SCIENTIFUR
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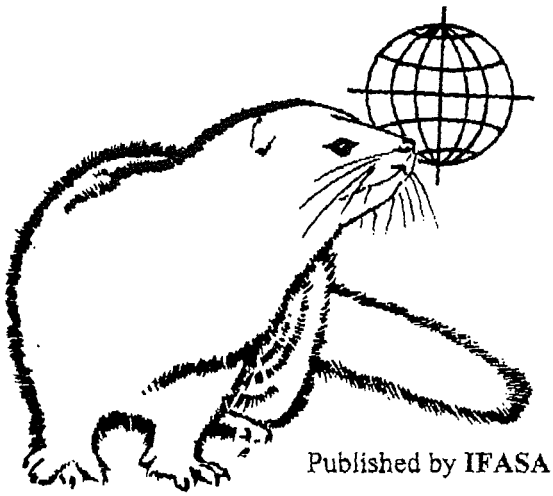

INTERNATIONAL FUR ANIMAL SCIENTIFIC ASSOCIATION

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Titles of other publications – not abstracted

Complete guide to ferrets. *James McKay. Book:Shrewsbury, England: Swan Hill Press, c1995, 160 pp. Code 14-O.*

Greasy kits: Incidence and therapy at Foulum in 1995. *Birthe M. Damgaard, M. Jørgensen, B. Houbak. Internal Report no. 59, pp. 51-55,1996, DIAS. In DANH. Code 9-5-M.*

Development of the digestive system in mink kits. *N.E. Hansen, J. Elnif. Internal Report no. 69,pp. 29-35,1996, DIAS. In DANH. Code 2-3-6-M.*

Let the female mink choose. *Dansk Pelsdyravl, Vol. 60 (4): 196-198, 1997. In DANH. Code 9-5-M.*

Stress and greasy kits. *J. Elnif, N.E. Hansen. Dansk Pelsdyravl, Vol. 60 (5): 230-231, 1997. In DANH. Code 3-5-M.*

Investigation of epidemiology in outbreak of greasy kits. *M. Chriel, E. Rattenborg, C. Hejlesen, S. Møller. Dansk Pelsdyravl, Vol. 60 (5):232-233, 1997. In DANH. Code 9-5-M.*

Practical experiences with family cages for mink. *J. Hansen. Dansk Pelsdyravl, Vol. 60 (5): 248-249, 1997. In DANH. Code 10-12-M.*

Stress in the weaning period. *T. Clausen. Dansk Pelsdyravl, Vol. 61 (6): 278, 1998. In DANH. Code 5-6-12-M.*

Factors affecting raccoon dog's growth. *H. Korhonen, M. Harri, J. Mononen. Finsk Pälstidskrift, Vol. 31 (8-9): 190, 1997. In SWED. Code 6-10-12-O.*

Adrenocorticotropic hormone (ACTH) but not alpha-melanocyte stimulating hormone (alpha-MSH) as a mediator of adrenalectomy induced hair growth in mink. *J. Rose. Journal of Investigative Dermatology 110 (4): 456, 1998. Code 2-3-M.*

Handling of manure from fur animal farms. *Anonymous. Booklet, 24 pp. Danish Fur breeders Association, February 1998, Langagervej 60, P.O. Box 79, DK-2600 Glostrup, Denmark. In DANH. Code 10-12-14-M-F-O.*

5. Nutrition & Nutritional Physiology

Titles of other publication – not abstracted

Influence of siting of the feed during lactation on the eating behaviour and development of the mink female and pups. *S. Møller. Internal Report no. 69 pp. 41-49, DIAS, 1996. In DANH. Code 5-6-12-M.*

Glucose metabolism in mink. *Christian F. Børsting. Internal Report no. 69, pp. 23-28, DIAS, 1996. In DANH. Code 3-6-M.*

Rates of urinary water electrolyte and nitrogen excretion in fed and fasted female mink (*Mustela vison*). *S. Wamberg, J. Elnif, A.-H. Tauson. Z. Ernährungsviss 36: 358, 1997. Code 3-6-M.*

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

Titles of other publications – not abstracted

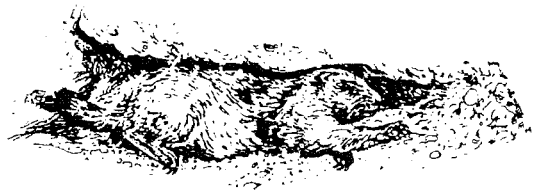
Who shall be with whom during summer: Varios combinations of discarded bitches, male and female puppies. *J. Hansen, J. Søe. Dansk Pelsdyravl, Vol. 61 (6): 276, 1998. In DANH. Code 10-11-12-M.*

7. Genetics & Breeding

Titles of other publications – not abstracted

Three polymorphic mink, *Mustela vison*, dinucleotide repeats. *K. Brusgaard, N. Shukri, S.N. Malchenko, O. Lohi, K. Christensen, T. Kruse. International Society for Animal Genetics 29: 150-160, 1998. Code 4-3-M.*

DNA technology in animal breeding. *K. Brusgaard, S. Malchenko. Internal Report no. 69, pp. 17-22, 1996, DIAS. In DANH. Code 3-4-M-F-O.*



8. Reproduction

Titles of other publications – not abstracted

Reproductive efficiency in mink (*Mustela vison*) Treated with the pesticides lindane, carbofuran and pentachlorophenol. *A.P. Beard, A.C. McRae, N.C. Rawlings. Journal of Reproduction and Fertility Ltd., Sept. 1997, Vol. 111 (1): 21-28. Code 5-M.*

IFASA

VIIth INTERNATIONAL SCIENTIFIC CONGRESS IN FUR ANIMAL PRODUCTION

Kastoria, Macedonia, Greece

13 - 15 September 2000

Second Announcement - Call for Papers

Organized by:

International Fur Animal Scientific Association

and

Prefecture of Kastoria

Prefecture of Kozani

Municipality of Kastoria

Chamber of Commerce & Industry of Kastoria

Greek Fur Trade Federation

Greek Fur Center

Kastorian Development Agency

EDIKA S.A.

Notes
Scientifur, Vol. 23, No. 4
November 1999

First of all I hope that my friend, Ejner Børsting, will forgive me the fatal error, not to bring the address of THE FIRST FREE ELECTRONIC "MEETING POINT" FOR FUR ANIMAL RESEARCHERS. Here is the address:

http://www.onelist.com/subscribe/fur

At this address you can meet your colleagues and exchange ideas, questions and experiences. See also the advertisement at one of the following pages.

This initiative, together with the possibilities which have opened up on the IFASA WEB-sites at the address:

http://www. IFASANET.ORG

- gives all the best possibilities for finding the right persons or groups to discuss with.

Hopefully it will all end up in very active groups within the different fields of science and production, making the everyday better for everybody and the international co-operation in all fields of fur animal production and science.

Especially we are considering the international scientific meetings, symposia and/or workshops regarding fur animal production. One international scientific congress every fourth year is too little. If the many scientific meetings on fur animal production that are going on in Scandinavia, Eastern Europe and in the USA/Canada were made more international for example under the auspices of IFASA, at least one international scientific meeting regarding fur animal production could be arranged each year. This would benefit the production and the international co-operation very much.

Therefore get on the Internet and promote your own research and thereby promote fur animal production.

In this the last issue of Vol. 23 (1999) we are publishing 6 original reports and 1 reviewed report. During 1999 it has been demonstrated that SCIENTIFUR is becoming more and more popular as the place, where original scientific reports regarding fur animal production are published.

It is very interesting to see how many readers we will get, when the reports are going to be published on the internet via IFASA's WEB-site. Thus we can check the number of readers of the reports. This is impossible in case of the printed matters because there may often be many possible readers of each copy of the journal. About 50 % of the SCIENTIFUR subscriptions are from libraries, institutes etc. Therefore it may be assumed that there are a lot more readers than subscribers, but how many we do not know.

On the pages after the Notes, you will find the second Announcement of the **VIIth INTERNATIONAL SCIENTIFIC CONGRESS IN FUR ANIMAL PRODUCTION in Kastoria, Macedonia, Greece 13 - 15 September 2000.**

Please help us and the congress organisers to reach all relevant persons who may have an interest in participating in this very important occasion for receiving scientific information and for meeting colleagues from all over the world, who are engaged in the fur animal production and/or science. Send them a copy of the announcement or the address of IFASA or the Congress Organisers on the e-mail address: **symvoli@yahoo.com**

Thank you in advance.

As advertised in the previous issue of SCIENTIFUR, the subscription price for 2000 is the same as in previous years, NOK. 500.- for members and NOK. 600.- for others. Fee for personal membership is NOK. 170.- for one year or NOK. 500.- for a 4 year membership. Please pay the 2000 invoice as soon as possible after you have received it at the beginning of the new millennium.

Dear Dorthe, Hanne and Janne, my sincere thanks for all your help in typing & laying out (Dorthe) and translating (Hanne) + language controlling (Janne & Hanne). Without this help there would be no SCIENTIFUR from my hand. Also thanks to the Norwegian Fur Breeders Association for housing IFASA/SCIENTIFUR and for printing the journal (Kristian Johansen) and for the very important economic support to IFASA/SCIENTIFUR. My thanks include both the Norwegian Fur Breeders Association and all Association members of the EFBA (European Fur Breeders' Association). Without this very massive economic support IFASA & SCIENTIFUR would not be found on the world map. THANK YOU ALL.

Finally we welcome you all to and wish you all the best in the new fur animal millennium which is approximately the 80th of fur animal production under farm conditions and the 24th of the life of SCIENTIFUR. As your ageing editor, I cannot imagine how the picture of it all will look on the border of the 21st millennium.

Merry Christmas and a Happy New Year to all of you.

Your Editor

Gunnar Jørgensen

VIIth INTERNATIONAL SCIENTIFIC CONGRESS IN FUR ANIMAL PRODUCTION

Kastoria, Macedonia, Greece
13 - 15 September 2000

Second Announcement- Call for Papers

GENERAL INFORMATION

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

CONGRESS SITE
The congress will be held at EDIKA Congress Center, at Kastoria.

CONGRESS STRUCTURE
The scientific program will consist of plenary sessions, oral presentations and posters. The papers will cover topics according to the 5 working groups of IFASA.
1. Breeding, reproduction and genetics.
2. Nutrition.
3. Pathology and diseases.
4. Behavior and welfare.
5. Fur properties.

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

SUBJECTS OF PLENARY SESSIONS
GENETICS
PATHOLOGY
REPRODUCTION
NUTRITION
FUR QUALITIES
Special plenary sessions are planned in two areas,
PLASMACYTOSIS and ANIMAL WELFARE.

SUBMISSION OF PAPERS

Titles and Abstracts
Titles and abstracts are due before January 30, 2000.
Titles and abstracts must not exceed 150 words, corresponding to 10 lines in print. Abstracts should indicate the research objectives, proposed methodology and expected outcome and should contain original material, which have not been published, presented or submitted to another conference. Undefined abbreviations should be avoided.

Manuscripts
Manuscripts must be submitted before March 30, 2000.
Manuscripts intended for oral presentation or posters (max. portrait size: 1,00 m by 2,00 m) are expected to follow the same pattern. Two copies of written version of manuscript are requested. In addition, we kindly request a computer floppy disk containing the material prepared as follows:

1. WORD for WINDOWS, ver. 2.0 or 6.0 editor,
2. Without formatting marks, except TAB marks,
3. Tables with TAB only, no more than 30 signs,
4. Figures in ink on paper or scanned in TIFF format,
5. Photos black/white only in TIFF format.

The manuscripts must be typewritten and double-spaced. The text, including tables, figures and references should not exceed 6 pages. The pages must be numbered consecutively beginning with the title page. Articles should where possible be organized as follows:
(1) Introduction, (2) Material and Methods, (3) Results and Discussion, (4) Conclusions

Title page:
The title page should contain:
1) A precise but brief title.
2) Full names of all authors.
3) Name and address of the institution and / or departments at which the research was carried out. Names of institutions must be given in English.

Tables:
Each table must be typed double-spaced on a separate sheet of paper. They should be numbered consecutively with Arabic numerals and have a concise heading. Abbreviations in tables should be explained in footnotes, using the following symbols in this order: 1), 2), 3).

Figures:
All illustrations, line drawings, and photographs are considered as figures. Figures should be numbered consecutively with Arabic numerals. Photographs should be submitted as near to their printed size as possible. Figure legends should be complete enough so that figure can be understood without reference to the text, and typewritten together on one sheet of paper.

References:
References in text will follow the Harvard style, with the author's name and year of publication:

Cholewa (1994), or (Cholewa 1994). A text reference to a paper by two authors is given by naming both authors. If there are three or more authors, the text reference is given by adding .,et al" to the first named author. In the list of references authors' names should be listed alphabetically. Cholewa R. 1994. Influence of some factors on variation in coat color of nutria. Applied Science Reports of Polish Society of Animal Production 15: 29-33.

The manuscripts will be read by qualified reviewers appointed by the members of the Scientific Committee. Minor editorial changes may be made prior to printing. The manuscripts will be sent back to authors if major changes are necessary. Proofs will be checked by the Scientific Committee.

REGISTRATION FEE

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

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

INFORMATION

For all information you may contact
SYMVOLI - Congress Organizers Ltd. Patmou 8, Kalamaria, 551 33 Thessaloniki, Greece.
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attention. VII IFASA Congress

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

PRELIMINARY PROGRAM

Tuesday 12/9	Wednesday 13/9	Thursday 14/9	Friday 15/9
	Registration Official Opening Ceremony **Plenary Lectures** Coffee break **and Poster Presentations** Lunch and Council Meeting	**Plenary lectures** Coffee break **and Poster Presentations** Oral Presentations Lunch	**Plenary lectures** Coffee break **and Poster Presentations** Oral Presentations Lunch and closing ceremony
Registration IFASA board meeting	**Oral Sessions** Sightseeing Welcome reception	**Plenary lectures and** Oral Presentations Gala	Fur fashion show Meeting of new IFASA board

Reviewed Report

Morphobiochemical blood indices in mink with chewed fur

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Received 01.01.99 **Accepted 04.08.99**

Summary

The occurrence of both males and female mink with chewed fur gave an impetus to the study of blood indices during the growing-furring period. During this period the key metabolic reactions of animals are weakened, as indicated by the decreased level of hemopoesis and activity of enzymes which regulate carbohydrate (LDH) and protein (ASAT) metabolism and SOD which controls lipoperoxidation. The rise in the amount of eosinophils in the blood of these animals is not statistically significant and is shown as a distinct tendency. The most substantial change observed in the immunoreactivity system of mink with chewed fur is a rise in NBT-reducing leucocyte activity. Changes in leucocyte metabolism provide evidence for disturbed homeostasis.

Keywords: mink, fur chewing, blood leucocytes, cytochemical indices.

Introduction

Fur chewing is a disturbance which causes a considerable damage to farm-bred mink stock. It also affects polar fox, chinchilla, sable, and silver fox. The occurrence of this phenomenon varies markedly from farm to farm: on some farms fur chewing is not observed, on others it affects the animal stock on a large scale. The fur of cubs is damaged by females who carry them; damaged fur is also observed after weaning (July-August), and does not depend on the time of weaning of the litter and social environment (*Damgaard & Hansen, 1996*). Adult animals chew the fur of each other when they are sexually active or aggressive (*Houbak & Hansen, 1996*). Fur chewing varies in intensity with season. During the day fur is chewed more vigorously when the motor activity of animals is at its highest level (*Malmkvist & Hansen, 1997*). Some authors note that this defect occurs at a certain time and is closely related to moulting (*Komarova, 1972; Lineitseva, 1981; Malmkvist & Hansen, 1997*).

The causes of the disturbance discussed are not clear yet. It does not occur in nature, but it could be brought about by the abruptly restricted mobility of farm-bred animals and indicates aggravation of their state. Transfer of mink to bigger cages has a positive effect on fur formation (*Pokk, 1963*). The increase of the defect is provoked by deficient feeding and the lack of trace elements and vitamins that result in disturbed metabolic processes (*Isayeva et al., 1981*). One can see from the literature that, unlike animals with undamaged hair, mink with

chewed fur show, on the one hand, a decline in some morphobiochemical blood indices, such as the amount of erythrocytes, hemoglobin and total serum protein, and, on the other hand, a rise in phosphorus and the absence of changes in the trace element content of the blood (*Isayeva et al., 1981; Lineitseva, 1981*). In some cases, improved feeding and addition of vitamins and biologically active compounds to rations normalizes the state of animals and decreases the number of animals with chewed fur (*Pokk, 1963; Pankovets et al., 1996*).

The aim of this investigation was to determine changes of morphobiochemical blood indices in mink with chewed fur and its connection with this disorder.

Materials and methods

The standard mink kits of both sexes breeding on the "Kondopogskii zverovod" Ltd. farm were used. The studies were made in September 1996 in relation with appearance of a number of mink with chewed fur. The animals were divided into three groups: I (n = 10) - mink with normal fur, II (n = 9) - mink with chewed fur, III (n = 11) - mink with restored fur after chewing.

Morphobiochemical and cytochemical blood analyses were made to get an estimation of the physiological state of the animals. Blood was sampled from the tail. One per cent heparin was used as anticoagulant. After centrifugation serum was frozen and stored at -25°C. Cytochemical analyses and determination of superoxide dismutase (SOD) activity were made immediately after blood sampling. The content of total protein and its comparison (albumins, α-globulins, β-globulins and γ-globulins), activity of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase (LDH), alkaline phosphatase (AP) of blood serum and indices of hemo- (erythrocytes, hemoglobin) and leucopoesis (leucocytes, leucoformula) were measured using standard method (*Berestov, 1971; Berestov & Kozhevnikova, 1981*). The SOD level in erythrocytes was measured according to Fridovitsh (1975). For cytochemical determination of leucocyte alkaline phosphatase (LAP) the method of asocoupling reaction was used (*Burstone, 1962*). The percentage content of phosphatase positive segmentonuclear leucocytes was estimated and based on the degree of intensity of coloring the average cytochemical coefficient (ACC LAP) was calculated. Reaction of nitroblue tetrasolium reduction (NBT-test) was carried out in intact blood, using the recommendations of the same authors (*Shubitch & Mednikova, 1978; Viksman & Mayanski, 1980*). The result was expressed in percentage of formasan positive leucocytes (NBT, %) and, depending on the amount of product of reaction in them, the cytochemical index of activity (CIA) was calculated.

Results and discussion

Weakening of key metabolic reactions is observed in all three groups of mink, as indicated by the decreased enzymatic activity of carbohydrate (LDH) and protein (ASAT) metabolism and a decline in the indices of hemopoesis (erythrocytes and hemoglobin) either to the lower limit of the normal value or, like SOD which controls lipoperoxidation, or even below (Fig. 1). AP activity, total protein content and the fractional composition of protein in mink with chewed and normal fur were normal. No reliable differences in the above indices between the groups were reported.

It has been shown by studying the composition of the leucocytes that there are small differences in the levels of some cellular elements, such as monocytes, neutrophils, lymphocytes and eosinophils, between mink with chewed fur and those with normal fur (Table 1). However, the relative blood content varies over a broader range in mink with chewed fur and in animals that restore their hair than in those with normal fur. Basophils are observed only in normal mink and in those restoring hair and are absent from the blood of mink with defect fur. However, it is not the subject to be discussed, because the level in the blood is generally low. Furthermore, the amount of eosinophils tends to increase in mink with indications

of chewed fur. The percentage of these cells is 1.5 times higher in chewed mink and 2.4 times higher in those restoring their hair cover than in mink with undamaged fur. The data obtained agree with evidence for a rise in the amount of eosinophils in the blood of mink with chewed fur available in the literature (*Jørgensen, 1995*). The author believes that this is the response of the organism to the presence of mites in straw material. However, subsequent studies failed to confirm a relationship between the above factors (*Malmkvist & Hansen, 1997*).

Table 1. The leucocytic composition, NTB test and LAP of blood in standard mink kits with normal and chewed fur

Indices	Group I		Group II		Group III	
	Lim	aver	Lim	aver	Lim	aver
Leucocytes, 10^9/l	3.45 - 10.55	5.56	3.65 - 11.65	6.56	2.75 - 8.00	5.53
Monocytes, %	1.00 - 6.00	3.80	2.00 - 10.00	4.70	1.00 - 6.00	3.30
Lymphocytes, %	28.00 - 55.00	39.70	13.00 - 65.00	47.70	14.00 - 64.00	34.20
Neutrophilic band, %	0.00 - 4.00	1.30	0.00 - 3.00	0.50*	0.00 - 2.00	1.00
Neutrophilic segmented, %	42.00 - 65.00	54.30	31.00 - 77.00	45.90	30.00 - 77.00	56.50
Eosinophiles, %	0.00 - 2.00	0.80	0.00 - 5.00	1.20	0.00 - 9.00	1.90
Basophiles, %	0.00 - 1.00	0.10	0.00 - 0.00	0.00	0.00 - 1.00	0.10
LAP, %	0.00 - 8.00	2.10	0.25 - 12.00	3.28	0.00 - 6.00	2.05
ACC LAP, Units	0.00 - 0.23	0.05	0.01 - 0.25	0.06	0.00 - 0.14	0.04
NBT, %	2.00 - 10.00	4.00	4.00 - 21.00	9.30*	2.00 - 16.00	7.60*
CIA, Units	0.02 - 0.18	0.05	0.04 - 0.42	0.16*	0.04 - 0.40	0.14*

* - The differencies were significant compared to group I (Kolmogorov-Smirnov test).

Cytochemical analysis has shown that the groups of animals studied differ most substantially in the functional activity level of leucocytes that are highly reactive and sensitive to internal changes in the organism. Increase of NBT-positive leucocyte amount was observed in mink with chewed fur. Particularly the increase of active and highly active leucocytes level was marked.

It is known from the data obtained from medical and biological studies that changes in the reactivity of leucocytes are caused by various factors, such as bacterial agents, circulating immune complexes, etc. (*Mayanski & Mayanski, 1989*). Additional evidence is needed to interpret the activation of the oxygen–dependent metabolism in minks with defect fur from the point of view of the effect exerted by the pathogen on the organism. However, changes in the leucocyte metabolism of mink with chewed fur, not observed in normal animals, are undoubtedly caused by disturbed homeostasis. This evidence is supported by data on pathological processes in the liver, kidneys, skin, connective tissue, and hair follicles of these animals (*Isayeva et al., 1981; Pankovets et al., 1996*).

Thus, in farm–bred mink hair "chewing" accompanies the decreased activity of enzymes, such as ASAT, LDH and SOD, and attenuation of hemopoesis. The rise in the amount of eosinophils in the blood of these animals is not statistically significant and is apparent as a distinct tendency. The most substantial change ob-

served in the immunoreactivity system of the organism of a mink with chewed fur is a rise in the NBT-reducing activity of leucocytes. Changes in leucocyte metabolism are regarded as evidence for disturbed homeostasis.

References

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

Berestov, V.A. & Kozhevnikova, L.K., 1981. Blood enzymes of fur bearing animals. Leningrad., "Nauka", 186 p. (In Russian)

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

Damgaard, B.M. & Hansen, S.W. 1996. Stress physiological status and fur properties in farm mink placed in pairs or singly. Acta Agric. Scand. Sect. A, Animal Sci. 46, 253-259.

Fridovich, I., 1975. Superoxide dismutase. Annu. Rev. Biochem., 44, N1, 147-159.

Houbak, B. & Hansen, S.W. 1996. Fur chewing in farm mink - temporal development and effect of social environment. Animal Production Review. 29, 77-81.

Isayeva, T.I., Evsikova, L.P., Kulikov N.E. 1981. Some biochemical and histological indices of mink organs with chewing fur. Biology and veterinary of fur animals and rabbits. Moscow. 26, 117-118. (In Russian)

Jørgensen, M. 1995. Fur biting in mink. Scientifur. Vol. 19, no. 4, pp, 288.

Komarova, L.G. 1972. Fur chewing in fur animals. Krolikovodstvo i zverovodstvo. N3. 24-25. (In Russian)

Lineitseva, E.G. 1981. About defects skin at alive American minks. Biology and pathology of fur animals. Petrozavodsk. Part 1, 143-144. (In Russian)

Malmkvist, J. & Hansen, S.W. 1997. Why do farm mink fur chew? NJF Seminar no. 280/ NJF Report no. 116, 211-216.

Mayanski, A.N. & Mayanski, D.N. 1989. Sketches about neutrophil and macrophag. Novosibirsk. 344 p. (In Russian)

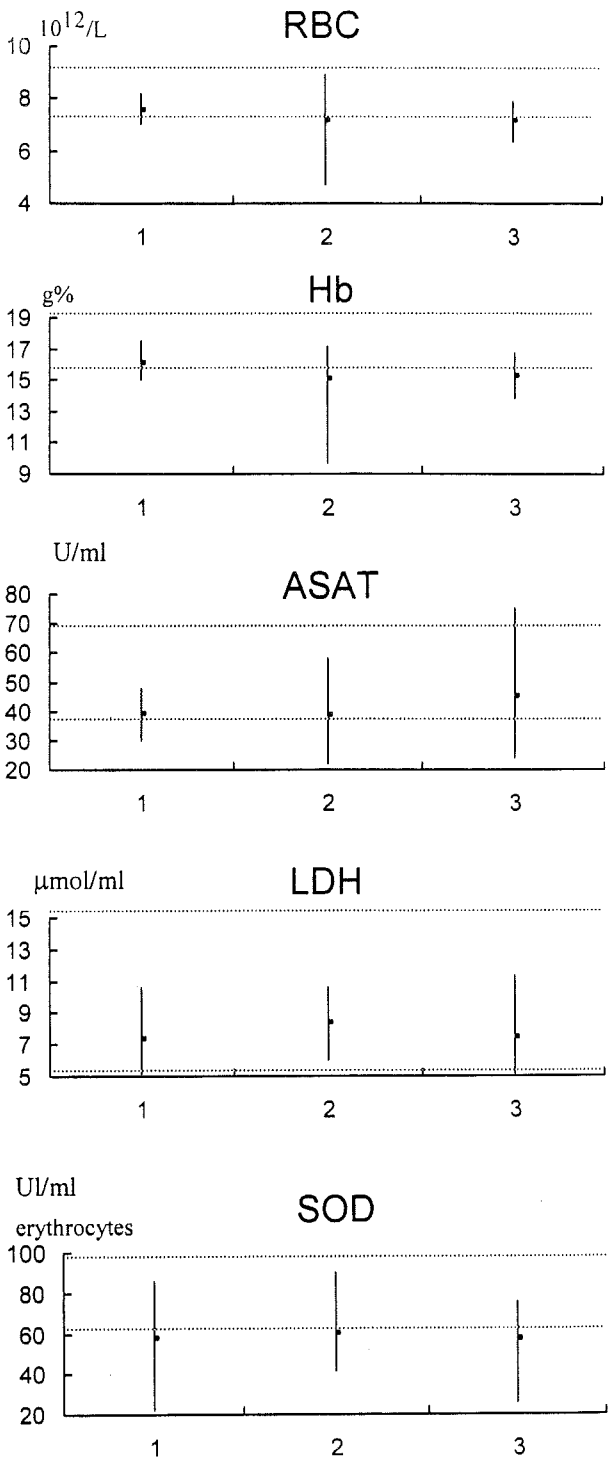


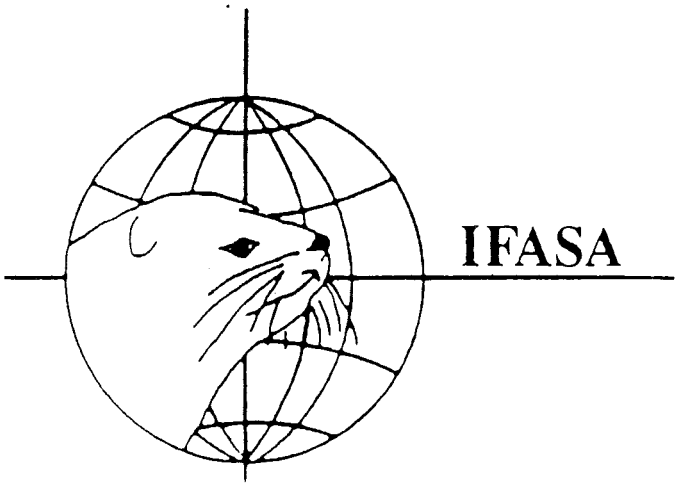
Fig.1. Variations in the morpho-biochemical blood indices of mink with chewed fur (limits and mean values). Group numbers are shown on the abscissa. Dotted lines indicate the confidence limits (0±SD) of normal values determined during 10-years investigation.

Pokk, E. 1963. Fur dissection and chewing in fur animals. Krolikovodstvo i zverovodstvo. N8. 26-27. (In Russian)

Pankovets, E.A., Krivenya, O.V., Trebukhina R.V. et al. 1996. The histochemical characteristic of a liver and skin of minks with chewing fur depending on therapy of vitamins and addition of premix M in a diet. Selskohozyaistvennaya biologia. N4. 115-116. (In Russian)

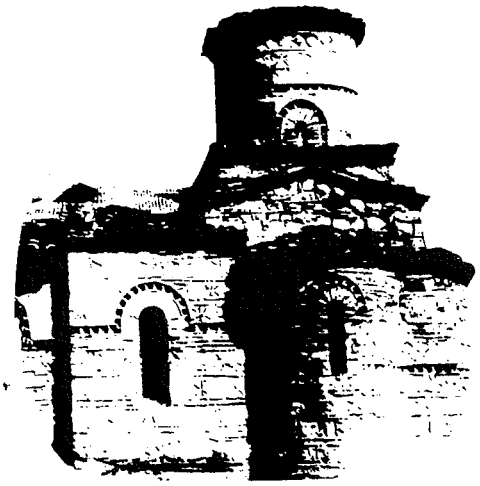
Shubitch, M.G. & Mednikova, V.G. 1978. The NBT-test at children in norm and with purulent - bacterial infections. Laboratornoye delo. 9, 515-517. (In Russian)

Viksman, M.E. & Mayanski, A.N. 1980. The characteristic of the opsonik factors at the reaction of nitroblue tetrazoliun reducing in human neutrophils. Bulleten eksperimentalnoi biologii i meditsini. 2, 214-215. (In Russian).



