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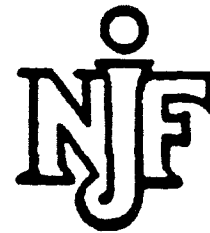


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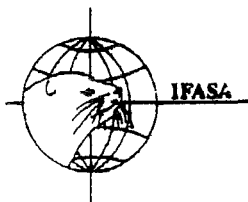
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Notes  
Scientifur  
Vol. 22, No. 1, 1998

Dear Readers,

First of all we wish to thank our many contributors and readers for the many Christmas and New Year Greetings we have received also this year.

Our hope for the new year is to enlarge the IFASA/SCIENTIFUR family which is also depending on a good economy on the fur animal production side. We are aware of the dark clouds on the economic skies of Asia, but as all of you we hope that the nature of these difficulties is only temporary and local - if that is at all possible in our world of today.

We are working hard to get hold of scientific reports regarding fur animal production published elsewhere. We do, however, have difficulties even though we send out approx. 300 requests for specific reports to editors every year. At least 30% of these do not answer our request, and as mentioned in Notes No. 4, 1997 very few scientists send in reprints to SCIENTIFUR for abstracting by themselves. Less than ten were received that way in 1997 - and this is very little compared to the total number of actual reports estimated to amount to more than 200 in 1997. We sincerely hope that we shall find these reports and receive them currently during 1998, but as readers I am sure you will agree with us that scientific news is the most important when it is new. Therefore: Colleagues, please send reprints of your reports to SCIENTIFUR on the very day you receive them and help us - and with us also the industry - to be updated with fresh information from day to day.

As you will see from this issue of SCIENTIFUR, the number of original reports is very high, i.e. eight, and the number of abstracts reduced correspondingly. The number of original reports underlines the significance of SCIENTIFUR as the information link regarding scientific literature in the fur animal world.

In this issue we publish an English translation of Internal Report No. 94, 1997 entitled Welfare in fur animals - behavioural and health perspectives, from the National Institute of Agricultural Sciences in Denmark. This report covers very recent information on research in fur animal welfare. We will bring one or two translated reports per issue of SCIENTIFUR and finally collect them all in a booklet which will be a very important help in the standing debate on welfare.

Also in this issue we start a series of scientific reports regarding "REPRODUCTION PHYSIOLOGY IN FUR ANIMALS". The series is edited by Dr. Ludmila Osadchuck and, as you will see from her introduction, it is to a certain degree a natural continuation of the series regarding domestication which we finished in No. 1, 1997. We highly welcome both initiatives because we feel that also this way we act as an essential link in the process of spreading important scientific information to the entire world.

As mentioned in the latest issue of SCIENTIFUR, we maintain the same prices for subscription etc. as the years before. New readers may always find information on prices etc. in the pages of SCIENTIFUR.

We are in the process of sending out invoices covering 1998 and hope that you will pay the bill as soon as possible in order to save us the time and money involved in sending out reminders. If for some reason you want to end your subscription and/or membership, please return the invoice with your comments.

With our best wishes for a successful coming season, we remain your link to important parts of the scientific world.

Best regards,  
Your editor

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*Translated Report***Reality and Future of Welfare Policy***Leif Lau Jeppesen**University of Copenhagen, Zoological Institute, Tagensvej 16, DK-2200 Copenhagen N*

The Council of Europe has agreed upon a convention for the protection of animals kept for farming purposes. The convention has a standing committee, which is continuously engaged in securing that the purpose of the convention, the protection of domestic animals, is being fulfilled. In 1991, after long time's work, the committee finally passed a set of recommendations for the keeping of fur animals. Member countries are committed to respect these recommendations. Every fifth year the recommendations are revised, hence the standing committee is once again working on recommendations for the keeping of fur animals. On behalf of the fur animal industry (EFBA), I have participated in the work of the standing committee, both in this round and prior to the agreement of the 1991-recommendations. This has given me occasion for the following considerations concerning the professional and political aspects of the welfare debate.

The public has a legitimate interest in knowing and being able to accept the living conditions of farm animals. The acceptance is culturally determined and very haphazardly dependent on momentary political fluctuations. The haphazardness is a problem. The national and supra-national courts for regulating the production of farm animals are a necessary protection against this haphazardness. However, these are also

very expensive organisations, which have the potentials of the entire political system to become self-perpetuating and self-sufficient and to lose touch with reality where production is going on, and where the values of society are being created.

The convention of The Council of Europe's standing committee is no exception. It is a necessary, but also an incredible institution that lives a life of its own, which by no means, in practice, has on its agenda to complete its work. In practice, its purpose is continuous change in the conditions of things, and political pressure in order to reach an agreement on "doing something" seems frightening and professionally unreasonable compared with European mink production, which is fundamentally satisfactory. It is my understanding that the committee has never considered whether a method of production is either good or acceptable, but basically works on the conviction that the conditions constantly can and must be improved.

The committee is now planning to recommend that mink should be kept in larger groups, that they should be weaned later, and that they should have access to swimming water. There is no unambiguous scientific evidence or practical experience, which shows that these changes will benefit the welfare of the mink.

Accordingly, the subjects are probably referred to continued research, which means that the industry is being pressured to pay for such research and simultaneously must live with the fact that these drastic changes might be recommended in 5 or 10 years. Seen in the light of the fact that mink welfare hardly can be any better, and that the mentioned proposal might just as well harm rather than benefit welfare, then it is a rather unreasonable situation for the industry. The situation could be regarded as reasonable if science could reach a final clarification, and if the committee were able to accept such a clarification and aim at a permanently applied recommendation. However, no guarantees can be given for any of these premises, which is what I shall attempt to illustrate in the following.

Unfortunately, the conclusion will be that it seems as though the costs of the political and technical welfare initiatives have come to stay. For a long time to come, they will probably be just as sure as the costs for buildings, feed, and medicine. And they are just as necessary. Because, in spite of the many faults and shortcomings of the institutionalised welfare debate, it is nevertheless more preferable compared with a random, anarchistic development in this area. Among the certain gains from the work in the ongoing series of meetings, one must mention that the present cage sizes seem to be approved of as minimum standards. The perspective for the future development is that they will also be passed on as EU regulations and thereby become legal standards in all countries, including countries that are now working with prohibitions against production of fur.

Professional agreement on the measurement of welfare is possible. It is, however, close to impossible to envisage that research in relation to the standing committee's needs is able to achieve a comprehensive agreement. At least until now it hasn't been the case. The international society for applied ethology, which, like the industry, has the status as an observer with the privilege to speak during the meetings of the standing committee, frequently produces technical "facts". Some are supportable by most

experts, others certainly are not. Arguments that farm mink are wild, frightened, and dangerous animals and that mink have a biological need for swimming do not enjoy great support from the experts. In the following, I shall keep within the discussion of the subjects of swimming water and needs.

It has been demonstrated that mink in their natural habitat live close to water and catch about half of their feed in water. It has also been pointed out that for long periods of time mink can choose to live exclusively on terrestrial animals and avoid seeking water. If swimming were a biological need in itself, they would seek water to swim in. On the farm, mink take time to swim if it is optional, and they work just as hard for access to water as for access to other diversions e.g. new objects or straw. Subsequently, it cannot be denied that swimming water is one among several potential environmental enrichments for farm mink. It can, however, according to my opinion, be discussed whether it is the best, and whether all thinkable enrichments necessarily should be squeezed into the cages. The negative effects of swimming water on farm hygiene, on the economy, and on the surrounding environment also ought to be weighed reasonably. And there is still no reason to believe that mink have a real biological need to swim. Lack of fulfilment of the need for a nesting box, correct weaning and genuine social contact is clearly reflected in reduced welfare. The presence or absence of swimming water, based on existing knowledge, does not have similar consequences for the welfare of the animals. Hence, swimming is not a biological need. Swimming is a way for the mink to move, and a means to get biological needs fulfilled.

Although it might be professionally unsatisfactory, it is still not a poor comparison to compare the biological assistance in the standing committee with the legal advice in a lawsuit. Here both the council for the defendant and the council for the prosecution are professionals, who basically refer to the same set of rules, regulations and technical facts. Thus, as there is no greater reason to hope or expect that

the committee will be confronted with a joint professional accept of some kind of housing system, it is impossible to test my suspicion that this would not necessarily lead to a political agreement either. The coherence between the political result and the professional background is, however, limited:

Results exist which show that groups of two pups, while growing up in the conventional cage system, do not develop stereotypies or do it later than isolated animals or groups of three animals. The reason for this development is assumed to be optimal stimulation in groups of two animals and under- and overstimulation, respectively, in the other groups. This was the background to why the committee in 1991 recommended that mink pups be kept in pairs, because they hereby obtain the most stable conditions. Later on, in Holland, they have experienced a political storm against the industry and without scientific arguments accepted that mink there now shall be kept in larger groups, in larger cages, and with a larger stocking density. On this still clearly political background, the Dutch delegate, at one of the later meetings, pleaded for a change saying that from now on the committee should refer to the fact that groups of animals from the same family obtained the most stable conditions.

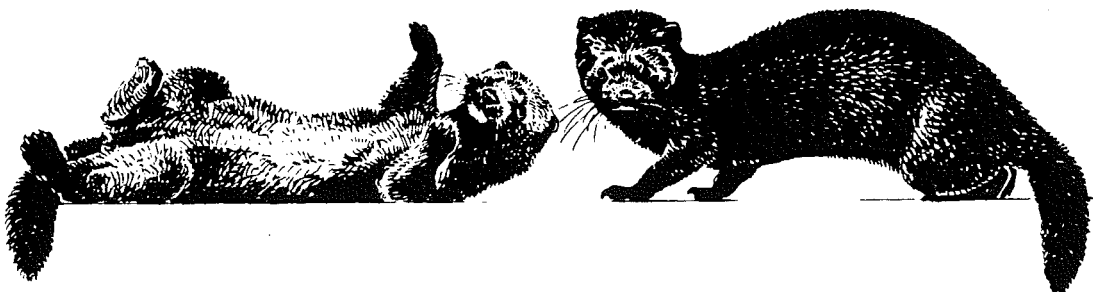
At the meeting this enjoyed the ordinary consent from a great majority of the national representatives: something new has to happen and consensus is good. However, there is still no scientific evidence that family relations has anything to do with social stability, and it is continuously the best recommendation for the

present cage sizes (which the committee has approved of) to raise the pups in groups of two animals.

The question of group size for mink is also more generally of interest to the understanding of the coherence between professionalism and political resolution in the standing committee. On this point, the technical background has not changed over the past 10 years. The political ideas, on the other hand, have changed: Because of the wild mink's solitary way of living it was, until 1991, difficult to gain a hearing for the fact that mink could go closely together, and for the fact that they as adults did not need extra visual separation between the cages. Now, there is as mentioned considerable pressure to introduce larger groups and larger densities.

The pure political interactions between countries are also a barrier against professionalism. Support to agreements that are inconvenient to some countries' production generates failing support from these countries when ones own production is being discussed. This is quite natural, and instead of being indignant I would rather see it as yet another little cost in the great game where we can gain uniform production rules in all member states and lower the degree of haphazardness in the development of welfare policy. Forget that we could do it better and cheaper if we were alone. We aren't.

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



2 International symposiums

**Physiological bases for increasing the productivity of predatory fur animals**

Petrozavodsk  
*September 15-17 1998.*

General biology department  
of RAS

Scientific council of RAS on  
the problems of research,  
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**Physiological bases for in-  
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THE CIRCULAR № 1

Dear colleague

The organizing committee invites you to take part in the second International symposium "PHYSIOLOGICAL BASES FOR INCREASING THE PRODUCTIVITY OF PREDATORY FUR ANIMALS", which will be held in Petrozavodsk on September 15-17, 1998

The following problems will be discussed at the symposium:

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2. *The effect of environmental factors on the organism and optimization of breeding.*
3. *Ways of stimulation of reproduction and development.*

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**The text** of the abstracts should be prepared in the Microsoft Word, or in any other editor for Windows on A4 sheet; margins: left - 25 mm., right - 25 mm., top - 30 mm., bottom - 20 mm; font «Times New Roman», 14 points, title of the abstract - 14 capital letters, line spacing - 1.0 (see example). Pages should be covered with the text from top to bottom and printed on jet or laser printer. The electronic version of the abstracts in ASCII codes can be sent to the address: [ILYUHA@MAZE.CENTRE.KARELIA.RU](mailto:ILYUHA@MAZE.CENTRE.KARELIA.RU)

**The registration fee** (50\$) including registration, conference papers and congress banquet. Registration fees should be paid at registration.

The organizing committee reserves the right to decide on the possibility consider an opportunity of including your report in the program of the symposium. Additional information will be given in the circular № 2.

see further on page 22

**Organizing committee**

*Original Report*

## Different Cage Sizes and Effects on Behaviour and Physiology in Farmed Silver and Blue Foxes

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### Introduction

Farmed foxes have been kept in a relatively stable cage environment for some decades. Recent legislation (Danish Veterinary Council, 1997; European Convention, 1991) demands that foxes are provided with an observation platform and that each growing fox has at least 0.5 m<sup>2</sup>, a breeding vixen 1 m<sup>2</sup>, and a breeding vixen with cubs at 4 weeks of age 2 m<sup>2</sup>. These cage sizes have been used for many years in Danish fox production, whereas the observation platform is a recent improvement. Several ongoing studies in both Denmark (a.o. Pedersen and Jeppesen, 1993) and Finland (a.o. Korhonen and Niemela, 1996; Mononen 1996) have provided evidence that farmed foxes use observation platforms, and especially the Finnish studies have shown that the design of the observation platform is important when evaluating the foxes' preferences (Mononen, 1996). Less impact has been put on the cage sizes or designs for singly kept animals, though these should be considered equally important in our efforts to improve welfare in farmed foxes. For mink it was recently shown that the cage size did not affect welfare parameters whereas the lack of a nest box had a reducing effect on welfare (Hansen and Brandt, 1989;

Hansen and Damgaard, 1991). This result gives some indication that quality of space might be more important for the well being of fur animals than quantity of space, but it remains to be examined in foxes at least.

The present study was performed in order to reveal the impact of different cage sizes on the welfare of farmed silver and blue foxes as reflected by behavioural responses to different levels of encounters with humans and reflected by the foxes' basal plasma cortisol concentrations. The two species were compared regarding all measured parameters.

### Materials and methods

#### *Subjects and housing*

One-hundred female silver fox and 100 blue fox cubs were tested for behavioural responses towards an approaching human in their standard 1-room fox cage (1m x 1.2m x 0.75m, LxWxH) at the age of 4 months. Twelve of the most aggressive and twelve of the most confident vixens of each species were then distributed singly into cages of either 1-, 2-, 4- or 8- rooms (1.2, 2.4, 4.8, 9.6 m<sup>2</sup>, respectively, Fig. 1), so that 3 aggressive and 3 confident foxes were represented in each

cage size. The cage sizes 4 and 8 were made by joining two or four double standard fox cages (2m x 1.2m x 0.75m, LxWxH), respectively. The foxes were kept singly throughout the experiment with one species occupying one fox house.

Feeding took place once a day, and the feed was always placed in a feedtrough in position 2. Water was available ad libitum by an automatic drinking system with a drinker in every even-numbered position of each cage.

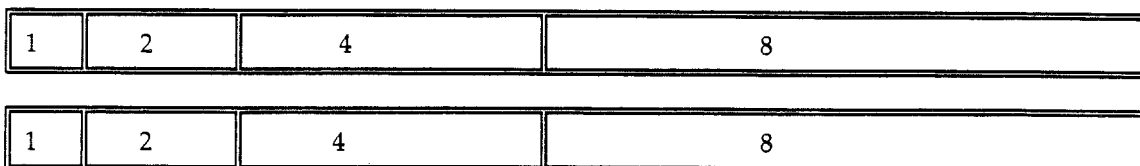


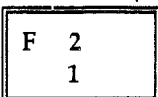
Figure 1. Schematic illustration of the different cage sizes in one fox house. The two rows continue with 2 more sections identical with the shown section, i.e. 3 x 4 cage sizes in each row and 6 x 4 cage sizes in each fox house. The numbers refer to the no. of rooms in each cage.

Scan-samplings

Once a week, six rounds of 10 min instantaneous scan-samplings were performed in the months of September to December, in all 19 times and a total of 114 scan-samplings. The fox's position in the cage in relation to the observer at the begin-

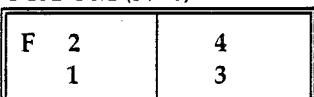
ning of each of the six rounds was recorded with a score system where increasing numbers reflected increasing distance to the observer (1-room=score 1-2, 2-room=score 1-4, 4-room=score 1-8, 8-room=score 1-16, Fig. 2).

1-ROOM (N=6)



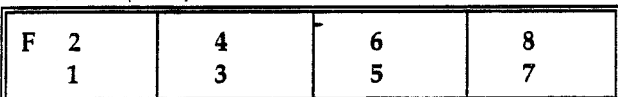
X →

2-ROOM (N=6)



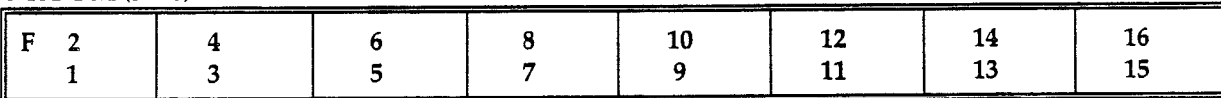
X →

4-ROOM (N=6)



X →

8-ROOM (N=6)



X →

Figure 2. The four different cage sizes (1-, 2-, 4-, 8-ROOM) with indications of the feeding site (F), the position of the observer (X), and the direction of the observations during scan-samplings (→). The numbers 1-16 are the scores that relate to the foxes' distance to the observer at the beginning of each scan sampling. Uneven numbers indicate a short distance to the route of the observer, even numbers the opposite. Automatic drinkers are placed in even-numbered positions.

The foxes' behavioural responses towards the approaching observer during the scan-samplings were recorded as being either 1) defensive/-offensive aggressive (evaluated by ear and body postures, threat displays, attacks and vocalisation as growling, jaw gape and hissing), 2) confident (evaluated by ear and body postures, the fox is sniffing towards and approaching the observer in a confident manner), 3) fearful (evaluated by ear and body postures, e.g. crouching, shivering, withdrawing or fleeing from the observer), or 4) passive (sleeping or sitting or lying awake, but with no obvious other reaction interpretable as belonging to the other 3 behavioural categories). For a more detailed description of the various behavioural elements see e.g. Pedersen and Jeppesen (1990).

#### Blood samplings

After 4 months in the different cage sizes, all foxes were exposed to a bleeding procedure in order to evaluate the concentration of basal plasma cortisol reflecting the general stress-level of the foxes. Each fox was cornered in one room of its cage and removed from the cage by a grip in its tail and using a pair of neck tongs. A 5 ml blood-sample was drawn from the right front leg of the fox and the fox was released into its cage. The blood samples were centrifuged and the supernatant removed and stored at -20°C until analysed by the Kodak-Amerlite-kit. The duration of the capture/bleeding procedure (the time from opening the cage door and until blood was flowing from the right front leg of the fox) was

noted as well as the fox's behaviour during the capture procedure according to the above mentioned behavioural categories.

#### Statistics

Data, which were found to approach a normal distribution, were analysed by a two-way ANOVA comparing cage sizes within species and between species. Frequency data were analysed by the Chi-Square Test or Fisher Exact Test when 25% of the cells contained expected values below 5 (SAS Institute Inc., 1988). Non-parametric data were analysed by the Wilcoxon-Mann Whitney U-test (SAS Institute Inc., 1988).

The initial behavioural responses of the foxes (12 aggressive, 12 confident of each species) were found to have no effect on or correlation to the later measured parameters in any of the species (SAS Institute Inc., 1988). Therefore these responses will not be mentioned further.

#### Results

##### Silver foxes

The behavioural responses of the silver foxes in 4- and 8- room cages changed during the course of the scan-samplings with significantly less flight responses in the sixth round compared to the first round (Table 1). No significant differences were found regarding this parameter in the cage sizes 1 and 2.

Table 1. Mean percentage of silver foxes (SILVER FOX) in four different cage sizes (1-, 2-, 4-, and 8-room cages) responding as either aggressive (AG), confident (CO), fearful (FE), or passive (PA) during the first round (1<sup>st</sup> round) and last round (6<sup>th</sup> round) in 6x19 (total 114) scan-samplings.

	SILVER FOX 1 <sup>ST</sup> ROUND (N=24)				SILVER FOX 6 <sup>TH</sup> ROUND (N=24)				
	AG	CO	FE	PA	AG	CO	FE	PA	P <
1-room cage	47	51	1	1	42	57	0	1	0.5
2-room cage	49	48	3	0	43	49	6	2	0.3
4-room cage	16	63	21	0	32	56	11	1	0.01
8-room cage	7	39	54	0	15	53	31	1	0.003

**Table 2.** Mean percentage of foxes (SILVER FOXES and BLUE FOXES) in four different cage sizes (1, 2-, 4-, and 8-room cages) responding either as aggressive (AG), confident (CO), fearful (FE), or passive (PA) during a total of 114 scan samplings.

	SILVER FOXES (N=24)				BLUE FOXES (N=24)			
	AG	CO	FE	PA	AG	CO	FE	PA
1-room cage	44	55	1	0	20	33	47	0
2-room cage	44	51	5	0	24	56	20	0
4-room cage	26	60	13	1	0	42	57	1
8-room cage	12	49	39	0	0	25	75	0

Statistics between cage size within species and between species within cage size can be found in the text.

The behavioural scores of the silver foxes during the total scan-samplings are shown in table 2. Individuals in 1- and 2-room cages did not differ in behavioural responses ( $P > 0.1$ ) whereas more foxes behaved with aggression in the 1- and 2-room cages compared to the 4-room ( $P < 0.005$ )

and 8-room cages ( $P < 0.0001$ ). In these later cage sizes fearful responses were observed more frequently. In the total scan-samplings, silver foxes mainly placed themselves near the route of the observer at a non-random order between cage sizes ( $P < 0.001$ , Fig. 3)..

**1-ROOM (N=6)**

F	2
	98

X →

**2-ROOM (N=6)**

F	1	1
	47	51

X →

**4-ROOM (N=6)**

F	1	0	2	2
	23	42	22	9

X →

**8-ROOM (N=6)**

F	1	1	0	2	1	1	1	2
	13	28	20	9	6	6	5	4

X →

**Figure 3.** The mean choice of stay in percent during 114 scan-samplings for 24 silver foxes kept in four different cage sizes (1-, 2-, 4-, 8-ROOM). N indicates the number of subjects/cage, 'F' indicates the feeding place for each cage size, 'x' indicates the position of the observer and '→' the direction of the observations during scan-samplings.

Foxes from 1-room cages mainly placed themselves in position 1, close to the observer. Foxes in 2-room cages placed them-selves more frequently in positions 3 and 1 as compared to positions 2 and 4. In 4-room cages, position 3 was again preferred and then positions 1 and 5, and in 8-room cages the same pattern was revealed (positions 3, 5 and then 1 with decreasing preference) The duration of the capture procedure prior to the blood sampling was lower in 1- and 2-room cages as compared to 8-room cages ( $P < 0.05$ , table 3). No other differences were found between cage sizes regarding this parameter. All silver foxes in the 1-room cages showed defensive aggression during the capture procedure (AGGRESSIVE=100%) and thus differed from foxes in the 4-room (AGGRESSIVE=33%; FEARFUL=67%,  $P < 0.01$ ) and 8-room (AGGRESSIVE=17%; FEARFUL=83%,  $P < 0.005$ ) cages. Silver foxes in the 2-room cages showed mainly aggression during capture (AGGRESSIVE=67%; FEARFUL=17%; PASSIVE=17%) and tended to differ from silver foxes in the 8-room cages ( $P = 0.07$ ).

**Table 3.** The mean duration  $\pm$  the standard deviation (STD) of the capture procedure (until blood is flowing) in seconds prior to blood sampling for 24 silver foxes (SILVER FOXES) and 24 blue foxes (BLUE FOXES) kept individually in four different cage sizes (1-, 2-, 4-, 8-room cages).

	SILVER FOXES (N=24)	BLUE FOXES (N=24)
1-room cage	29.3 $\pm$ 9.8ax	42.7 $\pm$ 10.8y
2-room cage	31.5 $\pm$ 12.0a	43.2 $\pm$ 14.4
4-room cage	54.0 $\pm$ 46.1	42.0 $\pm$ 10.9
8-room cage	63.7 $\pm$ 31.5b	48.8 $\pm$ 12.5

Statistical analysis performed by two-way ANOVA between cage sizes and between species. Different letters 'a,b' indicate significant levels below 5% between cage sizes within species and 'x,y' between species within cage size.

