta and Babusik (1993) have presented the number of spermatozoa 299.5 $x10^6$ in silver fox ejaculates obtained by using electroejaculation method under halothane narcosis. Jalkanen (1993) has reported an estimate of 322.4 $x10^6$ spermatozoa in silver fox selected for artificial insemination; the variation range was wide, from 61-1170 $x10^6$. Some discrepancy in average values above could be the result of an intensive selection of farmed fox males for semen quality for increased male fertility in Western countries.

The percentage of abnormal spermatozoa was 15.7%. Jalkanen (1993) has provided a lower estimate, 12.5%. In her study, silver foxes were selected for artificial insemination and certain structural defects, such as cytoplastic droplets, were not taken into account. In the present study, we established that defects of the tail (7.2%) are most frequent, making up 50% of all abnormalities. The estimate is in agreement with those reported (*Jalkanen, 1993*).

As is known spermatozoa continue to mature in the epididymus, and this is associated with morphological, biochemical and physiological changes (*Rajalakshmi, Prasad, 1979*). Migration and loss of cytoplasmic droplets, as well as reduction of the acrosomes, have been referred to morphological defects (*Rajalakshmi, Prasad, 1979*). Christiansen (1984) distinguished primary and secondary morphological defects. Primary defects result from abnormal spermatogenesis in the seminiferous tubules and secondary defects arise during sperm passage through the epididymus and ejaculation. According to Christiansen's classification, defects of the acrosomes and the end piece are referred to secondary abnormalities. Accepting his classification, most abnormalities we studied here in male silver foxes presumably may be secondary.

There are mechanisms allowing only structurally normal spermatozoa to pass to and through the reproductive tract of the female and reach the site of fertilisation. Nevertheless, there are observations indicating that structurally defective spermatozoa are also capable of fertilising ova (*Smith et al., 1970*). The sperm is of good quality if it contains less than 15% abnormal spermatozoa (*Fougner, Forsberg, 1987; Fougner, 1989; Jalkanen, 1993*). Of the foxes we analysed, 40.6% did not fulfil this criterion. However, there seems to be no direct relation between the number of defective spermatozoa and the fertilising ability of sperm. It was established that a sperm sample containing 20 $x10^6$ spermatozoa with 15% abnormal forms is the critical dose at which silver foxes retain their fertility at the control level (*Fougner, Forsberg, 1987; Fougner, 1989*).

Measurements of plasma testosterone level in silver foxes revealed a distinct pattern of changes, with the highest values in the middle of the breeding season. The values of the testosterone concentrations are in accordance with data obtained for adult silver foxes (*Osadchuk, 1993*). The data of this study revealed that a decrease in the testosterone level at the end of the breeding season is accompanied by a decrease in sperm production that is in accordance with an essential role of testosterone in the maintenance of normal spermatogenesis in mammalian species. However, in silver foxes, changes in plasma testosterone level during the breeding season did not correlate with changes in sperm quality, and sperm of good quality could be obtained at the end of the breeding season, when hormone concentration decreased almost tenfold.

It has been established that the presence of a female stimulates the pituitary-gonadal axis in males of some species (*Coquelin and Bronson, 1979; Jonston and Bronson, 1982; Osadchuk et al., 1985; Osadchuk, 1997*). The stimulating effect may be provided in mouse without tactile contacts with a female (Osadchuk et al., 1985) and also by the presentation of a nonreceptive female (*Coqulin and Bronson, 1979*), or an ovariectomised female (*Jonston and Bronson, 1982*). Sexual stimulation is associated with a rapid reflectory increase in plasma

level of luteinising hormone, followed by testosterone increase. The endocrine responses in males are elicited by pheromonal and possibly tactile signals emitted by a female (*Jonston and Bronson, 1982*). We showed here that silver fox females increase plasma testosterone levels in males at the onset of the breeding season. This complements and is in agreement with the data obtained for other animal species and for adult silver foxes (*Coquelin and Bronson, 1979; Jonston and Bronson, 1982; Osadchuk et al., 1985; Osadchuk, 1997*). However, introduction of a female to the male in the middle of the breeding season did not increase testosterone levels in male silver foxes. It is not immediately apparent why this is so. In an attempt to interpret this observation, it is well to recall that the reproduction system is simultaneously activated in many females in the middle of the breeding season on the farm. Assuming that females release pheromones activating the male hypophysis-gonadal axis, the accumulation of pheromones would reach maximum values in the middle of the breeding season, thereby presumably producing a further elevation in plasma testosterone level in male silver foxes. It is pertinent to note that the previous studies of the endocrine mechanism of sexual arousal were performed in conditions in which females were separated from males. In contrast, plasma level of testosterone increased in male rats in a mixed-sex colony (*Taylor et al., 1987*).

Females emit not only pheromonal, but also vocal signals, contributing to the stimulation of testicular endocrine function. Thus, in the blue fox (a species taxonomically closely related to the silver fox) the sounds emitted by the females in heat produce a significant increase in plasma testosterone level compared to males not exposed to the auditory stimulus (Sirotkina and Tyutyunnik, 1994).

Thus, the obtained results suggest that socio-sexual contacts have an influence on testosterone production in male silver foxes. There are no data concerning the role of olfactory stimuli in the regulation of the reproductive function in silver foxes. However, it was noted that the secretion of the supracaudal glands (*Fox, 1975*) and the products excreted with urine (*Henry, 1977*) may have an important role in olfactory communication in the fox.

Acknowledgements

This work was supported in part by grants from the Russian Foundation for Fundamental Research N 97-04-49941 and the Russian State Program "Frontiers in Genetics".

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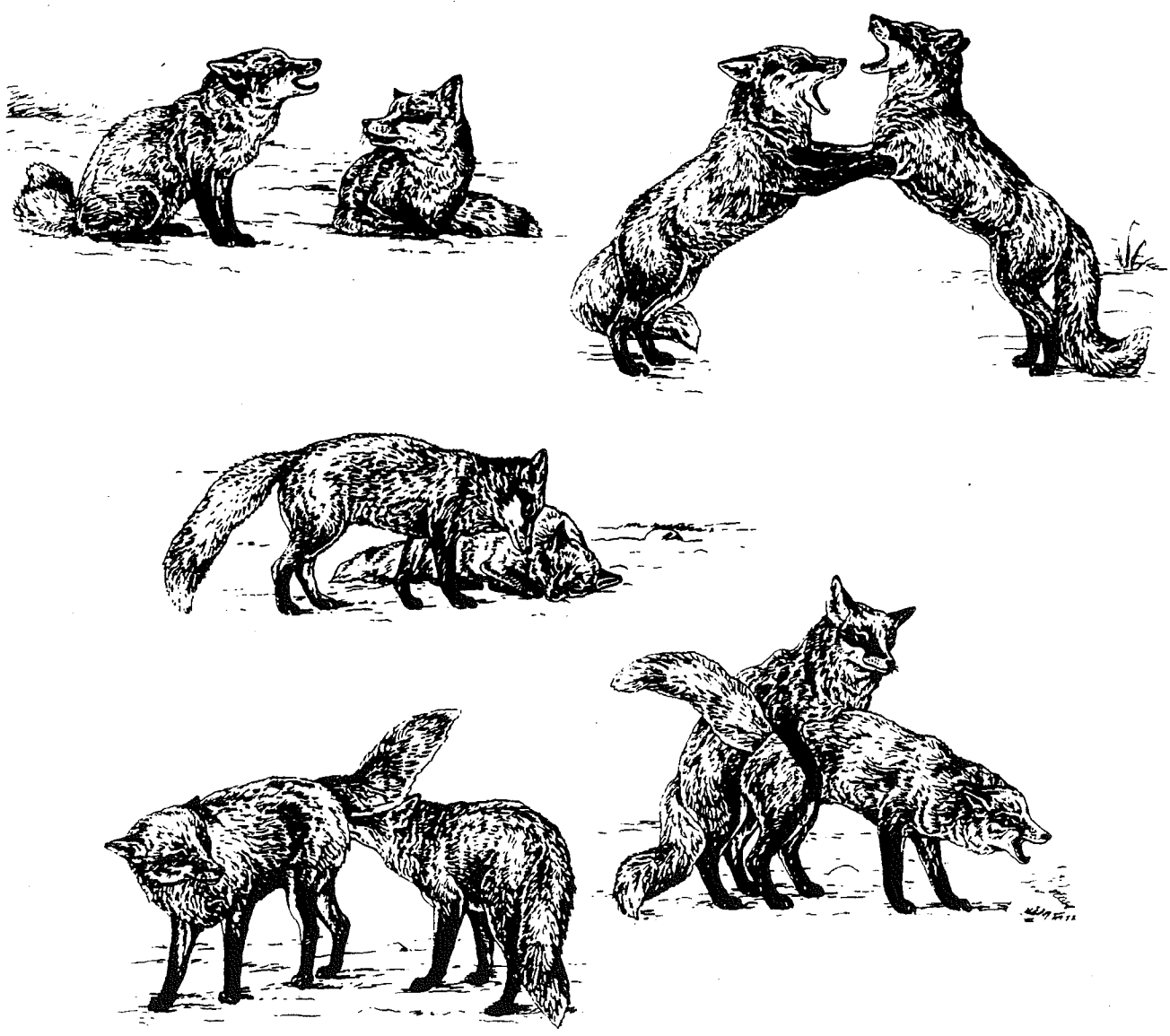

Original Report

The effect of prostaglandin analogues on sex hormone corticosteriod level in the blood and reproduction of mink

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Summary

The effects of prostaglandin (PG) analogues on the hormonal activity of mink were studied 7 days before the beginning of their mating period. 50 mkg/kg of the analogue of PG S_{16}, which belongs to groups E_1, were injected subcutaneously to mink during their early rut period (27 February) and 50 to 100 mkg/kg of body mass were injected on 3 March, which is a conventional rut time. Radio-immunologic methods were employed to determine the sex hormone and corticosteroid levels in the blood serum. The injection of 50 mkg PG S_{16}/kg of body mass before rut in mink resulted in a 1.5-fold increase in estradiol level and a 1.8-fold decrease in progesterone content. The injection of estuphalane in the amount of 5 mkg/kg one week before mating resulted in a 1.8-fold increase in cortisol level. Stimulation of mink by the analogues of $PGE_1 - M_{15}$ and BNC-β in the initial period of mating (3.03) at a dose of 25 and 50 mkg/kg decreased the level of progesterone, and an insignificant increase of estradiol, promoted more intensive follicle growth, increased the number of ova maturing and promoted an earlier beginning of mating.

Introduction

Prostaglandin and their analogues are mainly used in practical veterinary medicine for regulating the reproductive function of farm animals. Data on the involvement of both endogenic and exogenic PGs in reproduction have been obtained by studying the stimulation and timing of estrus, the fertility of males, generic activity, lactation and the commercial breeding of farm animals, mainly cattle, pigs and sheep (*Arrata & Issia, 1978; Smith et al., 1979; Tolstikov et al., 1989*). The involvement of endogenic prostaglandins in the reproduction of furbearing animals and the use of their synthetic counterparts are still poorly studied. It is known, for example, that endogenic PG-$F_2\alpha$ participate in luteolysis of corpus luteum in the ovaries of farm animals during estrus (*Hixon & Flint, 1987*). PGS, group E are of great importance during embryonic diapause in mink as they increase the secretion of luteinising hormone and prolactine into the blood by the hypophysis and hypothalamus, and support progesterone secretion by the corpus luteum. Simultaneously, an increase can be observed in estrogenic function of the ovaries and the animals' sexual activity (*Juyjo et al., 1976*). Application of PG-$F_2\alpha$ is not always justified as it can cause side-effects (tachicardia etc.). Preparations based on cloprostenole (estrumat, estrophan, estuphalan) have presently become very extensively used (*Abramchenko et al., 1986*). Bankov et al. (1982) injected 125 mkg of cloprostenole two times, with a 9-day interval, to sheep. The research data show that PG do not change hormone profiles in the blood whereas female fertility becomes higher than that in the control group of animals. Injection of 11-DPGE$_1$

to blue foxes in a dose of 50 mkg/kg before rut (24 February) caused an increase of estradiol levels (more than 3-fold) and a decrease of progesterone levels compared to the control (*Sirotkina et al., 1994*).

Materials and methods

We studied the influence of prostaglandin analogues on the activity of the hormonal function in dark-brown mink during mating periods in 1989-1994.

50 mkg/kg of the analogue of PG S_{16} and 5 mkg/kg estuphalan, were injected subcutaneously to mink during their early rut period (27 February) and 25 to 100 mkg/kg S_{16}, M_{15}, BNC-β were injected on 3 March which is a conventional rut time. The control animals were injected with equal amounts of physiological solution. Their blood was studied a day after the injection of the preparations. A radio-immunologic method using "Beloris" sets were employed to determine the sex hormone and corticosteroid level in the blood serum. The physiological state of the animals was indicated by visual observations, hemoglobin concentration, erythrocyte content as well as the amount of total protein, and its fractional composition pattern. The obtained results were analysed with the help of Student's test.

Results and discussion

When analysing the available data, the progesterone and estradiol level were found to change slightly in the serum of the animals treated with 100 mkg/kg Pg S_{16} during the period, which coincided with the beginning of rut (3 March), as compared with the control group. However, the injection of 50 mkg PGS_{16}/kg of body mass before rut (27 February) in mink resulted in a 1.5 fold increase in estradiol level ($P<0.01$) and a 1.8-fold decrease in progesterone content ($P<0.001$, Fig. 1). Nevertheless, no significant changes were found in the level of hemoglobin, erythrocyte content and total protein (Fig. 2).

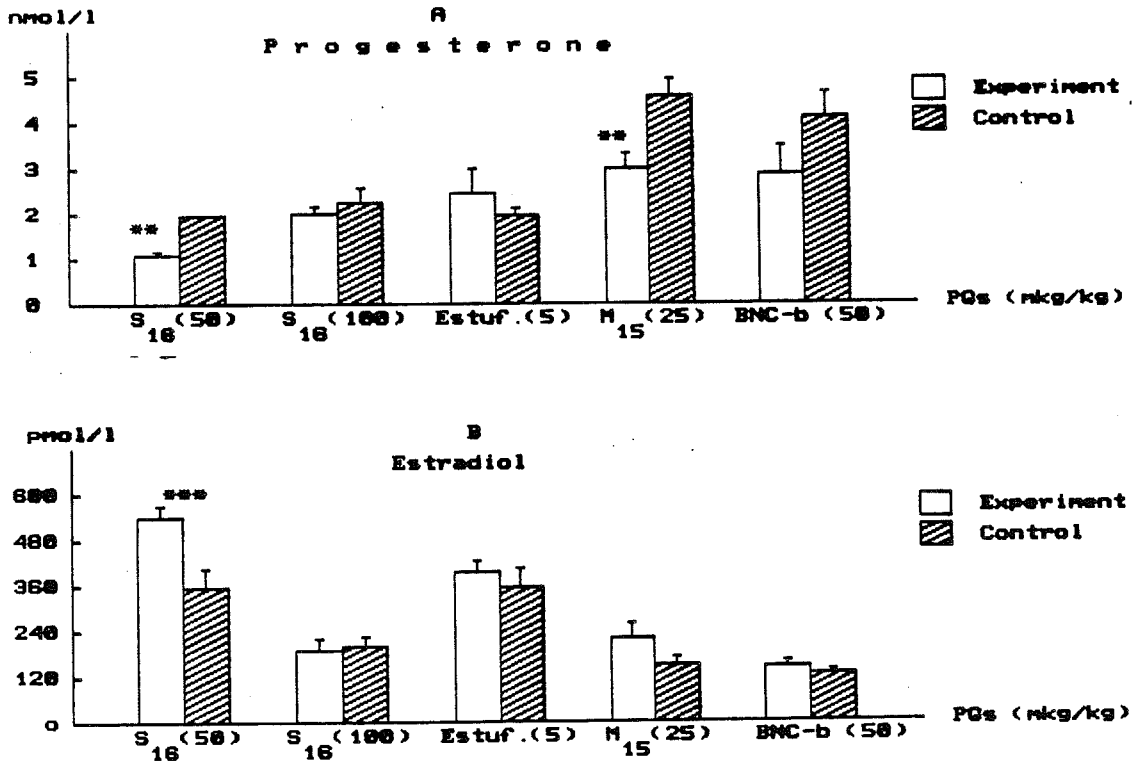

Fig. 1. The stimulation effect of prostaglandin analogues on progesterone (A) and estradiol (B) levels in the blood of female dark-brown minks. The values of differences between experiment and control are marked by asterisks: ** - P<0,01; *** -P<0,001.

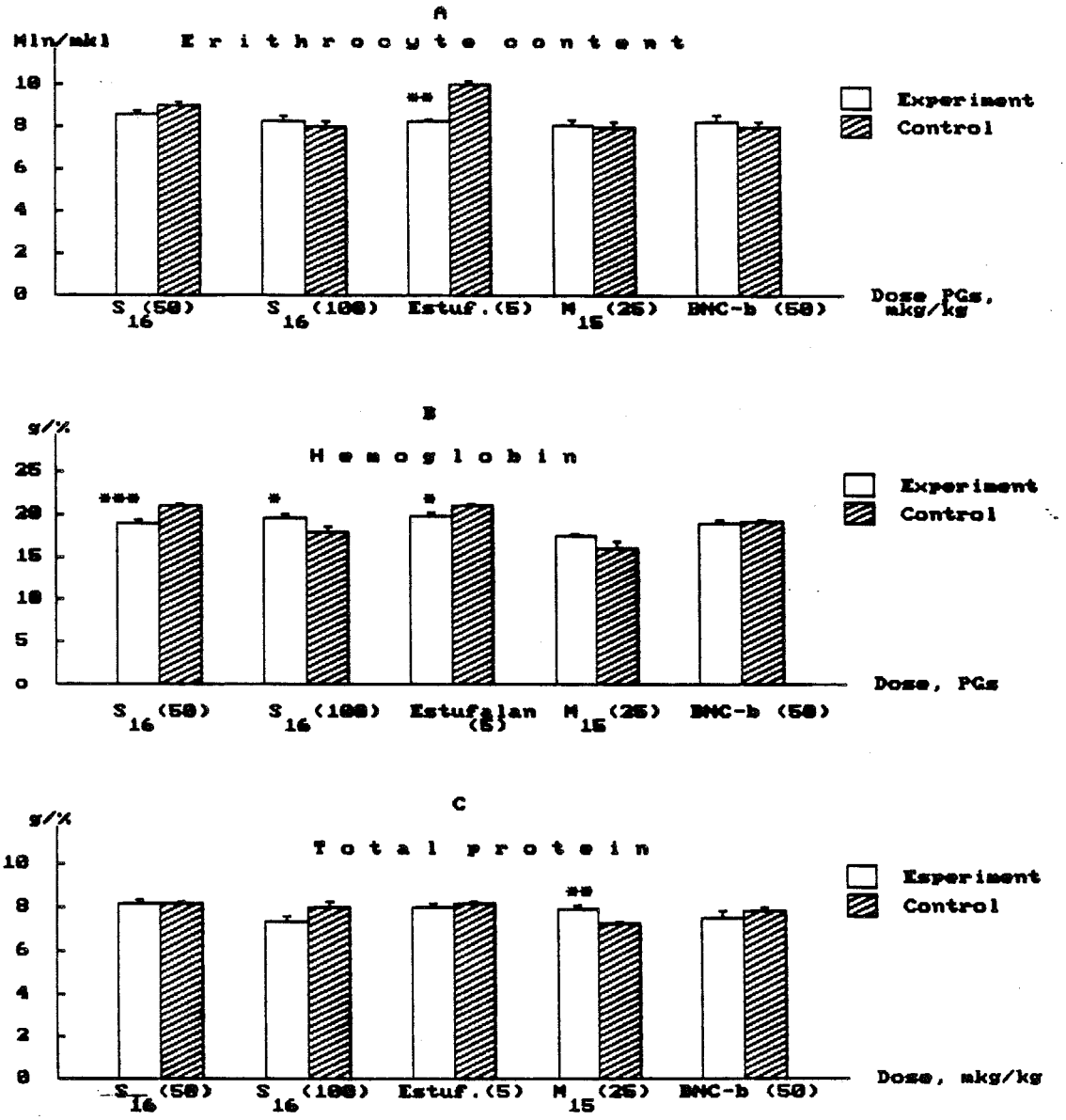

Fig. 2. The effect of prostaglandin analogues on hemoglobin (A), erithrocyte content (B) and total protein (C) levels in the blood of minks. The values of differences between experiment and control are marked by asterisks: * - P<0.05; ** - P<0.01; *** - P<0.001.