IFASA

VII Scientific Congress, Kastoria 2000



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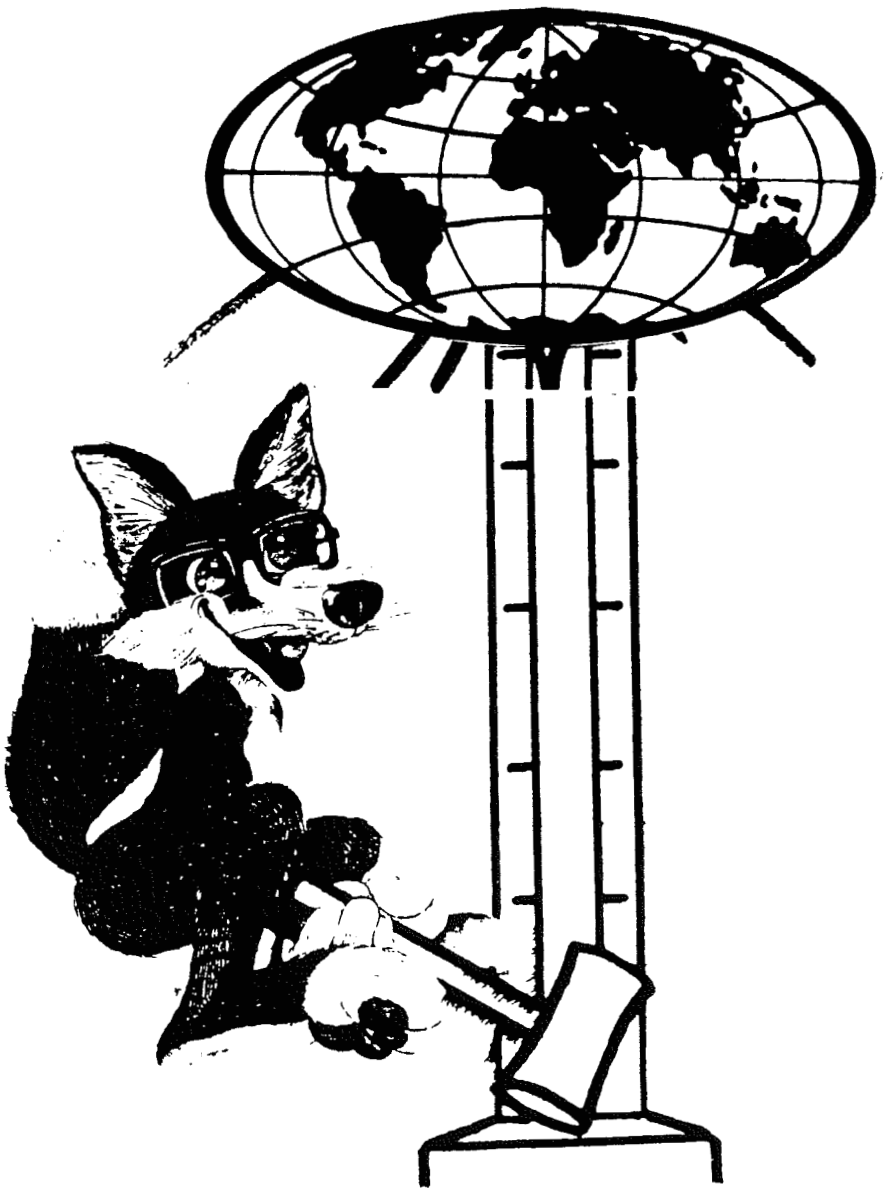

Original Report

Correlation between body weight at pelting and pelt length in chinchillas (*Chinchilla laniger*)

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Abstract

The experiment was carried out on the research chinchilla stock of the Faculty of Animal Science at Kaposvár. We observed the connection between body weight at pelting time and pelt length (n=125) and between body weight and pelt length (n=41) on chinchillas 8-12 months of age on the matured pelt. The average body weight at pelting was 539 g, the pelt length 384 mm and the body length 258 mm. No difference was observed between the sexes. 50% of the small sized chinchillas (under 539 g) were divided into small (under 350 mm) and medium (350-400 mm) size pelt length categories. The correlation coefficient between body weight and pelt length was r=0.65 ($p<0.0001$), between body length and pelt length r=0.48 ($p<0.01$).

Introduction

The profitability of fur production is determined by the size and quality of the pelt, i.e. the final product. The quantitative features can be improved through selection methods. Much research has been performed on other fur animal species to reveal the relationships between body weight, body length and pelt length. Close correlations(r=0.8-0.9) have been detected both between body weight and pelt length in mink (*Therkildsen, 1988; Hansen et al., 1992; Børsting and Therkildsen, 1992*) and in arctic fox (r=0.52) (*Piorkowska, 1996*) and between body length and pelt length in mink (r=0.72-0.74) (*Hansen et al., 1992*) and in arctic fox (r=0.62) (*Piorkowska, 1996*).

The number of chinchilla farms was approx. 800 in Hungary in 1998 and this number continues to increase. The research study to be presented in the following was intended mainly to provide further information on the selection of growing chinchillas. The objective of the author was to determine the correlation rates between body weight at pelting and pelt length and between body length and pelt length.

Material and method

This experiment was carried out on the standard chinchilla stock maintained at the Research Farm of the Pannon Agricultural University in Hungary. The chinchillas were housed in a confined room heated in winter and fitted with windows. Each animal was placed in a single wire-net cage (40x65x35 cm), in which the holes were 1.5 cm wide. The cages were lined with bedding or they were fitted with slatted floors. The cages were of the traditional type and were arranged in four floors. The relative humidity of the inside air was 40 to 60%, the temperature fluctuated between 16 and 18°C, in summer between 20 and 25°C. The youngs were weaned at the age of 56 to 60

days. The growing chinchillas were fed ad libitum from 2 to 7 months of age. Their feed ration contained a chinchilla pellet (mix) and grass hay. The feed supply was changed to portioned feeding after 7 months of age (approx. 20 g/day). Drinking water was available *ad libitum* from valved self-drinkers.

The chinchillas were killed according to the procedure approved by the EU (CEFBA, 1994, supplemented with regulations concerning herbivorous fur animals), i.e. by dislocation of the cervical vertebras. Pelting was carried out according to fur trade demands, described by Várady (1989).

The data on body weight and pelt length of 125 mature-hair growing chinchillas were evaluated. Body length was measured on 41 individually before pelting. The pelt was considered to be mature when its maturity rate was at least 70% on the surface between the front legs and tail. Only the data of individuals pelted after reaching their first adult fur coat (at 8 to 12 months of age) were included in the evaluations. Pelt length was measured on the dry pelt, body length with a ruler between nose and tail root on the freshly killed animal before pelting.

The experimental categories of body weight were defined according to four quartiles, as follows:
1. small (below 500 g),
2. medium (between 500 and 539 g),
3. large (between 540 and 565 g),
4. extra large (over 565 g).

The categories for pelt length were formed according to those used in fur trading:
1. short (below 350 mm),
2. medium (between 350 and 400 mm),
3. long (between 401 and 450 mm),
4. extra long (over 450 mm).

The statistical tools used for data processing were:

- frequency analysis (body weight and pelt length),
- t-test (between sexes),
- linear regression (correlations between body weight and pelt length and between body length and pelt length).

The computer programme used for data processing and statistical evaluation was SPSS 7.5 for Windows (1996).

Results and discussion

No significant difference was revealed between sexes in the characteristics measured (Table 1). The mean for body weight at pelting was 539 g, for pelt length 384 mm and for body length 258 mm. Figure 1 depicts the categories of growing chinchillas based on their body weight and pelt length at pelting. The ratio of pelts below 350 mm long was low (6.4%), and a high percentage of these pelts was produced by chinchillas of below average weight (539 g). More than 2/3 (68.8%) of the pelts fell into the medium range of pelt length (350-400 mm). The largest proportion of these pelts (44%) was produced by chinchillas of below average weight. In total 50% of the chinchillas producing short or medium long pelts belonged to the below average weight categories. One quarter of the pelts belonged to the category "long" (400-450 mm). A great majority of these (15.2%) were produced by animals of extra large body weight. There was no pelt of "extra" length (over 450 mm) in the population evaluated. The distribution data suggest that pelt size increased similarly to the increase in body weight at pelting. The relationship of body weight and pelt length was determined by single-factor linear regression analysis according to the following function:

pelt length (mm) = 266.5 + 0.22 body weight (g).

The correlation was medium (r=0.65, P 0.0001) between body weight and pelt length, and lower than medium (r=0.48, P 0.01) between body length and pelt length.

Table 1. Effect of sex on body weight, pelt length and body length at pelting (mean±SE)

Sex	n	Body weight (g)	Pelt length (mm)	n	Body length (mm)
Male	95	537±6	383±2	33	257±2
Female	30	545±11	385±4	8	259±4
p		n.s.	n.s.		n.s.

Notes: n= number of animals, p= level of significance; n.s.= non-significant difference (p>0.05)

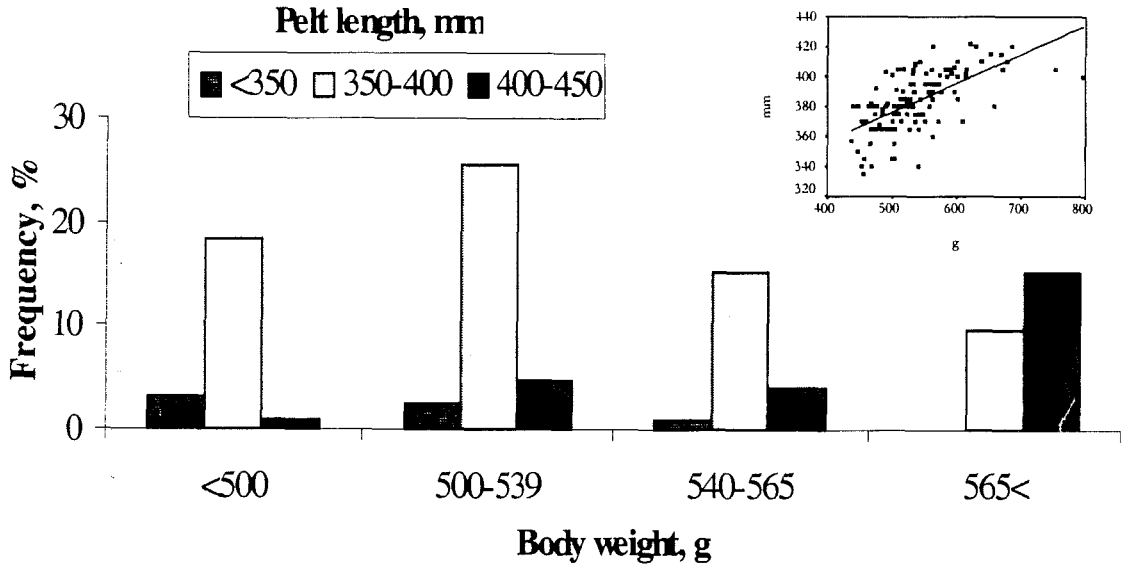

Fig. 1. Frequency of chinchillas depending on their body weight and pelt length at pelting

Conclusion

In a previous paper (Lanszki, 1996) the author reported that progeny born with lower weight in larger litters would also be smaller at the time of evaluation. These individuals would be in a disadvantaged position at pelting compared to those born with larger body weight and achieving better weight gain.

Feeding is also an essential factor. The pelt produced by fat animals is not necessarily longer although though these animals are heavier. Similar connections have been revealed in other species (*Therkildsen, 1988; Nurminen and Sepponen, 1996*). For this reason, when the animals reach their final/mature body length (in the closing period of rearing) it is advisable to introduce a portioned feeding regime. This is important not only to prevent the animals from putting on too much fat: pelting also becomes easier. That is the pelt obtained from animals of normal body condition does not split so easily and the fur does not get fatty during the pelting process.

These results underline the observation that heavier-bodied chinchillas belong to the category of long pelt producers in a higher ratio. This is similar to that found in other species. The correlation coefficient detected in this experiment was medium strong (r=0.65), which provides an indication of the chance for im-

proving pelt length fairly efficiently through selection for increase in body weight. An increase by 50 g in body weight measured at pelting time is accompanied by a 1.1 cm increase in pelt length. The correlation revealed between body length and pelt length was weaker (r= 0.48). What may be a partial background factor is that, in contrast to other fur animal species, body length of chinchillas cannot be measured following the body line (the animal being placed on its back) either on live or on killed animals because of the danger of losing much hair from the pelt. Therefore, the best method for finding the longest-pelted individuals is to base the consideration on body weight instead of body length.

Negative correlations have been detected between pelt length and pelt quality in mink: r= -0.17 *(Lohi, 1989, cit. Børsting and Therkildsen, 1992)*, r= -0.3 - 0.4 *(Børsting and Therkildsen, 1992)*, r= -0.6 - -0.9 *(Lohi and Christensen, 1985)*. However, classification performed on live animals produced positive relationship values in some cases. The influence of the level of animal care, housing conditions (such as cages and climatic environment *(Várady, 1989; Lanszki and Horváth, 1997)* and genetics on pelt quality can also be substantial. The importance of inheritance (good heritability) is obvious through some characteristics of the pelt determined in the progeny by the parents *(Lanszki and Pénzes, 1998)*.

References

Børsting, E., Therkildsen, N. (1992) Skin length and skin quality. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 613-619.

CEFBA (1994) Code of practice for the care and handling of farmed mink, fitch and fox in Europe.

Hansen, B.K., Lohi, O., Berg, P. (1992) Correlation between the development of mink kits in the lactation and growth periods, correlation to fur properties and heritability estimations. Norwegian Journal of Agricultural Sciences, Suppl. no. 9: 87-92.

Lanszki, J. (1996) The effect of litter size and individual weight at birth on the growth and mortality of chinchillas. Scientifur, 1: 42-46.

Lanszki, J., Horváth, P. (1997) A comparative study on the effects of different housing methods and diets on growing chinchillas (*Chinchilla laniger*). Scientifur, 1:11-18.

Lanszki, J., Pénzes, R. (1998) Pelt characteristics of chinchillas (*Chinchilla laniger*) determined by the mating combinations of their parents. 10th Hungarian Conference on Rabbit Production, 129-133.

Lohi, O., Christensen, K. (1985) Afkomsundersøgelser med mink. NJF Symp. Aalborg

Nurminen, L., Sepponen, J. (1996) Effects of fattening on the skin length of the farmed mink. Applied Science Reports. 29: 159-165.

Piórkowska, M. (1996) Relation between the body weight of arctic foxes and the physical parameters of their pelts. Applied Science Reports. 29: 197-174.

SPSS for Windows 1996. Version 7.5, Copyright SPSS Inc.

Therkildsen, N. (1988) Early weighing of mink provides information on pelt length. Deutsche Pelztierzüchter. (62) 8: 117-119.

Várady, B. (1989) Chinchilla breeding [A csincsilla tenyésztése].(In Hungarian) Cegléd, 1-96.

Original Report

Plasma cortisol concentration as an indicator of stress in mink dams at weaning

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Abstract

The plasma levels of cortisol, carbamide and creatinine were measured in blood samples obtained from 12 mink dams at days 35, 42, 43, 44, 46 and 49 post partum. The dams were separated from their kits on day 42 shortly after blood sampling. The day after weaning there was a 2-fold increase in plasma cortisol, but two days after weaning the cortisol concentration was identical to the levels before weaning. One week after weaning the cortisol level returned to the level found in unstressed resting mink. A lowering of plasma carbamide around weaning might indicate reduced feed consumption in this period.

Introduction

Female mink are exposed to several stress factors in the nursing period, due to the number of kits per litter and to a high milk production of up to 190 ml per day in weeks 3 - 4 of lactation (*Wamberg & Tauson, 1998*). The plasma cortisol level in dams before and after weaning showed a distinct stress reduction of the females one week after they had been removed from their kits (*Sørensen et al., 1997*). An investigation of the amount of eosinophilic granulocytes in the blood of females as an indicator of the stress level also showed a fast decline after weaning (*Jeppesen et al., 1988*). Weaning itself, however, is probably the most stressful event for the dams during the nursing period. In mink farming the females are abruptly removed from all the kits which is in contrast to the natural state where a gradual withdrawal of females from kits takes place (*Dunstone, 1993*). Furthermore, the females are taken to a new cage surrounded by unfamiliar females. In some occasions the drip-water system in the new cage is different from the conventional system. All such changes may cause refusal of eating by some dams the day after weaning and some of them may develop nursing sickness (*Clausen et al., 1992*). In the present investigation the plasma concentration of cortisol was measured in female mink on the days before and after weaning. Blood was drawn within 2 minutes after capturing of the dams since Moe & Bakken (1997) have shown an increase in plasma cortisol in foxes after this period due to handling of them. Furthermore, plasma carbamide and creatinine were determined for evaluation of feed consumption and kidney function.

Materials and methods

For the investigation of the 1997 nursing period, 12 two-year-old mink dams (standard black colour) with more than 5 kits per litter were used. The kits were born April 30 (day zero) and were weaned at day 42 post partum. On days 35, 42, 43, 44, 46 and 49 after birth blood samples stabilized with Na-heparin were taken from venae cephalica. On the day of weaning blood samples were taken before the females were transferred to new cages. To avoid an increase in plasma cortisol all blood samples were taken within two minutes after the dams had been trapped. In non-stressed female mink the cortisol level will decline to the normal level within a few hours after handling (*Damgaard, pers comm.*), indicating that blood sampling once a day is not deleterious for cortisol measurement. Carbamide and creatinine in plasma were determined as previously described (*Wamberg et al., 1992*), and cortisol was measured with a standard RIA kit (Coat-A-Count®, Diagnostic Products Corporation, USA) as described by Hansen et al. (1996). The reliability of the RIA kit was controlled by cortisol measurement on a pooled plasma sample from mink males euthanised in 1996. The levels of cortisol in this plasma sample were 181 ± 15 and 186 ± 5 nmol/l in 1996 and 1997, respectively, in the latter case corrected by 8 % according to the instructions given by the manufacturer. One female developed nursing sickness on June 6 and was treated with sodium chloride (0.9%), Vitamin B complex ® and antibiotics.

Results, discussion and conclusion

In mink and other carnivores the plasma concentrations of carbamide and creatinine vary considerably in response to feed intake, food composition and to renal function (*Watson et al., 1981, Tauson & Wamberg, 1998*). Thus, a high plasma concentration of urea may suggest that the animal has had a good appetite. On the other hand, the finding of the highest plasma urea concentrations at day 35 and day 49 (Table 1) may indicate that the females ate less food in the days around weaning. The high levels of plasma creatinine are due, in part, to the ingestion of a diet rich in animal protein, whereas the changes observed during the weaning period (day 35-49) may be taken as a combined measure of the effects of changes in feed intake and glomerular filtration of the kidneys (Table 1).

From day 35 to day 49 there was a decline in plasma cortisol level (Figure 1) corresponding to what was found in a previous investigation (*Sørensen et al., 1997*). The day after weaning, however, there was a distinct peak in plasma cortisol level, indicating that the weaning itself was very stressful event for the females. Two days after weaning the plasma cortisol level was reduced to the same level as before weaning. One week after weaning the plasma cortisol level corresponded to that observed in non-stressed animals outside the breeding period (*Damgaard, personal comm.*).

Table 1. Plasma creatinine and plasma carbamide in mink dams around weaning. Mean ± SEM

Days after birth	Number n	Creatinine µmol/l	Carbamide mmol/l
35	11	127 ±10	16.5 ±0.6 *
42	3	119 ±11	10.8 ±1.2 *
43	6	124 ±4	12.5 ±1.0
44	8	118 ±6	10.6 ±0.6
46	10	145 ±8	9.4 ±1.2 #
49	12	106 ±7	12.9 ±1.1 #
Heparin plasma males 22/3-96		70	10.6

* P<0.01
\# P<0.05

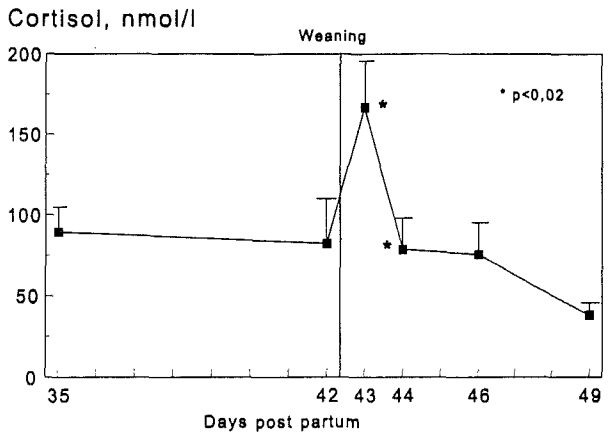

Figure 1. Plasma cortisol in nursing mink dams (n=12) around weaning (day 42). Bars indicate SEM

Cortisol level in a pooled plasma sample from male mink in 1996, and that measured in a previous investigation by Sørensen et al. (1997) were about twice as high as the values found in the present investigation. This may be due to the fact that the former blood samples were not taken within two minutes after the animals had been trapped. Irrespective of an apparently higher cortisol level due to the handling of the animals for a longer period (*Sørensen et al., 1997*), a significant decline of the cortisol concentration was seen one week after weaning.

In conclusion: the results showed that weaning at 6 weeks after birth is very stressful to the dam when she is abruptly removed from all her kits and transferred to unfamiliar surroundings. Some females may react by refusing to eat and by developing nursing sickness. Handling of the females in the days around and at weaning should be further investigated aiming at a minimum of stress to the females and avoiding development of nursing sickness after weaning.

References

Clausen, T.N., Olesen, C.R., Hansen, O. & Wamberg, S., 1992. Nursing sickness in lactating mink (Mustela vison) I. Epidemiological and pathological observations. Can J Vet Res, 56, 89-94

Dunstone, N., 1993. The mink. T & A D Poyser Ltd, London.

Hansen, O., Wamberg, S. & Clausen, T.N., 1996. Failure of loop diuretics to induce nursing sickness in mink at weaning. Can J Vet Res, 60: 277-280.

Jeppesen, L.L., Heller, K.E. & Houbak, B., 1988. Stress i forbindelse med fravænning hos mink. Faglig Årsberetning 1987, 208-215, Dansk Pelsdyravler Forening.

Moe, R.O. & Bakken, M., 1997. Effects of handling physical restraint on rectal temperature, cortisol, glucose, and leukocyte counts in the silver fox (*Vulpes vulpes*). Scientifur, 21, 2: 107-108.

Sørensen, B., Clausen, T.N., Wamberg, S. & Hansen, O., 1997. Minktævers vægtudvikling og stresspåvirkning i dieperioden ved tidlig (6 uger) og sen (7 uger) fravænning. Faglig Årsberetning 1996, 207-213, Pelsdyrerhvervets Forsøgs- og Rådgivningsvirksomhed.

Tauson, A.-H. & Wamberg, S., 1998. Effects of protein supply on plasma urea and creatinine concentrations in female mink (*Mustela vison*). J Nutr 128: 2584-2586.

Wamberg, S., Clausen, T.N., Olesen, C.R. & Hansen, O., 1992. Nursing sickness in lactating mink (*Mustela vison*). II Pathophysiology and changes in body fluid composition. Can J Vet Res, 56: 95-101.

Wamberg, S. & Tauson, A. H., 1998. Daily milk intake and body water turnover in suckling mink (*Mustela vison*) kits. Comp Biochem Physiol, 119 A: 931-939.

Watson, A.D.J., Church, D.B. & Fairburn, A.J., 1981. Postprandial changes in plasma urea and creatinine in dogs. Am J Vet Res 42: 1878-1880.

Mangement of the mink production

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We congratulate Dr. Steen H. Møller with the new title based on the first scientific reports ever regarding management of mink farms.

Summary

Due to the strictly synchronous annual cycle of mink production, the management differs significantly from the management of other intensive, yet continuous livestock productions, as for instance cattle, pigs and poultry. A distinction can be made between strategic, tactical and operational management, with time horizons of several years, months to years and hours to weeks, respectively. At the strategic level, the seasonal synchronisation has no effect on management. At the tactical level it has a marked effect, as the adjustment completing the feedback-loop is postponed for almost one year. With a year between repetition of a period, other production conditions, such as the weather, may have a large impact on the result. At the operational level, the amount and types of management routines change enormously throughout the various production periods. Due to the continuous alternation between annual production periods the farmer's routine and experience is built up slowly and stepwise. These conditions call for specific management routines and management decision support tools, at the operational and tactical level of mink production.

At the operational level there is a need for thoroughly tested and robust plans for management to ensure that in particular, new farmers are able to build their routine and experience on a safe basis. The drastic variation in fur prices has resulted in a significant variation in the number of mink farmers and consequently in the need for advice and guidance at all levels. The need is particularly distinct during the short and labour intensive periods such as mating, whelping and pelting periods, where mistakes can effect the production of the entire year, and advisers are busy, because many mink farmers seek advice at the same time. Also, in these periods there are often extra inexperienced farm hands that need guidance in the relevant routines on the farm.

In order to meet these needs, three systematic operation programmes (SOP) have been developed. Each of them describes a *period of time*, in which a predefined *observation* releases a certain *action*. Two of the programmes cover the short and labour-intensive mating and whelping periods, where as the third programme illustrates the incorporation of specific disease preventive initiatives in the usual farm routines. The programmes are tested and revised in close co-operation with potential users. They are primarily developed in order to support new farmers during their experience build-up, but they have also turned out to be useful to more experienced farmers, employees and consultants in various situations. To ensure an efficient and fair priced distribution, with the possibility of updating, the programmes have been published on the Internet. The increasing number of people visiting the SOP's on the WWW, as well as the response from Denmark and abroad, confirms that the Internet is applicable in this connection.

Mink farmers develop their management through changes in various routines from year to year. Because of variation in climatic condi-

tions, feed quality, animals and possible changes in other routines during the year, the effect of a single factor is difficult to define in comparison to the results from last year. At the tactical level there is, therefore, a need for:

- more systematic methods to examine the effect of new routines under one's own farm conditions
- methods to separate the effect of generally uncontrollable factors from the effect of management, when the course of the production and the need for adjustments is analysed

The applicability of farm experiments, in a systematic evaluation of management routines under production conditions, was investigated. Within two farmer groups, meeting regularly to discuss and exchange mink management experience, an experiment on skin length was conducted. The effect of postponing the time of pelting by two weeks from pelt prime, as well as the effect of weight development prior to pelting, was investigated. There was a marked difference in skin length per kg mink on the participating farms. Postponing the time of pelting had no effect on the skin length of a mink of average weight. The body weight of the mink at fur moulting in October/November had a larger effect on the skin length, than the weight development from October until pelting. This development of management procedures by production experiments resembles the industrial concept of "Evolutionary Operation" (EVOP). The large number of animals and the synchronous production makes the EVOP concept especially suited for mink production. By carrying out the experiments within farm groups, general and farm specific effects could be separated in a conjoint analysis of the results. The investigation demonstrated that farm experiments *can* be applied in the evaluation of new routines. The farmers conducted the experiment according to the plan and the results provided new knowledge of the tested routines. Thus, farm experiments within the right frames, can be valuable tools for the individual farmer, as well as for the entire industry.

When a production period is completed, the mink farmer must evaluate the results according to the scheduled goals. If deviations are significant, it may be necessary to adjust the goals, the plans or the implementation of plans. As the adjustment cannot be made until the following season, the farmer is to evaluate whether the cause for the deviation will apply again next year. Uncontrollable factors affecting the production make this evaluation difficult. Some uncontrollable production conditions like, for instance, climate will effect all farms in a geographic area. The effect may thus be analysed on the basis of production data from a number of farms. To examine this approach, the possibility of separating the effect of management from general uncontrollable factors and estimating the need for adjustments was analysed. Reproduction data from 27 mink farms over 7 years was used. The annual average litter size was calculated for each farm and compared to the average from all farms in the same geographic area. In this way the development on the individual farm was separated from the general development from year to year. Additionally, the effect of the current period of gestation, distribution of female age and litter size index was calculated for the individual farm in relation to the average distribution of these factors on all the farms.

The tool developed for decision support hereby provides the participating mink farmer with a comprehensive view of the development of litter sizes on his own farm compared to those of other farmers. If the litter size is unsatisfactory, he may examine the farm-specific effect of each of the three above-mentioned factors on the reduction of the litter size. If the combined effect is insignificant, the reason is to be found in other factors. A significant negative effect of either the length of gestation, female age distribution, or litter size index can give rise to adjustments in the next season, even in cases where the litter size is otherwise satisfactory.

Thesis, 172 pp, based on the following reports abstracted in this issue of SCIENTIFUR.

Article 1: *S.H. Møller, J.T. Sørensen.* A systems description of a strictly synchronised animal production: The case of mink production. 1999.

Article 2: *S.H. Møller, J.T. Sørensen, A.R. Kristensen.* The concepts of systematic operation programmes (SOPs) applied for the improvement of health and welfare management in mink. Livestock farming systems, More than food production. EAAP Publication No. 89: 277:281, 1997.

Article 3: *S.H. Møller.* Effects of weight development, pelting time, colour type and farm on skin length in mink. Acta Agric. Scand., Section A, Animal Sci. 49: 121-126, 1999.

Article 4: *S.H. Møller.* Application of a central database for mink farm management: Description of a decision support tool for reproduction in mink. Reviewed Report. Scientifur (accepted).

A systems description of a strictly synchronised animal production: The case of mink production

Steen H. Møller, Jan Tind Sørensen

The different characteristics of seasonally synchronous and continuos animal production systems are revealed by analysing the interaction between the management and the production system. A strictly synchronous animal production is illustrated by the annual cycle of mink production. By regarding the mink production as a cybernetic system, the interaction between the management system and the production system is analysed. Characteristic needs for management support are identified as tools to plan and prepare for the production period to come, to evaluate the effect of changes in management and to evaluate the need for adjustments from year to year.

Systematic Operation Programmes, On-farm experiments and Evaluation of production results between years are presented as tools to meet the need for management in mink production. The generality of the needs for management support and of the tools presented are discussed in relation to other seasonally synchronous animal productions.

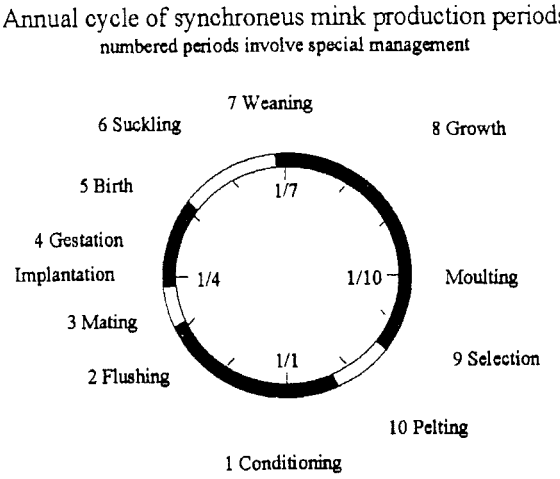

Fig. 2. The annual cycle of synchronous mink production periods. Numbered periods indicate special management routines.

1 table, 3 figs., 24 refs. Authors' abstract

The concepts of systematic operation programmes (SOPs) applied for the improvement of health and welfare management in mink

S.H. Møller, J.T. Sørensen, A.R. Kristensen

Due to a strict annual production cycle, experience is gained slowly and stepwise. Insufficient management in one period may therefore affect the whole annual cycle. Because of these characteristics of mink production, the need for management support is high. This paper describes the concept of systematic operation programmes (SOPs) as one way to meet this need. Based on the development and testing of three SOPs for different aspects of mink production, the role of SOPs in the process of learning and gaining experience is discussed. Situations where a SOP may be applied for the improvement of management are identified.

1 table, 10 refs. Authors' summary.

Effects of Weight Development, Pelting Time, Colour Type and Farm on Skin Length in Mink.

Steen Henrik Møller

The effects on skin length of pelting time, body weight in October/November and weight change until pelting were studied in four different colour types of young male mink on 9 private farms. The skin length was closely related to body size, expressed either as body weight or as body length and condition (weight/length). The regression of skin length on body size differed between farms and colour types. Within the pelting season from mid-November to mid-December, the time of pelting did not affect the skin length of an average size mink, but the regression of skin length on body size interacted with the time of pelting. The effect of body weight on skin length was 11.8 cm per kg at the usual time of pelting and 14.5 cm per kg at pelting 16 days later. The effect on skin length of the weight in October/November was 12.6 cm per kg, while the effect of a weight change between October/November and pelting was 5.5 cm per kg.

5 tables, 2 figs., 18 refs. Author's summary.