The cortisol concentrations are shown in table 4. The plasma cortisol concentrations were affected by sampling order in the sense that the first 2 samplings of each cage size showed a lower concentration of plasma cortisol compared to the next four samplings ( $P < 0.05$ ). The capture duration and behaviour during capture did not affect the cortisol concentrations ( $P > 0.1$ ) Generally, the plasma cortisol concentrations were high (range 18 to 324 nmol/l) and therefore concluded not to represent true base levels. Earlier studies have revealed that base levels of plasma cortisol in silver foxes lie within the range of 25 to 85 nmol/l (Jeppesen and Pedersen, 1991). Since there was no effect of the capture duration it is believed that the cortisol measurements reflect a general level of acute stress caused by the presence of the blood sampling team in the fox house. Different cage sizes had no significant influence on the concentration of plasma cortisol in the blood ( $P > 0.5$ ).

**Table 4.** Mean plasma cortisol concentration  $\pm$  STD in nmol/l in 24 silver foxes (SILVER FOXES) and 24 blue foxes (BLUE FOXES) kept individually in four different cage sizes (1-, 2-, 4-, 8- room cages).

	SILVER FOXES (N=24)	BLUE FOXES (N=24)
1-room cage	186.6 $\pm$ 103.9	169.2 $\pm$ 72.9
2-room cage	206.8 $\pm$ 109.9	138.8 $\pm$ 46.2
4-room cage	213.8 $\pm$ 88.3	150.2 $\pm$ 52.5
8-room cage	193.3 $\pm$ 58.6a	110.3 $\pm$ 54.4b
All cages	200.2 $\pm$ 86.8a	142.1 $\pm$ 57.7b

Statistical analysis performed by two-way ANOVA between cage sizes and between species. Different letters (a,b) indicate significant differences between species below 5% level.

#### Blue foxes

During the course of scan-samplings no significant change in behavioural responses of the blue foxes was observed (Table 5).

**Table 5.** Mean percentage of blue foxes (BLUE FOXES) in four different cage sizes (1-, 2-, 4-, and 8-room cages) responding as either aggressive (AG), confident (CO), fearful (FE), or passive (PA) during the mean first round (1<sup>st</sup> round) and mean last round (6<sup>th</sup> round) of 6x19 (total 114) scan-samplings.

	BLUE FOXES 1 <sup>ST</sup> ROUND (N=24)				BLUE FOXES 6 <sup>TH</sup> ROUND (N=24)				
	AG	CO	FE	PA	AG	CO	FE	PA	P <
1-room cage	17	32	28	23	18	32	22	27	0.70
2-room cage	24	55	13	8	24	58	13	5	0.95
4-room cage	0	42	58	0	0	43	54	3	0.21
8-room cage	1	24	75	0	0	28	72	0	0.47

Behavioural scores during scan-samplings were not randomly distributed concerning different cage sizes ( $P < 0.0001$ , Table 2). Two-way comparisons between cage sizes revealed more frequent fearful responses in the 1-room cages compared to 2-room cages ( $P < 0.0001$ ), and more frequent aggressive responses in the 1- and 2-room cages compared to the 4- and 8-room cages ( $P < 0.0001$ ) which in turn had a higher frequency of fearful responses. In the 4-room cages the foxes showed confident responses more frequently than foxes in the 8-room cages where fearful responses predominated ( $P < 0.001$ ).

In larger cage sizes (2-, 4-, and 8-room), blue foxes mainly placed themselves near the route of the observer but with some distance during scan-samplings and at a non-random order ( $P < 0.001$ , Fig. 4). Foxes in 1-room cages were as often observed in position 2 as in position 1. Foxes in the 2-room cages placed themselves most frequently in position 3. In the 4-room cages, position 5 was preferred and then positions 7 and 3. Position 5 was also preferred in the 8-room cages then positions 9, 7, 3 and 11 with decreasing preference.

Prior to blood sampling, the duration of the capture procedure was not affected by the differ-

ent cage sizes ( $P > 0.1$ , table 3). Blue foxes reacted predominantly with defensive aggression in the 1-room cages during this capture procedure (AGGRESSIVE=67%; PASSIVE=33%) and differed from individuals in the 2-room cages ( $P < 0.05$ ), which reacted mainly with fear responses (AGGRESSIVE=17%; FEARFUL=67%; PASSIVE=17%). In the 4-room cages, 50% of the foxes were aggressive, 17% were fearful and 33% were passive during capture, and in the 8-room cages the distribution of aggressive, fearful and passive foxes was 17%, 50% and 33%, respectively. Comparisons between the cage sizes 2-4, 2-8, 1-4, 1-8, and 4-8 in behavioural responses to the capture procedure revealed no significant differences ( $P > 0.1$ ).

The sampling order affected the concentration of plasma cortisol in the blood ( $P < 0.05$ , same pattern as in silver foxes) but capture time and behaviour did not ( $P > 0.1$ ). Generally, the plasma cortisol concentrations were high (range 50 to 241 nmol/l), and, as in silver foxes, concluded not to represent true base levels. Earlier studies have revealed base levels of plasma cortisol in blue foxes within the range of 30 to 85 nmol/l (Pedersen and Skovgaard, 1995). No significant differences between cage sizes in plasma cortisol concentrations were found in blue foxes ( $P > 0.1$ , table 4).

## 1-ROOM (N=6)

F	41
	58

x →

## 2-ROOM (N=6)

F	5	6
	33	56

x →

## 4-ROOM (N=6)

F	3	1	3	0
	1	14	54	22

x →

## 8-ROOM (N=6)

F	1	1	1	18	1	2	0	1
	5	12	20	18	18	11	6	5

x →

**Figure 4.** The mean choice of stay in percent during 114 scan-samplings for 24 blue foxes kept in four different cage sizes (1-, 2-, 4-, 8-ROOM). N indicates number of subjects/cages, 'F' indicates the feeding place for each cage size, 'X' indicates the position of the observer and '→' the direction of the observations during scan samplings.

### Comparison of species

Silver foxes were observed as being confident on more occasions than blue foxes in the first rounds of scan-samplings regardless of the cage size ( $P < 0.001$ ) and this difference was also observed in the last rounds of scan-samplings ( $P < 0.0001$ , Table 1 and 5), regardless of the cage size.

This result was also reflected in the total scan-samplings where more blue foxes showed fear responses in all four cage sizes compared to silver foxes which in turn showed either aggressive or confident responses ( $P < 0.001$ , all comparisons, Table 2).

During scan-samplings, blue foxes in 1-room cages were observed more frequently away from the observer compared to silver foxes ( $P < 0.001$ , Fig. 3/5). Silver and blue foxes did not differ in frequency of stays in the different positions in

the 2-room cages ( $P > 0.1$ ). Again, in both the 4-room and 8-room cages, blue foxes were observed more frequently further away from the observer compared to silver foxes ( $P < 0.001$ , both cage sizes).

The duration of the capture procedure prior to blood sampling was lower in silver foxes in the 1-room cages compared to blue foxes ( $P < 0.05$ , table 3). No other differences were found between species regarding this parameter ( $P > 0.1$ ). Behavioural responses to being captured did not differ between species from the same cage size ( $P > 0.1$ ).

The total (all cage sizes together) mean concentration of plasma cortisol in silver foxes was higher than in blue foxes ( $P < 0.01$ , table 4). When each cage size was compared between species, silver foxes showed a higher cortisol concentration in the 8-room cages ( $P < 0.05$ , table 4).

## Discussion

### *Silver foxes*

The study showed that by increasing cage sizes the level of flight responses increased and aggression decreased both towards the observer passing by, and towards being captured by humans. The proportion of confident foxes was fairly consistent (appr. 50%) and not affected by cage size. In the small cages, the foxes mainly responded with defensive aggression in both types of interactions with humans and this might be due to limited escape possibilities. Initially, the foxes in the larger cage sizes kept a slightly greater distance to the observer and only a few individuals responded with defensive aggression and twice as many with flight responses.

Fear towards humans is considered to be indicative of impaired welfare in husbandry animals. Therefore, it seems immediately as if the large cage sizes are not solutions for improving welfare in farmed foxes, at least on a short-term basis. But when single scan-samplings were compared in the present study, it was found that the foxes habituated to the presence of the observer during the course of the study; fear responses decreased. The larger cages might have given the fox some sense of control since it was able to flee from human presence, but when repeatedly exposed to humans, confidence overshadowed fear and the fox approached, knowing that it could flee if needed. Sense of control is important to animal welfare (Broom and Johnson, 1993). It could be argued that defensive aggression (as observed in the smaller cages) was as much an indicator of impaired welfare as fearfulness since this behaviour is often expressed when an animal is cornered and escape is not possible i.e. lack of control.

The duration of the capture procedure was lowest in the smallest cages, which showed that foxes are easier to handle in smaller cages. In this respect smaller cages are advantageous to the foxes. The plasma cortisol levels did not point on any cage size being better or worse regarding welfare, but might have reflected a general level of acute stress caused by the blood-sampling team, as stated earlier.

### *Blue foxes*

Defensive aggression was only observed in the smaller cage sizes and blue foxes in 2-room cages were the most aggressive and the most confident at human exposure whereas half or more of the animals were fearful in the other cage sizes. This fact could be interpreted in the direction that 2-room cages were better for blue foxes than both 1-, 4- and 8-room cages, since fear was reduced in this cage size. In blue foxes, no habituation to the presence of the human observer was found during the course of the experiment, which might indicate a general low motivation to socialise with humans. Blue foxes preferred to keep some distance between themselves and an approaching human if the cage size was larger than a 1-room cage, but they did not prefer the maximum distance available in the largest cages. They chose a position in between, having some distance to the observer and still being able to watch what was going on.

Different cage sizes seemed not to affect the duration of capture of blue foxes, which indicates that a larger cage size causes no practical problem when handling is necessary. The mean level of plasma cortisol decreased with increasing cage size which might indicate that the influence of the blood sampling team is less stressing when distance is great. This result was, however, not significant and consequently no physiological indication on improved or impaired welfare in different cage sizes was found in blue foxes.

### Comparison of species

Roughly, more than half of the silver foxes and less than 40% of the blue foxes reacted with confident responses in the scan-samplings, which might indicate a different level of adaptation to captivity in the two species or a species-specific difference in the motivation to socialise with humans. Both explanations could be supported by the fact that the blue foxes preferred a greater distance to humans in most cage sizes compared to silver foxes, and blue foxes more frequently reacted with flight or crouching, whereas silver foxes reacted with confidence or aggression. Last but not least blue foxes did not habituate to the

presence of humans during the course of the study, as silver foxes did in large cages. In an earlier study it was found that blue foxes would hide more often in a whole-year shelter than silver foxes during daytime disturbances (Pedersen and Jeppesen, 1993) supporting the findings of a general higher fear level in blue foxes concerning human proximity in the present study. In general, silver foxes have been regarded as the less adapted species, mainly due to its dynamic and active behavioural patterns. But regardless of the blue foxes passive and inactive behavioural patterns, the study of Pedersen and Jeppesen (1993) and the present study indicate that blue foxes might have difficulties in adapting to human presence and more so than silver foxes.

The capture procedure was extended in blue foxes compared to the same procedure in silver foxes, but this can be explained by the fact that it was harder to find a blood vessel to bleed from in blue foxes compared to silver foxes. Unfortunately, capture time was measured as the time passing from opening of the cage door and until blood flow, so the above statement cannot be confirmed in this study.

Silver foxes showed a higher total level of plasma cortisol than blue foxes. This might indicate a species-specific difference in physiological reactions to human presence. Any conclusion on relation between these levels and differences in experienced levels of stress in the two species is premature.

### Conclusion

This study revealed no solutions for improving welfare of farmed silver and blue foxes on the behavioural or physiological level by increasing the cage size from 1.2 m<sup>2</sup> up to 9.8m<sup>2</sup>. However, some indications were found that 2-room cages (2.2m<sup>2</sup>) reduced fear and increased confidence in blue foxes. Increasing space quantity induced fearfulness in both species and it should be taken into account that domestic animals need to be exposed to humans in order to habituate to them and that too much space might prevent adaptation to human disturbances, especially in species

where motivation to socialise to humans is low or fear of humans is high.

Detailed behavioural and physiological studies on a long-term basis are needed in order to confirm or affirm that more space would give some sense of control (improving welfare) and less space would induce lack of control (reducing welfare) as discussed in the present study. Behaviour without human presence should also be monitored in further studies and they should be performed on a long-term basis with the inclusion of reproductive measures and some interpretable physiological measures in order to conclude upon welfare and species-specific differences related to space availability and on a more general level.

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*Original Report*

## Effect of increased cage length on locomotor activity of juvenile blue foxes (*Alopex lagopus*)

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### Abstract

The present study aimed at clarifying to which extent doubling the cage length from 120 cm to 240 cm affects the amount and duration of locomotor activity in farm-raised blue foxes (*Alopex lagopus*). The experiments were carried out during October-December under conventional shed cage conditions. Experimental animals were juvenile male blue foxes (N=8) born in May. The experimental set-up employed a construction in which the foxes spent the first 2 weeks in a small cage measuring 120 cm long x 105 cm wide x 70 cm high. Thereafter, cage length was enlarged to 240 cm for another two weeks. The locomotor activity of the foxes was video recorded by the continuous recording method. Statistical analyses were based on the mixed-model approach to the repeated measurements. The results showed that the foxes' locomotor activity remained the same despite enlargement of cage space. Nor did the duration of activity change with increasing cage size. The mean length of activity was in each cage option rather short. The minimum required space for the fox is not only its body dimensions, but should also include possibilities to exercise adequately. Before it is possible to make final conclusions on the actual

space needs of farmed foxes; further studies on a larger space than a traditional shed can hold are necessary.

### Introduction

Animals have an apparent need for movement and they try to exercise frequently (Fraser & Broom, 1997). Farm animals are housed in confined conditions which are not necessarily large. Thus, there can be a justified doubt whether such conditions are sufficient in size to satisfy animals' locomotor needs or not (Hetts et al., 1992). Recently, traditional fox farming has dramatically faced this problem as a result of increasing public criticism against foxes' housing in small-sized cages (Bakken et al., 1994; Korhonen & Niemelä, 1997). The Standing Committee of European Convention on the Protection of Animals Kept for Farming Purposes (1991) has also emphasised that the housing environment of farm foxes should be developed to better enhance animal welfare. Actually very little is known about physical space requirements of farmed foxes. The current recommendations concerning cage size for commercial foxes are based on practical experience but not on scientific results. Some preliminary data are available comparing fox activity in

shed cages and in large ground floor enclosures. These data suggest that foxes are more active in enclosures (Korhonen, 1994; Korhonen & Alasuutari, 1994).

## Materials and methods

### *Animals and general management procedures*

The present study was carried out at the Fur Farming Research Station of Kannus, in western Finland (63.54 N, 23.54 E), during October–December, 1996. The subjects were 8 juvenile male blue foxes born in May 1996. They were randomly selected from the farm stock of 350 juvenile males. None of experimental animals were siblings. Before the experiments, they were housed singly in conventional farm cages (120 cm long x 105 cm wide x 70 cm high) in two-row sheds. Freshly-mixed fox feed manufactured by the local feed kitchen (Kannus Minkinrehu Ltd.) was supplied once a day (at 1 p.m.) from a feed machine. Feed portions were about 600 g/animal/day. The feed was mainly composed of slaughterhouse offal, fish and cereals (Berg, 1986). Water was freely available from water cups. All animals remained healthy throughout the study.

### *Experimental set-up*

Eight similar test cages were built up. They were placed in the same two-row shed, one after one in the left row. The right row was left open for video recording equipments. The experimental set-up employed a novel construction in which each individual animal spent the first 2 weeks in a conventional small cage size (option A; measuring 120 cm long x 105 cm wide x 70 cm high). Thereafter, cage size was enlarged from a length of 120 to 240 cm for a further 2 weeks (option B). This is the maximum length that a conventional farm shed can hold (Korhonen & Harri, 1988). The experimental wire-mesh cages were located 80 cm above ground level.

### *Video recordings and analyses*

The behaviour of eight test blue foxes, each in a similar test cage, was video-recorded simultaneously throughout the study. The recordings

ran non-stop 24-h all the time (a continuous recording method: Korhonen *et al.*, 1996; Fraser & Broom, 1997). The video system consisted of eight black and white video cameras (Computer FC 55) with wide angle lenses, two guads (Computar QS-MX) enabling each simultaneous recording from four cameras to a time-lapse video-recorder (Hitachi VT-L2000E) and two black and white monitors (Computar CEM-12). Videograms were recorded at a frequency of 1.25 per second. During the dark hours each cage was lit with two dim red lights (Philips E27ES, 60 W). The experiment started on Friday at noon when the experimental animals were placed into the smaller test cages. Test animals were accustomed to the test environment until Monday morning (9 a.m.) when the actual recordings started. The video recordings ended on the following Saturday morning at 9 p.m. (including 5 days' continuous recordings) and omitted until next Monday morning (9 a.m.) when recordings again started for the next following 5 days. Thus, each animal was video-recorded 10 working days in each cage alternative. Weekends were omitted because no employers were present on the farm then. Enlargement of cage size was made after a 2 week period on Saturday morning at 9 a.m. Thus, the animals were allowed to use a larger cage option over weekend.

The videotapes were analysed using a video tape recorder (JVC videocassette recorder HR-D560E) and a TV monitor (Philips). Analyses was made by two female investigators using the continuous analysis method (Martin & Bateson, 1986). The following behaviours were analysed: (1) locomotor activity (min/24 h), and (2) duration of active bout (min). The shortest bout of behaviour analysed was 1 min.

### *Statistical methods*

For each fox there were repeated measurements in two different cages. Any two observations for a given fox were correlated. This correlation was taken into account on selected models. The covariance structure of the repeated measurements was chosen by comparing several potential structures by Akaike's in-

formation criterion and Schwarz's Bayesian criterion. Analysis of activity level was based on the following model:

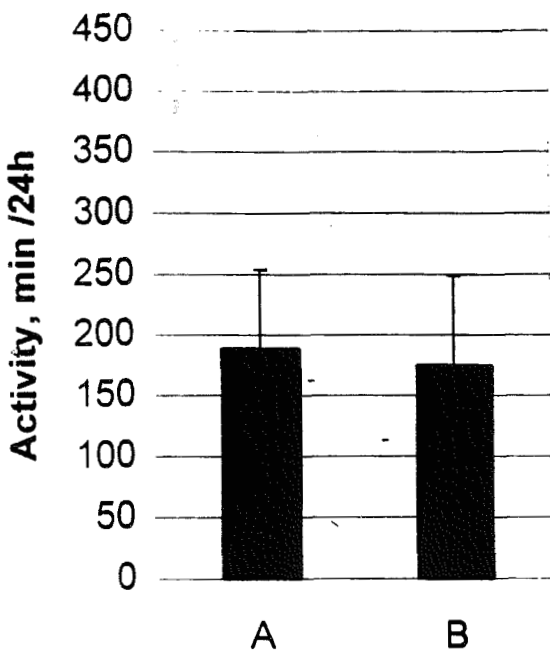
$$Y_{ij} = \mu + \rho_i + \tau_j + e_{ij}$$

where  $Y_{ij}$  is the locomotor activity for the fox in cage  $j$ ,  $\mu$  is a constant,  $\rho$  is random effect of fox,  $\tau$  is fixed effect of cage and  $e_{ij}$  is error. The model assumes the following:  $\rho_i$  are independent of  $N(0, \sigma_p^2)$ ,  $\sum \tau_j = 0$ ,  $e_{ij}$  are independent of  $N(0, \sigma^2)$ ,  $\rho_i$  and  $e_{ij}$  are independent. The model is equivalent to the standard model of the randomised complete block design where the covariance structure is compound symmetry. Assumptions of the models were checked using graphical methods: box-plot for normality of errors and plots of studentized residuals against fitted values for constancy of error variance (Neter *et al.*, 1996). It was also made sure that a difference between the persons who analysed the videotapes was not significant.

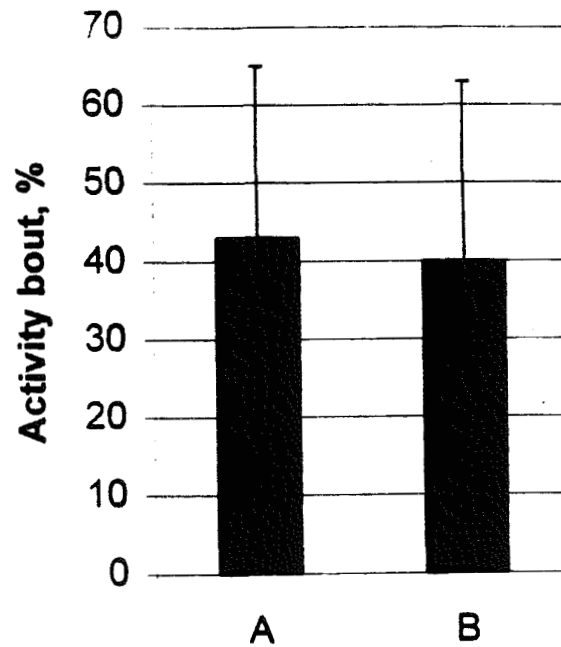
The parameters of the models were estimated by restricted maximum likelihood (REML) estimation method.

The duration of recorded active bouts of each fox was divided into three categories: short (1-2 min), medium (3-6 min) and long (>7 min). The categories are marked as S, M and L, respectively. The sums of percentages of these three groups is 100% for a given fox and cage. This kind of data is called compositional data and it were analysed according to Aitchison (1986) by defining two logarithms:  $\log(S/L)$  and  $\log(M/L)$ . Ternary-diagrams were drawn for ensure conclusions of analysis.

The analyses were performed using the SAS system for Windows release 6.12 using the following procedures: MIXED, GLM (SAS 1996), UNIVARIATE (SAS 1990a) and GPLOT (SAS 1990b).



Cage option



Cage option

Fig. 1. Left: Comparison of locomotor activity (min/24 h) in options A (120 cm long x 105 cm wide) and B (240 cm long x 105 cm wide). Data are presented as least square means  $\pm$  SD. N=8 juvenile male blue foxes for each option. Right: Percentage of activity bouts which lasted 3 min or longer (least square means  $\pm$  SD). (N=8).

## Results

An initial examination of the experimental data revealed a clear variation in the amount of activity between individuals but only a slight difference between study days. A comparison of locomotor activity between cage options A and B is shown in fig. 1. Mean daily activity in the smaller cage (option A) was  $205 \pm 68$  min/24 h and in the larger cage (option B)  $187 \pm 67$  min/24 h. The difference between these options was not statistically significant ( $F=2.23$ ,  $p=0.15$ ).

The duration of activity was typically rather short (fig. 1). Almost half of the activity lasted for 1 or 2 minutes. In addition, data analyses revealed that 75% of the bouts were no longer than 5-6 minutes in length. Bout length in option A did not differ from that in option B ( $F=1.72$ ,  $p=0.20$ ).

## Discussion

Farm foxes were previously housed in wire-mesh cages measuring 80 cm long x 105 cm wide x 70 cm high. Nowadays, however, farmers are recommended to use larger cages if possible. As a matter of fact, most foxes are now kept in shed cages, which are 120 cm in length. A third possibility would be to increase the cage length to the maximum that a traditional shed can hold, i.e. to 240 cm in length. Such maximum size-cages are not in general use on fur farms. The present study attempted to clarify if enlargement of conventional cage length to maximum size affects locomotor activity of foxes. Measurements of activity in this study included both quality and quantity aspects (Wiegand *et al.*, 1994).

A simple geometric calculation is a logical first step when estimating minimal space needs of farm foxes (Tennesen, 1989). The blue fox normally weighs 7-9 kg, its body length without tail is about 70 cm and height from floor to top of head is 40 cm. These measures are less than the dimensions of a cage. Secondly, the size of a fox cage should be so large that it enables nor-

mal body movements. If the size of a farm cage is too small, it does not enable the foxes to move or turn around normally, but forces them to walk with very short bouts. Thus, duration of an activity bout can be considered as one quality criterion of movement. The present results revealed that bout length did not increase with increasing cage size. This lets us suppose that also a smaller cage might fulfil the minimal space needs of foxes. On the other hand, mean bout length in each cage option was not very long. This might be an indication of certain difficulties to exercise in a normal way. The problem here is that there is no data available on bout length of foxes housed in larger spaces like enclosures. Before this data is available we actually cannot make final conclusions on sufficient cage space in foxes.

The present results did not confirm the basic hypothesis that enlargement of blue fox cage size from a length of 120 to 240 cm increases the quantity of locomotor activity. This result can be interpreted by the following means: (1) the smaller cage size already provides foxes adequate possibility to exercise and foxes thus do not need a larger space for exercise. Similarly, in other similar-sized canids like laboratory dogs and raccoon dogs, a moderate increase of cage size from the minimum standard has not increased the activity of these animals (Hite *et al.*, 1977; Korhonen & Harri, 1988; Hughes *et al.*, 1989). However, these results do not necessarily reject the hypothesis that dog-sized canids need larger space (Hetts *et al.*, 1992). As a matter of fact, there can be also the second explanation (2): even the larger space was too small to enable an appropriate level of exercise. Actually the latter conclusion is supported by preliminary observations from large ground enclosures showing that blue foxes seems to move more in enclosures than in cages (Korhonen, 1994; Korhonen & Alasuutari, 1994). However, proving (or disproving) the latter conclusion requires further studies in much larger housing space than presently used. Conclusions, particularly regarding wellbeing, should not be based solely on measures of activity, but other welfare variables must be further studied.

