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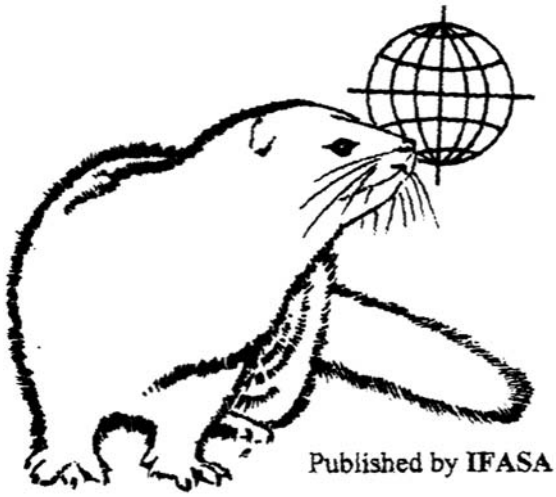

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IV – 1 RP

Stochastic simulation of breeding schemes to improve economic genetic merit in mink production

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Summary
In this study selection within farms was compared with selection across farms connected with an exchange of breeding animals following a circular pattern between farms.
We expected that a systematic exchange of animals in a group of farms would increase the total economic genetic merit. Genetic gain is influenced by several factors, among others population size, which can be increased by collaboration between several farms. The circular exchange of animals was analysed to test the effect of type, age and proportion of exchanged animals and the effect of the number of farms in a collaboration group. In all cases the breeding goal includes body weight and litter size, and the genetic gain is estimated using stochastic simulation. The total economic genetic merit per year is illustrated together with the rate of inbreeding during a period of 10 years.
Economic genetic merit varied from 7.6 to 8.5 DKK per mink per year. Increased weight genetic gain was obtained by exchange of yearling breeding animals. Furthermore, the annual increase of inbreeding was reduced from 0.86% per year in within farm selection to 0.42% per year with selection of animals across farms.
It is concluded that the economic genetic merit in a mink population can be improved by collaboration with other mink populations.

Introduction

In mink production each farm is a breeding unit, with its own breeding goal. Apart from selection among own stock some new breeding animals are bought based on auction or pelt show results. However these are not reliable indicators of genetic differences between farms. Most breeding units are small, which can lead to increased rate of inbreeding (Berg, 1997). In fur production high inbreeding tends to decrease reproduction (Nielsen & Berg, 1993; Nordrum, 1993; Berg, 1996), especially if the selection is on litter size in a small unit (Berg, 1997).

A group of fur producers with a common breeding goal could exchange breeding animals between farms to establish genetic links between farms. This increase of population size will consequently improve the genetic gain due to higher selection intensity, larger accuracy, and decreased rate of inbreeding.
In this project we used stochastic simulation to estimate the consequences of systematic exchange of animals within a group of farms on the total economic genetic merit. The economic genetic merit together with the genetic gain per year for litter size, body weight and pelt quality is presented in each scenario.
The aim of this paper is to test whether the economic genetic merit per year is higher when

- animals are selected within farms versus across farms
- males versus females are selected across farms
- larger proportions of animals are selected across farms
- more farms are included in a Breeder Group

Material and Methods

Statistical programmes
MINKSIM is a programme package for stochastic simulation specially applied to fur animal production. It is composed of a set of programmes developed as flexible tools for stochastic simulation of breeding schemes in farmed animals (e.g mink: Berg, 1997; sheep: Lauridsen, 1998, and cattle: Sørensen, 1999; Nielsen et al., 2001). The programme package MINKSIM simulates breeding work in a commercial farm and includes separate modules for each production period: -mating, -reproduction, -breeding value estimation and selection (Hansen & Berg, 2003).
The simulations of the data used in the analyses are based on the assumptions described in Table 1 and the scenarios we have chosen to illustrate alternative breeding schemes (see Collaboration pattern). Each scenario is repeated 25 times.

Table 1. Base values for some production parameters

Type of lines	Pure bred lines, registrations on all animals
Line size	200 dams + 40 sires
Mating	Random mating, 5 females per male
Percent of fertile dams	92 %
Mean values $_{(\pm std)}$ for traits	Litter size: 1st litter: $5.3_{\pm 2.3}$ 2nd litter: $5.6_{\pm 2.0}$ 3rd litter: $5.0_{\pm 2.5}$ Male weight: $2400_{\pm 250}$ Pelt quality: $3_{\pm 1}$
Selection principles	Truncation selection on an index: $I=0.07*ebv_{bw}+ 12*ebv_{ls}$, where ebv_{bw} and ebv_{ls} are estimated breeding values for body weight and litter size, respectively.
Simulation period	15 years, results from the last 10 years is used
Number of runs per year	25, except in the scenario (4) with different number of farms where 50 repetitions were used.

Simulation of animals

To produce a population that illustrates the situation on a commercial farm in a Breeder Group, each animal is simulated, with a corresponding breeding value and phenotypic observation. For each simulated animal genetic and phenotypic records are generated from predefined statistical distributions, taking the reduced variance due to Bulmer-effect and inbreeding into account (Bulmer, 1974). For details, see Sørensen, 1999; Sørensen et al. 1999.

Estimation of predicted breeding values

In the simulation program the prediction of breeding values is done once a year as in commercial mink farming. Breeding values for all animals from a Breeder Group are predicted simultaneously and the selection is based on these breeding values. Prediction of breeding values is carried out by univariate Animal Models using the programme package DMU (Jensen et al., 1997; Madsen & Jensen, 2000), corresponding to the model used in the Danish 'CFC-avl' Hansen et al. (1999), and including fixed effects of herd and year.

Genetic assumptions

When simulating breeding values including several traits, knowledge about genetic and environmental parameters for the traits simulated are needed. Genetic parameters known from earlier research (Hansen & Berg, 1997) and from literature in general (Berg, 1993a; Berg, 1993b) are used (Table 2). The habitability for litter size in each parity is adjusted according to the total phenotypic variance assuming the same genetic variation in all parities. The genetic correlation between 1., 2. and 3. litter is set to 1 and the genetic correlation between body weight and litter size to –0.30, and the genetic correlation between adult body weight and pelt quality to –0.04 (according to results of Lagerkvist et al., 1994).

Breeding goal

The aim is to maximize the economic index, which is based on a combination of the economic value of one unit of the studied traits. Skin length and litter size are both very important for the economic result of mink production and are therefore chosen as the

Table 2. Heritabilities (on the diagonal), genetic correlations (above) and phenotypic correlations (below the diagonal)

Trait	Litter_1	Litter_2	Litter_3	Body weight	Pelt quality
Litter_1	**0.08**	1	1	-0.30	-0.01
Litter_2	0.6	**0.11**	1	-0.30	-0.01
Litter_3	0.4	0.6	**0.07**	-0.30	-0.01
Body weight	0.09	0.09	0.09	**0.50**	-0.04
Pelt quality	-0.01	-0.01	-0.01	-0.10	**0.35**

first traits to be studied. Increasing skin length has a strong positive effect on skin price and increased litter size reduces production costs. Animals are ranked and selected due to this economic index.

The economic value of individual traits

Based on analyses of pelt prices of scanblack and scanbrown males in years 2000-2002 (Clausen, 2002) a mean value of 7 DKK per cm male pelt is estimated. As there is a high correlation between body weight in November and pelt size (Lohi & Hansen, 1989; Hansen & Lohi, 1990; Hansen et al., 1992), body weight at grading is here used as an estimate for skin size. 100 g extra body weight at pelting is calculated to yield an addition of 1 cm in pelt length (Møller, 2002). Applied to the above figures of pelt price per cm this gives an equation: 1 g extra body weight = 0.07 DKK extra price.

Litter size is recorded at the age of 14 days. According to Lagerkvist (1997) increased litter size will reduce the production costs with 12 SEK per skin when litter size increases from 6 to 7 at an assumed pelt price level of SEK 200 and feed price of 2 SEK per kg.

All three production traits, litter size, body size and pelt quality are simulated as continuous variables. Litter size is rounded to integers and set to zero if negative. Five percent of the females are randomly selected to be barren. Together with the distribution of litter size this results in approximately 10 % barren females in total. Consecutive records of litter size of the same female have a repeatability of 0.4 to 0.6, corresponding to a genetic correlation of 1. Five percent of both males and females are culled for reasons not correlated to the selection criterion (mortality or selection on other traits). All barren females are discarded. Variation in bodyweight for males and females is simulated to be similar, assuming that female weights are transformed to the scale of male weights.

Variation between farms is illustrated in the last year for each sub scenario (Table 4), and is used in relation to the economic genetic merit to compare the farms within a Breeder Group.

Rate of inbreeding. The inbreeding for each animal is estimated as the Wrights coefficient of inbreeding using the algorithm described by Meuwissen & Luo (1992). Rate of inbreeding in a sub scenario illustrates one of the consequences of the breeding scheme.

Collaboration pattern in a Breeder Group

To analyse the effect of collaboration four scenarios with sub scenarios are selected. Combinations are simulated with at least 25 replications. Within each replication breeding values and the realised observations for all animals are stored. From these data the average genetic merit, the average rate of inbreeding and the average genetic variation for animals born within the same year are calculated.

Scenario 1:
Flow of animals within and across farms:
a) Within farm selection, b) Selection across farm, c) Circular exchange of breeding animals between farms - within farm selection and exchange of a fixed proportion of animals.

Scenario 2:
Type of breeding animals to be exchanged:
a) Males or females, b)Yearling or adults

Scenario 3:
Proportion of exchanged breeding animals: - 15, 30 or 50 %

Scenario 4:
Size of the *Breeder Group:* 2, 3 or 5 farms in the group.

Statistical analysis of the collaboration effects

In each scenario different effects are studied by a univariate analysis. Simulation results from each year (se later) are analysed in a model considering the main effects and the corresponding interactions.

Scenario 1: $$Y_{ij} = \mu + F_i + e_{ij}$$

where Y_{ij} is the regression of the simulated trait on each year from each replication, e.g the annual economic genetic merit, μ is the general mean, F_i is the effect of flow of animals (i=1,2,3), e_{ij} the residual of the jth replication (j=1,……..25).

Scenarios 2 and 3:

$$Y_{klmn}=\mu+A_k+P_l+S_m+A_k*P_l+A_k*S_m+P_l*S_m+P_l*S_m*A_k+e_{klmn}$$

where Y_{klm} is the regression of the simulated trait on year from each replication, e.g the annual economic genetic merit, μ is the general mean, A_k is the effect of age of animals (k=1,2,3) males and females having two and three age groups, respectively, P_l is the effect of proportion of exchanged animals (l=15,30, 33/50) where adult males have a maximal proportion of 33%, S_m is the effect of sex of animals (m=1,2), A_k*P_l is the interaction between age and proportion, A_k*S_m is the interaction between age and sex, P_l*S_m is the interaction between

proportion and sex, e_{klmn} the residual of the *n*th replication (*n*=1,……..25). As males was only accepted for breeding in two years, a maximum of 33 % of adult males could be exchanged.

Scenario 4: $Y_{op} = \mu + N_o + e_{op}$

where Y_{op} is the regression of the simulated trait on each year from each replication, e.g the economic genetic merit, μ is the general mean N_o is the effect of number of farms (*i*=2,3,5), e_{op} the residual of the *p*th replication (*p*=1,……..50).

The comparison of scenarios is based on the total economic genetic merit (DKK) per year and compared with the sub scenario with 'Within farm selection'.

Results and Discussion

Scenario 1. The effect of different flow of animals across farms

Selection across farms results in a higher economic genetic merit, a lower rate of inbreeding and an increased genetic trend in body weight than selection within farms.

Response in Breeder Groups is compared in three sub scenarios. Two extremes: 'Within farm selection': in this case no animals are exchanged between farms meaning that the entire breeding stock each year is selected from own animals; 'Total exchange': is selection across farms. In the third situation 'Circular exchange' 15% of the yearling breeding stock and 5% of the adult breeding stock are purchased from one farm, and a similar proportion sold to a third farm in a circular pattern.

Economic genetic merit in Breeder Groups practising exchange of breeding animals is higher (8.37 and 8.48 DKK, respectively) than in the group with 'within farm selection' of animals (8.10 DKK), corresponding to a higher economic genetic gain of 3-5%.

Exchange of breeding stock, total or circular, reduces the variation between farms. Based on order statistics, the economic genetic merit per year on the best of the 3 farms in the group with within farm selection is 8,46 DKK, which means that in this case the best farm in the group is only as good as the average result for all farms with exchange of animals following a circular pattern.

The rate of inbreeding depends on the amount of exchanged breeding animals. Breeder Groups with within farm selection of animals have the highest rate of inbreeding and Breeder Groups with total exchange of breeding animals the lowest rate of inbreeding. The rate of inbreeding is reduced with approximately 50% when 20 percent of the breeding animals are exchanged and with 62% if all breeding animals are exchanged (Table 3).

Of the three traits studied only the genetic gain in body size is significantly influenced by the animal flow between farms. On average the genetic gain is 126 g per animal per year, being lowest in 'within farm selection' and 3% and 4% higher in the two other groups. Both in litter size and in pelt quality the genetic trend is negative and does not depend on the flow of breeding animals.

Table 3 Flow of breeding animals – selection within farm or across farms: total exchange or circular exchange The total economic genetic merit (DKK), rate of inbreeding, litter size (kits per litter), genetic gain in body weight (g) and genetic gain in pelt quality (percent change of a quality score).

	Selection			
	Within farms	Across farms		
	No exchange	Total exchange	Circular exchange	p-level
Economic genetic merit	$8.10_{\pm 0.05}$	$8.37_{\pm 0.05}$	$8.48_{\pm 0.07}$	***
Variation between farms[1)]	$0.18_{\pm 0.03}$	$0.01_{\pm 0.00}$	$0.03_{\pm 0.01}$	-
Rate of inbreeding	$0.0087_{\pm 0.0001}$	$0.0033_{\pm 0.0001}$	$0.0042_{\pm 0.0001}$	***
Genetic trend in:				
-litter size	$-0.058_{\pm 0.003}$	$-0.060_{\pm 0.003}$	$-0.051_{\pm 0.004}$	Ns
-body weight	$125.60_{\pm 0.50}$	$130.00_{\pm 0.69}$	$129.79_{\pm 0.83}$	***
-pelt quality	$-0.021_{\pm 0.003}$	$-0.015_{\pm 0.003}$	$-0.020_{\pm 0.004}$	Ns

[1)] *Variation of economic genetic merit between farms in the last year.*

Table 4. Total economic genetic merit, rate of inbreeding, and genetic gain in body weight, litter size and pelt quality related to type of animals, sex, age and proportion of exchanged animals. Average change per year (LSM) related to 'within farm selection'.

Type of collaboration and type of animals				Percent change per year in Breeder Groups with 'circular exchange' related to 'within farm selection' of animals					
collaboration	Sex	Age	Pro-portion	Total Economic	Variation between farms[1)]	In-breeding	Body weight	Litter size	Pelt quality
Within farm selection	-	-	-	8.09 DKK	$0.18_{\pm 0.03}$	0.0086%	125.53g	-0.058 kit	-0.021point
circular	Male	yearling	15	$+1^{ns}$	$0.06_{\pm 0.01}$	-51^{***}	$+2^{**}$	-10^{ns}	-10^{ns}
			30	$+4^{***}$	$0.03_{\pm 0.01}$	-55^{***}	$+4^{***}$	-6^{ns}	-3^{ns}
			50	$+3^{**}$	$0.02_{\pm 0.00}$	-61^{***}	$+4^{***}$	-10^{ns}	-7^{ns}
circular	Male	adult	15	-0.3^{ns}	$0.10_{\pm 0.02}$	-44^{***}	0^{ns}	$+3^{ns}$	$+27^{ns}$
			30	-3^{**}	$0.04_{\pm 0.01}$	-55^{***}	-2^{**}	-7^{ns}	$+16^{ns}$
			33	-4^{***}	$0.05_{\pm 0.01}$	-56^{***}	-4^{***}	$+5^{ns}$	-22^{ns}
circular	Female	yearling	15	$+2^{*}$	$0.08_{\pm 0.01}$	-47^{***}	$+2^{*}$	$+4^{ns}$	$+29^{ns}$
			30	$+3^{**}$	$0.05_{\pm 0.01}$	-58^{***}	$+3^{***}$	-0.3^{ns}	$+14^{ns}$
			50	$+2^{*}$	$0.03_{\pm 0.00}$	-61^{***}	$+3^{***}$	-4^{ns}	$+6^{ns}$
circular	Female	adult	15	-1^{ns}	$0.09_{\pm 0.02}$	-44^{***}	0^{ns}	-13^{ns}	-6^{ns}
			30	-2^{ns}	$0.08_{\pm 0.01}$	-56^{***}	0^{ns}	-14^{ns}	-12^{ns}
			50	-6^{***}	$0.03_{\pm 0.01}$	-59^{***}	-5^{***}	-10^{ns}	-0.6^{ns}

$^{ns}p>0.05$; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$
[1)] Variation of economic genetic merit between farms in the 15. year.

Scenarios 2 and 3. The effect of sex, age and proportion of exchanged animals

The results of different alternatives of 'circular change' are presented in Table 4 as the difference compared to the corresponding value if no breeding animals are exchanged. The main result is that it is preferable to exchange young animals as higher economic genetic merit and lower inbreeding can be expected than when exchanging adult breeding stock.

Economic genetic merit is influenced by age, proportion and up to some degree by sex of the exchanged animals.

Both in males and females, the exchange of young animals is more beneficial (Table 4) resulting in 2% to 4% higher economic genetic merit. The larger the proportion of adult males or females exchanged the smaller is the economic genetic merit per year.

Comparing exchange of young animals to adults the maximum difference in economic genetic gain in favour of young animals is 7 % and 5% in males and females, respectively.

The variation between farms is low in both alternative age and sex groups. Variation between farms decreases with increasing number of animals exchanged.

Generally there is no difference between the sexes in economic genetic merit per year. Thus it is the proportion of exchanged animals that determines the effect, and fewer males need to be exchanged to obtain the same change in response to selection.

Rate of inbreeding is increasing per year and is affected primarily by the proportion and slightly by the age of exchanged animals.

The rate of inbreeding in all Breeder Groups is significantly lower than if 'within farm selection' occurs (Table 4). The rate of inbreeding decreases with an increasing amount of exchanged breeding animals, despite the sex and age of the breeding animals.