Influence of birth weight on live weight development up to weaning in farm mink (*Mustela vison*)

Steffen Hoy, Uwe Mengs, Ulf D. Wenzel

Investigations with 79 litters and 525 liveborn kits and 237 individually controlled up to weaning kits showed a highly significant influence of birth weight (x) on weaning weight (y) in farm mink (*Mustela vison*) ($r = .404$; $y = 179.0 + 12.9\,x$; $p < .01$). Birth weight of weaned kits (10.0 g) was significantly higher compared to kits who were lost during the suckling period (8.3 g). Increasing litter size led to highly significant reduction both in birth weight from 11.7 g (litter size = 2 to 4) to 7.5 g (litter size = 11) and weaning weight from 424 g (litter size at weaning = 1) to 278 g (litter size = 9). Male mink kits had a higher birth weight (9.7 g) compared to female siblings (9.1 g).

Arch. Tierz., Dummerstorf 41: 497-504, 1998. In GERM, Su. ENGL. 4 tables, 2 figs. Authors' summary.

Growth Rates and Intraspecific Variation in Body Weights of Raccoons (*Procyon lotor*) in Southern Texas

Stanley D. Gehrt, Erik K. Fritzell

We estimated growth rates and sex and age-specific seasonal weight fluctuations for raccoons *(Procyon lotor)* in San Patricio Co., Texas. During 1990-1992 we recorded 248 weights for 167 raccoons. Growth rate parameters for raccoons <2-years-old differed ($P < 0.05$) between males and females. Females reached adult size as yearlings, while males did not reach adult size until they approached 2 yr of age. Mean weights of adult raccoons differed between sexes ($P < 0.0001$) and among months ($P < 0.0001$). Raccoons in a subtropical climate experienced seasonal fluctuations in body weight; however, these seasonal patterns differed in their timing and magnitude from those of raccoons in temperate climates.

Am. Midl Nat. 141: 19-27, 1998. 1 table, 2 figs., 32 refs. Authors' summary.

Studies of the physiology of chinchillas with particular regard to immunity and reproduction

Joanna Gromadzka-Ostrowska

In this paper wide references on chinchilla anatomy, embryology, physiology and medical and zootechnical utility, as well as the results of own investigations into chinchilla reproduction and immunity are reported. Chinchillas show a number of structural and functional modifications, which enable them to survive in an extremely harsh environment. This applies to the physiology of the gastrointestinal tract,

water relations, renal function, blood gas transport, blood composition and the unique arrangement of their hair. Chinchillas are able to produce highly concentrated urine and therefore they appear to be similar to desert rodent species in their ability to extract useful water from concentrated solutions of NaCl. A high average rate of transport of food and food residues through the digestive tract and a relatively high degree of digestibility allow these animals to live on food rich in fibre and low in easily digested nutrients. They are characterized by the ability to survive at high altitudes or in other hypoxic surroundings because they can extract and utilize oxygen at as low as 20 mm Hg partial pressure. Body hair of chinchillas is arranged in dense clusters of as many as 75 hairs. Each cluster consists of a single guard hair and two lateral groups of wool hairs.

According to the obtained results, the reproductive processes (estrus cycle, pregnancy, lactation and male sexual activity) just as several immunological indices change seasonally. Significantly higher concentrations of estrogens and progesterone, short regular estrus cycles and higher immunological indices are found between December and May, while in summer months lower concentrations of sex hormones, lower levels of humoral and nonspecific immunity factors, and poorer red blood cell parameters are recorded.Seasonal rhythms are under pineal control, which modulates their periodicity by the secretion of melatonin. Reproductive activity changes and parallel immunity alterations are connected with the duration of the photoperiod. It seems that in chinchillas the melatonin secretion under prolonged daylight conditions causes an increase in pituitary and gonadal hormonal secretion. It also appears that gonadal steroids interact with the development of expression of humoral immunity and modulate the immunological functions by influencing the growth and development processes and the differentiation of immunological cells. Changes in the levels of protein fractions during pregnancy and lactation as well as during growth and development may be due to the changes in antibody concentrations and may indicate that both β_2- and γ-globulin fractions in chinchillas are immunoglobulins. The described protein changes may also prove that β_2-globulins in this species are the major immunoglobulin fraction.

Thesis, Dr. Hab. 134 pp. Zeszyty Naukowe, Akademii Rolniczej im. H. Kollataja w Krakowie, Rozprawy nr 238, 1999. In POLH, Su. ENGL. Author's summary.

Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea pig, ferret and mink

Annelise Hem, Adrian J. Smith, Per Solberg

Fig. 1. A method for physical restraint prior to saphenous vein puncture.

A method is described for blood collection from the lateral saphenous vein. This enables rapid sampling, which if necessary can be repeated from the same site without a need for new puncture wounds. The method is a humane and practical alternative to cardiac and retroorbital puncture, in species where venepuncture has traditionally been regarded as problematic.

Laboratory Animals 32: 364-368, 1998. 3 figs., 17 refs. Authors' summary.

Changes in Estrogen Receptor Expression and Cell Activity of Lactotropes in Female Mink (*Mustela vison*) Pituitary in Response to Variations in the Gonadal Steroid Environment

Sergio Vidal, Maria del Mar Yllera, Albina Román, Lucas Moya

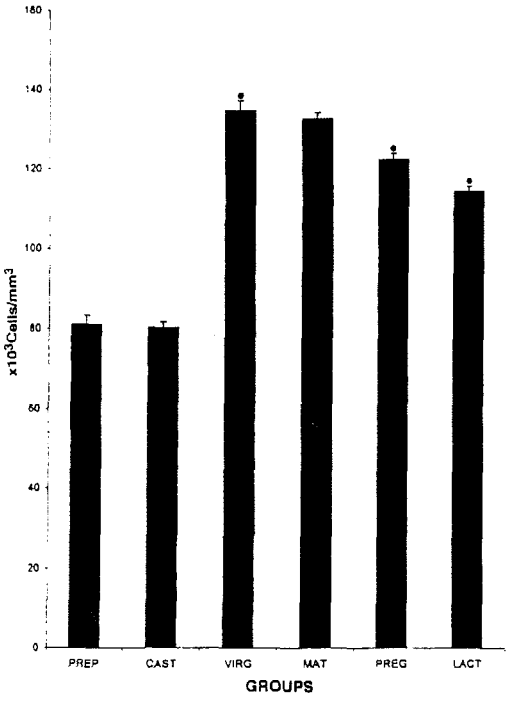

FIG. 17. Changes in the numerical density (N_V) of the female mink ER-immunoreactive cells (PREP, prepubertal; CAST, castrated; VIRG, virgin; MAT, mated; PREG, pregnant; LACT, lactating). The asterisks denote statistically significant ($P < 0.001$) differences in each group compared with the preceding group. Error bars represent SEM.

This study was undertaken to get new information on the role played by estrogen (E) on the activity of mink lactotropes. Immunocytochemistry for estrogen receptor (ER) and prolactin (PRL) was applied to assess modifications in the protein production that occur as a result of in vivo changes in the gonadal steroid environment. Variations in the functional activity of lactotropes were demonstrated from the ultrastructural characteristics and morphometric parameters (cellular area, numerical density, and secretory granular size). The present study documents the presence of ER in mink lactotropes revealing the ability of E to regulate the expression of ER in the mink pituitary. Furthermore, all morphological and morphometric parameters of lactotrope activity appeared significantly increased in intact females, killed during the mating period, compared with castrated females under the same photoperiodic conditions. Castration thus blocks the stimulatory effect of photoperiod on metabolic activity of mink lactotropes suggesting that E may participate in the photoperiodic regulation of PRL.

General and Comparative Endocrinology 114: 365-377, 1999. 17 figs., 52 refs. Authors' abstract.

Description of Two Types of Mammosomatotropes in Mink (*Mustela vison*) Adenohypophysis: Changes in the Population of Mammosomatotropes under Different Physiological Conditions

S. Vidal, A. Román, L. Moya

The present study was undertaken to clarify the existence of mammosomatotropes (MS cells) in the mink adenohypophysis and their possible involvement in the interconversion of mammotrope and somatotrope cells under different physiological conditions: prepubertal, pubertal and adulthood. Electron microscope immunocytochemistry was used to detect growth hormone (GH) and prolactin (PRL) immunoreactivities in the anterior pituitary gland of mink. Primary antisera raised in rabbit (human anti-GH, NIDDK-AFP-1613102481; human anti-PRL, NIDDK-AFP-55781789) were localized with appropriate species-specific antisera coupled to colloidal gold particles of different sizes. MS cells were most frequently observed in adult mink. Double labeling for GH and PRL was presented in two types of MS cells. MS_1 cells, observed only in adults, showed an irregular morphology, with many cytoplasmic processes. Within their cytoplasm there were numerous rounded secretory granules of approximately 135 nm mean diameter. In MS_1 cells three types of secretory granules were identified. The most numerous contained only PRL. The least frequent contained only GH and the third type contained GH and PRL and appeared in an intermediate quantity. MS_2

The protein content of the feed is one of the most important factors influencing the productivity of animal production. Proteins are built up by amino acids which can be divided into essential amino acids and non-essential amino acids. An animal's requirement for protein is therefore normally expressed both as its minimum need for nitrogen and its need for essential amino acids.

The need for protein depends on many factors such as energy intake, level of activity, physiological status, growth capacity, previous nutrition, individual differences and genetic differences. An estimated requirement always depends on the physiological activity for which the requirement is meant, as the protein level sufficient for one physiological process is not necessarily adequate for another physiological process. Most of the experiments in mink have aimed at deciding the need in kits in the growing-furring period to ensure an optimum pelt quality, growth and health.

The amount of protein necessary to meet the demand depends on the protein source, amino acid composition of the protein source, digestibility of the protein and energy distribution with regard to protein, fat and carbohydrates in the feed. In the growth period of mink kits from approx. 10 to 32 weeks a minimum of 30% of the metabolisable energy (ME) from protein is recommended under normal practical conditions in Scandinavia. In the same period a fat content of 35% to 55% of ME is recommended and a maximum carbohydrate content of 30% of ME. These recommendations are based on a study of experimental results showing the need of growing mink to ensure growth, pelt quality and health (*Hansen et al., 1991*).

Intermediary metabolisation of protein

In adult animals, the majority of proteins are absorbed as amino acids from the intestine, whereas newborn animals can absorb intact proteins in the first weeks or days of their lives. The majority of the absorbed amino acids are removed from the blood by the liver. The liver plays a central role in the metabolism of the primary nutrients in the organism, and it controls the anabolism and catabolism of proteins. Protein synthesis in the liver is essential, especially the synthesis of plasma proteins. Among the plasma proteins, the lipoproteins are important, as they are essential to the metabolisation of fats. Disturbances in the synthesis of lipoproteins may result in an accumulation of fats in the liver cells and thus lead to the development of fatty liver (*Alpers & Sabesin, 1987*), and examinations have shown that the main cause for the development of fatty liver is disturbances in the synthesis of lipoproteins.

Cats and other carnivores have a considerably higher protein requirement than omnivorous and herbivorous animals. Protein is an important source of energy for carnivores as they convert protein to glucose via gluconeogenesis (*MacDonald et al., 1984*).

Experiments with various protein contents in the feed

In a cooperation between the Danish Fur Breeders Research Centre and the Danish Institute of Agricultural Sciences, a number of experiments were initiated in 1992 to clarify the effect of various protein levels in the feed as well as the effect of adding amino acids to the feed on the production parameters of mink.

The experiments have naturally been divided into sub-projects dealing with pelt quality and growth (*Børsting & Clausen, 1996*) and sub-projects covering the effects on physiological parameters (*Damgaard et al., 1998a, 1998b*).

The overall purpose of the physiological examinations was to illustrate the effect of the protein level on the health status and physiological parameters in growing mink.

Experiments were carried out in three consecutive years (Experiments 1-3) with mink kits of the standard colour type (Scanblack) on the research farm Vest. The effects of dietary protein levels of 15% (15P), 20% (20P), 25% (25P), 30% (30P), and 35% (35P) of ME were

studied. The carbohydrate content was 17-18% of ME in all feed mixtures, so that changes in protein content were corresponded by changes in fat content varying between 47% and 67% of ME. Furthermore, in Experiments 2 and 3 there were groups that were given feed with a low protein content supplemented with essential amino acids to cover the estimated needs of growing mink (*Børsting & Clausen, 1996*). The animals were weighed at weaning, in September and at pelting. Blood samples were collected in September and at pelting, and liver samples were taken at pelting for physiological analyses.

Effects of dietary protein levels on health parameters

Liver variables

In the experiments performed, feeding with a low protein content in the feed in the growth period resulted in fat infiltration in the liver of the mink. With a decreasing protein content in the feed, the fat content in the liver increased. The reason was probably a defect in the synthesis of lipoproteins essential to the metabolisation of fat and thus to the transportation of triglycerides between liver, tissue and intestine. The cat has limited possibilities of adapting to a low protein content in the feed (*Rogers et al., 1977*). This is probably the reason why the cat has a high need for protein, as it cannot reserve protein for necessary life processes but has a high obligate loss of nitrogen. The high need for dietary protein in carnivores is primarily due to a high need for maintenance prior to growth, and the protein is transformed to energy via the gluconeogenesis (*MacDonald et al., 1984*). It is likely that the mink resembles the cat with regard to protein transformation.

Liver weight in relation to body weight increased with a decreasing protein content in the feed (Figure 1), and a high relative liver weight was connected to fat infiltration in the liver. The physiological background for the high liver weight cannot be explained on the basis of the experiments carried out but is probably due to fat infiltration of the liver. Supplementation of essential amino acids to feed with a low protein content to cover the requirements of mink did not change the fat content in the liver compared with feed without supplementation of amino acids. This indicates that, like the cat, the mink cannot adapt to a low protein content in the feed (*Rogers et al., 1977*).

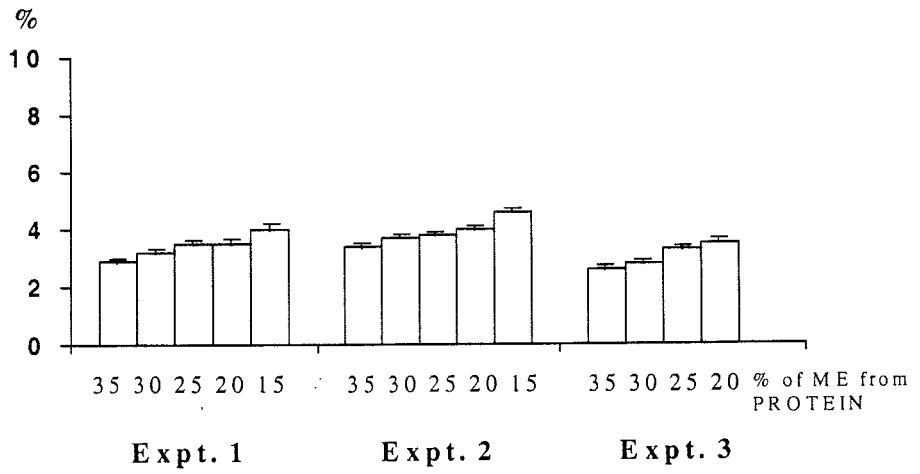

Figure 1. Liver weight in relation to body weight (average and SEM) at different levels of metabolisable energy (ME) from protein in Experiments 1, 2 and 3. n = 15 per group. (After Damgaard et al., 1998a).

Table 1. Plasma activity of ALAT at different levels of ME from protein (35P, 30P, 25P, 20P, 15P) at pelting i December in Experiments 1, 2 and 3. n = 15 per group. Values are average and SEM.

Group	35P	30P	25P	20P	15P	P-level[1)]
Exp. 1	4.4±0.67	5.4±.068	2.5±0.26	3.8±0.32	4.0±0.48	***
Exp. 2	3.1±0.46	3.3±0.34	3.6±0.28	4.1±0.48	5.2±0.87	***
Exp. 3	3.6±0.50	3.5±0.52	4.1±0.35	6.1±0.70	-	***

[1)] Effect of group; *** P<0.001.

Plasma activity of ALAT

The plasma activity of the enzyme ALAT increased with decreasing protein content in the feed (Table 1). High plasma activities of ALAT were connected with a high degree of fat infiltration in the liver, and it is therefore likely that accumulation of fat in the cells is the reason for the destruction of the cells so that ALAT is released. The enzyme ALAT is an intracellular enzyme that is relatively liver specific in mink (*Juokslahti et al., 1980*). Based on examinations of the correlation between plasma activity of ALAT and the degree of fat content in the liver, it was found that the plasma activity of ALAT is valuable for estimation of the incidence of fat infiltration in the liver (*Damgaard et al., 1998a*).

Mortality rate

The mortality rate was high in the groups fed low protein levels and the high mortality rate was probably due to liver insufficiency, as the most frequent diagnosis at the autopsy of dead animals was increased fatty liver. The mortality rate depended on the protein content of the feed, and 30% of ME from protein was necessary to ensure a low mortality rate in all experiments. These results are in agreement with previous results showing that 31% of ME from protein ensured normal growth and viability in mink kits (*Skrede, 1978a*). Supplementation of essential amino acids to feed with a low protein content to cover the estimated requirements of growing mink did not improve the survival rate. It is therefore likely that the amount of protein rather than the quality of protein is the limiting factor for the survival of mink kits.

Growth performance

To ensure normal growth performance in the growth period in all experiments, a dietary protein level of 25% of ME was needed. Skrede (1978b) found that the need for protein was higher in the early growth phases than in the later growth phases and it was proved that 31% of ME from protein, probably suboptimum in the early growth phases, resulted in compensatory growth in the later growth phases (*Skrede, 1978a*). Supplementation of essential amino acids to feed with a low protein content to cover the estimated needs of growing mink had an improving but not satisfactory effect on growth. This indicates that mink have a high need for protein like cats and other carnivores (*MacDonald et al., 1984*).

Blood variables

The plasma concentration of total protein, urea and creatinine was higher at the high protein contents than the low protein contents, and supplementation of essential amino acids to feed with a low protein content had an increasing effect on the variables. The variables examined are therefore supposed to be indicators of the metabolism of amino acids. The results are in agreement with previous experiments with mink showing that the plasma concentration of urea was lower when

feeding with a low protein level compared to a normal protein level (*Työppönen et al., 1986*).

Conclusion

Based on the experiments performed it was concluded that mink is a carnivore and therefore has a need for a high protein content in the feed. Besides essential amino acids, mink have a special requirement for glucogenic amino acids. At a low protein content in the feed, fat infiltration in the liver, a high plasma activity of ALAT, a high mortality rate and reduced growth performance were found. The plasma concentration of total protein, urea and creatinine was affected by the protein content of the feed. To ensure a low mortality rate and a normal growth performance of mink kits in the growth period, the feed must contain at least 30% of ME from protein.

References

Alpers, D.H. & Sabesin, S.M. 1987. Fatty liver: Biochemical and clinical aspects. In: Schiff, L. & Schiff, E.R. (eds.) Diseases of the liver. 6th ed. J.B. Lippincott Co., Philadelphia, USA, pp. 949-978.

Børsting, C.F. & Clausen, T.N. 1996. Requirements of essential amino acids for mink in the growing-furring period. Polish Soc. Animal Prod. Appl. Sci. Rep. 28, 15-24.

Damgaard, B.M., Clausen, T.N. & Dietz, H.H. 1998a. Effect of dietary protein levels on growth performance, mortality rate and clinical blood parameters in mink (*Mustela vison*). Acta Agric. Scand. Sect. A, Animal Sci. 48:38-48.

Damgaard, B.M., Clausen, T.N. & Børsting C.F. 1998b. Effects of dietary supplement of essential amino acids on mortality rate, liver traits and blood parameters in mink (*Mustela vison*) fed low protein diets. Acta Agric. Scand. Sect. A, Animal Sci. 48:175-183

Dunstone, N. 1993. The mink. T & A D Poyser Ltd, 24-28 Oval Road, London NW1 7DX, UK, 232 pp.

Juokslahti, T., Lindberg, P. & Työppönen, J. 1980. Organ distribution of some clinically important enzymes in mink. Acta Vet. Scand. 21, 347-353.

Hansen, N.E. 1978. The influence of sulfuric acid preserved herring on the passage time through the gastro-intestinal tract in mink. Z. f. Tierphysiol., Tierernährg. u. Futtermittelkde. 32, 233-239.

Hansen, N.E., Finne, L., Skrede, A. & Tauson, A.-H. 1991. Energiforsyningen hos mink og ræv (in Danish). NJF-utredning/rapport no. 63, DSR forlag, Den Kgl. Veterinær- og Landbohøjskole, København, Denmark, 59 pp.

Jensen, B.B. 1993. The possibility of manupulating the microbial activity in the digestive tract of monogastric animals. 44th Meeting of the European Association for Animal Production. National Institute of Animal Science, Denmark. Abstracts, Vol. 1, pp. 312-313.

MacDonald, M.L., Rogers, Q.R. & Morris, J.G. 1984. Nutrition of the domestic cat, a mammalian carnivore. Ann. Rev. Nutr. 4, 521-562.

Kainer, R.A. 1954. The gross anatomy of the digestive system of the mink. II. Am. J. Vet. Res. 15, 91-97.

Rogers, Q.R., Morris, J.G. & Freedland, R.A. 1977. Lack of hepatic enzymatic adaptation to low and high levels of dietary protein in the adult cat. Enzyme 22, 348-356.

Ruckebusch, Y., Phaneuf, L.-P. & Dunlop, R. 1991. Physiology of small and large animals. B.C. Decker, Inc., Hamilton - Philadelphia, USA, 672 pp.

Skrede, A. 1978a. Utilization of fish and animal byproducts in mink nutrition. I. Effect of source and level of protein on nitrogen balance, postweaning growth and characteristics of winter fur quality. Acta Agric. Scand. 28, 105-129.

Skrede, A. 1978b. Utilization of fish and animal byproducts in mink nutrition. II. Effect of source and level of protein on female reproductive performance, and pre-weaning growth and mortality of the progeny. Acta Agric. Scand. 28, 130-140.

Stevens, C.E. 1977. Comparative physiology of the digestive system. In: Swenson, M.J. (ed.) Dukes' physiology of domestic animals. 9th ed. Cornell University Press, Ithaca, New York 14850, USA, pp. 216-232.

Szymeczko, R. & Skrede, A. 1990. Protein digestion in mink. Acta Agric. Scand. 40, 189-200.

Työppönen, J., Valtonen, M. & Berg, H. 1986. Low-protein feeding in mink: Effects on plasma free amino acids, clinical blood parameters, and fur quality. Acta Agric. Scand. 36, 421-428.

Direct Measurements of Daily Intake in Suckling Mink (*Mustela vison*) Kits

Søren Wamberg, Anne-Helene Tauson

In this study, the tritiated water dilution technique was found to be a useful and reproducible method for measurement of daily water turnover and milk intake in mink kits with a minimum of interference in the mother-young relationship. During postnatal wk 1-4, the calculated energy output of the daily milk yield of each dam corresponded well with the estimated value for daily energy requirements for growth and maintenance of the kits.

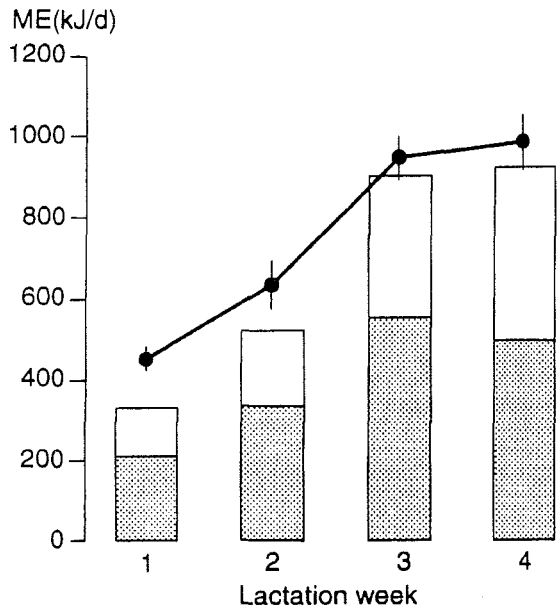

FIGURE 2 Mean (± SEM) daily energy output [ME; kJ/(dam · d)] in mink milk (●) compared with the estimated daily energy requirements for body growth (hatched columns) and maintenance (open columns) of the kits during the first 4 wk of lactation. For details, see text.

American Society for Nutritional Sciences. J. Nutr. 128: 2620-2622S, 1998. 1 table, 2 figs., 10 refs. Authors' conclusion.

Accuracy of Quantitative Collection of Urine in Carnivores

Søren Wamberg, Anne-Helene Tauson

The experimental method described in this paper, using implanted osmotic pumps for continuous release of specific urinary markers, to assess the accuracy of quantitative collection of urine in small, strictly carnivorous mammals, was shown to be feasible and highly reproducible. The technique may also be used in experimental studies on renal clearances and water turnover rates in animal species in which permanent catheterization is not easily performed. Finally, 24-h urinary excretion of endogenous creatinine is a poor index of the accuracy of daily urine collection in mink.

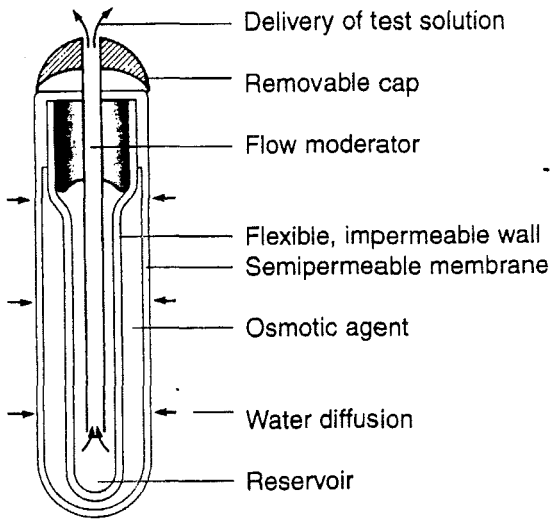

FIGURE 1 Cross section of the osmotic pump, consisting of a flexible drug reservoir, surrounded by an osmotic agent, which is encapsulated in a rigid semipermeable membrane. The flow moderator and the semipermeable membrane are the limiting factors for the actual pumping rate. The dimensions of the pump (Alzet model 2ML1) are as follows: total length, 54 mm; diameter, 14 mm; weight (empty), 5.7 g; volume (reservoir), 2 mL; nominal pumping rate, 10 μL/h.

American Society for Nutritional Sciences J. Nutr. 128: 2758S-2760S, 1998. 1 table, 3 figs., 9 refs. Authors' conclusion.

Energy Metabolism, Nutrient Oxidation and Water Turnover in the Lactating Mink (*Mustela vison*)

Anne-Helene Tauson, Henriette Juul Sørensen, Søren Wamberg, André Chwalibog

Mink kits are born very immature physiologically; they are blind, nearly hairless, devoid of their own thermoregulatory capacity and have very limited locomotor ability. Moreover, they have almost no mobilizable energy reserves be-

cause the fat content in the body at birth is only 1% (Tauson, 1994). On the other hand, they have the capacity for rapid growth during the suckling period, with an average relative growth rate of 12%/24 h during the first 3 wk of life (Tauson 1994), a period in which the kits are totally dependent on the mother's milk for nourishment. For these reasons, and because litters usually are large (commonly averaging>6 kits), the lactation period is very demanding on the energy resources of the dam. Despite a substantial increase in feed intake, dams with large litters are unable to sustain their energy needs by feed consumption and have to mobilize body fat reserves; weight losses of 20% frequently occur during the lactation period (Hansen 1997). Furthermore, female mink have been shown to be in negative energy balance during late gestation (Tauson and Elnif 1994); therefore, a profound additional weight loss during lactation may lead to nursing sickness (Wamberg et al. 1992). It is of utmost importance, therefore, to stimulate energy and water turnover lactation performance and animal health. This study aimed to increase knowledge regarding the specific features of metabolism and water turnover in lactating mink and to estimate the milk yield by the use of a factorial approach.

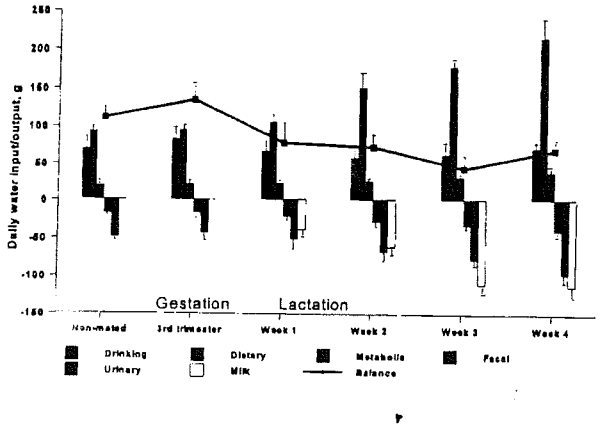

Fig. 1.. Daily water input/output relationships for three female mink that were not mated, and for 10 females that were mated and subsequently gave birth. Results are based on measurements carried out in the last trimester of gestation, and in wk 1, 2, 3 and 4 of lactation. Output of water in milk was calculated by a factorial approach. Values are means and SD.

This study has clearly demonstrated that lactating mink are not able to sustain their energy requirements by feed intake after wk 2 of lactation, but have to mobilize fat reserves from the body. The milk yield can be considered very high in relation to body size; our data indicate that a female of 1100 g with a litter size of 5 kits produces 3000 g milk during the first 4 wk of lactation. Water to sustain milk production is provided mainly by the feed, provided that conventional wet diets are fed, but metabolic water contributes 10% of the total water input. Water output in urine increases substantially in lactating animals, reflecting the need for excretion of excess nitrogen from deaminated protein via urine. To sustain the metabolic needs of high yielding female mink, palatable diets with a high energy concentration must be provided, as well as an ample water supply.

American Society for Nutritional Sciences. J. Nutr. 128: 2615S-2617S, 1998. 1 table, 1 fig., 10 refs. Authors' introduction and conclusion

Effects of Protein Supply on Plasma Urea and Creatinine Concentrations in Female Mink (*Mustela vison*)

Anne-Helene Tauson, Søren Wamberg

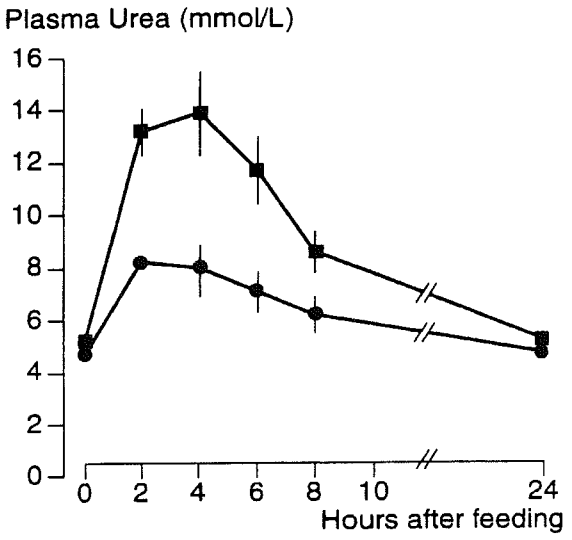

FIGURE 2 Changes in plasma urea concentration (mean ± 2 sd) in adult female mink after ingestion of a high protein (■, HP; n = 5) or a low protein (●, LP; n = 5) test meal.