### Acknowledgements

This study was a part of a comprehensive Finnish co-project entitled "Alternative housing environment for farmed foxes". Great thanks to Mrs. Tiina Huuki, Mrs. Elisa Tavasti and Mrs. Tellervo Suikkola for analysing the video tapes and to Mr. Pekka Siirilä for computer assistance. Thanks to Mr. Pekka Eskeli and Mr. Jaakko Huuki for video recordings and Mr. Pekka Toikkanen, Mr. Aimo Joki-Huuki and Mr. Terho Lindqvist for care of the animals.

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1. To promote the knowledge of all aspects of fur animal science and the fur industry.
2. To act as a formal link between scientists, Fur Breeders Associations and government agencies on an international level.
3. To be responsible for arranging international fur animal congresses and other international meetings within the field of fur animal science.
4. To cooperate with other international organizations to achieve these aims.

To reach these goals, a board has been elected by the council, and 5 working groups have been established. These working groups are responsible for the scientific cooperation within their specific fields which are:

1. Breeding, reproduction, genetics.
2. Nutrition and nutritional physiology.
3. Pathology and diseases.
4. Behaviour and welfare.
5. Fur-properties.

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*Short communication*

## Activity and digging motivation of farm foxes with ground exposure

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### Introduction

Housing arrangements of farmed blue foxes (*Alopex lagopus*) have been widely criticized recently. Firstly, because the cage floor is constructed of wire-mesh and is therefore considered harmful. Secondly, it is thought that foxes housed within barren wire-mesh cages cannot adequately satisfy all their behavioural needs. For example, wild foxes are terrestrial animals which are known to dig and occupy subterranean burrows (MacDonald, 1988; Meia & Weber, 1993). On farms, digging on wire-mesh floor is difficult. An obvious welfare enhancement would be to provide farm foxes with access to an earthen floor which would enable better opportunities to perform species-specific behaviour like digging (European Convention, 1991).

The present study employed an experimental set-up in which foxes simultaneously had free access to wire-mesh and earthen floors (Korhonen & Niemelä, 1997). The aim was (1) to study foxes' preference for these two floor sections, and (2) to evaluate their digging motivation.

### Materials and methods

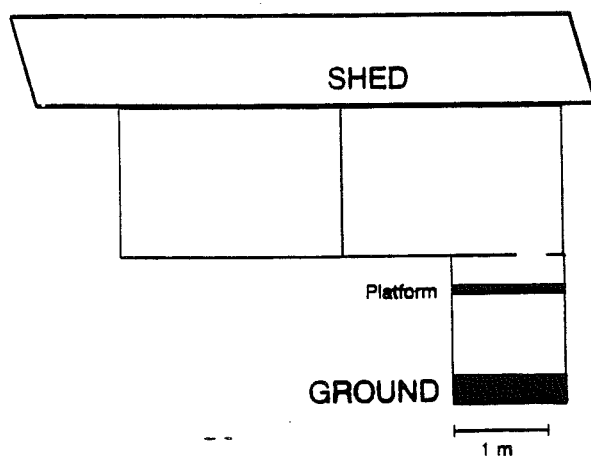
#### *Subjects and general management procedures*

The present experiment was carried out at the Fur Farming Research Station of Kannus, between November-December, 1996. The subjects were 8 juvenile male blue foxes born in May 1996. All experimental animals were randomly selected from the farm stock of about 350 juvenile males. Before the experiments began all experimental animals were housed in pairs in conventional farm cages (120 cm long x 105 cm wide x 70 cm high). None of the foxes had any previous experience with an earthen surface. Fresh-mixed fox feed manufactured by the local feed kitchen was supplied once a day. Feed portions were about 650 g/animal daily. Water was freely available from water cups.

#### *Experimental set-up*

Eight similar test cages were employed. The cages were placed in the same two-row shed, one after the other in the left row. The right row was left free to accommodate the video equipment. The present experimental set-up

employed a novel design in which each animal spent the first 2 weeks in wire-mesh floored cages measuring 240 cm long x 104 cm wide x 70 cm high (option A). The cages were located 80 cm above the ground in a conventional shed. After 2 weeks, the animals were also given free access to an earthen level cage (120 cm long x 105 cm wide x 80 cm high) located on the ground directly below the experimental wire-mesh cages (option B). The earthen cage had an earthen floor (fig. 1). Wire-mesh was placed at a depth of 20 cm under the ground to prevent the foxes from escaping. The earthen cage also contained a wire-mesh platform (25 cm wide x 110 cm long) situated about 25 cm below the shed cage floor. This platform allowed the foxes easy access from the shed cage to the ground cage and *vice versa*.



**Fig. 1.** Schematic diagram of the experimental set-up. First, foxes were housed in a large wire-mesh shed cage measuring 240 cm long x 105 cm wide x 70 cm high. Thereafter, an opening was made in the cage floor, allowing the foxes free access to the earthen floor cage (120 cm long x 105 cm wide x 70 cm high) situated on ground level.

### Recordings and analyses

The behaviour of the test blue foxes was video-recorded. The recordings ran non-stop over a 24-h period (Fraser & Broom, 1997) by a video system comprising eight black andwhite video

cameras (Computar FC 55) equipped with wide angle lenses, two quads (Computar QS-MX) each enabling simultaneous recording from four cameras into a time-lapse video-recorder (Hitachi VT-L2000E) and two black-white monitors (Computar CEM-12). Videograms were recorded at a frequency of 1.25 per second. During darkness each cage was lit with two dim red lights (Philips E27ES, 60 W). The experiment started on Friday at noon when the experimental animals were individually placed into the shed cages. The test animals were allowed to become accustomed to the test environment until Monday morning (9 a.m.) when the actual recordings were begun. Video-recordings were ended at 9 p.m. on Saturday morning of the same week (including the 5 days' continuous recordings) and omitted until next Monday morning (9 a.m.) when recordings were resumed for the next 5 days. Thus, each animal was video recorded 10 workdays in each test cage option. Weekends were omitted because no employees were present on the farm then. Enlargement of the cage size was made after the 2 week period on Saturday morning at 9 a.m. Thus, the animals became accustomed to a larger cage option over the weekend. Videotapes were analysed using a video tape recorder (JVC video cassette recorder HR-D560E) and a TV monitor (Philips). The following behaviours were analysed: (1) time spent on wire-mesh and earthen sections, and (2) time spent digging.

### Results

Activity of the foxes when access to the earthen floor was obstructed (option A) averaged  $187 \pm 67$  min/24 h (mean  $\pm$  SD). After providing foxes access to the earthen floor (option B), activity averaged  $183 \pm 67$  min/24 h. Preferences of experimental foxes for earthen and shed cage floors were evaluated in option B. Foxes spent time on the wire-mesh section 127 min/24 h and on the earthen section 56 min/24 h (fig. 2). Total time spent on wire-mesh and earthen sections was 1367 min/24 h and 72 min/h, respectively (fig. 2). All foxes, except fox number 4 were observed to visit the earthen floor. Visits to the ground level were made

daily. Besides moving, foxes were occasionally seen resting on the ground level. However, resting frequency was low, and foxes numbers 4 and 8 did not rest on the earthen floor at all. As concerns activity, the foxes spent on average 68.8% and 30.5% of their active time on the wire-mesh and earthen floor levels, respectively. In addition, 0.7% of the time was used for rapid activity to-and-fro between the shed and earthen levels.

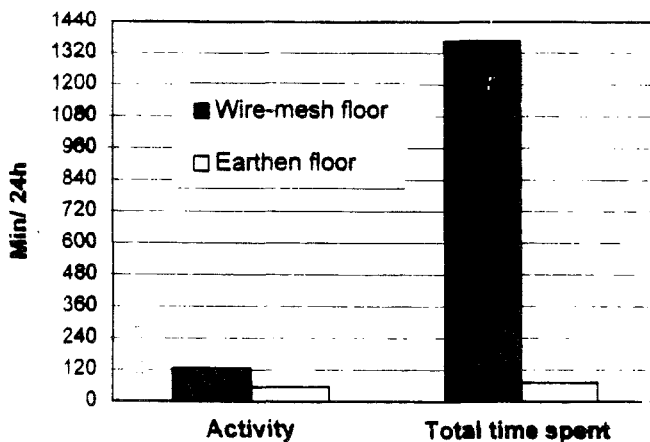


Fig. 2. Comparison of activity and total time spent between wire-mesh floor (at a higher level in shed cage) and earthen floor (on the ground).

The foxes did not dig at all in option A. After providing foxes free access to the earthen level (option B), only foxes number 2 and 6 dug during the 2 week period. Fox number 2 spent altogether 145 min digging divided among three different days (days 3,4 and 8). Fox number 6 was observed digging on two different days (total time 29 min; days 1 and 6).

### Conclusions

The present results revealed that foxes spent only a few hours per day on the earthen floor section. Thus, they strongly preferred the wire-mesh floor. There are at least two explanations for this result: (1) the foxes had earlier been accustomed to living on a wire-mesh floor and thus had no actual need for an earthen floor; (2)

because the exposure time on the earthen section was no longer than two weeks, the foxes did not learn to live on the earthen floor.

It can be assumed that if digging is a crucial need of farm foxes, access to the earthen surface will highly motivate the foxes to dig (Korhonen & Niemelä, 1997). This hypothesis is based on the theory that if an animal has not had the opportunity to perform an innate behavioural need for an extended period of time, the drive to perform that behaviour becomes more intense or "dams-up" (Lorenz, 1981). However, digging motivation of foxes in the present study was very slight. Reasons for this finding may be: (1) the slight need of digging behaviour; or (2) the fact that foxes did not learn to use earthen material for digging because of short exposure time. It is obvious that further studies with longer exposure times on earthen material must be conducted.

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### Ultrastructural and immunocytochemical studies of prolactin-secreting cells in adeno-hypophysis of the mink (*Mustela vison*)

Sergio Vidal, Matilde Lombardero, Lucas Moya

This investigation aimed to identify, by the double immunogold procedure, the ultrastructural characteristics of prolactin (PRL) cells in the mink. Such cells showed a marked pleomorphism and had a close topographic relationship with growth hormone cells. A common morphological characteristic of PRL cells in all stages of mink development was the presence of round secretory granules, in contrast to changes in the ultrastructural characteristics of PRL cells with physiological state and photoperiod. Thus PRL cells in prepubertal, pubertal, and sexually inactive adult mink, killed under a short-day photoperiod, showed little development of the organelles but a significantly increased cytoplasmic electron density. In sexually active mink and in lactating females under long-day conditions, PRL cells had a highly developed cytoplasmic organelle structure consisting mainly of rough endoplasmic reticulum. The morphometric study demonstrated that the mean diameter of the secretory granules similarly varied in both sexes. Pubertal mink had PRL cells with smaller secretory granules (female  $74.1 \pm 0.6$  nm, male  $80.4 \pm 1.7$  nm), whereas adult mink killed under a long-day photoperiod presented PRL cells with larger secretory granules (female  $194.5 \pm 2.2$  nm, male  $203.3 \pm 1.7$  nm). The changes in the ultrastructural characteristics of PRL cells during the annual cycle suggest a photoperiodic influence upon these cells. In addition the heterogeneity in ultrastructural characteristics and storage characteristics of PRL in some adult mink may suggest a varying metabolic role for PRL under certain, as yet not fully characterised, conditions.

*General and Comparative Endocrinology* 197, 311-321, 1997. 1 table, 8 figs., 50 refs. Authors' summary.

### Choices of farm foxes for raised wire mesh cage and ground pen

Hannu Korhonen, Paavo Niemelä

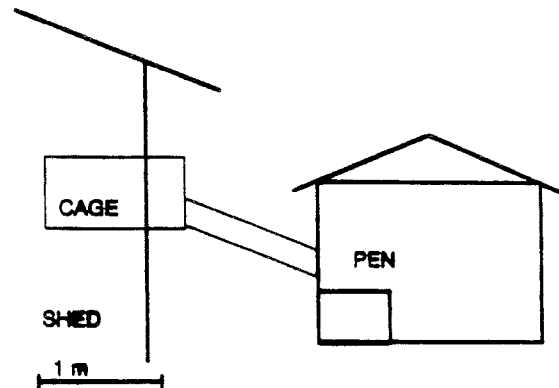


Fig. 1. Schematic picture of the cage-pen test set-up from a lateral perspective. A wire mesh tunnel connected the standard shed cage to the ground pen. Inside the pen was also a wooden box from the roof of which the fox could reach the tunnel.

Time-budget distribution for a raised wire mesh cage and a ground-level solid-floored pen were assessed in male silver foxes (*Vulpes vulpes*) (N=8 adults) and blue foxes (*Alopex lagopus*) (N=10 adults and N=10 juveniles) in a combined cage-pen housing arrangement employing 24 h infrared activity detectors and video recordings. The experimental set-up comprised a ground floor pen (2 m wide x 4 m long x 1.5 m high) equipped with a 1 m long wire mesh tunnel (diameter 30 cm) which was connected to the standard shed cage (110 cm long x 107 cm wide x 60 cm high). Time spent for locomotion, sitting and standing in silver foxes was of the same order of magnitude in both sections. However, the cage floor was utilised significantly more ( $p < 0.001$ ) than the ground floor for resting. The total time spent in the cage section was also significantly higher ( $p < 0.001$ ) than that in the pen section. Silver foxes spent 48 min/24 h for digging which occurred only in the pen section.

The feeding location did not significantly influence the choice of section due to the short amount of daily time used for eating. In adult

blue foxes the time used for locomotion was about the same in both sections. However, juvenile blue foxes spent more time in the cage section ( $p < 0.01$ ). It can be concluded that farm foxes originally born and raised in shed cages do not reject wire mesh flooring.

*Applied Animal Behaviour Science* 54, pp. 243-250, 1997. 1 table, 4 figs., 21 refs. Authors' abstract.

### Inter- and intraspecific competition between the fox species *Alopex lagopus* and *Vulpes vulpes*: an evaluation trial under penned conditions

Hannu Korhonen, Sakari Alasuutari, Auli Mäkinen, Paavo Niemelä

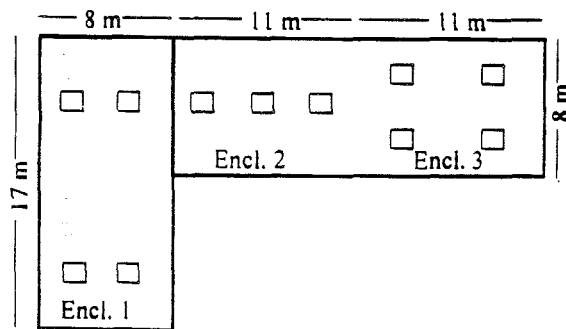


Fig. 1. Schematic picture of the experimental enclosures. Sites of wooden nestboxes are given. Enclosures (encl.) 2 and 3 were connected on 26 September 1994 by removing their common wall.

This study compared competition capacity and dominance relations between arctic foxes (*Alopex lagopus*) and red foxes (*Vulpes vulpes*). Experiments were carried out in semi-natural earthen floor enclosures using farm-bred colour types of both species (blue fox and silver fox) as subjects. Results of the dominance scoring and open field behaviour after weaning in August-September showed that blue foxes dominated over silver foxes. Thereafter, the situation gradually became reversed and silver

foxes were dominant during the breeding and whelping seasons. Housing both species together from weaning produced more curious animals as compared to when these species were placed in common quarters after the autumn equinox. In the case of blue foxes, the male dominated highly over all females. In silver foxes, the difference in dominance between the sexes was, however, less pronounced. The most dominant individuals in the study groups were typically among the heaviest. Breedings and whelpings succeeded better in silver than in blue foxes. However, none of the litters born survived more than one week. The present results support the conclusion that when both fox species are housed together. *Vulpes vulpes* tends to dominate over *Alopex lagopus*.

*Polar Biol* 17, pp. 330-336, 1997. 3 tables, 4 figs., 26 refs. Authors' abstract.

### Systematic operation programmes for the improvement of mink management distributed on the WWW

Steen H. Møller

A Systematic Operation Programme (SOP) systematises the management in a production period by describing all relevant management routines as a set of periods in which observed situations release actions. Mink production is characterised by annual cycles of highly different production periods with regard to length, management and labour intensity. Consequently, experience is gained slowly and stepwise. To meet the needs for transfer of knowledge, SOPs for the short labour intensive mating and nursing periods have been developed and tested on commercial farms. In order to distribute the SOPs effectively in terms of availability, updating, tailoring and cost, they have been published on the WWW.

*Proceedings from The European Conference for Information Technology in Agriculture, Copenhagen* 15-18 June, 1997. 4 pp, 4 refs. Author's abstract.

### Trends in breeding and skin production of polar foxes in the Bydgoszcz breeding district

*Stanislaw Kubacki, Henryka Bernacka, Malgorzata Przegalinska-Goraczkowska*

Data concerning two breeding seasons (1991/92 – 2385 skins and 1992/93 – 1338 skins) were investigated. Basic data describing the number of farms and their size, colour types, sizes and classes of skins were obtained from the Local Association of Breeders in Bydgoszcz. A decrease in the number of private farms (about 28%) as well as herd size were observed during the period under investigation. A positive increase in percentage of the most desirable colour types and qualities of skins was observed (from ex pale to medium). Sale percentage of qualities and sizes of skins is given in the table.

*Anim. Prod. Rev. (Poland). Appl. Science Reports 15, pp. 215-216 (poster), 1994. In POLH, Su. ENGL. 3 tables. Authors' summary.*

### Correlation between colour type and performance traits in raccoon dogs

*Grzegorz A. Niezgoda*

There are three colour types within the standard variety of raccoon dogs i.e. "grey", "brown" and the intermediate "mixed" type. The research concerned the frequency with which the particular colour types occurred in the breeding stock as well as among young animals and also the correlation between the colour type and some performance traits.

The particular colour types appeared in the breeding stock with the following frequencies:

Colour	Females	Males
Grey	39.1%	100%
Mixes	43.4%	
Brown	17.5%	

The proportions in the case of young animals were 41.5%, 35.4% and 23.1%, respectively,

which was found out during evaluation. All evaluated animals were also weighed. Statistical analysis proved considerable differences between the average body weight of mixed (7.92 kg) and gold brown individuals (7.98 kg) as opposed to that of silver grey type (7.75 kg). Whelping date also influenced the body weight – highly significant correlation of  $-0.19$ . Assessment was also influenced by whelping date, the lowest total scores being given to individuals belonging to the latest born litters. The colour type of an evaluated animal, as well as that of its mother, influenced the average scores for a particular trait, no influence was found, however, on the total score. Litter size at birth and weaning were not found to depend on the mother's colour type. No differences were found concerning the length of gestation or the estrus season.

*Anim. Prod. Rev. (Poland). Appl. Science Reports 15, pp. 221-222 (poster), 1994. In POLH, Su. ENGL. Author's summary.*

### Preliminary observations on maintaining nutrias in cages with different floors

*Ryszard Cholewa*

Nutrias of Standard and Greenland varieties were kept on different floors. The reproduction results were similar with regard to number of females giving birth and litter size, independent of the kind of floor in their cages.

The kit losses were lowest when kept with their dams, while in the weaned young animals, they were highest when animals were kept in cages with the concrete floor. As regards the raw skins obtained from young animals reared on the concrete floor, their quality was inferior to those from other rearing variants. These observations encourage further investigations which would be carried out on some selected groups of animals.

*Anim. Prod. Rev. (Poland). Appl. Science Reports 15, pp. 229-230 (poster), 1994. In POLH, Su. ENGL. 3 tables. Author's summary.*

### Variability of arteries of the aortic arch in silver fox

C. Wiland, I. Kubica, B. Zawadzinska

Investigations concerning the exit, course and matability of the main stems of the artery of the aortic arch were made on 47 silver foxes. Silver fox is a variety of *Vulpes vulpes* L.

The observations proved that the aortic arch in silver fox behaved similarly as in other carnivorous animals.

Two vascular variations have been found. One of these consisted in reduction of the branchiae cephalic artery, and second one in the deviation of the left subclavian artery from the branchial - cephalic one.

Within the arteries of the aorta arch bicarotid trunk has been in 8.4% of the cases.

*Zeszyty Naukowe No. 204 - Zootechnika 28, 35-40, 1996. In POLH, Su. ENGL. 4 figs., 15 refs. Authors' summary.*

### The case of variability of the arterial circle in silver fox

C. Wiland, B. Zawadzinska

During research on basilar arteries of the brain of 44 foxes was found an arterial variation. One case was found with a hard reduction in the unilateral caudal communicating artery. At the same time between the contralateral basal artery, the caudal communicating artery and the restal cerebellar artery there exists a buttonhole formation. On that case unilateral caudal cerebral artery branching of the basilar artery was found.

*Zeszyty Naukowe No. 204 - Zootechnika 28, 29-33, 1996. In POLH, Su. ENGL. 2 figs., 8 refs. Authors' summary.*

### The multiple renal arteries in the silver fox

Witold Brudnicki, Ryszard Jablonski, Benedykt Skoczylas, Cezariusz Wiland

The renal arteries of 29 silver foxes were studied. In 17.2% of the cases multiple renal arteries were found and all at the left side. Most of the cases (13%) were male. In 93% of the cases truncus phrenic caudal artery and abdominal cranial artery originated from the right renal artery.

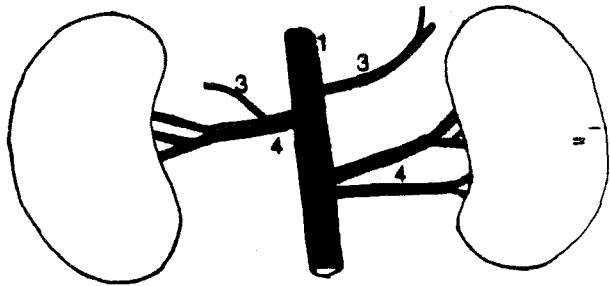


Fig. 3. Cases of multiple renal arteries.

*Zeszyty Naukowe No. 204 - Zootechnika 28, 23-27, 1996. In POLH, Su. ENGL. 3 figs., 13 refs. Authors' summary.*

### Do large males inhibit the growth rate of small females?

Ulla Lund Nielsen

200 pairs of mink were weighed at weaning on 4 July, before being placed in cages containing 1 male and 1 female and immediately before pelting on 18 Nov., in an attempt to determine whether being housed with a large male had an adverse effect on the growth of small females. It was concluded that body weight at weaning and date of birth were the most important factors affecting body weight at pelting, and that the initial body weight of the heaviest mink in the cage had no significant effect on that of its smaller partner.

*Dansk Pelsdyravl 58 (7), pp. 285, 1995. In DANH. CAB-abstract.*

### Fleas and flies on fur farms

Reidar Mehl

Parasite control, fly control, and economic importance of fleas and flies on fur farms in Norway are described. *Ceratophyllus gallinae*, *Monopsyllus sciurorum* and *Nosopsyllus fasciatus* were the fleas identified from samples from mink farms. The occurrence of houseflies (*Fannia canicularis* and *Musca domestica*) and larder beetle (*Dermestes lardarius*) required control measures on mink farms.

*Norsk Pelsdyrblad* 69 (3), pp. 22-24, 1995. In NORG. CAB-abstract.

### Some thoughts concerning behaviour and the environment in the production of fur bearers

Gudbrand Bakken

A discussion of the effects of housing and management on the behaviour and welfare of mink and foxes.

*Vara Pälsdjur* 67 (5), pp. 101-105, 1996. 1 fig. In SWED. CAB-abstract.

### Whelping results in 1996

Kaj Lindh

In 1996, in Finland, litter size averaged 4.66 kits for mink, 5.94 for polecats, 6.3 for blue foxes, 2.9 for silver foxes and 5.59 for raccoon dogs. Results were better than those in 1995 in all species.

*Finsk Pälstidskrift* 30 (8-9), pp. 203, 1996. In SWED. CAB-abstract.

### Breeding results in 1996

Per Clausen

Of the mink, blue fox and silver fox females mated in 1996 in Denmark, 7.4, 19.2 and 16.2%, respectively, failed to produce a litter, and the number of young weaned per mated female averaged 5.29, 5.47 and 3.13. Data are tabulated by colour type, farm size and district, and results are compared with those in previous years.

*Dansk Pelsdyravl* 59 (9), pp. 386-388, 1996. 9 tables. IN DANH. CAB-abstract



*Introduction review*

## **Reproductive physiology in fur animals**

*L. V. Osadchuk (Editor)*

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Novosibirsk, Russia*

### **Preface**

This book was designed to continue the report series published by *Scientifur* and to highlight the fur animal research carried out at the Institute of Cytology and Genetics of the Siberian Department of the Russian Academy of Sciences. The first issue of the series was devoted to different aspects of fox and mink domestication (*Experimental domestication of fur animals*, Eds. L.N. Trut and L.V. Osadchuk, *Scientifur*, Oslo 1997). The present series of reports is concentrated on the physiological aspects of fur animal reproduction.