A somewhat different decrease was observed after the injection of estuphalane (cloprostenol-bearing preparation of group $F_2\alpha$) in the amount of 5 mkg/kg body weight one week before rut began in mink (27 February). Tests on the blood serum done one day after the injection indicated a simultaneous but insignificant rise n estradiol and progesterone levels, whereas cortisol content increased 1.8 times (Fig. 1). The corticosteroid concentration in mink treated with PG S_{16} was similar to that in the control animals. The effect of the analogues of prostaglandins M_{15} and BNC-β on the hormonal status of mink was studied in two groups of animals. On 3 March, i.e. at the beginning of rut, they were treated with the preparations M_{15} and BNC-β in a dose of 25 and 50 mkg/kg of body mass, respectively. Their blood was tested 24 hours after treatment. The estradiol concentratin in both groups was slightly higher than in the control females. The estradiol concentration increased more appreciably

in mink treated with PG M_{15}, whereas progesterone concentration decreased considerably after the females were treated with M_{15} and BNC-β (Fig. 1).

Thus, our experiments have shown that the effect of the above preparations was dependent on the dosage and time of prostaglandin injection. The data obtained indicate that the doses of prostaglandins are not toxic. The hormonal acticity of mink can be stimulated by treating the animals with 50 mkg prostaglandin S_{16} per 1 kg body weight before rut because estrogenic activity increased and progesterone level declined under these conditions. The effect of the analogue of prostaglandin S_{16} was similar to the effects of luteotropic group E centralaction preparations. They increase luteinizing hormone (LH) in the blood and maintain the secretion of progesterone by the corpus luteum, thereby increasing the estrogenic function of the ovaries, promoting more intensive follicle growth, maturation of a larger number of ova and the earlier beginning of mating.

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Studies on reproduction in female mink (*Mustela vison*) exposed to polychlorinated biphenyls

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New doctor in the family. We congratulate Britt-Marie Bäcklin, Dr. Vet. Med. with the new title and the comprehensive scientific work it is based on.

Abstract

High levels of organochlorines such as polychlorinated biphenyls (PCB) and dichlorodiphenyltrichloroethane (DDT) in Californian Sea Lions and Ringed seals from the Bothnian bay as well as in the Baltic grey seal, have been correlated with low reproduction rate. In common with the Baltic seal population, the European otter population has also declined. A disease syndrome has been described in the Baltic seals which includes enlarged adrenal glands and uterine occlusions. In an experimental study in the 1970s, using mink as a model animal, it was shown that PCB but not DDT, reduced the number of whelps.

The mink was chosen as an experimental model in this study since it is a fish-consuming mammal and shows reproductive similarities with seals and otters. Ovulation and implantation seem to proceed normally in mink exposed to PCB but impaired fetal growth and fetal deaths are commonly observed. The mechanisms behind these effects have, so far, not been elucidated.

Since uterine occlusions and stenoses have been observed in the Baltic seals, the uterine gross morphology as well as the uterine and ovarian histology were examined post-partum, in mink, after exposure to commercial PCBs or fractions of the commercial mixtures.

Two studies were performed in order to determine which fraction of CB congeners, in the commercial product, contributes most to the toxic effects. There was no significant difference in the number of placental sites, i.e. implantation, in exposed compared to control animals, but the two commercial PCB products used strongly reduced the number of whelps born. The CB congener fractions only reduced the number of whelps born when they were given in combination. The post-partum observed uterine histological changes were interpreted as being secondary to PCB-induced fetal death.

To study the effects of PCB exposure during the implantation- and early post-implantation period, uteri and ovaries were histologically examined and endocrine parameters were measured. To investigate the effects of pregnancy itself, the study was performed on mated and non-mated, but ovulated, female mink. PCB exposure of both mated and gonadotrophin-releasing hormone (GnRH-) treated mink resulted in impaired growth of the uterine glands during the implantation period. The PCB-exposed groups displayed a peak in plasma oestrone sulphate concentration that was not observed in the control groups. The observed histological and hormonal changes did not prevent implantation in the PCB-exposed animals, but post-implantation, PCB-exposed mink displayed more fetal deaths and less developed placentae as compared to the control animals. At least 85% of the fetuses in the PCB-exposed group died during early gestation.

Mid to late-gestation placentae, with viable fetuses from PCB exposed animals, were examined morphologically. In the exposed groups, lesions in the maternal vessels of the placental labyrinthine zone were observed. The lesions comprised degenerate endothelial cells, loss of endothelial cells, and thrombi in maternal vessels. The interstitial layer, surrounding the maternal vessels, showed an increased frequency of bridges to the syncytiotrophoblasts. These results suggest that fetal death in PCB-exposed mink, at least in part, is due to lesions in the placenta which may result in insufficient supply of nutrients and gases to the fetus.

The Baltic seals have shown enlarged adrenals and mink have shown elevated excretion of cortisol during early pregnancy. These results indicate that PCBs may increase the production of glucocorticoids in seals and mink. Glucocorticoids have experimentally been shown to suppress levels of insulin-like growth factor (IGF) II mRNA in rodents. This growth factor has been shown to be important for mammalian embryogenesis. Therefore, cDNA for the mink IGF-II was cloned and sequenced, and the expression in adult and fetal tissues was determined after PCB exposure. The amount of IGF-II transcript in the adult liver decreased in response to the PCB dose while the amount in the whole fetus and placenta was seemingly unaltered by the exposure.

In conclusion, the present studies on PCB exposed mink indicate that implantation may be normal, although PCB altered both glandular growth and hormone levels in the dams. The reproductive impairments due to PCB exposure, such as reduced fetal growth and fetal death, are likely to be caused by changes in the placenta. The main part of fetal loss in PCB-exposed mink seemed to occur in the first half of the gestation period.

Thesis: Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine, Department of Pathology, Uppsala, 1996. 46 pp. 2 tables, 2 figs., 148 refs. Author's abstract.

This thesis is based on the following papers, which will be referred to in the text by their roman numerals (I-VI):

I Bäcklin, **B.-M. and Bergman,** A. Morphological aspects on reproductive organs in female mink (*Mustela vison*) exposed to polychlorinated biphenyls and fractions thereof. *Ambio 21, 596-601, 1992.* **Abstracted in Scientifur, Vol. 18, No. 2, pp. 95, 1994.**

II Bäcklin, B.-M. and Bergman, A. Histopathology of post-partum placental sites in mink (*Mustela vison*), exposed to polychlorinated biphenyls or fractions thereof. *APMIS, in press, 1995.* **Abstract in this issue of Scientifur.**

III Bäcklin, B.-M., Madaj, a. and Forsberg, M. Histology in ovaries and uteri and levels of progesterone, oestradiol-17β and oestrone sulphate during the implantation period in mated and Gonadotrophin-Releasing hormone-injected mink (*Mustela vison*) exposed to polychlorinated biphenyls. *Submitted.* **Abstract in this issue of Scientifur.**

IV Bäcklin, B.-M., Persson, e. and Dantzer, V. Morphological studies of the placenta in polychlorinated biphenyl-exposed mink (*Mustela vison*). *Manuscript.* **Abstract in this issue of Scientifur.**

V Ekström, T., Bäcklin, B.-M., Lindqvist, Y. and Engström, W. Insulin-like growth factor II in the mink (*Mustela vison*): Determination of a cDNA nucleotide sequence an developmental regulation of its expression. *General and comparative endocrinology 90, pp. 243-250, 1993.* **Abstracted in Scientifur, Vol. 19, No. 4, pp. 290, 1995.**

VI Bäcklin, B.-M., Gessbo, Å., Forsberg, M., Schofield, P., Rozell, B. and Engström, W. Expression of the insulin-like growth factor II gene in polychlorinated biphenyl-exposed female mink (*Mustela vison*) and their fetuses. *Manuscript.* **Abstract in this issue of Scientifur.**

II. Histopathology of post-partum placental sites in mink (*Mustela vison*), exposed to polychlorinated biphenyls or fractions thereof

B.-M. Bäcklin, A. Bergman

Polychlorinated biphenyls (PCB) cause reproductive failure in mink. Ovulation and nidation occur, but the fetuses die during gestation. The toxicity of different chlorinated biphenyl (CB) congeners differs markedly. Dioxin-like congeners with no (0-*ortho* CBs) chlorine in the *ortho* position to the biphenyl bond are considered to be highly toxic. Altogether, 13 groups of 10 female mink (*Mustela vison*) were exposed to PCB or CB fractions thereof during the reproductive season of 1988 and 1989. In 1988, one group of mink received 2 mg/day of Clophen A50 and five groups received single fractions thereof or synthetic 0-*ortho* CB in their diet. In 1989, one group received 1.64 mg/day of Aroclor 1254 and six groups received combinations of fractions thereof. The daily amounts of the fractions administered per animal were equivalent to those present in 2 mg of Clophen A50 or 1.64 mg of Aroclor 1254. After administration for 3 months in both experiments, the animals were killed 5 days after parturition. Histological examination focused on the placental sites.

The most involuted placental sites were those of early fetal death in primiparous, non-whelping animals in the 1989 experiment. The least involuted placental sites, displaying a hyperplastic and pleomorphic uterine luminal epithelium, in which proliferating cell nuclear antigen (PCNA) was present, were those of late fetal death in biparous, non-whelping animals in the 1988 experiment. The survival of the fetoplacental unit was related to PCB exposure. The histology of the placental sites seemed only to be related to the survival time of the fetuses and to the number of former reproductive seasons.

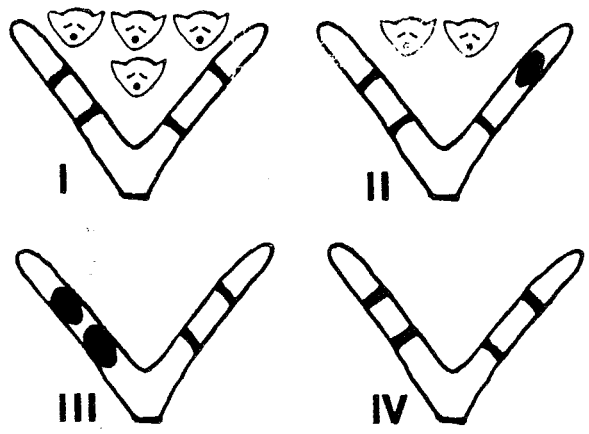


Fig. 1. The uterine classification. Class I: whelping animals showing only post-parturient placental sites. Class II: whelping animals showing early and late fetal death. Class III: non-whelping animals showing early and late fetal death. Class IV: non-whelping animals showing early fetal death.

APMIS 103, pp. 843-854, 1995. 3 tables, 11 figs., 28 refs. Authors' summary.

III. Histology in ovaries and uteri and levels of progesterone, oestradiol-17β and oestrone sulphate during the implantation period in mated and Gonadotrophin-Releasing hormone-injected mink (*Mustela vison*) exposed to polychlorinated biphenyls

B.-M. Bäcklin, A. Madej, M. Forsberg

Earlier studies have shown that polychlorinated biphenyls (PCB) do not prevent ovulation, fertilisation and implantation, but exposure of female mink during gestation caused fetal death. To understand this phenomenon, 30 PCB-exposed female mink and 30 controls were mated or induced to ovulate without fertilisation by treatment with gonadotrophin-releasing hormone (GnRH) and correlations were measured between reproductive, morphological and endocrine parameters. The exposure to PCB (Aroclor 1254) started before ovulation. Equal numbers of animals from each group were killed on days 10, 17 and 26 post-coitum or on GnRH-injection days. Plasma concentrations of progesterone, oestradiol-17β and oestrone sulphate in sampled blood were then measured. Ovaries, uteri and placentae were examined histologically.

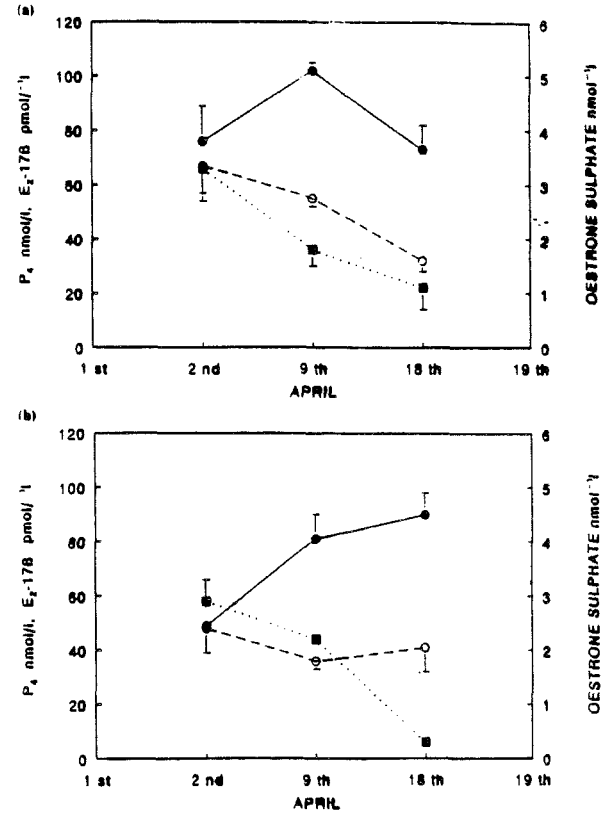


Fig. 4. Plasma concentrations of progesterone (P_4, ●) oestradiol-17β ($E_{2,3}$, ○) and oestrone sulphate (■) 10, 17 and 26 days after GnRH injection in (a) control and (b) PB-exposed mink.

Compared with controls, exposure to PCB during the reproductive season resulted in significantly smaller uterine glandular diameters before implantation or at the end of the experiment in both mated and GnRH-treated mink. The GnRH treatment increased the sizes of the ovarian corpora lutea and oesterone sulphate 10 days after treatment but the increase in uterine glandular diameter was significant only in GnRH-treated control animals. Both GnRH-treated and mated PCB-exposed groups displayed a peak in oesterone sulphate concentration that was not observed in any of the control groups. Possible actions of PCB are discussed. The observed

histological and hormonal changes in the mated PCB-exposed group did not prevent implantation. Exposure to PCB increased fetal mortality. This effect was associated with an effect on placental development.

Journal of Applied Toxicology, Vol. 17 (5), pp. 297-306, 1997. 2 tables, 4 figs., 42 refs. Authors' summary.

IV. Morphological studies of the placenta in polychlorinated biphenyl-exposed mink (*Mustela vison*)

B.-M. Bäcklin, E. Persson, V. Dantzer

Polychlorinated biphenyls (PCB) have been shown to cause fetal death in mink and to cross the placenta barrier. No indications of impaired implantation have been reported. In order to study the effects of PCB on placental morphology in mink, twelve animals served as controls and ten animals each were orally exposed to Clophen A50 at two dose levels, 0.65 (low dose) and 1.3 mg (high dose)/day for 54 days, starting before mating. Placental samples were collected during mid to late gestation. The evaluation was performed by using both light and electron microscopy. In the control group 11% of the placentae were degenerated. In PCB-exposed groups, the percentage of degenerated/dead fetus placentae was 31% in the low dose- and 64% in the high dose group. All animals in the control group exhibited sites of implantation. In contrast, one animal in the low dose group and four animals in the high dose group exhibited no sites of implantation. However, there was no difference between PCB-exposed and control animals in number of placentation sites in implanted animals. There was a marked increase in the occurrence of fetal death in the PCB-exposed animals, among which twelve animals had viable fetuses or a mixture of viable, and dead fetuses. In nine of these animals, the labyrinthine zones of placentae related to viable fetuses displayed maternal vascular lesions. The lesions comprised thrombi, degenerate endothelial cells and loss of endothelial cells as well as haemorrhages. Some of the maternal endothelial cells were lower than others and contained electron-dense granules. The formation of thrombi seemed to be acute since there were no signs of placental adaptations to ischaemia. In placentae of PCB-exposed animals, cytoplasmic bridges of either maternal or syncytiotrophoblast origin crossed the interstitial layer with a higher frequency than seen in control material. Extracellular fluid was present between microvilli of syncytiotrophoblast and the interstitial layer. The possible mechanisms of PCB action are discussed.

1 table, 5 figs., 55 refs. Authors' abstract.

VI. Expression of the insulin-like growth factor II gene in polychlorinated biphenyl-exposed female mink (*Mustela vison*) and their fetuses

B.-M. Bäcklin, Å. Gessbo, M. Forsberg, P. Schofield, B. Rozell, W. Engström

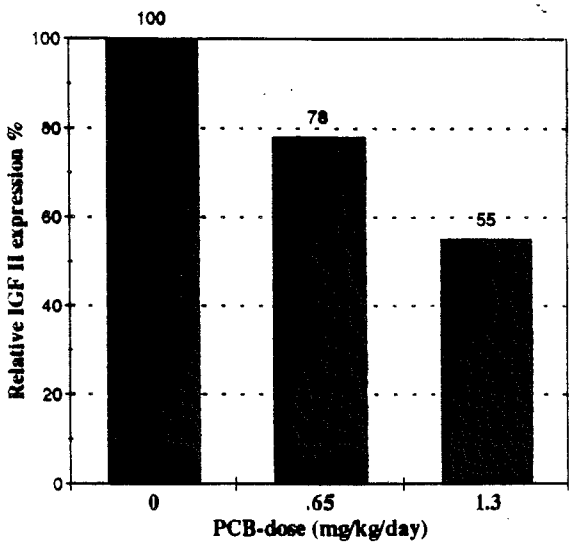


Fig. 1. Relationship between PCB exposure and IGF-II cDNA expression in adult liver. After hybridisation with an IGF-II cDNA probe, filters were stripped of bound probe and rehybridized with a HMG CoA reductase control probe. The relative intensity of the bands was determined by scanning densitometry and the relationship calculated by dividing the integrated areas.