In clinical veterinary medicine, single or serial measurements of plasma concentrations of urea and creatinine are widely used to evaluate the functional status of the kidneys. In carnivores such as dogs, cats and mink, however, the diagnostic value of these measures may be limited or uncertain, because they are markedly affected by nonrenal factors, and particularly by the amount and quality of dietary protein intake. Thus, Watson et al. (1981) showed that the plasma response in dogs was different when the animals were fed raw vs. heat-treated meat; the postprandial response in plasma urea was more affected by processing than that of creatinine. Changes in plasma concentrations of urea and creatinine, therefore, even in the postabsorptive state, must be interpreted with caution. The aim of this study was to evaluate the influence of dietary protein level on the postprandial changes in plasma concentrations of urea and creatinine in adult female mink given a single test meal.

This experiment underscores the importance of feed-induced changes in plasma concentrations of urea and creatinine in carnivores. Interpretation should be made with caution and the effects of sampling time in relation to feeding and quantity of dietary protein intake taken into consideration.

American Society for Nutritional Sciences. J. Nutr. 128: 2584S-2586S, 1998. 1 table, 3 figs., 9 refs. Authors' introduction and conclusion

Can gas exchange measurements be used for calculation of nutrient oxidation in mink (*Mustela vison*) exposed to short-term changes in energy supply?

Anne-Helene Tauson, Rikke Fink, A. Chwalibog

Nutrient oxidation was calculated from gas exchange measurements for 6 control and 12 flush fed female mink, measured in six consecutive, one week periods. The energy supply to controls and flushed animals in periods 1 and 6 was approx. 850 kJ ME/day, and during restriction and flush feeding, it was approx. 450 kJ ME/day and approx. 1300 kJ ME/day, respectively. Over the total experimental period the energy intake was similar in both groups, but it differed significantly between periods in the flushed group. Protein, fat, and carbohydrate oxidation averaged 39, 38, and 21 %, of the total heat production (HP), respectively in the control group. During restriction, protein oxidation was approx. 35% of HP in flushed animals, then increasing to 55% of HP during the first period of refeeding. High values for fat oxidation were recorded during restriction because of fat mobilization and values were low when feed supply was ample. It was concluded that the calculation method was a good indicative method, but some short-comings were discussed.

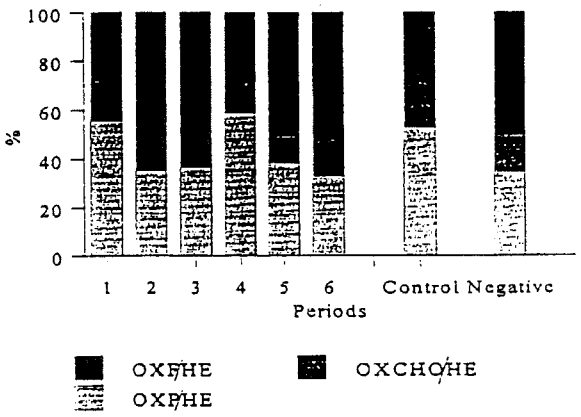

Fig. 1 Heat from oxidation of protein (OXP/HE), fat (OXF/HE) and carbohydrate (OXCHO/HE) in relation to total heat production (HE) for the flushing group in the 6 balance periods and as average for the whole experiment for the control groups

Z Ernährungswiss 36: 317-320, 1997. 3 tables, 1 fig., 13 refs. In ENGL, Su. GERM. Authors' summary.

Long-term effects of tryptophan on behavioural response and growing-furring performance in silver fox (*Vulpes vulpes*)

Kirsti Rouvinen, Shannon Archbold, Sandy Laffin, Mikko Harri

The effects of dietary tryptophan (TRP) supplementation on behavioural response, body weight, feed consumption, and winter fur de-

velopment was assessed on silver fox pups from July 28 until December 5. Ten males and ten females received a commercial fox ration (control) and 10 males and 10 females the same ration supplemented with TRP (1.2 g/MJ ME). Dietary TRP supplementation increased the consumption of protein and gross energy in September and November and total DM in September. The male foxes also consumed more feed and gained more weight than the females throughout the trial. Dietary TRP supplementation did not affect body weight gain, initiation of winter fur growth or fur quality in the test groups. There was a trend toward later priming of fur in the TRP supplemented group.

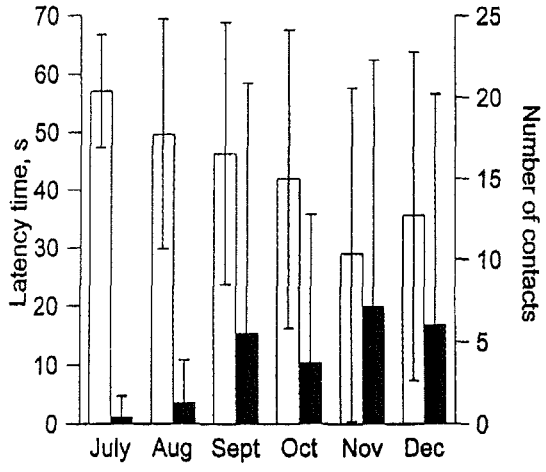

Fig. 2. Overall latency time and number of contacts with the ball during a monthly 1 min novel object test with juvenile silver foxes from July 28 (baseline, before dietary treatments) until December 5 (end of dietary treatments). Presented are means±SD. Number of foxes per test, n = 40. Latency time P = 0.0001, number of contacts P = 0.0002, Kruskal-Wallis test.

The number of contacts with the novel object increased and the latency time until contact with the tidbit and the novel object reduced towards the end of the experiment. In the tidbit test, dietary TRP supplementation reduced the latency time of the females (40.4 s) compared with the non-supplemented females (58.0 s), the TRP supplemented males (51.7 s), and the non-supplemented males (47.6 s, P = 0.001). In the novel object test, the latency time of the TRP females (32.5 s) was likewise reduced compared with the control group females (46.9 s) and TRP group males (44.0 s) being comparable to the control group males (38.5 s, P = 0.029). It appears that dietary TRP supplement reduces fear and enhances exploratory behaviour in the female silver fox. This is likely due to the female being more sensitive to the imbalance between TRP and other large neutral amino acids, the supplement leading to increased brain serotonin synthesis. Further research needs to elucidate the effects of dietary TRP on the pineal function due to potential interference with seasonal breeding and furring controlled by the photoperiod.

Applied Animal Behaviour Science 63: 65-77, 1999. 2 tables, 5 figs., 39 refs. Authors' abstract.

Digestibility by mink and storage stability of feedstuffs made from raw ground, acid-treated or fermented dogfish *(Squalus acanthias)*

M. B. White, D. M. Anderson , K.I. Rouvinen

Dogfish was evaluated in a 3 x 4 factorial design experiment conducted to determine digestibility coefficients (DC) of dry matter (DM), crude protein (CP), crude fat (CF), gross energy (GE) and amino acids (AA) in raw ground dogfish (RGD), acid (ASD) and fermented (FSD) dogfish silages for mink. The ASD was prepared with the addition of 2.5% (wt:wt) formic acid (conc. 85%) and 200 mg kg^{-1} antioxidant (ethoxyquin) to the raw ground fish. The FSD was produced with the addition of the commercial biopreservative Marisil®, (Finn Sugar) (1 %) and extruded wheat (15%) to the raw ground fish. In the digestibility trial, consisting of three, 11-d periods, each having a 6-d adjustment followed by a 5-d collection, 12 mature standard type mink were confined to metabolism cages. Using the total collection (TC) method, where graded levels (0, 15, 30 and 45%) of the test feedstuff were substituted into a basal diet, an extrapolation using regression analysis was done to estimate digestibility of

each test feedstuff. The AD of DM, CP, CF and GE was RGD: 81.8, 92.2, 96.2 and 89.6%; ASD: 87.1, 92.6, 100.0 and 93.2%; FSD: 86.0, 93.3, 98.1 and 90.4%, respectively. The AD of AAs was generally highest in the fermented dogfish feedstuff. A quality evaluation of the silages was conducted in a completely randomized design with four replications (two replications for AAs), to determine storage stability of the silages, on 10 different sampling days (days 1, 3, 6, 9, 15, 30, 90, 180, 270 and 360), (three sampling days for AAs) post-manufacture. The ASD was stable up to 180 d, after this storage time it underwent increases in pH and total volatile nitrogen (TVN) and decreases in N and AA content. The preservation of dogfish with a fermentation method (FSD) was judged to be unsuccessful with this feedstuff having a high initial pH (6.4) and large increases in TVN content early in storage. It was concluded that feedstuffs made from dogfish would provide a source of highly digestible nutrients for mink; however, more research is required before a fermented dogfish feedstuff can be incorporated into practical mink diets.

Can. J. Anim.Sci. 78: 633-640, 1998. 6 tables, 32 refs. Authors' summary.

Stable isotopes in adipose tissue fatty acids as indicators of diet in Arctic foxes (*Alopex lagopus*)

C.M. Pond, I. Gilmour

It is suggested that the key to successful nutrition of captive animals is understanding the species' natural diet, and isotopic methods of feed-web analysis are described as they offer several advantages over conventional methods of analysis. Fatty acids are particularly suitable as indicators of long-term dietary habits. Tracing the ecological origins of biological materials such as fatty acids can be further refined by studying the presence of stable isotopes. The Arctic foxes on Svalbard Island in the Arctic ocean, and chemical and isotopic analysis of triacylglycerol-fatty acids, are further discussed. It is suggested that compound-specific isotope analysis of lipids be used for studies on species that are known to include suitable combinations of feeds from contrasting sources in their diets.

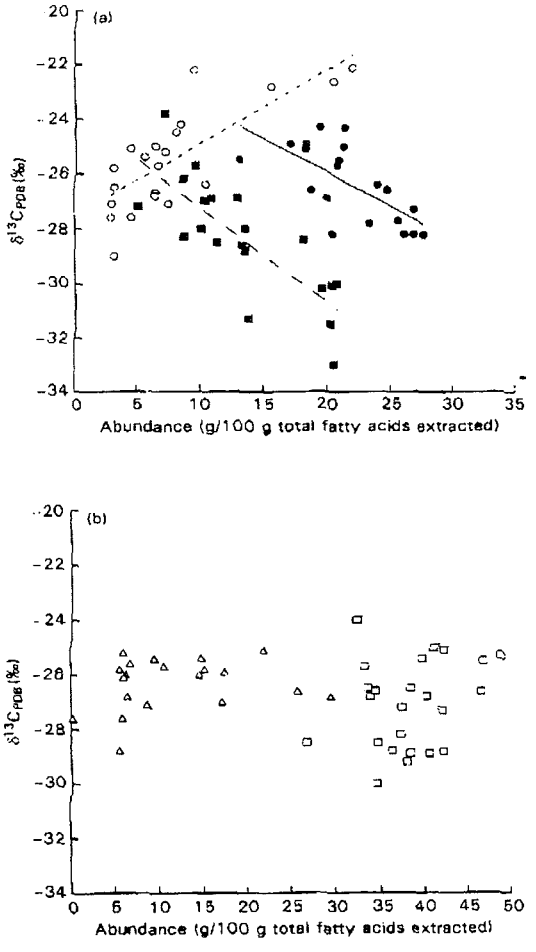

Fig. 1. The carbon isotopic composition, measured as the ratio $\delta^{13}C$ relative to the Peedee belemnite standard ($\delta^{13}C_{PDB}$) of various fatty acids *v.* their abundance relative to the total fatty acids extracted. (a) (•), Palmitic acid ($C_{16:0}$) *r* - 0·68, *P*<0·01; (■), stearic acid ($C_{18:0}$), *r* - 0·79, *P*<0·01; (○), palmitoleic acid ($C_{16:1n-7}$) *r* - 0·77, P<0·01. (b) (□), Oleic acid ($C_{18:1n-9}$); (△), gondoic acid ($C_{20:1n-9}$). Neither shows any significant correlation with abundance (*r* 0·25, *P*<0·2). (Redrawn from Pond *et al.* 1995 and Gilmour *et al.* 1995).

Symposium on Nutrition in wild and captive wild animals, held at Edinburgh Zoo, 16-18 May 1997. Proceedings of the Nutrition Society, 56, 3, pp. 1067-1081, 1997. 2 figs., 74 refs. CAB-abstract.

Effects of Formalin on Bacterial Growth in Mink Feed, Feed Consumption and Reproductive Performance of Adult Mink, and Growth of Mink Kits

K.C. Li, D.C. Powell, R.J. Aulerich, R.D. Walker, J.A. Render, R.K. Maes, S.J. Bursian

Feed that is typically used on commercial mink ranches is an ideal environment for bacterial growth because of the raw animal by-products used as ingredients. Recently, formaldehyde was approved for use as an antimicrobial agent in poultry feed. Experiments in our laboratory were carried out to investigate the effects of incorporating different concentrations of formalin into the feed of mink on the growth of gram-negative and gram-positive bacteria. Feed containing 0, 550 or 1100 ppm formalin was kept refrigerated for up to 7 d and the number of colony forming units of gram-negative and gram-positive bacteria derived from the feed was determined each day. Colony forming units in the formalin-treated feed were significantly fewer than colony forming units in untreated feed. In the second trial, feed containing the same concentrations of formalin was maintained at 30°C for 24 h and cultured bacterial colonies were counted at 0, 12 or 24 h of feed incubation. Both concentrations of formalin were effective in significantly reducing the number of colony forming units. A feed consumption trial determined if mink (*Mustela vison*) preferred formalin-treated feed to non-treated feed kept refrigerated for up to 7 d. Consumption of feed treated with 1100 ppm formalin was significantly lower than consumption of the non-treated feed on d 1, 2, 4 and 5, but body weight was not affected. A long-term feeding trial determined the effects of formalin on mink reproduction, early growth of offspring and quality of fur. Mink were fed formalin at concentrations of 0, 550 or 1100 ppm for approximately 140 d beginning 1 mo prior to mating until kits were weaned at 6 w of age. Mating success was not affected by consumption of formalin-treated diets, but kit survival at birth was adversely affected in mink consuming 1100 ppm formalin. Hemoglobin concentration, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin were significantly decreased in 6-w-old kits, but there were no significant differences in any of these parameters between the kits exposed to 0 and 550 ppm formalin. In a second phase, some kits and their dams were continued on their respective dietary treatments from weaning through pelting (approximately 220 and 320 d, respectively). At pelting, hematocrits and hemoglobin concentrations for the kits fed 1100 ppm formalin were significantly less compared to the control and 550 ppm formalin groups. There were no significant differences in body weights among female kits or adult female mink. The body weights of male kits in the 1100 ppm formalin group became significantly less than the body weights, of male kits in the control and 550 ppm formalin groups as the trial progressed. The quality of fur was highest for mink in the control group and lowest for mink in the 1100 ppm formalin group. While dietary 1100 and 550 ppm formalin were effective in suppressing bacterial growth in the feed of mink, the deleterious effects of 1100 ppm formalin on kit survival, hematologic parameters, body weight, and quality of fur preclude formalin use at this concentration.

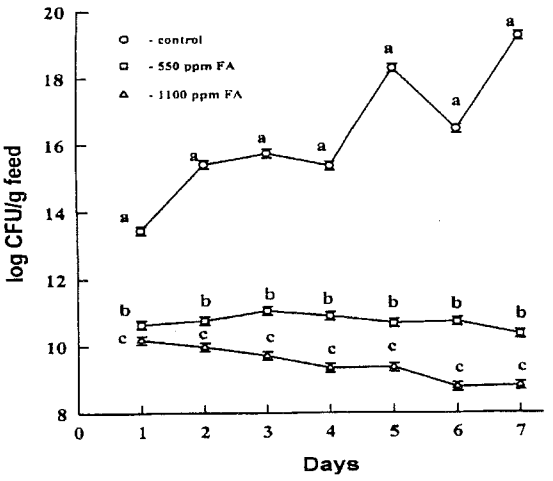

Figure 1. Colony forming units of gram-positive and gram-negative bacteria in formalin-treated feed refrigerated for up to 7 d. Each point represents the mean±standard error of 3 samples. Means with different superscripts at each d are significantly different ($p<0.05$).

Vet Human Toxicol 41 (4), pp. 225-232, 1999. 4 tables, 4 figs., 34 refs. Authors' abstract.

Behavioural Tests in Welfare Research of Foxes

Teppo Rekilä
University of Kuopio
P.O. Box 1627
Fin-70211 Kuopio, Finland

New doctor in the family. We congratulate Dr. Teppo Rekilä with the new title and the comprehensive work it is based on.

Abstract

Farmed blue foxes *(Alopex lagopus)* and silver foxes *(Vulpes vulpes)* have been housed in wire mesh cages with a wire mesh floor without any furnishings in recent decades. The main arguments against keeping foxes in the present systems have been barrenness of the cage and fear of humans resulting from insufficient adaptation to the housing environment or/and to the presence of human.

A total of nineteen tests have been used to measure behavioural characteristics in foxes. These include for example the open field test, which is commonly used for laboratory and farm animals, and several tests measuring reaction of the animals to the presence of humans, either directly or indirectly. Most of the tests are considered to measure fearfulness.

In the present study, a behavioural test measuring fear in foxes was developed and its relationship to some other welfare parameters was assessed, the possibility of selecting against fear in blue foxes was studied and the effects of different environmental variables inside and outside the cage on the welfare of foxes were examined.

The feeding test, developed in this study, is based on the hypothesis of hyponeophagia, i.e. that fearful animals do not start to eat when exposed to the human presence. The feeding test provides a feasible, reliable and valid measure of fear of humans in farmed foxes. Fearful blue and silver foxes had a stress level as assessed using urinary cortisol:creatinine ratio. In silver foxes, the same was observed in blood cortisol in an unstressed situation and following ACTH administration.

In the genetic selection experiment, blue foxes were selected according to their behaviour in the feeding test, with the selection carried on over three generations. Subsequently, the number of fearful individuals decreased. Thus, the behaviour of blue foxes in the feeding test is heritable.

In addition, they had reduced stress levels as assessed by urinary cortisol:creatinine ratio and reproductive performance seemed to improve.

Despite being widely used and providing ease of obtaining data using an automatic system, the validity of the open field test as a fear test is hampered by the several motivations affecting the animal's behaviour in the test, and by the lack of validation of the test for foxes. The present results show that the open field test measures general activity drive of the foxes, rather than their fearfulness.

Fear in foxes can be reduced by selecting for less fearful animals and by promoting positive human-animal relationships. Provision of cages with nest boxes or platforms affect the behaviour of the foxes only minimally, and to a

lesser extent than the environment outside the cage.
The environment outside of the cage should be considered as an essential part of the foxes' environment.

Thesis, 52 pp. 3 tables, 1 fig., 121 refs. The thesis is based on the following publications.

I. Harri M, Rekilä T, Mononen J: Factor analysis of behavioural tests in farmed silver and blue foxes. Appl Anim Behav Sci. 42: 217-230, 1995. *Abstracted in this issue of SCIENTIFUR.*

II. Rekilä T, Mononen J, Harri M: Effect of inside-cage and outside-cage environment on behaviour test performance of blue foxes *(Alopex lagopus).* Acta Agric Scand Sect A, Animal Sci 46: 247-252, 1996. *Abstracted in SCIENTIFUR, Vol. 22, No. 2, pp 202, 1998.*

III. Rekilä T, Ahola L, Mononen J, Harri M: Effect of the environment inside and outside the cage on activity and behaviour test performance of silver foxes *(Vulpes vulpes). Agric* Food Sci Finland 7: 13-19, 1998. *Abstracted in this issue of SCIENTIFUR.*

IV. Rekilä T, Harri M, Ahola L: Validation of the feeding test as an index of fear in farmed blue *(Alopex lagopus)* and silver foxes *(Vulpes vulpes).* Physiol Behav 62:805-810, 1997. *Abstracted in this issue of SCIENTIFUR.*

V. Rekilä T, Harri M, Jalkanen L, Mononen J: Relationship between hyponeophagia and adrenal cortex function in farmed foxes. Physiol Behav 65: 779-783, 1999. *Abstracted in this Issue of SCIENTIFUR..*

Factor analysis of behavioural tests in farmed silver and blue foxes

M. Harri, T. Rekilä, J. Mononen

We recorded and analysed behaviour of farmed silver and blue foxes in the open-field with a Computer-based system. In addition, the reaction towards humans and the capture time were recorded for the animals in their home cage. Factor analysis with VARIMAX rotation produced four independent factors. In the silver fox, the first factor was loaded from general activity in the open-field and accounted for 42% of the variance; the second factor was mainly loaded from initial activity (24%). The third factor was related to the reaction towards humans (19%) and the fourth was the capture time (15%). In the blue fox, the factors were: general (41%) and initial activity in the open-field (26%), capture time (17%) and reaction towards humans (15%). A 5 min exposure to the open-field was sufficient to provide the information needed. In the spring in comparison with the autumn a higher proportion of silver foxes did not come out of the start box to the open-field at all, while fewer young blue foxes emerged than old ones, such reaction thus being an additional behavioural test. The open-field Factor 2 (initial activity) was higher in adult silver foxes and in the autumn compared with juveniles or the same adults about 5 months earlier. The adult individuals in this group were more passive towards humans in the spring. Adult blue foxes were more active in the open-field than the young and their initial activity was higher in the spring. In the spring there were more passive blue foxes while in the autumn the number of fearful individuals was greater. In the autumn, juveniles were more aggressive than adults. Adult blue foxes were more easily accessible to a capturer than young blue foxes.

The effect of cage environment on behaviour of the foxes was assessed in animals housed in empty wire-mesh cages or in cages provided with resting platforms and nest boxes or platforms only. There were no other differences between these groups in their open-field behaviour except that the initial activity was lower in the control blue foxes. Blue foxes in the control group were more fearful and passive towards humans, whereas no differences were found among silver foxes. The capture time was always longest in the nest box groups, due to the obstacles that the nest boxes presented to the capturer. Our results confirm that behaviour of

foxes is subject to changes detectable by the behavioural tests used. However, in this study the behaviour of both fox species appeared to be affected more by season and age than by housing conditions.

Applied Animal Behaviour Science 42: 217-230, 1995. 4 tables, 1 fig., 25 refs. Authors' abstract.

Effect of the environment inside and outside the cage on the activity and behaviour test performance of silver foxes

Teppo Rekilä, Leena Ahola, Jaakko Mononen, Mikko Harri

On the basis of daily activity in the home cage and the open field test the effect of the internal design and location of cages on the behaviour of silver foxes *(Vulpes vulpes)* during a growth period was evaluated. The inclusion of platforms in cages increased the daytime activity of silver foxes in their home cage, but the inclusion of nest boxes did not. Silver foxes housed at the front of the animal barn were less active during the working day and more active in the evening than were animals housed at the rear. The results of the open field test did not differ significantly between animals housed in cages differing in design. This study demonstrates that the behaviour of silver foxes was only minimally affected by the interior environment of the cage, and that attempts to improve housing design should also take the environment outside the cage into account.

Agricultural and Food Science in Finland, Vol. 7: 13-19, 1998. 1 table, 2 figs., 16 refs. Authors' summary.

Validation of the Feeding Test as an Index of Fear in Farmed Blue *(Alopex lagopus)* and Silver Foxes *(Vulpes vulpes)*

Teppo Rekilä, Mikko Harri, Leena Ahola

The reliability and validity of the eating behaviour in the presence of man (Feeding test) as an index of fear were assessed in farmed blue *(Alopex lagopus)* and silver foxes *(Vulpes vulpes)*. Repeatability of the Feeding test was good in both species. No further habituation occurred after the fourth successive test in either species. In addition, the behaviour of both species was independent of the person who performed the test. The normal feeding interval, i.e., 24 h, between feed deliveries, was long enough to provide reliable results. The presence of a cage mate did not influence the blue foxes' response in the Feeding test.

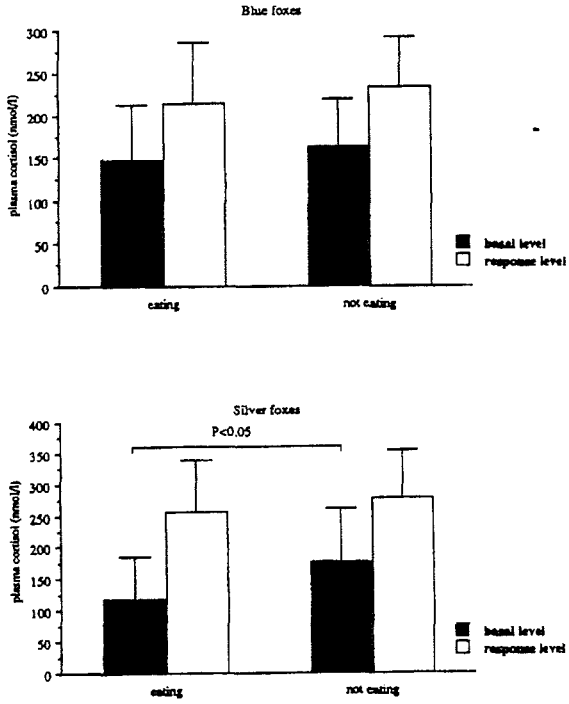

FIG. 1. Basal level and response level of cortisol (M ± SD) in blue and silver foxes eating and not eating during the Feeding test. The response level of cortisol is higher than basal level in both groups of blue foxes (ANCOVA with sampling order as the covariate, $p < 0.01$) and in both groups of silver foxes ($p < 0.001$). Basal level and response level samples of plasma cortisol were drawn within 2 and 20 min of the start of the capture of the fox, respectively. Foxes were released in the period between these two samples.

A significant relationship between the results of the Feeding test and the Tit-bit test in both species and between the Feeding test and the fearfulness score in silver foxes indicate that all these tests measure similar features, most probably foxes' fear of humans. Those silver foxes that did not eat in the Feeding test had higher base levels of cortisol than the animals that did eat, providing further support for the above conclusion. The present study demon-

strates that the Feeding rest is a reliable, i.e., repeatable and free of random errors, and fairly valid fear test for blue and silver foxes. The Feeding test seems likely to give good results in measuring fear in farmed blue and silver foxes, but further investigations will be needed to fully validate it, especially for blue foxes.

Physiology & Behaviour, Vol. 62, No. 4, pp. 805-810, 1997. 1 table, 2 figs., 40 refs. Authors' summary.

Relationship Between Hypotieophagia and Adrenal Cortex Function in Farmed Foxes

Teppo Rekilä, Mikko Harri, Liisa Jalkanen, Jaakko Mononen

The adrenal cortex function of farmed blue *(Alopex lagopus)* and silver foxes *(Vulpes vulpes)* differing in their reaction in the feeding test were assessed. The urine cortisol:creatinine ratio was lower for those animals eating in the feeding test in comparison to those not eating in both species. In addition, eater silver foxes had lower baseline serum cortisol concentration and also lower serum cortisol concentration 2 h after ACTH administration than noneaters. There were no differences in any serum cortisol levels between the eater and noneater blue foxes. The weights of body and adrenals did not differ between confident and fearful animals in either species. The present study demonstrates that animals not eating in the feeding test may have higher fearfulness and be more stressed than animals eating.

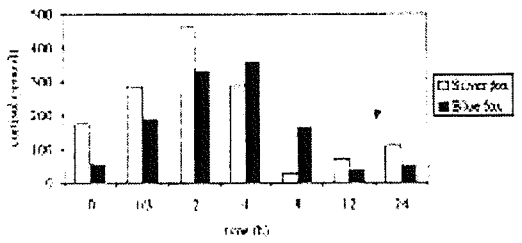

FIG. 1. Blood cortisol level before and after ACTH administration (t.zt.) in silver foxes and blue foxes.

Physiology & Behavior, Vol. 65, Nos. 4/5, pp. 779-783, 1999. 1 table, 1 fig., 38 refs. Authors' abstract.

Factor analysis of behavioural tests in farmed silver and blue foxes

Mikko Harri, Teppo Rekild, Jaakko Mononen

We recorded and analysed behaviour of farmed silver and blue foxes in the open-field with a computer-based system. In addition, the reaction towards humans and the capture time were recorded for the animals in their home cage. Factor analysis with VARIMAX rotation produced four independent factors. In the silver fox, the first factor was loaded from general activity in the open-field and accounted for 42% of the variance; the second factor was mainly loaded from initial activity (24%). The third factor was related to the reaction towards humans (19%) and the fourth was the capture time (15%). In the blue fox, the factors were: general (41 %) and initial activity in the open-field (26%), capture time (17%) and reaction towards humans (15%). A 5 min exposure to the open-field was sufficient to provide the information needed.

In the spring in comparison with the autumn a higher proportion of silver foxes did not come out of the start box to the open-field at all, while fewer young blue foxes emerged than old ones, such reaction thus being an additional behavioural test. The open-field Factor 2 (initial activity) was higher in adult silver foxes and in the autumn compared with juveniles or the same adults about 5 months earlier. The adult individuals in this group were more passive towards humans in the spring. Adult blue foxes were more active in the open-field than the young and their initial activity was higher in the spring. In the spring there were more passive blue foxes while in the autumn the number of fearful individuals was greater. In the autumn, juveniles were more aggressive than adults. Adult blue foxes were more easily accessible to a capturer than young blue foxes.

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forms only. There were no other differences between these groups in their open-field behaviour except that the initial activity was lower in the control blue foxes. Blue foxes in the control group were more fearful and passive towards humans, whereas no differences were found among silver foxes. The capture time was always longest in the nest box groups, due to the obstacles that the nest boxes presented to the capturer. Our results confirm that behaviour of foxes is subject to changes detectable by the behavioural tests used. However, in this study the behaviour of both fox species appeared to be affected more by season and age than by housing conditions.

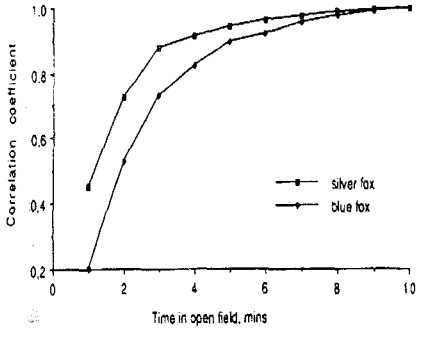

Fig. 1. Correlation between cumulative and total open-field activity in silver and blue foxes.

Applied Animal Behaviour Science 42: 217-230, 1995. 4 tables, 1 fig., 25 refs. Authors' abstract.

Experiments and a model examining learning in the area-restricted search behavior of ferrets *(Mustela putorius furo)*

David G. Haskell

Area-restricted searches have been described as important components of the foraging behavior of many organisms. It is unclear, however, whether individual foragers can use learning to fine-tune their searches, or even whether these searches are efficiently performed. I used a simulation model to make qualitative predictions about search behavior in a laboratory system. The simulation model indicates that the sinuosity and path length of searches strongly affect search efficiency. The model predicts that, for a rate-maximizing forager, path length should increase and search sinuosity should decrease as prey become less clumped. Foraging animals may therefore be selected to learn the path length and sinuosity of searches in response to changing degrees of clumping of prey. These predictions were tested in a laboratory system involving ferrets *(Mustela putorius furo)* foraging for oil-drop "prey items." Search paths changed in a graded manner to experimental manipulations of the clumping of prey. As predicted by the model, ferrets learned to perform longer and less sinuous search paths as prey became less clumped. This study provides the first evidence that area-restricted search behavior is learned and can be fine-tuned to efficiently exploit different spatial distributions of food.