Exchanging 15 percent of breeding animals will reduce the rate of inbreeding with 44% to 51%.

In Breeder Groups with 15 percent exchange of yearling males, the rate of inbreeding is lower than in the Breeder Groups with corresponding proportions of adult males or adult or young females.

In females there is no difference between the rates of inbreeding due to the age.

The genetic gain in body weight per year is significantly higher in groups exchanging young breeders (2% to 4%) compared to corresponding exchange of adult breeders (-2% to -5%) (Table 4). Already with 15 percent exchange of young breeding animals the genetic gain per year is higher than with a corresponding exchange of adult animals.

The genetic gain in litter size is decreasing in all cases and is not affected by factors included in the full model. There is no difference between any of the sub scenarios or compared to the Breeder Group with within farm selection of breeding animals.

The genetic gain in pelt quality varies between the different alternatives but no significant differences were found.

Scenario 4. Breeder Groups with different number of farms.

The advantage of more farms in Breeder Groups is the reduced rate of inbreeding. No difference was found in economic genetic merit, or in the three traits litter size, body weight or pelt quality. This indicates that increasing population size beyond 400 females is not advantageous.

Conclusion

Increased proportion of exchanged young animals increases the economic genetic merit.

Increased proportion of exchanged adult animals will decrease the economic genetic merit gradually.

The rate of inbreeding decreases with increasing amount of exchanged young animals and increasing number of farms in a Breeder Group.

Table 5. Number of farms in a Breeder Group with exchange of 30 % adult breeding males. Total economic genetic merit (DKK), rate of inbreeding, litter size (kits per litter), genetic gain in body weight (g) and genetic gain in pelt quality (percent change of a quality score). (50 repetitions)

Per year	No. of farms in a Breeder Group			
	2	3	5	p-level
Economic genetic merit (DKK)	$7.99_{\pm 0.05}$	$7.88_{\pm 0.04}$	$7.97_{\pm 0.03}$	ns
Variation between farms[1)]	$0.03_{\pm 0.01}$	$0.06_{\pm 0.01}$	$0.08_{\pm 0.01}$	-
Rate of inbreeding	$0.0055_{\pm 0.0001}$	$0.0041_{\pm 0.0001}$	$0.0041_{\pm 0.0001}$	***
Genetic trend in:				
- litter size	$-0.054_{\pm 0.004}$	$-0.062_{\pm 0.002}$	$-0.057_{\pm 0.002}$	ns
- body weight	$123.50_{\pm 0.54}$	$123.17_{\pm 0.41}$	$123.52_{\pm 0.40}$	ns
- pelt quality	$-0.019_{\pm 0.0031}$	$-0.017_{\pm 0.002}$	$-0.020_{\pm 0.002}$	ns

[1)] *Variation between farms in the last year in economic genetic merit*

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IV – 2 RP

Inbreeding in a commercial fur animal breeding program

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Abstract
A number of mink populations from 6 farms, which all uses the Norwegian field data recording system, 'Pelsdyrkontrollen', have been analysed for level of inbreeding and its possible effect on the reproduction results. Pedigree information is available for 51.900 mink over a period of 6 to 10 years, and 3.237 females have litter size recorded in 2003. The regression of litter size on inbreeding was –0,02678 ± 0,01564 (p=0,0869).
Calculations of average inbreeding coefficient on all whelps compared to the same calculations for those which are selected as new breeders shows that selection for reproduction seem to work against the general tendency to increase the inbreeding coefficient.
Approximately 150 Norwegian farmers use 'Pelsdyrkontrollen' for selection of new breeding animals. The module for selection based on live grading, which is used by approximately 1/3 of these farmers, has been enhanced with a tool that can help the farmer to maintain as high effective population size as possible on the farm. This makes it easier to control the rate of inbreeding.

Introduction

Inbreeding is the mating of animals that have ancestors in common, such that at a particular locus their progeny may be homozygous for an allele, which belonged to one ancestor (Cameron, 1997).
The most important consequence of elevated levels of inbreeding is the fact that inbred animals generally perform poorer than their non-inbred counterparts. Some of this poorer performance can be ascribed to the effects of negative recessive genes. More importantly, it seems that higher levels of inbreeding affect the fitness traits (reproduction and survival) the most and this is highly detrimental in any herd or breed.
The most used method of determining inbreeding is the inbreeding coefficient, introduced by Wright in 1921. It's called F and is defined as the correlation between genetic values of gametes.

The prediction of rate of inbreeding is often referred to as the simplified equation (Falconer, 1989)

$$\Delta F \approx \frac{1}{8N_m} + \frac{1}{8N_f}$$

where N_m and N_f are the numbers of breeding males and females in the population.

In fur animal farms the number of males is normally restricted compared to the females, thus the effect of population size is determined much by the number of breeding males. This will reduce the effective population size and increase the rate of inbreeding (ΔF).
In a closed population the rate of inbreeding will increase regardless of which selection and mating system is chosen. That means that it is important to know more about an individual than it's grandparents, to be able to reduce or control the rate of inbreeding.
Most breeding programs in principal result in a reduction in the genetic variation caused by the estimating model of breeding values, as the programs have tendencies to pick animals from the same families. In small populations the risk of fixation of genes and elimination of genes by random drift is higher than in larger populations and will increase the risk of building up homozygous loci, i.e. inevitably reduce the genetic variation. On the other hand the possible negative effect of inbreeding on the reproductive performance will tend to have the opposite effect i.e. the inbred animals, which performs poorer, will get lower indices and will contribute to fewer new breeding animals in the next generation.
The normal mink farm in Norway is rather small, with an average of approximately 500 breeding females, often divided into two or three "purebred" colour types. Many of the farms are situated at long distances from each other making the exchange of animals between farms difficult. The danger of contagious diseases, such as plasmacytoses also

makes the exchange of animals complicated. Most of the farms operate their own breeding programme, thus a mink farm may often be looked upon as a closed population with a rather small effective population size.
This investigation has been performed to make an overview of the general situation of a typical Norwegian mink farm and give the breeders a tool to monitor their own situation and have some practical control on the rate of inbreeding in their population.

Material and methods
Data from 6 different mink farms in the central database of the Norwegian field control system 'Pelsdyrkontrollen' have been used in this investigation.
The distribution of the pedigree information in the material is shown in table 1.
The data contains pedigree of approximately 10 years, which gives almost 10 generations of mink. The database was not originally designed for calculations and monitoring of inbreeding, thus there was made an extraction of the pedigrees and the data was transformed to be handled by Microsoft® Excel and analysed by SAS® Stat. Software.
The rate of inbreeding was calculated by Proc.Inbred of SAS® Stat. as the inbreeding coefficient designed by Wright. The calculations were made within each farm.
The effect of inbreeding on the reproductive performance of the minks was investigated by analysing the possible effect of inbreeding on litter size. Both litter size at birth and litter size at 3 weeks were used in the analyses.
However the initial analyses showed that analysing on numbers of kits at 3 weeks gives more consistency in the material. This is to be expected, as many breeders are more likely to make an accurate count of live kits at 3 weeks, than they do at birth. A total of 3237 females with litter size > 0 at 3 weeks in 2003 were included in the calculations.
Data were analysed with a linear model with farm, year of birth (female age) and mink type as fixed effects. The regression of the inbreeding coefficient of the female on her litter size in 2003 was calculated.

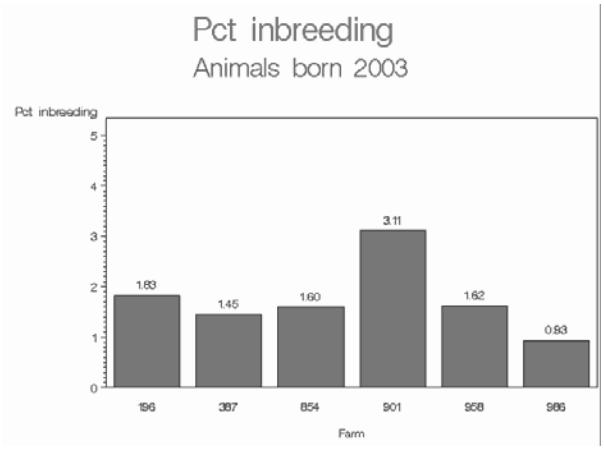
Table 1: Distribution of animals in the pedigree on year of birth and farm

No.	Year											All
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	animals
Farm												
196	1	6	31	145	228	334	749	890	881	5185	4282	12732
387		2	13	20	63	79	126	139	203	910	1104	2659
854			17	87	134	186	405	351	385	2679	2444	6688
901	1	21	46	102	166	168	359	484	599	3609	3841	9396
958	1	6	24	86	142	189	435	580	486	3629	3346	8924
986		6	16	95	182	337	726	892	798	4517	3932	11501
All	3	41	147	535	915	1293	2800	3336	3352	20529	18949	51900

Results

The average degrees of inbreeding in the farms are shown in fig.1.

Figure 1: Average inbreeding per farm of animals born in 2003



The general impression is that there is no alarmingly high degree of inbreeding in the farms, on the other hand there are some differences between the farms. It is essential to remark that the inbreeding coefficients in the material are results of the pedigrees as they look at this moment, and does not include any earlier inbreeding in the animals forming the "base population".

The effect of inbreeding on reproduction in the total material of the 6 farms was calculated with litter size at birth and at 3 weeks, shown in table 2.

Tabel 2. Regression of litter size on degree of inbreeding

	N	b	st.d.	p
Litter size at birth	3407	- 0,02533	0,01720	0,1410
Litter size at 3 weeks	3237	- 0,02678	0,01564	0,0869

The results are in good accordance with similar results from other publications on fur animals (Berg, 1996; Nordrum, 1993) and similar data from other farm animals (Pirchner, 1983).

The effect of inbreeding on reproduction (litter size at 3 weeks) can also be described by the effect of the actual selection. From data for 2002 we calculated the average inbreeding coefficient for all whelps and the average of those who were selected as breeders. In all 6 farms the inbreeding coefficient of the whelps selected as breeders was lower than the total whelp population in the same farm, as shown in table 3. This indicates that even though the indices might give an effect of inbreeding, in this material it seems that the negative effect of inbreeding on reproduction works in contradiction to the tendency from the indices to pick animals that are from the same family.

Table 3. Average inbreeding for all whelps and for the new breeding animals chosen amongst the whelps

Farm	All whelps	Selected breeders
196	1,4	1,0
387	1,9	1,6
854	1,1	0,8
901	3,1	2,5
958	1,0	0,9
986	1,5	1,0
Average	1,67	1,17

Discussion

The farms included in this investigation have approximately 200 to 1000 breeding females each thus giving a good picture of the mink farms in Norway. The results shows a clear effect from inbreeding on reproduction traits. The level of influence from inbreeding on litter size at 3 weeks is in accordance with similar results in other reports on inbreeding. The level of inbreeding was not high in the material, and as far as one can tell the rate of increase of inbreeding is not high. The effect of low rates of inbreeding can often be eliminated by selection. As an example 12 generations of breeding with a rate of 1% inbreeding, would create an inbreeding of 12%. The negative effects of this, however, will normally be eliminated over the same time by means of selection. 12 % inbreeding is the same as one generation of half sib matings, but this of course will have much greater effect, as it will not be subject to selection along the way.

The evidence of effects on reproduction from inbreeding in this material, and similar but mostly smaller effects on other production traits (Berg, 1996), still makes it relevant for commercial mink breeders to pay some attention to the effect of inbreeding in their breeding program.

Possible means of controlling the rate of inbreeding are (Cameron, 1997)

- Selection on biased predicted breeding values (increased heritability will reduce the tendency to select related animals)
- Breeding values corrected for genetic relations
- Control and monitoring of the family structure

Farmers using 'Pelsdyrkontrollen' are offered an overview of the level of inbreeding in their population and the effect of it on the reproduction. A new Excel® based service, has recently been introduced and made available on the Internet without extra cost for the members of 'Pelsdyrkontrollen'. An extract of the database is transformed to an Excel-file and located at a given Internet-address from where it may be downloaded to the farmers own PC. By a few mouse-clicks the Excel sheet can produce the statistics shown in table 4. This gives the actual level of inbreeding and the development over time.

Another statistic, which can be obtained by a new mouse-click is shown in table 5. Here the breeding animals are grouped according to type, year of birth and three categories of inbreeding. The results gives number of females in each group and average litter size at 3 weeks per group.

Table 4: Actual report on inbreeding distributed on mink colour type and year of birth. The numbers of animals of each type in the farm must be a part of the evaluation of the situation.

Average inbreeding	Year of birth							
Mink colour type	1997	1998	1999	2000	2001	2002	2003	Totalt
100	1,6	3,3	3,6	3,4	3,5	3,8	4,0	3,8
109		0,0	0,0	1,4	3,7	1,7	3,4	3,2
200		0,0	1,0	1,7	0,7	2,5	3,4	2,8
210	0,0	0,0	0,9	0,5	0,1	0,1	1,3	0,6
221	0,0	0,0	2,4	1,6	0,9	1,0	2,5	1,9
222		0,9	0,4	1,1	1,5	1,1	2,3	1,8
223			0,6	1,0	0,9	1,6	2,3	2,0
224				0,6	0,4	1,8	2,7	2,5
421		0,0	1,4	1,7	2,2	1,2	1,8	1,7
500	0,0	0,0	1,1	2,0	3,0	2,3	3,4	2,8
Total	1,0	1,1	1,6	2,1	2,4	2,5	3,1	2,8

Table 5: Reproduction performance (litter size at 3 weeks) of scanblack females grouped in 3 different categories of inbreeding and in year of birth. Numbers of females in the groups is to be considered.

			Inbreeding			
Type	Year	Data	**a=0-5**	**b=6-15**	**c=16-**	Total
100 (scanblack)	1998	Number of females	15	9		24
		Average litter size		**5,0**		5,0
	1999	Number of females	45	11		56
		Average litter size	**5,5**	**3,5**		5,2
	2000	Number of females	149	44	2	195
		Average litter size	**6,4**	**6,7**		6,4
	2001	Number of females	135	65	4	204
		Average litter size	**6,6**	**6,4**	**4,0**	6,5
	2002	Number of females	112	53	1	166
		Average litter size	**5,8**	**5,5**		5,8
Numbers of females			472	186	7	665

These statistics may be used by the breeder to decide if there are any problems regarding inbreeding in his farm, and aid in the decisions regarding the necessity of import or other measurements to avoid negative effects of inbreeding.
The Excel-tool for selection, based on fertility indices and live grading results, also supplied by 'Pelsdyrkontrollen', has now been enhanced by a facility to control the numbers of animals selected per sire. The consequences of the restriction-choices for family structure made by the breeder are shown immediately and the breeder may decide to accept the results or go back and recalculate with other restrictions on family structure. In this way the breeder may to a certain degree control and reduce the rate of inbreeding and at the same time know how much it costs in loss of progress in the production traits.

Conclusions and remarks

This investigation was meant to be a practical way of using the field data from the central database in 'Pelsdyrkontrollen' to investigate the situation regarding inbreeding in Norwegian mink farms and the possible effect of inbreeding on reproduction. Furthermore the goal was to give the farmers the option to "scan" their populations of minks, find the level of inbreeding and offer a tool to monitor the rate of inbreeding. At the same time evaluate the costs of the reduced rate of inbreeding on the reduced progress of the production traits.
The Norwegian members of 'Pelsdyrkontrollen' will be offered these options from the 2004 production season. The system will be available through the Internett, in accordance with the system for live grading launched in 2000 based on the use of Microsoft® Excel.

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Genetics of litter size, age at first insemination and animal size in blue fox (*Alopex lagopus*)

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Abstract
Skin size of blue fox has increased considerably in Finland during the last decade. This may have lead to decreased fertility through unfavourable genetic correlation. The average number of pups per mated females has slightly decreased after the mid-90's. The objective of this study was to estimate the genetic parameters of the first three litter sizes, female age at first insemination and animal size using REML with single and multitrait animal models. The data was obtained from the Finnish Fur Breeders' Association. In the single trait analysis data and pedigree had 30268 and 44297 animals in litter size, 46295 and 62035 animals in age at first insemination and 68108 and 78775 animals in animal size, respectively. Multitrait analysis had 9126, 5115, 2525, 15381 and 23574 observations in 1st, 2nd and 3rd litter size, age at first insemination and animal size, respectively. Pedigree had 32356 animals in the multitrait analysis. Heritability estimates were 0.08, 0.08 and 0.07 for first, second and third litter size, respectively. Heritability estimates in single and multitrait analysis were 0.16 and 0.18 for age at first insemination and 0.24 and 0.25 for animal size, respectively. The genetic correlations between animal size and age at first insemination and first, second and third litter size were -0.20, -0.40, -0.40 and -0.23, respectively. Genetic correlations between first and second litter size were 0.62, between first and third 0.51, and between second and third 0.60. This study supports the conclusion that there is an antagonistic genetic correlation between fertility and animal size.

Introduction

During the last decade, one of the main goals in Finnish blue fox breeding has been to increase animal size. The average skin size has considerably increased during the last $10-15$ years. While the skin size has increased, the average number of pups per mated female has slightly decreased after mid-90's. Because few studies have been made about the genetic correlation between animal size and fertility of blue foxes, consequences of selection on skin size to fertility is unclear. However, Lagerkvist et al., 1994, found low antagonistic genetic correlation between fertility and animal size. The objective of this study was to estimate genetic parameters between litter size, age of female at first insemination and animal size. The main objective was to study if there is an antagonistic correlation between fertility and animal size. The secondary objective was to study how genetic increase in animal size affects the sexual maturity of young females.

Material and Methods

Data was obtained from the Finnish Fur Breeders' Association. The analyzed data set was a subset having 18 farms with observations from years 1989-2001. The farms were known to have breeding cooperation. In the data only purebred blue foxes were accepted.

The studied fertility traits were the first three litter sizes (LS) and females age at first insemination (AFI). Litter size observations from females with $1-20$ pups after $2-3$ weeks from whelping were accepted. However, litter size observations from females mated with more than one male per breeding season were excluded. Observations from litter sizes were excluded, if an observation from animal size was missing. Also observations from later (second and third) litter sizes were excluded if observation from an earlier litter size was missing. AFI was defined as number of days between date of birth and first recorded mating. Observations under 274 and over 367 days were assumed to be incorrect and were therefore excluded. The animal size (AS) was a subjective grading measurement made by the farmer. In Finland, the grading scale for animal size goes from 1 to 5 so that 1 is smallest and 5 is biggest. The grading is done so that the average size is approximately 3 in the farm each year. In Finland, grading is done to young animals mainly in October, just before the pelting season.