The Institute of Cytology and Genetics has a long tradition in fur animal science, and reproductive physiology of fur bearing species is one of the most popular topics. Research programmes of the Institute in the area of fur animal science are directed towards improving the efficiency of fur animal production, most notably fox and mink, through better understanding of the influence of genetic and environmental factors on reproduction and by the development of advanced breeding technologies. The high scientific activity in this field is reflected in many publications in Russian and international scientific journals.

Investigators commonly deal with an isolated problem in animal reproduction. This specialisation is of course inevitable for a subject such as reproduction. However, the fragmentation has a cost. There is still a lack of overviews that can point to the species-specific features of reproductive physiology in farmed fur animals. The latter years have shown a significant increase in the number of articles in the field of physiology in fur-bearing species such as foxes and mink but reports concerning the physiological mechanisms of reproduction in other fur animals, such as nutria, are scant. However, studies of reproductive physiology in farmed fur species are needed to gain better insight into the general patterns of mammalian reproduction, as well as to promote the development of new methods for breeding commercially valuable species.

Although this series of reports cannot claim to provide a wide look into reproductive physiology of fur bearing animals, it can serve to summarise the knowledge obtained on this topic in recent years at the Institute. Only part of the work which was done at the Institute in recent years is presented here. A number of

excellent studies have been omitted from the present reports. Unfortunately, this is inevitable in such a collection of reports. Every collection of this type reflects the strengths and interest of its editor. First, the articles have been selected on the grounds that they were connected to the hormonal regulation of reproduction. Second, I felt it was important to show the variety of studies at the Institute of Fur Animal Reproduction. Third, some of the studies presented here arose from my interest in reproductive endocrinology in fox, mink and nutria. In any case, the authors who have chosen to collaborate in the writing of this collection of reports are men who have intimate contact with the progress in fur animal science. Most of the studies presented here have been carried out at the Institute of Cytology and Genetics while some of them have been done in collaboration with researchers from the University of Kuopio (Finland), the Agricultural University in Ås (Norway) and the Research Institute of Animal Production in Nitra (Slovakia).

Most of the studies are concerned with mink (*Mustela vison*) as the object of investigation. Effects of different colour mutations on folliculogenesis, sex hormone production and brain level and metabolism of some neurotransmitters are described in several articles. Of particular interest is the study of polyploidy in different tissues including germ line tissue in mink with normal and low fertility due to a possible close relationship between polyploidy frequency and the hormonal status of the organism. Another part of the reports presents studies on the influence of man-animal contacts on the development of adrenocortical and gonadal steroidogenesis in the fox. Recently a new project was initiated to investigate reproductive endocrinology in nutria (*Myocastor coy-*

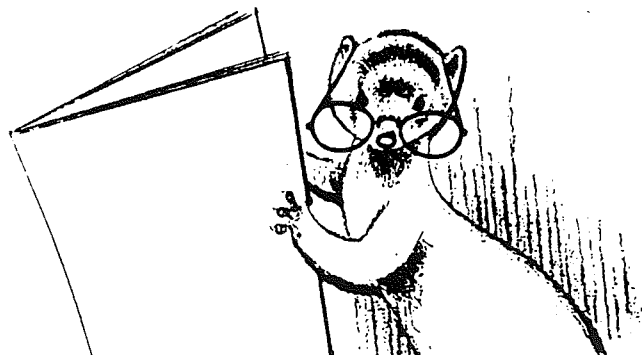
*pus*), a very popular species in fur animal production. The results of the first investigations are presented here.

This series of reports, which eventually will be collected and published as a book will allow the reader to learn in detail which studies are in progress at the Institute at the present time. I assume that the reader may need information regarding the methods and points of view of the authors and have therefore presented the papers as traditional scientific publications. There are style differences among the articles and I hope that this is not too distracting. Certainly, more research is needed to understand the physiological mechanisms of reproduction in species which are relevant for the fur industry. However, only by objectively presenting and reviewing what is currently known in this respect can one ask the important questions in future research.

I am very grateful to all contributors for their willingness to write articles for this series. I would like to acknowledge the assistance and support I have received from my collaborators and co-authors from Norway, Slovakia and Finland. Finally, I am especially indebted to Gunnar Jørgensen for invitation to publish this collection of reports.

The book will hopefully be of interest to those studying the physiological aspects of reproduction. It is also intended for scientists, managers and farmers who are professionally involved in the breeding of fur animal species.

Novosibirsk  
October 1997  
Ludmila Osadchuk



*Original Report*

## **Polyploid cells in bone marrow and spermatogenic epithelium in mink males with lower fertility**

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### **Summary**

The frequency of polyploid cells both in bone marrow and spermatogenic epithelium was examined in 21 two year-old mink males with normal karyotype; 9 of them exhibited a normal fecundity in the preceding breeding season, and 12 males exhibited a high level of embryonic and early postnatal losses of their progeny. The decrease in fecundity was found to be related to the significant increase in the frequency of polyploid cells in the bone marrow and that of polyploid spermatogonial metaphases and II of meiosis. Anomalies of mitosis including endoreduplication as well as cell fusion are proposed to lead to formation of polyploid cells. A close correlation between the frequencies of polyploidy occurrence in somatic and generative tissues has been found. The mechanisms underlying the relationship between the increased polyploidy in somatic and generative tissues, and the reduced fecundity are discussed.

### **Introduction**

In previous studies we have shown that in mink with normal diploid karyotype and high levels of embryonic and postnatal mortality, the frequencies of polyploid cells in the bone marrow of females and males and in the spermatogenic epithelium of males are increased compared to mink with normal fertility (Isakova et al., 1976; Isakova et al., 1977). The aim of this study was to further examine polyploidy frequency in the bone marrow, as well as in spermatogenic epithelium cells at different stages of spermatogenesis in mink males of normal and decreased fertility.

### **Material and methods**

The mink males studied were bred at the Experimental Fur Farm of the Institute of Cytology and Genetics, Novosibirsk. Two-year-old Standard Dark, Sapphire and Pearl mink males were used. The criterion of "good" reproduc-

tive performance was mating of a male with 2 females, producing not less than 4 pups each. Performance was considered "poor" if the majority of females mated with the male were either barren or produced not more than 2 pups per litter. Table 1 shows that there is a difference between males with "good" and "poor" reproductive performance; their polygamy coefficients were 3.1 and 4.5, respectively

( $p < 0.05$ ). These males did not differ in the number of newborn per whelped female (5.4 and 5.1, respectively). There is a significant increase in barren vixens in the "poor" group which was due to total embryonic losses (Belyaev, Zhelezova, 1968). Males showing good reproductive performance also differed in early postnatal losses of their progeny from males whose performance was poor (17.1% and 52.1%, respectively,  $p < 0.05$ ).

Table 1. Reproductive performance in mink males with good (I) and lowered (II) fertility.

Fertility	Male	Number of mated females			Number of pups		Early postnatal mortality	Genotype
		total	barren	whelped	total	litter size		
I	374	3		3	14	4.7	2	Standard
I	376	4		4	30	7.5	8	Standard
I	382	2		2	10	5.0	-	Pearl
I	388	2		2	10	5.0	-	Pearl
I	455	5	1	4	28	7.0	9	Sapphire
I	482	2		2	8	4.0	1	Standard
I	463	5	2	3	12	4.0	3	Sapphire
I	506	3		3	18	6.0	-	Sapphire
I	507	2		2	10	5.0	1	Pearl
Total		28	3	25	140	5.4	24	
		10.7%*					17.1%*	
II	378	6	6	-	-	-	-	Standard
II	381	3	1	2	12	6.0	10	Pearl
II	453	6	4	2	11	5.5	4	Pearl
II	457	6	2	4	25	6.2	9	Sapphire
II	461	6	-	6	31	5.1	17	Sapphire
II	468	6	1	5	22	4.4	15	Sapphire
II	470	6	5	1	9	9.0	1	Sapphire
II	472	3	-	3	11	3.7	7	Sapphire
II	473	6	4	2	5	2.5	2	Sapphire
II	484	3	2	1	7	7.0	5	Standard
II	511	2	1	1	5	5.0	2	Pearl
II	602	1	-	1	4	4.0	2	Standard
Total		54	26	28	142	5.1	74	
		48.1%					52.1%	

Asterisks here and in further tables designate significant differences between groups I and II ( $p$  at least  $< 0.05$ ).

Spermatogenesis in mink is known to extend from the beginning of December to the second half of March (Tiba et al., 1968). From our observations, mitotic divisions of spermatogonia dominate in December and January, as well as in the end of March, while the meiotic stage most frequently occurs in February. For this reason, we took part of the material at the end

of January and the beginning of February, and the rest at the beginning of March. All the animals studied had normally developed testes. Chromosome preparations were made from the bone marrow of the femur epiphyses and from the seminiferous tubules according to the methods described (Isakova, 1989). The frequency of polyploidy was estimated at a mag-

nification of 10x40, and the number of chromosome sets at a magnification of 10x100 using an Ergaval microscope (Zeiss, Jena). The number of bone marrow cells examined per mink was 300-500 and those of spermatogonial metaphases, meiotic metaphases I and II were from 120-140. Student's t-test was used to compare the percentages for the two animal groups studied.

## Results

All the animals studied had normal diploid karyotype (2n, XY). Table 2 shows the distribution of bone marrow cells for the number of chromosomes in each animal. The frequency of aneuploid, most notably of hypodiploid, cells was increased in males with reduced fertility. The same has been observed for female mink (Isakova et al., 1976).

**Table 2.** Distribution of chromosome number in bone marrow cells of mink males with good (I) and lowered (II) fertility.

Fertility	Male	No. of cells examined with chromosome number			Total
		28-29	30	31-32	
I	374	1	29	-	30
I	376	2	28	-	30
I	382	2	28	-	30
I	388	-	30	-	30
I	455	-	30	-	30
I	482	1	28	1	30
I	463	1	29	-	30
I	506	1	29	-	30
I	507	1	29	-	30
Total		9	260	1	270
		3.4%*	96.2%	0.4%*	100%
II	378	4	29	2	35
II	381	4	31	-	35
II	453	3	27	-	30
II	457	4	26	-	30
II	461	3	27	-	30
II	468	-	30	-	30
II	470	-	30	-	30
II	472	2	27	1	30
II	473	2	28	-	30
II	484	1	29	-	30
II	511	-	30	-	30
II	602	-	30	-	30
Total		23	344	3	370
		6.2%	93.0%	0.8%	100%

### *Polyploidy in bone marrow.*

Polyploid cells were observed to contain from 4 to 32 chromosome sets. We counted separately cells with low (4n) and high (>4n) level of ploidy. The mean frequency of polyploid cells occurrence was 0.9% and 1.4% in males with normal and low fertility ( $p < 0.05$ ). The tetraploid (4n) cells dominated in both animal groups (Table 3).

### *Polyploidy in spermatogonial epithelium.*

Analysis was performed at the following stages of spermatogenesis: spermatogonia (spermatogonial metaphases), spermatocytes I (meiotic metaphase I) and spermatocytes II (meiotic metaphase II).

a). Spermatogonial metaphases. At this stage, most of the cells had a normal diploid chromosome set, occasionally tetraploid and hexaploid. Table 4 presents the results of the analysis of the frequency of cells with different ploidy levels. There was an increase in the frequency of tetraploid and hexaploid spermatogonial metaphases in mink with low fertility ( $p < 0.05$ ).

b). Diakinesis - metaphase I. In diploid spermatocytes, mink chromosomes formed 15 bivalents at the meiotic stage diakinesis - metaphase I with the sex chromosomes pairing end-to-end. The sex bivalent stains weaker and is less despiralized than the autosomal bivalents. No cells with polyvalents, sex univalents, or anomalies of chromosome structure were found. The chromosome sets were diploid (15 bivalents) in most spermatocytes I and single tetraploid cells with a double number of bivalents were observed. Table 5 demonstrates that the number of polyploid cells is the same at this stage in males showing good and poor performance (2.6% and 2.5%, respectively).

c). Metaphase II. Cells with 15 or 30 chromosomes were encountered among spermatocytes II (meiotic metaphase II). The results of the analysis of the frequencies of hyploid and diploid cells are given in Table 5. The average numbers of diploid metaphases II were, respectively, 1.83% and 3.4% ( $p < 0.05$ ) in males with normal and reduced fertility.

Table 3. Frequency of polyploid cells in the bone marrow of mink males with good (I) and lowered (II) fertility.

Fertility	Male	Total	Number of cells			%
			Polyploid		subtotal	
			4n	>4n		
I	374	500	2	2	4	0.80
I	376	400	-	-	2	0.50
I	382	400	2	1	3	0.75
I	388	400	5	2	7	1.75
I	455	400	3	1	4	1.00
I	482	400	1	2	3	0.75
I	463	400	2	-	2	0.50
I	506	400	2	-	2	0.50
I	507	400	4	2	6	1.50
Total		3700 (100%)	21 (0.6%)	12 (0.3%)	33 (0.9%)*	
II	378	500	2	1	3	0.60
II	381	500	2	2	4	0.80
II	453	400	3	4	7	1.75
II	457	400	4	2	6	1.50
II	461	400	5	4	9	2.25
II	468	400	4	-	4	1.00
II	470	400	2	3	5	1.25
II	472	400	2	2	4	1.00
II	473	400	4	2	6	1.50
II	484	400	4	3	7	1.75
II	511	400	8	-	8	2.00
II	602	400	5	2	7	1.75
Total		5000 (100%)	45 (0.9%)	25 (0.5%)	70 (1.4%)	

Table 4. Frequency of spermatogonial metaphases in mink males with good (I) and lowered (II) fertility.

Fertility	Male	Total	Number of cells			%
			Polyploid		subtotal	
			4n	6n		
I	374	340	25	7	32	8.6
I	376	370	25	1	26	7.0
I	382	400	18	-	18	4.5
I	388	280	18	-	18	6.4
I	455	400	31	-	31	7.8
I	482	200	12	-	12	6.0
I	463	330	26	-	26	7.9
I	506	200	9	-	9	4.5
I	507	200	12	-	12	6.0
Total		2750 (100%)	176 (6.4%)	8 (0.3%)	184 (6.7%)*	
II	378	400	30	3	33	8.3
II	381	400	36	3	39	9.8
II	453	400	32	3	35	8.8
II	457	380	40	3	43	11.3
II	461	400	46	6	52	13.0
II	468	400	36	2	38	9.5
II	470	400	29	-	29	7.3
II	472	370	31	1	32	8.6
II	473	400	30	4	34	8.5
II	484	340	23	-	23	6.8
II	511	220	26	-	26	11.8
II	602	200	12	1	13	6.5
Total		4310 (100%)	371 (8.6%)	26 (0.6%)	397 (9.2%)	

**Table 5.** Frequency of cells with different ploidy level in metaphase I and metaphase II in mink males with good (I) and lowered (II) fertility.

Fertility	Male	Total	Metaphase I			Total	Metaphase II			
			2n	No	4n %		n	No	2n %	
I	374	240	230	10	4.2	220	214	6	2.7	
I	376	270	258	12	4.4	200	195	5	2.5	
I	382	230	228	2	0.9	240	239	1	0.4	
I	388	250	240	10	4.0	160	157	3	1.9	
I	455	230	223	7	3.0	220	215	5	2.3	
I	482	260	255	5	1.9	130	129	1	0.8	
I	463	250	247	3	1.2	140	137	3	2.1	
I	506	220	219	1	0.5	240	236	4	1.7	
I	507	230	224	6	2.6	120	118	2	1.7	
Total		2180	2124	56	2.6	1670	1640	30	1.8*	
II	378	240	230	10	4.2	170	163	7	4.1	
II	381	300	296	4	1.3	170	166	4	2.4	
II	453	250	244	6	2.4	240	233	7	2.9	
II	457	360	350	10	2.8	250	243	7	2.8	
II	461	230	216	14	6.1	220	209	11	5.0	
II	468	260	254	6	2.3	280	273	7	2.5	
II	470	250	246	4	1.6	220	215	5	2.3	
II	472	220	214	6	2.7	180	177	3	1.7	
II	473	260	255	5	1.9	120	117	3	2.5	
II	484	250	246	4	1.6	220	216	4	2.0	
II	511	230	226	4	1.7	230	212	18	7.8	
II	602	200	196	4	2.0	100	95	5	5.0	
Total		3050	2973	77	2.5	2400	2319	81	3.4	

**Table 6.** Polyploidy at different stages of spermatogenesis in mammals and poultry.

Species	Reproductive formance	per-	Polyploid cells, %			References
			I	II	III	
Man	Poor, age 14-31 years		6.8	2.6	6.4	Sasaki, Makino, 1965
	Poor, age 40-79 years		7.1	2.5	6.9	
Man Chinese ham- ster	Poor		6.2	1.6	9.1	McIlree et al., 1966 Hulten et al., 1970a
	Unknown		8.9	1.7	1.3	
Man	Good		4.8	1.3	3.6	Skakkebek et al., 1973
	Poor		4.8	1.3	10.6	
Cockerel	Unknown		9.8	2.0	5.8	Pollock, Fechheimer, 1978
Mink	Good		6.7	2.6	1.8	Present paper
	Poor		9.2	2.5	3.4	

Note: I - Spermatogonial metaphases; II - Metaphase I; III - Metaphase II

Fig. 1 is a graphical representation of polyploidy frequency during spermatogenesis. The percentage (proportion) of polyploid cells was highest for spermatogonial metaphases. It was much lower for spermatocytes I. The frequency of polyploid cells was somewhat lower among spermatocytes II as compared with spermatocytes I in males with normal fecundity. It was increased in males showing poor performance, being 3.4% and 2.5%, respectively ( $p < 0.05$ ). Moreover, males with normal and reduced fertility differed significantly in polyploidy frequency at the spermatogonial stages and in spermatocytes II.

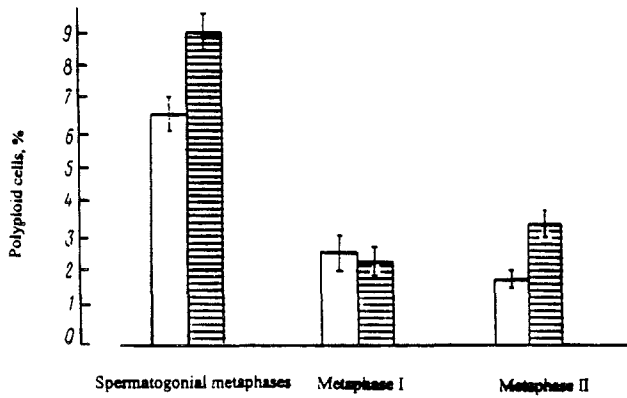


Fig. 1. Frequency of polyploid cells at different stages of spermatogenesis in mink males with different fertility. Open bars represent males with good fertility; hatched bars - mink with lowered fertility.

The relation between polyploidy frequency in bone marrow and spermatogenic epithelium. It follows from the obtained data that the frequencies of polyploid cells in bone marrow and in spermatogenic epithelium at the spermatogonial stages and in spermatocytes II was increased in males with reduced fertility compared to males with normal fertility. The relation between polyploid cells in bone marrow and spermatogonia, as well as in bone marrow and spermatocytes, is graphically represented in Fig. 2. The relation is positive and close to linear in both groups. The correlation coefficient be-

tween polyploidy frequencies among the bone marrow cells and spermatogonial metaphases is  $0.46 \pm 0.17$  ( $p < 0.05$ ), and between the frequencies of polyploid cells in bone marrow and diploid metaphases II is  $0.54 \pm 0.16$  ( $p < 0.01$ ).

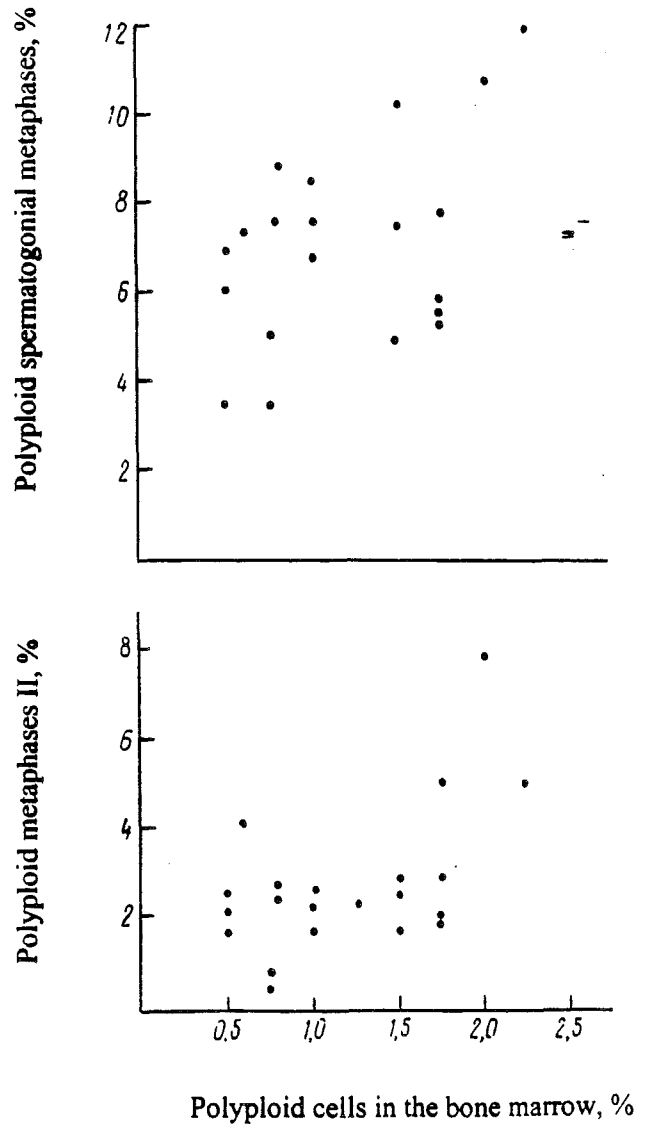


Fig. 2. Frequency of polyploid spermatogonial metaphases and metaphases II in mink males with different polyploid levels in their bone marrow.

## Discussion

The presence of polyploid cells in mammalian bone marrow is a common event related to its specific function (Brodsky, Uryvaeva, 1985). Our data indicate that the frequency of polyploid cells is sufficiently enhanced in mink males to expose increased embryonic and early postnatal mortality of their progeny (1.4% vs. 0.9% in control animals,  $p < 0.01$ ). A similar pattern has been observed for 1.5-year-old mink females (Isakova et al., 1976). These data also indicate that the sex difference in the level of somatic polyploidy exists. It is noteworthy that the number of cells with low ploidy level ( $4n$ ) was increased in females whose fertility was low, while the ratio of cells with low ( $4n$ ) and high ( $>4n$ ) ploidy was the same in males differing in fertility.

The polyploidy level in spermatogenic epithelium, particularly in spermatogonial metaphases, has been found to be significantly increased as compared with bone marrow (6.7% and 9.2% in normal and infertile males, respectively). What may be the origin of polyploid spermatogonia? We carefully selected round or oval (intact) metaphase spreads for the study, and this might have considerably excluded the proportion of artificial polyploid cells resulting from the fusion of cells during the processing of the material. A spermatogonial metaphase spread was observed (male N 473, table 1). This pattern was evidence that endoreduplication may be one of the mechanisms underlying the formation of polyploid spermatogonia. However, the presence of hexaploid ( $6n$ ) cells was convincing evidence that at least part of polyploid spermatogonia resulted from the fusion of cells, such as of three diploid cells or of one diploid and one tetraploid cell. Electron microscopic studies on spermatogenic tissue support this assumption: intercellular cytoplasmic bridges are formed during the differentiation of the sex cells in the testicles of invertebrate and vertebrate species (Dym, Fawcett, 1971). The authors suggested that the bridges may play a role in the synchronisation of differentiation of this cell group, and that both retention of intercellular septa and normal narrow-

ing of the bridges depends to a large extent on the state of the generative cell cytoplasm; conditions altering the properties of the cytoplasm can widen the bridges and make the adjacent cells fuse. The increase in the proportion of hexaploid cells and in the total number of polyploid cells in males with reduced fertility is presumably evidence that spermatogenic cells fuse more frequently in comparison with males of normal fertility. Polyploid spermatogonial metaphases have been described in man (Sasaki, Makino, 1965; McIlree et al., 1966; Hulten et al., 1970a; Skakkebek et al., 1973), mouse (Fechheimer, 1961), Chinese hamster (Hulten et al., 1970b), cattle (Popescu, 1971) and cockerels (Pollock, Fechheimer, 1978). Polyploidy frequencies in all these species were quite comparable to the one estimated for mink.

Of particular interest are the data on polyploidy frequencies at the different stages of spermatogenesis (fig. 1). The percentage of polyploid cells was highest in spermatogonia, and their number sharply decreased in spermatocytes I. As indicated before, no polyvalents, whose formation would be expected in the case of meiotic division of tetraploid spermatogonia, were found in tetraploid metaphases I. The absence of polyvalents has been observed by other investigators (Ford, Evans, 1971; Pollock, Fechheimer, 1978), and this gave all the more reason to suggest that polyploid cells are mainly formed during the making of preparations. Table 6 summarises the literature data on polyploidy at spermatogonial stages in different species. It will be noted that the course of changes in polyploidy frequency during spermatogenesis in all the compared species is the same as in mink: negative selection of most polyploid cells before meiosis, followed by an increase in the frequency of diploid metaphases II compared to that of polyploid metaphases I. This pattern of changes in polyploidy frequency in spermatogenesis conforms to the expectations. It is known that cell death in metazoan spermatogenesis is genetically determined (apoptosis), and meiosis and the early postmeiotic period are the critical stages (Roosen-Runge, 1973).