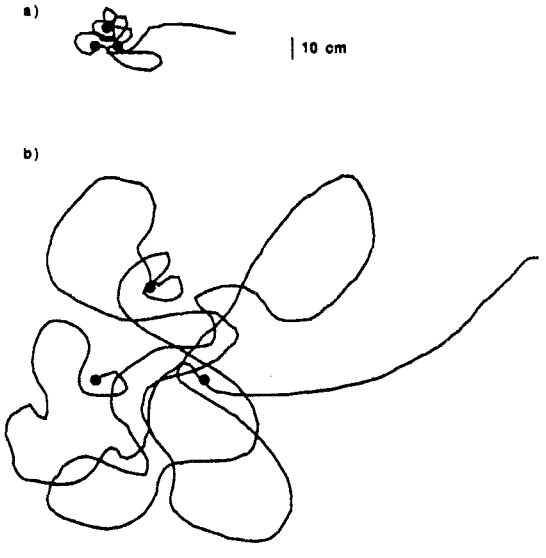

Figure 6
Example of ferret foraging paths (a) when oil drops were 11 cm apart and (b) when oil drops were 55 cm apart.

Behavioral Ecology, Vol. 8, No. 4: 448-455, 1996. 6 figs., 32 refs. Author's abstract.

A comparison of the use of resting platforms and nest boxes in growing farmed silver foxes *(Vulpes vulpes)*

Jaakko Mononen, Hannu Korhonen, Mikko Harri, Sari Kasanen

The use of the interior and roof of a nest box and the use of various types of resting platform were studied in 50 juvenile silver foxes *(Vulpes*

vulpes) of both sexes housed singly in traditional fox cages measuring 115 x 105 x 70 cm (L x W x H). The experiment was carried out from early July to the end of December. The use of the nest boxes and platforms by the silver foxes was video-recorded for one 24-h period in August, October and December. Furthermore, the use of these furnishings was observed 14 times per week for the whole experiment by a person walking past the experimental cages in the daytime. The video recordings showed that silver foxes spent an average of 2.0 ± 2.7% (median 1.3%) of their daily time in the nest boxes.

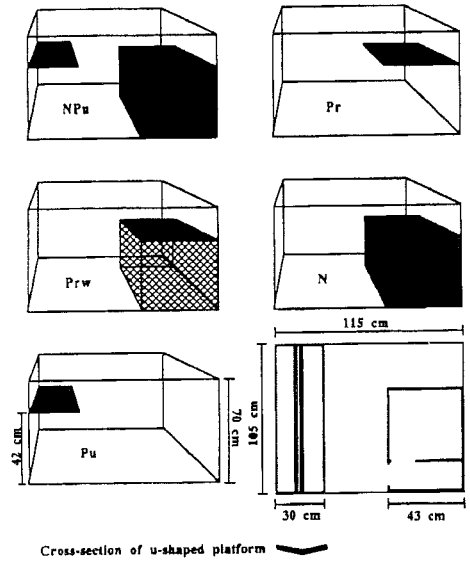

Fig. 1. Schematic drawings of the experimental cages, nest boxes and platforms. For clarity, NPu-type cage is shown also from above (bottom right). For technical reasons, the u-shaped platforms in NPu and Pu cages seem to be flat, but their true bottom shape is as indicated in the cross-section. In the daytime observations, the observer approached each cage from the right.

The foxes were observed in the nest boxes in 0.9 ± 2.7% (median 0.3%) of the direct daytime observations. The average use of the platforms and the roof of the nest box (i.e., one type of platform) varied from 24 to 84% of daily time and from 17 to 92% of the daytime observations depending on the month and the platform or cage type. The roof of the nest box was used more than platforms of corresponding shape and size, and the use of the roof decreased less in the course of autumn than the use of the platforms. This was possibly due to the nest box obstructing the view from the cage floor and restricting the free floor area of the cage. In the early winter, the silver foxes preferred the flat and wide platform to a narrower platform with a slightly u-shaped bottom. This may be due to the wider platforms enabling the foxes to assume more easily the curled resting posture typical in cold weather.

Applied Animal Behaviour Science 58: 383-396, 1998. 4 figs., 15 refs. Authors' abstract.

Choice between floor type and floor level in farmed silver foxes

M. Harri, J. Mononen, S. Kasanen, L. Ahola

The debate about whether or not wire mesh floor is aversive for animals is as old as the history of fur farming. Due to a lack of scientific evidence, argument is mainly based on emotions; wire mesh floor is not natural. The available scientific evidence is scarce and does not give clear evidence for or against. Some experiments have shown that, when given a choice, adult silver foxes distributed their time about equally between a solid bottom and wire bottom (c.f., Bakken et al., 1994), whereas some experiments showed that, unexpectedly, silver and blue foxes spent even more time in a high-mounted wire-mesh cage than in a 4-times larger earth-floor pen (Korhonen and Niemelä, 1994). This and many other results (c.f., Bakken et al., 1994) support the hypothesis that silver foxes prefer to stay on a higher level. However, in addition to floor type, also other key features of the cage environment influence their choices. The present paper is a part of the experimental series in which those key features have been examined. This time silver foxes had to weigh the importance of the floor level against the importance of the floor type. In earlier studies silver foxes did not show any preference for solid floor (cf. Bakken et al., 1994; Korhonen and Niemelä, 1994), whereas in the present study some preference for sandpeat floor was found. However, this preference was not exclusive but was influenced by several intervening factors. The preference for wire mesh floor as a resting place at once increased as it was lifted higher and the preference further increased with time. These observations support the conclusion that a surface for active behav-

iour and rest are selected using different criteria. For resting place, level is important. Unexpectedly, the preference for wire floor as a resting place did not decrease during the third period although the wire floor was returned to its initial lower level. Exceptionally hot weather may explain this as well as a lower activity of the animals during the last two periods. Furthermore, preference of active behaviours for sand floor is greatly influenced by experimental set-ups. Korhonen and Niemelä (1994) used a long tunnel to connect the cages, whereas in the present study a simple hole between the cage pairs served the purpose. As a result, what the foxes chose in the study of Korhonen and Niemelä (1994) may be going through the tunnel, rather than a quality of surface at the end of tunnel explaining the equal activity counts in the two cages connected with the tunnel. In this study going through the hole was perhaps not as attractive as an object of preference, maybe going through the hole even disturbed normal locomotory patterns to such an extent that the foxes distributed their activity more inside one and the same cage. Selection of wire floor for defecation was, however, exclusive.

Proceedings of the 29th International Congress of the International Society for Applied Ethology, Exeter, UK, 3-5 August 1995,pp. 171-172. 1 table, 2 refs. Authors' introduction & conclusion.

Housing design for farmed foxes based on key features of the environment

M. Harri, L. Ahola, S. Kasanen, J. Mononen, T. Rekilä

In this study we tested preferences of farmed foxes, the silver fox *Vulpes vulpes* and the blue fox *Alopex lagopus*, for different details in their cage design in an attempt to detect those key features which are the targets of the choices. Unexpectedly, blue foxes chose a wire mesh platform as often as the wooden one. Both species avoided resting platforms with walls, a finding confirmed by preference tests: Both species almost exclusively preferred the cage half without opaque walls. The foxes used an interior of a nest box located at floor level less than 10 % of the time while spending a major part of their resting time on its roof. By contrast, a nest box placed at the top of a cage was highly preferred but lost its favor as soon as one of its walls was removed. The preference for cage floor material was dependent on the way the cages were connected and other factors. Compared to a mesh floor, there was no consistent preference for a sand floor, whereas there was a preference for an elevated floor of any type. These examples demonstrate that the focus of animals' preference may be a minor feature in the environment, rather than the environment as humans experience it.

Scand. J. Lab. Anim. Sci. No. 1, Vol. 23, pp. 107-112, 1996. 26 refs. Authors' summary.

Choice between cages with and without nest boxes in farmed foxes

J. Mononen, M. Harri, T. Rekilä

The barrenness of the cages of farmed silver foxes *(Vulpes vulpes)* and blue foxes *(Alopex lagopus)* is one of the major complaints against fox farming. Traditionally, only breeding females have been provided with breeding boxes for giving birth and nursing the pups. Otherwise foxes have lived in their cages without any furnishing. Whole-year nest boxes and resting platforms have been recommended as a possibility to improve foxes' welfare (European Convention, 1991). However, the available scientific evidence to support this recommendation is scanty and controversial. In the present study the strength of the need of juvenile silver and blue foxes for a wooden box was assessed in a preference test including a deprivation from access to the nest box. From the present results it might be concluded that silver foxes prefer a solid surface to the wire mesh as a resting place. However, this conclusion is not supported by earlier results (Bakken *et al.*, 1994; Korhonen & Niemelä, 1995). The preference for resting sites situated high up in the cage has been observed earlier in silver foxes (Bakken *et*

al., 1994). Thus, it is possible that the silver foxes in the present study chose the roof of the nest box as a resting site because it was the highest suitable place in the double cage system. We recognize the weaknesses of preference tests, but share the opinion of Hutson *et al.* (1993) that these tests provide a useful tool for identifying the environmental features that animals prefer. Assuming that greater use indicates greater need, silver foxes should benefit from the nest boxes more than blue foxes. These two apparently similar fox species with these obvious differences in their preferences offer an excellent opportunity to study the relationships between preference, need and animal welfare by also employing methods other than simple preference tests.

Proceedings of the 29th International Congress of the International Society for Applied Ethology, Exeter, UK, 3-5 August 1995,pp. 203-204. 1 table, 4 refs. Authors' introduction & conclusion.

Effects of Top Nest Box on Growth, Fur Quality and Behaviour of Blue Foxes *(Alopex lagopus)* During their Growing Season

M. Harri, J. Mononen, T. Rekilä, H. Korhonen, P. Niemelä

The effects of a top nest box on production-related parameters and behaviour were examined in a field trial on four groups of 24 growing blue fox cubs from weaning until pelting. Control animals had a wire mesh platform. There were no differences in weight gain between groups. Dirtiness of nest boxes decreased towards the end of the season. By October, over 90% of the boxes were scored as acceptable. Fur quality tended to be lower in nest box groups, due to staining from faeces and urine. Use of the nest boxes seemed to depend more on the individual animal rather than its species. Three individuals (out of 20) accounted for almost one half (48%) of the total use, while the 12 least enthusiastic users accounted for less than 5% of the total use (as assessed by video recording). The enthusiastic users were mainly in the box at night. In the feeding and disturbance tests, more individuals from the nest box groups showed fear-related reactions. A transparent front wall did not reduce the number of timid individuals. Fearfulness resulting from nest box history is a negative feature from both practical and animal welfare points of view.

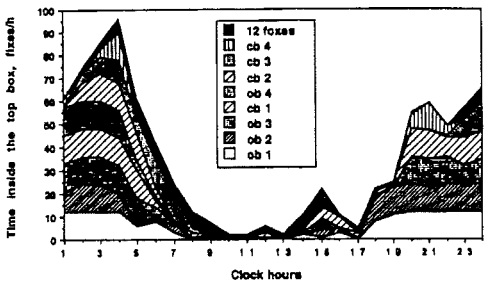

Fig. 2. Circadian distribution of cumulative nest box use by 20 blue foxes on October 16th. The animals are ranked in descending order of magnitude of 24-h use, except that values for 12 individuals using the boxes least are pooled. The use is calculated on an hourly basis (max = 12 fixes/h per animal, 100% for 20 animals = 240 fixes). ob = open box, cb = closed box.

Acta Agric. Scand., Sect. A, Animal Sci. 48: 184-191, 1998. 6 tables, 2 figs., 30 refs. Authors' summary.

The Behaviour of Farm Mink with Unlimited Access to Water for Swimming

Claus Peter Bjælke Hansen, Leif Lau Jeppesen,

Since birth eighty mink have been kept in an environment designed to investigate the possibility of swimming being a behavioural need in farm mink. Half of the animals have free access to water-filled basins, the other half have never experienced this. In addition, the design includes two different cage sizes. As shown previously, no effect of water could be detected with regard to the level of stereotype behaviour. The group with large cages showed significantly less stereotype behaviour than the group with small cages.

Of the forty animals with water, eleven were selected for 24-hours video recordings. This revealed a large individual variation with regard to water utilization. Frequency of swimming bouts ranged from 0 to 177, which was posi-

tively correlated with the amount of water loss from the basins during that week. The amount of time spent in the water varied from 0 seconds to nearly 40 minutes and average duration of swims from 2 to 37 seconds. No relation between swimming frequency or duration and stereotype behaviour was detected.

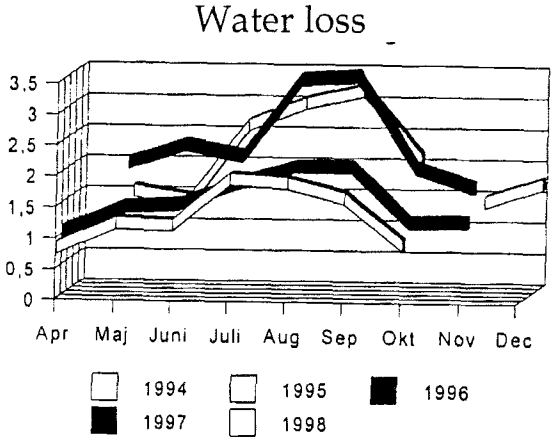

Fig. Five year monthly average for water loss in liters/mink/week.

The data from the four animals using the water the most were investigated further. Dividing the swimming bouts into five second intervals and plotting them against frequencies, the distributions were shown to follow Poisson distributions. This indicates that the duration of a swim is independent of the time already spent in the water. This is not expected if swimming is a behavioural need.

The water losses from the water basins were positively correlated with average monthly temperatures. This indicates a correlation between temperature and amount of swimming bouts. In conclusion, it is suggested that swimming by farm mink is determined by external factors such as temperature and is not a behavioural need. It is recommended that the relation between swimming and temperature and between cage size and stereotype behaviour is investigated further.

Technical Year Report, pp. 169-175, February 1999. 4 tables, 1 fig., 9 refs. In DANH. Authors' summary.

Resource distribution, female home range dispersion and male spatial interactions: group structure in a solitary carnivore

Stanley D. Gehrt, Erik K. Fritzell

We monitored 74 (41 male, 33 female) radiocollared raccoons *(Procyon lotor)* from February 1990 to July 1992 on the Welder Wildlife Refuge, Texas, to relate male and female space use to each other and to the spatial distribution of water, a critical resource for raccoons. Female home ranges were spatially aggregated early in the study, when standing water occurred in only a few, widely separated patches on the study site, but were randomly distributed during seasons when water was more widely distributed. Adult females generally foraged and rested independently of other adults. Most adult males were arranged in spatial groups whose home ranges overlapped little with those of adjacent groups. These groups were usually composed of three to four individuals; Doncaster's (1990, *J. theor. BioL,* **143,** 431-443) test for dynamic interaction and visual observations showed that group members tended to associate positively during resting and foraging activities in all seasons. Among raccoons, as among many other mammals, female spatial patterns are apparently determined primarily by resource distribution, whereas male patterns are influenced by the distribution of females.

Anim. Behav. 55: 1211-1227, 1998. 3 tables, 7 figs., 52 refs. Authors' summary.

Hormonal and Experiential Factors in the Expression of Social and Parental Behavior in Canids

Cheryl S. Asa

Canids are frequently cited as being unusual among mammals for their tendency toward monogamy (Kleiman 1977). Yet, there are other features of their reproductive biology that are not typical of most mammalian species but that are important to understanding their unique

reproductive systems. These features include monestrum, obligate pseudopregnancy in adult females that fail to conceive, incorporation of postpubertal offspring into the social unit, behavioral suppression of reproduction in subordinates so that only the dominant pair produces young, possible inbreeding avoidance, the production of altricial young and parental care by other group members including adult males. This chapter will discuss the unique interplay of social organization and physiology that appears to have evolved in canids that may enhance both social accord and reproductive success.

The advantages of cooperative hunting may have selected for the extended family organization seen in so many canid species. In turn, several unusual features of their reproductive biology seem to favor this form of sociality or perhaps are necessary for it to succeed. For example, synchronous monestrum may serve to minimize antagonistic interactions in the pack. The obligate pseudopregnancy in the absence of conception not only prevents further estrous cycles but also primes nonpregnant subordinate females to display maternal behavior toward the dominant female's pups and perhaps increase their chances of survival. Furthermore, seasonal prolactin elevations in males as well as females, coincident with the birth of pups, may stimulate parental behavior in the males and reinforce maternal behavior of the females. Roughly similar social systems have been described for some primates without the concomitant alterations in reproductive cycles exhibited by canids. However, the level of accord and cooperation that may be necessary for coordinated hunting by these carnivores might not survive the social strife that accompanies polyestrous reproductive cycles in other species.

Book: Cooperative Breeding in Mammals, Edited by Nancy G. Solomon & Jeffrey A. French. Pp. 129-149. 4 tables, 3 figs., 93 refs. Authors' introduction & conclusion.

Early development of pituitary-gonadal axis in prenatally stressed blue foxes

L.V. Osadchuk, B.O. Braastad, M. Bakken, O.N. Kozlova, I. Huhtaniemi

As previously reported, handling is a stressor for farmed blue foxes. This study investigated the influence of prenatal stress on body, gonadal and pituitary weights, ano-genital distance, gonadal steroid production and LH pituitary content in blue fox offspring whose mothers exposed to handling in the last third of gestation. The 10-d-old cubs (n=68) were sacrificed by decapitation. The gonads were frozen or incubated *in vitro* in absence or the presence of hCG (2.5 IU per sample). The gonadal homogenates or incubates were analysed for the content of testosterone and estradiol by RIA and the pituitary homogenates for LH by IFMA assay. No significant differences between control (C) and prenatal stressed (PS) groups were detected for body and pituitary weights, LH pituitary content and testicular testosterone production. The gonadal weights were decreased by stress (ovaries: PS 50.6±1.8 mg *vs.* C 65.7±4.3 mg, $p<0.001$; testes: PS 17.7±1.0 mg *vs.* C 23.2±1.0 mg, $p<0.001$). The ano-genital distance was also decreased in stressed female foxes (PS: 0.89±0.03 cm *vs.*C: 1.07± 0.04 cm, $p<0.05$). The ovaries of stressed cubs produced significantly less estradiol (PS: 32.6±3.7 pg/ovary/hr vs. C: 43.5±3.5 pg/ovary/hr, $p<0.05$) and testosterone (PS: 0.12±0.02 ng/ovary/hr *vs. C:* 0.40±0.16 ng/ovary/hr, $p<0.05$) than those of controls. In contrast, the gonadal contents of steroids tended to be higher in prenatally stressed cubs of both sexes. It is concluded that prenatal handling stress causes an alteration in the neonatal maturation of the pituitary-gonadal axis in the blue fox and has more profound effects in female offspring.

Journal of Reproduction and Fertility: Abstract series, No. 23, 1999, pp. 37. Only abstract received. Authors' abstract.

Archaeological records of the extinct Sea Mink, *Mustela macrodon* (Carnivora: Mustelidae), from Canada

David W. Black, Joanna E. Reading, Howard G. Savage.

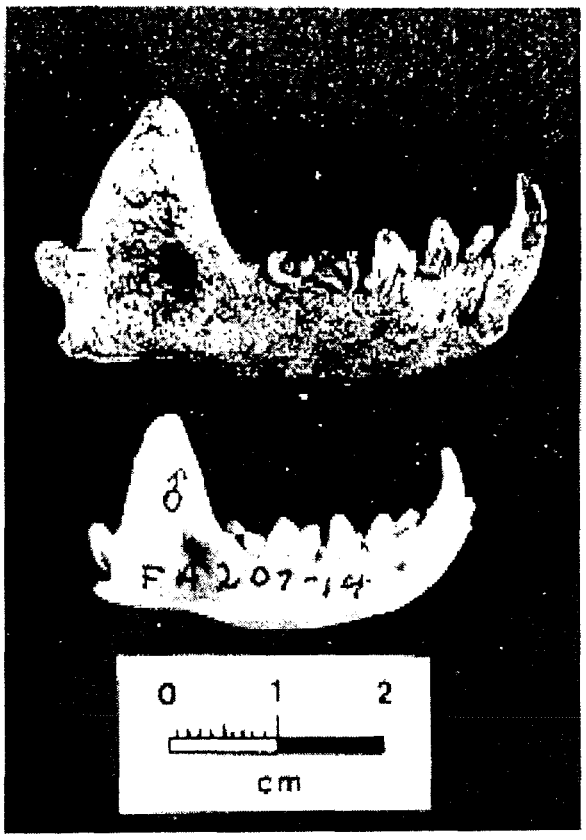

Fig. 2. The right mandible of Sea Mink (*M. macrodon*) from the Weir site compared with the right mandible of an adult male Mink (*M. vison*). Above: *M. macrodon* (BgDq6:2794-I) collections of the Department of Anthropology, University of New Brunswick. Below: *M. vison* (FA207-14) collections of the Faunal Osteo-archaeology Laboratory, Department of Anthropology, University of Toronto.

The extinct Sea Mink, *Mustela macrodon*, is reported from bones found in prehistoric archaeological sites in southern New Brunswick. The former range of this species, based on historical records and archaeological remains, is considered to have included coastal Maine, coastal New England as far south as Massachusetts, the southern coasts of the Maritime Provinces, and possibly Newfoundland. The association of some Sea Mink bones reported here with flaked lithic materials from geological sources in Maine suggests the bones were brought to New Brunswick by Native people, rather than representing a population of Sea Mink living on the New Brunswick coast in the past.

Canadian Field-Naturalist 112 (1): 45-49, 1998. 1 table, 2 figs., 26 refs. Authors' abstract.

Creation of the red-fox type suitable for farm conditions

M. Barta, D. Mertin

In a breeding experiment the reproductive characteristics, fecundity of wild red and silver foxes and their crosses over a course of five years are evaluated. The mean number of live-born offspring in the group of red foxes was 1, and in silver foxes 3.87.

In the group of crosses the mean number of weanlings/female was 1.33 in 1987, 4.84 in 1990, 0.44 in 1989, and 3.50 in 1990, respectively.

VÚZV, Nitra 8.-9. Oktober, pp. 269-271, 1997. 2 tables, 3 refs. In SLOV, Su. ENGL. Authors' summary.

Genetic and phenotypical parameters of polar blue fox (*Alopex lagopus* L) fur

Socha Stanislaw

The heritability and genetic, phenotypical and environmental correlations for body size and fur quality in polar blue foxes (*Alopex lagopus* L.) were evaluated using the animal model method. The studies were performed for 7 years on about 15,000 animals. The coefficients of heritability were: 0.280 for body size, 0.555 for colour intensity, 0.296 for colour clarity, 0.415 for fur density, 0.461 for hair length, 0.779 for general appearance and 0.423 for total score number.

The correlations between particular traits were mostly low (positive and negative). For example: genetic correlations between body size and colour intensity, colour clarity, fur density, hair length, general appearance and total score were -0.069, -0.011, 0.130, -0.107, -0.008 and 0.190, respectively. The phenotypical and environmental correlations were at similar levels. Such differentiation of correlation coefficients between particular traits in foxes make it difficult to select the animals for further breeding.

VÚZV, Nitra 8.-9. Oktober. pp. 297-300, 1997. 1 tables, 10 refs. In SLOV, Su. ENGL. Authors' summary.

Folliculogenesis and blood estradiol content in mink of different color types

D.V. Klochkov, R. G. Gulevich, L. V. Osadchuk

The authors studied the process of folliculogenesis and estradiol concentration in the plasma of peripheral blood of mink with standard and sapphire coloring at postnatal ontogenesis in age 3,7 and 11 months. During ontogenesis in both genotypes the number of follicles in the early stages of development (growing) is reduced but in the late stages (maturing) increases. The latter reach the maximal amount at the time of sexual activity, at age 11 months (on average in one ovary in standard and sapphire mink, respectively, 4.4 and 5.7). The main difference of folliculogenesis of mink of sapphire coloring in comparison with the standard coloring is a decreased number of primordial, growing and atrefic follicles. Folliculogenesis in mink of standard coloring depends on the season with a tendency to an increasing number of different types of follicles in September at age 5 months. Estradiol concentration in the blood of both genotypes is at its maximal level just before mating.

Agriculture Biology 16: 68-75, 1998. 1 table, 2 figs., 20 refs. In RUSS, Su. ENGL. Authors' summary.

Studies on fetal testosterone in silver foxes with reference to the effects of domestication

L.V. Osadchuk

In previous investigations it was found that selection of silver foxes for reduced aggressiveness towards humans (domestic behaviour) was associated with a number of endocrine changes in adult gonadal function. It was suggested that shifts in the developmental formation of gonads can be of crucial importance for changes of this function in adulthood. The purpose of the present study was to determine the effects of behaviour selection for domestication on fetal gonadal function in silver foxes. The serum levels, gonadal contents and *in vitro* gonadal productions of testosterone with or without hCG were studied on days 35, 40, 45 and 50 of prenatal life in domesticated and control silver foxes. Testosterone was measured by RIA. An effect of behaviour selection on body and gonadal weights in both sexes was shown. At the end of pregnancy (on days 45 and 50), the weights of fetuses and gonads from the domesticated groups were lighter than from the control. The serum and gonadal contents and *in vitro* baseline production of testosterone by the testes did not differ between domesticated and control animals during embryogenesis. When the fetal testes were stimulated by hCG *in vitro*, the hCG responsiveness appeared earlier in the domesticated group than in the control (day 40 vs. day 50) in the course of embryonic life. In addition, the clear sexual differences in the serum levels and gonadal contents of testosterone were demonstrated as being lower in females compared to males at all times of prenatal life studied. The data obtained suggest that behaviour selection of silver foxes has led to a timing shift in maturation of some parts of the hypophyseal-testes axis during embryonic life at least concerning the testicular responce to hCG stimulation.

17th Joint Meeting of the British Endocrine Societies, 23-25 March 1998. Only abstract received. Author's abstract.

Hormonal effects of selection for domestic behaviour in silver foxes

V. L. Osadchuk

Silver fox *(Vulpes vulpes)* bred in captivity show dominance of defensive responses to human contact. Genetic selection for elimination of aggression and fear towards humans (domestic behaviour) produced a population of foxes behaviourally resembling dogs. In earlier studies some changes in reproductive function of domesticated animals have been shown. The data suggested that the changes could result from hereditary modification of hormonal systems controlling reproduction. The aim of this study was to investigate gonadal hormonal function in silver foxes after long-term selection for domestic behaviour. Levels of sexual steroid hormones and *in vitro* steroid biosynthesis in gonads were measured in silver foxes of both sexes throughout the annual reproductive cycle. The increased progesterone levels in domesticated vixens during pregnancy indicated that the hormonal mechanism involved in increased fertility has been developed by selection. The significant decrease in the testosterone level associated with the decreased number of mountings was found in domesticated males during the mating season. The shorter period of hormonal testicular activity in domesticated males was accompanied by their lower sexual activity. In addition, it was demonstrated that timing shifts in the developmental formation of pituitary-gonadal axis in selected foxes during embryonic and postnatal life. The data obtained show that hereditary reorganisation of silver fox behaviour resulted in a complex of hormonal changes of gonads. The research leads to the conclusion that selection for reduced aggression against humans that may be carried out unconsciously by man with wild species bred in captivity can bring about within a short time period a sharp destabilisation in the reproductive system, in particular in its hormonal part.

XVIIIth International Congress of Genetics, August 10-15, 1998, Beijing, China. Only abstract received. Author's abstract.

Inheritance of red, silver and silver cross colours of fur coat in the common fox

Grazyna Jezewska, Janusz Maciejowski

The matings of silver, red and silver cross foxes have been performed within as well between colour variety groups. The results of segregation of colour varieties in the offspring enabled verification of the so far accepted hypothesis on genetic determination of coat colour. The new hypothesis has been formed that two pairs of genes A,a and B,b determine the silver, red and silver cross fox colour but the homozygotic system aa affects epistatically locus B making animals aa silver independent of the glue arrangement in locus B. The cross fox colour phenotype is determined by the heterozygotic arrangent Aa with the homozygotic bb genotype.

Annales Universitatis Mariae Curie-Sklodowska Lublin-Polonia, Vol. XIV, 37, pp. 237-240, 1996. In POLH, Su. ENGL. 1 table, 6 refs. Authors' summary.

Inheritance of white color in raccoon dogs

Grazyna Jezewska

White raccoon dogs maintained in Poland are mated between one another or crossbred with standard raccoon dogs (wild type color). Analyzing the results of color splitting in progeny originating after differently colored parents, the attempt to assess the heredity of a fur cover color was undertaken. In studies, only those litters in which females were mated with the same male in a season, were taken into account. Due to the crossbreeding of standard raccoon dogs with white ones (112 litters, 611 young animals), splitting into standard (54.5%) and white (45.5%) raccoon dogs was obtained in progeny. According to literature data, white and standard progeny should be born at the same frequency (splitting ratio 1:1) due to the crossbreeding of white ("Ww") and standard ("ww") raccoon dogs. In presented studies, it was shown the deviation from expected split-

ting ratio and it was statistically confirmed (χ^2 = 4.95 > $\chi^2$0.05 = 3.84). Similar results were obtained in a case of crossbreeding the white raccoon dogs between themselves. Expected splitting ratio should be equal to 3:1 or, if the suggestion about probable lethality of "W' gene in a homozygotic system is taken into account, the phenotypical splitting 2:1 should be expected. In presented studies, 51.5% white raccoon dogs and 48.5% standard ones were born from the mating between white raccoon dogs themselves (15 litters and 68 young animals). Too small observation number did not let estimate the proper splitting scheme, nevertheless obtained results points that perhaps white color of fur cover in raccoon dogs is much more conditioned than by one gene pair.

Annales Universitatis Mariae Curie-Sklodowska Lublin-Polonia, Vol. XIV, 38, pp. 241-244, 1996. In POLH, Su. ENGL. 3 tables, 4 refs. Author's summary.

Assessment of genetic variability in captive and wild American mink *(Mustela vison)* using microsatellite markers

A. M. Belliveau, A. Farid, M. O'Connell, J.M. Wright

The genetic variability of 212 black mink from four ranches, and 20 each from wild mink trapped in Eastern Canada, pastel and brown (wild-type) was assessed using seven microsatellite loci. The average number of alleles per locus and expected heterozygosity (He) in the entire sample were 6.57 and 0.63, respectively. The estimates of H_E were comparable among the black mink herds (0.53 to 0.61), and between black and the wild mink (0.50), indicating a considerable level of genetic variability within black mink, despite high levels of uniformity that have been achieved in fur quality traits as a result of many years of intense selection. Brown mink had the highest H_E among populations (0.65), which could be the cause or the effect of their higher vigor and reproductive performance compared with the black mink. All the populations showed a higher level of homozygosity than expected from the Hardy-Weinberg (H-W) proportions at several loci (positive FIS), perhaps as a result of linebreeding and positive assortative mating commonly used in the mink industry. Excess of homozygosity in the wild mink may indicate breeding between related individuals occupying adjacent territories, and a limited movement of mink in the wild. The black mink herds were closely related to each other, as were the pastel and brown. Gene flow from common sources to all the herds and infusion of the Jetblack allele into all the ranches were likely the causes of relatedness of the black mink herds. The black mink herds were more closely related to the wild mink than to the colored mink. This panel of microsatellites correctly classified black and nonblack mink into their respective groups with 91 to 97% accuracy. Between 70 and 88% of the black mink were correctly assigned into their herd of origin.

Can. J. Anim. Sci. 79: 7-16, 1999. 6 tables, 1 fig., 42 refs. Authors' summary.