(Co)variance components were estimated both in single trait and multitrait animal models using the DMU program (Madsen & Jensen 2000) that relies

on restricted maximum likelihood (REML) method in variance component estimation. In the single trait analysis variance components of 1st LS, AFI and AS were estimated by model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wc} + \mathbf{Za} + \mathbf{e} \qquad (1)$$

where **y** is vector of observations in 1st LS, AFI or AS and **b** is a vector of fixed effects, and **c**, **a** and **e** are vectors of random litter, animal and residual effects, respectively. **X**, **W** and **Z** are the incidence matrices for fixed, litter and animal effects, respectively. Model (2) was used in multitrait analysis.

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\\ \mathbf{y}_3\\ \mathbf{y}_4\\ \mathbf{y}_5\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2 & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{X}_3 & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_4 & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_5\end{bmatrix} \begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\\ \mathbf{b}_3\\ \mathbf{b}_4\\ \mathbf{b}_5\end{bmatrix} + \begin{bmatrix}\mathbf{W}_1 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{W}_2 & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{W}_3 & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{W}_4 & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{W}_5\end{bmatrix} \begin{bmatrix}\mathbf{c}_1\\ \mathbf{c}_2\\ \mathbf{c}_3\\ \mathbf{c}_4\\ \mathbf{c}_5\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_1 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_2 & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{Z}_3 & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{Z}_4 & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{Z}_5\end{bmatrix} \begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\\ \mathbf{a}_3\\ \mathbf{a}_4\\ \mathbf{a}_5\end{bmatrix} + \begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\\ \mathbf{e}_3\\ \mathbf{e}_4\\ \mathbf{e}_5\end{bmatrix} \qquad (2)$$

where the trait numbers 1 -5 correspond to AS, AFI and 1st,2nd and 3rd LS, respectively. Fixed and random effects were the same in both single and multitrait analysis. Fixed effects for each trait are presented in table 1. Covariance matrices of random effects in single and multitrait models were assumed to be:

$$\text{var}\begin{bmatrix}\mathbf{a}_s\\ \mathbf{c}_s\\ \mathbf{e}_s\end{bmatrix} = \begin{bmatrix}\sigma_a^2\mathbf{A} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \sigma_c^2\mathbf{I} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \sigma_e^2\mathbf{I}\end{bmatrix} \text{ and}$$

$$\text{var}\begin{bmatrix}\mathbf{a}_m\\ \mathbf{c}_m\\ \mathbf{e}_m\end{bmatrix} = \begin{bmatrix}\mathbf{G}_0 \otimes \mathbf{A} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{C}_0 \otimes \mathbf{I} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{R}_0 \otimes \mathbf{I}\end{bmatrix}$$

respectively, where s and m correspond with single and multitrait analysis, respectively, and σ_a^2, σ_c^2 and σ_e^2 are additive genetic, litter environment and residual variance, respectively. **A**, **I**, $\mathbf{G}_0$, $\mathbf{C}_0$ and $\mathbf{R}_0$ are numerator relationship matrix, identity matrix, 5×5 additive genetic covariance matrix, 5×5 covariance matrix for litter environmental effect and 5×5 covariance matrix for residual effects, respectively. Heritability and proportion of litter variation were calculated as

$h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$ and

$c^2 = \sigma_c^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$, respectively.

Results and Discussion

Mean, standard deviation (s.d), coefficient of variation (CV), minimum and maximum in 1st, 2nd and 3rd LS, AFI and AS for the analyzed data are presented in table 2.

The average of 1st LS is about 2.5 pups smaller than in 2nd and 3rd LS. The standard deviations were similar in 1st, 2nd and 3rd LS. Nevertheless, the coefficient of variation in 1st LS is slightly larger than in 2nd and 3rd LS due to smaller mean in 1st LS. The difference in CV between 1st LS and later LS's is probably caused by strong selection between 1st and 2nd LS. The average of AFI is 319.98 days, which is about 10.7 months. The standard deviation of AFI is quite low (10.84) which causes considerably lower coefficient of variation (0.03) than in other traits studied $(0.18 - 0.49)$. The mean of AS was 3.99, showing, that above average values are given more often than desired.

Table 1. Fixed effects in single and multitrait analysis for 1st, 2nd and 3rd litter sizes (LS), age at first insemination (AFI) and animal size (AS).

	LS				
	1st	2nd	3rd	AFI	AS
farm-year	X	X	X	X	X
time of birth (for animal)[1)]	X			X	X
mating method[2)]	X	X	X		
number of matings[3)]	X	X	X		
age of dam[4)]					X
sex[5)]					X

[1)]4 classes (104-129, 130-144, 145-160 and 161-180 days from the beginning of the year)
[2)]2 classes (natural or artificial insemination)
[3)]2 classes (1 or >1 matings/season)
[4)]3 classes (1, 2 or 3-15 years old)
[5)]3 classes (male, female or pup)

Table 2. Mean, standard deviation (s.d), coefficient of variation (CV), minimum and maximum of 1st, 2nd and 3rd litter size (LS), age at first insemination (AFI) and animal size (AS).

trait		mean	s.d	CV	Minimum	Maximum
LS[1)]	1st	6.14	3.01	0.49	1	18
	2nd	8.68	3.38	0.39	1	19
	3rd	8.72	3.25	0.37	1	17
AFI[2)]		319.98	10.84	0.03	271	361
AS[3)]		3.99	0.72	0.18	1	5

[1)] number of pups 3 weeks after whelping, [2)]days, [3)] size points

Table 3. Animals in data and in pedigree, phenotypic variances (σ_P^2), litter environmental effect (c^2) and standard errors (s.e) and heritabilities (h^2) and their standard errors (s.e) in 1st litter size (LS), age at first insemination (AFI) and animal size (AS) in single trait analysis.

trait	Animals		σ_P^2	$c^2 \pm s.e$	$h^2 \pm s.e$
	in data	in pedigree			
LS	30 268	44 297	9.20	0.03± 0.01	0.08±0.01
AFI	46 295	62 035	96.97	0.26±0.01	0.16±0.01
AS	68 108	78 775	0.67	0.10±0.00	0.24±0.01

Heritabilities and proportion of litter variation in single trait analysis are in table 3. The heritability estimate of 0.08 for litter size was low, which agrees with Valberg Nordrum, 1993, and Nikula, 2000. In the literature, AFI has not been estimated for blue foxes. However, in pigs and dairy cattle the subject is widely studied. Raheja et al., 1989, had slightly smaller estimate of heritability (0.11) for heifers than in the present study for blue foxes (table 3). On the other hand Hanenberg et al., 2001, had higher estimates of heritability for gilts (0.32). In our study, the litter variation in AFI was 0.26, which is considerably larger than the heritability (0.16). This is probably due to large effects of dams nursing ability, competition between pups within litter and the location of the litter in the shed. When the litter effect was excluded, estimate of heritability was over 0.40.

Heritability of AS was smaller than in Kenttämies & Smeds, 2002. Wierzbicki, 2000, had higher heritability when data was not transformed. After probit transformation of data Wierzbicki, 2000, had heritabilities similar to the present study. However, the studies are not entirely comparable, because Wierzbicki, 2000, had no litter environment as a random effect in the model. In the present study, litter environment was 0.10, so it accounts for quite a large amount of variation in AS.

In multitrait analysis, number of observations was largest in AS whereas 3rd LS had smallest amount of observations (table 4). However, every trait pair had always over 2400 observations. The pedigree had 32356 animals in multitrait analysis. The heritabilities in multitrait analysis and genetic and phenotypic correlations between 1st, 2nd and 3rd LS, AFI and AS are in table 5. Heritabilities in 1st LS (0.08), AFI (0.18) and AS (0.25) were close to those in the single trait analysis. Heritabilities in 2nd and 3rd LS were close to 1st LS. Standard errors were slightly higher in multitrait analysis than in single trait analysis, which was probably due to smaller amount of observations per trait.

The genetic correlations between AFI and 1st, 2nd and 3rd LS were 0.26, 0.34 and 0.26, respectively. Thus genetically early sexually maturing animals had less pups three weeks after whelping than later maturing animals.

Genetic correlations between AS and 1st, 2nd and 3rd LS were -0.40, -0.40 and -0.23, respectively. Despite the smaller genetic correlation between AS and 3rd LS than between AS and 1st and 2nd LS, and despite standard error increasing along with parity number, the genetic correlation between AS and first three LS is clearly antagonistic.

The genetic correlation between AS and AFI was -0.20. Because grading is done in October when young animals are still growing, the shape of growth curve has a big impact on the grading size of blue fox. It seems, that the selection based on the grading size, increases the growth rate, which again via moderate genetic correlation makes animal mature sexually earlier.

The genetic correlation between 1st and 2nd LS was 0.62 between 1st and 3rd LS 0.51 and 2nd and 3rd LS 0.60. Correlations were high but still they supported the conclusion that 1st, 2nd and 3rd LS are at least partly different traits.

Table 4. Number of animals in each trait and trait pairs in multitrait analysis of 1st, 2nd and 3rd litter size (LS), age at first insemination (AFI) and animal size (AS).

		LS				
		1st	2nd	3rd	AFI	AS
LS	1st	9126				
	2nd	5115	5115			
	3rd	2525	2525	2525		
AFI		9105	5106	2523	15 381	
AS		8919	4969	2423	13 415	23 574

Table 5. Heritabilities and their standard errors (diagonal), genetic correlations and their standard errors (upper triangle) and phenotypic correlations (lower triangle) in 1st, 2nd and 3rd litter size (LS), age at first insemination (AFI) and animal size (AS) in multitrait analysis.

		LS				
		1st	2nd	3rd	AFI	AS
LS	1st	0.08±0.02	0.62±0.17	0.51±0.24	0.26±0.10	-0.40±0.09
	2nd	0.18	0.08±0.03	0.60±0.27	0.34±0.13	-0.40±0.12
	3rd	0.16	0.23	0.07±0.04	0.26±0.18	-0.23±0.16
AFI		0.08	0.04	0.00	0.18±0.02	-0.20±0.06
AS		-0.07	-0.07	-0.01	-0.02	0.25±0.02

Conclusion

Heritability of litter size was estimated to be low. The heritability of female age at first insemination was higher than in litter size. Large effect of the random litter effect in age at first insemination indicates that maternal effects may be considerable. In the future, it might be reasonable to estimate the genetic parameters by including maternal effects in the model. The heritability of animal size was moderate and lower than in previous studies made for Finnish blue foxes. Moreover, the moderate estimate of heritability in animal size cannot totally explain significant increase of skin size in the Finnish blue fox population during the last decade.

If the antagonistic genetic correlations between animal size and 1st, 2nd and 3rd litter size are not accounted, genetic increase in animal size will decrease the average litter size. However, because of the low heritability of litter size, the phenotypic impact may be quite small. The negative genetic correlation between animal size and age at first insemination indicates, that genetic increase in animal size will decrease age at first insemination. In the future it would be reasonable to study how different components of growth (fat, protein, water) and the shape of growth curve relate to the age at first insemination. This is interesting, because there was positive genetic correlation between age at first insemination and first three litter sizes. If large amount of breeding efforts is put to increase animal size, it will decrease litter size. Moreover, the litter size will decrease also indirectly via decrease in age at first insemination. It supports the conclusion that it might be reasonable to include age at first insemination in multitrait breeding value evaluation to support improvement in traits with economical importance.

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IV – 6 RP

Diapause, implantation and placentation in the mink: A critical role for embryonic signaling

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Abstract
During the first six days following mating and ovulation, the mink embryo follows the usual mammalian pattern of development to the blastocyst stage. These embryos then undergo a period of obligate developmental arrest, known as diapause. We have studied the mechanisms regulating the sequence of events between the escape from diapause to the postimplantation invasion of the uterus. We have demonstrated marked increases in embryo diameter within 24 h, and in DNA and protein synthesis beginning as early as 48 h after the reinitiation of development. Culture of cells harvested from embryos at intervals demonstrated that the trophoblast proliferated more readily during the early reactivation phase, while the inner cell mass proliferated later. The signal for trophoblast proliferation was fibroblast growth factor-4 (FGF4) presumed to be produced by the inner cell mass, and acting on its cognate receptors in the trophoblast. Embryos reached approximately 2.0 mm in diameter prior to implantation into the uterus, some 11-12 days after reactivation. During reactivation, the blastocyst produces prostaglandins, particularly PGE_2, which then acts on uterine receptors of the EP-2 and EP-4 subtypes. The vascular endothelial growth factor (VEGF), a promoter of angiogenesis, is strongly expressed by the trophoblast cells of the implanting embryo, and transcription of the VEGF gene was induced by PGE_2 and PGD. The embryo is necessary for the local expression of both known forms of the VEGF receptor associated with the early stages of vascularization of the placenta. Our investigations indicate that, following the escape of the mink embryo from its arrested state, cascade of embryonic signals promote trophoblast development, blastocyst invasion, and vascularization of the placenta.

Introduction
Mink gestation is characterized by a discontinuity in development of the embryo, occurring at the blastocyst stage. This condition, known as embryonic diapause, evolved as a strategy for the timing and syncronization of parturition at time favorable to the survival of offspring (Thom, Johnson *et al.* 2004). The developmental trajectory of the mink embryo was described by Hanssen in his comprehensive investigation of reproduction in this species (Hanssen 1947). Following fertilization, which occurs within the first 72 h following mating-induced ovulation, the embryo develops to a blastocyst of 300-400 cells over the next four days. From fertilization through implantation, the mustelid embryo remains encapsulated in the acellular glycoprotein zona pellucida of the oocyte (Enders, Schlafke *et al.* 1986). As in other species, two components of the blastocyst are recognizable, the inner cell mass that will become the embryo proper and the trophoblast, that will become the fetal component of the placenta, and will contribute to the extraembryonic membranes. Developmental arrest ensues, and early investigations indicated that embryo growth, fluid uptake and cell replication were absent during diapause (Baevsky 1963; Daniel 1967).
The length of diapause can vary substantially between animals, with periods as brief as a few days to more than 40 days under certain experimental conditions (Murphy and James 1974). Embryo survival rates, and thus numbers of offspring, are believed to be inversely related to the length of diapause. This provides a compelling rationale for the investigation

of the mechanisms of developmental arrest, embryo reactivation and early implantation. Herein we discuss the events and potential regulation of the escape of the mink embryo from diapause and its consequent implantation into the uterus.

Materials and Methods

Animals were bred to two fertile males according to usual commercial farming procedures. To investigate the termination of diapause, embryos were collected from mated mink at 7-9 days after the final mating, or at intervals through the 10 days preceding implantation from animals treated with 1 mg/kg/day ovine prolactin (Sigma, Oakville ON) to terminate preimplantation delay. Embryos were collected by flushing of the uterine horns as previously described (Moreau, Arslan *et al.* 1995). Some embryos from each collection date were incubated overnight in the presence of 100 µM bromodeoxyuridine 5'-triphosphate (BrdU, Sigma Chemicals) to determine DNA synthesis through embryo re-activation. Following removal of the capsule, embryos were incubated with anti BrdU antibodies, and nuclei undergoing active DNA synthesis were visualized by means of a fluorescein isothionate labeled second antibody. To estimate protein synthesis, newly flushed embryos were incubated in TC-199 medium in the presence of 10 mCi/ ml ^{35}S-L-methionine (New England Nuclear, Guelph ON) at 37 C for 2 h. The incorporation of ^{35}S-methionine incorporation was determined as described by Bell et al. (1997).

Groups of five mink embryos in diapause or 9 days after activation were incubated in 500 µl INRA Menezo B2 medium (Pharmascience, Paris, France) supplemented with 5% FBS (Gibco) for 48h, with or without mink uterine epithelial cells (Moreau, Arslan *et al.* 1995). An aliquot of 100 µl of the embryo conditioned medium was used to evaluate the concentrations of PGE_2 by radioimmunoassay, according to the method described in (Xiao, Liu *et al.* 1998) using PGE_2 antiserum from Assay Design (Ann Arbor, MI). It had a percentage of cross-reactivity with PGE, $PGF_{1\alpha}$, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ of 70%, 1,4%, 0,7% and 0.6% respectively. The assay sensitivity was 4 pg/ 100 µl. The intra-assay variation calculated between duplicates, ranged from 0.04 and 7.17%.

Embryos taken at various times after initiation of embryo reactivation were dissected and placed in separate cultures of the trophoblast and the inner cell mass (ICM) on a mouse fetal fibroblast feeder monolayer. The entire ICM was plated in mouse embryonic stem cell medium (Betts, Bordignon *et al.* 2001), while trophoblast was cultured in trophoblast stem (TS) cell medium (Tanaka, Kunath *et al.* 1998). Confirmation of trophoblast lineage was achieved by RT-PCR amplification of the marker genes Cdx2, Eomes, FGFR2, and Hand1 over time in culture in trophoblast cells derived from embryos taken at day 5 after activation. To estimate trophoblast proliferation rate, we harvested vesicular outgrowths of the trophoblast monolayer and replated them in fibroblast-conditioned TS medium in the presence and absence of 25 µg/ml FGF4. Following incubation, cells were stained with 4'-6-diamino-2-phenylindol (DAPI, Sigma Chemicals) and counted under fluorescence microscopy.

For RT-PCR amplifications, all the collected tissues were frozen in liquid nitrogen, and subsequently disrupted in RLT buffer (Qiagen, Mississauga, ON, Canada) with 0,12M □-Mercaptoethanol (Sigma). RNA was purified using an RNeasy Protect Mini Kit (Qiagen) as recommended by the manufacturer, or with minor modifications to the protocol for the single blastocysts (Desmarais, Bordignon *et al.* 2004). For the uterine tissue 1.5 µg/ sample of total RNA was used for reverse transcription (RT) with Omniscript RT kit (Qiagen), according to the instructions of the manufacturer. For the blastocysts and the ICM and trophoblast cells, isolated RNA was reverse transcribed into cDNA with Superscript Rnase H- (Invitrogen, Carlsbad, CA) following the manufacturer instructions. Primers for FGF4, Cdx2, Eomes, FGFR2, PGE synthase and PGE receptors EP-2 and EP-4 were designed based on homologous sequences of human and mouse. Mink-specific primers for glyceraldehydes 3-phosphate dehydrogenase (GAPDH) were used as a control. 1 U of Taq polymerase (Amersham Biosciences Corp., Baie d'Urfe, QC, Canada) per microliter of reaction was used to amplify the cDNA from each sample. PCR products were separated in 1.2% agarose gel and stained with ethidium bromide for visualization. At least three independent samples were sequenced for verification of transcript identity.