Exposure of pregnant mink to PCB causes fetal death. The mechanisms are largely unknown. Elevated levels of excreted cortisol have been reported in pregnant mink during PCB exposure. Since glucocorticoids have been shown to suppress expression of the insulin-like growth factor (IGF-) II gene in other species, the present study was performed to examine the possible involvement of altered IGF-II expression in mink after PCB exposure.

Ten female mink each were exposed to 1.3 or 0.65 mg Clophen A50/day, respectively, during the reproductive season. Ten unexposed animals served as controls. There were no significant differences in the number of implantation sites between implanted control and exposed animals. The number of viable fetuses decreased in the exposed animals, in a dose dependent way, compared to controls. The expression of the IGF-II gene in adult livers from PCB-exposed animals was decreased, compared to control animals, also in a dose dependent way. In contrast, the IGF-II expression in placentae and fetuses was unaltered. Furthermore, the maternal excretion of U-cortisol increased in both exposed groups during the implantation period. The results suggest that expression of the IGF-II gene is down-regulated by PCB exposure in the adult liver. They also indicate that the regulation of the gene differs between adult and fetal life.

2 tables, 1 fig., 26 refs. Authors' abstract.

Control of luteal function in the mink (*Mustela vison*)

B.D. Murphy, K. Rajkumar, A. Gonzalez Reyna, D.W. Silversides

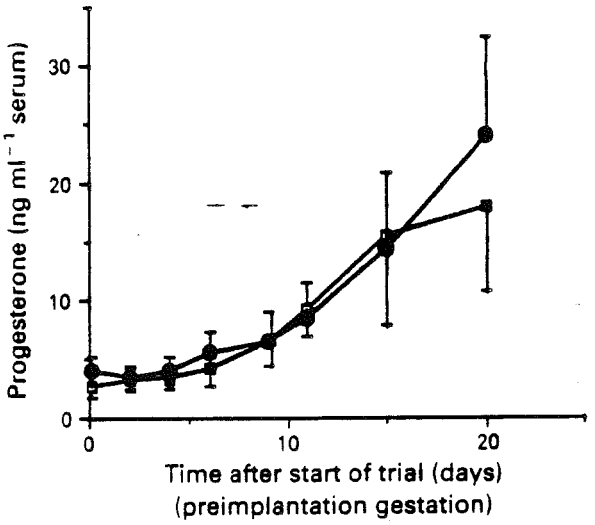


Fig. 1. Peripheral concentrations (mean ± SEM) of progesterone in the serum of two groups of 12 mink treated with 10 daily doses of monoclonal antibodies against gonadotrophin-releasing hormone (⊡) or irrelevant monoclonal antiserum (●) starting 6 days after the last of two matings.

The ranch mink was studied to determine the role of pituitary luteotrophins on corpus luteum (CL) function before and after implantation. Twelve mink were treated with monoclonal antiserum against gonadotrophin-releasing hormone (GnRH), and 12 with an irrelevant monoclonal antibody during embryonic diapause. Activation of the CL, plasma progesterone concentration and embryo implantation were unaffected by this treatment. In a second trial, groups of ten mink were treated with GnRH antibodies, bromocriptine, bromocriptine plus 0.5 mg prolactin per day per animal, or ethanol vehicle. Comparison of the consequent profiles of progesterone indicated that both bromocriptine and anti-GnRH compromised postimplantation luteal cells from ovaries of mink at 21-24 days after implantation with either LH or prolactin increased the accumulation of progesterone over 2 h. Addition of 25-hydroxy-cholesterol (25OHC) as substrate increased basal levels and the progesterone accumulation stimulated by LH and prolactin; the increases induced by luteotrophins were additive. There was an apparent synergistic interaction between prolactin and canine low-density lipoproteins (LDL) in the stimulation of progesterone secretion *in vitro*. The results are interpreted to indicate that LH/FSH are not required for luteal support during embryonic diapause, or for luteal activation. Prolactin is necessary for luteal activation, and LH and/or FSH and prolactin are obligate luteotrophins during the postimplantation period in mink.

J. Reprod. Fert., Suppl. 47, pp. 181-188, 1993. 1 table, 6 figs., 23 refs. Authors' summary.

Spermatogenic activity in male mink prior to the breeding season

K.D. Seo, J.H. Lee, T.H. Byun, K.S. Shim, S.H. Lee

Twenty-four male mink reared in the Taekwalryung area were used to establish spermatogenic activity occurring prior to the breeding season from October to March the following year. Changes in testes morphology, spermatogenic cells, cell differentiation and appearance of spermatids in the seminiferous tubules were examined using cytochemical, biological and biochemical methods. The size and weight of the testes gradually increased regardless of a sharp decrease in body weight from

October to March. In particular, the testis weight increased sharply from December to March. The overall ratio of testis to body weight increased up to 12-fold during the 6 months. Cytochemical analysis using PAS staining demonstrated that the acrosomes in spermatids are fully developed in January. Viable spermatozoa appeared in the caudal region from February and March, at concentrations of 2.3-3.8x10^7/ml and 5x10^7/ml, respectively although spermatozoa appeared in the caput of the epididymis. Finally, electrophoretic analysis showed that biochemical changes also occurred in the lumen of each microenvironment of spermatogenesis. The results should be useful for selection of male mink in reproductive performance and breeding programmes.

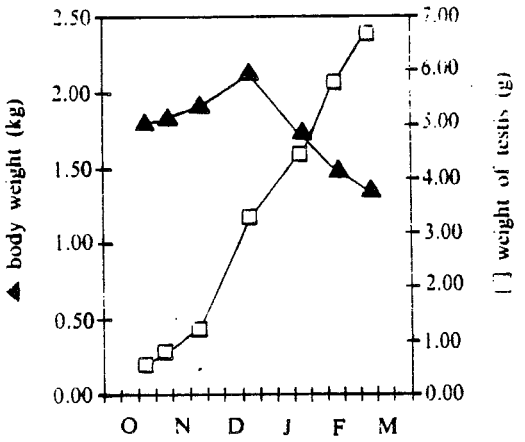


Fig. 4. Changes in average body and testis weight during the study. Note the sharp decrease in body weight in December and continuous increase of testes weight up to early March.

Korean Journal of Animal Sciences 35, 1, pp. 24-31, 1993. In KOREAN, Su. ENGL. 1 table, 9 figs., 16 refs. Authors' summary.

Luteal and placental characteristics of carnivore gestation: expression of genes for luteotrophic receptors and steroidogenic enzymes

D.A. Douglas, J.-H. Song, A. Houde, G.M. Cooke, B.D. Murphy

Experiments were carried out to investigate the abundance of mRNA for luteotrophic receptors and steroidogenic elements in the ovaries and corpora lutea of mink during the embryonic diapause, peri-implantation and postimplantation pregnancy. The second aim was to determine whether the mink placenta synthesized progesterone. Homologous cDNA probes for the mink LH and prolactin receptors were generated by the polymerase chain reaction. Heterologous cDNA probes for steroidogenic acute regulatory protein (StAR), cytochrome P450 side chain cleavage (P450scc) and 3β-hydroxysteroid dehydrogenase-Δ^4-Δ^5 isomerase (3βHSD) were also used. The abundance of mRNA encoding the prolactin receptor was low during the period of embryonic diapause and increased concurrent with circulating progesterone. The abundance of LH receptor messages reached peak values during the peri-implantation period followed by maintenance of a steady-state after implantation. The abundance of StAR and P450scc messages appeared not to vary during gestation, while that for 3βHSD was correlated with changes in circulating progesterone. There was no evidence of 3βHSD activity or transcripts in the placenta. These results indicate that prolactin and LH are necessary for activation of the corpus luteum during the period of embryonic diapause, and for its maintenance during postimplantation gestation. The mink placenta does not synthesize progesterone.

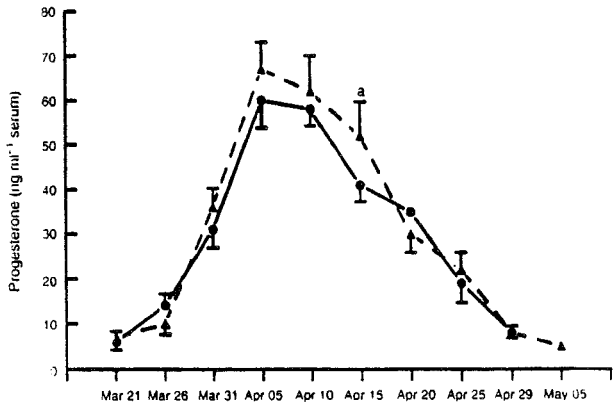


Fig. 1. Composite peripheral progesterone concentrations in nine intact (▲) and nine hysterectomized (●) mink in the preimplantation phase of gestation. Significant difference between the two means at $P<0.05$.

Journal of Reproduction and Fertility Supplement 51, pp. 153-166, 1997. 10 figs., 61 refs. Authors' summary.

Prolactin in canine and feline reproduction – review

W Jochle

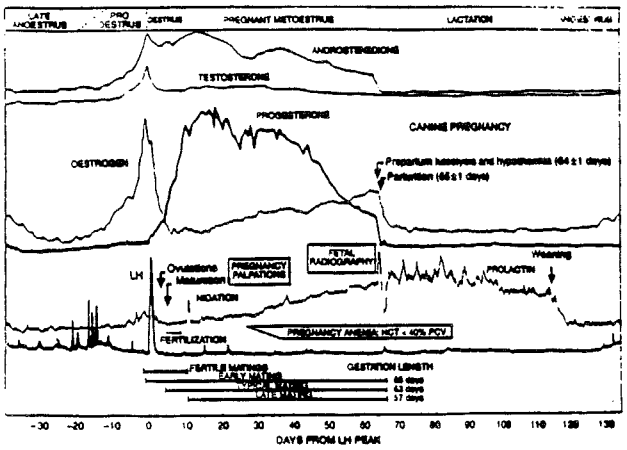


Fig. 2. Typical hormonal blood profiles in the pregnant bitch during the sexual cycle. This includes information on the length of gestation due to early, typical and late matings, on ovulation and nidation, on pregnancy anemia and pregnancy detection methods, as well as blood profiles during lactation (*Concannon, 1986*).

Prolactin (PRL), a pituitary hormone, exerts a significant influence on reproductive functions in dogs and non-domestic canines (wolf, fox, coyote et al.). Pseudopregnancy is obligatory for all non-pregnant females in these species, which ensures their capability of caring for and even nursing a litter. This is caused by a PRL rise during the second half of pregnancy, which in the dog is quantitatively equal in pregnant and overt pseudopregnant animals; in covert pseudopregnant bitches this rise in PRL is significantly lower. Consequently, these animals do not show the enlargement of the mammary glands and their secretions, and the typical distorted behaviour seen in overtly-pseudopregnant bitches. The use of potent PRL-inhibitors, mostly dopamine agonists like bromocriptine, metergoline and cabergoline, has revealed that PRL is the luteotrophic hormone from day 30 of pregnancy onward and that PRL is essential for the preparation of the mammary glands for lactation, the commencement of lactation and its maintenance, and for the maternal (and paternal) care of the litter. Hence, these PRL-inhibitors are in use for induction of abortion after mid-gestation, for the treatment of overt pseudopregnancies and to stop unwanted lactation. Male and female dogs and wolves show almost identical seasonal changes in PRL blood concentrations with peak levels before mid-year and the nadir just before the year's end. In non-domestic canines with one oestrus annually in late winter/early spring the annual PRL peak coincides with the need to care for the litter late in spring/early in summer.

Females that were pregnant or pseudopregnant are ready to nurse and take care of whelps and simultaneously, the seasonally peaking PRL blood concentrations seem to smooth over social tensions between males and ensure their essential participation in the care of the litter. In the bitch, pseudopregnancy has become an atavism and overproduction of PRL causes anestrus. Hence, PRL-inhibitors can be used for the treatment of anestrus and for shortening the estrous interval as well.

The pseudopregnant cat does not form additional PRL, but in the pregnant cat, PRL is an essential luteotropin during the second half of pregnancy. Hence, cats can be aborted during this time period with PRL-inhibitors and these compounds are useful in order to stop lactations.

Reproduction in Domestic Animal 32 (4), pp. 183-193, 1997. 1 table, 11 figs., 59 refs. Author's summary.

Exogenous chorionic gonadotrophin and breeding efficiency

I. Heimler, R.J. Hutz, D.M. Voltz, W.B. Wehrenberg

The mink breeding season occurs annually and lasts for approximately three weeks. Therefore, on the commercial mink ranch this is a very work-intensive period. We sought to reduce this work-intensity by increasing breeding efficiency through estrus synchronisation with human chorionic gonadotrophin (hCG) administration. Mink were injected with either saline or 250 IU of hCG between March 8th and 12th. Saline-treated animals were then immediately subjected to normal breeding practices, whereas in hCCG-treated animals breeding was attempted 8 days following hCG injection. Breeding efficiency, defined as the number of successful matings divided by the total number of breeding attempts, was significantly improved by 14% with hCG administration. There was no significant difference between the two groups in the number of animals that bred, but is was observed that a signifi-

cantly lower percentage of hCG-treated mink whelped. Thus, while exogenous hCG was effective in synchronising estrus in female mink and in facilitating breeding efficiency, it also resulted in a significant decrease in the total number of offspring produced. We conclude that commercial application of estrus synchronisation in mink may not be of economic value to the commercial mink industry.

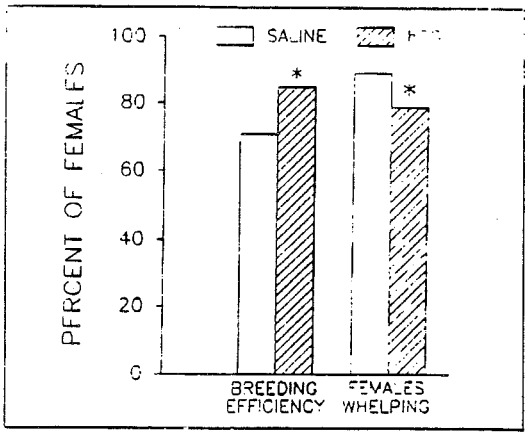


Fig. 1. The percentage of breeding efficiency and whelping in saline and hCG-treated female mink is illustrated. Breeding efficiency is defined as the number of successful breeds divided by the total number of breeding attempts (successful plus unsuccessful). The breeding efficiency was significantly greater for hCG-treated females ($p<0.05$). There was a significantly lower precentage of hCG-treated mink whelping ($p<0.05$).

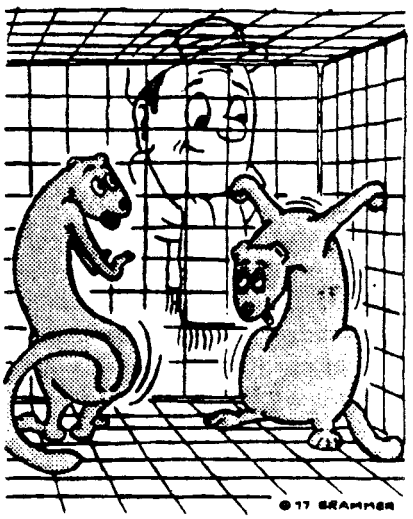
Norwegian Journal of Agricultural Sciences 10, pp. 179-186, 1996. 2 tables, 1 fig., 20 refs. Authors' summary.

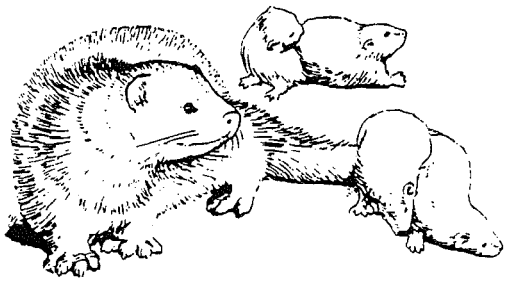
Infertility is affected by the number of matings

Michael Sønderup

Data on 71,440 mink females mated in Denmark in 1995 were analysed. Of adult females mated 1, 2 and 3 times, 14.03, 6.43 and 13.64% respectively failed to give birth to a litter vs. 12.87, 5.83 and 0% of females aged 2 years and 23.3, 7.6 and 10.42% of primiparous females. Litter size at birth averaged 5.87, 6.32 and 5.45% respectively for adult females in the 3 mating groups (of which 5.11, 5.8 and 4.55 were viable) vs. 5.85, 6.43 and 6.91 for females aged 2 years (5.59, 6.19 and 6.77) and 4.65, 6.06 and 6.17 for young females (4.37, 5.76 and 5.47).

Dansk Pelsdyravl 58 (10), pp. 395, 1995. 1 table. In DANH. CAB-abstract.





Original Report

Indices of thiamine metabolism in mink in different physiological periods

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Abstract

Some indices of thiamine metabolism in mink blood that depend on the amount of the vitamin supplied in different physiological periods were studied. In intact animals that were not supplied with additional thiamine a slight thiamine deficiency was observed during premating and mating periods. It became more acute in the course of gestation, as compared to the test group supplied with additional vitamin. A substantial decrease in transketolase and thiamine triphosphatase acticity was observed during pregnancy in both groups. Moderate thiamine deficiency, indicated by the size of thiamine diphosphate effect, was revealed in intact mink that were not supplied with additional vitamin.

Introduction

Vitamin B_1 (thiamine) affects many functions in the organism. Its diphosphorus ether is involved in carbohydrate, lipid, protein, and nucleic acid metabolism.

Recently, vitamins have been used mainly to actively affect some metabolic processes that are often unrelated to the coenzymatic function of vitamins (*Novikov & Bortnovsky, 1985; Tumanov & Trebukhina, 1987*). It is known thate there is no thiamine synthesis in the organism of carnivores (*Helgebostad, 1981*). It is especially interesting, therefore, to study the metabolic processes of thiamine in mink in different biological periods. The condition of the organism can thus be assessed, depending on its physiological requirement of thiamine with regard for life cycle periodicity, a characteristic of mink responsible for differences in the intensity of metabolism in carnivores.