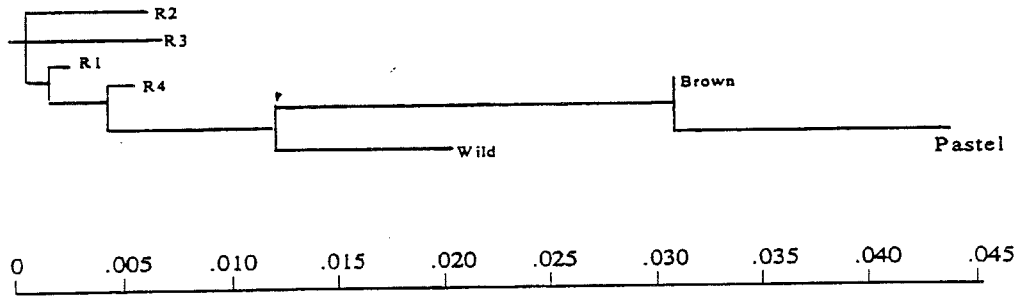

Fig. 1. Neighbor-joining dendogram for the black mink herds (R1, R2, R3, R4), brown, pastel and wild mink derived from the matrix of Nei's genetic distances.

Original Report

Embryonic mortality in the American mink: a morphological analysis of preimplantation loss

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Summary

Based on morphological studies of 270 early American mink embryos (Standard, +/+), it may be estimated that the proportion of embryonic death at the stages of zygote, cleavage and blastocyst were about 22%, 12% and 25%, respectively. The zygote and blastocyst stages are the most vulnerable in preimplantation development. Abnormal zygotes were, as a rule, unfertilized. Embryos die at the blastocyst stage by preferential damage of either the embryoblast or trophoblast. The major causes of preimplantation loss appear to be impairment of the fertilization process, influence of the maternal hormones, and, possibly, the interplay of the spermatozoa of the first mating.

Key words: preimplantation development, embryo mortality, mink

Introduction

The problem of embryonic mortality in mink (*Mustela vison*) has been raised by Hansson (1947); Enders (1952) and Sundqvist et al., (1989). The 25% percent prenatal and early postnatal losses in mink (see Murphy 1992) are unacceptably high. They reduce the observed (actual) fertility (*Evsikov 1987*). The issues of mink mortality have not been resolved to the present day. Shortage in mink hampers the use of this particular carnivoran species as a tool of biotechnology and limits the chances for obtaining results.

It is known that various factors may affect embryonic mortality in American mink: the food ration of the females, their hormonal status, the date of their mating, the species-specificity of gametogenesis and embryogenesis, the genotype of the mother and fetus, among others (*Enders 1952; Vagin 1983; Isakova 1983; Evsikov 1987; Sundqvist et al., 1989; Tauson and Gustafson 1994*). High embryonic loss occurs from the early postimplantational to the fetal periods (*Belyaev et al., 1978*). However, successful application of modern biotechnological methods in carnivores requires more knowledge about why and how embryos die before implantation. With this in mind, the aim of this study was *(1) to estimate the level of embryonic mortality, (2) to highlight the most vulnerable stages and (3) to describe the possible mechanisms whereby embryos are eliminated before implantation.*

Materials and methods

Animals. It has previously been shown that in American mink coat color mutations exert a pleiotropic effect on the reproductive function. A decrease in fertility is, as a rule, observed in homozygotes (for example *ppaa; ppaakk*); heterozygosity for certain coat color genes in turn leads to heterosis for fertility (*Belyaev et al., 1972; Evsikov 1987*). To obtain assurance that effects related to the embryonic and maternal genotypes are excluded, we recovered embryos from Standard (+/+) females mated to Standard (+/+) males. The mating scheme was traditional (1+2 after 7 days). The dates of successful matings were optimal because they took place in the second half of March. Females were of the same age. They were not pretreated with hormones.

Embryos. Embryos were flushed with DPBS solution (*Kizilova et al., 1999*). An inverted microscope Labovert with Nomarsky-optics and phase-contrast (Leitz, Germany) was used for observing, photographing and morphometric measurement of live embryos. The embryos were then fixed in 2.5% glutaraldehyde and 2.5% formaldehyde in standard buffer phosphate solution (pH 7.6 - 7.8), washed in the same buffer, then postfixed in 1% OsO_4. Dehydration and embedding in Epon were standard for the mustelid embryos (*Enders et al., 1986*). Two μm thick sections were cut with an ultramicrotome Reichert. The sections were stained with fuchsin, pyronine, methylene blue, toluidine blue or safranin O. Total preparations were made from part of the blastocysts that were fixed in the same way. Chromatin was subsequently stained with safranin O (1% stock stain solution in a 10% ethyl alcohol for 5-10 min); staining intensity was controlled visually. The preparations were then embedded in a 10% glycerine solution.

Two hundred seventy early embryos from fertilization to implantation were studied by the above morphological methods.

Results

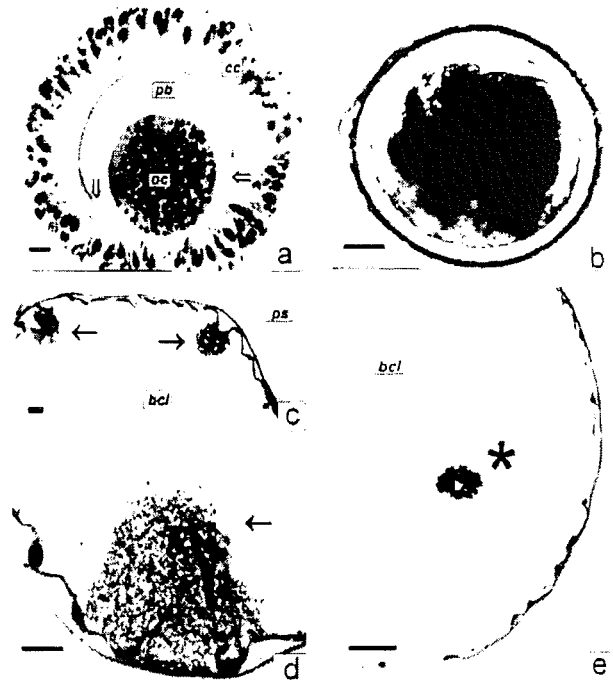


Fig.1 Abnormal development to the activation stage

a) an oocyte that had not passed the denudation stage by 52-54 h.p.c., its *zona pellucida* is damaged in several sites (⇒);

b) an abnormally dividing embryo, in all likelihood, only the first cleavage division was completed normally, one of the two blastomeres fragmented later;

c) and d) local «explosive» damage (→) of the trophoblast of the diapausing blastocysts;

d) very infrequently (1.6%) 1-2 blastomeres are found in the blastocoel of the diapausing blastocyst and this appears to be a consequential abnormality;

Designation: ***oo*** - oocytes; ***cc*** - cumulus cells; ***pb*** - polar body; ***ps*** - perivitelline space; ***bcl*** - blastocoel; scale 20 μm.

The percentage of abnormal embryos by 44-46, 48-50 and 52-54 hours *post coitum* (h.p.c.) was 8%, 39%, and 22% respectively. Pronuclei and the second polar body were not observed in these zygotes. Consequently, this group con-

sists mainly of unfertilized oocytes. The denudation stage in American mink is normally completed by 50-52 h.p.c. (*Kizilova et al., 1999*). We observed unfertilized oocytes (4 oocytes in the same female) that did not get free of the cumulus by 52-54 h.p.c. (Fig. 1 a). In these oocytes, there was extensive damage of the *zona pellucida* and the cumulus cells were invaded with unidentified bacteria. Another abnormal zygote had three pronuclei and presumably resulted from dispermic fertilization.

During cleavage, compaction and cavitation, the percentage of embryos with pronounced abnormalities did not exceed 8-10% (5 of the 58 embryos).

There was extensive damage of plasmalemma in the abnormal embryos. Marked vacuolization of the cytoplasm, coagulation of lipid granules and frequently their extrusion into the perivitelline space were also observed (Fig. 1 b).

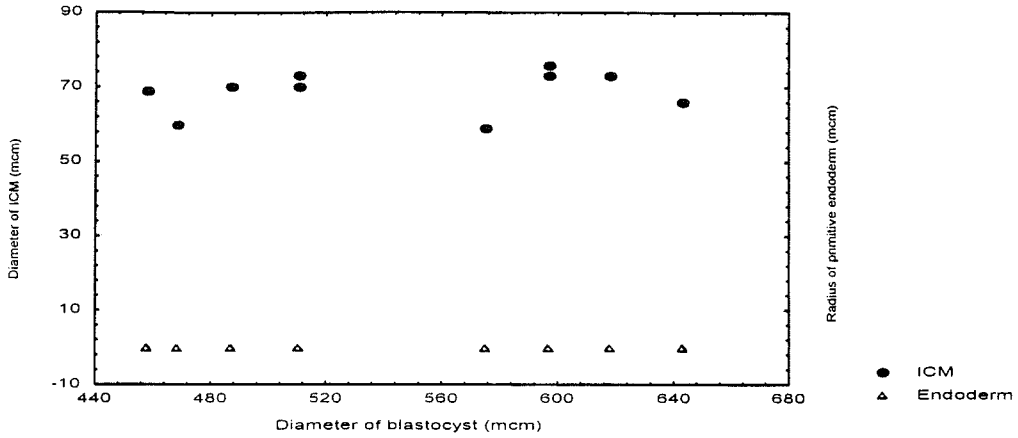
Among the diapausing blastocysts, at the stages of activation or the formation of the embryonic disk, the proportion of the abnormal embryos was 11%, 34% and 8%, respectively.

To make the description of the embryonic loss at the blastocyst stage more correct, the embryos were distributed within each litter. Data obtained from the morphometric measurement of blastocysts were taken as criteria. Examples of such a distribution are given in Fig. 2. The blastocysts recovered from the same female were distributed according to their diameter and radius of the primitive endoderm. This distribution tendency is noted from diapause onset (8-9 days *post coitum*, d.p.c.) (Fig. 2 a) and it is most distinct at the stage of the activation and formation of the embryonic disk (Fig. 2 b, c). The morphology of the embryos that formed the modal classes was, as a rule, normal. The deviating blastocysts, smaller or larger in size, than those of the modal class, were frequently abnormal.

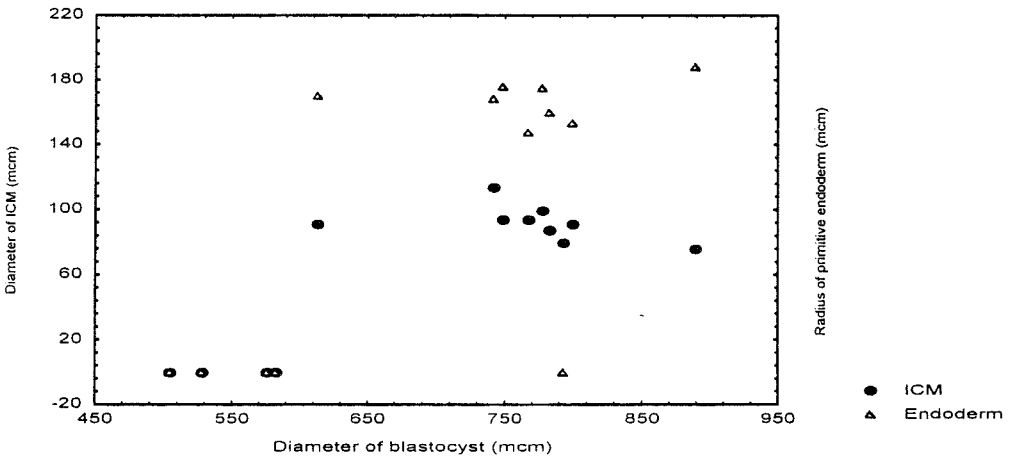
Blastocysts with two internal cell masses (ICM) were referred to the group of normal embryos. Their percentage was low, being 6.3% (8 of the 127 blastocysts). Both ICMs became synchronously activated, each was encircled by its own primitive endoderm. Have such embryos resulted from precisely this wayward development? This may be a uniovular (enzygotic) twin dividing at the cavitation stage. Or are they, possibly, the result of the dispermic fertilization of the oocytes and the polar body subsequently developing under a common trophoblast? In the former case, such a blastocyst would develop into a pair of monochorionic twins, in the latter, the blastocyst would turn out be a genetically mosaic, a so-called spontaneous chimera. Monochorionic twins and spontaneous chimeras have been previously described in mink (*Belyaev and Isakova, 1988; Fechhaimer et al., 1984; Isakova 1989*).

Blastocysts with lagging blastomeres in the blastocoel (Fig. 1 e) were rarely encountered. It appears that these embryos are also variants of normal development.

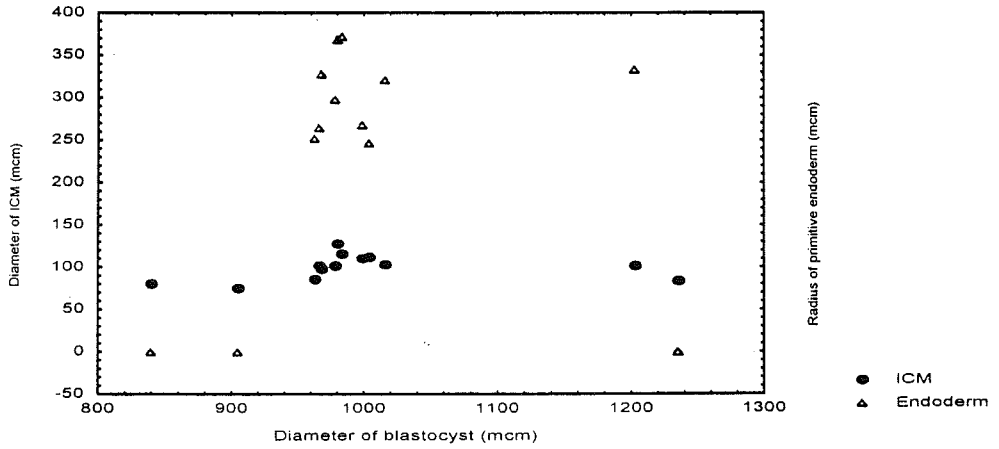
There were embryos with specific locally restricted damage among the diapausing blastocysts (one female, 5 embryo, modal class) (Fig. 1 c, d). Single trophoblast cells "exploded" out, breaking apart, and extruded large amounts of osmiophilic particles. These particles were of the same shape and size and smaller than 1 μm in their diameter. The nature of damage, its restriction to particular sites and the fact that damaged embryos were recovered from the same female all suggested that an infectious agent probably was in cause.



a) diapause;



b) activation;



c) embryonic disc begins to form;

Fig 2. Distribution of the blastocysts within each litter

The diameter of the abnormal embryos assigned to the first group was small and they resulted from abnormal morphogenesis: their ICMs were strongly deformed, or destroyed (Fig. 3), the primitive endoderm was often missed (Fig 2 b, c). The degeneration of the ICM was most likely provoked by disaggregation of the germ cells (Fig. 3 a, b) or by the coagulation of lipid granules followed by lysis of the germ cells (Fig. 3 c, d). These changes led to the death of the embryoblast, with the trophoblast retained and, as a result, the trophoblast bubbles formed.

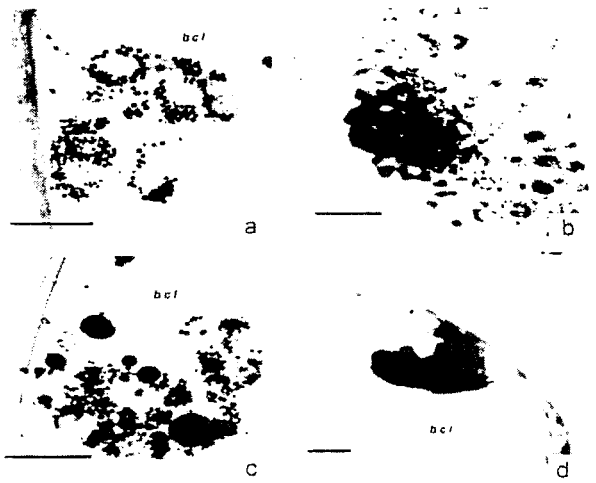


Fig. 3 Damage of the ICMs by cell disaggregation (a, b) or coagulation of lipid granules (c, d)
a and d - appearance of semithin sections; b and c - photograph of live cells;
bcl - blastocoel; scale 20 μm.

Blastocysts with a long diameter that frequently completely collapsed (Fig. 4 a, b) were referred to the second group. In this case, the integrity of the trophoblast and primitive endoderm was lost, although no evidence for visible damage of ICM was found.

It should be noted that the trophoblast is continuous in the normal blastocysts, dead cells and breaks in the layer are only occasional. Dividing cells lie, as a rule, next to these damaged sites (Fig. 5 a, b). In contrast, trophoblast damage is very extensive in the large abnormal blastocysts. Mitotic activity of the trophoblast in such blastocysts is 2-4-fold that in the normal, but repair, in all likelihood, lagging behind, cannot make the damaged trophoblast recover its normal spatial organization. As a result, the trophoblast falls apart into clumps (Fig. 5 c, d). Furthermore, the mitotic spindles may be frequently oriented in a disorderly fashion. As a consequence, there appear groups or bands of cells protruding from the trophoblast layers' plane that is also an abnormality (Fig. 5e).

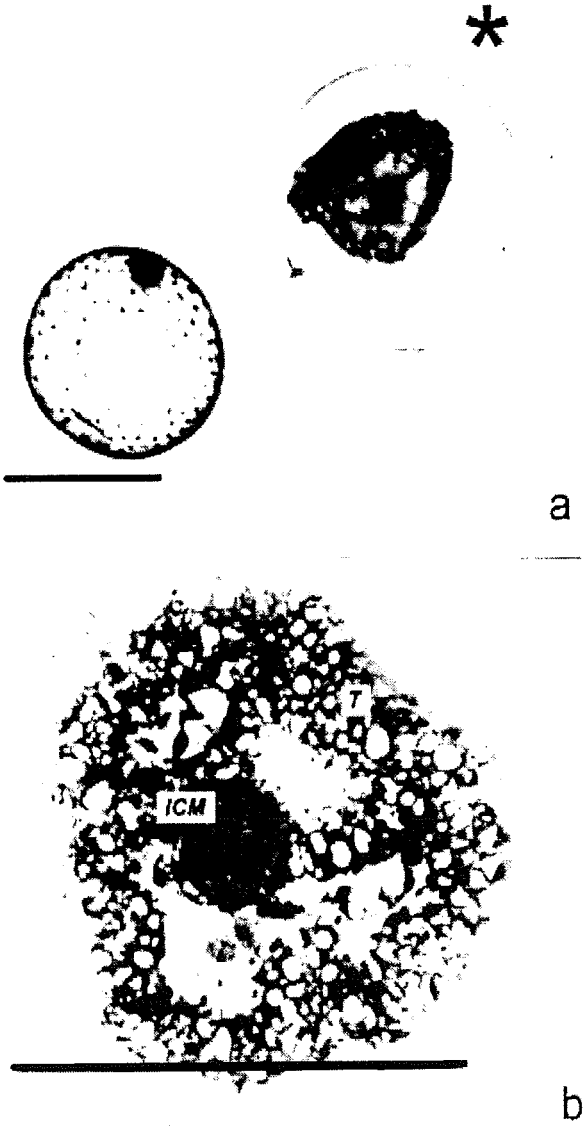


Fig. 4. An irreversibly collapsed blastocyst (*)
a - photograph of live cells ; b - appearance of semithin sections;
Designation: *T* -trophoblast; *ICM* - internal cells mass; scale 20 μm.

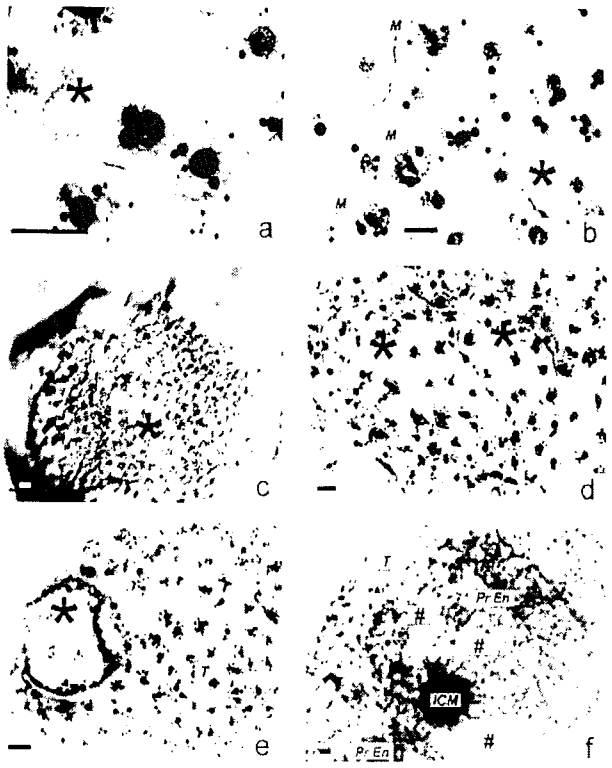


Fig. 5. Damage of the trophoblast (*) and of the primitive endoderm (#)
a and b) regenerating trophoblast in normal blastocysts;
c and d) extensive breaks of the trophoblast in the blastocyst with large diameter;
e) abnormal spatial organization of the trophoblast resulting from impaired normal orientation of the division spindles;
f) extensive damage of the primitive endoderm in the blastocysts with large diameter;
c - photograph of a live embryo; a, b, d, e, and f - total preparation of a blastocyst;
Designation: *T* - trophoblast; *M* - mitotic cells; *ICM* - internal cell mass; *PrEn* - primitive endoderm; scale 20 μm.

Because 7 blastocysts were obtained from 5 females together with the zygotes recovered after the second mating, the origin of these blastocysts raises no doubt. Spermatozoa (Kim et al., 1979) were found in 4 of 7 blastocysts in the perivitelline space, the trophoblast cells and in the blastocoel (Fig. 6 a, b, c). Mink sperm retains its fertilizing capacity for no more than 72 h.p.c. Because the age of the blastocysts was 7-8 h.p.c., the detected spermatozoa were not thought to be recovered after the first mating. All the blastocysts were degenerated and their trophoblasts were damaged.

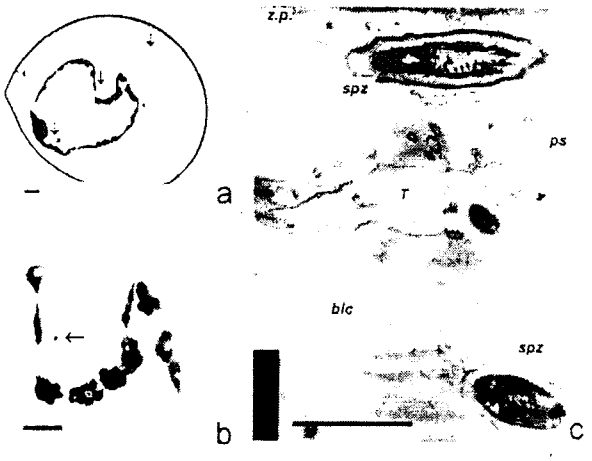


Fig. 6. Spermatozoa recovered after the second mating are detected in the blastocysts resulting from the first mating
a and b - appearance of semithin sections; c - appearance on ultrathin sections;
Designation: *z.p.* - zona pellucida; *ps* - perivitelline space; *T* - trophoblast; *bcl* - blastocoel; *spz*, → - spermatozoa; scale: a, b - 20 μm; c - 1 μm.

Discussion

Thus, the proportion of embryonic death at the stages of zygote, cleavage and blastocyst were about 22%, 12% and 25%, respectively. Taken together, preimplantation loss in Standard (+/+) mink is normally about 50-60 %. Zygote and blastocyst are the most vulnerable stages.

A comparatively low fertility of mink semen (Sundqvist et al., 1989) may induce the mortality of zygotes retained unfertilized. According to our data, embryonic loss at the blastocyst stage results from preferential damage of either the embryoblast or the trophoblast. Genetic factors may be implied such as genetic mosaicism, aneuploidy, and triploidy caused by dispermic fertilization of the oocytes cannot be excluded. Genetic impairment prevents the proliferation of the ICM cells and makes impossible its normal reorganization. It is worthy to note that a relatively high proportion of genetic abnormalities is a distinctive feature of mink

embryogenesis. It will be recalled that embryonic mortality in mink is related to the occurrence frequency of heteroploidy and aneuploidy in embryos (*Belyaev and Isakova 1983; Fechhaimer et al., 1984*).

It appears that the extensive damage of the trophoblast is the physiological effect of the second ovulatory wave or of «unsuccessful» termination of diapause. Endogenous maternal growth factors and hormones most likely affect mink trophoblast. EGF, TGFα, IGF-1, $TGF\beta_{1\text{-}2\text{-}3}$, CSF-1, LIF, PDP, GM-CSF, IL-1, and IL-2 have been implicated as putative regulators of the expansion, hatching and attachment of blastocyst in carnivores (*Kanzaki et al., 1991; De et al., 1992; Haimovici and Anderson 1993; Dalton et al., 1994 - cited from Moreau et al., 1995; Murphy 1992*). In western spotted skunk, epidermal growth factor receptor expresses in the preimplantation uterus and blastocyst (*Paria et al., 1994*). Hormonal signals that elicit change in the state of growth factor receptors have not, so far, been identified (*Paria et al., 1994*). However, it has been suggested that not estrogens, but rather progesterone, LH or prolactin accomplish the role of these signals (*Paria et al., 1994; Moreau et al., 1995*).

Offspring may occasionally be obtained from the first «initiating» mating. However, the blastocysts resulting from the first mating are subjected to preferential elimination. The estrogen peak during the second ovulation wave has a detrimental effect traditionally implied as causative in this elimination (*Enders 1952*). According to our data, spermatozoa that have gained access to the female reproductive system after the first mating may damage those of the second mating and cause their degeneration. At any rate, the multiple primary damage incurred to the trophoblast as a result of «breakthroughs» of the spermatozoa may develop later into extensive confluent intercellular breaks and lacunae.

Irreversible collapse may lead to embryo death. It cannot be excluded that the process we observed may be an artifact of female sacrifice or of the flushing procedure itself. However, the proportion of collapsed diapausing blastocysts is lower than of that at the stage of expansion. It is well known that blastocoel volume periodically fluctuates. However, there are no comparative data giving an idea of how the collapse may enfold *in vivo* in the carnivores.

The loss of early embryos as a result of bacterial invasion was unexpected. It has been established that *Mycoplasma canis* and *M. felis* inhabit the carnivoran reproductive *system (Borhsenius and Chernova 1989*). Virus of the C-type has been identified in the perivitelline and interblastomeral space of early baboon embryos (*Panigel et al., 1975*). To our knowledge, these are no reliable data about bacterial infections of carnivoran preimplantation embryos. Consequently, findings warrant further checking and thorough analyses using molecular and immunological toolkits.

Acknowledgments

We thank Dr. Sergey Ya. Amstislavsky for the valuable discussion. We are also grateful to Denis M. Larkin and Alexander V. Dubynin for computer assistance and Anna N. Fadeeva for translating the paper from Russian into English.

References

Belyaev D.K., Evsikov V.I., Matysko E. The genetics of fertility in animals. Comm. 3: The effect monohybrid heterosis on fertility and viability in mink and prospects for it use in breeding. Genetika. **1972.** V.8(1). P. 62-70. (In Russian)

Belyaev D.K., Isakova G.K., Evsikov V.I. The genetics of fertility in animals. Comm.4: Heteroploidy in embryogenesis and its role in decrease of fertility in mink Genetika. **1978.** V.14(2). P.242-249. (In Russian).

Belyaev D.K. and Isakova G.K. Spontaneous chimeras in early postnatal development in mink. Genetika. **1983.** V.19(9). P. 1539-1544. . (In Russian).

Borhsenius S.N., Chernova O.A. Mycoplasms: molecular and cell biology, pathogenicity

and diagnostics. **1989**. Leningrad: «Nauka». 127 P. (In Russian).

Enders A.C. Reproduction in mink (*Mustela vison*). Proc. Am. Phil. Soc. !**952**. V. 96. P. 691-755.

Enders A.C., Schlafke S., Hubbard N.E. and Mead R.A. Morphological changes in blastocyst of the western spotted skunk during activation from delayed implantation. Biol. Reprod. **1986**. V. 34. P. 423-437.

Evsikov V.I. The genetics and evolutionary aspects of the problem of the homeostasis of fertility in mammals (exemplified by minks). Genetika. **1987**. V. 23(6). P. 988-1003. (In Russian).

Kim J.W., Kitts W.D., Ahmad M.S., Krishnamurti C.R. The ultrastructure of mink (*Mustela vison*) spermatozoa. Can. J. Zool. **1979.** V. 57(4). P. 924-933.

Moreau G.M., Arslan A., Douglas D.A., Song J., Smith L.C., Murphy B.D. Development of immortalized epithelial and stromal cell lines from the mink (*Mustela vison*) uterus and their effect on the survival *in vitro* of mink blastocysts in obligate diapause // Biol. Reprod. **1995.** V. 53. P. 511-518.

Panigel M., Kraemer D.C., Kalter S.S., Smith G.C., Heberling R.L. Ultrastructure of cleavage stages and preimplantation embryos of the baboon // Anat. Embryol. **1975.** V. 147 (1). P. 45-62.

Paria B.C., Das S.K., Mead R.A. et al. Expression of epidermal growth factor receptor in the preimplantation uterus and blastocyst of the western spotted skunk // Biol. Reprod. Fertil. **1994.** V. 51(2). P. 205-213.

Fechheimer N.C., Isakova G.K., Belyaev D.K. The mechanisms of the spontaneous diploid-triploid chimerism in mink (*Mustela vison*) and hens (*Gallus domesticus*). **1984**. Genetika. V. 20(12). P.2048-2054. . (In Russian).

Hansson A. The physiology of reproduction in the mink with special reference to delayed implantation. Acta Zool. **1947.** V.28. P.1-136.

Isakova G.K. Karyological analysis of early mink embryos **1983**. Genetika. V.19(10). P.1742-1743. (In Russian).

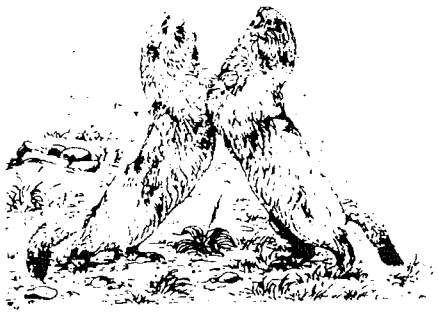
Kizilova H.A., Golubitsa A.N., Zhelezova A.I., Maximovski L.F., Kerkis A.Yu., Baiborodin S.I. and Serov O.L. Development of the zygote and visualization of the pronuclei in mink (*Mustela vison*). Scientifur, **1999**. v23(3)

Sundqvist C., Amador A.G. and Bartke A. Reproduction and fertility in the mink (*Mustela vison*). J. Reprod. Fert. **1989.** V.85. P. 413-441.

Tauson A-H., Gustafsson H. Effect of flushing on embryos and early developmental stages in mink (*M. vison*) // Acta Agricult. Scandinavia Sect. A. Anim. Sci. **1994**. V. 44. P.43-49.

Vagin Yu. V. Intrauterine selection of mink embryos and factor determining its direction and intensity. DAN SSSR. **1983.** V. 273(4). P. 997-1000. (In Russian).

Murphy B.D. Progress and challenges in the physiology of reproduction in furbearing carnivores. Norv. Jorn. Agricult. Science. **1992.** Suppl. 9. P. 17-29.