The heteroploid cells were also shown to die during these periods (Roosen-Runge, 1973). It may be assumed that most of the polyploid spermatogonial cells are eliminated in this way (prezygotic selection). A sharp decrease in the proportion of polyploid spermatocytes I compared with spermatogonia is presumable evidence that most polyploid spermatogonia are unable to divide meiotically. The kinetics of spermatogenesis is proposed to be determined not only by the mother's level of sex hormones and gonadotrophins, but also by the functional activity of the genome of developing generative cells (Baranov, Dyban, 1968). Thus, a proper level of sex hormones and the ability of developing sex cells to respond to maternal hormonal stimuli are necessary to ensure the normal differentiation process. It is thus evident that cells with chromosome disbalance have a smaller chance to enter meiosis than normal diploid cells. The facts that spermatogenic cells with structurally abnormal karyotypes die during meiosis, even during the postmeiotic period, while most polyploid cells are subjected to negative selection before meiosis indicate that polyploidy results in greater gene disbalance in cells leading to their earlier death. It is of interest that mammalian tetraploid embryos also die at the earlier stages of embryogenesis than embryos with other types of anomalies of chromosome set, such as translocation, aneuploidy or triploidy (Snow, 1973; Dyban, Baranov, 1987).

Polyploidy frequency is increased at the spermatocyte I - spermatocyte II stages, as the data in Fig. 1 and Table 5 show. Conditions favouring the formation of polyploid cells can presumably arise again in the course of meiosis. It may be assumed that polyploid spermatocytes may result from failures during the anaphase of meiotic division I (karyokinesis not followed by cytokinesis). In fact, it has been shown that about 80% of diploid spermatozoa in man have an XY chromosome set (Beatty, 1977).

Thus, in mink, the frequency of polyploid spermatogonia and spermatocytes II is increased as compared with polyploid spermatocytes I. The correlation coefficient between

polyploidy frequency among spermatogonia and spermatocytes II is  $0.65 \pm 0.22$ ,  $p < 0.01$ . There may possibly be a common cause (or mechanism) of the emergence of polyploidy at these stages of spermatogenesis. The data in Table 6 show that the frequency of diploid spermatocytes II was increased relative to polyploid spermatocytes I in a group of infertile men compared to controls (normal reproductive function). The same pattern was observed in mink with reduced fertility.

What may be the fate of heteroploid spermatocytes II? It is quite possible that their viability is lower compared to normal, and that most of them die before reaching the stage of the mature spermatozoa. Furthermore, diploid spermatozoa preferentially die during their passage from the uterus to the oviduct (Krzanowska, 1974). However, the motility and viability of some of the diploid spermatozoa may be normal (Beatty, Fehheimer, 1972; Stolla, Gropp, 1974). Diploid sperm have been found in man (Sumner, 1971; Carothers, Beatty, 1975), rabbit (Beatty, Fehheimer, 1972; Carothers, Beatty, 1975), mouse (Maudlin, Fraser, 1977), bull (Esnault, Ortavant, 1967) and mink (Belyaev et al., 1962). The percentage of diploid gametes was not more than 1.0% in all the cases. The nonviable triploid embryos can result from fertilisation by diploid spermatozoa. However, the increased proportion of abnormal gametes in mink with reduced fertility does not seem high enough to cause a significant increase in the mortality rate of progeny. Experience with artificial insemination of rabbits has shown that the incidence of triploid embryos is not dependent on the amount of diploid gametes in the semen; also, a high portion of diploid spermatozoa was noted to be correlated to the small total number of gametes, i.e. oligospermia (Beatty, Fehheimer, 1972; Fehheimer, Beatty, 1974).

Cytophotometric analysis of sperm DNA content in men with oligospermia revealed high heterogeneity in sperm DNA content compared to normal subjects (Sarcar et al., 1978). The same was observed in men with disorders of the reproductive function: an increase in the

number of diploid spermatocytes II was correlated to spermatogenic arrest and a decrease in the number of mature spermatozoa (Fechheimer, 1961; Hulten et al., 1970a). An increase in cell death in meiosis and oligospermia was observed in mice heterozygous for chromosome translocation T6 (Baranov, Dyban, 1968). The same is presumably true for mink: judging by the time course of changes in the frequency of polyploid cells, cell loss is much higher during the premeiotic period in animals with reduced fertility, and this may affect the number of mature spermatozoa. Considering all the data obtained, it may be suggested that an increase in the frequency of somatic polyploidy indicates not only a probable increase in the proportion of unbalanced gametes, but also a general impairment of spermatogenesis.

#### Acknowledgements

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### Determination of the requirement for methionine and cystine in the growing period

Tove N. Clausen, Niels Therkildsen, Christian Børsting

The determination of the requirement for methionine and cystine in the growing and furring period was continued in 1995. We used 8 groups of each 54 male and 54 female kits. Two groups were fed with 20 vs. 30 percent of the metabolizable energy from protein and 6 groups were fed varied methionine and cystine contents in the diets fulfilling the requirements of the other essential amino acids. The levels of methionine were 0.16 and 0.18 grams of apparently digestible methionine per 100 kcal, and the levels of cystine were 0.06, 0.08 and 0.10 grams of apparently digestible cystine per 100 kcal. The investigations confirmed the conclusions from previous years, namely that the requirement for mink kits in the growing and furring period as a whole is 0.06 gram of apparently digestible cystine per 100 kcal and 0.16 grams of apparently digestible methionine per 100 kcal.

*Technical Year Report 1996, pp. 27-45, 1997. In DANH. 6 tables, 4 refs. Authors' summary.*

### Fatty acid composition in body fat and milk fat in mink

Tove N. Clausen

Investigations were carried out on the formation of body fat depots in mink females fed with different types of fat and varying levels of carbohydrates from the first of January to the first of July. Furthermore, we investigated the importance of the fatty acid composition in the body depots to the fatty acid composition in milk fat. We used groups of mink females fed with fish oil and two groups fed with swine fat. Another 5 females were fed with fish oil until birth, then with swine fat for the rest of the period.

The day after birth, we took body fat biopsies of five females per group. A further five females per group, with 6 kits per litter, were milked once a week during the whole nursing period. The milk samples, body fat samples and feed samples were analysed for fatty acid composition.

There were only small variations in the fatty acid composition in the milk from females fed the same feed. Females who changed from fish fat to swine fat the day after birth had, during the whole nursing period, a fatty acid composition in the milk, corresponding to the females fed with swine fat in the whole period, and very different from the females fed with fish oil in the whole period.

*Technical Year Report 1996, pp. 47-56, 1997. In DANH. 4 tables, 5 figs., 4 refs. Author's summary.*

### Continued investigations on the use of slaughterhouse offal and industrial fish in the nursing period

Tove N. Clausen, Carsten Hejlesen, Niels Therkildsen

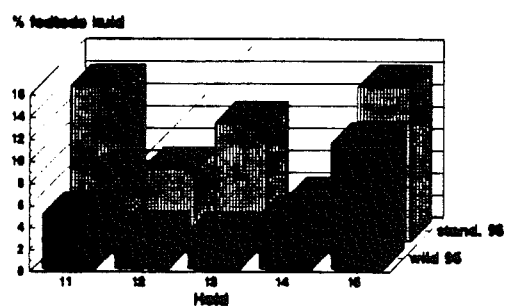


Fig. 3. Per cent greasy kits in wild type mink 1995 and standard mink 1996.

The use of slaughterhouse offal instead of industrial fish was investigated in the nursing period 1996. We used 5 groups with each 137 scanblack female mink. The females were feed varying levels of slaughterhouse offal (0 - 17.5 - 35%) and industrial fish (35 - 17.5 - 0%). The

energy distribution was protein (P): fat (F): carbohydrate (c), 55:35:10. A further two groups were fed with 35% industrial fish and an energy distribution of P:F:C 65:25:10 vs. 45:45:10.

Females fed a feed with the energy distribution P.F.C. 55:35:10 had more kits than the other females, but the litter size was low in all groups. There was no difference between groups in the female weight loss from birth to weaning. The groups fed slaughterhouse offal had the best kit weights at weaning, but slaughterhouse offal could not prevent greasy kits.

*Technical Year Report 1996, pp. 67-77, 1997. In DANH. 7 tables, 3 figs. Authors' summary.*

**On the use of high levels of fatty fish for mink in the growing period**

*Tove N. Clausen, Niels Therkildsen, Carsten Hejlesen, Christian F. Børsting, Birthe M. Damgaard, Richarda Engberg, Søren Krogh Jensen*

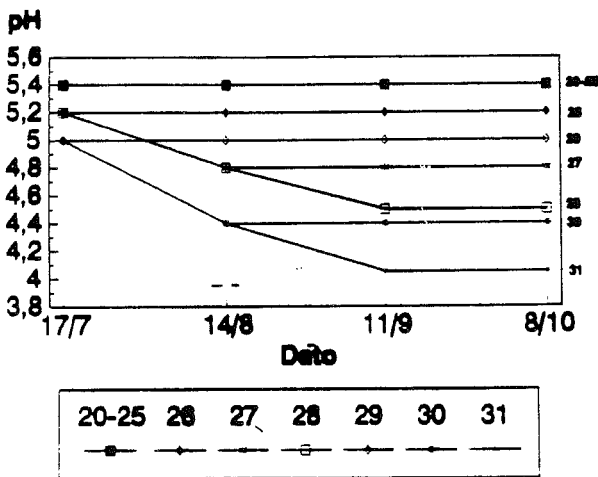


Fig. 1. pH in the different feed groups.

Investigations on the use of fat mackerel and fat herring scrap, defatted silage of herring scrap and mackerel for mink kits in the growing and furring periods were carried out. We used 12 groups of 76 males and 76 females of the wild type of mink. Three groups were fed with increasing levels of fat herring scrap, three

groups with increasing mackerel, three groups with increasing defatted herring scrap silage and, finally, three groups with increasing defatted mackerel silage. The highest levels of defatted herring scrap and mackerel silage were stopped in September because of reduced appetite. The high level of silage decreased the pH in the feed to 4.1.

Up to 50 per cent of the fat in the feed from fatty fish products (herring and mackerel), could be used without any negative effects on skin size, skin quality and the animal health. Feeding 70% of the fat of marine origin gave a reduced skin size and quality, whereas the health was unaffected.

*Technical Year Report 1996, pp. 101-123, 1997. In DANH. 10 tables, 2 figs. Authors' summary.*

**Herring scrap and defatted herring scrap silage for mink in the reproduction and nursing periods**

*Tove N. Clausen, Niels Therkildsen, Carsten Hejlesen, Christian F. Børsting, Birthe M. Damgaard, Richarda Engberg, Søren Krogh Jensen*

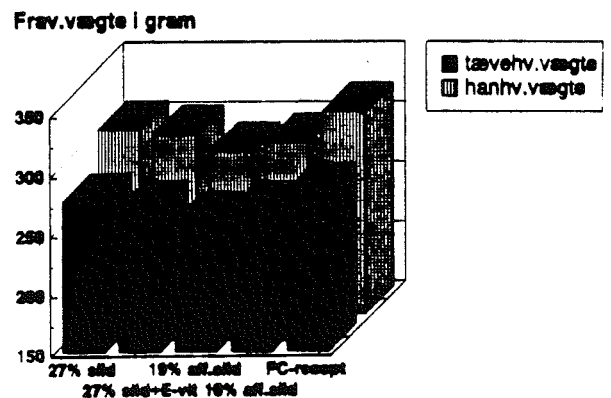


Fig. 1. Male and female kit weaning weights.

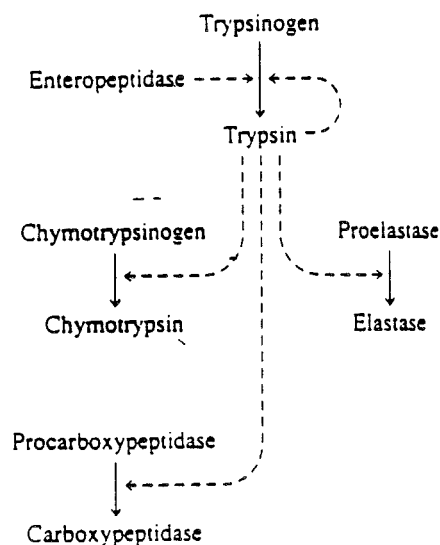
In the winter and nursing periods we investigated the use of 27% herring scrap with a natural high fat content (13.5% fat) with 60 vs. 120 mg vitamin E per kg feed, and two levels of defatted herring scrap silage in groups of each 137 wild type female mink. These 4 experi-

mental groups were compared to a control group which were not fed any of the two herring products. The products could be used for the females without any negative effect, but the kits had lower weights at weaning compared to the control group, especially in the groups receiving defatted herring scrap silage. This is most likely due to a reduced palatability of the feed, caused by a low pH in the diet in these groups. The blood content of thrombocytes was reduced with increasing levels of fat from herring, but the plasma tocopherol concentration was high in all groups, which showed that it was not necessary to increase the addition of vitamin E beyond 60 mg when we used 27% fat herring scrap.

*Technical Year Report 1996, pp. 125-134, 1997. In DANH. 8 tables, 1 fig. Authors' summary.*

### Proteinases, $\alpha$ -amylases, and lipases in mink-pancreas

*Charlotte Bjerregaard, Kirsten Mortensen, and Hilmer Sørensen*



**Fig. 2.** Aktivering af pancreas proenzym (zymogener) i tyndtarmen. Det ses, at trypsin aktiverer de forskellige proteinaser.

Mink feed is produced from varying amounts of fish products and other animal and vegeta-

ble feedstuffs under consideration of the price of the raw material. Utilisation of the feed by the mink imply hydrolysis of the individual feed components followed by absorption from the intestinal system, and as mink have a relatively short digestive tract compared to the body length, and also a quick feed passage rate, there is limited time for these processes. A study of the activity of the pancreatic enzymes in mink (lipase,  $\alpha$ -amylase, chymotrypsin, and trypsin) therefore calls for attention. Inhibition studies using trypsin from pig, rat, and mink have revealed a considerable difference in the sensibility of enzymes from these animals towards trypsin inhibitor from peas. The inhibition of mink trypsin was thus a factor 10 higher than for pig trypsin, indicating that mink may be seriously affected by the presence of hydrolyase inhibitors in the feed (Arentoft *et al*, 1992). Further studies will reveal whether these differences in enzymatic activity concern all the pancreatic hydrolases.

Evaluation of mink feed quality may be performed *in vitro* by the EDOM-method (Enzyme Digestible Organic Matter), based on an imitation of the digestion processes existing in the stomach and intestine (Boisen and Fernández, 1992). According to the previous results, the experimental conditions of the EDOM method should, however, be adjusted to mink (enzymes, incubation time etc.), as the method developed for monogastrics in general may lead to erroneous conclusions.

*Technical Year Report 1996, pp. 139-150, 1997. In DANH. 1 table, 3 figs., 19 refs. Authors' summary.*

### Nitrogen balance in adult female mink (*Mustela vison*) in response to normal feeding and short-term fasting

*Anne-Helene Tauson, Jan Elnif, Søren Wamberg*

Ten adult female mink (*Mustela vison*) were studied in a 7 d balance experiment consisting of a 2 d pre-surgery feeding period, followed by surgery, 1 d of recovery, 4 d of *ad libitum* feeding, and a 2 d fasting period. In this ex-

periment (Expt A) the animals had osmotic pumps implanted for continuous release of radioactively-labelled *p*-aminohippuric acid (*p*-aminobenzoyl-2-<sup>3</sup>H]glycine; [<sup>3</sup>H]PAH; *n* 10) and <sup>14</sup>C-labelled inulin ([<sup>14</sup>C]IN; *n* 5). Repeated 24 h collections of urine, corrected to 100% [<sup>3</sup>H]PAH or [<sup>14</sup>C]IN recovery, were used for accurate determination of N balances, 24 h urinary excretion of urea, creatinine, and total N, and calculation of mean 24 h renal clearance rates for endogenous creatinine and inulin. N balances were slightly below zero, but not significantly different between feeding and fasting periods, indicating that correction to 100% [<sup>3</sup>H]PAH recovery resulted in slight overestimation of the final balances. During fasting, withdrawal of the dietary water and protein loads resulted in a dramatic decline in 24 h urinary volume, and urea and creatinine excretion. Large individual variations in 24 h urinary creatinine excretion (with relative variation coefficients up to 30%) confirmed that this is an unreliable index of the completeness of urine collection. In this respect, recovery rates of [<sup>3</sup>H]PAH proved far more consistent. Renal clearance values obtained in fed mink were in fair agreement with published data from cats, dogs and ferrets (*Mustela putorius furo*). Inulin clearance was about 30% higher than endogenous creatinine clearance, although its decline in response to fasting was not significant. In a separate study (Expt B) another ten female mink were equipped with osmotic pumps containing [<sup>3</sup>H]PAH for determination of 24 h excretion rates of purine derivatives. During feeding, allantoin accounted for more than 97% of the excretion of purine derivatives in urine, uric acid making up less than 2.5%, and xanthine and hypoxanthine less than 1%. In fasted animals, urinary excretion of each of these purine derivatives declined to less than 50% of the feeding value. In conclusion, an experimental technique is presented for efficient and accurate measurements of daily urinary excretion of nitrogenous constituents, which allows for correct determination of N balances in adult mink and, presumably, in other mammalian species.

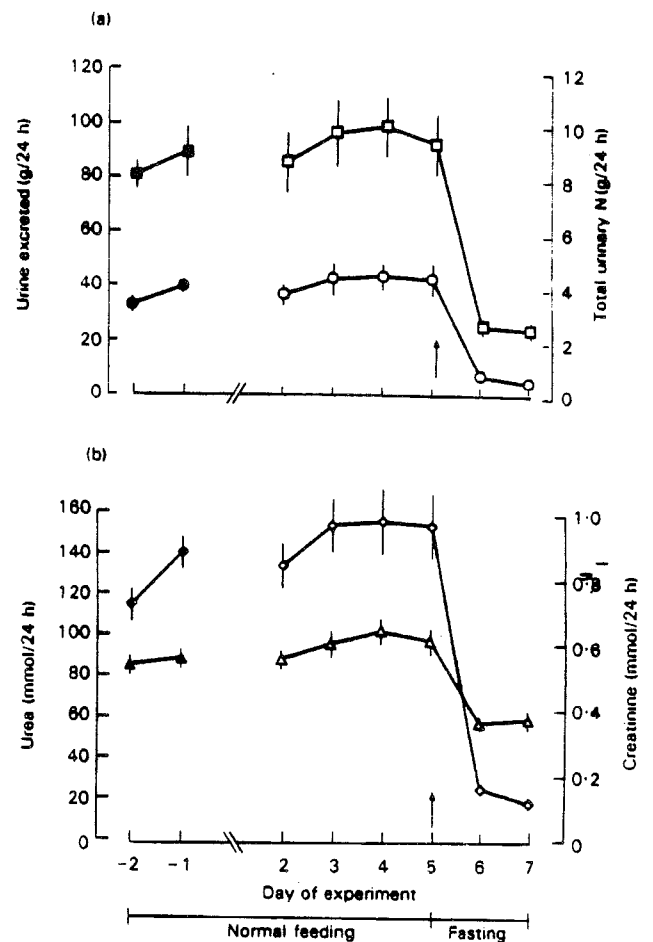


Fig. 1. Expt A. (a) Daily urinary output (□) (g/24 g) and total nitrogen in urine (○) (g/24 h), corrected to 100% radioactively-labelled *p*-aminohippuric acid (*p*-aminobenzoyl-2-<sup>3</sup>H]PAH) recovery and (b) urinary excretion rates (mmol/24 h) of urea (◇) and creatinine (△), corrected to 100% [<sup>3</sup>H]PAH recovery, in ten female mink during normal feeding (pre-experimental days -2 and -1 (■, ●, ◆, ▲); and experimental days 2-5) and short-term fasting (days 6-7). ↑, The onset of fasting. Values are means with their standard errors represented by vertical bars. The animals had the osmotic pump implanted on day zero, and no collections were made on day 1.

British Journal of Nutrition 78, pp. 83-96, 1997. 4 tables, 1 fig., 59 refs. Authors' summary.

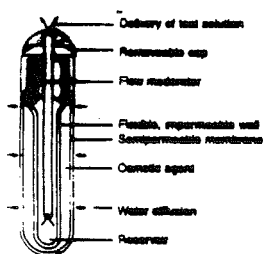
### Accurate collection of urine in carnivores

Søren Wamberg

In studies of animal nutrition, accurate determination of daily (24-h) urinary excretion of dietary constituents, xenobiotics, and end-products of metabolism is a prerequisite for clinical and experimental evaluation of diseases and nutritional requirements. In strictly carnivorous animals, such as cats, ferrets and mink, nitrogen balance, and hence protein requirements, are frequently over-estimated because of incomplete collection of a highly concentrated urine and because of the animals' habit of squirting.

In this study, the accuracy of quantitative urine collection was assessed in conscious female mink by repeated measurements of the recovery in urine of two well-known urinary markers (*p*-aminohippuric acid and inulin), released by an intraperitoneally implanted osmotic pump.

The experimental technique presented in this study, using osmotic pumps for continuous release of specific urinary markers to assess the accuracy of quantitative urine collection in mink, was shown to be valuable and reproducible. In strictly carnivorous mammals, renal glomerular filtration rate and the rates of urinary volumen and solute excretion are highly influenced by dietary protein and water intake.



Cross-section of the Alzet® osmotic pump (model 2ML1). The dimensions are: Total length, 54 mm; Diameter, 14 mm; Weight (empty), 5.7 g; Volume (reservoir), 2 ml; Nominal delivery rate, 10  $\mu$ l/h.

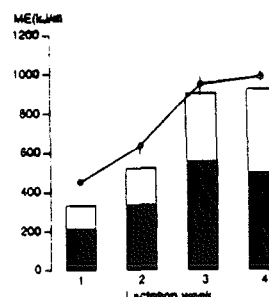
Poster: "XXXIII International Congress of Physiological Sciences" in St. Petersburg 30/6-5/7 1997. 2 tables, 3 figs., 4 refs. Author's Introduction, Aim and Conclusions.

### Accurate measurements of daily milk intake in suckling mink (*Mustela vison*) kits

Søren Wamberg, Anne-Helene Tauson

The daily milk production of mink dams during the first 4 weeks of lactation is uncertain, although some estimates have been made by factorial methods. Direct measurements of the milk yield of female mink have been reported in only a single study at peak lactation (Ofte-dal, 1981). Therefore, we measured the daily milk intake of 42 mink kits during the first 4 weeks of postnatal life using the tritiated water dilution technique.

- The THO dilution technique is suitable for direct measurement of milk intake in mink kits.
- The daily milk yield of the mink dam increased as lactation progressed, reaching a maximum of about 30 g/kit per day, or more than 200 g/dam per day.
- The calculated total milk energy output by the dam, in excess of 1 MJ/day, corresponded well with the estimated daily energy requirements for growth and maintenance of the kits.



Mean daily energy output (ME; kJ/dam/day) in mink milk (♦) compared to the estimated daily energy requirements for body growth (dark blue) and maintenance (light blue) of the kits during weeks 1-4 post parturition.

Poster: "The Waltham International Symposium on Pet Nutrition and Health in the 21st Century" in Orlando, Florida, Maj 26-29, 1997. 1 table, 2 figs., 4 refs. Authors' Introduction and Conclusions.

**The influence of supplementary feed loose-fat ERAFET on productivity parameters of polar foxes**

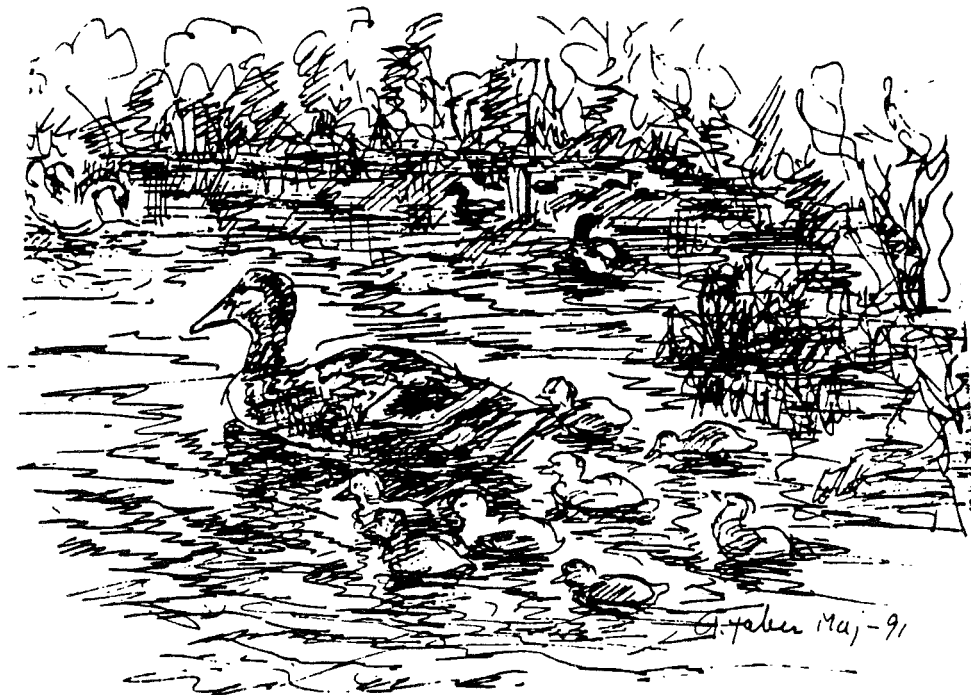
*Manfred Oskar Lorek, Andrzej Gugolek*

With the aim of determining the usability of loose-fat ERAFET in feeding polar foxes, the influence of that preparation was examined on the basis of some productivity indices. The study was carried out on 120 young foxes from weaning to slaughter. The animals were divided into 3 equal groups. Group I, which served as a control, consisted of animals fed without the addition of ERAFET. Groups II and

III, the experimental animals, were fed the same diet as the animals from the control group but supplemented with ERAFET in the following proportions: 4% (group II) and 8% (group III).