A mink ovarian tumor cell line was used to test the effects of PGE on VEGF promoter transactivation. A 2 Kb construct of the proximal mink promoter region

was used in a luciferase reporter assay. Briefly, the proximal promoter region was inserted in a pGL2 vector and mink cells were transfected using the Effectene reagent (Qiagen). Transfected cells were then treated with 10µM PGE (Sigma) for 24 h. Renilla luciferase control vector pRL.SV40 was used to normalize results for transfection efficiency.

Data were analysed by means of least square analysis of variance in the General Linear Model procedures of SAS. Following confirmation of a significant F value, comparisons among means were made by the Tukey HSD test. Regression analysis was performed for in vitro proliferation experiments. Significance was established at $p<0.05$.

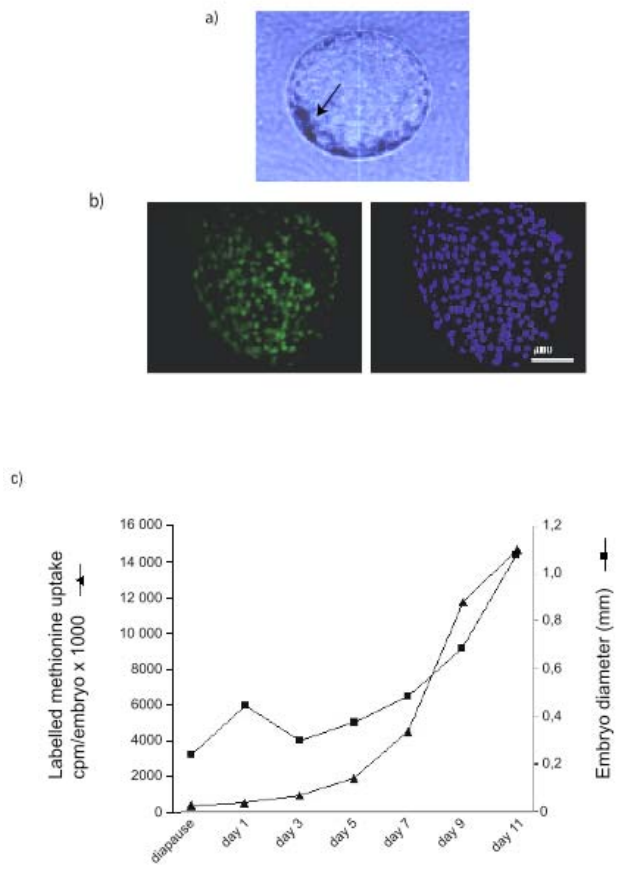
Results

Embryos in diapause displayed the carnivore phenotype including the presence of the oocyte derived capsule, the trophoblast and an inner cell mass (Fig. 1a). They did not take up BrdU, indicating an absence of significant DNA synthesis. At 72 h after the initiation of activation, most cells in the embryo were positive for BrdU, and thus, in the S-phase of the cell cycle (Fig. 1b). Although there appeared to be embryo expansion within 24 h after activation, the first statistically definable increase was present at 72 h, and there was a consequent increase in embryo diameters through day 11 (Fig. 1c).

The embryos found in the uteri at day 13 after initiation of reactivation in approximately half of the treated animals were attached and undergoing implantation (data not shown). The onset of protein synthesis occurred somewhat approximately 24 h following initiation of expansion, while the first discernable increase in the 35S-methionine uptake was evident at 96 h after the initiation of reactivation (Fig. 1c). Embryos collected at subsequent times displayed large scale increases in protein synthesis from days 7-11 (Fig. 1c).

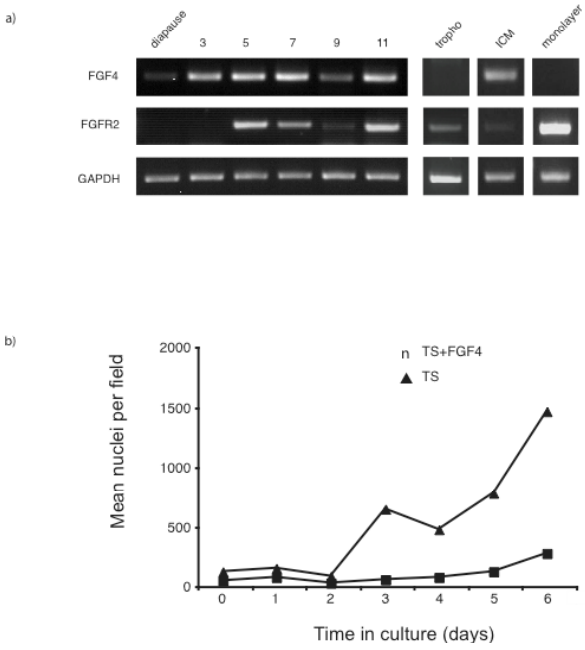
Neither ICM nor trophoblast cells taken from embryos in diapause were capable of proliferation in vitro. Trophoblast cells proliferated readily at day 5 after embryo activation while ICM colonies grew more quickly at day 9 (data not shown). FGF4 is believed to be expressed by the ICM and to affect the proliferation of the trophoblast. RT-PCR analysis revealed that FGF4 transcripts were present in the embryo from day 3 after initiation of activation (Fig 2a). FGF receptor mRNA first appeared on day 5

Figure 1(a). The mink embryo in diapause showing the capsule and the trophoblast. The darkened cellular mass at 8 o'clock is the inner cell mass (arrow).
(b) The mink embryo at day 3 after reactivation showing BrDU uptake (left panel) and DAPI staining to identify nuclei (right panel).
(c) Concurrent increases in embryo volume as measured by embryo diameter and embryo protein synthesis as indicated by ^{35}S-methionine uptake following embryo activation.



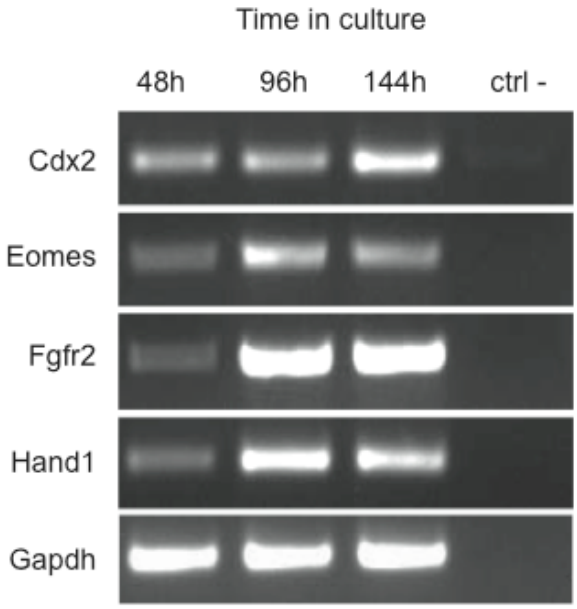
after reactivation (Fig 2a) and both transcripts persisted through implantation. Examination of trophoblast and ICM cultures indicated that the latter was the source of FGF4, and that the trophoblast expressed the receptor (Fig 2a). Trophoblast proliferation in vitro was dependent on the presence of FGF4 (Fig. 2b).

Figure 2 (a). RT-PCR evaluation of embryo expression of fibroblast growth factor 4 (FGF4) and its cognate receptor in whole embryos during reactivation (diapause through day 11) and in trophoblast, ICM and fibroblast feeder layer cultures.
(b) Proliferation of trophoblast cells in vitro in the presence or absence of FGF4.



A series of expression markers were examined to establish whether cell lines derived from mink embryos were, in fact, derived from the trophoblast (Fig. 3).
The results demonstrate that all markers, with the exception of Pal31 are present in low abundance in recently derived cell cultures, there is significant upregulation of the expression of each over time. The most prominent increase was in FGFR2, the putative effector of FGF4 effects on trophoblast proliferation.
We then undertook to determine if embryos in diapause had the capability to synthesize and secrete molecules to signal the uterus. Figure 4a depicts the accumulation of PGE_2 in medium of cells (control) and embryos cocultured with cells over 48 h of incubation. It reveals that embryos collected in diapause had no apparent capability to synthesize and

Figure 3. Semi-quantitative RT-PCR analysis demonstrating the evolution of the occurrence of markers for trophoblast during embryo reactivation. Trophoblast cells were separated from the ICM and cultured for 48, 96 and 144 h prior to isolation and reverse transcription of RNA.



secrete PGE_2. Embryos harvested on day 9 after reactivation had six-fold greater PGE_2 secretion relative to diapause embryos, cells alone, or dead embryos. To examine whether cells were required for PGE_2 synthesis, embryos were incubated alone (Fig4b).
The results indicate that the source of the prostaglandin synthesis is the live embryo, as concentration of the hormone is several fold greater in medium containing embryos.
To further explore the potential for embryo signaling to the uterus of the mink, we identified mRNA for PGE synthase, and for two of the PGE_2 receptors of the EP subtype (EP-2 and 4) in the postimplantation mink uterus (Fig 5a). We then tested the potential for PGE_2 to affect the transcription of VEGF, an important downstream gene in placental development. The results indicated that PGE_2 treatment induced a precipitous increase in the VEGF promoter activity (Fig. 5b).

Figure 4 (a). Accumulation of prostaglandin E2 (PGE_2) in media derived from incubation for 144 h of mink embryos in diapause or following activation. Embryos showing cell death have been included for comparison. Embryos were cultured over a layer of mink uterin stromal cells.
(b) Incubation of embryos in the absence of the feeder layer of uterine cells for 144 h resulted in several-fold increases in $PGE_2$2 accumulation.

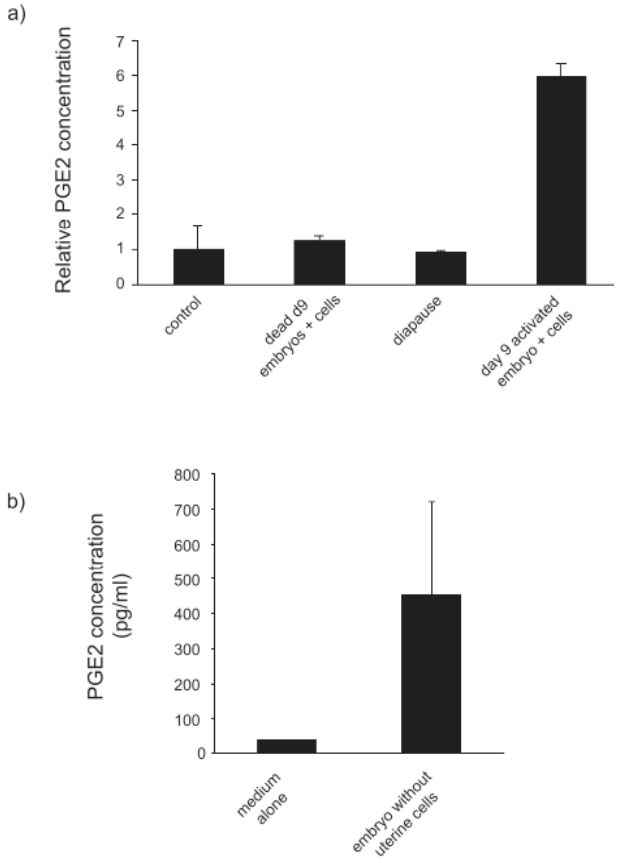
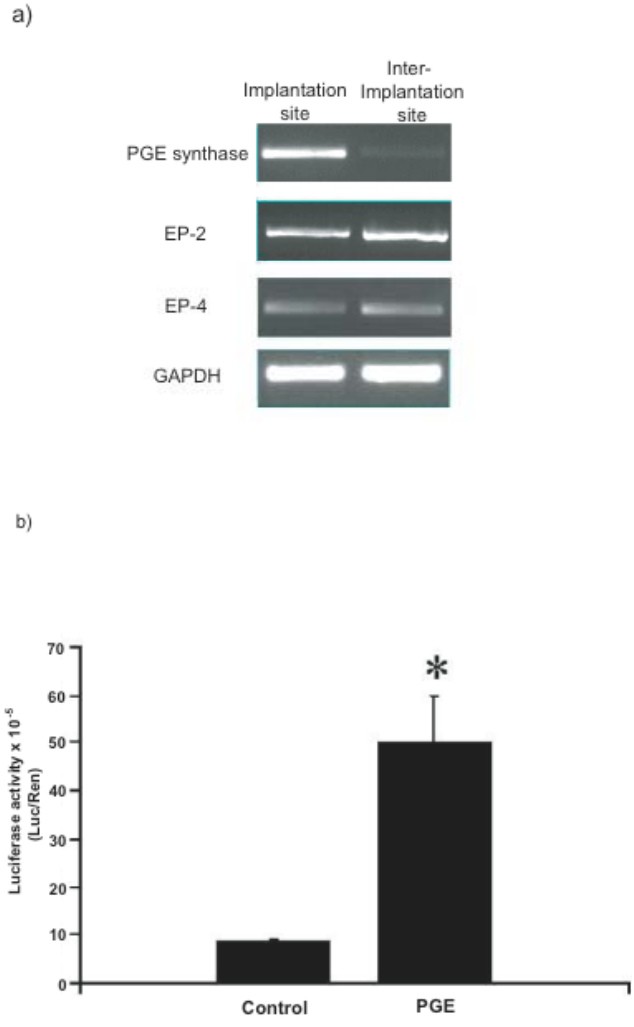


Figure 5 (a). RT-PCR analysis of transcript abundance of PGE-synthase and the PGE_2 receptors, EP-2 and EP-4 at and between implantation sites during early invasion of the mink uterus by the trophoblast.
(b) Luciferase transcription in a transient transfection assay demonstrating that PGE_2 has the capacity to stimulate promoter activity of the mink VEGF gene in mink cells.



Discussion

The results of the present study support the long held view that the cells that comprise the mink embryo in diapause have exited from the cell cycle. It is only after reactivation that DNA synthesis occurs in the trophoblast and the ICM. It is of interest to note that cells derived from either embryonic compartment during diapause are likewise incapable of proliferation in vitro. It is known that the maternal environment influences that persistence of diapause (Chang 1968), indicating that the uterus either prevents reactivation or the uterus during diapause lacks elements that allow for the continuation of embryonic development. It is known that growth of embryos collected in the diapause state can occur in vitro, in the absence of maternal signals (Moreau, Arslan *et al.* 1995), providing support for the view that maternal inhibition maintains diapause. This notwithstanding, acceleration of development can be induced in

embryos recovered after reinitiation of development (0.4 mm or greater) by prolactin (Polejaeva, Reed *et al.* 1997), which argues for positive control by maternal factors.

In fact, the complex pattern of events that bring about the reactivation, trophoblast attachment to the uterus and consequent invasion of the trophoblast in the implantation process remains largely undetermined. We have endeavored to assemble some of the pieces of the puzzle based on the hypothesis that signaling between cells in the embryo and between the embryo and the uterus induces embryonic maturation and postimplantation events in the uterus. In the first instance, we have shown that activation induces early increases in both embryo volume and embryo protein synthesis. Activation is also characterized by expression of FGF4, and the acquisition of its cognate receptor by the embryo. In vitro studies with trophoblast cells indicate that, as in the mouse (Tanaka, Kunath *et al.* 1998), FGF4 is an important stimulator of proliferation. In the mouse, it has been shown that FGF4 is expressed by the ICM (Yuan, Corbi *et al.* 1995). Our results support this view, in that FGF4 expression was restricted to ICM cultures while the FGF4 receptor was found in trophoblast cells. We suggest that the ICM of the mink embryo expresses FGF4 early in activation and signals the trophoblast causing the preimplantation proliferation that we have observed.

The significance of the cyclo-oxygenase (COX) enzymes that synthesize prostaglandins to embryo implantation has been demonstrated in a number of species (Das, Wang *et al.* 1999). We have shown that the mink embryo and endometrium express COX-2, the regulated version of the rate limiting enzyme for prostanoid synthesis at the time of embryo attachment (Song, Sirois *et al.* 1998). The present results indicate that the activated embryo synthesizes PGE_2, independent of uterine influence. RT-PCR amplification of transcripts from uterus at the time of implantation revealed that attachment and invasion sites, but not inter-implantation sites express PGE-synthetase, the key enzyme in PGE_2 synthesis. Further, the transcripts for the PGE_2 receptors, EP1 and EP2 were abundant in the early postimplantation uterus. Together these results argue for signaling by the activated and early implanted embryo to the uterus as a component of the attachment and invasion process.

There is a growing body of evidence to indicate that eicosanoids are important regulators of angiogenesis in a number of tissues, including the uterus (Fujiwaki, Iida *et al.* 2002). Given that PGE_2 appears to be synthesized by the activated mink embryo and by the trophoblast at the site of implantation, it was deemed interesting to determine whether PGE_2 could induce in vitro transcriptional activation of the principal angiogenic element, VEGF. Our results, using the gene promoter from mink VEGF transiently transfected into mink cells demonstrate a marked PGE_2 stimulation of transcription.

In summary, we have investigated the potential signaling cascades that regulate the proliferation of the mink trophoblast, its invasion, and its capacity to induce angiogenesis. While much of the overall pattern remains unresolved, the present investigation indicates that embryo reactivation engenders synthesis of FGF4 by the embryo which has a paracrine effect on proliferation of the trophoblast. The activated embryo also secretes PGE_2, both before and after implantation, and this hormone has the capacity to induce angiogenic factors that are important to the invasion process. The embryo-uterine signaling in embryonic diapause, embryo reactivation and implantation is a fertile ground for further investigation.