Behavioural aspects of the raccoon mating system: determinants of consortship success

Stanley D. Gehrt, Erik K. Fritzell

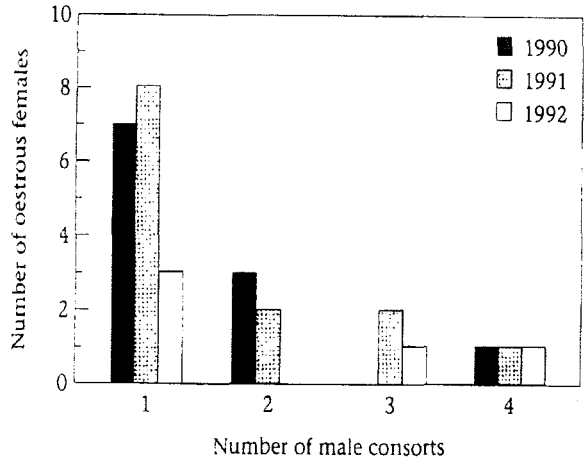


Figure 2. Distribution of the number of males with which each female raccoon consorted during an oestrous cycle. Data are for the 1990–1992 mating seasons on the Welder Wildlife Refuge, Texas. Some females occur in multiple years.

We monitored raccoons, *Procyon lotor*, in southern Texas during the 1990-1992 mating seasons to describe mating behaviour and identify factors affecting consortship success. During most of this study, raccoons were spatially aggregated, with female home ranges congregated around permanent water sources and larger home ranges of male groups encompassing each female group. Consortship success varied among males and ranged from zero to six females per male within a mating season. Individual females consorted with one to four different males during an estrous period; however, most (62%) females consorted with only one male during their estrus. Dominance through overt conflict appeared to influence male consortship success. During two mating seasons, one male from each group consorted with females on more days than all other males combined. Body weight of males was positively correlated with number of consortship days. As synchrony of estrus increased, variance in number of consortship days among males decreased, and access to estrous females increased for subordinate males. Wounding among males increased during the mating season, and was more frequent for males than for females. The mating system, as determined by consortship behaviour, appeared to shift between, polygyny and promiscuity, and possibly varied annually as a result of the timing of estrous cycles.

Animal Behaviour 57: 593-601, 1999. 2 tables, 2 figs., 64 refs. Authors' summary.

Canid Reproductive Biology: an Integration of Proximate Mechanisms and Ultimate Causes

Cheryl S. Asa, Carolina Valdespino

The canid reproductive system includes many features that are unusual or even unique among mammals. Focusing on gray wolves, for example, these include monogamy, monestrum with exceptionally long proestrus and diestrus phases, a copulatory lock or tie, incorporation of adult young into the social group, behavioral suppression of mating in these subordinate young, obligate pseudopregnancy in subordinate females, and alloparental care. These features can be analyzed on the levels of both proximate and ultimate causation by considering them in the context of the reproductive system as a whole. First, when assessing possible proximate mechanisms, monestrum appears to be pivotal. It is probably accomplished by the extremely long luteal or desirous phase, which is followed by a seasonal peak in prolactin. Two sequellae of the extended diestrus (or pseudopregnancy) in non-pregnant subordinate females are to 1) suppress any subsequent cycles, and 2) hormonally prime them to behave maternally. The prolactin peak in all adult pack members, coincident with the birth of pups, also may stimulate parental behavior. The risk of monestrum (limited chance for conception) appears to be reduced by the relatively long proestrus and estrus periods, as well as by monogamy. The adaptive value, or ultimate cause, of this reproductive system is most obvious for the more social canid species, such as the gray wolf. That is, advantages to sociality,

such as cooperative hunting, may have driven development of the anomalies of the reproductive system.

Amer. Zool., 38, pp. 251-259, 1998. 2 tables, 46 refs. Authors' summary.

Second estrus and late litters in raccoons

Stanley D. Gehrt, Erik K. Fritzell

We used observations of resting behavior by 38 radiocollared raccoons *(Procyon lotor)* to document late mating for a free-ranging population in southern Texas during 1990-1992. Male-female consortships (n = 67) exhibited a bimodal distribution each year. In each instance, a second late estrus (n = 11) occurred after a female failed to produce a litter, or lost a litter soon after parturition. The mean (±SD) number of days between first and second estrus was 78.6 ± 17.6. Six litters were born between 30 July and 4 September from second matings. Raccoons born as a result of a second estrus may be relatively common, although survivorship of late-born litters is likely low.

Journal of Mammalogy 77 (2): 388-393, 1996. 2 figs., 24 refs. Authors' summary.

Reproduction in foxes: current research and future challenges

W. Farstad

Basic information on fox reproduction, such as endocrinology, oocyte maturation, artificial insemination, fertilisation and embryo development, ovarian and testicular function, parturition, milk production and neonatology has been gained from studies of farmed animals. Fox farming is an industry with considerable economic importance in countries such as Norway and Finland, and the use of farmed animals as models to study wild canine species has proven valuable. This paper reviews some major research accomplishments and new knowledge and identifies future challenges in research regarding both the wild and domestic variants of the fox species.

Animal Reproduction Science 53: 35-42, 1998. 49 refs. Author's abstract.

Luteotropic Hormone Receptors in the Ovary of the Mink (*Mustela vison*) during Delayed Implantation and Early-Postimplantation Gestation

D.A. Douglas, A. Houde, J.H. Song, R. Farookhi, P.W. Concannon, B.D. Murphy

The reproductive cycle of the mink displays rigid seasonality and obligate embryonic diapause. After ovulation, the corpus luteum (CL) involutes, and it secretes basal progesterone until activated prior to implantation. To study changes in the relative abundance of luteal prolactin and LH receptor mRNA through gestation, ovaries and serum were collected from pregnant female mink at 2-day intervals (n = 3 per date) through embryonic diapause and CL activation (March 19-31) and at 5-day intervals through implantation and early-postimplantation gestation (March 31-April 15). To determine the effect of endogenous prolactin, mink received Alzet osmotic minipumps releasing 2 mg/day 2-bromo-α-ergocryptine (bromocriptine) or saline on March 19. Ovaries and serum were taken from 3 animals every 2 days until March 31. Prolactin receptor mRNA in ovaries was low during CL activation but increased 3-fold through embryo implantation. Its abundance correlated with prolactin binding to ovarian membranes and with circulating prolactin. Bromocriptine suppressed endogenous prolactin levels and prevented the increase in prolactin receptor mRNA.

There was a transient peak in LH receptor mRNA in the ovaries at March 19-23, which declined to basal levels by March 25 and remained constant through midgestation. Bro-

mocriptine prevented the preimplantation peak in LH receptor mRNA and reduced its abundance below pretreatment levels. The results suggest that prolactin up-regulates its receptor and maintains the LH receptor in the mink CL. The pattern of LH receptor mRNA argues for a role for LH in CL reactivation and termination of embryonic diapause in mink.

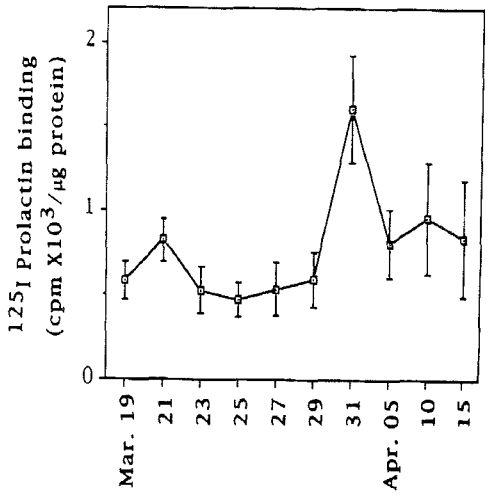


FIG. 4. Mean ± SEM of ^{125}I-labeled prolactin binding to homogenates of ovaries from pregnant mink collected between March 19 and April 15 (n = 3 per sampling date). Results are expressed as mean ± SEM cpm bound per mg protein.

Biology of Reproduction 59: 571-578, 1998. 5 figs., 65 refs. Authors' abstract.

Cloning, Developmental Expression, and Immunohistochemistry of Cyclooxygenase 2 in the Endometrium during Embryo Implantation and Gestation in the Mink (*Mustela vison*)

Jian H. Song, Jean Sirois, Alain Houdet, Bruce D. Murphy

Cyclooxygenase (COX) is the first rate-limiting enzyme in the biosynthesis of PGs. There are two isoforms, COX- 1, a constitutive enzyme and COX-2, the induced form, products of two different genes. In this study, we report COX-2 complementary DNA cloning, uterine expression, and immunohistochemical localization in the mink uterus during postimplantation gestation. The open reading frame of mink COX-2 contains 1812 nucleotides encoding 604 amino acids. The homologies are 86%, 83%, 83%, 83%, 85%, and 84% in nucleotides and 86%, 87%, 87%, 85%, 86%, and 88% in amino acids with human, mouse, rat, guinea pig, sheep, and rabbit, respectively. All domains associated with biological activity are conserved in the mink. Northern analysis revealed a transcript of 4.2 kb for COX-2 in mink uterus and adrenal. Semiquantitative RT-PCR showed that COX-2 messenger RNA is not present during diapause. The abundance of COX-2 messenger RNA reached its maxima ($P < 0.05$) on days 3-5 of postimplantation, gradually decreased through day 9, and was not present thereafter. By immunohistochemistry, COX-2 was present in uterine epithelium, stroma, and necks of endometrial glands at sites of implantation. COX-2 expression appears to be induced in the endometrium by the embryo and may play a role in implantation and placentation in the mink.

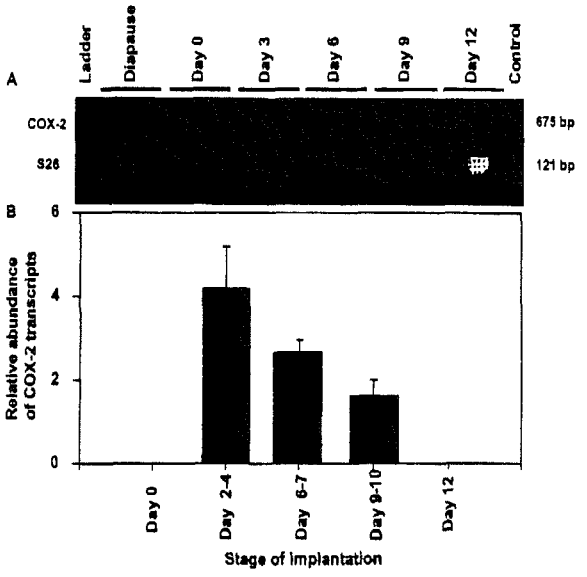


FIG. 4. Illustration of semiquantitative RT-PCR for COX-2 transcripts in the mink uterus during diapause and postimplantation gestation. A, A representative agarose gel demonstrating migration of triplicate RT-PCR products (*upper band*, 20 µl COX-2; *lower band*, 10 µl S26). Samples were subjected to the same RT, amplified in separate tubes, and then combined for electrophoretic analysis. The first lane on the *left* is the 1-kb DNA ladder. B, Mean ± SEM of the dimensionless ratio of COX-2 to S26 amplicons from embryo-uterus complexes of various postimplantation ages in the mink. Day 0 represents the day of embryo expansion before attachment. All means were different from each other at $P < 0.05$.

Endocrinology, Vol. 139, No. 8: 3629-3636, 1998. 5 figs., 53 refs. Authors' abstract.

Cloning of Leukemia Inhibitory Factor (LIF) and Its Expression in the Uterus During Embryonic Diapause and Implantation in the Mink (*Mustela vison*)

Jian H. Song, Alain Houde, Bruce D. Murphy

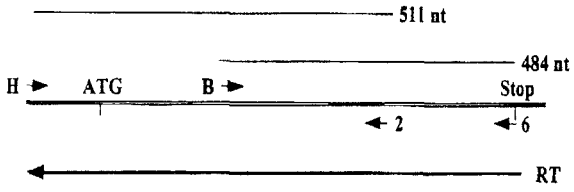


Fig. 1. Cloning strategy for mink leukemia inhibitory factor (LIF). First strand cDNA was generated by reverse transcription (RT) of total uterine mRNA from the pregnant mink uterus. PCR amplification was achieved by sense primers mink LIF-H (H) and mink LIF-B (B) and antisense primers mink LIF-6 and mink LIF-2. Double lines represent the mink LIF cDNA, overlapping clones are represented above, and the dark line indicates the direction of reverse transcription.

Leukemia inhibitory factor (LIF) is essential for embryo implantation in mice. Whether LIF plays a role in termination of embryonic diapause and initiation of implantation in carnivores, especially in species with obligate delayed implantation such as the mink, is not known. The objectives of this study were to clone the LIF coding sequence in the mink and determine its mRNA abundance in the uterus through embryonic diapause, implantation, and early postimplantation. We show that the mink LIF cDNA contains 609 nt encoding a deduced protein of 203 amino acids. The homologies are 80.6, 90, 88.2, 87.6, and 86.8% in coding sequence and 79.2, 90.1, 91, 90.1 and 85.4% in amino acid sequence with mouse, human, pig, cow, and sheep respectively. Glycosylation sites and disulfide bonds present in other species are generally conserved in the mink LIF sequence. Quantitation by polymerase chain reaction amplification indicates that LIF mRNA is expressed in mink uterus just prior to implantation and during the first two days after implantation, but not during diapause or later after implantation pregnancy. The abundance of LIF mRNA was significantly higher in the uterus at the embryo expansion stage ($P < 0.05$) than at days 1-2 of postimplantation. By immunohistochemical localization it was shown that LIF is expressed in the uterine epithelial glands at time of embryonic expansion and in early postimplantation. The coincidence of LIF expression with implantation in this species suggests that LIF is involved in the implantation process, and may be a maternal signal which terminates obligate embryonic diapause.

Molecular Reproduction and Development 51: 13-21, 1998. 5 figs., 50 refs. Authors' abstract.

Transcervical Artificial Insemination in the Domestic Ferret (*Mustela putorius furo*)

J.D. Kidder, R.H. Foote, M.E. Richmond

The research was undertaken to develop a successful nonsurgical procedure for artificially inseminating ferrets. A fiberoptic endoscope used in conjunction with a specially designed speculum and catheter permitted cervical catheterization and intrauterine insemination. Sperm were collected from the cauda epididymides of 10 discarded breeder males; the number of sperm in diluted samples used for insemination ranged from 4.4-13.6 x 10^6/100 µl with progressive motility of sperm ranging from 40 to 60%. Sperm collected from each male were diluted with an egg-yolk extender (TEST) and used to inseminate 8-12 females, with deposition of sperm intravaginally or transcervically into the uterine body 0 or 24 hr after an ovulatory injection of human chorionic gonadotropin (hCG). The vaginal inseminations were used as a control, and no pregnancies resulted after insemination of 26 females. Intrauterine inseminations resulted in 4/24 (17%) of the ferrets pregnant when hCG administration was coincident with insemination, and 19/24 (79%) of the ferrets were pregnant when inseminations were done 24 hr after hCG administration. All inseminated females were euthanized on day 20 after insemination to count fetuses. The mean number of fetuses was 3.1 (range, 1-8). The number (millions) of motile sperm inseminated (X) had a significant effect on the percentage of fetuses (Y). Regression analysis indicated a linear relationship between the two variables, with an R^2 value of 0.99 and a line of best fit described by the

equation $Y = 0.029 + 0.034\ X$. This paper is the first report of transcervical artificial insemination in the domestic ferret *(Mustela putorius furo)*. The method can serve as a model for application to ferrets and other mammals, particularly endangered species.

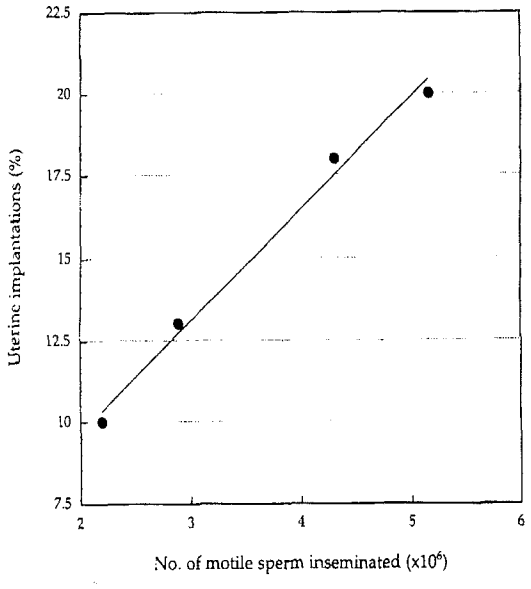


Fig. 3. Relationship between the percentage of fetuses (Y) ($n = 60$) and the number of progressively motile sperm (X) inseminated 24 hr after hCG administration in the domestic ferret. ($Y = 0.029 + 0.034\ X$, $R^2 = 0.99$, $P < 0.001$.)

Zoo Biol 17:393-404, 1998.3 5tables, 3 figs., 22 refs. Authors' summary.

Allocation of Inner Cell Mass and Trophectoderm Cells to the Preimplantation Blastocyst of the Domestic Ferret, *Mustela putorius furo*

Jeffrey D. Kidder, James R. Giles, Robert H. Foote, Milo E. Richmond, Michelle Salerno

The growth of ferret preimplantation blastocysts in vivo, collected between 156 and 240 hr post coitum, was investigated. A technique, combining immunosurgery and differential fluorochrome staining, was used to discriminate between inner cell mass (ICM) and trophectoderm (TE) cells. Using the stains propidium iodide and bisbenzimide (Hoechst 33342), the ICM was stained blue and the TE was stained pink. The ICM and TE counts for 90 blastocysts, respectively, averaged 25 and 63 at 156 hr and increased exponentially to 2077 and 4137 at 240 hr. The Box-Cox procedure was used for choosing a transformation that minimized the error sum of squares for a linear regression of Y (cell count) on X (time in hr). Logarithmic transformations of the ICM, TE and total cell count gave a good fit, but the following equations obtained by the Box-Cox procedure provided the best fit, where Y is cell count and X is time in hours. For inner cell mass: $Y = [(176.06 + 2.45X)/-899.44 + 1]^{-3.33}$; trophectoderm: $Y = [(301.38 + 14.48X)/-6863.42 + 1]^{-10}$; and total: $Y' = [(2266.97 + 17.OX)/-7837.21 + 1^{1-5}$. The R^2 values were 0.73, 0.84, and 0.84, respectively. The exponential growth of the ferret embryo during the time interval that measurements were made fits the general pattern described for other mammalian embryos. This report is the first to characterize the pattern of cell allocation and growth in preimplantation blastocysts of the ferret, and the first such report for a carnivore. The pattern of in vivo development provides a standard for judging the quality of in vitro produced and matured ferret embryos and, concomintly, a means to evaluate culture systems.

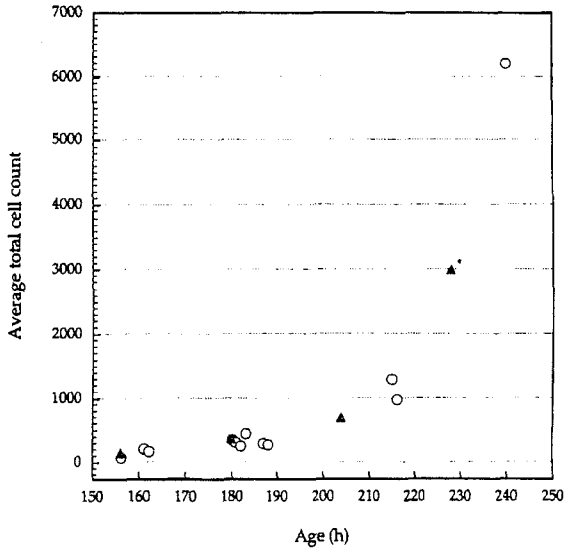


Fig. 2. Average total number of cells in ferret blastocysts 156–240 hr post coitum. (▲) Average total cell counts of ferret blastocysts reported by Daniel ('70). (*) Estimated value.

Journal of Experimental Zoology 283: 202-209, 1999. 1 table,

Delayed implantation in the marbled polecat, *Vormela peregusna syriaca* (Carnivora, Mustelidae) evidence from mating, parturition, and post-natal growth

M. Ben-David

In many species of carnivores, pregnancy is longer than expected based on maternal body size, as a result of delay in implantation. In this study on the marbled polecat *(Vormela peregusna syriaca),* pregnancy length, and associated environmental factors were investigated from 1985 to 1989, in Israel. Data on mating dates, parturition dates, and post-natal development from captive and wild polecats revealed the existence of a long and variable pregnancy length (243-327 days) characteristic of delayed implantation. Post-natal development of the captive born cubs resembled that described for other small mustelids. Results from a multiple linear regression model suggested that parturition date was related to average minimum temperature during the period preceding implantation (r^2 = 0.37). This result suggests that delayed implantation offers the female the ability to track environmental conditions and allows greater flexibility in the timing of parturition.

Mammalia, t. 62, no. 2, pp. 269-283, 1998. 4 tables, 4 figs., 43 refs. Author's summary.

Effects of Photoperiod on Annual Reproductive and Hormonal Rhythms and Fertility in Silver Fox Females

L. V. Osadchuk

The silver fox is a strictly seasonal breeder and photoperiod is a main factor that regulates reproduction in this species. The aim of the present work was to study the effects of different skeleton photoperiods on the induction of early estrus, hormonal activity of gonads and fertility in anestrous silver foxes. Adult females (n = 48) were exposed to short days (9.5L:14.5D) from September until March. Females (n = 12) kept under the natural daylight conditions served as the control. The experimental groups received illumination in two fractions: 7- and 2.5-hour long. The 7-hour period comprised natural daylight from 10:00 to 17:00 and an additional light pulse at night (00-02:30, group 1), in the morning (06:00-08:30, group 2) and in the evening (17:30-20:00, group 3). There are three types of response in group 1. A half of animals showed signs of estrus in November-December, 2.5-3.0 months before the natural reproductive season. The second part displayed no changes until the end of the experiment indicating photorefractoriness. The third part had estrus during the natural reproductive season. Photoperiod 2 and 3 did not affect the temporal pattern of, reproductive rhythm, All artificial photoperiods increased the estradiol level and decreased the progesterone, cortisol and testosterone levels during the anestrous phase and suppressed the ovarian hormonal activity during the follicular and luteal phases but photoperiod 1 had the most pronounced effects. Fertility was decreased in all experimental groups. Our results suggest that the photoperiod with a light pulse at night can induce early estrus and ovarian hormonal activity in anestrous females. The data obtained suggest that decreased fertility under these conditions could be due to altered ovarian hormonal secretion.

Proc. Of Acad. Of Sci. Biology no 2, pp. 191-200, 1999 + abstracted in the 18th Joint Meeting of the British Endocrine Societies, 12-15 April 1999, Bournemouth, p. 202. In RUSS, Su. ENGL. 2 tables, 4 figs., 32 refs. Author's summary.

Original Report

Study on Morphology and Structure of Hairs of Three Species of Foxes

Fei Rongmei, Jing Songyan

Northeast Forestry University, Harbin 150040 P.R.C.

Abstract

With Scanning Electron Microscope, we observed morphology and structure of coat hairs of silver fox, blue fox and blue frost fox. According to morphology, coat hairs can be divided into four types: straight guard hair, lanceolate guard hair, pine guard hair and underfur. From the cross section, a hair can be divided into three layers: cuticle scale layer, cortex layer and medulla layer. The main scale types of straight guard hair of silver fox are long petal, in blue fox sundry petal, and in blue frost fox sundry waved. The medulla pattern of straight guard hairs in the three species of foxes are all Network--C, and of underfur all Ladder- - A. The morphology of melanine granules of hairs of all three species of foxes are different.

Forewords

Silver fox *(Vulpes fulvus)* and blue foxes (*Alopex lagopus)* are farm bred fur animals. Blue frost fox is a sterile crossing of male silver fox and female blue fox by artificial insemination and kept only for one season. It is also an important commercial fur animal. The first blue frost fox was born in 1990 in China (*Kong, 1990*). Blue frost resembles its parents in general features (*Blomstedt, 1987*). The blue frost foxes are all fairly dark and silvery with a typical intermediate hair type. In colour they often resemble totally the silver fox and can be distinguished only by the shorter type of hair (*Nes et al., 1989)*. Study on morphology and structure of hair of these three species would be helpful to breeding of foxes and other fur animals, and would help us to obtain high quality pelts.

Materials and Methods

Materials

All the foxes came from Harbin Wildlife Farm of Hei Longjiang Province Foreign Trade Company.

Methods

(1) Observation of morphology , scale layers, and medulla layers of hair. 0.5g hairs were taken from the back of foxes, degreased with 95% alcohol:ether (1:1) for 30 mins, then washed with 100% alcohol. After that, we could study their morphology, and measure the length and width of the hairs by mathematical statistics. We fitted hair on sample platforms with double rubberized tape, cut the hairs along the vertical section with a razor blade, spourted Platinum (Pt.) , and observed the scale structures and medulla patterns with a type S--520 SEM at 19KV.

(2) Observation of melanine granules: 2 g of hair were taken from the three species of foxes, degreased with 95% alcohol:ether (1:1) for 1 hour, hydrolysed with 3N HCI, centrifugated, filtered with filter paper

and dried. We fitted filter paper which had melanine granules on a sample platform with double rubberized tape, spurted Platinum (Pt.), observed and measured with a type S--520 SEM at 19KV.

Results

1. Morphology

According to the morphology, all hairs of three species of foxes can be divided into 4 types generally: straight guard hair, lanceolate guard hair, pine guard hair and underfur. Straight guard hair and underfur are the main types of coat hair, their length and width are seen in Table 1. The tip region of the straight guard hairs of all three species is black, the middle region is white and the root region is grey. The black tip region length is about 1/3--1/4 of that of a whole hair in silver fox. 1/4-1/5 in blue fox, and 1/7--1/8 in blue frost fox. The black tip region is mingled with white guard hairs in silver fox, and few black guard hairs in blue frost fox. The underfur is grey in silver fox and blue fox, and blue grey in blue frost fox. Straight guard hairs of blue frost fox are the shortest and thickest in these three species.

2. Structure

According to cross section, from outer to inner, a hair can be divided into cuticle scales layer, cortex layer and medulla layer. There are a lot of melanine granules in the cortex and medulla layers.

The type of scale structure varied with the different array of scales cells. The structure of the hair scales of the three species are different (Figs. 1-6 and Table 2).

Table 1. Length and width of hair in three species of foxes

Species	Types	Length (mm)	Width (μm)
Silver fox	Straight guard hair	97.39±6.21	89.80±7.02
	Underfur	55.43±7.01	21.44±4.80
Blue fox	Straight guard hair	81.67±3.17	72.16±8.66
	Underfur	45.46±3.49	23.97±5.31
Blue frost fox	Straight guard hair	48.15±6.57	86.52±10.25
	underfur	30.09±3.50	15.26±3.06

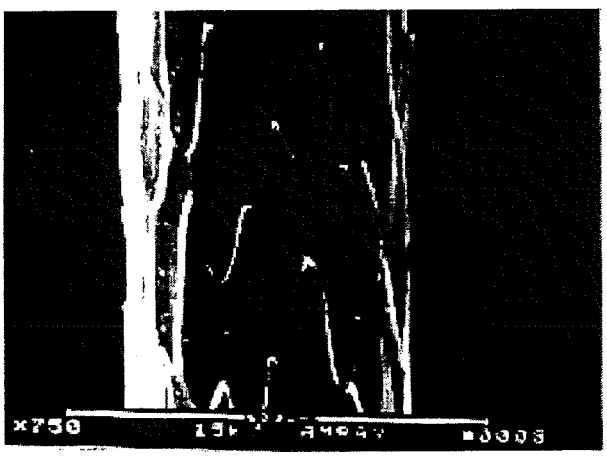


Fig. 1. Long petal of scale of straight guard hair of silver fox. X 750x1.5.

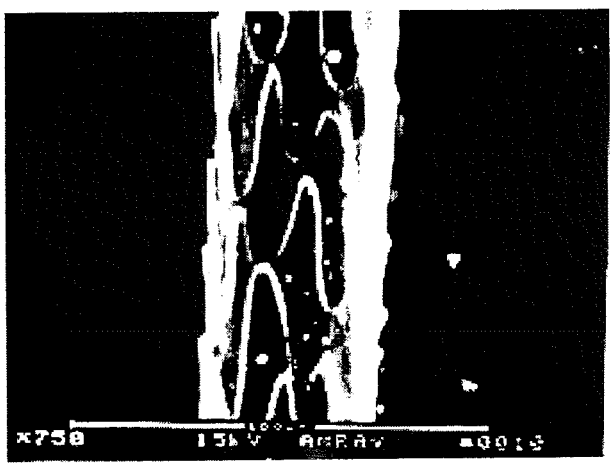


Fig. 2 Long petal of scale of underfur of silver . X 750x1.5

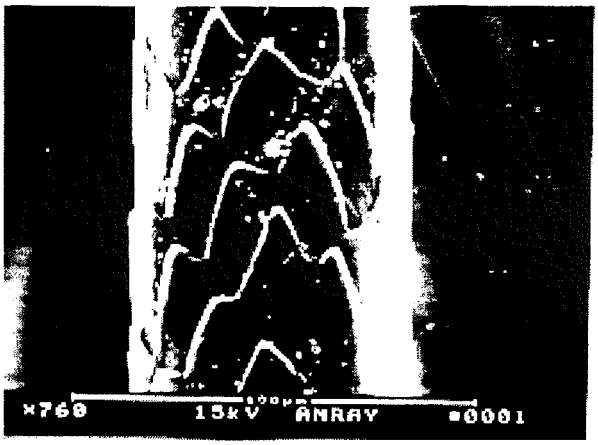


Fig. 3. Sundry petal of scale of straight guard hair of blue fox. X 760x1.5.

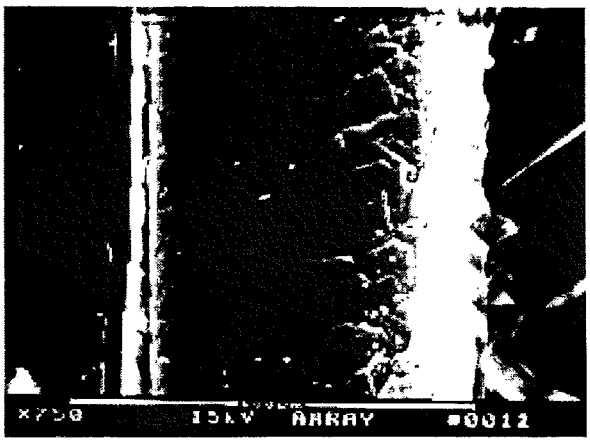


Fig. 5. Sundry waved of scale of straight guard hair of blue frost fox. X 750x1.5.

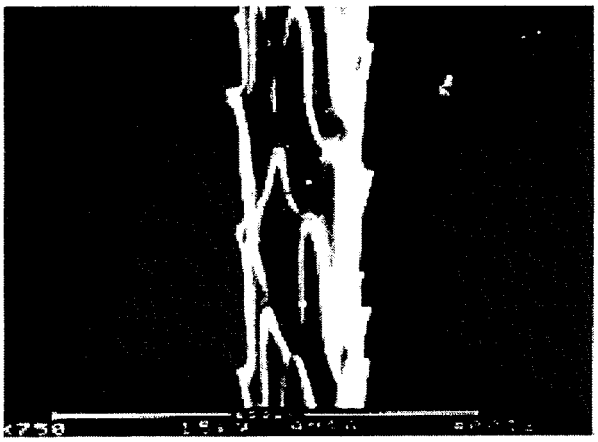


Fig. 4. Long petal of scale of underfur of blue fox. X 750x1.5.