The results obtained proved the possibility of using loose-fat ERAFET in feeding polar foxes with some advantageous influence on the examined productivity indices.

*Anim. Prod. Rev. (Poland). Appl. Science Reports 15, pp. 153-163, 1994. In POLH, Su. ENGL. 5 tables, 14 refs. Authors' summary.*



*Original Report***Indices of blood metabolic profile in foxes  
with lung-heart syndrome\****Leon Saba<sup>1</sup>, Antoni Kopczewski<sup>2</sup>, Hanna Bis-Wencel<sup>1</sup>**Leszek Tymczyzna<sup>1</sup>, Bożena Nawakowicz-Debek<sup>1</sup>*

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**Summary**

In the mid-eighties in Canada a new, unknown, disease syndrome was described and defined as congestive cardiomyopathy or congestive cardiac insufficiency in young foxes. Some authors consider a taurine deficiency as an etiological factor. Yet there is no confirmed evidence for this. In the present investigation we have determined basic blood indices of sick and healthy foxes as an element of the pathogenetic course of the disease. It is evident that lung-heart syndrome in young foxes has the character of a metabolic disease. Despite a rapid level of clinical symptoms the changes in the organism appear gradually and slowly. Functional failure of liver, kidney, and heart in young foxes affected by the lung-heart syndrome is one of the elements of this disease pathogenesis and the changes stated in hematologic indices give evidence of damage of the red blood cell system.

**Introduction**

In the mid-eighties in Canada a new, then unknown disease was described and defined by Fernst and Clark and Onderka as congestive cardiomyopathy or congestive cardiac insufficiency in young foxes (3,5). In Poland this disease was called "lung-heart" syndrome (6). Its etiology had not been known so far; however, contagious and parasitic factors were excluded (6).

Some authors consider a taurine deficiency as an etiological factor (6). However, there is still no confirmed evidences for this point. The available bibliography does not contain any data on the changes in the metabolic profile in sick foxes in comparison to healthy ones.

It is advisable to determine basic blood indices of sick and healthy foxes as an element of the pathogenetic course of the disease.

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\* The work financed by the Committee for Scientific Research (CSR) No 5S 310 006 07

## Material and methods

The analyses were made in two farms (A and B) on black-silver foxes over two years time where cases of lung-heart syndrome have appeared with various intensity for many years. The examinations included 30 sick foxes every year in animals who showed evident clinical symptoms of lung-heart syndrome, and 30 healthy foxes of the same age from the same farms.

The blood was collected from sick foxes in the final stage of the disease. The hematologic indices were fixed after standard methods in the full blood. Biochemical parameters i.e. level of urea, glucose, bilirubin, total cholesterol,

creatinine and general protein were established according to monostests from the firm Cormay s.c. (Poland). Activity of AspAT, ALAT, LDH, CPK was determined after the monostests of the firm POGH-Gliwice (Poland). Levels of Ca, Mg, Na were fixed using ASA. The results were calculated as the means, standard deviations and significant differences between sick and healthy animals were defined.

## Results and discussion

The hematologic indices are presented in Table 1. It proves that lung-heart syndrome causes clear changes in the indices of the erythrocyte system. Characteristically, there were no changes in the leucocyte count (1).

Table 1. Hematologic indices

FARM	Year	Hematocrit l/l				Erythrocytes $10^{12}/l$				Leucocytes $10^9/l$				Hemoglobin mmol/l			
		sick foxes		healthy foxes		sick foxes		healthy foxes		sick foxes		healthy foxes		sick foxes		healthy foxes	
		X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
A	I	0,38 a	0,05	0,48 b	0,06	5,02 a	0,9	8,3 b	0,6	8,6 a	1,0	8,9 a	1,8	5,0 a	0,5	7,8 b	0,6
	II	0,36 a	0,04	0,50 b	0,07	5,7 a	0,5	8,9 b	0,4	9,1 a	2,1	8,8 a	1,3	5,3 a	0,6	8,2 b	0,7
B	I	0,35 a	0,05	0,55 b	0,06	6,0 a	0,4	7,9 b	0,6	8,9 a	0,8	9,1 a	1,0	5,5 a	0,4	8,1 b	1,1
	II	0,33 a	0,04	0,54 b	0,07	5,8 a	0,5	8,2 b	0,9	9,0 a	0,9	9,5 a	1,3	6,1 a	0,5	9,3 b	0,8

## Explanations

The examinations were made on 30 sick foxes and 30 healthy ones every year. The means denoted with different letters show significant statistical differences.

Table 2. Biochemical indices of fox blood serum

FARM	Year	Urea mmol/l				Glucose mmol/l				Bilirubin $\mu$ mol/l			
		sick foxes		healthy foxes		sick foxes		healthy foxes		sick foxes		healthy foxes	
		X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
A	I	8,4 a	1,3	5,0 b	0,4	5,6 a	1,1	6,9 b	0,6	3,6 a	1,3	0,5 b	0,1
	II	9,1 a	0,9	5,3 b	0,8	4,9 a	0,9	7,2 b	1,1	4,1 a	1,5	0,6 b	0,2
B	I	8,6 a	1,2	4,8 b	0,9	5,8 a	1,3	6,9 a	1,0	4,5 a	1,4	0,5 b	0,1
	II	9,4 a	0,8	5,2 b	0,7	5,1 a	0,8	7,3 b	0,8	4,9 a	0,9	0,7 b	0,3
		Cholesterol mmol/l				Creatinine $\mu$ mol/l				General protein g/l			
		sick foxes		healthy foxes		sick foxes		healthy foxes		sick foxes		healthy foxes	
		X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
A	I	7,1 a	1,3	6,0 a	1,3	91 a	5,6	51 b	6,1	75 a	5,0	61 b	4,0
	II	8,3 a	1,4	5,8 b	0,5	89 a	7,8	42 b	6,0	77 a	4,0	59 b	5,0
B	I	7,5 a	1,5	6,1 a	1,6	87 a	6,1	45 b	10,1	81 a	3,2	65 b	4,8
	II	7,9 a	0,9	5,8 b	1,7	96 a	6,3	51 b	8,9	72 a	4,1	69 a	3,2

The biochemical parameters established indicate evident differences in the level of urea, bilirubin, and creatinine between healthy and sick animals. Some changes also occurred in the levels of glucose, cholesterol and general protein (Table 2). That gives evidence of liver and kidney damage throughout the disease course (4, 6).

The data mentioned above were confirmed by the examinations of the enzyme activity given in Table 3. The activity of transaminases, lactate dehydrogenase and creatine kinase reveal the damage of liver and kidneys already mentioned as well as of muscles. With regard to mineral element level in the fox serum (Table 4)

there were changes in K, Mg and Na concentrations (2). The data presented indicate that the changes in the lung-heart system appearing spontaneously together with deterioration of circulatory and respiratory insufficiency produce secondary changes due to venous congestion and kidney anoxia. Kidney insufficiency that then occurs causes disturbances in the sodium-potassium equilibrium, which in turn brings about myocardial insufficiency. It is significant that the Canadian authors, Ferns, Clark (3) and Onderka (5) dealing with the question of lung-heart syndrome do not record any pathologic changes in kidneys in the course of the disease discussed.

Table 3. Activity of some enzymes in fox serum

F A R M	Year	Asp AT μkat/l				AlAT μkat/l				LDH U/l				CPK U/l			
		sick foxes		healthy foxes		sick foxes		healthy foxes		sick foxes		healthy foxes		sick foxes		healthy foxes	
		X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
A	I	0,88 a	0,08	0,31 b	0,04	0,180 a	0,011	0,120 b	0,025	180 a	10,0	90 b	9,6	75 a	5,0	42 b	4,0
	II	0,91 a	0,06	0,41 b	0,03	0,190 a	0,021	0,118 b	0,031	160 a	12,1	110b	8,3	87 a	4,5	50 b	5,1
B	I	0,82 a	0,07	0,32 b	0,04	0,190 a	0,031	0,135 b	0,042	175 a	14,2	95 h	9,5	90 a	5,6	45 b	5,3
	II	0,87 a	0,09	0,35 b	0,04	0,180 a	0,032	0,141 b	0,043	180 a	10,0	110b	8,3	74 a	4,8	49 b	4,8

Table 4. Mineral elements in fox blood

F A R M	Year	Ca mmol/l				K mmol/l				Mg mmol/l				Na mmol/l			
		sick foxes		healthy foxes		sick foxes		healthy foxes		sick foxes		healthy foxes		sick foxes		healthy foxes	
		X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
A	I	2,3 a	0,5	2,4 a	0,4	3,3 a	1,0	5,1 b	0,6	0,7 a	0,3	1,2 b	0,1	135 a	10,0	151 b	4,1
	II	2,5 a	0,6	2,8 a	0,3	3,5 a	0,9	5,3 b	0,8	0,8 a	0,4	1,3 b	0,2	132 a	9,2	149 b	4,2
B	I	2,7 a	0,7	2,6 a	0,2	3,0 a	0,8	5,6 b	0,7	0,8 a	0,3	1,4 b	0,3	134 a	8,3	152 b	4,3
	II	2,6 a	0,8	2,5 a	0,3	3,2a	0,9	5,7 b	0,6	0,7 a	0,4	1,4 b	0,1	132 a	10,1	159 b	4,5

According to many authors the course of this disease is quite rapid (3, 5, 6). However, the changes in the blood metabolic profile show that the process takes a slow course and its biochemical manner confirms that lung-heart syndrome is of a metabolic disease character.

The results have shown that

1. In the light of the data presented it is evident that lung-heart syndrome in young foxes has the character of a metabolic disease.
2. Despite a rapid level of clinical symptoms the changes in the organism appear gradually and slowly.
3. Functional failure of liver, kidney, and heart in young foxes affected by lung-heart syndrome is one of the elements of this disease pathogenesis.
4. The changes found in the hematologic indices give evidence of damage of the red blood cell system.

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*Original Report***Taurine in serum, bile and feed of foxes  
with lung-heart syndrome\***

Leon Saba<sup>1</sup>, Antoni Kopczewski<sup>2</sup>, Jerzy Slawon<sup>2</sup>, Hanna Bis-Wencel<sup>1</sup>, Leszek Tymczyna<sup>1</sup>

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**Abstract**

Alimentary deficiency of taurine causes congestive cardiomyopathy in cats. A disease with a similar course appears in juvenile foxes. Ferns and Clark (2) stated as first that taurine deficiency may be a possible cause of congestive cardiomyopathy as there is only slight amount of it in some types of feed, besides which feed thermal processing clearly decreases the taurine level. Therefore, an assumption emerges that taurine deficiency in foxes may be conducive to lung-heart syndrome in juvenile foxes.

That is why the objective was to determine taurine level in the serum, bile and feed of foxes at the ranches where lung-heart syndrome occurred.

**Material and methods**

The investigations were made in two farms with black and silver foxes where lung-heart cases have appeared with various intensity for many years.

Amino acids were fixed in 85 blood serum samples from animals aged 2-3 months affected by clinical signs of lung-heart syndrome and the blood samples were collected at the final stage of the disease. Another 85 blood samples were gathered from non-affected foxes coming from the same ranches and of the same age.

Taurine concentration was established in 50 samples of biliary fluid from dead foxes affected by this disease and 50 samples from foxes experimentally slaughtered coming from the same ranches.

Moreover, taurine concentration was determined in 60 feed samples from four affected ranches where cases of the disease was noted and in the same number of feed samples coming from the farms free from this disease, yet in the same region.

Taurine determination was made after a colorimetric method according to Curzon and Giltrow (1).

## Results and discussion

The data on taurine concentration in blood serum and bile of foxes are presented in Table 1 and 2. They demonstrate that in both fluids the amino acid amount was lower in foxes affected than in non-affected ones coming from the same ranches. As for now it can not be stated what taurine level in serum and biliary fluid is physiological.

**Table 1.** Taurine amount in blood serum of black and silver juvenile foxes in  $\mu\text{mol/ml}$

Ranch	n <sup>x</sup>	n <sup>xx</sup>	Affected foxes		Non-affected foxes	
			x	SD	x	SD
A	45	45	1.4 <sup>a</sup>	0.2	2.0 <sup>b</sup>	0.3
B	40	40	1.5 <sup>a</sup>	0.3	2.1 <sup>b</sup>	0.2

n<sup>x</sup> - sick animals (test size)

n<sup>xx</sup> - healthy animals (test size)

a; b - means differ significantly if marked with different letters

**Table 2.** Taurine content in biliary fluid of black and silver foxes in  $\mu\text{mol/ml}$

Ranch	n <sup>x</sup>	n <sup>xx</sup>	Affected foxes		Non-affected foxes	
			x	SD	x	SD
A	27	27	0.8 <sup>a</sup>	0.1	1.5 <sup>b</sup>	0.2
B	23	23	0.9 <sup>a</sup>	0.2	1.3 <sup>b</sup>	0.3

n<sup>x</sup> - sick foxes (test size)

n<sup>xx</sup> - foxes slaughtered experimentally (test size)

a; b - means differ significantly if marked with different letters

Ferns and Clark (2) maintain that as foxes belong to the canid order, one can expect that taurine synthesis in foxes is more similar to their synthesis in dogs than in cats.

Taurine synthesis in the cat is very slow, considerably slower than in the dog and is linked to a low activity of enzymes that metabolize cysteine to taurine. The question is whether this

effect appears also in foxes. Cardiomyopathies are a result of difficult metabolisation of taurine and some authors (3, 5) consider them to be one of predominant diseases of the circulatory system in cats and some other animals from Felidae i.e. lions.

As late as in 1987 a relation between congestive cardiomyopathy in cats and their taurine deficiency was discovered. That may have drawn the attention of Onderka (4), Ferns and Clark (2) to a possibility that so called lung-heart syndrome in juvenile foxes can be connected with taurine deficiency in some cases.

Yet, there is one definite difference namely ecstatic cardiomyopathy in cats which, as a rule, involves older animals that are eight-ten years old and manifests itself with circulatory insufficiency due to a disease of the two ventricles of the heart.

It is interesting that in foxes the same symptoms occur in two or four months-old animals and disappear with their growth period completion.

It seems worth considering if, in the presence of earlier confirmed long processes of taurine metabolisation, a probable deficiency of taurine in a fox dam is not reflected in this amino acid level in juvenile foxes resulting in the appearance of symptoms of cardiomyopathy.

This suggestion is presented because in some farms plant products which constitute a significant percentage of the feed ration do not contain taurine. In turn Odle et al. (3) found in their accurate dietary examinations that taurine coming from poultry waste is absolutely not digestible for cats. It is also known if this is also the case for foxes.

Moreover, due to thermal processing of all the feeds of animal origin the amount of taurine destroyed reaches up to 50%.

The hypothesis on a probable etiological factor that there may be a taurine deficiency in lung-heart syndrome is put forward by the present

authors and can be confirmed by the data demonstrated in Table 3. It is evident that the taurine level in feeds used in the ranches where lung-heart syndrome appears is statistically lower compared to the farms where the syndrome has not yet been recorded.

**Table 3.** Taurine concentration in feeds in  $\mu\text{mol/g}$

Ranches	n <sup>x</sup>	x	SD	n <sup>xx</sup>	x	SD
A	15	1.07 <sup>a</sup>	0.2	15	1.93 <sup>b</sup>	0.2
B	15	1.09 <sup>a</sup>	0.3	15	2.01 <sup>b</sup>	0.3
C	15	1.08 <sup>a</sup>	0.2	15	2.02 <sup>b</sup>	0.3
D	15	2.1 <sup>a</sup>	0.3	15	2.03 <sup>b</sup>	0.2

n<sup>x</sup> - number of feed samples from affected ranches

n<sup>xx</sup> - number of feed samples from unaffected ranches

a; b - means differ significantly if marked with different letters

As already mentioned data on the dietary requirement for taurine in foxes is not available so it is impossible to relate to this observation.

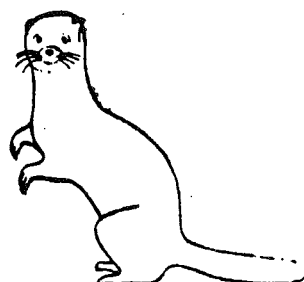
**Results**

1. It was found that taurine concentration in blood serum of affected black and silver juvenile foxes showing lung-heart syndrome symptoms was significantly lower compared to non-affected animals of the same variety and age from the same ranches.

2. It was proved that taurine concentration in biliary fluid of black and silver foxes affected by lung-heart syndrome was lower than in non-affected ones of the same age and from the same ranches.
3. The investigations on taurine content in feeds from the ranches where lung-heart disease appeared demonstrated lower taurine content than in feeds from the unaffected farms.
4. Taurine content in physiologic salines and feeds administered to the foxes may be evidence of its contribution to the etiology or pathogenesis of lung-heart disease, yet research in this field is still in the early stage and needs further work.

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*Original Report*

**The residues of methylbrompheninfos (Polwet 5 and Polwet 20)  
in the tissues and organs of polar foxes after action  
against external parasites**

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=

### Summary

The investigation aimed at evaluating the residues of methylbrompheninfos used in the preparations Polwet 5 and Polwet 20 in polar foxes in the doses required for action against the external parasites *Sarcoptes scabiei* v. *canis*, *Otodectes cynotis*, *Chaetopsylla globiceps*.

The residues of methylbrompheninfos in the tissues were determined by the gas chromatography method in 3, 7, 14 and 28 days after the last application of the preparations.

The methylbrompheninfos (Polwet 5 and Polwet 20) residues 3 days after the last administration of the preparation fell within the limits 0.088-0.006 mg/kg b.w.

28 days after the last Polwet 5 and Polwet 20 exposition the methylbrompheninfos residues were reduced. The highest reduction was observed in the fat tissue – from 0.015 to 0.007, in the muscle tissue, liver and kidneys it fell within the limits 0.009-0.003 mg/kg b.w.

### Introduction

Methylbrompheninfos is one of two original enolphosphates synthesized in Poland. It shows a relatively low toxicity, especially dermal toxicity and thus can be used against external parasites in both farm livestock and domestic animals (*Sciesinski, 1977*).

Our earlier investigations (*Sciesinski, 1995*) confirmed a high efficiency of Polwet 5, Polwet 20 and Polwet aerosol (methylbrompheninfos) against *Sarcoptes scabiei* v. *canis*, *Otodectes cynotis* and *Chaetopsylla globiceps* in polar fox and silver fox.

The aim of the present investigation was to determine the methylbrompheninfos residues in the tissues and organs of polar fox after the administration of Polwet 5 and Polwet 20 in the doses recommended against external parasites. Although fox carcasses are not used for human consumption they are often fed to foxes and pigs, thus re-entering the food chain and the acaricides and insecticides which they contain may affect the health of the animals.

## Material and methods

The investigation was performed on a cooperative farm with polar foxes aged 4 to 8 years with a body weight of about 6 kg during the December-January period.

In the first group comprising 8 polar foxes (half of them males and half females), the animals were rubbed with 1% aqueous Polwet 5 emulsion (5 times with 3 day intervals) containing 5% methylbrompheninfos in the doses recommended against itch mite (*Sarcoptes scabiei v. canis*) in polar foxes.

In the second group also comprising 8 polar foxes (half of them males and half females), the animals were rubbed (5 times with 3 day intervals) with the mixture containing 1 part Polwet 5 and 1 part liquid paraffin in the doses used against the auricular itch mite (*Otodectes cynotis*).

The third group comprised 8 polar foxes (half of them males and half females) which were sprayed twice, with a 10 day interval, with 0.1% aqueous emulsion prepared with Polwet 20 (containing 20% methylbrompheninfos) in the doses applied against fleas (*Chaetopsylla globiceps*).

On the third day after the last application of the preparations 2 animals from each group (one male and one female) were diagnostically slaughtered. The next slaughters took place on the 7th, 14th and 28th day after the last application of the preparations.

Samples for the investigations were collected from the muscles *Musculus trapezius thoracis*, *M. gluteus superf.*, *m. psoas major*, and *M. psoas minor*, as well as subcutaneous fat from the lumbar region, peritoneal and perirenal fat as well as the liver and kidneys.

The method of methylbrompheninfos determination in the tissues and organs was adapted by Knapek and Utracki from the Institute of Organic Industry and is based on the method for the brompheninfos determination in ani-

mal material worked out by Juszkiewicz et al. (1971). The method aims at determining methylbrompheninfos in the muscular tissue, kidney and liver with the help of gas chromatography. The lower detectability limit amounts to 0.003 mg/kg (Sciesinski et al. 1984).

The method consists in the repeated extraction of methylbrompheninfos from the muscles, fat, liver and kidneys using acetonitrile. After dilution with a 2% aqueous solution of sodium sulphate the remnants were re-extracted with petroleum ether and after concentration was purified in a florisil column.

Petroleum ether was used in the methylbrompheninfos extraction from the fatty tissue. Then the remnants were re-extracted to acetonitrile and after dilution with a 2.5% aqueous solution of sodium sulphate it was captured in chloroform.

After chloroform evaporation the extract was purified in the florisil column. After evaporation of the eluate the remnants were dissolved in acetone and its methylbrompheninfos contents were determined using the gas chromatography method.

## Results and discussion

No clinical symptoms were observed in the investigated animals after the application of the preparations.

The results of the determinations of the methylbrompheninfos residues (Polwet 5 and Polwet 20) in the tissues of the polar foxes are presented in Table 1.

The methylbrompheninfos residues (Polwet 5) applied in a 1% emulsion in the doses controlling itch mites (*Sarcoptes scabiei v. canis*) in the subcutaneous fatty tissue of polar foxes 3, 7, 14 and 28 days after the last application of the preparation fell within the limits from 0.086 to 0.015, in the muscular tissue from 0.060 to 0.005, in the liver from 0.045 to 0.005, and in the kidney from 0.009 to 0.005 mg/kg b.w.

Table 1. Methylbrompheninfos residues in muscular and fatty tissues as well as liver and kidneys of polar foxes

Group		Methylbrompheninfos residues			
		No. of days after last application			
		3	7	14	28
1		2	3	4	5
<u>Polwet 5 1% aqueous emulsion</u>					
Fatty tissue	control	0.003	0.003	0.003	0.003
		0.081	0.051	0.015	0.016
		0.092	0.042	0.025	0.015
	mean	0.086	0.046	0.020	0.0155
Muscular tissue	control	0.003	0.003	0.003	0.003
		0.031	0.028	0.040	0.003
		0.060	0.013	0.035	0.009
	mean	0.045	0.0205	0.037	0.006
Liver	control	0.004	0.003	0.003	0.003
		0.035	0.010	0.030	0.006
		0.009	0.008	0.010	0.005
	mean	0.022	0.009	0.020	0.0055
Kidney	control	0.003	0.003	0.003	0.003
		0.010	0.015	0.012	0.006
		0.009	0.020	0.018	0.004
	mean	0.0095	0.017	0.015	0.005
<u>Polwet 5 2.5% emulsion with liquid paraffin</u>					
Fatty tissue	Control	0.003	0.003	0.003	0.003
		0.080	0.030	0.035	0.010
		0.097	0.021	0.020	0.015
	mean	0.088	0.025	0.027	0.012
Muscular tissue	control	0.004	0.003	0.003	0.003
		0.086	0.021	0.020	0.010
		0.081	0.026	0.028	0.009
	mean	0.083	0.023	0.024	0.0095
Liver	control	0.003	0.003	0.003	0.003
		0.040	0.010	0.019	0.007
		0.035	0.015	0.020	0.004
	mean	0.037	0.012	0.019	0.005
Kidneys	control	0.003	0.003	0.003	0.003
		0.012	0.010	0.090	0.006
		0.010	0.009	0.015	0.007
	mean	0.011	0.009	0.052	0.006

The methylbrompheninfos residues in the doses effective against auricular itch mites (*Otodectes cynotis*) in the polar fox 3, 7, 14 and 28 days after the last application of the preparation amounted to: 0.088-0.005 in the fatty tissue, 0.083-0.009 in the muscular tissue, 0.037-0.005 in the liver and 0.011-0.006 mg/kg b.w. in the kidney.