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IV – 7 RP

The effects of air pollutants on the cortisol and progesterone secretion in polar fox (*Alopex lagopus*)*

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Abstract
The aim of the present work was to show the effect of air pollution on a level of cortisol and progesterone at blue foxes on 25-35 days following the mating. The animals from a farm situated in the south eastern Poland were maintained at the pavilion system and constituted the control. The experimental group was made up by the females kept in the chamber with limited air movement, thus exposed to air contaminants. The air monitoring confirmed the occurrence of higher concentrations of gaseous substances in the chamber. In the females exposed to the air pollutants there were recorded higher levels of the hormones released. The dams exposed to the pollutants showed higher levels of the hormones secreted. The mean values of progesterone in the females of the experimental group were 72,32 ng/ml, whereas in the control – 42,62ng/ml. The mean values of cortisol in the experimental group recorded were 210,88nmol/l and proved to be substantially higher than in the control. This fact proves the activation of the defensive mechanisms as well as the impact of exogenous agents. The statistical analysis involved the test of double cross classification.

Introduction
Polar foxes (*Alopex lagopus*), commonly called the blue, have been "domesticated" for nearly one hundred generations. The appropriate environmental conditions were created for them so they could live and reproduce. Through the several-stage selection process the individuals were obtained with highly functional qualities and decreased susceptibility to stress. These assumptions have been continued by means of the breeding methods improvement, providing a high level of animal welfare as well as a proper contact between man and animal. Under the undesirable environmental conditions, apart from the stress resulting from welfare depression, there is recorded inactivation of, among others, the hypothalamus-adrenal system. The presence of generally perceived stressors may lead to some changes in physiology, reproduction and behaviour as shown in the investigations carried out at the laboratory animals [Braastad et al, 1998; Nowakowicz-Dębek et al, 2003 and 2004; Smith, 1998].
The aim of the following work is to determine the effects of air pollutants on the level of cortisol and progesterone at foxes.

Material and methods
The investigations were performed at a polar fox (*Alopex lagopus*) farm situated in the south-eastern part of Poland. The animals were caged in open air according to the pavilion system made the control (group A). To show the influence of released air contaminants on foxes, a female group was placed in a chamber with limited air movement (ranging from 0,1 to 0,2 m/s), yet at permanent outside air inflow and outflow (group B). Throughout the experimental period the animals were provided with veterinarian and zootechnical service; air quality was monitored by gas chromatography and colorimetric techniques [Nowakowicz-Dębek et al, 2003; Rodel and Wolm, 1992].
Blood for the cortisol and progesterone determination was collected from the female foxes on 20-35d following the mating. The material for cortisol analysis was taken for not longer than 2-3 minutes. At both cases blood was collected from the foot vein (*vena saphena parva*), simultaneously in every group. The level of the mentioned indices was fixed by the immunoenzymatic method with the kits from BioMerieux.
The statistical analysis was performed with the double cross classification test.

Results
The feeding conditions and animal maintenance are vital for their health state, efficiency and

reproduction parameters. Air pollution as one of the microclimate factors induces the disturbance of animal homeostasis and as a consequence, a decline of animal welfare. One of the systems affected by these changes is, beside the immune system, the neurohormonal system. Therefore, apart from hormone secretion measurements, air monitoring is carried out. Air chromatographic and colorimetric analysis showed higher levels of gaseous pollutants in the chamber compared to the farm. Fairly high levels were recorded for ammonia and sulphur compounds (especially mercaptans, sulphides).

Tab.1. Mean levels of sulphur compounds and ammonia over the analyzed period

Name of compound group	Farm ($\bar{x}$ ±SD)	Chamber
Inorganic sulphur compounds ($\mu g/m^3$)	0,75± 0,30	1,86± 1,65
Mercaptans ($\mu g/m^3$)	2,06± 1,00	19,31± 26,80
Sulphides ($\mu g/m^3$)	0,73± 0,42	1,16 ± 1,11
Ammonia (mg/m^3)	0,37± 0,17	1,37± 1,66

$\bar{x}$ - *the mean*
SD – standard deviation

A part of the analysed and published findings was also presented in Fig.3, where high concentrations of phenol, ethylbenzene, naphthalene, methane and other gases were shown in the chamber [Nowakowicz-Dębek et al, 2003 and 2004]. The chamber microclimate differed markedly from the conditions recorded at the farm.

In the examined foxes the progesterone level was differentiated on 20-35d after mating. In the females maintained at the chamber, its mean values were substantially higher (72.32 ng/ml) as against the control (farm-42.63 ng/ml). The circulating levels of progesterone observed at the successive collections varied between the examined groups.

The I collection exhibited a marked progesterone increase at the foxes from the experimental group (B) compared to the control (A). Progesterone release at II collection, however, decreased and the difference was greater than in the experimental group.

Fig.1.Progesterone levels at dams at the successive collections at the farm and chamber (ng/ml)

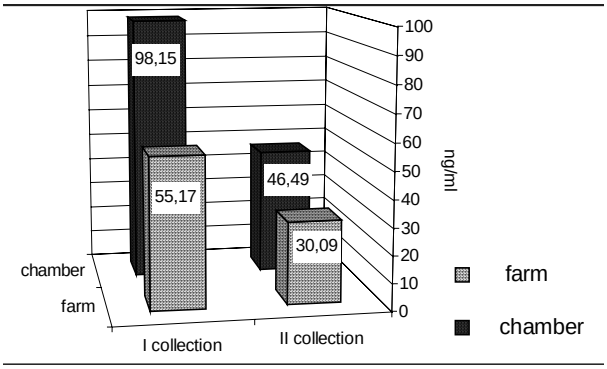
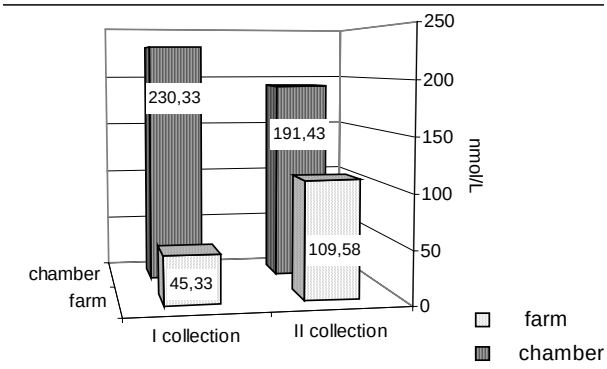


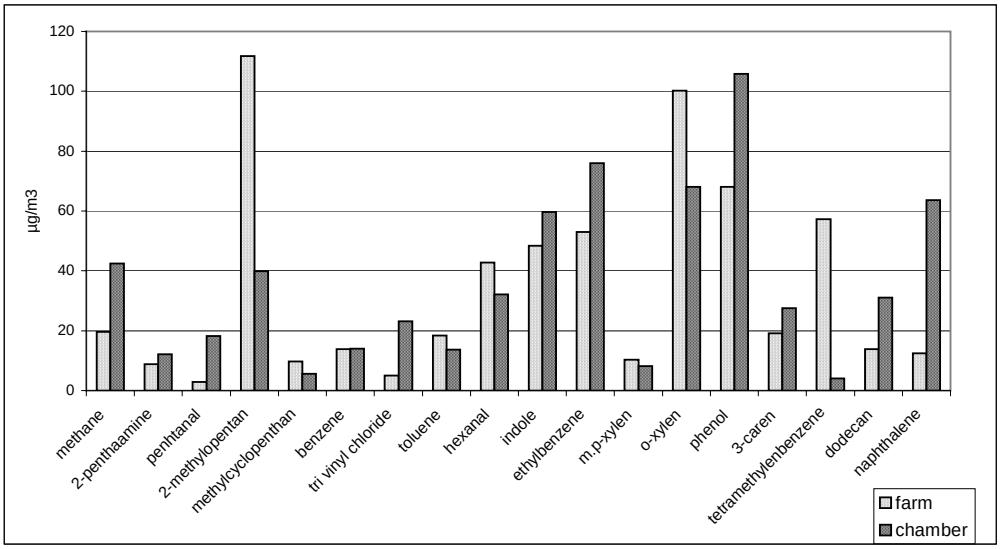
Fig.2.Cortisol levels at dams at the successive collections at the farm and chamber (nmol/L)



The cortisol values from both fox treatment groups are presented in Fig. 2. The highest values were detected for the foxes kept in the chamber (mean – 210.88 nmol/L), i.e. those exposed to gaseous pollutants (Fig. 3).

At successive collections, different fluctuations of cortisol were reported. In group A, its mean values had an upwards tendency at the successive collections, whereas in B a decline was noted. Comparing the mean values of cortisol of the foxes from group A and B some substantial differences were recorded subject to a collection. In I collection it reached 230 nmol/L, whereas at II – 81,85 nmol/L; the differences were statistically significant. In the face of the fact that the air examinations exhibited a higher level of gaseous pollutant release in the chamber in relation to the farm, an attempt was made to study an organism response to the harmful compound activity. Basing on the above mentioned data it may be assumed that the differences in the secretion of particular hormone might have been induced by an experimental factor, that is air pollution.

Fig.3.Mean values of other gaseous compounds identified at the farm and chamber in µm^3 [acc. to Nowakowicz-Dębek et al., 2004]



Discussion

The optimum breeding and rearing conditions is an essential requirement to show balance in the environment and animal welfare.

The investigations performed revealed the environment-animal relations. By means of depression and change of the basic microclimate parameters a tendency was to exhibit occurrence of disturbances in animal organism, particularly in the first trimester of pregnancy when dam sensitivity increases and embryos are implanted. A hormone essential for pregnancy maintenance proves to be progesterone and blocking its activity results in abortion. It is also indispensable to provide the appropriate development of the uterus mucosa that constitutes the base for implantation and new organism growth. Like estrogens, it affects the cells and tissues of whole organism, stimulating their metabolism. The pregnancy time is often termed a period of profound hormonal changes that manifest themselves with fluctuations of levels and hormone metabolism. An increase of sex hormones lead to, among others stimulation of respiration and diastole of bronchi, because progesterone is a stimulator of this center in the central nervous system. In the case of the respiratory system impairment progesterone can be used as an agent stimulating the ventilation, yet the researches have not been confirmed [Braastad et al, 1998; MnLean et al, 1995; Schatz, 1999; Smith, 1998].

The exhibited great differences in the progesterone in circulation indicate the influence of the environmental factors and inactivation of defensive mechanisms preventive of various disturbances. However, the present state of research does not allow to explain or to indicate the initiators of the disorders. The identified compounds are, among others, xenobiotics that may lead to the changes in organism functioning. Here the toxic operation of ammonia should be mentioned as it is present in the chamber air. Prolonged exposure to ammonia may lead, among others to some damage and paralysis of the respiratory center. Whereas, exposition to contaminants, like sulphur compounds recorded in the chamber air should be considered as the respiratory tracks irritation or even disturbances of the peripheral and central nervous system. The toxic impact of many pollutants like that have been documented, whereas no works available discussing their influence on a hormone release mechanism, at pregnancy in particular. It should be pointed out that in the polluted farm air there appears full mixture of harmful gases. It is not expected to analyse them separately because as it is well known their operation in the gas mixture can get enhanced or attenuated.

Another important mediator in organism proves to be cortisol. It is often considered a stress hormone occurring at endosystemic disturbances to mobilize organism. It shows, among others anti-inflammatory

activity, increased lymphocyte count in blood, inhibits immune response as well as reaction of allergen and antibody that inhibits allergic reaction. Cortisol, being a hormone of adrenal cortex, also affects the reproduction system. Generated by the fetal adrenal gland, it stimulates lungs development and maturity. Beside, it is one of factors of the parturition-inducing mechanism [Kazimierczak, 2001; McLean et al 1995; Smith, 1998].

In numerous works concerning behavior and the parturition mechanism of animals the authors refer to the secretion of cortisol. Osadchuk et al. [2003] reports that improper conditions of blue fox maintenance during pregnancy or stress brought about by inappropriate service may be manifested with an increase of cortisol secretion in dam`s adrenal gland or fetus plasma cortisol. Dam`s stress results in disturbances of sex steroid formation at fetuses. The changes were reported to appear especially at the morphometrical measurements of female fetuses [Osadchuk et al, 2003]. The prenatal stress effects of blue fox cubs were also described by Braastad et al. [1998]. The authors indicated the postnatal consequences of its activity on dam`s organism over the last trimester of pregnancy.

The glucocorticoid levels change under various stress forming situations. Rekila et al. [1999] claim that elevated response is differentiated and depends on a species as well as individual psychic constitution. The authors imply that there is a broad individual variation, which is significant at animal selection for farm population.

Hormones of a developing organism regulate metabolism, control the metabolic processes and homeostasis, i.e. stability of the internal conditions crucial for animal life. The disturbance of this equilibrium caused by the undesirable conditions of microclimate is likely to have negative effects on animals as it has been demonstrated in the present work.

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IV – 8 P

The influence of antioxidant emicidin on minks' physiological condition and reproduction

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Abstract
Antioxidant emicidin has an expressed quality to connect free radicals, it stabilizes cells' membranes and helps to increase the indices of animals' productivity. Emicidin was injected (enterally and orally) in doses 7, 25 and 50 mg an animal a day.
It was found that emicidin assists to increase the safety of cubs at the rate of 7-16 % and has growth assisting effect (males and females both). It also helps to increase, has growth the quality of skins by 5-17 % in males. Emicidin also normalizes the level of general protein of blood serum in whelped female minks, increases their lactation and absolutely excludes females' lactational exhaustion. The strongest effect of using antioxidant emicidin was checked when injecting it orally to mature females in dose 25 mg an animal a day during the periods of whelping and lactation.

Introduction
In modern animal industries biologically active substances with antioxidantal properties are beginning to find wide using. One of the substances with the expressed ability to connect free radicals, to inhibit processes of peroxidal oxidation of biomembranes' lipids and to reduce intensity of oxidizing processes in organism is emicidin.
Emicidin - 2 ethil, 6- methyl, 3-oxipiridin sukcinate represents a crystal white powder with a cream shade, easily soluble in water, with pH from 4,3 up to 4,9.
Due to the mechanism of action, emicidin has a wide spectrum of pharmacological effects and influences basic key parts of pathogenesis of various diseases associated with processes of free radical oxidation.
There was found growth stimulating and antistressful action of emicidin on pets' organism.
Results of our preliminary researches on application of emicidin in minks breeding indicate its positive influence on a number of productive parameters of animals when injected enterally.
Therefore there is a doubtless scientific and practical interest in the further studying of influence of antioxidant emicidin on viability of females minks weakened due to of lactation, especially having many cubs and also on the growth of suckling and young minks, taken from mothers.
The present research was carried out with the purpose of receiving objective estimation of efficiency of use of antioxidant emicidin in minks breeding.

Materials and Methods
Researches were conducted on sapphire and brown minks of wild type.
In experiments on the base of «Rodniki» of the Moscow region (Russia) there were used 280 lactating females and 1768 cubs received from them.
There was estimated influence of antioxidant emicidin on physiological
condition and productive parameters of minks in the various biological periods: lactation (April - June), active growth of young animals (June - August), at various ways of injection (peroral, parenterally) in dozes 7, 25 and 50 mg on a head per day under the developed circuit.
Experiments were executed according to the accepted requirements on formation of experimental groups, maintenance and feeding of animals (Balakirev & Yudin, 1994).
Biochemical and patomorphological researches were conducted with the use of standard methods (Berestov, 1976).
The following parameters were used as criteria of an estimation of animals' physiological condition: viability of females and safety of cubs, concentration of the general protein of blood serum, the size of adrenal glands of cubs at slaughter.

Efficiency of animals was estimated by parameters of absolute and relative gain of body weight of suckling (males and females both) and young animals (males), taken from mothers.

Results and Discussion

In the present work results of 5 experiments are used.

Experimental data have allowed to define positive influence of antioxidant emicidin on physiological condition and productivity of minks, receiving various dozes of antioxidant with feed composition or parenterally, both during the reproductive period and during active growth of young minks (Table 1).

Results of researches have shown, that injection of emicidin caused the increase of milk yield and, as a result, the increase of safety of cubs of minks' females with many cubs on 10-12 % . Animals were injected by antioxidant first ten days of lactation.

The relative gain of body weight of suckling cubs to 20-day's age was higher on 4-5 % and cubs were more viable.

The parameters of safety received by parenterally and peroral ways of injection of emicidin were similar in efficiency, at the same time, the second method of injection appeared to be less toilful and, therefore more acceptable for technology of fur farming.

Most of experimental researches were carried out with the use of this way of injection of antioxidant.

Positive influence of emicidin on the viability of lactating females is confirmed. It has ability to prevent the exhaustion of females in lactational period whereas 12% of intact females perish with the diagnosis «lactational exhaustion».

Safety of suckling young animals receiving emicidin by the time of taking them from females has received 84,7-94,8 % against 69,0-88,2 % - in the case of intact females. The best effect was in the group with doze 25 mg / head.

Table 1 The results of using emicidin (united data of 5 experiments)

Age group	Number of animals	Dose mg	Indexes	Methods of injection			
				parenterally	control	peroral	control
Suckling cubs	458	25	Safety by taking from mother, %	88.3	76.6-78.4	85.4	69.0
		25	Average increase of body weight by 20 day	165	160-161	-	-
	1483	7	Safety by taking from mother, %	-	-	87.1	88.2
		25		-	-	85.4-94.8	69.0-88.2
		50		-	-	84.7	69.0
		7	Including from litters with 7-11 cubs	-	-	87.0	84.2
		25		-	-	85.4-94.4	69.0-84.2
		50		-	-	84.7	69.0
Mature females	217	7	Safety after taking cubs, %	-	-	100	88.2
		25		-	-	100	88.2
		7	Including females with many cubs	-	-	100	76.3
		25		-	-	100	76.3
Cubs taken from mothers	216	7	Average increase of body weight, %	-	-	98.5	100.5
		25		-	-	106.2	100.5
		50		-	-	95.9	91.9
	216	7	Skins without defects, %	-	-	14.8	0
		25		-	-	14.8	0
		50		-	-	22.9	0
	216	7	Quality index	-	-	94.8	88.8
		25		-	-	89.4	88.8
		50		-	-	99.3	82.2

Influence of antioxidant was best revealed on females having 7-11 cubs. In this group no female died after the cubs were taken from them, while the safety of intact females was 76,7 % only.

Viability of cubs of experimental females with many cubs was much higher, than in the control - 84,7-94,4 % against 69,0-84,2 %. At the same time best result was taken from females, receiving emicidin in a doze 25 mg / head.

There was marked more intensive growth of cubs taken from mothers, receiving emicidin in dozes 25 and 50 mg / head in comparison with intact animals.