Material and methods

The metabolic indices of thiamine were studied during some physiological periods in the life of mink, such as premating, mating, pregnancy, lactation, winter fur formation and rest. To provide a positive vitamin status at the preliminary stage of the experiment, the dark-brown females of the test group were supplied intramuscularly with a 6% thiamine bromide solution, the dosage accepted being 1 ml per animal. Intact mink were offered the usual farm rations.

Some indices, such as the enzymatic activity of transketolase (TK), the size of thiamine diphosphate effect (TDP-effect), thiamine triphosphatase (TTPase) activity and a total thiamine level in the blood, were used to characterize thiamine metabolism. The size of TDP-effect was estimated by a rise in TK activity after preincubation of blood samples with thiamine diphosphate (*Dreyfus & Lundquist, 1962*). It is considered that the size of TP-effect ranging from 0 to 15% indicates adequate

provision, that of 15 to 30% shows a slight thiamine deficiency, that of 30 to 40% suggests a moderate deficiency, and that in excess of 40% is indicative of a heavy shortage of thiamine. The total thiamine level in the blood was determined by fluorimetric method, TTPase – by the decrease in the content of thiamine diphosphate inoculated into the incubation mixture as substrate (*Penttinen & Uotila 1981*).

Results and discussion

Our study has shown TK activity to be high in both groups of mink during the premating period. The TDP-effect was 15.5% in the experimental group of mink and 21% in intact mink. There were no well-grounded differences in total thiamine level and TTPase activity between the groups.

Table 1. Indices of thiamine metabolism in mink blood in different physiological periods (M±m)

Periods	Experimental group			Intact group		
	Total thiamine mkmol/l	TK mkmol/s*l	TTPase nkat	Total thiamine mkmol/l	TK mkmol/s*l	TTPase nkat
Premating	0.62±0.02	13.35±0.21	22.70±2.92	0.54±0.05	13.13±0.33	25.14±2.37
Mating	0.66±0.02*	11.83±0.35	5.63±0.32*	0.49±0.03	11.36±0.49	4.25±0.24
Pregnancy	0.82±0.03*	8.70±0.61	6.84±0.67	0.61±0.03	7.30±0.57	5.00±0.96
Lactation		9.23±0.24			9.40±0.32	
Winter fur formation	0.54±0.02	9.91±0.41	13.30±0.51	0.48±0.03	8.77±0.59	13.08±0.80
Rest	0.60±0.03*	10.99±0.35	13.98±0.74	0.49±0.03	10.27±0.63	15.54±0.18

* difference is significant in comparision to intact group

TK activity was observed to decline slightly in the course of mating (Table 1). The TDP-effect decreased to 17.5% in intact mink, and no thiamine deficiency was reported for the experimental group. The groups clearly differed in total thiamine level ($p<0.01$). TTPase activity was observed to diminish substantially in both groups of mink, but it was higher in the experimental group than in the intact group ($p<0.01$). Degradation enzymes are known to activate once there is an excess of free thiamine phosphates (*Chernikevich et al., 1995*) the low level of which during pregnancy caused enzymatic activity to decline. It is presumably the adaptive response of the organism which helps retain the active forms of thiamine. In this case, TK activity did not respond practically to a drop in thiamine level because the percentage of the vitamin is not related directly to enzymatic activity under thiamine deficiency conditions over long periods of time (*Ostrovsky, 1971*). The response of TTPase was more obvious during that period.

A marked decline in TK activity accompanied by a rise in the TDP-effect observed in intact mink during pregnancy. The size of the TDP-effect was twice as much in the intact animals as in the experimental mink, indicating an apparent thiamine deficiency (TDP-effect 35.5%) avoided by the animals supplied with additional vitamin (Fig. 1). Total thiamine and TTPase activity, which indicated a high level of thiamine triphosphate degradation, were higher in experimental mink.

During lactation TK activity rose, and the size of the TDP-effect showed that both groups of mink were equally provided with the vitamin. Obviously, the vitamin was supplied exogenously in sufficient quantities during that period, and its addition did not affect the indices studied.

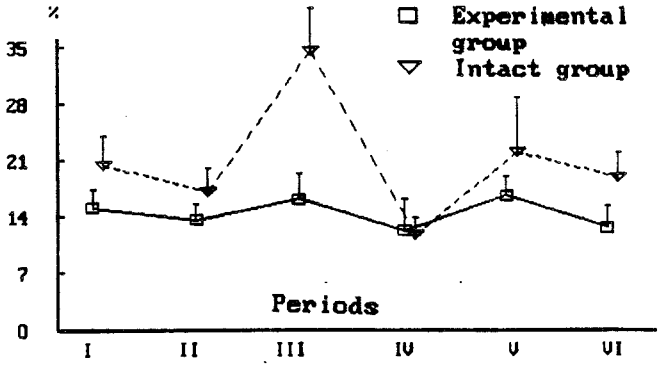

Fig. 1. The size of TDP-effect in mink blood in different physiological periods (I – premating, II – mating, III – pregnancy, I – lactation, V – winter fur formation, VI – rest).

TK activity varied slightly in the course of winter fur formation. In intact mink, the TDP-effect was 25.5%, indicating a slight shortage of thiamine. TTPase activity and the total thiamine level were practically identical in both groups, but enzymatic activity was much higher than in the previous study.

During rest TK activity increased and the TDP-effect decreased. A slight thiamine deficiency

(TDP-effect 19.4%), observed in intact mink, corresponded to a lower total thiamine level ($p<0.001$). Experimental animals were adequately provided with thiamine (TDP-effect 13.0%).

Our studies have shown that the thiamine-dependent indices of mink change most substantially in biological periods during which vitamin B_1 was actively used by the organism. At this time thiamine-dependent enzymatic activity declined. The TDP-effect responded clearly to the amount of thiamine supplied to the organism. It has been shown that additional introduction of thiamine had a stabilizing effect on the retention of the vitamin status over the entire study period. A slight and even moderate thiamine deficiency, not apparent clinically, causes no visible deviations from physiological norm. However, the loss of the vitamin in biologically significant periods during which a lot of energy is used can have a negative effect on the reproductive functions of animals in the future. Although mink are highly adaptive, specific thiamine-dependent reactions should be studied to be able to correct metabolic processes in the organism.

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Content and digestibility of amino acids in feeds for mink

Rikke Fink, Aloys Lau, Christian F. Børsting

In 1997, digestibility trials were done on various raw materials especially fish products in order to examine whether the existing values of especially the content of digestible amino acids in "The Danish Feedstuff Table for Mink Feed" are in accordance with the new values measured in digestibility trials with the raw products of today.

Quite significant differences were found among the fish products analysed in the amount of digestible protein and digestible essential amino acids per 100 g. The quantity of digestible protein per 100 g. product was up to 30% lower in herring and mackerel offal compared to sand eel, ensiled sand eel and offal from cod and plaice. On the other hand, the content of essential amino acids per 16 g N in offal from cod and plaice was considerably lower than in both sand eel, ensiled sand eel, herring and mackerel offal. The low content of digestible protein in herring and mackerel offal meant that these products contained the lowest quantities of digestible amino acids per 100 g. The content of digestible amino acids in offal from cod and plaice was in general a little higher than in herring and mackerel offal. Sand eel and ensiled sand eel had the highest content of digestible amino acids per 100 g. In general, the content of digestible cystine and tryptophan per 100 g. measured in offal from cod and plaice, sand eel and ensiled sand eel, respectively, was lower than the existing values in the "The Danish Feedstuff Table for Mink Feed" for fish offal low in fat, fish waste and ensiled fish.

South American fish meal contained more ash and less crude fat that the equivalent Danish product (Raw material No. 322) but the amount of digestible protein, cystine, lysine and methionine in g. per 100 g. product corresponded in general to the level in Danish fish meal. As expected, poultry offal with 20% feathers had a higher content of digestible cystine than the poultry offal used until now with 12% feathers (Raw material No. 244), whereas the content of digestible methionine was a little bit lower. The content of digestible protein and the content of most of the other essential amino acids were a little higher in comparison with the old table values. The digestibility was furthermore examined in wheat gluten, the digestibility of which has not been examined in mink earlier. Compared to maize gluten (Raw material No. 521), the apparent digestibility of crude protein was 13% higher in wheat gluten. The amount of digestible methionine per 100 g. was 23% higher in wheat gluten than in maize gluten, whereas the content of cystine was approx. 54% lower in wheat gluten.

Technical Year Report 1997, pp. 11-24. 3 tables, 11 refs. In DANH. Authors' summary translated by Hanne Artved.

Acid-preserved raw materials and addition of acid to mink feed

Søren Wamberg, Tove Clausen, Otto Hansen

A survey is given of the different theoretical and practical aspects of the use of acid-preserved raw materials and/or addition of strong acids in the production of mink feed. Based on the natural composition of the feed and the special, high protein transformation of the mink, the natural (so-called "endogenous") formation and excretion of acid (and base) equivalents with the urine when feeding with ordinary farm feeds has been explained. Furthermore, a theoretical calculation has been made regarding the additional acid stress to which the animals are subjected when acid-preserved raw materials or feed mixtures added strong inorganic acids such as for instance sulphuric acid and phosphoric acid are used.

Technical Year Report 1997, pp. 27-35. 1 table, 11 refs. In DANH. Authors' summary translated by Hanne Artved.

The use of large amounts of carbohydrates in the winter/nursing period

Tove N. Clausen, Carsten Hejlesen

In the winter and nursing period of 1997, the significance of a high carbohydrate level in the feed to the performance of the females and the kits in the nursing period was studied. For the study, two groups of 115 standard females per group were

used. The energy distribution in the feed for the control group (protein:fat:carbohydrates) was 56:32:12. For the experimental group, the distribution was 44:35:21. In the period from May 21 to 28, the feed for half of the females and the kits in the experimental group was changed to 45:45:10. The results showed that a high content of carbohydrates in the feed in the winter period reduced litter size at birth, whereas a high carbohydrate content in the feed in the nursing period gave good kit weights at weaning. Furthermore, it was found that a powerful change in feed in the period from May 21 to 28 was not appropriate for the kits. The study of the energy distribution in the period in question will continue to procure more significant results.

Technical Year Report 1997, pp. 37-42. 4 tables. In DANH. Authors' summary translated by Hanne Artved.

Fat fish products for mink in the growth period - Examinations in 1996

Tove N. Clausen, Carsten Hejlesen, Christian Friis Børsting, Birthe M. Damgaard, Ricarda Engberg, Søren Krogh Jensen

Studies of the use of fat fish products of herring offal and mackerel offal as well as defatted ensiled herring offal and defatted herring offal were carried out in the growth period of 1996.

For the study, groups of an equivalent number of wild mink male kits and wild mink female kits were used (74 or 102 pairs per group), three groups with 30, 50 and 70%, respectively, of the fat from herring offal and three groups with 30, 50 and 70%, respectively, of the fat from mackerel offal. Furthermore, two groups with defatted ensiled herring offal and three groups with defatted herring offal and a control group took part in the experiment. The experiment confirmed the conclusion of the experiments of previous years, i.e. that up to 50% of the fat from fish fat can be used without a negative effect on the health of the animals, skin length and quality. The animals that were given 50% of the fat from mackerel offal did, however, have a poorer quality than the control group but they were somewhat longer. Defatted hering offal could be used with a good result with up to 32% in the feed. Defatted ensiled herring offal may probably be used up to a certain level after which the acidity and taste of the feed affect the feed intake of the animals.

Technical Year Report 1997, pp. 43-59. 9 tables, 4 refs. In DANH. Authors' summary translated by Hanne Artved.

The use of ethoxyquin in the highest allowable quantities

Tove Nørgaard Clausen, Carsten Hejlesen

In experiments with the highest allowable quantity of ethoxyquin (150 ppm) in the feed for mink females in the winter period as well as in the nursing period, there was a tendency towards a higher kit loss from birth to weaning and lower kit weights at weaning in the group with the high content of ethoxyquin. Furthermore, the females in the group given the high quantity of ethoxyquin weighed significantly less than the control females from February 20.

Technical Year Report 1997, pp. 61-65. 5 tables, 1 refs. In DANH. Authors' summary translated by Hanne Artved.

Changes in the urine pH of mink kits (7-9 days old) when given different feeds

Tove Clausen, Søren Wamberg

A study of the significance of the feed on the urine pH in mink kits in the early growth period was done. Three groups of ten male kits were given control feed and control feed added extra magnesium oxide (0.144%) or ammonium chloride (0.35%), respectively. pH was measured in the urine. The urine pH of the control group was 6.9. The Mg addition resulted in an increase in urine pH to 7.1 and an increased excretion of crystals in the urine. Addition of NH4Cl in a quantity of 0.35 p.c. resulted in a decrease in urine-pH to 6.1 and no secretion of crystals was seen. Ammonium chloride in the quantity used had no negative effect on feed intake and growth of the kits. A linear correlation

between feed BE (base excess) and the urine pH-value was found. pH = 5.04 + 0.087*BE (in mmol/100 g. of wet feed).

Technical Year Report 1997, pp. 67-75. 5 tables, 3 figs., 5 refs. In DANH. Authors' summary translated by Hanne Artved.

Fat herring offal and defatted herring offal for mink females in the winter and nursing periods

Tove Nørgaard Clausen, Carsten Hejlesen, Christian Friis Børsting, Birthe M.Damgaard, Ricarda Engberg, Søren Krogh Jensen

For the experiment, five groups of 90 young wild mink females were used. In two groups 20% herring offal was used with 63 and 78%, respectively, of the fat from fish fat, and in two groups 20 and 29%, respectively, defatted herring offal in the feed with approx. 20% of the fat from fish. These groups were examined versus a control group with 45% of the fat from fish. The females had been given the corresponding raw materials in the preceding growth and moulting periods. The results showed that these products can be used for kits in the growth period and for young females in the following winter and nursing periods without any negative affect on the reproduction results of the females. Kits that were given defatted herring offal had the best weights at weaning, and in the two groups given herring offal decreasing kit weights at weaning were seen with increasing content of fish fat in the feed.

Technical Year Report 1997, pp. 77-83. 8 tables, 4 refs. In DANH. Authors' summary translated by Hanne Artved.

Light feeding of female kits to improve their later milk production

Carsten Hejlesen, Tove N. Clausen

It was studied whether light feeding of standard female kits in the period from September 3 to pelt grading and/or light feeding of the females in April had any effect on the weight loss of the females during nursing and on kit weight on days 28 and 42. Four groups of 100 females were used. Feed allocation for the four groups in the fall/April was ad lib./ad lib., ad lib./light, light/ad lib. and light/light. A significantly higher percentage of barren females was found with light feeding from September 3 to pelt grading and with light feeding in April compared to ad libitum feeding in the two periods.

Neither light feeding from September 3 to pelt grading nor light feeding in April affected the weight loss of the kits from birth to day 42. But females fed lightly from September 3 to pelt grading had a significantly lower weight loss from birth to day 28 than females fed ad libitum in the same period. Kits from females fed lightly from September 3 to pelt grading had the same weight on day 28 but a higher weight on day 42 than kits from females fed ad libitum in the same period. Kits from females fed lightly in April had the same weight on day 28 and on day 42 as kits after females fed ad libitum in April.

Technical Year Report 1997, pp. 93-99. 3 tables, 6 refs. In DANH. Authors' summary translated by Hanne Artved.

Phase feeding of mink kits in the growth period (protein reduction)

Carsten Hejlesen, Tove N. Clausen, Niels Therkildsen

It was examined whether the content of protein in the feed for mink kits could be reduced from 30 to 25% of metabolizable energy on selected dates between August 15 and October 15 without any negative consequences to skin size and pelt quality. 7 groups of 74 kits were used plus a control group of 100 wild mink male kits. When reducing the amount of protein to 25% of ME, synthetic methionine and cystine were added so that the content in the feed of these amino acids corresponded to the content in feed with 30% of ME from protein. Weight at pelting and skin length were influenced in a positive direction, the later the reduction in protein took place until October 1, but at the same time a negative effect was seen on pelt quality.

Technical Year Report 1997, pp. 101-105. 3 tables. In DANH. Authors' summary translated by Hanne Artved.

Feed with 0.5% acetic acid for mink in the nursing period

Carsten Hejlesen, Tove N. Clausen

It was examined whether addition of acetic acid up to a total content of 0.5% affected the course of the nursing period in relation to the weight loss of the females and the growth of the kits. The experiment included 3 groups of 115 standard females which from May 6 were given feed containing totally 0.20%, 0.35% and 0.50%, respectively, of acetic acid (pH was 6.5, 6.1 and 5.8, respectively).

Neither the weight loss of the females from birth to day 28 and to day 42, nor the weight of the kits on day 28 and day 42 were affected by the experimental treatment. The number of females with nursing disease was not affected by the experimental treatment. The number of females with greasy litters was higher when given 0.35 and 0.50% acetic acid in the feed compared to a total content of 0.20% acetic acid.

Technical Year Report 1997, pp. 111-114. 2 tables, 2 refs. In DANH. Authors' summary translated by Hanne Artved.

Determination of tocopherols in food and feed by Supercritical Fluid Chromatography

Steen Buskov and Hilmer Sørensen

The group of fat-soluble vitamins consists of compounds from the vitamin A-, D-, E- and K-group and are important constituents of food and feed due to their nutritional importance. The vitamin E-group consist of both α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol of which α- and γ-tocopherol are the dominating tocopherols in vegetable oils like rape seed and soyabean oil. Tocopherols are important due to their good antioxidative capability of which one is the function as lipid soluble antioxidant, i.e. to prevent oxidation of double bonds in the unsaturated fatty acid carbon chains in triacylglycerols. Determination of the four tocopherols, α-, β-, γ- and δ-tocopherol and α-tocopheryl acetate has mostly been done by reversed phase HPLC with UV- or fluorescence detection, however with more or less successful separation of the β- and γ-tocopherol isomers. Supercritical Fluid Chromatography (SFC) is a relatively new separation method of which some of the features are shorter analysis time, higher column efficiency, more degrees of freedom in the optimisation step and faster equilibration between column and mobile phase, compared to HPLC. Successful separation of α-, β-, γ- and δ-tocopherol together with α-tocopheryl acetate on a Spherisorb S3 ODS2 150 x 4.6 mm I.D. column has been achieved in about 15 minutes with the SFC-technique. The method has been adopted to determination of tocopherols in various products after extraction by SFE (supercritical fluid extraction) and the combination of SFE and SFC seems to be a promising combination of environmental friendly laboratory methods.

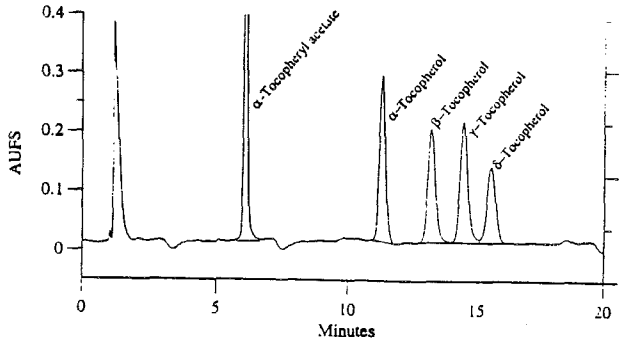


Fig. 1. SFC-chromatogram of a mixture of α-Tocopheryl acetate and α-, β-, γ- and δ-tocopherol.