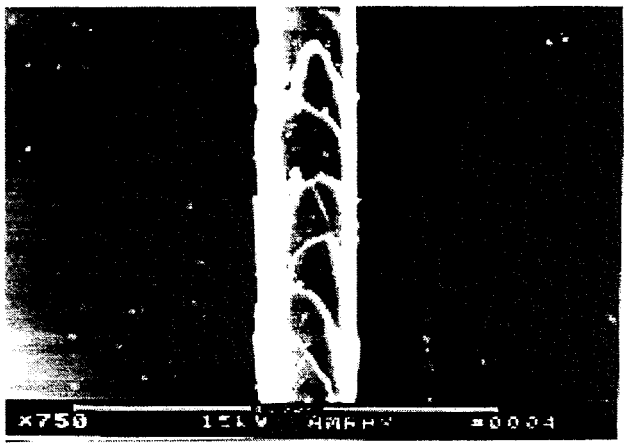


Fig. 6. Oblique petal of underfur of blue4 frost fox. X 750x1.5.

Table 2. Structure of scale of three species of foxes

Species	Type	Type of scale (from tip to root)	Main type
Silver fox	Straight guard hair	Coronal – sundry waved – ovate petal – long petal – sundry	Long petal
	Underfur	Coronal – long petal – sundry coronal	Long petal
Blue fox	Straight guard hair	Coronal – sundry waved – ovate petal – long petal – sundry petal – sundry coronal	Sundry petal
	Underfur	Coronal – long petal – sundry colonal	Long petal
Blue frost fox	Straight guard hair	Coronal – sundry waved – long petal – ovate petal – sundry coronal	Sundry waved
	Underfur	Obilque petal – long petal – sundry coronal	Oblique petal

Table 3. Structure and morphology of melanine granules of three species of foxes

Species	Morphology	Length (μm)	Width (μm)
Silver fox	Long rod, ellipse	8.610±1.34	2.969±0.58
Blue fox	Long rod	8.889±1.01	2.976±0.24
Blue frost fox	Ellipse, round	8.134±1.27	3.260±0.95

Different medulla patterns in the medulla layer come from different arrays of medulla cells in the medulla. The medulla patterns of straight guard hairs in the three species of foxes are all Network-C (Fig. 7), the pattern of underfur are all Ladder--A (Fig. 8). The structure and morphology of the melanine granules of the three species of foxes are different (Table 3 and Fig. 9).

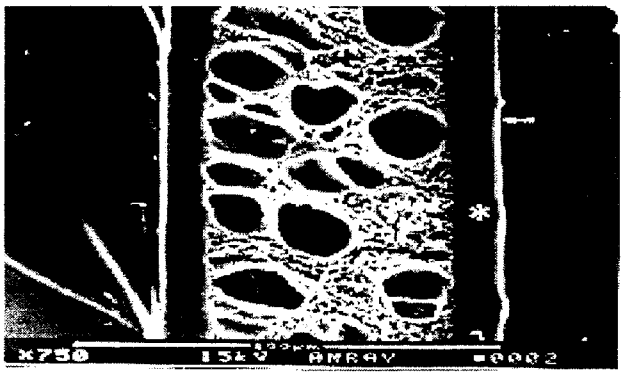


Fig. 7. Network-C of medulla pattern of straight guard hair of silver fox. X750x1.5. Med:Medulla,, *:Cortex, →:Scale.

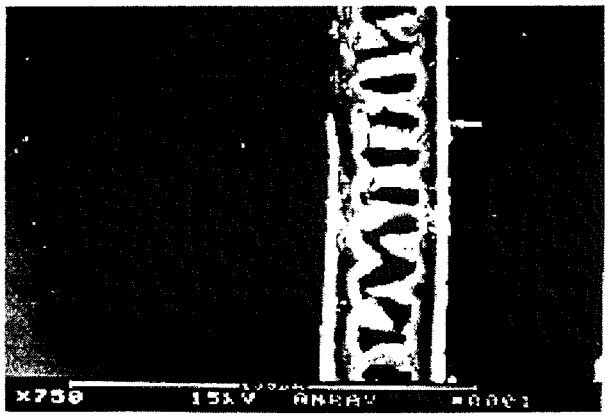


Fig. 8. Ladder-A of medulla pattern of underfur of silver fox. X 750x1.5. Med:Medulla, *:Cortex, →:Scale.

Fig. 9. Morphology of melanine granules of hair of blue frost fox. X 8.000x1.5. ⇒:Melanine granules.

Discussion

1. The coat hairs of the three species of foxes can be divided into 4 types. Blue frost fox is a hybridized progeny of silver fox and blue fox, and a lot of features are similar to its parents.

2. Generally, type of scale of a hair is coronal in the tip region, sundry coronal in the root region, and other types in the middle region. There are main types and transitional type scales in a hair.

3. The medulla patterns in straight guard hairs and underfur of the three species are identical. This indicated that the medulla patterns of animals in one Family is identical. The relationship of animals could be probed by the type of medulla pattern.

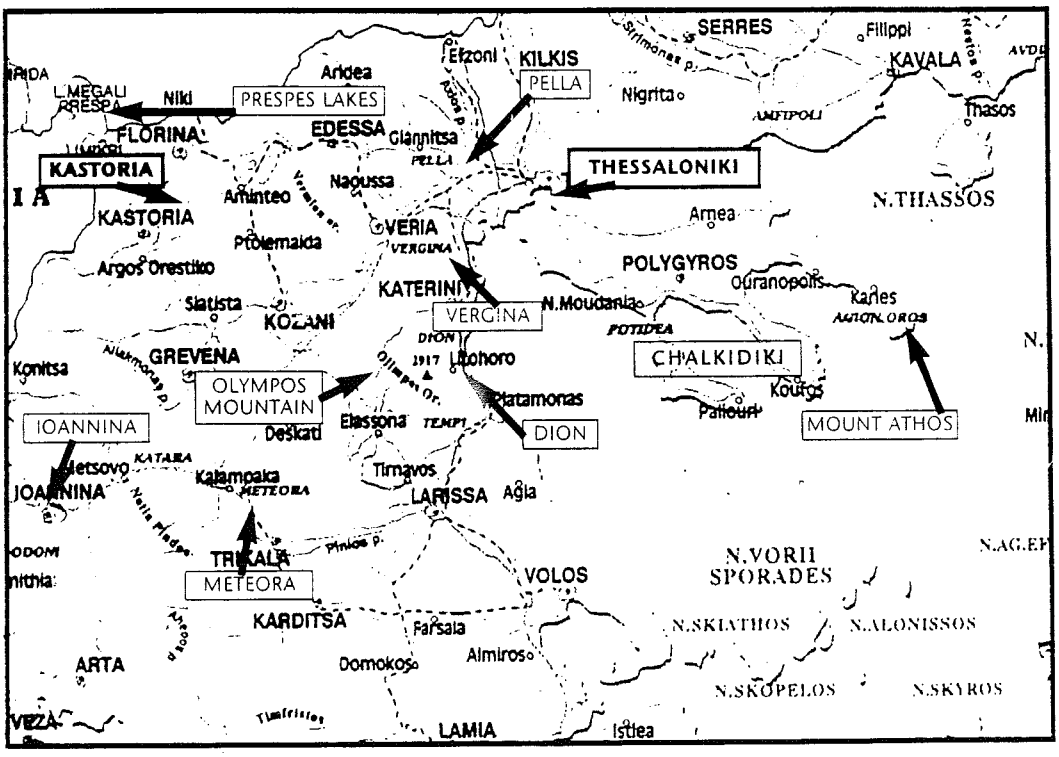
4. The morphology and size of the melanine granules of the three species of foxes are different. The sizes and morphology could be used as reference for classification of the animals. It will be good for research of breeding and improvement of colour of fur animals.

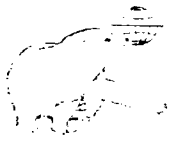
References

Blomstedt, Leena. 1987. Hair structure and development of fur in young mink, silver fox, blue silver fox and blue fox. Scientifur, 11 (3):264—265.

Kong, Oingsong. 1991. A brief report on artificial insemination of foxes in China. Chinese Wildlife 60 (2):27 (In Chinese).

Nes, N.N., Einarsson, E.J. O. Lohi. 1989. Beautiful Fur Animals and Their Colour Genetics. Scientifur.





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Original Report

Mink skin tyrosinase exhibits extraordinary activity that fluctuates with molt

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Abstract

To characterize mink skin tyrosinase during autumn molt, we prepared anti-tyrosinase antiserum and investigated the changes in the activity of tyrosinase with or without reaction with the antiserum. Mink skin tyrosinase solution was separated into a supernatant and precipitate after reaction with antiserum. Tyrosinase showed incubation time-dependent two-phase activity; i.e., an initial low-reaction phase followed by a high-reaction phase. This two-phase activity remained even after immunoreaction, and the tyrosinase activity increased in both the supernatant and precipitate in a dose-dependent manner. The activity increase in the supernatant was quite different from that observed in B-16 melanoma tyrosinase, in which activity decreased with the addition of antiserum. Further, the degree of antiserum-induced activation differed between anagen and telogen samples. These results suggested that mink skin tyrosinase has a peculiar nature and that its activity and quantity changes during autumn molt.

Introduction

The mink is a fur-bearing animal that exhibits a typical seasonal molt in spring and autumn. Molt is not only part of the hair growth cycle but also a total skin event that includes changes in the skin components.

Melanogenesis is a process closely coupled to hair growth and contributes to hair and skin coloring. Melanin production is specifically observed in the differentiated melanocytes of neural crest origin. Tyrosinase (EC 1.14.18.1) is a rate-limiting enzyme in melanin biosynthesis that catalyses the first two steps: the conversion of tyrosine to DOPA, and of DOPA to DOPA-quinone (*Hearing and Jimenez, 1987*). Indeed, two types of melanins are synthesized during melanogenesis: pheomelanins, which are yellow to reddish pigments, and eumelanins, which are black to brown. Recently, two other enzymes were shown to be involved in melanin production: tyrosinase-related protein-1 (TRP-1) and TRP-2, which appear to be involved mainly in eumelanin production

(*Kameyama et al., 1993; Orlow et al., 1994; Tsukamoto et al., 1992*). There are many genetic loci influencing melanogenesis, and there is therefore a great variation in coat color in mammals. In mice, for example, coat color is regulated by more than 150 alleles at over 60 loci (*Silvers, 1979*).

However, studies on melanogenesis and its regulatory mechanism have generally used mice or humans as the experimental model. Although tyrosinase is the key enzyme for melanin production in all mammals, the hair cycle of mice and humans is not common to other mammals. Most mammals have a seasonal molting cycle, and studies on pigmentation of seasonal molting animals are quite limited (*Blumenkrantz and Blomstedt, 1987; Weatherhead and Logan, 1981*). We previously reported (*Fukunaga et al., 1992*) that tyrosinase activity in mink skin was closely correlated with seasonal molt and that mink skin tyrosinase has unique catalytic activity compared to that of humans or mice. To further characterize mink tyrosinase, we prepared anti-tyrosinase antiserum and examined the interaction between mink skin tyrosinase and the antiserum.

Materials and methods

Cultured B-16 melanoma cells were inoculated into C57BL mice and the grown melanoma tissue was excised for preparation of tyrosinase purification material. Tyrosinase was purified according to the method of Nishioka (*Nishioka, 1978*) and Laskin and Piccinini (*Laskin and Piccinini, 1986*). Briefly, melanoma tissue was homogenated and ultracentrifuged at 100,000 *g* for 1 hour. The resultant supernatant was applied to a DEAE Sepharose CL-6B column and eluted by a 0 - 0.5 M NaCI gradient. Tyrosinase-positive fractions were then applied to a Con-A Sepharose CL-4B column and eluted by a 0 - 2.0 M a-methyl-D-manoside gradient. Positive fractions were collected and concentrated as purified tyrosinase. This purified tyrosinase was immunized to rabbit and raised anti-tyrosinase antiserum. The reaction of the prepared antiserum to mink skin tyrosinase was confirmed by western blotting.

Male dark mink skin samples were taken from the dorsal skin during the anagen and telogen of autumn molt. A cryosection of each skin sample was prepared and the molting stage was confirmed histologically (*Fukunaga et al., 1991 and 1992*).

Crude tyrosinase solution obtained from the mink skin was prepared as described previously (*Fukunaga et al., 1992*). Briefly, mink skin that had 1 cm^2 surface area was punched out and homogenized in 0.1 M phosphate buffer (pH 6.8) containing 1% Triton X-100, and then ultracentrifuged at 100,000 g for 1 hour. The resultant supernatant was used as the mink skin tyrosinase solution in the experiment. Various amounts of anti-tyrosinase antiserum (0-160 µl) were added to 800 µl of mink skin tyrosinase solution for immunoreaction. The final volume of this mixture was adjusted to 1 ml with PBS. After overnight incubation at 4C°, the mixture was centrifuged at 10,000 g for 20 min. The separated precipitate was washed twice with PBS and then suspended in 1 ml of 0.1 M phosphate buffer (pH 6.8) containing 1% Triton X-100. Both the supernatant and precipitate were added to 4 ml of 1 mM DOPA in 0.1 M phosphate buffer (pH 6.8) as a tyrosinase reaction substrate. This reaction mixture was incubated at 37C°, and DOPA oxidase activity was measured from changes in optical density at 475 nm every 3 hours for 24 hours.

Cultured B- 16 melanoma cells were harvested and homogenated in 0. 1 M phosphate buffer (pH 6.8) containing 1% Triton X-100, and then centrifuged at 10,000 g for 20 min. The resultant supernatant was used as B-16 melanoma tyrosinase. Immunoreaction was performed using 50 µl of tyrosinase solution and various amounts (0 - 80 µl) of antiserum. After incubation and separation, as for mink tyrosinase, DOPA oxidase activity was measured as follows. A 70 µl sample of the supernatant and 630 µl of DOPA solution were mixed and the change in optical density was monitored for 10 minutes. The precipitate was suspended in 1 ml of DOPA solution, incubated for 24 hours before the optical density was measured.

Results

As we previously reported (*Fukunaga et al., 1992*), mink skin tyrosinase showed two-phase catalytic activity dependent on the incubation period. There was an initial low-reaction phase followed by a high-reaction phase (Fig. 1). This two-phase activity was maintained even after the tyrosinase solution reacted with the antiserum. Moreover, this activity tended to increase with the addition of antiserum in a volume-dependent manner. This phenomenon was observed not only in the precipitate (ppt) fractions (Fig. 1B) but also in the supernatant (sup) fractions (Fig. 1A). Two-phase activity and the increase in activity resulting from the addition of antiserum were detected in all samples used in this experiment. When antiserum used to blank, no tyrosinase activity was detected and normal rabbit serum had no effect on the activation of tyrosinase activity (data not shown).

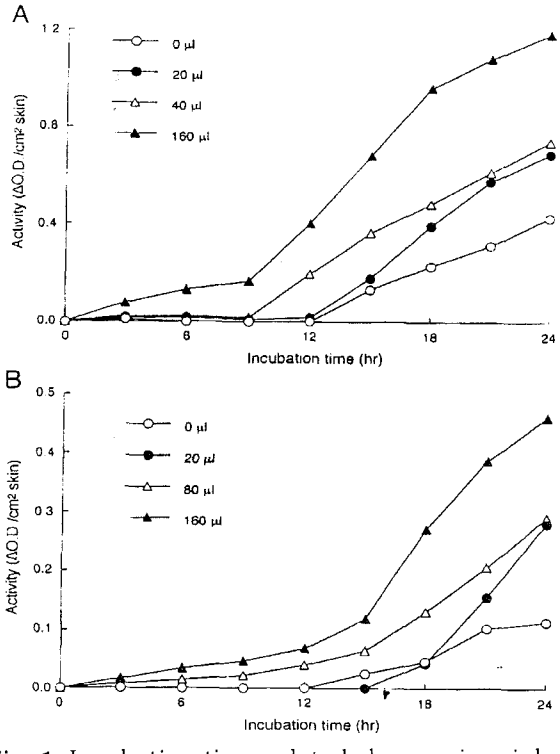

Fig. 1. Incubation time-related changes in mink skin tyrosinase activity after reaction with anti-tyrosinase antiserum. Crude tyrosinase extracted from mink skin (1 cm^2) sampled on Aug. 7 was incubated with DOPA at 37C°. Details are provided in Materials and Methods. Numbers in the figure represent the amounts of added antiserum.

In anagen samples, both sup and ppt fractions showed an increase in tyrosinase activity with the addition of antiserum in a volume-dependent manner (Fig. 2). Antiserum-induced activation of tyrosinase activity was observed for all incubation periods. The ppt fractions were more strongly activated than were the sup fractions. A sup fraction incubated without antiserum showed relatively high tyrosinase activity from early in the incubation period, and antiserum-induced tyrosinase activity was observed but at levels lower than those in the ppt fractions (Fig. 2A). In ppt fractions, tyrosinase activity was increased 5- to 10-fold by the addition of the antiserum (Fig. 2B), but the activity in sup fractions only increased 1.5-fold, at most.

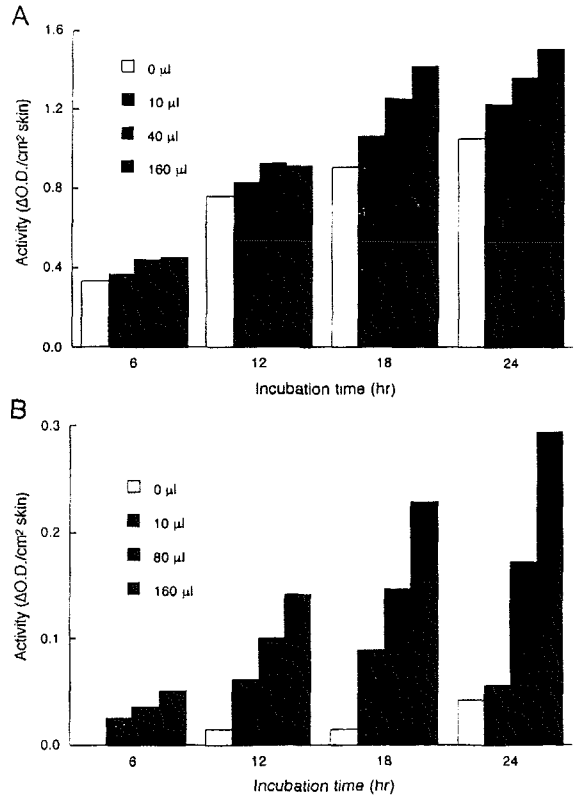

Fig. 2. Effect of the addition of antiserum on tyrosinase activity in anagen samples. Tyrosinase extracted from anagen mink sampled on Sept. 12. Activity of both the supernatant (A) and precipitate (B) measured every 6 hours. Number in the figure represent the amounts of added antiserum.

In telogen samples, no tyrosinase activity was detected in sup fractions incubated without antiserum for 6 and 12 hours. However, samples to which antiserum was added showed tyrosinase activity during these incubation periods and this activity increased with the addition of antiserum in a volume-dependent manner (Fig. 3A). There was a higher increase in tyrosinase activity in the telogen sup fractions than in the anagen fractions. The increase in tyrosinase activity was 2- to 3-fold higher in samples to which antiserum was added than that in samples without antiserum for all incubation periods. Ppt fractions without antiserum showed very low tyrosinase activity throughout the incubation period. When antiserum was added, there was almost no tyrosinase activity detected in early incubation periods, but rapid and marked increases were detected in later incubation periods (18 and 24 hours) (Fig. 3B).

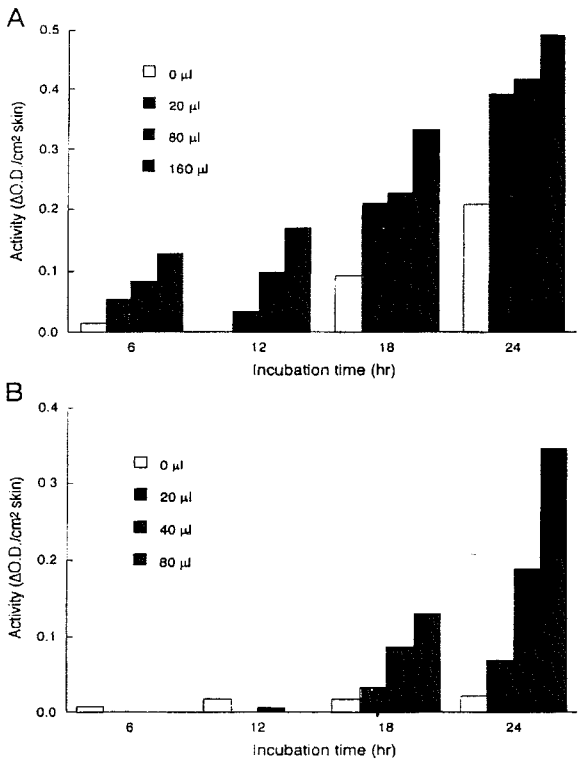

Fig. 3. Effect of the addition of antiserum on tyrosinase activity in telogen samples. Tyrosinase extracted from telogen mink skin sampled on Dec. 12. Activity of both the supernatant (A) and precipitate (B) measured every 6 hours. Numbers in the figure represent the amounts of added antiserum.

After reaction with antiserum, the tyrosinase activity of the B-16 melanoma cells showed a different pattern from that of mink skin tyrosinase. While the activity increased in ppt fractions with the addition of antiserum in a volume-dependent manner (Fig. 4B), the tyrosinase activity in sup fractions decreased as the volume of antiserum added increased (Fig. 4A).

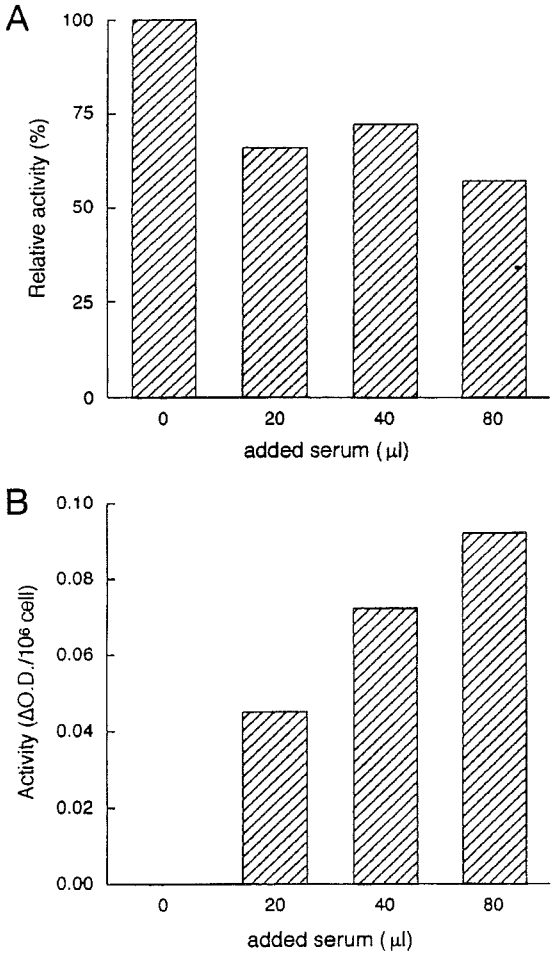

Fig. 4. Effect of the addition of antiserum on B-16 melanoma tyrosinase. Activity of the supernatant (A) is a relative value of which the activity of an antiserum free sample represents 100%. Activity of the precipitate (B) was determined by the measured changes in optical density as details in Materials and Methods.

Discussion

In this study, mink skin tyrosinase showed two-phase activity dependent on the incubation period, an initial low-reaction phase and a

late high-reaction phase, as previously reported (*Fukunaga et al., 1992*). Unexpectedly, the activity of mink skin tyrosinase increased with the addition of anti-tyrosinase antiserum. When crude mink skin tyrosinase solution was reacted with the antiserum, both the resultant supernatant and precipitate showed a dose-dependent increase in activity. The increased activity in the precipitate was thought to be that of tyrosinase bound to the antibody and which increased in proportion to the increases in antiserum. However, the increase of tyrosinase activity in the supernatant can not be explained so simply. Antiserum alone showed no tyrosinase activity and normal rabbit serum had no effect on the activation of tyrosinase activity. Conversely, B-16 melanoma tyrosinase activity in the supernatant decreased in proportion to increases in antiserum. It was reported that a tyrosinase inhibitor might exist in human and mice skin or melanocytes (*Babu et al., 1998; Kameyama et al., 1993; Wong and Pawelek, 1975*). Therefore, it is suspected that the increase in mink skin tyrosinase activity in the supernatant resulted from the competition between an internal tyrosinase inhibitor and the antiserum for the tyrosinase, not from any activation factor that might exist in the antiserum. After reaction with antiserum, the precipitate was thought to be an affinity-purified tyrosinase by antibody and this fraction still demonstrated two-phase activity. Therefore, this two- phase reaction was thought to be an inherent characteristic of mink skin tyrosinase, while the tyrosinase activity of melanoma cells or mice skin increased linearly in a time-dependent manner until it reached a plateau. We first thought that semiquantitative determination could be done by using the antiserum, but the results were more complicated than we expected. Since we used crude tyrosinase solution in this study, it is possible that some other factor that affects tyrosinase activity, such as TRP-1 or -2, exist in the solution.

Melanogenesis is closely linked to hair growth; melanin synthesis begins early in the anagen then declines and ceases during the catagen and no pigmentation occurs during the telogen (*Slominski et al., 1991; Slominski et al., 1994*). Our results showed that tyrosinase activity was detected in telogen samples in later incubation periods. But inactive melanocytes have been observed in the secondary germ region of resting murine follicles (*Sugiyama and Kikuta, 1979*). Our previous data indicated that the low-reaction phase might simulate the actual mink skin condition (*Fukunaga et al., 1992*). Therefore, it is probable that the tyrosinase activity detected in the telogen samples in later incubation periods was caused from residual but not active tyrosinase in the skin.

Differences in tyrosinase activity were observed between anagen and telogen precipitates after reaction with the same volume of antiserum. This indicates that the amount of tyrosinase that reacted with a certain amount of antiserum fluctuated during molt. This notion is supported by the fact that both anagen and telogen supernatants showed higher tyrosinase activity than did the respective precipitates, although the difference in activity between the supernatant and precipitate was smaller in telogen samples than in anagen samples. Tyrosinase was shown to be present in amelanotic melanoma cells (*Bouchard et al., 1989*), and there was no linear correlation found between the amount of tyrosinase and its level of activity (*Burchill et al., 1988; Fuller et al., 1987; Jimenez et al., 1988*). In mice, tyrosinase changed its activity, the amount of enzyme and mRNA expression during the hair cycle (*Slominski et al., 1991*); it is suspected that tyrosinase changed not only its activity but also its amount in mink skin during molt.

In this study, we demonstrated that mink skin tyrosinase has unique catalytic activity. To elucidate the nature of mink tyrosinase, it is necessary to purify tyrosinase from mink skin and to obtain genetic information. In addition, the roles of other factors such as TRP-1 and TRP-2 must also be characterized in order to clarify the mechanisms of melanogenesis in seasonal molting mammals.

References

Babu, B. R., Diwakar, G., Ramaiah, A., Fatma, T. 1998. Effect of an abundant human skin mlanosomal 66 kDa protein (Mp 66) on murine tyrosinase - its physiological implications on melanogenesis. J. Biosci., **23**, 125-129.

Blumenkrantz, N., and Blomstedt, L. 1987. Chelators depigment and inorease elasticity of mink skin. Acta Agric. Scand., **37**, 375-395.

Bouchard, B., Fuller, B. B., Vijayasaradhi, S., and Houghton, A. N. 1989. Induction of pigmentation in mouse fibloblasts by expression of human tyrosinase cDNA. J. Exp. Med.,**169**, 2029-2042.

Burchill, S. A., Virden, R., Fuller, B. B., and Thody, A. J. 1988. Regulation of tyrosinase synthesis by α-melanocyte-stimulating hormone in hair follicular melanocytes of the mouse. J. Endocrinol., **116**, 17-23.

Fukunaga, S., Kohno, K., Nakamura, F., and Kondo, K. 1991. A quantitative histological study in melatonin induced autumn molt in mink skin. Scientifur, **15**, 107-113.

Fukunaga, S., Kohno, K., Nakamura, F., and Kondo, K. 1992. Two autumn molts in mink skin caused by melatonin treatment. J. Anim. Sci. Technol., **63**, 694-703.

Fuller, B. B., Lunsford, J. B., and Iman, D. S. 1987. α-Melanocyte-stimulating hormone regulation of tyrosinase in Cloudman S-91 mouse melanoma cell culture. J. Biol. Chem., 262,4024-4033.

Hearing, V. J., and Jimenez, M. 1987. Mammalian tyrosinase: the critical regulatory control point in melanocyte pigmentation. Int. J. Biochem., **19**, 1141-1147.

Jimenez, M., Kameyama, K., Maloy, W. L., and Tomita, Y. 1988. Mammalian tyrosinase - biosynthesis, processing, and modulation by melanocyte stimulating hormone. Proc. Natl. Acad. Sci. USA, 85,3830-3834.

Kameyama, K., Takemura, T., Hamada, Y., Sakai, C., Kondoh, S., Nishiyama, S., Urabe, K., and Hearing, V. J. 1993. Pigment production in murine melanoma cells is regulated by tyrosinase, tyrosinase-related protein-1 (TRP-1), DOPAchrome tautomerase (TRP-2), and a melanogenic inhibitor. J. Invest. Dermatol., 100, 126-131.

Laskin, J. D., and Piccinini, L. A. 1986. Tyrosinase isozyme heterogeneity in differentiating B 16/C3 melanoma. J Biol Chem 261, 16626-16635.

Nishioka, K. 1978. Particulate tyrosinase of human malignant melanona; solubilization, purification following trypsin treatment, and characterization. Eur. J. Biochem., **85**, 137-146.

Orlow, S. J., Zhou, B. K., Chakraborty, A. K., Drucker, M., Pifko-Hirst, S., and Pawalek, J. M. 1994. High-molecular-weight forms of tyrosinase and the tyrosinase-related proteins: evidence for a melanogenic complex. J. Invest. Dermatol., **103**, 196-201.

Silvers, W. K. (ed) 1979. The coat colors of mice: a model for mammalian gene action and interaction. New York, Springer Veriag.

Slominski, A., Paus, R., and Costantino, R. 1991. Differential expression and activity of melanogenesis-related proteins during induced hair growth in mice. J. Invest. Dermatol., **96**, 172-179.

Slominski, A., Paus, R., Plonka, P., Chakraborty, A., Maurer, M., Pruski, D., and Lukiewicz, S. 1994. Melanogenesis during the anagen-catagen-telogen transformation of the murine hair cycle. J. Invest. Dermatol., **102**, 862-869.

Sugiyama, S., and Kikuta, A. 1979. Mode of redifferentiation and melanogenesis of melanocyets in mouse hair follicles. J Ultrastruct Res 67, 40-54.

Tsukamoto, K., Jackson, 1. J., Urabe, K., Montague, P. M., and Hearing, V. J. 1992. A second tyrosinase-related protein, TRP-2, is a melanogenic enzyme termed DOPAchrome tautomerase. EMBO J., 11, 519-526.

Weatherhead, B., and Logan, A. 1981. Interaction of a-melanocyte-stimulating hormone, melatonin, cyclic AMP and cyclic GMP in the control of melanogenesis in hair follicle melanocytes in vitro. J. Endocrinol., **90**, 89-96.

Wong, G. and Pawelek, J 1975. Melanocyte-stimulating hormone promotes activation of pre-exisiting tyrosinase molecules in Cloudman S91 melanoma cells. Nature, **255**, 644-646.

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