By the end of August (the end of intensive growth) distinctions in body weight of animals in experimental groups (on the average) and the control reach authentic value: 1766 ± 21 against 1622 ± 22 g ($P < 0,001$), gradually smoothing out to slaughter (November). The body weight was 2248 ± 47 g against 2186 ± 51 g, length of a body – 48,8 ± 0,34 sm against 48,6 ±031, accordingly.

Females, receiving emicidin, had more intensive increase of concentration of the general protein of blood serum during the first 20 days after whelping (after large blood loss). The relative gain of level of the general protein of blood serum in them in this period has made 36,8 % against 18,2 % in intact animals.

Patomorphological researches of gastroenteric system of the cubs taken from mothers receiving and not receiving emicidin during active growth, have revealed pathological changes of organs accordingly at 40 % and 60 % of the surveyed animals.

At the animals receiving emicidin, the weight of adrenal glands was less 12,5-20 %, than at intact, so it is possible to be considered as the consequence of display of antistressful action of the substance. It is corresponded with data taken by L.Osadchuk (2001) which specify the connection of the sizes of adrenal glands and level of produced corticosteroids, the production of which amplifies in reply to influence of stresses - factors.

Conclusion

The given data indicates the positive influence of antioxidant emicidin on viability of lactating females and young minks.

The greatest effect from using of antioxidant emicidin reveals during its peroral injection to the adult mink females in a doze of 25 mg on a head in a day. At the same time the most essential effect of emicidin reveals on the viability of female minks with many cubs and their cubs.

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IV – 9 RP

The measurements of the skin electrical conductivity in the acupuncture points affecting reproduction in female polar foxes (*alopex lagopus*) during the estrus period

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Summary
The aim of the investigation was the measurement of electrical conductivity (electric potentials in μA – 1 x 10^{-6} A) in the acupuncture points in female polar foxes during diestrus and estrus.
The measurements were taken in the acupuncture points situated on the urinary bladder meridian (points B_{22}, B_{23}, B_{25}, B_{31}, B_{32}) and on the meridian of the main beck regulator (points Lg_2, Lg_3, Lg_4) which are stimulated in cases of reproduction disturbances (during parturition and postpartum period) and additionally the acupuncture points situated on the large intestine meridian (points LI_4 and LI_{11}) affecting the immune system. The mean range of the skin electrical conductivity in the chosen points during estrus amounts to 81.2 - 88.1 μA on the urinary bladder meridian (points B_{22}, B_{23}, B_{25}, B_{31}, B_{32}) and to 86.0 - 87.7 μA on the meridian of the main back regulator (points Lg_2, Lg_3, Lg_4). The values are much higher than those observed during the diestrus period ($p<0.01$).
The values of the skin electrical conductivity measured in the points situated on the large intestine meridian (LI_4 and LI_{11}) affecting the immune system amounting to 68.7 - 70.0 μA and don't differ statistically from the results during diestrus period in female polar foxes.

Introduction
The electroacupuncture diagnosis is used in human medicine and there are some attempts at using the electroacupuncture in animal production (Hyodo, 1979). The aim of the investigation was the measurement of electrical conductivity (electrical potential in μA - 1 x 10^{-6} A) in the acupuncture points affecting reproduction results in the polar foxes females during estrus and compare them with the results from diestrus period.
The measurements were taken in the acupuncture points situated on the urinary bladder meridian (points B_{22}, B_{23}, B_{25}, B_{31}, B_{32}) which are stimulated in cases of disturbances during estrus, parturition and postpartum period and on the meridian of the main beck regulator (Lg_2, Lg_3, Lg_4) which are stimulated during parturition and postpartum period. In addition the measurements of electric potential in the points situated on the large intestine meridian (LI_4 and LI_{11}), affecting the immune system stimulation were taken during estrus and diestrus periods.

Material and methods
15 females 2-4 years old, with similar condition were chosen for the experiment. The measurements of electrical potential in acupuncture points during diestrus, right before estrus (March – April) were taken. The measurements were repeated on 10 females during estrus period: only the females in heat, showing the sexual receptivity were chosen for the investigation. The experiment was performed in March - April when the air-temperature was +4 to +10ºC. The measurements were taken with the Diagnoscope EAP 871 constructed in the Institute of Biocybernetics and Biomedical Engineering from Polish Academy of Sciences in Warsaw.
The measurements of electrical conductivity were taken at the following parameters: direct current 6V and the intensity of the short-circuit current 200 μA. Electrical conductivity in the chosen points is presented conventionally in the units of electric current intensity (μA – 1 x 10^{-6}A).
The passive electrode of the diagnoscope was fixed to the wetted ear of the female fox with physiologic salt solution and the measurement points were localized by an active electrode. The active electrode was adapted to the skin at a right angle and the measurements were taken with a slight steady pressure. The time of the measurement was regulated automatically and the apparatus disconnected itself after 3 seconds.

Table 1. Localization and description of acupuncture points chosen for the experiment

Acupuncture points	Localization	Description
B_{22}	between 1-2 transverse processes of the lumbar vertebra	stimulated in cases of estrous disturbances
B_{23}	between 2-3 transverse processes of the lumbar vertebra	stimulated in cases of estrous disturbances
B_{25}	between 4-5 transverse processes of the lumbar vertebra	stimulated in cases of parturition and postpartum period disturbances
B_{31}	openings on the sacral bone from the dorsal side	stimulated during the parturition and postpartum period
B_{32}	openings on the sacral bone from the dorsal side	stimulated during the parturition and postpartum period
Lg_2	between the sacral bone and the first coccygeal vertebra /along the back/	stimulated during the parturition and postpartum period
Lg_3	between the sacral bone and the last lumbar vertebra /along the back/	stimulated during the parturition and postpartum period
Lg_4	between 2-3 spinous processes of the lumbar vertebra /along the back/	stimulated during the parturition and postpartum period
Li_4	between the thumb and the metacarpus	stimulation of that point affects the immune system
Li_{11}	on the anterolateral surface of the forearm in the depression above the radial bone epiphysis	stimulation of that point affects the immune system

10 acupuncture points: 8 stimulated in cases of estrus, parturition and postpartum disturbances (B_{22}, B_{23}, B_{25}, B_{31}, B_{32}, Lg_2, Lg_3, Lg_4) (Kothbauer & Meng, 1983) and 2 affecting immune system (LI_4 and LI_{11}), (Sciesinski, 1988, Sciesinski, 1996) were chosen for the experiment. The localization and description of chosen points are presented in the Table 1. For statistical analyses, a 2-sample t-test was used to test differences between females in estrus and diestrus periods.

Results and discussion

The results of the skin electrical conductivity (μA) in the chosen acupuncture points stimulated in cases of estrus, parturition and postpartum disturbances are presented in Table 2.

The electric potential in all checked points lying on urinary bladder meridian (B_{22}, B_{23}, B_{25},B_{31}, B_{32}) and on the main back regulator (Lg_2, Lg_3, Lg_4) was much higher ($p<0.01$) during estrus than during diestrus. Higher potential suggests that mentioned points at that time are more sensitive for any impulse. The acupuncture stimulation of chosen points, as described by other authors (Westermayer 1979, Kothbauer & Meng 1983) can improve reproduction results.

The results of skin electrical conductivity points responsible for immune system activity in polar fox females are presented in Table 3.

There were no statistical differences in electrical conductivity in acupuncture points Li_4 and Li_{11} situated on the large intestine meridian located on forearm, between diestrus and estrus periods, even the conductivity measured during estrus appeared higher. As it was described in literature (Sciesinski, 1988; Sciesinski, 1996), stimulation of these points affects immune system. When the animals are healthy and in good condition, the electrical conductivity of specific points should not differ statistically (Sciesinski, 1996).

The presented results illustrate the differences in the skin electrical conductivity between female polar foxes in the diestrus period and those being in heat. The higher values of the skin electrical conductivity in female polar foxes during estrus are affected by physiological processes in their organisms.

Table 2. The comparison of electrical conductivity acupuncture points (µA) affecting reproduction results during diestrus and estrus in polar fox females

Acu-puncture point	Electrical conductivity (µA, 1 x 10^{-6} A)			
	During diestrus		During estrus	
	x	Sd	x	Sd
B_{22}	62.1A	4.91	87.3B	3.63
B_{23}	62.7A	4.49	87.6B	5.02
B_{25}	61.7A	5.46	88.1B	4.46
B_{31}	62.8A	5.33	81.2B	3.37
B_{32}	60.4A	6.06	87.0B	4.71
Lg_2	57.8A	5.34	86.2B	4.33
Lg_3	58.9A	6.87	87.7B	3.87
Lg_4	59.1A	10.27	86.0B	5.62

A,B – difference at $p<0.01$

Table 3. The comparison of electrical conductivity (µA) points stimulated during immune system disturbances, between diestrus and estrus periods, in polar fox females

Acu-puncture point	Electrical conductivity (µA, 1 x 10^{-6} A)			
	During diestrus		During estrus	
	x	Sd	x	Sd
Li_4	56.4	4.50	63.5	8.08
Li_{11}	54.4	4.77	61.2	7.07

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IV – 10 RP

Isolation of microsatellite markers for American mink *(Mustela vison)*

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Abstract
The objective of this study was to isolate and characterize microsatellite markers, especially tetranucleotide repeats, for American mink. A size-selected mink genomic library was constructed, and recombinant colonies (n=2435) were screened with two pools of probes. One pool included $(AAAC)_8$, $(AAAT)_8$, $(AACC)_8$, $(ATGG)_8$ and $(AC)_{15}$, and the other pool contained $(AAAG)_8$, $(AAGG)_8$, $(AGGG)_8$, $(ATAG)_8$ and $(AG)_{15}$ oligonucleotides in equal amounts. Positively hybridized colonies were bi-directionally sequenced. Thirteen of the recombinant colonies (0.53%) contained a microsatellite. One GTTT, one GGAT, four AG and seven AC repeats were detected, which may represent the relative abundances of these repeat motifs in the mink genome. One locus could not be amplified by the polymerase chain reaction. Variability of other loci was determined by genotyping 86 unrelated mink of three color types (black, brown, pastel) and wild mink trapped in northern New Brunswick (Canada). Two of the loci were monomorphic, and the other 10 generated 2, 3, 4, 5, 6, 7, 7, 9, 10 and 11 alleles (average of 6.4). Seven of the primer sets amplified DNA of American pine marten *(Martes americana*).

Introduction
Microsatellites are markers of choice for evolutionary and conservation studies, paternity testing, assignment of individual animals to specific subpopulations (Belliveau *et al.* 1999), as well as for the construction of linkage maps which are valuable tools in animal genetic improvement. Despite the economic importance of American mink (*Mustela vison*) in North America and northern Europe, information on the mink genome, compared to most other farm animal species, is very scarce. Fewer than 100 microsatellite markers have so far been identified for mink (O'Connell *et al.,* 1996; Brusgaard *et al*., 1998 a,b,c; Davis and Strobeck,1998; Fleming *et al.,* 1999; Vincent *et al.,* 2003), and more are needed to construct a rough linkage map of the mink genome. The objective of this work was to isolate and characterize microsatellite markers, particularly tetranucleotides, for mink. Although tetranucleotides are less abundant than dinucleotides in mammalian genomes (Lander *et al.,* 2002), they can be more easily and accurately scored on gels or electropherograms as a result of low intensity of stutter bands (Urquhart *et al.,* 1995; Walsh *et al.,* 1996).

Materials and Methods
Approximately 30 µg of genomic DNA from one female black mink was digested to completion overnight with *Sau*3AI. Digested fragments were size separated on a 1% agarose gel, and fragments of 300 to 800 bp were recovered from the gel and purified by phenol extraction and ethanol precipitation (Sambrook *et al.* 1989). Size-selected fragments were ligated into *Bam*HI-digested dephosphorylated pGEM-3Z vector (Promega, Madison, WI, USA). The ligated products (2 µL) were used to transform 50 µL of maximum efficiency competent *E. coli* (JM109, Promega) and were plated out on LB/ampicillin/IPTG/X-gal media and cultured overnight. Recombinant colonies were transferred onto duplicate LB/ampicillin plates and were lifted onto Hybond-N^+ nylon membranes (Amersham, Piscataway, NJ, USA) after overnight growth. Lifted colonies were fixed on membranes by baking for 2 hours at 80°C under vacuum. Cell debris were removed by incubating membranes in 100 mL of a digestion solution (100 µg/mL proteinase K, 50 mM Tris Cl, pH 7.6, 0.1% SDS and 50 mM NaCl) at 37°C with gentle agitation for at least 6 h, and were rinsed in 100 mL of 2X SSC.
Membranes were hybridized with two pools of oligonucleotide probes using a chemiluminescence DNA detection kit (Amersham) according to the manufacturer's instructions. One pool contained 50 ng

each of $(AAAC)_8$, $(AAAT)_8$, $(AACC)_8$, $(ATGG)_8$ and $(AC)_{15}$ oligonucleotides and the other contained the same amount of $(AAAG)_8$, $(AAGG)_8$, $(AGGG)_8$, $(ATAG)_8$ and $(AG)_{15}$. The concentration of each probe was doubled when membranes were re-hybridized. Pre-hybridization and hybridization were performed at 42°C in a rotisserie hybridization oven for one and three h, respectively. Membranes were exposed to the Kodak Bio-Max MR-1 X-ray films, and positively hybridized colonies were re-plated and re-hybridized for confirmation. A few bacterial cells from each confirmed colony were directly transferred to a PCR cocktail, and the DNA insert was amplified using the T7 and SP6 universal primers (Promega) at 50°C annealing temperature. Amplified DNA inserts were bi-directionally sequenced. Sequence alignment and editing were performed using the Sequencher software (Gene Codes Corp., Ann Arbor, MI), and search for repeats was performed by the Tandem Repeat Finder program (Benson, 1999). Primers for the amplification of microsatellite loci were designed using the Oligo Primer Analysis Software, Version 6 (Molecular Biology Insight, Cascade, CO).

Following optimization, forward primers were fluorescently labeled with NED (Applied Biosystems), 6-FAM or HEX (Invitrogen, Burlington, ON). Amplifications were performed in 15.0 µL total volumes containing (final concentration) 0.1% Tween 20, 1X PCR buffer, 0.2 mM each dNTP, 800 nM each primer, 0.24 unit of *Taq* polymerase (Roche, Laval, QC) and 20 to 50 ng of genomic DNA. All loci were amplified using the 2-step PCR protocol in an Eppendorf Master Cycler (Hamburg, Germany), which was programed at 95^{B}C initial denaturation for 4 min, followed by 30 cycles of denaturation at 94^{B}C and primer-specific annealing temperature (Table 1), each for 30 s.

The Mvi804, Mvi1008, Mvi1010, Mvi1012, Mvi1014 and Mvi1017 loci yielded split peaks on electropherograms and unstable allele sizes, which were caused by the nontemplated addition of adenosine to the 3' end of the PCR products (Hu, 1993; Brownstein *et al.,* 1996; Magnuson *et al.,* 1996). Leaving PCR products of the Mvi804 and Mvi1017 at room temperature for at least two weeks, and those of Mvi1008 and Mvi1012 for at least one week, prior to genotyping, which resulted in the addition of adenosine to PCR products, resolved the problem. Leaving PCR products of the Mvi1010 and Mvi1014 for at least two weeks partially resolved the problem, and the use of TaKaRa LA *Taq* polymerase (Fisher Scientific, Ottawa, ON) improved stability of the Mvi1010 peaks.

Polymorphism at each locus was determined by genotyping 86 mink; 25 black, 20 pastel, 20 brown and 21 wild. Black mink originated from four large breeding ranches in Nova Scotia, and were unrelated to each other for at least one generation. Samples of pastel and brown mink were from large-size breeding ranches in Prince Edward Island, and wild mink were trapped in a 40 km^2 area in northern New Brunswick (Belliveau *et al.* 1999). Genotyping was performed using an ABI Prism 377 DNA sequencer equipped with the GeneScan and Genotyper software (Applied Biosystems, Inc., Foster City, CA). Diluted amplicons and a size marker (400 HD ROX, Applied Biosystems) were denatured at 94^{B}C for two minutes prior to loading (1.5 µL) onto the gel slot. Genotypes of all mink were determined at every locus. Observed and expected heterozygosities were computed using the Popgen software (http://www.ualberta.ca/-fyeh).

Results and discussion

Eighteen of the 2435 recombinant colonies were positively hybridized and DNA inserts were sequenced. Thirteen DNA inserts contained a microsatellite (0.53% of recombinant colonies) and five inserts had fewer than five uninterrupted dinucleotide repeats, which were excluded from further analysis. Searches of GenBank with the flanking sequences of the microsatellites confirmed that none has been previously reported. The repeat motifs and GenBank accession numbers of the loci are shown in Table 1. Although 80% of the probes were tetranucleotide-specific oligonucleotides, only two loci containing tetranucleotide repeats were revealed (one GTTT and one GGAT), while four AG and seven CA repeats were detected. The number of each type of repeat identified may reflect the relative abundance of each type of repeats in the mink genome, and are consistent with those in the human (Lander *et al.,* 2001). Excluding Mvi1017, which contained six repeats (CCT, TCC, CTT and GA), the mean numbers of the longest uninterrupted repeating units were 13.9 for AC, 11.0 for AG and 5.5 for tetranucleotides (Table 1).

Table 1. Repeat motifs and GenBank accession numbers of microsatellite loci.

Locus	Repeat motif	GenBank Accession number
Mvi804	$(GTTT)_6$	AY602193
Mvi1001	$(TC)_{11}N_{184}(TC)_5(CT)_2$	AY602194
Mvi1006	$(CA)_{16}$	AY602195
Mvi1007	$(GC)_5(CA)_{12}$	AY602196
Mvi1008	$(CA)_6(TA)_2(CA)_6TG(CA)_4CG(CA)_3N_{70}(TG)_{11}$	AY602197
Mvi1009	$(AC)_{12}$	AY602198
Mvi1010	$(GT)_4(TC)_{13}$	AY602199
Mvi1012	$(GGAT)_3GAAT(GGAT)_5$	AY602200
Mvi1013	$(AG)_9GATA(AG)_6$	AY602201
Mvi1014	$(TG)_{16}$	AY602202
Mvi1015	$(AC)_8AA(AC)_{15}$	AY602203
Mvi1016	$(GT)_{15}$	AY602204
Mvi1017	$(CCT)_3TCT(CCT)_4(TC)_2(CT)_2(TCC)_{12}N_{26}(TTC)_4(TCC)_9$ $(TTC)_3TGCT(CTT)_{12}N_{43}(GA)_6GGGCAT(GA)_7$	AY602205

The mean number of AC repeating units falls within the 13.4 to 15.1 reported by others for mink (O'Connell *et al.,* 1996; Fleming *et al.,* 1999; Vincent *at al.,* 2003).