Technical Year Report 1997, pp. 191-201. 1 table, 9 figs, 20 refs. In DANH. Author's summary.

Determination of water soluble vitamins by Micellar Electrokinetic Capillary Chromatography

Steen Buskov, Hilmer Sørensen, Jens Christian Sørensen, Susanne Sørensen, Preben Lisby Wesseltoft

Vitamins are a structurally heterogeneous group of essential constituents of food, which are required in small amounts for normal growth, maintenance and functioning of animal tissues. Further, many of them are precursors for enzyme cofactors. In addition, vitamins are often relatively unstable and losses therefore occur easily during food preparation, storage and processing, which gives a need for gentle and efficient methods of analysis. These re-

quirements may be fulfilled by capillary electrophoresis methods, and previous work in this field both employ CZE and MECC for separation of vitamins.

The separation of water-soluble vitamins and vitamin co-factors was investigated by micellar electrokinetic capillary chromatography (MECC) and diode array detection using sodium cholate as the micellar phase. A successful separation of 17 water-soluble vitamins and vitamin co-factors was achieved. The linearity and repeatability of the MECC-method was good with correlation coefficients generally greater than 0.999. The separation efficiency was satisfactory with a good resolution ranging from 2 to 45 and a theoretical number of plates varying from 200000 to 480000.

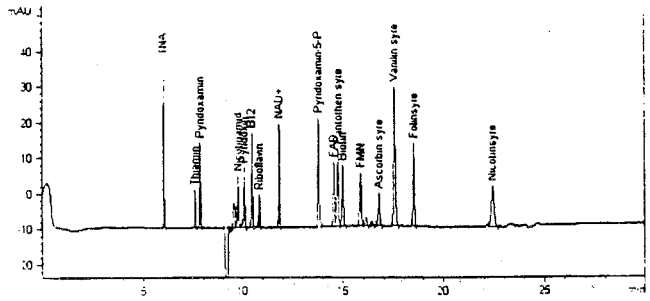


Fig. 1. Electropherogram of a test mixture of vitamins and vitamin cofactors using the MECC system.

Technical Year Report 1997, pp. 203-211. 2 tables, 5 figs, 11 refs. In DANH. Author's summary.

Pathological effects of dietary zearalenone and/or tamoxifen on female mink reproductive organs

B. Yamini, S.J. Bursian, R.J. Aulerich

The efficacy of dietary tamoxifen (TAM) to alleviate the hyperestrogenic effects of the mycotoxin zearalenone (ZEN) was assessed by pathological examination of the reproductive organs of female mink (*Mustela vison*). Mink were fed 20 mg/kg ZEN, 10 mg/kg TAM, or 20 mg/kg ZEN + 10 mg/kg TAM from about 2 mo prior to breeding until the kits reached 3 w of age. All female mink fed ZEN mated, but only 25% whelped. No mink fed TAM or TAM + Zen mated. Postmortem examination revealed moderate to severely distended uteri filled with caseated necrotic substances in the TAM, ZEN and ZEN + TAM fed mink. Histologic examination revealed mild to severe endometrial hyperplasia to uterine atrophy, endometritis, metritis and pyometra. Ovarian follicles were atrophied and degenerated. TAM was not effective in alleviating the hyperestrogenic effects of ZEN but was a potent estrogen agonist in mink.

Fig. 1. Uterus; mink, group 4 dosed with 20 mg/kg ZEN + 10 mg/kg TAM. Extensively distended uterine horns with serosal hemorrhage.

Vet Human Toxicol 39, pp. 74-78, 1997. 8 figs., 15 refs. Authors' abstract.

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

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

We investigated the folate-dependent toxicity of formate to mink to better understand the use of formic acid in fur animal feeds. Folic acid supplemen-

tation (0, 1, 5, 10, and 20 mg/kg of DM) in the feed of weanling mink for 4 wk resulted in hepatic tetrahydrofolate (H_4folate) concentrations of 3.94, 8.51, 9.15, 10.4, and 15.0 nmol/g, respectively (SE 1.03). Oxidation tests in metabolic chambers, preceding a single injection of sodium [^{14}C]formate (500 mg/kg BW), showed that the nonsupplemented mink oxidized formate into CO_2 at a rate of 37% less than that of the supplemented mink. The oxidation rate increased with supplementation level and was maximal, 54.2 mEq·kg^{-1}·h^{-1}) (SE 3.0), at 10 mg of folate/kg; at the highest level of supplementation (20 mg/kg), CO_2 production tended to be lower. Concentration of hepatic ^{14}C increased with the hepatic H_4folate, and its accumulation continued after the highest point of oxidation. These observations indicate that mink oxidize formate readily but at a slightly lower rate than do rats. However, if extra folate is not supplemented in the feed during the period of early intensive growth, hepatic H_4folate level may decline to the levels found in humans and monkeys, which are susceptible to formate accumulation. Average daily weight gain improved with each increase in supplementation of folic acid; however, only the differences between the nonsupplemented diet and the two highest levels of the vitamin reached statistical significance (P<.05).

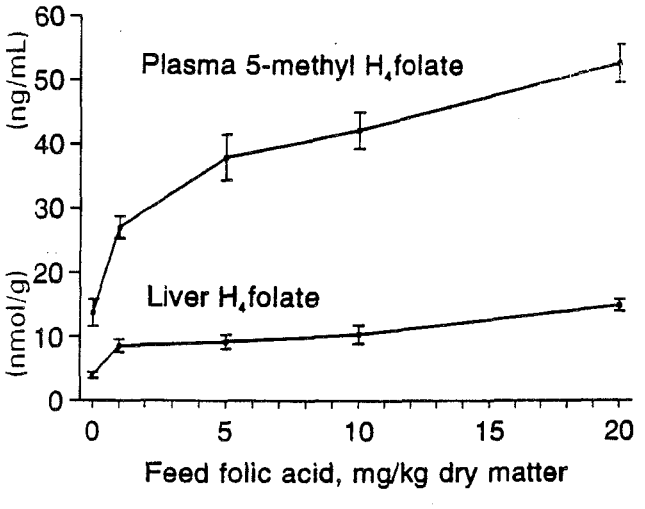


Figure 1. Effect of supplemented folic acid on plasma (5-methyl tetrahydrofolate [H_4folate]) and hepatic folate (H_4folate) concentrations in mink. Each value represents the mean of six animals ± SE.

J. Anim. Sci. 75, pp. 1569-1574, 1997. 2 tables, 5 figs., 21 refs. Authors' summary.

Influence of phytase supplementation on growth and nutrient deposition in monogastrics

C. Wecke, Rosemarie Köhler, F. Liebert, F. Reinisch

In growth and balance experiments with chickens, piglets and rats the influence of phytase supplementation to diets based on maize, soybean meal or combinations of both feedstuffs on live weight gain, feed conversion and phosphorus resp. crude nutrient deposition was estimated. Total P-content of all diets amounted 2,5 ... 3,6 g/kg and percentage of phytate-bound phosphorus was between 58 and 76%.

Generally, phytase supplementation resulted in higher live weight gains and better feed conversion rates. P-balance and P-utilization were markedly improved in all trials and in chickens a significantly higher nutrient deposition was obtained.

Vitamins and additives in human and animal nutrition. 5th Symposium, Jena/Thuringen 28-29 September 1995, pp. 422-427. In GERM, Su. ENGL. 5 tables, 1 ref. Authors' summary.

Effect of storage and treatment on tocopherol content of rapeseed and linseed

F. Schöne, Maria Matthey, G. Flachowsky

Rapeseed and linseed were differently treated and stored for 112 days – treatments intact seed (1) untreated, (2) moistened to 80% dry matter, DM, (3) moistened + 10 g kg^{-1} seed propionic acid, treatments ground seed (4) untreated, (5) + 10 g kg^{-1} propionic acid with (6) 30 g kg^{-1}, (7) 150 g kg^{-1} mineral premix and (8) 150 g kg^{-1} mineral premix + 10 g kg^{-1} propionic acid. At the beginning and at the end of storage the tocopherols α, β, γ, δ and the 8-plastoquinone were analysed by HPLC. The vitamin E of rapeseed (341 mg kg^{-1} DM) consisted of one third α-tocopherol and two thirds γ-tocopherol. The vitamin E of linseed consisted only of γ-tocopherol. There was no storage effect in case of intact seeds, moisture diminished only γ-tocopherol concentration of linseed by a quarter. In ground rapeseed with high mineral premix addition (150 g kg^{-1}) the loss was 57% α-tocopheraol and one fifth γ-tocopherol

during storage period. Propionic acid had no effect. Ground linseed with mineral premix addition diminished γ-tocopherol content by 45% during 112 days storage.

Vitamins and additives in human and animal nutrition. 5th Symposium, Jena/Thuringen 28-29 September 1995, pp. 183-186. In GERM, Su. ENGL. 2 tables. Authors' summary.

Histological detection of the autofluorescence of vitamin A in tissue of pig and mink

Katharina Bonitz, Gerda Gutte, Ingeborg Buchholz, Florian J. Schweigert

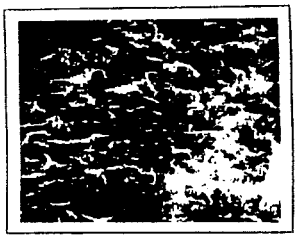


Abb.1: Vitamin A-Fluoreszenz in der Leber vom Schwein (500x)

Abb.1a: Vitamin A-Fluoreszenz in der Leber vom Schwein nach 30 Sekunden Anregung mit UV-Licht (500x)

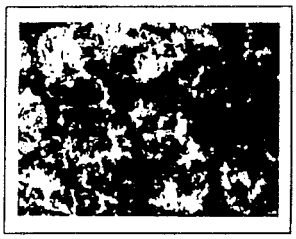


Abb.2: Vitamin A-Fluoreszenz in der Nebenniere vom Nerz (500x)

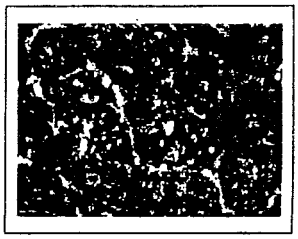


Abb.2a: Vitamin A-Fluoreszenz in der Nebenniere vom Nerz nach 30 Sekunden Anregung mit UV-Licht (500x)

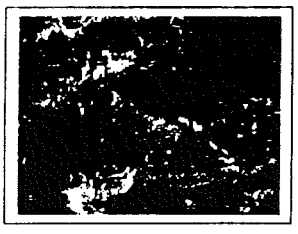


Abb.3: Vitamin A-Fluoreszenz im Uterus vom Schwein (500x)

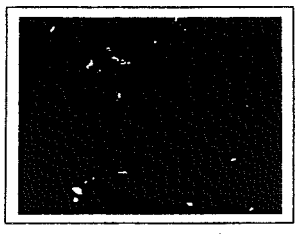
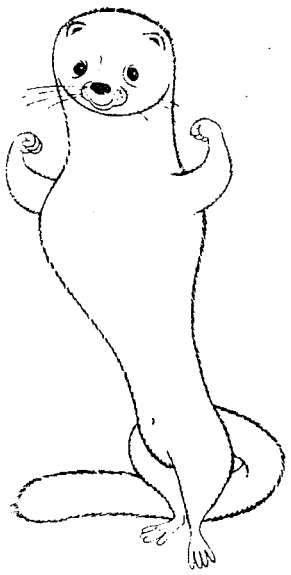


Abb.3a: Vitamin A-Fluoreszenz im Uterus vom Schwein nach 30 Sekunden Anregung mit UV-Licht (500x)

The histologic distribution of the autofluorescence of vitamin A in native cryosections of liver, kidney, adrenal, testis and uterus of pig and mink was detected. Based on the intensity of the yellow-green fluorescence obvious quantitative differences between species and tissues were observed. When comparing tissues of pig and mink a generally higher intensity of fluorescence was found in tissues of mink. In both species the highest intensity of fluorescence was observed in the liver, followed by adrenal, kidney, testis and uterus. In the liver fluorescence was located in the perivascular region, in the adrenal in spongiocytes, in the uterus in secrete of uterine glands. In testis and kidney fluorescence was found but not clearly associable with any cellular structure.

Vitamins and additives in human and animal nutrition. 5th Symposium, Jena/Thuringen 28-29 September 1995, pp. 67-72. In GERM, Su. ENGL. 1 fig., 11 ref. Authors' summary.



Original Report

Reversion to Virulence of an Attenuated Mink Distemper Virus Vaccine Induced by Rapid Serial Passage in Ferrets

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Summary

A commercial chicken embryo adapted mink distemper virus vaccine was reverted to virulence by serial passage in susceptible ferrets. While the febrile response of the ferrets increased in succeeding passages, clinical signs of distemper were not apparent until the 14th serial passage. By the 21st passage all of the inoculated ferrets succumbed, and distemper inclusions were demonstrated in the urinary bladder and bronchi. The passaged virus was transmissible by contact to susceptible ferrets. A considerable difference between the early and late passage viruses was observed in the sequential replication of CDV in ferrets. The fraction of single virulent pocks collected from the chorioallantoic membrane increased concurrently with ferret back passages. The 35th ferret passaged virus, however, lost its virulence for ferrets by 4 serial reattenuation passages in fertile eggs.

Introduction

It is a constant concern to all proponents of live virus vaccines that it is theoretically possible for any attenuated live virus vaccine to revert to virulence. The purposeful reversion of live rinderpest vaccine to virulence in a natural host may have been the initial published report. Hale and Walker[1] reverted egg adapted rinderpest vaccine to virulence following 6

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back passages in calves. Nakamura and Kishi[2] enhanced the virulence of lapinized/egg propagated rinderpest vaccine after 25 serial passages in rabbits.

Torrey *et al*[3] reported that 4 rabbit origin hog cholera vaccines regained their virulence for swine after 5,9,17, or 18 passages in separate trials. Pigs vaccinated with 4 vaccines shed virus and susceptible pigs in contact with vaccinated pigs were infected. An attenuated vaccine for Venezuelan equine encephalomyelitis vaccine (TC-83) was serially back passed in horses[4]. The clinical response increased and a higher level of viremia was recorded during 5 back passages. Goto *et al*[5] reverted a commercial canine distemper virus (CDV), embryonated egg adapted, vaccine to high virulence by 14 serial back passages in ferrets. Reversion to virulence of the Rockborn dog kidney cell attenuated CDV vaccine virus was demonstrated after 6 serial passages in dogs[6] and 10 passages in dog lung macrophages.

Woolcock and Crighton[7] reviewed previous duck hepatitis virus reversion trials that demonstrated reversion to virulence with several chicken embryo passaged strains that had the potential for duck hepatitis vaccines. The enhanced virulence was always detected within 4 serial duckling passages. An infectious bursal disease live virus vaccine of about the 50th chick fibroblast passage increased bursal lesions after 6 serial passages in one day old chicks. The passaged virus was also spread to contact birds[8]. Six serial passages of 2 infectious bronchitis vaccine strains in 21 day old chickens enhanced the capacity of the vaccine to increase the incidence and severity of *Mycoplasma synoviae* airsacculitis[9]. Witter and coworkers reported 2 attenuated Marek's disease vaccine viruses that showed increased pathogenicity during 5 serial back passages in chickens[10].

There has been at least one report of a live human vaccine back passaged in children. After 20 consecutive transfers through the intestinal canal of healthy susceptible children, Types 1, 2, and 3 oral poliomyelitis vaccine strains had increased neurotropism for monkeys[11].

The present work describes the procedures used to revert an attenuated chicken embryo propagated distemper vaccine to virulence in ferrets and its reattenuation in chicken embryos.

Materials and methods

Ferrets. European ferrets (*Mustela putorius*) of both sexes, from the Washington State University stock colony in which canine distemper (CD) had not previously been recognized, were used at approximately 12 months of age. Sentinel ferrets from the colony had no CDV serum neutralizing antibody. All ferrets were kept in isolation, except where contact infection trials were conducted.

Viruses. The original inoculum for the reversion trials was 1.0 ml of a commercial egg adapted distemper vaccine (64th passage of the Wisconsin FXNO strain) containing $10^{5.5}$ 50% egg infective doses (EID_{50}). Green's distemper strain, which is highly virulent for ferrets, was used as the challenge virus in determining immunity[12]. Criteria for distemper in ferrets included purulent conjunctivitis, nasal exudate, skin rash, profound depression, anorexia, and death. All diagnoses were confirmed by demonstrating distemper inclusion bodies in microscopic sections of epithelium of the urinary bladder and bronchi strained with hematoxylin and eosin and trichrome staining[13].

Inocula for serial passage and virus titration. A 20% (w/v) homogenate of lung and spleen from inoculated ferrets was made with Earl's solution containing 0.5% lactalbumin hydrolysate and antibiotics. The suspension was centrifuged at 4,000 xg for 10 minutes. The supernatant fluid was kept at -60°C for further passage or virus titration. Suspensions of spleen, lung, liver, kidney, mesenteric lymph nodes, and brain were prepared in a similar manner and were used to record viral distribution and replication.

Method of serial passages. For each of 35 serial back passages, 3 ferrets were inoculated intraperitoneally (i.p.) at 7 day intervals with 1.0 ml of a 20% spleen and lung suspension pooled from 2 of the 3 previously passaged ferrets. The 3rd ferret for each passage was used as an indicator ferret, and if it remained normal for 30 days, it was later challenged i.p. with 0.5 ml (100 50% lethal doses) of virulent CDV to determine if the ferret was immune to challenge.

Virus titration in embryonated eggs. Ten-fold serial dilutions were made from tissue homogenates from each ferret, and 0.2 ml of each dilution was inoculated by the stab method[14] into 7-day-old developing chick embryos (8 eggs per dilution). After incubation at 37°C for 7 days, the chorioallantoic membrane (CAM) was examined in a Quebec colony counter and a membrane with identifiable pocks was considered to be infected with CDV. Virus titers were expressed as EID_{50} per ml of 20% tissue homogenate. Using 10 fold dilutions and 8 eggs per point, Lo *et al*[15] determined the 95% confidence interval for EID_{50} to be $10^{0.63}$ EID_{50}. Therefore viral titers differing by more than $10^{0.63}$ were considered significantly different.

Determination of the virulence for ferrets of heavily infected CAM's or single pocks from an infected CAM. Six to 8 infected CAM's from the 1st to 25th passages were inoculated onto the CAM of embryonating eggs. The infected CAMs were harvested and homogenized with Earl's solution, and 1.0 ml of the homogenate of each passage was inoculated i.p. into 2 ferrets to determine the virulence of the passaged virus.