In the last group of polar foxes which received a 0.1% emulsion of Polwet 20 in the doses used against fleas (*Chaetopsylla globiceps*), the methylbrompheninfos residues 3, 7, 14 and 28 days after the last application fell within the limits of 0.037-0.007 in the fatty tissue, 0.021, 0.004 in the muscular tissue, .006-0.003 in the liver and 0.007-0.004 mg/kg b.w. in the kidney.

The investigations of the methylbrompheninfos (Polwet 5 and Polwet 20) residues in the polar fox tissues in the concentration controlling external parasites in polar foxes showed that in 3 days its concentration in the fatty tissues fell within the limits of 0.088-0.037, in the muscular tissue 0.083-0.021, in the liver 0.045-0.006 and in the kidney 0.22-0.007 mg/kg b.w. 28 days after the last application of the preparations the residue level decreased assuming the following values: 0.015-0.003 in the liver and 0.006-0.004 mg/kg b.w. in the kidney.

The highest level of the methylbrompheninfos residues in the polar foxes 28 days after the application of Polwet 5 and Polwet 20 were observed in the fatty tissue where it amounted to 0.015-0.007 mg/kg b.w. In the muscular tissue, liver and kidney they amounted to 0.009-0.003 mg/kg b.w. thus nearly within the same limits as the methylbrompheninfos residues in the animals of the control group.

The methylbrompheninfos residue level in the polar foxes agrees with the results obtained in other species, such as pigs, cattle and sheep (Sciesinski, 1997).

In view of the lack of knowledge of methylbrompheninfos tolerance, the withholding period cannot be calculated for these preparations

for polar foxes in case of the fox meat is fed to other animal species.

The problem of feeding foxes with carcasses contaminated with methylbrompheninfos will be the subject of the next report.

### Conclusions

1. The investigations of methylbrompheninfos (Polwet 5 and Polwet 20) residues in the tissues of polar foxes given concentrations controlling external parasites (*Sarcoptes scabiei* v. *canis*, *Otodectes cynotis*, *Chaetopsylla globiceps*) showed that 3 days after the 1st application they fell within the limits of 0.088-0.006 mg/kg b.w.

The highest amounts of the residues were observed in the fatty tissue – 0.088 and muscular tissue – 0.083 mg/kg b.w. when applying a 2.5% emulsion Polwet 5.

2. 28 days after the last application of Polwet 5 and Polwet 20 the level of the methylbrompheninfos residues decreased with the highest amounts observed in the fatty tissue – from 0.015 to 0.007 and in the muscular tissue, liver and kidney from 0.009 to 0.003 mg/kg b.w.

### Acknowledgement

The author wants to express his warmest gratitude to Dr. Jerzy Motz from the Breeding Farm LAS at Skolimow and to Prof. Dr. Stanislaw Byrdy from the Institute of Organic Industry at Pszczyna for making this investigation possible.

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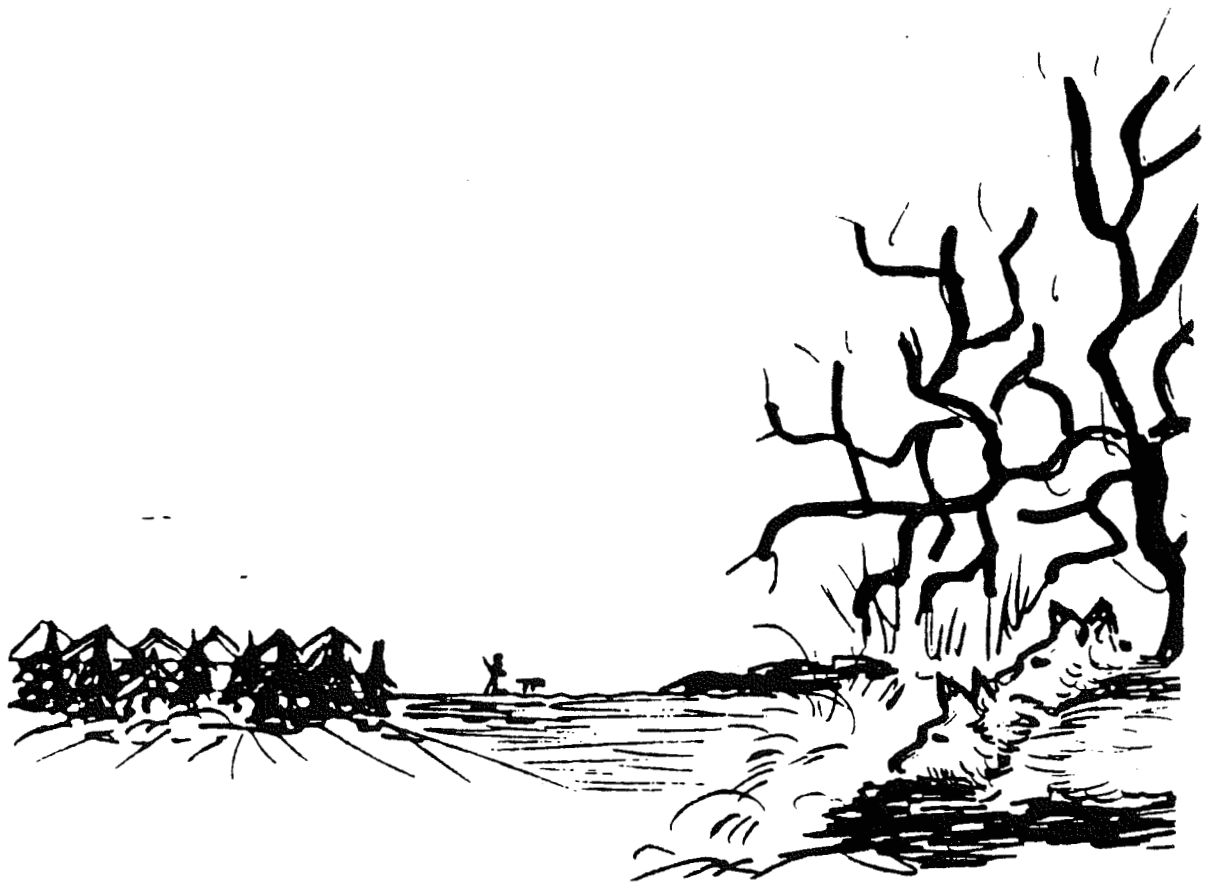
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ylbrompheninfos) against external parasites (*Sarcoptes scabiei* v. *canis*, *Otodectes cynotis*, *Chaetopsylla globiceps*) in polar and silver foxes. *Scientifur*, Vol. 20, No. 1.

Sciesinski, K. 1997. Pozostalosci krajowych preparatow z grupy enolofosforanow w tkankach i narzadach zwierzat gospodarskich oraz w mleku. Praca habilitacyjna SGGW.



**Influenza A virus, H10N4,  
Naturally pathogenic for mink (*Mustela vison*)**

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New doctor in the family. We congratulate Lena Englund, Vet. Med. Dr. with the new title and the excellent scientific work the thesis is based on.

The thesis summarizes and discusses the results from studies of an influenza A virus isolated from an outbreak of pneumonia among farmed mink in Sweden. This new disease in mink was described based on clinical, serological, and pathological investigations and the causality established by experimental infection of mink. The virus was identified and named A/mink/Sweden/84 (H10N4). Serological investigations showed that this virus was not present in mink in other areas of the country. A direct transmission of virus from birds to mink was suggested, since the subtypes H10 and N4 had previously only been isolated from birds. The genetic relationships between the mink-virus and three avian derived influenza viruses of subtype H10 were analysed by oligonucleotide mapping. The minkvirus was shown to be closely related to two avianderive H10N4 viruses and less related to the prototype avian H10 strain, A/chicken/Germany/49 (H10N7). Experimental infection of mink with these four H10 influenza strains showed that all four viruses stimulated an antibody-mediated immune response. All three H10N4 viruses also caused clinical disease in mink and spread through contact, whereas the H10N7 virus only caused mild lung lesions but no clinical disease or contact transmission. Experimental aerosol infection of mink was used to study the early lesions in the respiratory tract caused by the H10N4 virus from mink and the prototype avian H10 virus. Through immunohistochemistry, morphometrical analysis of the pneumonia, histopathology and virus culture, marked differences in pathogenicity were observed

between the two viruses. The H10N4 virus was re-isolated from all infected mink, whereas no H10N7 virus could be re-isolated. Both viruses caused a broncho-interstitial pneumonia in the infected mink. However, the spread of the virus within the respiratory tract and the area density of pneumonia peaked on day two for the H10N7 virus, whereas the H10N4 virus from mink continued to spread all through the one-week observation period, ultimately killing one of the infected mink on day seven. An additional study indicated that the differences in virus spread *in vivo* could be modelled *in vitro* in mink lung-cell cultures.

*Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala 1997, 52 pp. 2 tables, 2 figs., 145 refs. Author's abstract.*

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Klingeborn, B., Englund, L., Rott, R., Juntti, N. & Rockborn, G. 1985. An avian influenza virus killing a mammalian species – the mink. *Arch. Virol.* 86: 347-351. Abstracted in *Scientifur*, Vol. 10, No. 2, pp. 132, 1986.
- II. Englund, L., Klingeborn, B. & Mejerland, T. 1986. Avian influenza virus causing an outbreak of contagious interstitial pneumonia in mink. *Acta vet. scand.* 27: 497-504. Abstracted in *Scientifur*, Vol. 11, No. 4, pp. 345, 1987.

- III. Berg, M., Englund, L., Abusugra, I.A., Klingeborn, B. & Linné, t. 1990. Close relationship between mink influenza (H10N4) and concomitantly circulating avian influenza viruses. *Arch. Virol.* 113: 61-71. Abstracted in *Scientifur*, Vol. 15, No. 2, pp. 154, 1991.
- IV. Englund, L. & Hård af Segerstad, C. Two avian H10 influenza A virus strains with different pathogenicity for mink (*Mustela vison*). Submitted. Abstracted in this issue of *Scientifur*.

#### Two avian H10 influenza A virus strains with different pathogenicity for mink (*Mustela vison*)

L. Englund, C. Hård af Segerstad

ADP (%)

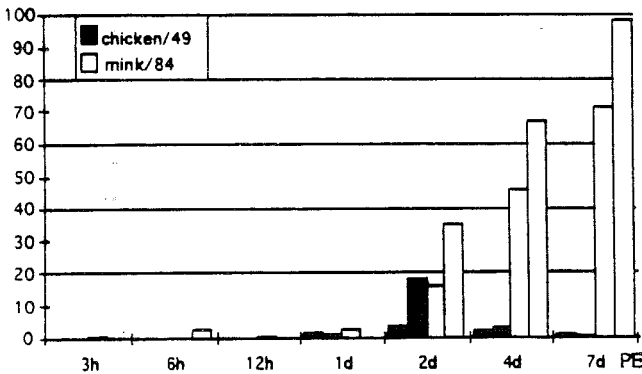


Fig. 4. Area density of pneumonia (ADP) for each mink exposed to influenza A virus mink/84 (open bars) and chicken/49 (filled bars), respectively. Time PE refers to the interval between exposure to virus aerosol and euthanasia. All ADPs for the other infected mink, sham and control mink were below 0.15% and thus not plotted in this diagram.

Influenza virus infections are endemic in horses and swine but not in other mammalian animal species. Avian influenza viruses are normally non-pathogenic to mammals but do occasionally cross the species barrier. One such crossover with avian influenza, H10N4, caused an outbreak of severe respiratory disease in farmed mink. We compared two strains of avian influenza A viruses of subtype H10 by exposing mink kits to aerosols containing ei-

ther A/mink/Sweden/3900/84 (H10N4) or A/chicken/Germany/N/49 (H10N7). Both virus strains caused pneumonia and antibody production in exposed mink but only mink/84 virus was re-isolated. The lesions after exposure to mink/84 were more severe with higher area density of pneumonia, lower daily weight gain, and more abundant virus presence in the tissues as shown by immunohistochemistry. Both virus strains infected epithelial cells in the respiratory tract and the results indicated that mink/84, but not chicken/49 virus, established multiple cycle replication. The reasons for the differences in pathogenicity may, thus, primarily be found among factors influencing virus replication and spread rather than among those regulating initial virus adherence and entry.

Paper IV submitted, 26 pp. 4 figs., 59 refs. Authors' summary.

#### The health condition of farm fitches on some Polish farms

Olga Szeleszczuk, Rafal Przybyla, Piotr Niedbala

The aim of this study was to estimate the health condition of farm fitches with particular reference to Aleutian disease (plasmacytosis) and its diagnosing by the use of non-specific Iodine Agglutination Test (IAT), a specific test, Counter Current Immunoelectrophoresis (CIEP) and anatomopathological examination. The research was done on 275 young fitches on three farms situated in the Rzesow district (Farm A), Czestochowa (Farm B) and Suwalki (Farm C).

The evaluation of salubrity of farm fitches on the farms under study showed that 64.8% of the animals were ill, including 2.3% with Aleutian disease. Plasmacytosis in farm fitches is of a chronic nature, its course being mild without the characteristic anatomopathological lesions such as those observed in mink.

*Anim. Prod. Rev. (Poland). Appl. Science Reports* 15, pp. 193-199, 1994. In POLH, Su. ENGL. 3 tables, 18 refs. Authors' summary.

### The usefulness of moxidectin (Cyanamid) for the control of ear scab in rabbits

Alexandra Balicka-Laurans

The studies were carried out on 28 rabbits naturally infected with *Psoroptes cuniculi*. It was found that moxidectin (Cyanamid) administered in a single dose of 400 mg/kg of body weight was 100 per cent effective against ear scab in rabbits. Moxidectin diluted 1:10 in distilled water (the basic solution contained 1% of active substance) facilitated the administration of the drug and did not cause any lesions at the site infection.

*Anim. Prod. Rev. (Poland). Appl. Science Reports 15, pp. 201-205, 1994. In POLH, Su. ENGL. 2 tables, 10 refs. Author's summary.*

### Feral mink (*Mustela vison*) and their potential as disease vectors in Ireland: An investigation in Co. Wicklow

Kevin O'Crowley, James G. Wilson

Mink have been farmed in Ireland since 1951, and feral animals have now been reported in all 21 counties. Although their home range is quite small (2-3 km), juveniles will range up to 50 km in search of territory, and this, in conjunction with opportunistic feeding habits, makes the mink a potentially important reservoir and vector of a number of pathological conditions. In this survey, 15 mink taken along the Slaney river in Co. Wicklow were subjected to a range of parasitological, bacteriological, haematological and immunological investigations.

In general, the animals were in good condition, apart from one individual over two years old. Few ectoparasites or endoparasites were found and haematology indicated little of significance. No evidence was found of tuberculosis or brucellosis infection, nor was *Salmonella* detected in any of the animals.

Seven adult animals had positive titres for *Toxoplasma gondii*, but cysts were not found on

histological examination of the brains. All juveniles tested were negative. From this survey there is little evidence that mink are important vectors of disease or infection for domestic livestock. Whether this is due to the life-style of the mink, or to the location, should be the subject of further investigation.

*Irish Veterinary Journal 44, pp. 71-74, 1991. 2 tables, 1 fig., 12 refs. Authors' summary.*

### Granular cell tumour in the central nervous system of a ferret (*Mustela putorius furo*)

J.M. Sleeman, V.L. Clyde, K.A. Brenneman

A 4-year-old castrated ferret was presented with a 3-week history of intermittent, progressive right head tilt, circling to the right and ataxia which had progressed to seizures. Although treatment with diazepam controlled the seizures initially, the ferret was destroyed when increasing doses of diazepam failed to control the seizures. The most significant finding at PM examination was a large expansile mass located in the medial portion of the right forebrain. Histological examination revealed a granular cell tumour. This appears to be the first report of a primary neoplasm of the central nervous system in a ferret.

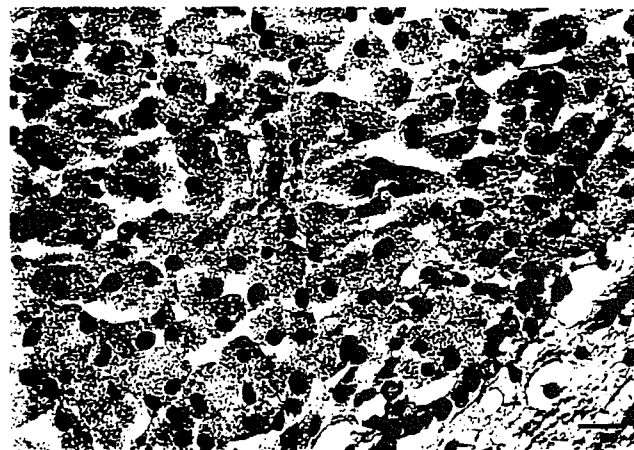


FIG 2: Photomicrograph of a granular cell tumour in the brain of a ferret showing a sheet of polygonal cells with abundant, finely granular cytoplasm bordered by a compressed, oedematous internal capsule and a short segment of ependyma. Haematoxylin and eosin. bar = 15 µm

*Veterinary Record 138 (3), pp. 65-66, 1996. 2 figs, 11 refs. CAB-abstract.*



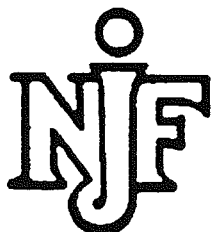
SCANDINAVIAN  
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Fur Animal Division

**NJF-seminar on "Reproduction, stress and  
animal welfare"  
May 22-23 1997 in Norway**

THE PROCEEDINGS CAN BE OBTAINED AT

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PROCEEDINGS

### **Reproduction, stress and welfare. Summary of the spring seminar**

The ethological group under NJF organised a seminar on the subjects reproduction, stress and welfare, mainly for researchers in applied ethology. Thirty-eight researchers, consultants and industry people attended the meeting. Five 1-hour lectures were given by invited guest speakers: P. Wiepkema, the Netherlands, E.Röskaft, Norway, B. Braastad, Norway, O.Vangen, Norway and J. Ladewig, Denmark. In addition 10 of the participants presented their research work on topics related to the theme of the seminar. On the last day of the seminar the participants were divided into 5 groups which had two hours to discuss and answer topics and questions related to all presentations. These answers were then discussed all together, before the end of the seminar.

The five lectures were interesting and inspiring. Among other things, the value of using nature as a model in applied research, the value of positive experiences in husbandry animals, different definitions of stress and the limitation of using cortisol as a welfare parameter, was discussed. We were educated in evolutionary adaptive strategies and the value of implementation of this theory in applied research. We heard about the new direction of welfare where the animals' "eigen value" has a major impact and thus forbidding transgenetic experiments, and once again the importance of a positive man-animal relationship was stressed. An appeal to entering a new field for ethologist's was forwarded, namely to implement psycho-farmaca in applied and basic research, since it has given interesting results in the treatment of behavioural problems in pet animals. We were also confronted with some of the negative secondary-effects that are found in husbandry animals selected for high yield and the effects of pre-mating or prenatal stress of the mother on the cubs' later behaviour and physiology.

The seminar was intense and efficient with many presentations and it was held in a nice atmosphere and with an eager level of questioning the lectures. The Leangkollen environment did not dissappoint us and we enjoyed the delicious food, the beautiful rooms and the efficiency and helpfulness of the staff. In all, it was a very successful seminar also due to the economic support from NJF and the Norwegian Furbreeders Association. Thank you.

*Vivi Pedersen*

### **Undesirable side effects of selection for high production efficiency in farm animals**

*W.M. Rauw*

The breeding goal in most livestock species is to create a population with high economic production efficiency, i.e. high production combined with relatively low feed intake. Genetic selection has indeed increased production levels considerably. However, apart from disired effects of genetic selection, selected animals seem to be more at risk for behavioural, physiological and immunological problems.

Selection for high production efficiency may result in correlated responses in other traits. The observed phenotypic association between two traits is a result of the underlying genetic relationship and the environmental relationship. Two causes of genetic correlations between traits can be distinguished: linkage and pleiotrophy. Linkage is the situation in which different loci which influence separate traits are situated close together on the same chromosome, preventing the genes from segregating independently at meiosis. Pleiotrophy is the situation where a single gene affects two or more different traits. Production traits are usually composed of many underlying cooperative metabolic processes, each of which is more or less genetically determined. The biochemical

reactions and control mechanisms on which a gene and thus genetic selection acts, are likely to influence more than one trait. Correlated responses in metabolic, reproduction, health traits and stress-susceptibility in broilers, pigs and dairy cows will be discussed.

Biological explanations for the occurrence of negative genetic correlations have been proposed by several authors. In 1954, Lerner discussed what he called 'genetic homeostasis': with natural selection, negative genetic correlations between traits will result in intermediate optima for many characteristics in order to maintain homeostasis in a wide range of environments. In 1963, Rendel suggested that negative genetic correlations may occur in a resource limiting situation where two characters have to share resources for their development. Goddard and Beilharz (1977) combined these ideas by relating the total amount of resources available to an animal to fitness: fitness is composed of several components, such as 'number of parities' and 'average litter size', which are suggested to be related multiplicatively. The total amount of resources consumed by these and other processes are suggested to be related additively, since resources consumed by one process can not be allocated to another process. In a limited resource situation, fitness will decrease if one of its component increases in combination with an increased allocation of resources to this trait. Fitness will reach a limit with optimal intermediate values for its components (Beilharz et al., 1993).

Artificial selection for a particular trait may lead to the situation in which resources are used to the maximum and no buffer is left to respond to unexpected stresses and challenges.

Moreover, the animal may be 'genetically pre-programmed' to allocate disproportionately many resources to the trait selected for, leaving the animal lacking in ability to respond to other demands. The population may lack the time required to adapt to changes imposed on it by selection: homeostatic balance will be lost and animal welfare will be impaired.

Without knowledge about underlying physiological processes on which genetic selection acts, genetic improvement through selection is essentially a black box technique. Speeding up genetic increase with application of modern reproduction techniques into a system that is essentially a black box, is very likely to lead to unfavourable and improperly understood side effects, if not to disorder (Luiting, 1993). Understanding biological backgrounds and non-linearity of selection will offer the opportunity to understand and anticipate to negative side effects of selection. This requires a holistic, interdisciplinary approach.

*Only abstract in proceedings. 3 refs. Author's abstract.*

#### **Stress and foetal loss in dairy goats**

*Inge Vogt Engeland, Øystein Andresen, Erik Ropstad, Harald Waldeland*

Foetal loss from non-infectious causes constitutes a substantial loss in Norwegian goat husbandry. A study included 1439 dairy goats from 22 different herds in Norway was carried out to define the nature of these losses. The reproductive performance was surveyed in these herds during one breeding season. Various herd factors related to disease, environment and management procedures were recorded and related to the incidence of non-infectious foetal loss in the herds. Effect of factors pertaining to the individual goats on the outcome of the pregnancy were studied in 515 goats from seven of the 22 herds. Altogether 285 of these goats were blood sampled on a weekly basis throughout pregnancy. The association between some blood parameters and foetal loss in 80 of these goats, i.e. 40 goats with a normal pregnancy and 40 goats with non-infectious foetal loss, was also examined.

Both environmental condition like a building design with large (in mean more than 21 goats in each pen) and crowded pens (in mean less than 0.6 m<sup>2</sup> per goat) and factors pertaining to

the individual goat like low social status and pregnancy with  $\geq 3$  fetuses were associated with foetal loss. Such factors may constitute a continuous stress condition that may influence pregnancy.

The mean level of glucose was elevated and the mean oestrone sulphate level was decreased in goats that experienced foetal loss in the present study. It is well known that an elevated level of glucose may be induced by cortisol. The level of cortisol and progesterone were slightly higher in goats with foetal loss than in the control goats during the last five weeks before question for further studies to determine if this difference is significant concerning foetal loss. Elevated level of glucose and cortisol and low level of urinary oestrogens have also been reported in habitual aborters in Angora goats. It is suggested that adrenal hyperfunction is associated with this loss.

There was a significant association between mother and daughter in foetal loss. Previous foetal loss was also associated with present foetal loss. These results suggest that there may be a hereditary predisposition for this problem in goats. The habitual abortion in Angora goats is suggested to be linked to mohair production, and that the physiological mechanism behind this abortion is thought to be an altered adrenal status (hyperadrenalism). In the present status no significant association was found between foetal loss and the main product of Norwegian goats, namely milk. However, the main trend was for an increased risk of foetal loss with increasing milk production.

Limiting this type of foetal loss under farming conditions may be difficult. Emphasis should be placed on routine management procedures and building construction that may minimise stress. Selection against all goats with foetal loss for non-infectious causes should be practised. When selecting bucks and goats for breeding, the reproductive performance of their mothers should be considered.