The unique sequence flanking the repeating unit of the locus Mvi1015 was too short, resulting in the failure of the primer set to produce a specific band. Sequences of the primers for the remaining 12 microsatellites are shown in Table 2. Ten of the loci were polymorphic in the panel of 86 mink, and generated between 2 (Mvi1013) and 11 (Mvi1016) alleles. Observed heterozygosity (H_o) ranged from 0.15 (Mvi1013) to 0.84 (Mvi1016), and expected heterozygosity (H_E) ranged from 0.25 (Mvi1013) to 0.85 (Mvi1016) (Table 2). The means of number of alleles, H_o and H_E of the polymorphic loci were 6.4, 0.54 and 0.67, respectively. Although estimates of the number of alleles, H_o and H_E are the characteristics of each locus, they also reflect the diversity of the animals that were genotyped. The estimates suggest that at least six of these loci, each with six or more alleles, are very useful for population genetics studies. Tetranucleotides had a lower level of variability than dinucleotides in this study, as one (Mvi1012) was monomorphic and one (Mvi804) had only 3 alleles.

Table2. Primer sequence, annealing temperature (T_A), number of alleles, allele sizes, and observe (H_O) and expected (H_E) heterozygosities of the mink microsatellites[1]

Locus	Primer sequence (5'-3')	T_A, ^BC	No. of alleles	Allele size, bp	H_O	H_E
Mvi804	F: GGAAATACCTATCATGGC R: AAGAGTTGTAAGGAAGTTCCAG	59.1	3	149-157	0.43	0.57
Mvi1001[2]	F: AGTGCAAGAAGGACGTAATGTG R: AGAGACCGAGAGAGCATGTATG	59.1	1	152	0.0	0.0
Mvi1006	F: CCAAGCAGGATTCAGCCTATTC R: AAGGCCATGCACTAGGTAA	59.1	10	149-167	0.79	0.79
Mvi1007	F: TAAGAGGCTTGCCGTGTTCA R: TCAGGACTGTCTCTTCGGGATG	59.1	4	248-254	0.59	0.64
Mvi1008[3]	F: GATGGGGATAAACCTGCTAATC R: CCCCAAATGAACCTCCATACAA	59.1	5	210-218	0.59	0.68
Mvi1009	F: CAAGCCTCCACAACTGT R: ACAATGGTGCTATGTTAGTTA	62.2	6	152-162	0.41	0.64
Mvi1010	F: ATCAAGCCCCACGTCATACTCCC R: GGCAGCCGCTTCATGACTGAGACAC	66.7	7	167-181	0.63	0.71
Mvi1012	F: ACTGATGCCTGCCATAGCTC R: TACCCAGCCTGGAGTAGTAGTTTG	59.1	1	258	0.0	0.0
Mvi1013	F: GCTCCATACTTGTCCAACAACTTCC R: CTGCTTCTCCCTCTCACCCTACC	59.1	2	166-170	0.15	0.25
Mvi1014	F: TCTGCATGTAAAATATGGGATA R: TCACAGGTCCTTGCTTGAACAC	56.5	9	136-152	0.71	0.81
Mvi1016	F: CTGCTTCTCTGCCTACTTCT R: TTGTTCCCTTCCTATTATCTGT	59.1	11	218-238	0.84	0.85
Mvi1017[4]	F: TCCTCTCATGTGTCTTTGGGTTAT R: TGCTCTTCAGGGAGTCTGCTTCT	66.0	7	326-349	0.28	0.76

1- Optimum $MgCl_2$ concentration is1.5 mM for all the primer sets (supplied in the PCR buffer), except for the Mvi1009, which requires 1.0 mM additional $MgCl_2$.
2-The first repeat of Mvi1001 is too close to the 5' end of the sequence, and is not included in the amplified segment.
3- Amplified segment contains both repeats of the Mvi1008.
4- Amplified segment contains all repeats of the Mvi1017

Seven of the primer sets (58%) generated specific PCR products in American pine marten (*Martes americana*). Two loci were monomorphic, and five loci generated between two and five alleles in six related individuals (Table 3). Interestingly, the Mvi1012, which was monomorphic in mink, generated two alleles in pine martens. Amplification of pine marten DNA with primers designed for mink, which belong to the same subfamily *(Mustelinae),* has been reported by O'Connell *et al.* (1996), Vincent *at al.* (2003), and Fleming *et al.* (1999). Likewise, 3 of the 13 microsatellites developed for American marten amplified mink DNA (Davis and Strobeck, 1998). The cross-species amplification of microsatellite primers is a fast and inexpensive method of developing genetic markers in the members of *Mustelidae* family.

Table 3. Annealing temperature (T_A), number of alleles and allele sizes of mink primers that amplified DNA of American pine marten[1]

Locus	T_A,°C	Number of alleles[2]	Allele size (bp)
Mvi804	51.4	1	153
Mvi1001	59.4	1	151
Mvi1006	65.0	4	156, 162, 164, 166
Mvi1007	56.5	5	259, 261, 263, 265, 267
Mvi1008	59.4	4	215, 219, 223, 227
Mvi1012	51.4	2	270,274
Mvi1014	56.5	2	118,122

1- Optimum $MgCl_2$ concentration is1.5 mM for all the primer.
2- In six related individuals.

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IV – 11 RP

Litter size, weaning success, and nursing mortality in chinchillas (*Chinchilla lanigera*) in relation to cage illumination

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Abstract

The aim of this study was determine effects of light intensity on litter size, number of weaned per litter and mortality rate during nursing. Study was performed on a reproduction farm in western Poland, during1999-2003. The analysis covered reproduction performance of 250 females of the standard variety. Light intensity was measured with a photoelectric light meter LS-200 and ranged between 0 and 270 lx. The chinchillas were assigned to 9 groups, 30-lx interval each. No statistical differences were found between the groups in relation to light intensity. Both litter sizes and number of weaned per litter grew along with increasing light intensity. The lowest mortality was found at the highest light level, i.e. 241-270 lx (group IX) – 4.17%. Nursing mortality showed a falling trend with growth in cage illumination level. The range between 241 and 270 lx was the optimal range of light intensity in this study.

Introduction

Wild chinchilla is a nocturnal species inhabiting rock cracks and hollows of mountain slopes of the Andes. The species, however, has been observed to live also a diurnal life in its natural habitat [Hoefer, 1994; Mohlis, 1983; Walker, 1975]. Barabasz [2003] also found that farmed chinchillas exhibit, as a result of domestication, increased activity during morning hours and during the day.

Reproduction of chinchillas and other farmed animals alike depends heavily on climatic conditions and, in the case of indoor housing, on the microclimate of the sheds, primarily on temperature, humidity, light, and feeding [Bernard et al. 1999]. Effect of light on reproductive processes consists in regulation of gonad activity. Growth of the ovarian follicles is controlled by folitropin, or the follicle stimulating hormone (FSH), whose synthesis and secretion from the anterior pituitary is stimulated by gonadoliberin (FSH/LH-RH), a decapeptide hormone released by the hypothalamus. Information on the amount of hypothalamus-released hormones is conveyed via thermal and light stimuli [Turner and Bagnara, 1978].

Chinchilla sheds should be dry, well illuminated, well ventilated, without draughts, and free of fungi [Barabasz, 1996]. The optimal temperature should remain within the range 16-22°C, relative humidity 50-70%, and air flow between 0.2 and 0.3 m/s [Barabasz, 1996; Felska, 1999; Parker, 1982]. Little has been reported, however, on the light conditions that are optimal for chinchillas. At present, artificial illumination is becoming more and more common in chinchilla sheds, and no adverse changes in the organisms of the animals have been found that would be a consequence of lack of sunlight. Artificial illumination depends on the number, type, power, and distribution of lamps. Usually, incandescent lamps of 60, 75, or 100 W, as well as fluorescent lamps of 25-40 W are used to illuminate the sheds; mercury or sodium vapour lamps are rare. The colour of artificial light must resemble the spectrum of natural light.

Due to chinchilla reproduction specificity, i.e. their low fertility in terms of number of litters and their sizes [Gromadzka-Ostrowska et al., 1985], intensive research has been in progress to improve reproduction parameters, often through enhancement of microclimate of the sheds, also through finding optimal light illumination.

The aim of the study was to determine the effect of light intensity on litter size, weaning success in terms of the number of weaned young, and pre-weaning mortality rate in chinchillas.

Material and Methods

The studies were performed on Alex Chinchilla Farm in Nowogard, one of the largest chinchilla breeding farms in Poland, during 1999-2003. Chinchillas on this farm are housed only indoors, in polygamous breeding system cages arranged in four-level sets. A polygamous set is composed of four females and a single male. Reproduction performance of 250 standard chinchilla females was evaluated. The females were at ages 2-5 years, i.e. in

the most fertile period of life. The first litters of the females were excluded from the analyses.

The chinchillas were managed in a shed with controlled constant temperature and relative humidity. In order to evaluate the microclimate of the shed, the following measurements were done: air temperature and humidity, air evaporative cooling, air flow, and light intensity. The temperature remained within 18-20°C, relative humidity within 50-60%, air evaporative cooling was 12.4-16.7 mW/cm^2, while air flow was 0.2 m/s.

The shed lacked windows; artificial light, produced by 40-W fluorescent lamps, was the only source of illumination. In 1998, a 12-hour light regime was introduced on Alex Chinchilla Farm, with average illumination intensities being about 5 W/m^2. In the beginning of 2001, in a selected part of the shed, 5 new lamps of 116 W were mounted. The average illumination of this part was 10 W/m^2.

Light intensity was measured inside the chinchilla cages by means of a photoelectric light meter LS-200 (Sonopan, Poland). Light was measured along the six planes: right, left, front, hind, up and down, and the mean for a particular cage was calculated from these six measurements. Light intensities in the cages ranged between 0 and 270 lx. The animals were distributed into nine 30-lx groups. The number of chinchillas in each illumination group and the ranges of light intensity is presented in Table 1.

Table 1. Number of females in each illumination group

Group	Light intensity [lx]	Number of females
I	0-30	62
II	31-60	37
III	61-90	28
IV	91-120	29
V	121-150	31
VI	151-180	16
VII	181-210	18
VIII	211-240	19
IX	241-270	10
Total	0-270	250

In each group, the number of offspring born and weaned from the litter and death rate during maternal nursing were analysed. The data were then computed and statistically processed using a spreadsheet and Statistica 6.0 software package. The following descriptive statistics were calculated: arithmetic mean (M), standard deviation (SD), and coefficient of variability (CV). The non-parametric Kruskal-Wallis test was applied for testing significance of differences, since the variables were of ranked character and were not normally distributed.

Results

Table 2 presents statistical characteristics of mean litter size and number of weaned young per litter.

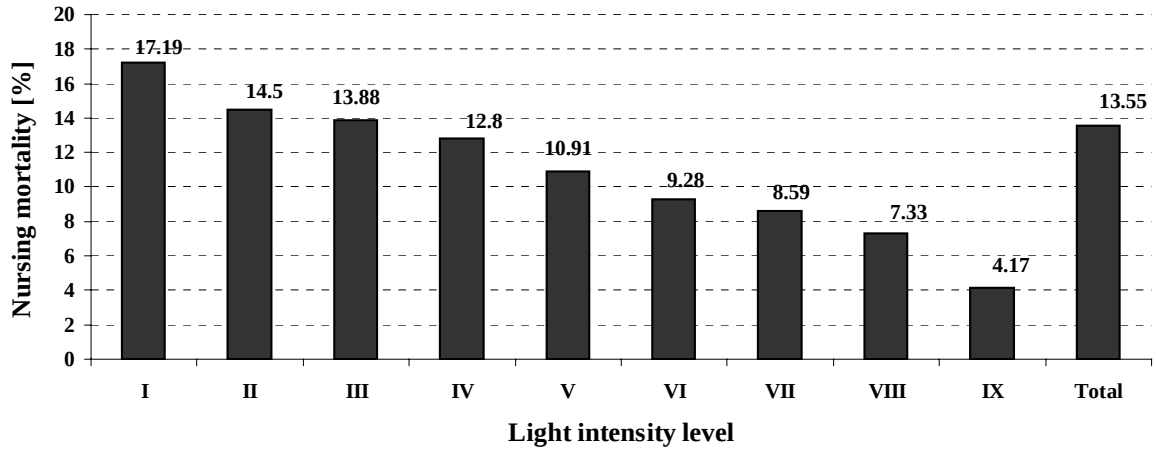
The highest numbers of both born and weaned offspring per one litter were recorded for the highest light intensities, i.e. at 241-270 lx (group IX), 2.25 and 2.17 young respectively. In contrast, the lowest numbers of born and weaned per one litter were recorded in the group of 0-30 lx (group I) and 211-240 lx (group VIII), respectively 2.03 and 1.84 (born) and 1.72 (weaned). The differences, however, proved statistically non-significant. The largest litters and the best weaning rates were achieved under light intensities ranging between 181 and 270 lx.

Figure 1 depicts the mortality of young during maternal nursing. The testing did not yield any statistically significant differences in deaths during nursing between the illumination groups; however, the highest mortality rate was found under the lowest light intensities, 0-30 lx (group I), 17.19%, while to lowest was found under the highest light intensities, 241-270 lx (group IX), 4.17%. The overall nursing mortality was about 13.5%. Despite no significant differences, the light intensity apparently influenced the pattern of nursing mortality; the mortality dropped with increasing illumination intensities.

Table 2. Statistical characteristics of chinchilla births and weaning

Group	Born chinchillas per litter			Weaned chinchillas per litter		
	M	SD	CV	M	SD	CV
I	2.03	0.79	38.92	1.72	0.91	53.18
II	2.15	0.73	34.10	1.83	0.84	45.74
III	2.08	0.82	39.36	1.76	0.77	43.64
IV	2.18	0.80	36.71	1.85	0.78	43.47
V	2.18	0.69	31.69	1.90	0.71	37.70
VI	2.20	0.79	36.05	1.98	0.82	41.52
VII	2.21	0.74	33.44	2.06	0.90	43.64
VIII	1.84	0.47	25.68	1.72	0.54	31.49
IX	2.25	0.61	27.02	2.17	0.76	35.14
Total	2.16	0.74	34.20	1.95	0.77	39.64

Figure 1. Nursing mortality in each illumination group



Discussion

Chinchillas belong to polyoestrous animals, i.e. having more than one sexual cycle in one year. The number of produced pelts, and thus the profit of the breeder, depends on the number of born and weaned chinchillas. The chinchillas seem to have a large reserve of unused reproductive potential. An adult female produced about 16 ovarian follicles in one sexual cycle, of which only 4 maturate during the oestrous stage of the cycle and, hence, given environmental and genetic conditions are good, four young per litter should be achieved [Barabasz, 2001]. Puzder and Novikmec [1992] have stated that the number of maturating egg cells per cycle ranges between 4 and 16, and the annual number of born offspring is 4-8. According to Socha et al. [2001a, 2001b], litter size may range from 1 to 5. Most often, however, 2 young are born in a litter [Barabasz, 1997; Puzder and Nowikmec, 1992; Socha et al., 2001a; Sulik, 1994]. This has been confirmed in this study. Lanszki et al. [1998] obtained 2.04 born and 1.84 weaned young chinchillas.

Our results correspond to those reported by Garcia et al. [1989], who stated that light influences chinchilla reproduction, since females kept in better-illuminated cages gave births to larger litters compared to those managed in the cages with lower light intensity. According to Garcia et al. [1989], the highest mortality rate is during the first two weeks of life and reaches as high as 20%. Such high mortality may result from insufficient milk supply by dams as well as from low tolerance of the young to lower temperatures, below 10°C. Lanszki [1996] observed that the highest mortality occurs during the first week of postnatal life and reaches about

15.3%. Felska et al. [2002] states, however, that pre-weaning death rate ranges between 10.4% to 17.1%, which corresponds to the results of this study. It has been found in this study that higher light intensities have a positive effect on raising young chinchillas. These results correspond to those published by Garcia et al. [1989], who observed that pre-weaning mortality in the cages placed at the 4th level, where light intensity was higher than at lower levels of cages, was lower compared to that recorded at the 3rd and 2nd levels of cages.

The elevated intensity of illumination applied in our experiment resulted in higher numbers of born and weaned offspring per litter as well as in lower death rate of young chinchillas during their maternal nursing period. The range of light intensity between 241 and 270 lx represented the optimal range in this study.

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IV – 12 RP

Evaluation of pastel fox breeding results in poland - production traits

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Abstract
Material for study was females of common fox different color varieties maintained in 1978-1997, from which 4155 progenies with pastel fur were observed.
In order to estimate the efficiency of selection, evaluation of genetic and phenotypic trends were applied. Phenotypic trends were estimated as changes of phenotypic trait mean values in time. The basis for genetic trends estimation in population studied was the solution for the birth year of an individual describing the changes of genetical quality in time. Calculations were made using software BLUPf90, applying multi-trait animal model.
Selection differences for reproduction and conformation traits taken into account during selection were calculated to estimate the intensity of the process. They were accepted as differences between mean phenotypic value of a trait among young foxes chosen to general herd and mean phenotypic value of a trait for all young.
On a base of results achieved, the conclusion was drawn that positive values of genetic trends for conformation traits and number of reared animals testify to proper direction of breeding work. However, their low values point out to low efficiency of selection. This can be a result of large number of traits considered during selection. For all investigated traits with exception of litter size at birth, an increasing tendency was found during the years under investigation which proves, the breeding work was conducted properly in this herd.