Embryonating eggs were also inoculated with inocula from the 1st, 8th, or 30th passages, and isolated single pocks removed and ground individually in 1.5 ml of 5% glycerin phosphate buffer, pH 7.4. One ml of each suspension was then inoculated i.p. into ferrets to determine the virulence of a individual pock. Also, 0.2 ml of the same isolated pock suspension was inoculated onto the CAM of embryonated eggs to determine the viability of the pock.

Results

Serial Back Passage In Ferrets To Select For Virulent CDV Variants

The reversion trial was instituted by inoculating each of 3 ferrets with 1.0 ml ($10^{5.5}$ EID_{50}) of a chicken embryo adapted CDV vaccine. From then on three ferrets (a,b,c) were inoculated with the spleen/lung homogenate of the previous passage. Two ferrets (a,b) were killed on the 7th day to provide inoculum for the succeeding passage. An incubation period of 7 days was selected because multiplication of the attenuated embryonated egg adapted CDV peaks at about 6-9 days in the vaccinated ferret[16]. One ferret (c) was not killed in order to observe the course of the disease and to serve as an indicator of a particular passage. If the indicator ferret showed no CD signs it was challenged with virulent CDV to determine its immunity. If the indicator ferret succumbed to CD, the passage level inoculum was considered to contain virulent CDV.

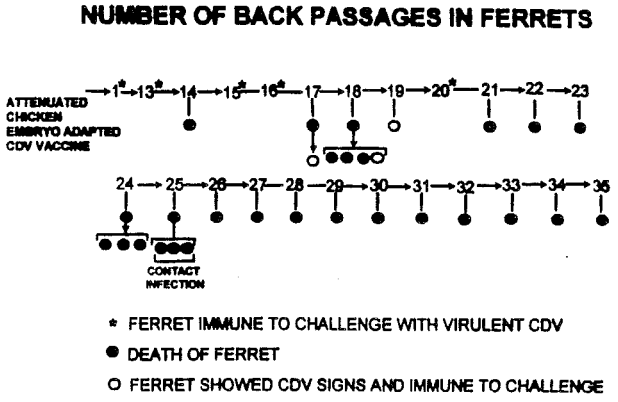

Fig. 1. An attenuated CDV vaccine which produced no signs of CD infection in ferrets, mink, and dogs was used as a source of CDV to initiate ferret back passages.

The results of serial back passage of attenuated CDV in ferrets are given in FIGURE 1. Clinical signs of distemper were not observed in passaged ferrets of the 1st through the 13th serial back passages, and all passaged indicator ferrets were immune when challenged with virulent CDV.

The indicator ferret of the 14th back passage showed clinical signs and succumbed to distemper. Ferrets inoculated with the 15th and 16th back passage inocula were not affected and were immune to challenge. The indicator ferret that received 17th back passage inoculum developed CD signs and died. A spleen-lung suspension from this ferret was inoculated into a susceptible ferret that showed signs of CD, subsequently recovered and was immune to virulent CDV challenge. The ferret inoculated with the 18th back passage inoculum died of CD, and a spleen-lung suspension from this ferret was inoculated into 4 susceptible ferrets. Three died of CD, but the 4th ferret showed CD signs, recovered and was immune to virulent CDV

challenge. The 19th passage ferret also showed signs of CD and was immune to challenge. The 20th passage indicator ferret showed no CD signs and was immune to challenge. From the 21st through the 35th back passages, all indicator ferrets exhibited clinical signs and died of distemper within 12-17 days p.i.

Contact transmission by shedding of CDV was demonstrated by placing the indicator ferret of the 25th back passage in the same pen with 3 susceptible ferrets. All three contact exposed ferrets later died of distemper.

The Temperature Response Of Inoculated Ferrets.

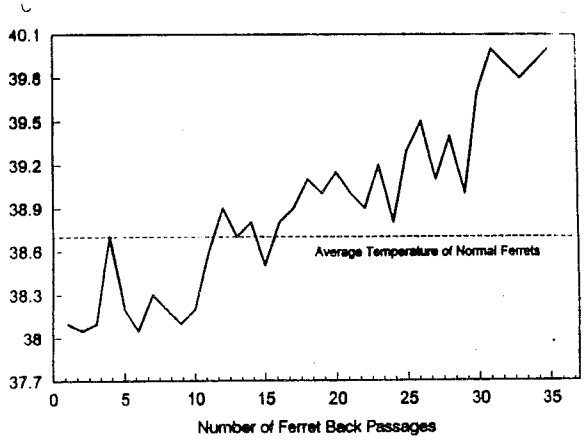

Fig. 2. The average rectal temperature of three ferrets used for each of the 35 serial back passages. The temperatures were only recorded on the 7th day after inoculation.

Rectal temperatures were taken from the 3 (a,b,c) ferrets used in the serial passage trial on the 7th day p.i. and averaged for passages 1 - 35 (FIGURE 2). The average temperature of normal ferret is ~ 38.7°c. The trend in temperatures increased concurrently with the increasing number of back passages. From the 1st to the 17th back passage, the average temperature was lower or nearly the same as the temperature of normal ferrets. At the 18th - 26th passage level, the average temperature ranged from 39°c to 39.3°c. After passage 27, the temperature response increased to a maximum of 40°C -- a temperature that is consistent with ferrets infected with highly virulent CDV.

Virus Titers Of The Inocula Used For Passaging CDV In Ferrets

Virus titrations of spleen-lung mixtures from passages 1 - 35 were recorded (FIGURE 3). Values increased gradually from $10^{3.9}$ to $10^{4.2}$ EID_{50} per ml (abbreviated to $10^{4.2}$ hereafter) during the 1st to 7th passages. The titer of the 10th passage reached a maximum of $10^{5.3}$, and this value was maintained with slight variation through the 19th passage, after which titers decreased to approximately $10^{3.0}$ at the 35th passage.

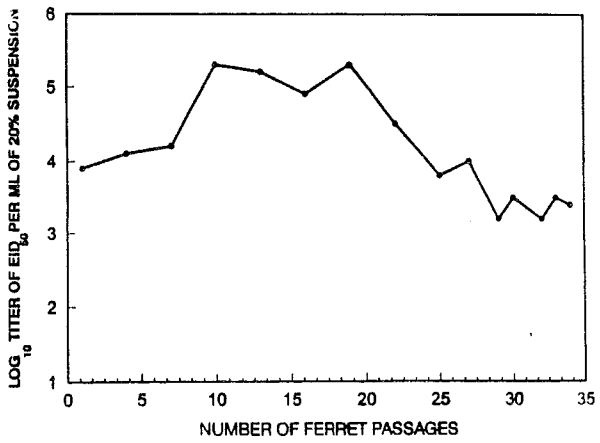

Fig. 3. The virus titers of the spleen-lung inocula for passages 1-35 when titrated on the CAM of developing chicken embryos.

CDV Titers In Tissues Of Ferrets Inoculated With Passaged Virus

Selected tissues from ferrets inoculated with the 1st, 15th, and 30th passages of CDV had virus titers which reached a maximum at the 15th passage and declined at higher passages. Virus titers in the spleen, lung, liver, and kidney of a ferret inoculated with the 30th passage of CDV were lower than those measured at the 1st and 15th passages, while titers of the mesenteric lymph nodes and brain remained nearly the same (TABLE 1).

Table 1. Replication of CDV in selected ferret tissues with increasing back ferret passages

Tissue*	EID_{50} titer(log_{10})/ml of 20% suspension from ferrets at indicated back passage		
	1st	15th	30th
Spleen	3.7	4.1	2.5
Lung	4.2	4.4	2.7
Liver	2.0	2.3	1.2
Kidney	2.2	2.4	1.5
Mesenteric lymph nodes	4.7	5.2	4.9
Brain	1.8	2.0	1.8

*All ferrets were killed 6 days after inoculation of a tissue suspension.

Replication Curves Of Early And Late Passaged Viruses In Ferrets

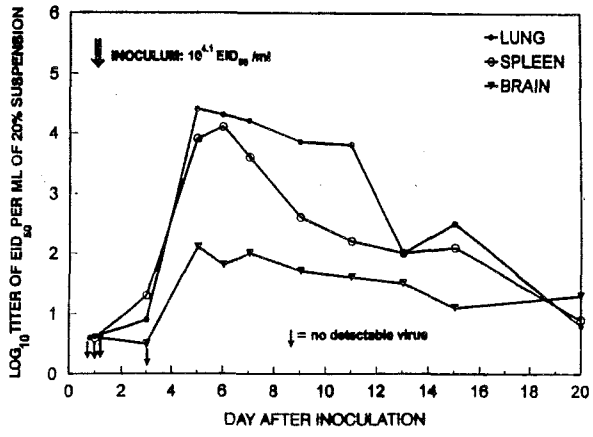

Fig. 4. Replication of the 4th serially back-passaged CDV in ferrets.

The 4th and 27th ferret passaged viruses were selected for sequential replication studies. The spleen, lung, and brain of ferrets killed at 2-day intervals (1 ferret/interval) were titrated in embryonated eggs. The virus titers of the spleen, lung, and brain from ferrets inoculated with the 4th passage increased rapidly between 3 - 5 days p.i. With peak titers of $10^{4.2}$ occurring in 5 - 7 days p.i. $10^{4.4}$, and $10^{2.2}$ respectively, and declined thereafter (FIGURE 4). The virus titers for the spleen and lung inoculated with the 27th passage reached a peak of $10^{3.7}$ and $10^{4.1}$ respectively, in 7 - 11 days p.i. and remained at high values until death occurred on the 13th day p.i. (FIGURE 5). The virus titer of the brain increased gradually to $10^{2.2}$ on day 7, reached a maximum of $10^{3.1}$ on day 11, and declined to $10^{2.2}$ on day 13.

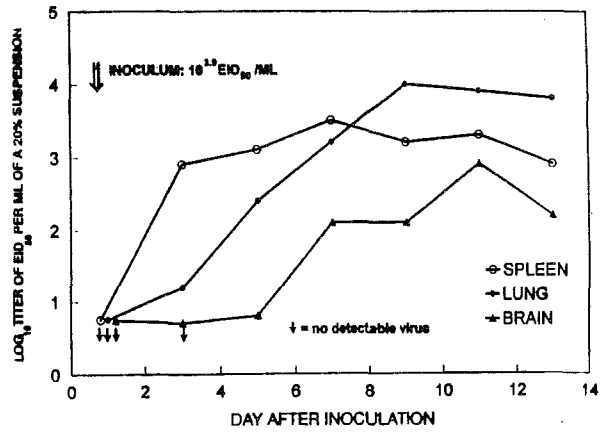

Fig. 5. Replication of the 27th serially back-passaged CDV in ferrets.

Infectivity of CAM's Inoculated With Ferret Passaged CDV

Suspensions prepared from heavily infected CAMs inoculated with the 1st, 10th, 13th, 16th, 19th, 22nd, and 25th passages were each inoculated into 2 susceptible ferrets. From CAM passages 1 - 13, no clinical signs were noted, and the ferrets were immune to the virulent CDV challenge. At passage 16, distemper signs developed in both inoculated ferrets, however they recovered and were immune to virulent CDV when challenged. At passage 19, 1 of 2 ferrets had distemper signs and succumbed; the other was immune to challenge. Both inoculated ferrets that received the 22nd and 25th back passage inocula died of distemper.

Inoculation Of Ferrets With Single Isolated Pocks Collected From The CAM.

Results of the CAM single pock experiment are shown in Table 2. When inocula prepared from each of 40 pocks obtained from passage 1 were inoculated into a ferret, all 40 ferrets remained clinically normal and were resistant to challenge with virulent CDV. At the 8th passage, 1 pock of 14 (7%) caused CD. Four of 13 ferrets (31%) inoculated with single pocks of the 18th passage and 11 out of 18 (61%) ferrets inoculated with 30th passage single pocks either showed clinical signs of CD or died.

Table 2. Results of inoculation of single pocks from CAM's inoculated with a spleen/lung suspension of back passaged CDV

Passage level in ferrets	Number of ferrets inoculated with single pocks*	Result of inoculated ferrets		
		Immune**	CDV Death	% CDV Lethal Pocks
1st	40	40	0	0.0
8th	14	13	1	7.0
18th	13	9	4	31.0
30th	18	7	11	61.0

*A live single pock was confirmed by reinoculation on the CAM.

**Immunity determined by challenge with virulent CDV.

Reattenuation Of Ferret Passaged CDV In Embryonated Eggs

Reattenuation of the 30th ferret back passaged CDV was accomplished by serial passage in embryonated eggs (FIGURE 6). A homogenate of the heavily infected CAMs of each passage was inoculated into 2 susceptible ferrets. At passages 1 - 4, all the ferrets showed CD signs and died within 11 - 14 days p.i. Ferrets inoculated with passages 5 - 11, showed no CD signs and were immune to challenge with virulent CDV.

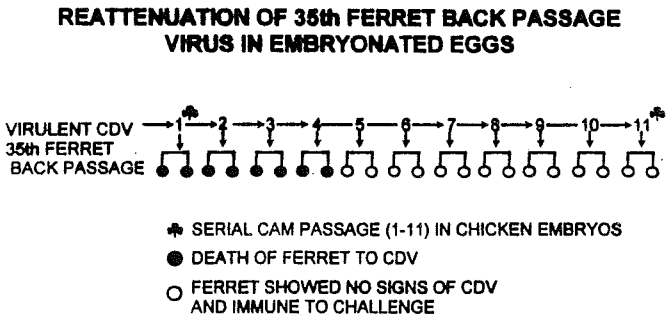

Fig. 6. The highly virulent 35th ferret back-passage lost its virulence for ferrets after 4 serial passages in embryonated eggs.

Discussion

It is apparent that rapid serial passage in ferrets reverted an attenuated canine distemper vaccine to virulence. The first evidence of reversion was at the 14th passage when the inoculated ferret died of distemper. After the 16th passage, virulent virus predominated in the passage inocula. While transmissibility to contact ferrets was demonstrated at the 25th passage, there is little doubt that shedding of virulent virus was possible at 14th or perhaps at an earlier passage. The temperature response was recorded only on the 7th day after inoculation of the ferrets employed for passage. The temperature response increased with the passages which correlates with enhanced virulence.

It was desirable, but not feasible, to titrate the inocula at each back passage in both ferrets and embryonated eggs. Avirulent and virulent CDV replicates on the CAM, but for a more accurate estimate of the revertant virulent virus load, ferret titrations are necessary.

A suspension prepared from a single isolated pock was considered to contain virulent virions if an inoculated ferret exhibited signs or succumbed to CDV. If a similar suspension immunized a ferret, the virion population was considered avirulent. There is little doubt that individual pocks contain both avirulent and virulent virions but we assume that the predominating virion population causes disease or immunizes.

The present investigation was done prior to the availability of procedures to determine genetic markers for virulence or attenuation during CDV replication in ferrets or embryonated eggs. Current studies have examined the molecular aspects of neurovirulence factors in measles -- a related morbillivirus[17].

The virus titer in EID_{50} of the spleen/lung inocula used in each succeeding back passage declined as well as the titers in the spleen, lung, liver, and kidney. On the other hand, the percentage of pocks containing virulent virions at a level sufficient to kill ferrets increased markedly with passages, which

suggests that virulent virions selectively replicated in ferrets. No pocks were lethal at the first ferret passage, but at the 8th back passage 7% caused CD, at the 18th passage 31% and at the 30th passage 61% of the pocks caused CD when inoculated into ferrets. It is not clear whether the reduction in CAM titer can be explained by the CAM being a less sensitive indicator for virulent virions.

The replication of CDV in the lung, spleen, and brain of ferrets inoculated with the 4th passage virus was determined by CAM titration. The CDV peaked at days 5 - 7 p.i. and declined due to the ferrets immune response. Conversely, the pathogenesis following the inoculation of 27th passage inocula was consistent with that of virulent distemper, i.e. there was an increase in the virus titer of the spleen, lung, and brain until death ensued on the 13th day p.i.

The changes from a CDV virulent field isolate to attenuation in embryonated eggs, then virulence for the ferret and then reattenuation could be explained by genetic changes in the viral population or by the presence of virus types that can selectively replicate in embryonated eggs or ferrets with the resultant predominant viral population determining virulence or attenuation. In these trials it seems less likely that genetic changes in the virus occurred. Since reattenuation only required 4 serial passages in embryonated eggs. However, differentiation between there two mechanisms would require genomic sequences of biologically active cloned strains. That this attenuated CDV would revert to virulence after a few serial passages in ferrets is a safe assumption.

Attenuation without loss of immunizing potential is of critical importance in formulating live virus vaccines. Unpublished anecdotal experiences have suggested that when chicken embryo propagated CDV was passaged excessively past the attenuation point, the CDV replicated well but the vaccine failed to effectively immunize. Conversely, consideration might be given to the prospect of using CDV vaccine containing a limited number of virulent virions. Vaccines prepared from seed virus near the attenuation point might more effectively immunize young dogs or mink particulary if they have low levels maternal antibody at the time of vaccination. The USDA Animal Plant Health Inspection Service requires a minimum of 5 serial back passages of the seed virus used for producing vaccines[18]. The most susceptible animal species must be used to provide assurance that the vaccine will not revert to virulence and shed to contact animals. Despite the reversion to virulence at the 14th passage of the CDV vaccine used in the present trial, this vaccine would meet the USDA back passage requirement which assures the safety of the vaccine.

This research was supported in part by the Mink Farmers Research Foundation.

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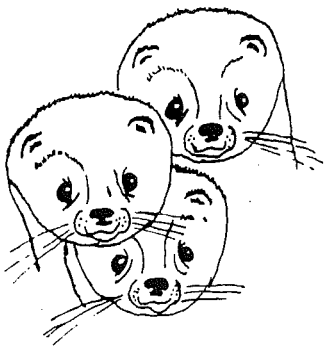

Original Report

Villous atrophy in the small intestine of mink kits with diarrhea

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Abstract

A severe diarrhea of unknown etiology often affects young mink kits in many Swedish mink farms. The small intestines of 33 kits from 15 litters with diarrhea were analyzed histologically. All the animals had microscopical lesions predominantly localized to the intestinal villi. Degeneration and desquamation of epithelial cells at the tips of the villi were seen in 25 kits (76 %) and severe atrophy of the villi appeared in 16 animals (48 %). Other lesions observed were fusion of villi, subepithelial edema, inflammatory cells in the villous propria and eosinophilic inclusions in the cytoplasm of epithelial cells. One animal showed epithelial cell hyperplasia at the tips of the villi. The appearance of the intestinal lesions suggests that they possibly could have been caused by a rotavirus. Further studies of the etiology and pathogenesis are, however, required.