*Only abstract in proceedings. Authors' abstract.*

### **Effects of chronic intermittent stress on reproductive physiology and behaviour at puberty in gilts**

*L.J. Pedersen, K.H. Jensen, E. Jørgensen, A.M. Giersing*

The age at which gilts reach puberty and the success of inducing puberty by boar exposure appear to vary considerably within herds as well as among herds. The causal relationship in this variation are poorly understood. It has been suggested that the variation as well as the problems with delayed puberty may be attributed to nonspecific physiological reactions to long term stress. The purpose of the present study was therefore to elucidate the effect of exposure to a standardised psychological stressor during the growing period on reproductive physiology and behaviour.

Forty-eight prepubertal gilts were used in the study of which 24 served as controls and 24 were subjected to chronic intermittent stress. The stressor consisted of daily exposure to inescapable and uncontrollable electroshock from 115-165 days of age. At 165 days of age all gilts were relocated and given daily boar contact. Behavioural observations of social reaction to the first boar exposure as well as of sexual receptive and sexual appetitive behaviour shown in the home pen were made. Twenty-four of the gilts were catheterized the day prior to initial boar exposure. Frequent blood sampling (every 15 minutes for 6 hours) were made to assess LH profiles day -1, 1, 2 and 4 after initial boar contact. The preovulatory LH and oestradiol pattern were assessed from blood samples collected every 4th hour from day 3 following initial boar exposure to the end of oestrus. Samples taken daily at noon were analysed for plasma cortisol.

The electroshock treatment significantly increased the age at puberty ( $p=0.04$ ) and tended to decrease the mean LH concentration prior to the preovulatory LH surge ( $p=0.08$ ) and the maximal concentration of LH during the preovulatory LH surge ( $p=0.07$ ). No significant

differences between treatments were found for any behavioural measures. The apparent down regulation of LH was not associated with increased activity in the hypothalamus-pituitary-adrenal-axis in that the basal concentration of cortisol was not affected by treatment. This indicates that other physiological mechanisms are involved in stress-induced suppression of LH.

*Only abstract in proceedings. Authors' abstract.*

### Cortisol in mink kits

*Jan Elnif*

**Introduction.** The ontogeny of the digestive tract is influenced by both exogenous and endogenous stimuli. Among endogenous factors, glucocorticoids have been shown to stimulate the development of digestive organs. Data on plasma cortisol concentrations in mink kits 1 to 10 weeks old and their influence on the gastrointestinal tract are presented and discussed.

**Material and methods.** One hundred-and-ten newborn mink kits from 20 litters (pastel colour type, litter size = 4-7) were used in the experiments. The 20 litters were randomly divided into 5 age groups each consisting of 4 litters, which were 1, 3, 5, 7 or 9 weeks at age at the start of the experiment. Kits from each litter were marked individually by nail cutting and split into 4 treatment groups resulting in 4-7 kits per treatment at each age. Each of these groups had a similar sex distribution: Group 0 consisting of kits only handled, group S consisting of kits treated with saline (0.9% NaCl), group A consisting of kits treated with ACTH (50 µg/kg/day), and group H consisting of kits treated with hydrocortisone-acetate (50 mg/kg/day). In each group, the injection volume was 2 ml/kg body weight and the animals were injected intramuscularly for seven consecutive days between 9 and 10 a.m.

On day 7, the last day of treatment, the kits were weighed, anaesthetised by intramuscular injections with ketamine hydrochloride and xylazine and subsequently bled by heart puncture within 2-3 min after injection. The animals were killed between 2½ and 7½ hours after the last treatment. An additional 64 mink kits were used to test the cortisol response following a single injection of ACTH (50 µg/kg body weight) or saline at 1 week or 4 weeks of age. These kits were killed either before injection or 1½, 3 or 6 hours after injection and blood was collected as above. Concentrations of cortisol were additionally measured in plasma and milk from mink bitches in their third week of lactation. Plasma cortisol concentrations were determined by an immunoluminescence-assay (Amerlite).

**Results.** Plasma cortisol did not change significantly with age in the two control groups. Plasma cortisol was highly increased (5-15 fold) in the hydrocortisone treated animals (group H) at the age of 2 and 4 weeks compared with control kits ( $P < 0.01$ ). The cortisol concentration in group H decreased with increasing kit age after 4 weeks but remained higher than in the control group at all ages ( $P < 0.05$ ). In 2-6 week old kits treated for 7 consecutive days with ACTH (group A) plasma cortisol at the time of sacrifice was not different from the hydrocortisone treated animals (group H), and in 10 week old kits it was significantly higher in group A than in the control group ( $P < 0.01$ ). The estimated biological half-life ( $t_{1/2}$ ) for cortisol in the continuously ACTH treated mink kits at the age of 2 and 10 weeks were approximately 180 min and 60 min, respectively. At 4 weeks there was no apparent change in plasma cortisol in response to the time after ACTH injection.

**Conclusion.** Our results indicate that mink kits have a stress non-responsive period (SNRP) at the age of 3-4 weeks, which is later in life than e.g. in rats where this period occurs at an age of

2 weeks. From the concentrations of cortisol in plasma ( $349 \pm 130$  nmol/L) and milk ( $163 \pm 50$  nmol/L) from the mothers it is estimated that mink kits in their 4th week of life get substantial amounts of cortisol via milk. The cortisol status of the mothers could thereby influence the development of the gastro-intestinal tract in mink kits.

*Only summary in proceedings. Author's summary.*

### **An analysis of perinatal cub losses in blue foxes**

*M. Harri, V. Ilukha, T. Rekilä*

Economy of fur production depends on fur size and quality and reproductive performance of the animals. The first two objectives have been subject of intensive and successful selection. However, despite a farming history of several decades, our knowledge on reproductive performance of foxes is still scarce. This is due to the fact that most farmers are unwilling to open the nest boxes before the cubs are old enough to come out voluntarily. Thus estimates on conception rate and pre- and postnatal mortality of cubs are only approximate. In the present study we were able to collect basic data for the different components of reproductive performance of blue foxes under farm conditions in a Russian research farm. The foxes were mated naturally and perinatal mortality was carefully recorded three times a day. The data is based on altogether 2413 vixens from four consecutive years. Thirty-nine percent of vixens were primiparous while 11.1% were 5-years-old or older. Altogether 2047 (84.8% of total) gave birth to 22941 cubs, but 5.9% of the cubs were stillborn and 11.4% died before weaning. Only in a very few cases (1.3%) was the whole litter lost, and, more commonly there were some cub losses (46.9%). Abnormal birth and abortion of a part of a litter contributed most to prenatal reproductive failure of the vixen. Infanticide played a minimal role as a cause of postnatal cub mortality (0.3% of died cubs), and the death of the vixen was extremely rare. Half of all parturitions occurred between May 14 and

May 28 and May 8 and May 20 with the median on May 20 and May 15 for primiparous and multiparous vixens, respectively. Thus for multiparous vixens, the parturitions peaked 5 days earlier ( $p < 0.001$ , median test) and with a narrower time span. The litter size was smaller (7.96-9.06) and cub losses, including stillborn cubs, were higher (19-24%) for primiparous vixens than for multiparous ones (9.99-10.55, 15-17%, respectively, while no significant differences were found between parities 2 to 5. On the other hand, litter size and cub mortality were independent of father's age and of the date of birth. Fractional cub mortality increased with increasing litter size. However, this increase was modest in extent, and indication that a probability of any particular cub to die before weaning is about constant but, of course, this probability hits larger litters more often than smaller litters. Postnatal cub mortality (y, %) decreased with the age of the cubs (x, days) and can be described by a simple log equation:  $y = 15.3 - 11.2 \log x$ ,  $r^2 = 0.933$ . This equation fits rather well the equation describing postnatal development of thermoregulatory capacity of the cubs. As thermoregulatory capacity is dependent on the size of a newborn and the degree of maturation of its nervous system, so are also its chances of survival. It is interesting to note that in the more southern farms cub losses generally are higher. This shows that blue foxes benefit rather than suffer from cold spring. The present results also emphasise that infanticide as a cause of cub mortality is very rare in blue foxes, and that results obtained from silver foxes cannot be directly transferred to blue foxes. The present results also suggest that the reproductive performance of blue foxes does not suffer from the inspection of nest box interiors pre- and postpartum, several times a day.

*Only summary in proceedings. Authors' summary.*

### **Periparturient behaviour in blue foxes**

*Teija Paavola, Jaakko Mononen, Teppo Rekilä*

The breeding goal in fur animal production is to produce a skin of the highest size and qual-

ity with the lowest cost. In practice this means that body size, fur quality and reproductive performance are the most important breeding objects. However, the knowledge about the subcomponents of reproductive performance is scarce.

This study aimed at describing and providing basic data on reproductive behaviour in farmed blue foxes. Periparturient behaviour of 8 vixens, kept under traditional management conditions, was video-recorded inside the breeding box. A quantitative analysis of behaviour was made by using instantaneous sampling with one minute sampling interval. Separate analyses were made in six different periods: 5 days and 1 day prior to parturition; the parturition period, and the next three days after parturition. Behaviour was divided into 16 categories and some categories were pooled together for later calculations.

Vixens were more active inside the box the day prior ( $11 \pm 3$ ; mean  $\pm$  SD) to parturition than 5 days ( $5 \pm 6$ ) prepartum ( $p < 0.05$ , Wilcoxon's non-parametric test). The parturitions were distributed quite uniformly around the clock. Parturition period lasted  $270 \pm 90$  min while the interval between subsequent deliveries was  $28 \pm 21$  min. Some vixens delivered first cubs on the wire net floor of the cage and then carried them into the nest box. Vixens gave birth to several cubs rapidly one after the other and followed by a longer period for rest and cub-care. In total, the parturient time was mainly spent having labour contractions, licking genital area and helping the cubs to be born. The percentage of time spent outside the nest box was only  $2 \pm 2\%$ . During the three days after parturition the time spent outside the box and cub grooming in standing position increased ( $p < 0.05$ , Freeman's nonparametric test), while the time spent for cub-care decreased (from 18 to 13%), although the vixens spent  $81 \pm 2\%$  of their time resting or sleeping with their cubs. Infanticide was not observed although some cub losses existed, most of them, during the first three days after parturition.

Only summary in proceedings. Authors' summary.

## Differences between blue and silver foxes in fear response

Teppo Rekilä

Farmed silver (*Vulpes vulpes*) and blue (*Alopex lagopus*) foxes are often referred to in a collective concept. However, they belong to different genera with differences in their biology, such as feeding habits, sociality, temperament and behavioural response. Hyponeophagia, i.e. hesitation of eating in a strange situation is shown to be valid measure of fear in rodents. Feeding test, which is based on hyponeophagia, is proposed to measure fear of foxes towards human. In the present study, we evaluated differences between silver and blue foxes in their reaction in the feeding test.

Blood samples were taken and 24-hour urine samples were collected from 40 animals (20 males and 20 females) which hesitate to eat in the Feeding test and from 40 animals (20 males and 20 females) which ate in the Feeding test. Each fox was caught from its home cage and the blood samples were drawn from the cephalic vein within 2 min of capture (base level) and 20 min after the first sample (response level). The increase in cortisol production 2 h after ACTH administration was analysed in another test. The levels of serum and urinary cortisol (nmol/l) were analysed by a competitive immunoassay technique and the level of urine creatinine (mmol/l) by kinetic Jaffe's reaction.

Base level and response level of cortisol following handling and ACTH administration were  $86 \pm 57$  vs.  $134 \pm 96$  ( $P = 0.064$ ),  $153 \pm 40$  vs.  $173 \pm 69$  ( $P > 0.05$ ) and  $600 \pm 111$  vs.  $728 \pm 113$  ( $P < 0.01$ ), respectively, for silver foxes eating and not eating in the feeding test. Corresponding values for blue foxes eating and not eating in the feeding test were  $49 \pm 29$  vs.  $5831$  ( $P > 0.05$ ),  $84 \pm 35$  vs.  $82 \pm 37$  ( $P > 0.05$ ) and  $389 \pm 51$  vs.  $371 \pm 54$  ( $P > 0.05$ ), respectively. The effect of the blood sampling order was found to contribute significantly to basal level ( $r^2 = 42\%$ ,  $P < 0.001$ ) and response level ( $r^2 = 39\%$ ,  $P < 0.001$ ) of cortisol in blue foxes but not in silver foxes.

Urinary cortisol:creatinine ratio for silver foxes eating and not eating were  $4.4 \pm 1.1$  vs.  $6.0 \pm 3.0$  ( $P < 0.05$ ) and for blue foxes  $5.9 \pm 1.3$  vs.  $7.4 \pm 2.0$  ( $P < 0.05$ ), respectively.

Differences in cortisol secretion between species may be attributed to differences in foraging strategy of these species. The silver fox is a reactive animal which hunts and searches for its food, while the blue fox in its natural habitat often follows polar bears, wolves and humans in the hope of scavenging some prey left by these larger predators. In a situation when the polar bear eats a seal, the strategy of the small blue fox is not to show its excitement, but rather to stay as close and as calm as possible. This is supported by telemetric heart rate measurements on farmed blue foxes showing that heart rate of an animal can rise in situations of excitement (feeding) without the animal showing any visible changes in behaviour. Under these circumstances, it may experience stress without revealing it in its behaviour. This natural behaviour may also explain the lack of association between the results of feeding test and blood cortisol in blue foxes.

*Only summary in proceedings. Author's summary.*

#### **Mating willingness and litter size in farm mink selected for confident or timid behaviour**

*Jens Malmkvist, Steffen W. Hansen*

Farm mink (*Mustela vison*) have been selected on the basis of their behaviour towards man since 1988 at Research Centre Foulum. This controlled selection of breeding animals has created a segregation of mink into groups with clearly different reactions towards humans, either in a confident or timid way compared with a control group. Besides, also differences in physiological response have evolved, where the timid group has a higher level of cortisol in plasma after handling, but same cortisol response in an ACTH-challenge test, compared with the confident group. As no differences in housing or handling procedures were imposed

on the groups, the observed differentiation in behaviour is regarded as hereditary.

The animals in this study belonged to three groups: A: selected for curious/confident reactions, B: selected for timid reactions, and C: selected without any demands on reaction towards humans. The total number of breeding animals in the three groups was 52 males plus 220 females in 1996 and 45 males plus 191 females in 1997. Only breedings within each group (A, B, C) was tried, after the so-called 1+8 mating system, which is standard practice on mink farms. The last two seasons the animals were offered the possibility to mate one to two weeks earlier than the usual fixed breeding season. The objective was to investigate if the behaviour-related selection has affected the reproduction of the farm mink, measured as mating willingness and reproductive success.

In 1996, the time when an average of 50% of the population were mated were 3.6 days for group A; thus the confident were mated 1.7 to 2.1 days earlier than the timid (B) and the control (C) animals (significant difference). The time span in minutes from introduction of female to male until 1st mating was shortest for group B animals, but not different between the groups during 2nd matings. Group C had a lower kit mortality from birth to day 50 (11.3%) than group A and B (20.4 to 21.2%). No significant differences were found between the groups regarding the frequency of successful matings, the ratio of remated females, number of interrupted matings, barren females or litter size.

Over eight years' selection on the basis of their behaviour towards humans has led to the development of reproductive differences primarily in the time of mating readiness, so that the group of confident mink can be mated earlier than groups of fearful or non-selected animals. The hypothesis that reproductive success, measured as the number of kits born per female, is greater for those individuals that are most domestic-like in their behaviour, is not supported by the results from the present study, even though earlier results indicated such an effect. In 1997 investigation will focus

on behavioural differences in the maternal care between the three groups, which maybe can explain the observed difference in kit mortality. The differences seen in kit loss may alternatively be related to random effects (including genetic drift), rather than effects of inbreeding depression or behavioural differences.

*Only summary in proceedings. Authors' summary.*

### **Reproduction management in the mink – theory and practice**

*Steen H. Møller*

As the mink is a strictly seasonal breeder, mink production is taken place in an annual cycle of distinct production periods including all animals on the farm. In the Northern hemisphere the breeding season is in March and problems in this period will affect the whole year's production. Compared with other farm animals, the reproduction management of the mink is very different. Ovulation is induced by copulation, but implantations are delayed, allowing for a curious pattern of repetition of copulation and ovulation during pregnancy. The mink shows no clear sign of heat, but the willingness to mate increases steadily during the mating period. Due to the complex reproduction in the mink, which is still not fully understood, different mating systems and routines have developed, partly based on experience, partly on knowledge. As the reproduction period is short and occurs only once a year, experience is gained slowly and stepwise. In order to help mink farmers to be well prepared for the labour intensive mating season, a Systematic Operation Programme (SOP) has been developed and tested on commercial farms. The (SOP) systematises the management in the reproduction period by describing all relevant management routines as a set of periods in which observed situations release actions. The programme consists of three action plans, each giving priority to individual actions in a part of the reproduction period. The actions suggested are based on a review of the knowledge about mink man-

agement, physiology and behaviour. In order to distribute the SOPs effectively in terms of availability, updating, tailoring and cost, they have been published on the WWW. The actionplans used in the daily operation are linked to the review, in order to make the programme coherent and documented.

*Only summary in proceedings. Authors' summary.*

### **The importance of stressor predictability**

*Randi Oppermann Moe*

Measurement of species-specific stress responses provide an important means to assess animal welfare. The following presentation is based on some results of my thesis "Investigation of methods to assess stress in farmed silver foxes" (Moe, 1996). The fact that handling and blood sampling is stressful (Moe & Bakken, 1996) raised the need to develop methods to assess effects of short term stress without having to enter the farm environment. One such method is to obtain data on increases in deep body temperature, termed "stressinduced hyperthermia" (SIH) by means of surgically implanted radio telemetry devices (Moe et al., 1995). SIH is evoked rapidly after onset of stress. The SIH response is closely linked with an activation of the SAM system and the HPA axis, coinciding with increases in blood glucose contents and plasma cortisol (Moe & Bakken, 1997a, b). Furthermore, since anxiolytic drugs prevented SIH, anxiety pathways may be involved (Moe & Bakken, *in press*). The remote data sampling of SIH obtained with transmitters, and videorecording of behaviour, showed that the presence of humans and handling, and social stressors, were perceived as stressful (Moe & Bakken, 1997a; Bakken et al., *submitted*). Does this mean that humans inevitably are perceived as stressful? Many studies in laboratory rodents have illustrated that psychological factors such as predictability help the animals to cope with the stressor. We attempted to investigate whether predictability could influence on the stress response towards humans (Bakken & Moe, *in preparation*). After

two weeks of conditioned learning, the silver foxes expecting a dog biscuit (signalled by a person wearing an overall) showed a reduced SIH compared with those expecting to be caught with a neck tong (signalled by a person wearing a coat). However, the greatest SIH response was observed when the foxes expected a biscuit, but were caught with a tong (by a person wearing an overall). This implies the importance of a psychological dimension of stressor controllability and predictability. Thus, the uncertainty of the humans intention when entering the farm environment may by one important component of what silver foxes experience as stressful.

*Only summary in proceedings. 8 refs. Authors' summary.*

**Stress, reproduction and animal welfare. Based on the review: The emotional vertebrate**

*P. Wiepkema*

Vertebrates have common ancestors, hence they share highly comparable strategies and mechanisms for maintaining internal and external homeostasis. The key concepts underlying homeostasis are predictability and controllability of relevant *Umwelt*-states and events. Coping activities of vertebrates are characterised by at least two elements: 1) motivation derived from the difference between what is and what should be, and 2) emotion corresponding to how well an organism trusts its means to restore or to maintain its homeostasis. The biological significance of these concepts is discussed.

*8 pp, 33 refs. Author's abstract.*

**Why do some animals choose not to reproduce in nature?**

*Eivin Røskaft*

*Review without summary. 12 pp.*

**Biological limits to selection – what is selection experiments telling us?**

*Odd Vangen*

*Review without summary, 4 pp. 8 refs.*

**Effects of prenatal stress on behaviour and reproduction in mammals**

*Bjarne O. Braastad*

Evidence mainly from studies of rodents and primates indicate strongly that prenatal stress can impair the stress-coping ability and produce a disruption of behaviour in aversive or conflict-inducing situations in juvenile and adult offspring (*Barbazanges et al., 1996; Weinstock, 1997*). Effects may be found on their sex-ratio at birth, on locomotion, play, exploratory behaviour, fearfulness, learning ability, social behaviour, aggression, sexual behaviour, and maternal behaviour, and on their reproductive success in the first, and sometimes also in the second, generation. In normal situations behavioural effects of prenatal stress are frequently not seen. Individual variation in the susceptibility to prenatal stress may exist. Behavioural inhibition and anxiety when exposed to novelty are typical results which may underlie the effects of prenatal stress on learning and various behavioural responses. This seems to be related to increased or prolonged activity in the HPA axis produced by impaired negative feedback in the hippocampus. Whether this is the whole story is less certain (*Holson et al., 1995*).

Since behavioural and neuroendocrine effects of prenatal stress in rodents are quite similar to those found in depressed humans, and since increased fearfulness and frustration is implicated, it may be predicted that farm animals subjected to prenatal stress will show a reduced ability to cope with a difficult environment and increased propensity for developing behavioural disturbances and reduced welfare. Recent results on farmed foxes, and indications in other farm species, show that prenatal stress

may affect the behavioural development of farm animals. As knowledge in this area is scarce, more research is warranted.

*Proceedings NJF Seminar on "Reproduction, Stress and Welfare", 16 pp. 102 refs. Author's summary.*

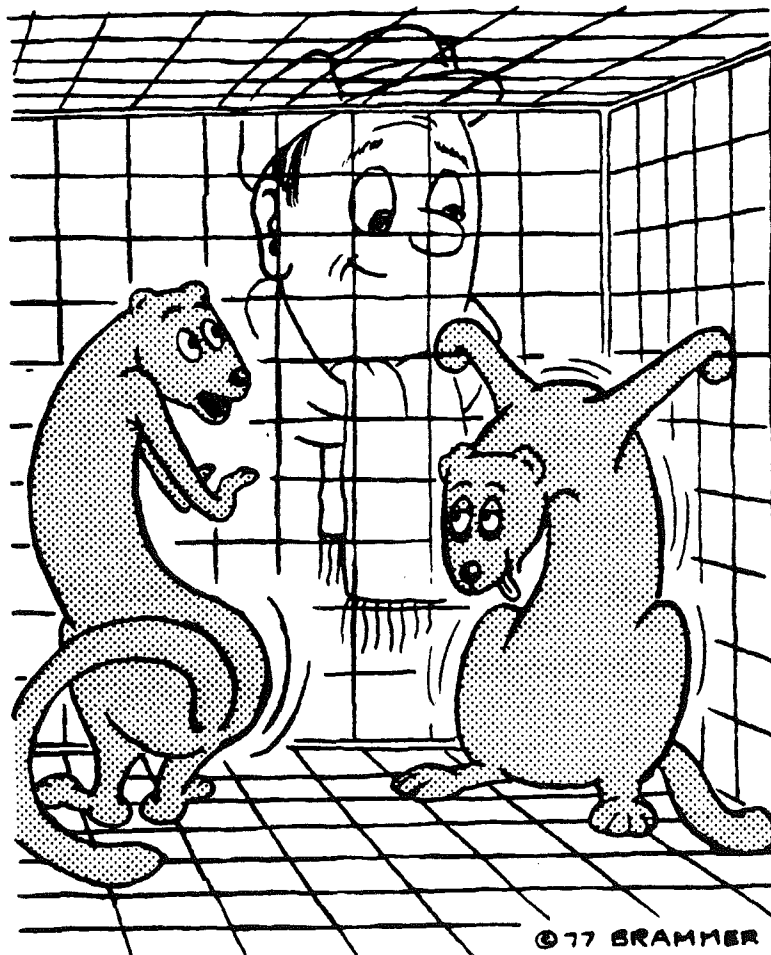
#### **Annual report (5) on Reproduction, cortisol-responses to an ACTH-challenge and behaviour in a tit-bit test**

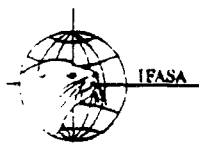
*Vivi Pedersen, Leif Lau Jeppesen, Kit Skovgaard*

This report concerns data on reproduction for both silver and blue foxes in 1996. In addition, results from an ACTH-challenge in blue foxes and a tit-bit test in both silver foxes and blue foxes are presented. The foxes are kept in three different housing systems which have been described in details in the 1. annual report (Jeppesen, 1994). The procedures concerning oestrus evaluation, mating and surveillance of cubs were performed as described in earlier reports (Pedersen, 1994; Pedersen and Skovgaard, 1995).

Regarding the year 1996, system 1 and 3 showed the best reproductive output in silver foxes and in system 1 the vixens showed less fear in a tit-bit test. In blue foxes, system 3 showed the best reproductive output if only pregnant vixens are considered important, but for the farmer the cost of having vixens around which do not reproduce are high. System 3 vixens were slim and they gave birth to many cubs maybe just because of that reason. But in relation to man, system 3 vixens were fearful and system 1 vixens were more confident. It should be emphasised that this is a half-year report on in the longitudinal study of different housing systems to silver and blue foxes. A comprehensive conclusion of these data are not meaningful until all data from the years 1992-1996 are put together in the full report which are expected to be published in June 1997.

*Proceedings NJF Seminar on "Reproduction, Stress and Welfare", 17 pp. 14 tables, 8 refs. Part of authors' introduction and conclusion.*





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