Introduction

In many Swedish mink farms young kits are often suffering from a disease with diarrhea at the age of about 1 - 5 weeks. The animals may be severely affected and even die if not properly treated. As the skin of the diarrhetic animals also becomes moist, the sick animals are sometimes called "wet kits", "sticky kits" or "greasy kits". Extensive studies of the disease have been made from the microbiological points of view (*Henriksen 1988, Svansson 1991, Riis Olesen et al 1992, Dietz & Rattenborg 1996, Jörgensen et al 1996).* Thus, a variety of bacterial organisms as well as reovirus, calicivirus and coronavirus have been isolated from the faeces of diarrhetic kits. In spite of that, however, no single factor has been claimed to be the cause of the disease. Instead, a multifactorial etiology has been suggested *(Henriksen 1987, 1988, Hyldgaard-Jensen 1989, Aldén & Mejerland 1997).*

According to the literature, the morphology of the disease has not been the subject of more extensive studies. However, a non-specific catarrhal enteritis with hydropic epithelial cell degeneration at the upper third of the intestinal villi and mononuclear cell infiltration in the villous propria have been described (*Henriksen 1987, 1988, Riis Olesen et al 1992).*

The aim of this study was to make a systematic histological investigation of the intestines of mink kits with diarrhea in order to shed some light on the etiology and pathogenesis of the disease.

Material and methods

The study was performed on animals suffering from diarrhea in 5 different mink farms in the southern part of Sweden. From each of 15 different litters with diarrhea, 1 - 3 kits in bad condition were sacrificed by the local veterinarian. The thoracic and peritoneal cavities were opened and the entire bodies were put in 10 % formalin for transportation to the laboratory. Totally 33 animals from the age of 17 - 32 days were studied. From each animal forty cm of the posterior part of the small intestine, comprising a major part of the jejunum and ileum, was dissected and divided in 5 equal pieces, aborally numbered 1 - 5. From each of these 8 cm long intestinal sections 6 cross-cut samples were taken at regular intervals, processed according to conven-

tional histological technique and stained with hematoxylin & eosin and periodic acid according to Schiff (PAS). Some samples were also taken for frozen sectioning and stained with Scharlach rot for lipids.

Results

Histologically, intestinal lesions were found in all the animals. The lesions were of different kinds, and appeared with great variation between the animals as well as between different intestinal sections within the same individual animal. The lesions were mostly localized to the intestinal villi.
Mainly four different types of prominent lesions were noted.

1. Degeneration and desquamation of epithelial cells, predominantly at the tips of the villi (Figs. 2, 5 [see legends]), were seen in 25 animals (76 %). The epithelial cells were low columnar or cuboidal in shape. Their nuclei were pycnotic or karyorrhectic and the cells had sometimes lost their connection with the basal membrane. This process made the tips of the villi irregular in shape with single cells or group of cells protruding from the villous tips. Some of these degenerating cells had become detached from the villi and appeared in the intestinal lumen, as single cells or in sheets of cells, and sometimes caught in stripes of mucus or covering lumps of mucus (Figs. 1, 2). Some villi were partially fused (Fig. 7). There was often a subepithelial edema in the villi (Figs. 2, 8) and in the villous propria slight amounts of inflammatory cells, granulocytes and/or mononuclear cells, sometimes appeared. No lesions were observed in the epithelium of the crypts.

2. Atrophy of the villi (Figs. 3, 4, 7) was seen in 16 animals (48 %). The villi appeared thin, short and blunt and were often fused at their lateral surfaces. The villous epithelial cells were poorly differentiated and low columnar, cuboidal or squamous in shape. The epithelial cytoplasm sometimes contained droplets of fat. In the crypts, there were no signs of degeneration of the epithelial cells. Instead, the epithelium in the crypts was often hyperplastic with an increased number of mitotic figures and increased cytoplasmic basophilia. In the villous propria a few granulocytes and/or mononuclear cells were often seen (Fig. 4). There were great variations in appearance, severity and distribution of the atrophy between different intestinal sections of the same animal, but sometimes also within the same intestinal section of the individual animal (Fig. 7).

3. Intracytoplasmatic eosinophilic and PAS-positive inclusions were seen in a great number of epithelial cells of mostly non-atrophic villi in 6 animals (18 %, Fig. 8). The inclusions were localized within vacuoles. They varied in size and were homogenous in structure. They were unaffected by treatment with diastase.

4. Focal hyperplasia of villous epithelial cells was seen in intestinal sections numbers 4 and 5 of one animal (Fig. 6). At the tips of the villi there were groups of cells with large foamy cytoplasm and irregularly shaped and often basally located nucleus. The hyperplastic cells seemed to encroach upon the space of the regenerating cuboidal epithelial cells.

Other lesions observed less frequently included:

- Partial lateral fusion of non-atrophic villi (Fig. 7).
- Slight amount of mononuclear cells and/or granulocytes in the propria of the villi (Figs. 6, 7).
- Subepithelial edema in the villi (Figs. 2, 8).
- Increased number of goblet cells and increased amount of mucus in the crypts and in the intestinal lumen (Figs. 1, 2, 7).
- Multiple small accumulations of mucus with some leukocytes in distended intestinal crypts forming so called crypt abscesses, in one case.

Discussion

The most severe lesion noted was atrophy of the intestinal villi. This villous atrophy is a well known phenomenon in animals as well as in man. It is a consequence of contraction of the villi, which in turn is a result of an insufficient amount of epithelial cells available for covering the villi *(Barker et al 1992)*.

Normally, the villous epithelial cells are derived from a proliferative compartment in the intestinal crypts. From there the cells move along the villi towards the tips. After some time at the tips the epithelial cells are lost by shedding into the intestinal lumen. Normally, there is a dynamic equilibrium between the rate of movement of the epithelial cells from the crypts and onto the villi, and the rate at which the cells are lost from the tips of the villi, re-

sulting in a quite stable topography of the intestinal mucosa *(Barker et al 1992)*.

A deficiency of epithelial cells for covering the villi may principally depend on decreased production of cells in the crypts or increased loss of cells at the villous tips. In this study, there were no degenerative lesions or signs of decreased proliferative activity in the crypts. Instead, in sections with villous atrophy, crypt epithelial cell hyperplasia was seen indicating an increased rate of cell turnover. At the tips of the villi, degeneration and desquamation of epithelial cells appeared in the majority (76 %) of the animals and degenerated and shedded epithelial cells could also be seen in the intestinal lumen. Thus, the observed villous atrophy seems to be a result of increased loss of apical epithelial cells. In spite of the increased proliferative activity in the crypts, the irregularity of the shape of the tips may be a consequence of the fact that the regenerative capacity for replacing shedded epithelial cells in time was insufficient.

The accumulation of lipid droplets in the cytoplasm of enterocytes on atrophic villi probably reflects deficiency in the mechanism of fat absorbtion (*Andrews 1993)*.

In humans, villous atrophy in its most severe form is seen par excellence in gluten-sensitive enteropathy (*Whitehead 1985*). In young domestic animals atrophy of intestinal villi usually is associated with intestinal infection with certain viruses. Intestinal infection with bacteria may also cause villous atrophy, which then, however, is usually combined with other more severe mucosal lesions of vascular and cellular nature. Bacterial induced intestinal lesions are usually also rather homogenous in appearance in contrast to those caused by virus. The great variation in appearance, severity and distribution of the intestinal lesions in this study is thus more similar to those following infection with virus (*Barker et al 1992)*.

The most common viral infections leading to intestinal villous atrophy in young domestic animals are those caused by corona- and rotaviruses. In principle, both these infections give similar morphological lesions, and to separate them only on morphological grounds is not possible. At least in the calf, however, the coronavirus, but not the rotavirus, is said to affect also the crypt epithelium, impairing the regeneration of villous epithelium (*Blood & Radostits 1989*). In this study, the crypt epithelium was normal or hyperplastic. Thus, if the reactions in the mink are comparable with those in the calf, the lesions found here were more similar to those caused by rotavirus than to those caused by coronavirus.

Intestinal villous atrophy may also be seen in mink virus enteritis (MVE, *Krunajevic' 1970*), an infection with parvovirus 2. In MVE infection, however, the primary lesions are located to the epithelium of the crypts (*Macartney et al 1984*), which was not the case in this study. The young kits in this study were also expected to be protected from MVE infection as their mothers were prophylactically vaccinated against MVE as kits *(Mejerland 1997)*.

The result of villous atrophy is a reduced area of functional epithelial cells, causing malabsorption. The net effect of this is diarrhea resulting in a life threatening condition of dehydration, electrolyte depletion and acidosis *(Barker et al 1992)*.

The eosinophilic inclusions in the villous epithelial cells of six animals (18 %) were localized to intracytoplasmatic vacuoles and mostly present in non-atrophic villi. They were unaffected by diastase treatment and thus did not contain glycogen. *Dietz & Rattenborg (1997)* found similar inclusions in mink kits with diarrhea but also, in higher frequency, in healthy mink kits. Similar inclusions have been observed also in the piglet (*Järplid,* unpublished observation). The nature and significance of these inclusions has not yet been determined

Conclusion

The etiology of the severe diarrhea in young mink kits is still unknown. Multiple etiological factors like virus, bacteria, imbalance in feed composition and hygienic conditions, and a combination of such factors, have been suggested (*Henriksen 1988, Hyldgaard-Jensen 1989, Riis Olesen et al 1992, Aldén & Mejerland 1997)*. The appearance of the intestinal lesions reported here suggest that they possibly could have been caused by a rotavirus. Further studies of the etiology and pathogenesis of the disease are, however, required.

Acknowledgements

The assistance of Dr Ann-Charlott Ekholm, Skara, is gratefully acknowledged.

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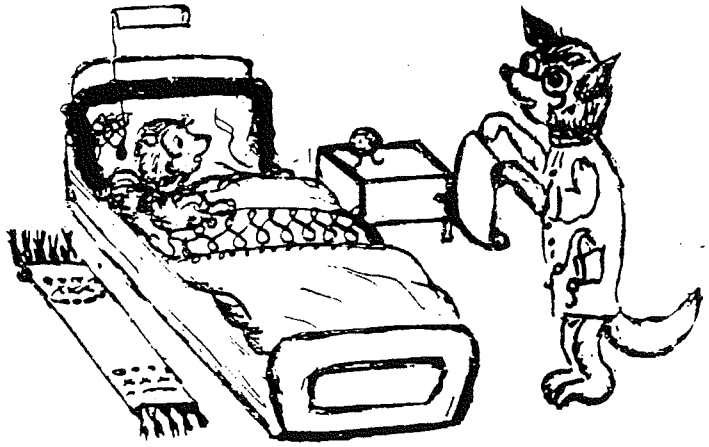

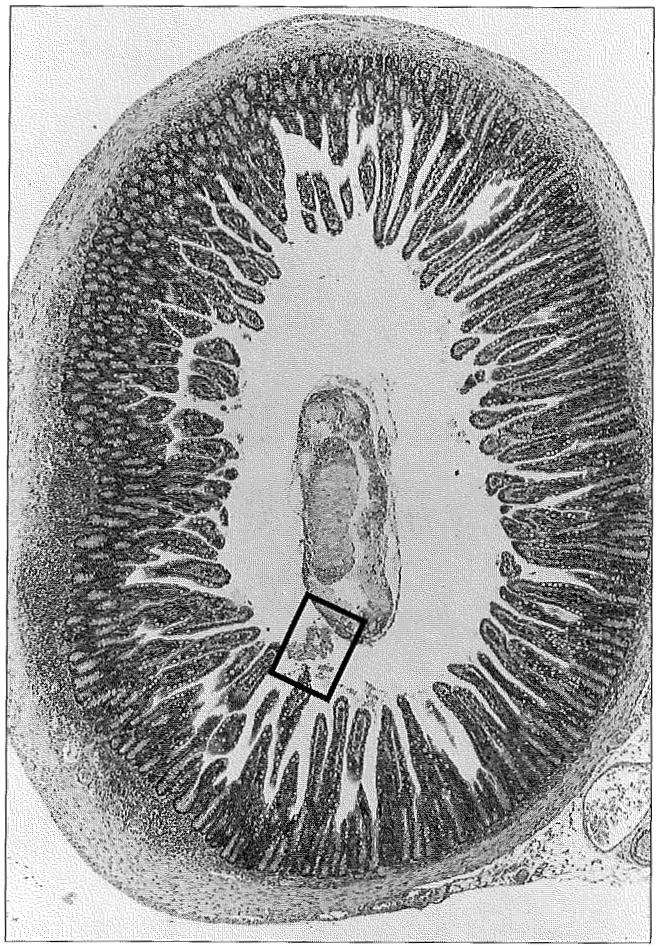

Fig. 1. Small intestine. The villi are slightly shorter than normal. In the lumen a lump of mucus is mixed with sheets of desquamated epithelial cells. H&E x 35.

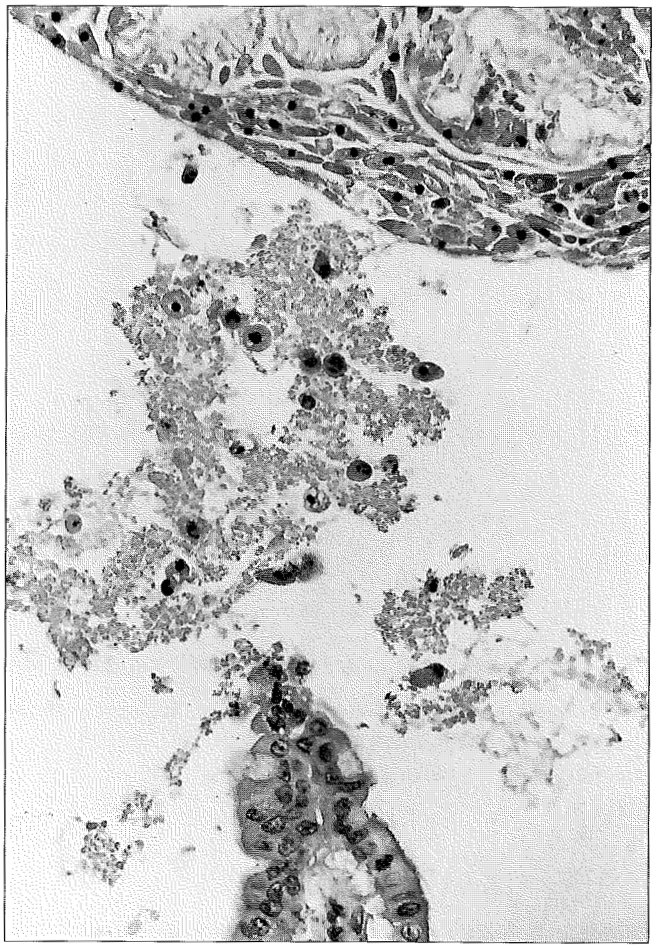

Fig. 2. Detail of Fig. 1. Tip of a villus (bottom) with degenerated epithelial cells. Shedded epithelial cells are free in the lumen (centre) or caught in the mucus (top). Edema in the villous propria. H&E x 350.

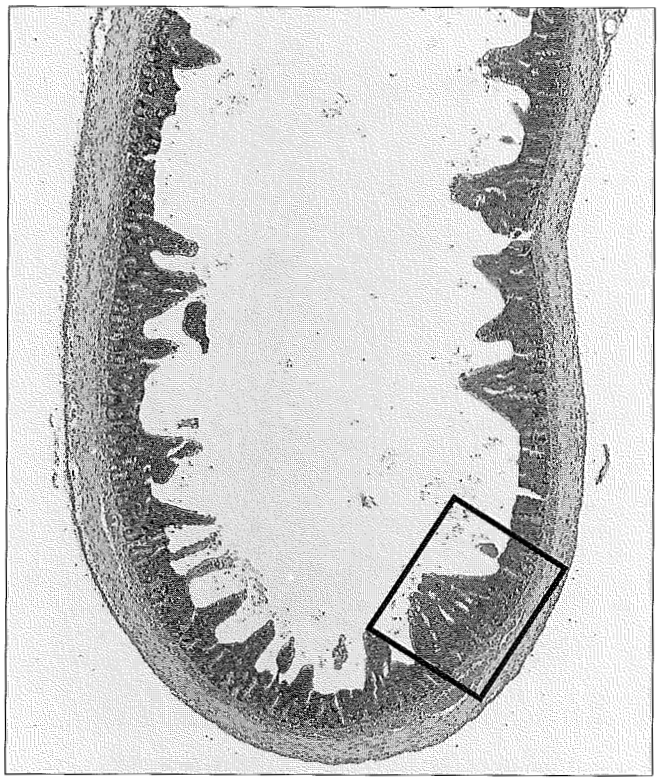

Fig. 3. Small intestine. Severe villous atrophy. The villi are thin, short, blunt and sometimes fused. H&E x 35.

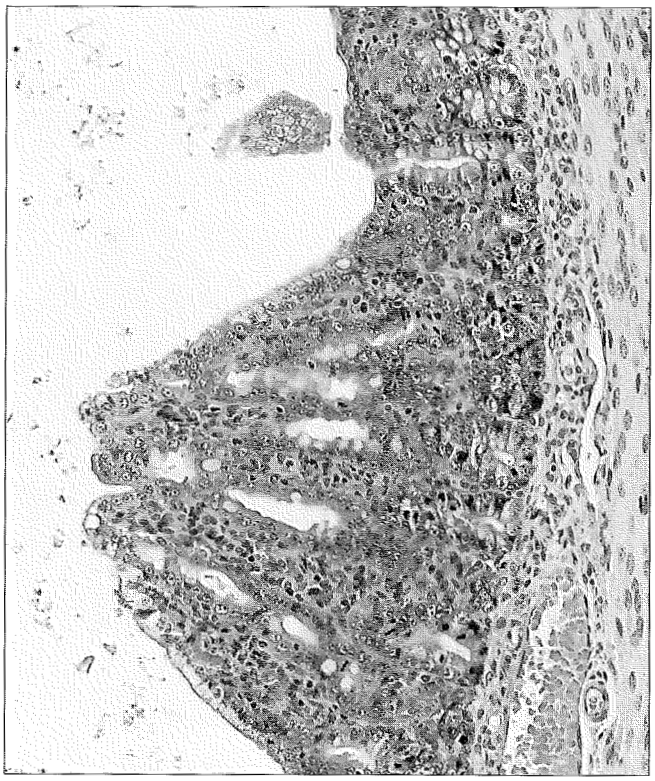

Fig. 4. Detail of Fig. 3. The atrophic villi are partially fused. The surface epithelial cells are cuboidal or low columnar in shape. Their cytoplasm is partly vacuolated. Mononuclear cells are present in the villous propria. H&E x 175.

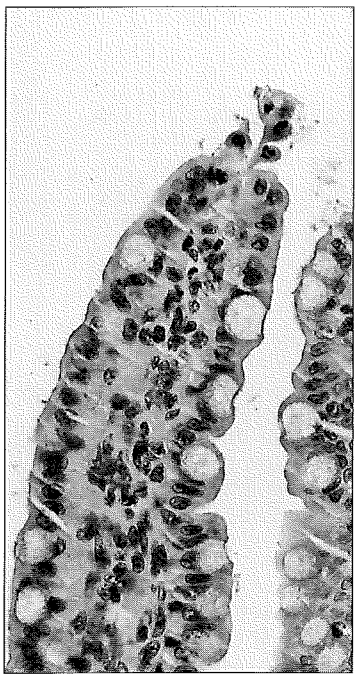

Fig. 5. Small intestine. Villus with degenerated epithelial cells shedding from the apical surface. H&E x 350.

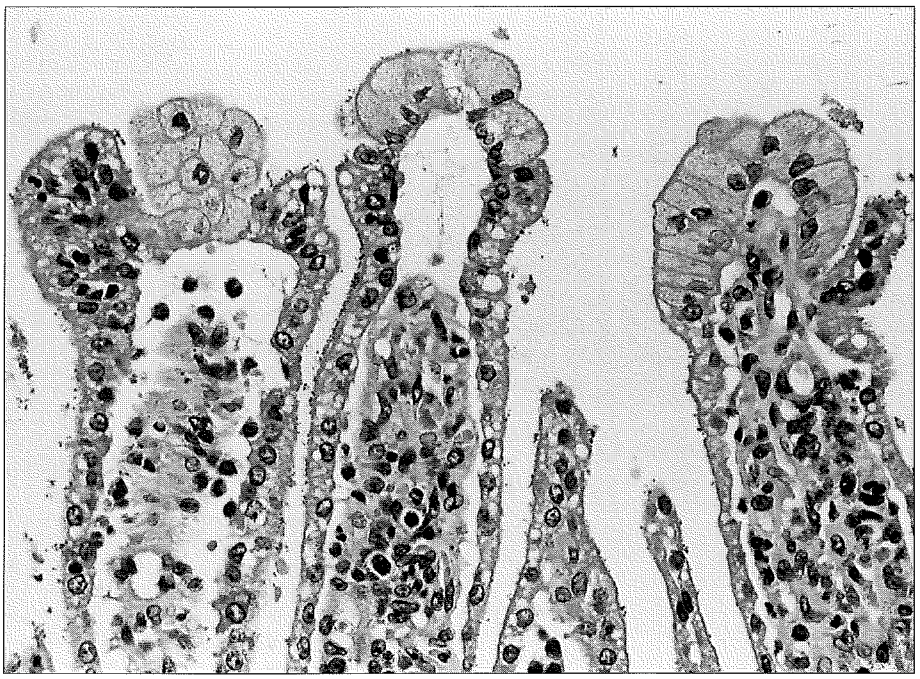

Fig. 6. Small intestine. Tips of villi with hyperplastic epithelial cells. H&E x 380.

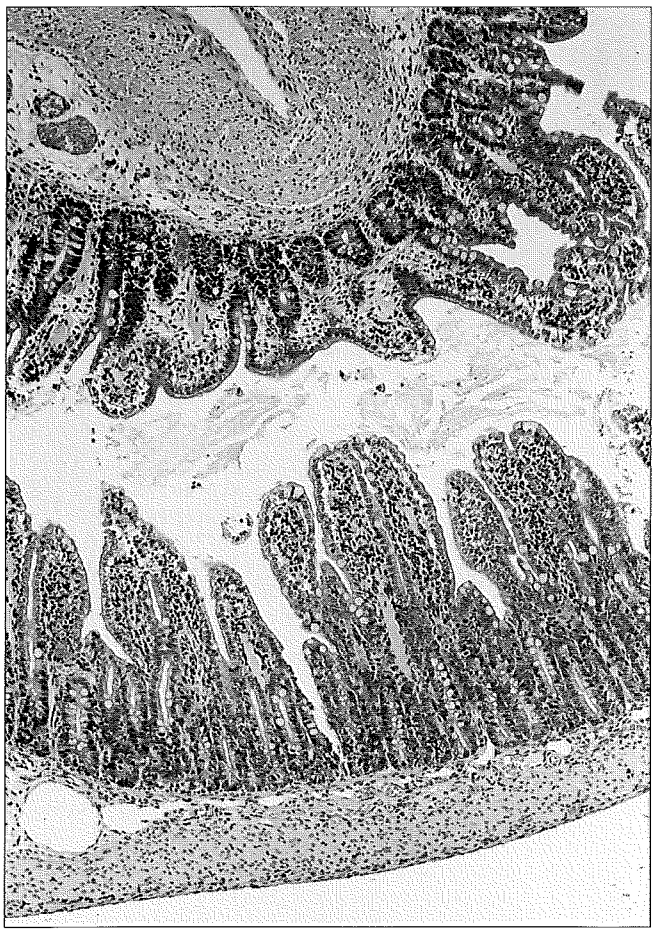

Fig. 7. Small intestine. Great variation in appearance and distribution of atrophy and fusion of villi within the same section of the intestine. Mucus in the lumen. H&E x 95.

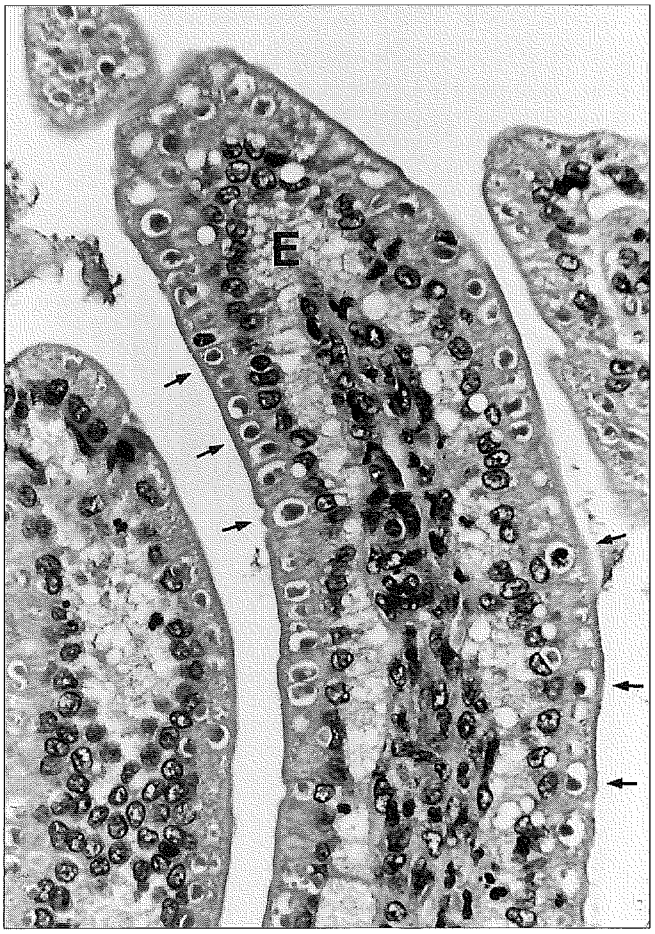

Fig. 8. Small intestine. Villus with several eosinophilic inclusions in cytoplasmatic vacuoles (arrows) of the epithelial cells. H&E x 400.

Sole Minkfoder field tests of greasy kits

Ejner Børsting

The tests showed that

- problems with greasy kits are increasing, the outbursts increase in strength, and from 94 to 96 they start earlier but tend to decline in strength in 1997
- the problem tends to "stick" to the farm
- the problem is most serious on large farms
- the problems are only to a limited extent connected with large litters
- farms with greasy kits have a slightly lower feed allocation in the latter half of April.

Technical Year Report 1997, pp. 169-178. 10 figs. In DANH. Author's summary translated by Hanne Artved.

Variation in the occurrence of gastro-intestinal diseases in mink at various levels and the significance of individual factors at farm level

Erik Rattenborg and Hans Henrik Dietz, Mariann Chriél

The variation in the occurrence of multifactorial gastro-intestinal diseases in mink after weaning was examined at the level of feed kitchens, farms and months.

The largest variation was found at the monthly level followed by farm level. The level of the feed kitchen was insignificant. This means that besides factors related to the age of the animals or the time period as such, the most important risk factors are to be found on the individual farm. Odds ratio for problems with gastro-intestinal diseases was 3.8 if there had been problems on the farm the preceding year. An odds ratio of 2.3 was found if there had been problems with "greasy kits" in the nursing period. A large number of other conditions relating to farm and management did not prove to have any significance as individual factors.

Technical Year Report 1997, pp. 185-189. 3 tables, 3 refs. In DANH. Authors' summary tramslated by Hanne Artved.

Evaluation of pathogenicity determinations of *Yersinia pseudotuberculosis* strains isolated from chinchillas from the western Pomerania area

Wieslawa Emirsajlow-Zalewska, Antoni J. Furowicz, Stojanka Aleksic, Danuta Czernomysy-Furowicz

The aim of the paper was evaluation of the pathogenicity of 6 strains of *Yersinia pseudotuberculosis* isolated from chinchillas from the Western Pomerania area on the basis of five tests considering specific features of bacterial cells possessing the virulence plasmids, like the cell surface hydrophobicity, autoagglutination at 37°C, calcium dependency at 37°C, absence of pyrazinamidases synthesis at 28°C and production of LT enterotoxin.

Advances in Agricultural Sciences, Vol. V, Fasc. 1, pp. 19-24, 1996. 4 tables, 17 refs. Authors' summary.

***Yersinia pseudotuberculosis* infections in animals**

Antoni J. Furowicz, Danuta Czernomysy-Furowicz

Yersinia pseudotuberculosis and *Yersinia enterocolitica* are ingested in contaminated food or water. The bacteria adhere to and invade columnar epithelial cells lining the lumen of the small intestine. The organisms are subsequently transported to the lamina propria where they generate an inflammatory response (gastroenteritis or mesenteric lymphadenitis) and encounter local macrophages. The bacteria survive within the phagolysosome of macrophages but not polymorphonuclear neutrophils (PMNs).

Advances in Agricultural Sciences, Vol. V, Fasc. 1, pp. 5-10, 1996. 1 table, 9 refs. Authors' summary.

Enterotoxigenic strain of *Yersinia pseudotuberculosis* as the cause of yersiniosis in chinchillas

Antoni J. Furowicz, Danuta Czernomysy-Furowicz, Marzena Misiura, Marzena Kowalczewska

On the chinchilla farm (1000 animals) many cases of sickness and mortality (8%) were noted. Twenty dead chinchilla were examined. Symptoms of *diar-*

rhea, dehydration and *pyometritis* were noted. *Pseudotuberculosis hepatitis, enteritis catarrhalis, lymphadenitis mesenterica, splenitis haemorrhagica* and interstitial pneumonia were found during autopsy. A profuse growth of enterotoxigenic strain of *Yersinia pseudotuberculosis* (RPLA test) was obtained from the internal organs of the chinchillas. Clinical signs, gross lesions and bacteriological examinations suggest a septicaemic nature of the disease. All chinchilla from the farm were treated with an immunomodulator (*Propionibacterium acnes*, t. I) and then vaccinated with a formalin inactivated culture of *Y. pseudotuberculosis*. In conclusion the efficacy of immunoprophylaxis of yersiniosis by means of an immunomodulator (*P. acnes*) and *Y. pseudotuberculosis* vaccine was clinically demonstrated.

Medycuna Wet. 52 (2), pp. 116-118, 1996. 3 tables, 17 refs. In POLH, Su. ENGL. Authors' summary.

Isolates of *Encephalitozoon cuniculi* from farmed blue foxes (*Alopex lagopus*) from Norway differ from isolates from Swiss domestic rabbits (*Oryctolagus cuniculus*)

A. Mathis, J. Åkerstedt, J. Tharaldsen, Ø. Ødegaard, P. Deplazes

Encephalitozoon cuniculi has a wide host range among mammals, but whether it represents a homogeneous species is a subject of controversy. We have isolated, cultivated (in human MRC-5 cells) and, for the first time, characterized by immunological and molecular biological methods four isolates of *E. cuniculi* from Norwegian blue foxes with a history of encephalitozoonosis. The isolates were compared with nine isolates from domestic rabbits from Switzerland. Two *E. cuniculi* subtypes were identified according to their host species. A 5'-GTTT-3' tetranucleotide repeat was present twice in the rDNA intergenic spacer in all isolates from foxes as opposed to three times in all isolates from rabbits. Furthermore, random amplified polymorphic DNA analysis showed one polymorphic band among the subtypes, and Western-blot analysis using serum from an infected fox discriminated between the two subtypes on the basis of their banding patterns in the 31-33 and 38-40 kDa. The 5'-GTTT-3' tetranucleotide repeat is a valuable genetic marker for these two subtypes of *E. cuniculi* and will be of use in continued studies on the molecular epidemiology of this parasite.

Parasitol Res 82, pp. 727-730, 1996. 2 figs., 17 refs. Authors' abstract.

Progression of Aleutian disease in natural and experimentally induced infections of mink

M. Keven Jackson, LeGrande C. Ellis, John D. Morrey, Zhi-Zhong Li, Dale L. Barnard

Obectives.To study temporal changes in amounts of viral DNA in blood leukocytes over long periods, and to determine whether severity of the disease is greater in experimentally induced, compared with natural, infection.

Animals.18 naturally and 6 experimentally infected black mink; 26 naturally infected brown mink.

Procedure. Polymerase chain reaction amplification to detect viral DNA in blood and counter-immune electrophoresis to detect serum antibody were performed at regular intervals.

Results. In naturally infected black mink, amounts of viral DNA were initially high, but after the appearance of antibody, viral DNA fluctuated and, in some instances, was undetectable. In other mink, small amounts of viral DNA were infrequently detected during the course of the infection. Amounts of viral DNA in leukocytes in late stages of the disease correlated with renal lesions in brown mink, but black mink had more severe lesions associated with smaller amounts of viral DNA. Severity of the disease was not enhanced in experimentally inoculated black mink.

Conclusions. After infection, leukocyte viral DNA is initially present in large amounts, but, in most mink, decreases markedly in association with the appearance of antibody. There is no difference in the progression and severity of the disease between black mink infected experimentally or naturally. Transmission of the disease may be enhanced by use of contaminated toenail clippers for blood collection.

Am J Vet Res 57, pp. 1753-1758, 1996. 2 tables, 3 figs., 21 refs. Authors' summary.

Investigation of an outbreak of Aleutian disease on a commercial mink ranch

M. Keven Jackson, Scott G. Winslow, Larry d. Dockery, Jedd K. Jones, Donald V. Sisson

Objective. To determine the modes of transmission of Aleutian mink disease in a natural outbreak.
Animals. 5,580 black and 9,087 brown mink from a ranch with an outbreak of Aleutian mink disease.

Procedure. Each mink had serum tested by counter-electrophoresis for Aleutian disease antibody. If a mink was seropositive for Aleutian disease virus by counter-electrophoresis, it was considered to be infected. Correlation of prevalence of the disease in kits and parents was determined. Spatial arrangement of infected and uninfected mink also was studied.

Results. Infected black dams were more likely to produced infected kits than were uninfected dams. In contrast, infected black sires were less likely to produce infected kits than were uninfected sires. In brown mink, in which prevalence of Aleutian disease was lower, transmission from infected dams and sires to kits was apparent. Infected black mink appeared to be more efficient in transmitting the disease horizontally than were infected brown mink. Although the spatial arrangement of infected mink indicated that mechanical transmission of the disease may be the most efficient mode of horizontal transmission, airborne transmission also occurred.

Conclusions and clinical relevance. Infected sires with nonprogressive Aleutian disease may confer protection to their kits in the face of a severe outbreak. Brown mink may be less able to transmit the virus horizontally than are black mink. Airborne transmission is substantial, but may not be as efficient as mechanical transmission.

Am J Vet Res 57, pp. 1706-1710, 1996. 5 tables, 1 fig., 16 refs. Authors' abstract.

Congenital ectromelia infection in fur-bearing animals caused by *Orthopoxvirus muris*

H. Mahnel, J. Holejsovsky, P. Bartak, C.-P. Czerny

Orthopox virus infection is endemic in farms with fur-bearing animals in the Czech Republic (*Bohemia and Moravia*). This disease is called ectromelia of silver foxes and mink. The infection is congenitally transmitted and manifests itself in reproductive disorders, stillbirth or birth of sick neonates. Adult animals are usually free of clinical symptoms. The infective agent was isolated from recent outbreaks and was identified as a mouse pox virus (*Orthopoxvirus muris*) by its cultural and immunological characteristics. The significance of this pox virus infection, hitherto not described in Western Europe, is discussed.

Tierärztliche Praxis 21, 5, pp. 469-472, 1993. 2 photos, 16 refs. In GERM, Su. ENGL. Authors' summary.

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Melatonin: physiology and uses in domestic animals. *B. Simon. 1993; Nantes (France), 108 p., 146 refs., 11 tables, 18 graphes. Ecole Nationale Veterinaire de Nantes (France). In FREN.*

Studies of the effects of aircraft noise on the peri-partal and post-partal losses in farm-raised mink (*Mustela vison* F. dom). *E. Stephan. 1991, vi, 137 (18), ill.*

Estimates of heritability, repeatability and non-genetic effects on the expression of reproductive traits and liveweight of chinchilla laniger Gray. *Ricardo Antonio H. Scheu. 1988, Santiago (Chile), 165 p., 94 refs. Universidad de Chile, Santiago. Fac. de Agronomia. In SPAN, Su. ENGL, SPAN.*

Diseases of the stone marten *Martes foina* (Erxleben, 1777) and the pine marten *Martes martes* (Linne, 1758). *Odward Geisel. 1992, 134 p., ill. Advances in veterinary medicien = Fortschritte der Veterinarmedizin, 0931-4229, no. 43. In GERM, Su. ENGL.*

Mink parvovirus infections. *D.D. Porter, A.E. Larsen. CRC handbook of parvoviruses, Vol. II, 87-101, 1990, 107 refs. Boca Ration, Florida, USA, CRC Press.*

Vitamins and additives in human and animal nutrition

These proceedings of the 5th conference on vitamins and additives in human and animal nutrition, held in Jena, Germany, on 28-29th September 1995 contains a general overview on the current importance of vitamin enrichment of foods, and sections on Vitamin A and carotenes (16 papers), Vitamins D and E, antioxidants (16 papers), B vitamins, ascorbic acid and vitamins generally (30 papers), Enzymes (17 papers), and Probiotics, antibiotics and other additives (23 papers). Each section includes aspects on human as well as animal nutrition and papers have a bibliography and English summary.

Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier

Herausgeber:
Rainer Schubert
Gerhard Flachowsky
Roland Bitsch

ISBN 3-00-000361-4

Technische Bearbeitung:
Gisela Sallen, Maritta Heyer

Gesamtherstellung:
Buch- und Kunstdruckerei Keßler GmbH
Erfurter Str. 19
D-99423 Weimar

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