Introduction
The beginnings of original Polish pastel fox breeding are in 1972 when a female of silver hair in one of Poznań farms born a litter consisting of black-silver (so-called "standard") and beige animals. The female along with the litter was purchased by ZHZF in Jeziory Wielkie [4, 6, 8]. This mutant was initially called "pearl of lakes" and then pastel fox as an analogy to coypu and pastel mink.
In the first years of pastel fox breeding (1972-1975), the general goal was to reproduce brown animals as soon as possible. It had to be done by reproduction of the mutated gene. Therefore, animals were mated related to one another. It led to the increase of inbreeding due to small number of individuals and common primary origin from the same foxes. Inbred depression that caused negative biological effects was a result. This variety of animals was characterized with hyperexcitability (timidness, aggressiveness, abnormal mobility) and brown-colored females damaged their litter more often than others. Also cases of submaxilla breaking due to strong squeezing the jaws on forks for animal catching were observed, which proved both great irritability and the fragility of their bones [4, 5, 6]. The problem that arose was to determine whether the symptoms are associated with new color genes (e.g. due to pleiotropic action) or are they a result of much advanced inbreeding. The thesis was drawn that reproduction and breeding problems did not result from unfavorable interaction of pastel color gene, but they were effect of strong inbreeding of animals. It appeared that inbreeding at foxes easily invoke inbred depression, which is usually manifested with the decrease of animal viability, condition worsening and decrease of fertility.
Janusz Maciejowski began organized breeding upon new mutation variety in 1976 [6]. The herd consisted of 13 pastel males and 9 females. Moreover, some silver foxes were vectors of brown color genes. Analysis of these animals' origin revealed profound inbreeding, because rapid reproduction of mutated gene was the initial aim of breeding. Decision of suspension of the pastel fox mating among themselves was a remedy.
At the first stage (1976-1980), intensive reproduction of new color variety genes was performed through mating the pastel with silver foxes and at the same time, avoiding of mating among themselves due to the threat of inbreeding effects [8]. No selection among pastel variety was made except from the culling of some animals because of their bad health in this period [4]. At the same time, mating according to the following scheme was performed:

♀ silver × ♂ pastel
♀ pastel × ♂ ½ pastel*
♀ ½ pastel* × ♂ pastel
♀ ½ pastel* × ♂ ½ pastel*

** ½ pastel – common fox of different color varieties, vectors of pastel gene to a minimum extent related with partners*

Mating of pastel males with females – vectors of pastel gene as well as reciprocal mating: males – vectors of pastel gene with pastel females, was the most preferred.
Pastel foxes were crossbred among themselves again in 1980 when the herd consisted of 55 females and 69 males on a basis of individually prepared mating schemes in which animal relation was taken into account.
Since 1981, selection among animals with pastel hair has been conducted in order to achieve foxes with positive fur traits. It can be concluded that general directions of selection had to be as follows: color type (the most desired shade), structure of hair cover, body structure traits, fertility, prolificacy, maternal solicitude and soft temper.
Elaboration of the structure assessment standard for pastel fox by Maciejowski, Sławoń and Dąbrowska in 1984 [9] was an important moment in new variety breeding. Despite changes introduced, directions of the color variety improvement were not changed. Besides commonly accepted fur traits (density, length of hair, hair uniformity, elasticity, silkiness), dark brown animals of blue shade were accepted as the most desired type, because (apart from aesthetic virtues) animals with darker fur show much less susceptibility to become turned red and faded color as compared to light brown individuals [3, 4].
The present paper is aimed to evaluate the results of breeding upon pastel fox in 1978-1997 through estimation the selection differences as well as genetic and phenotypic trends of some performance traits.

Methods

Material for study was originated from fur animal farm in Jeziory Wielkie near Poznań. Females of common fox of different color varieties maintained in 1978-1997, from which 4155 progeny with pastel fur were observed. On a base of breeding documents, data from reproductive performance of general herd animals as well as results of young animals rearing and structure assessment were collected. Young pastel foxes were assessed after achieving full maturity of hair cover in a context of their conformation every year [9]. In total, 1066 litters, in which at least one individual of pastel color from each mating occurred, were taken into account.
Reproduction and conformation traits covariance components were estimated by means of the REML method based on a multitrait animal model using VCE 4.2.5 computer programme by Eildert Groneveld [2].
In order to estimate the efficiency of breeding, evaluations of genetic and phenotypic trends were applied. Phenotypic trends were estimated as changes of phenotypic trait mean values in time. The basis for genetic trends estimation in population studied was the solution for the birth year of an individual describing the changes of genetic quality in time. Calculations were made using software BLUPf90 by Ignacy Misztal [7], according to following model:

- in a case of conformation traits

$$\mathbf{y_{ijklm} = \mu + R_i + PW_j + RS_k + a_l + e_{ijklm}}$$

where: y_{ijklm} – vector of traits analyzed
μ - mean value of traits for population
R_i – constant effect of birth year
PW_j – constant effect of interaction sex * mother's age
RS_k – constant effect of interaction birth year * whelping season
a_l – random effect of an individual
e_{ijklm} – random error

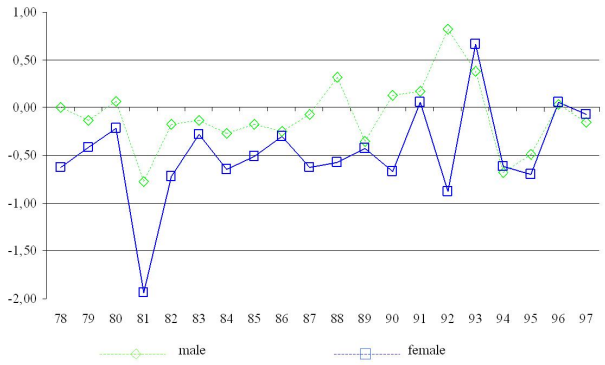
- for reproduction traits

$$\mathbf{y_{ijklm} = \mu + R_i + W_j + RS_k + a_l + p_l + e_{ijklm}}$$

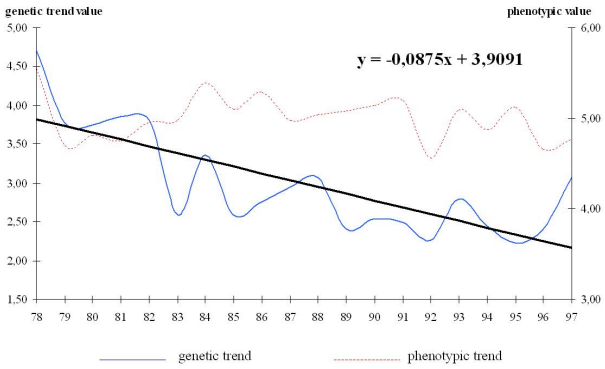
where: y_{ijklm} – vector of traits analyzed
μ - mean value of traits for population
R_i – constant effect of birth year
W_j – constant effect of animal's age
RS_k – constant effect of interaction performance year * whelping season
a_l – random effect of an individual
p_l – random effect of constant environment for a given animal
e_{ijklm} – random error

Selection differences for reproduction and conformation traits taken into account during selection were calculated to estimate the intensity of the process.

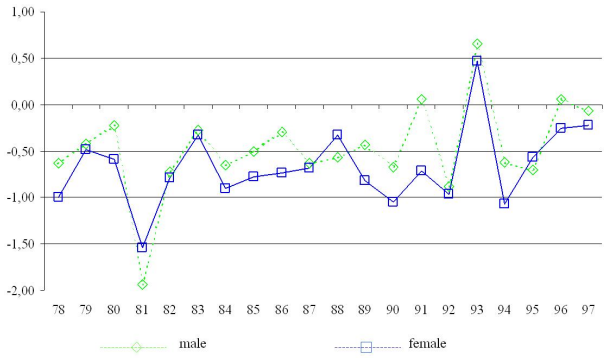
Ryc 1. Selection differential for litter size at birth



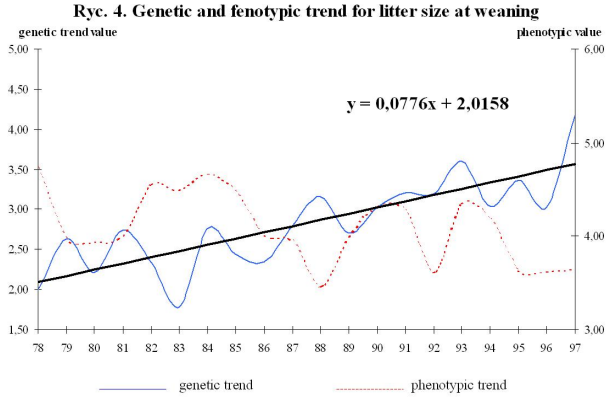
Ryc. 2. Genetic and fenotypic trend for litter size at birth



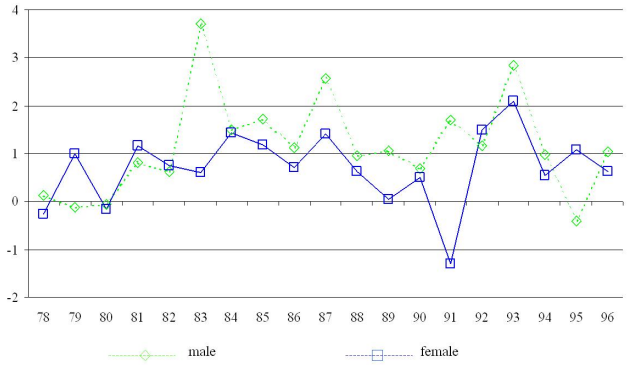
Ryc 3. Selection differential for litter size at weaning



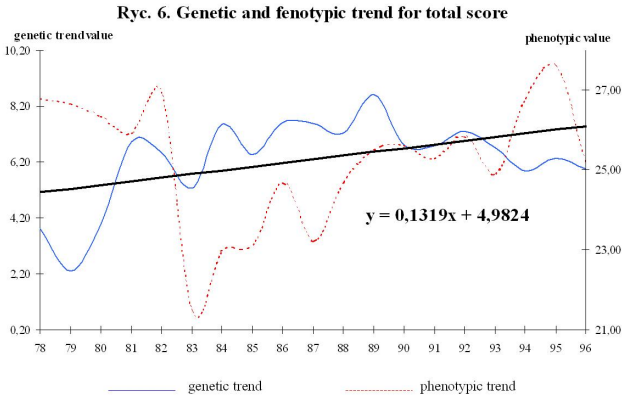
Ryc. 4. Genetic and fenotypic trend for litter size at weaning



Ryc 5. Selection differential for total score



Ryc. 6. Genetic and fenotypic trend for total score



They were accepted as differences between mean phenotypic value of a trait among young foxes chosen from the general herd and mean phenotypic value of a trait for all young animals (all animals that achieved fur maturity were subjected to assessment every year). Selection was achieved using the method proposed by Maciejowski and Jeżewska [5].

Results

Table1 presents the statistical characteristic of the traits (means and coefficients of variability) regarding the year of evaluation. In order to properly characterize and assess breeding performed in 1978-1997, it was necessary to evaluate selection differences and to calculate genetic and phenotypic trends for all traits taken into account during selection. Calculated selection differences and

trends for number of born and reared young foxes as well as for total conformation assessment are presented on Figures 1 - 6. Selection differences for number of animals born archieved the most favorable values between 1988 and 1993. Over the entire period, they were higher for males The lowest values were recorded in 1981 corresponding to the beginning of mating of mainly pastel animals among themselves. It can be observed that selection carried out for that trait did not give sufficient effects, because in the first years of the period individuals of high prolificacy were mated, and at negative selection differences there was no opportunity to achieve positive trend. However, the phenotypic trend persisted at constant level, which results from the improvement of environmental conditions (Figure 2). Figure 3 presents selection differences for number of reared young foxes. The lowest values were recorded in 1981 and positive values were observed only in 1991 for males and in 1993 for both sexes. During all period in question, selection differences for males exceeded values for females, which was consistent with commonly accepted rule that more intense selection is applied to males because of higher genetic potential of interaction with population. Maciejowski and Jeżewska presented similar method of selection difference calculation. Despite of lower selection differences, a positive genetic trend was achieved, which can be the result of environmental condition improvement and decreasing the level of herd inbreeding. However, in spite of positive genetic trend, no significant improvement of number of reared young foxes was achieved (Figure 4).

Table 1. Characteristic of vixens' reproduction and total number of scores indices in subsequent years

Year	Body conformation total scores			Female's reproduction traits				
					Mean litter size per female at birth		Mean litter size per female at weaning	
	n	**X**	**CV%**	**n**	**X**	**CV%**	**X**	**CV%**
1978	40	26.77	6.61	87	3.83	29.56	2.10	34.31
1979	91	26.67	6.90	146	3.08	31.85	1.62	32.56
1980	112	26.35	6.30	174	3.59	38.89	1.94	43.97
1981	127	25.92	7.91	204	2.56	37.59	1.76	40.36
1982	213	26.95	5.34	159	3.36	33.93	2.81	35.73
1983	243	21.44	14.13	175	4.03	29.66	3.15	33.91
1984	277	22.97	9.97	325	3.86	31.13	2.82	39.15
1985	473	23.12	10.03	283	3.80	32.48	2.92	35.35
1986	496	24.69	7.86	274	4.05	30.29	2.52	39.48
1987	496	23.22	8.23	273	3.76	30.51	2.87	38.63
1988	134	24.69	7.61	274	2.64	32.15	1.30	40.53
1989	447	25.51	5.49	272	3.88	29.52	2.66	39.81
1990	152	25.64	4.95	249	3.96	30.32	2.70	37.31
1991	80	25.3	6.28	190	4.33	28.89	2.58	41.10
1992	45	25.84	6.70	182	3.12	33.23	1.43	43.48
1993	154	24.91	8.11	115	4.42	30.72	2.97	40.53
1994	60	26.78	6.05	103	3.98	32.24	2.82	39.03
1995	84	27.58	4.02	117	3.61	33.33	1.78	45.13
1996	131	25.16	8.43	144	3.28	31.92	1.98	39.76
1997	-	-	-	157	3.21	33.37	2.15	41.76
Total	3855	26.67	7.42	3903	3.62	32.08	2.34	39.09

In a case of conformation assessment, changes are the best observed on a base of total evaluation. During all period studied, selection differences were positive. The only exception is 1991 when cardiac-pulmonary syndrome occurred on farm as well as 1995 (Figure 5). The highest values were recorded in 1983, 1987 and 1993. Thus, results plotted on Figure 6 presenting positive values of genetic and phenotypic trends do not differ greatly.

Selection differences and trends estimated in present paper for number of born and reared young foxes are difficult to compare, because there is few data in literature addressing that problem. Usually, mean numbers of born and reared young foxes in following years are given [1]. Negative selection differences for prolificacy were due to the fact that all young foxes of pastel color were incorporated to the herd regardless from how large litters from which they originated. The main selection stress was made on conformation traits. Negative selection differences for body size during period studied can be accounted for different factors. First of all, when determining the general herd every year, the best animals regarding to hair cover quality were chosen (animal's size was the secondary factor). There is a conviction among fox breeders that an animal achieves its real size at the second year of life. Another reason of negative selection differences was fact that all progeny that achieved fur maturity were retained for further breeding. Frequent observations made on farm in Jeziory Wielkie allowed the conclusion that pastel fox is characterized with slightly smaller body size as compared to silver or flame fox. It is perhaps caused by their curious variety or the fact that the level of the trait was neglected for many years of breeding.

Discussion

The first pastel foxes that were reproduced on farm were characterized with light brown color with slight red shade. Since the beginning of breeding, achievement of animals with dark brown hair cover with blue shade has been the goal. Selection pressure on that color type was intense and only young foxes with structure traits similar to standard were incorporated to general herd. Visible difference refers not only to color intensity of particular individuals, but also number of color animals similar to standard. The fact that almost all population of pastel foxes has hair cover color similar to standard (i.e. brown with blue shade) is the effect of such great selection stress. At the beginning of breeding, number of animals with color similar to standard was very low. The effect was achieved not only due to darkening of cover hair, but also lowering the level of cover silvering.

The crisis of fur market and changes in worldwide fashion significantly inhibited development of the color variety breeding. Nevertheless, breeding conducted for many years allowed achieving animals with intensive brown color with blue shade, silvering of 50% up to 70% and quite good fur quality traits. Since 1995, the pastel fox is under national protection in order to store and maintain existing state of animals within the protection of genetic resources of domestic breed populations.

Conclusion

On a base of results achieved, the following conclusions were drawn:

1. Positive values of genetic trends for conformation traits and number of reared animals testify to proper direction of breeding. However, their low values point out to low efficiency of selection. It can be a result of large number of traits considered during selection.
2. For all investigated traits, with exception of litter size at birth, an increasing tendency was found during the years under investigation which demonstrates , the breeding work was conducted properly in this flock.

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IV – 13 RP

Genetic variability of chosen conformation traits in Chinchilla

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Summary
The purpose of the studies was to evaluate genetic determination in chosen performance traits in chinchilla. Nine generations of standard chinchilla population was taken into consideration. Conformation evaluation carried on 1565 animals (59 males and 1506 females). Body size, colour type, fur colour purity, fur quality and belly-belt were taken into account.
The components of the co-variance of conformation traits were evaluated by REML method, basing on the multiple-traits animal model by the DMU. Genetic analysis of particular traits were performed with respect to: random additive genetic effects of animal, random additive genetic effects of animal's mother, permanent effects of year and month of whelping, sex and regression on the number of weaned offspring.
Heritability coefficients ranged from 0.071 to 0.389. The highest value concerned colour type (0.389) as well as fur colour purity (0.363). The mother's additive effect on the level of examined conformation traits oscillated from 0.054 to 0.672.

Introduction
The profitability of farm breeding of fur animals, including chinchilla, with the production and sale of skins is connected. Both the number and the quality of the obtained product condition reaching the maximum profit. Constant improvement of the animals is possible only owing to adequately conducted breeding, the aim of which ought to be transforming the existing variability into the possibly highest raising progress. The basis of the effective breeding program should be a reliable control of the usability of the animals as well as the evaluation of their breeding value [Łukaszewicz & Krencik, 1992]. It requires learned the variability of the features improved by a breeder. The variability described by variance components based on which genetic and environmental parameters are calculated. The absence of clearly visible genetic variability may largely impede arriving at breeding progress. The value of the received evaluation is therefore the prognostic of the efficiency of the performed selection.
The objective of the conducted studies was the evaluation of genetic variability of the most important traits of body conformation of chinchilla standard variety.

Material and Methods
The material for the studies came from the documentation of a pedigree farm of chinchilla. The studies covered a nine-generation population of the animals, standard variety. In the research years, were the 1.565 animals (59 male and 1.506 female) evaluated. There were no yearly changes in the conditions the animals were maintained in on the farm. Chinchillas were fed according to the norm recommended for the phytophagous fur animals [9] and were subjected to appropriate prophylactic and veterinary regime.
Two different standards were used for evaluation of animal body conformation during the years of studies. Therefore, in the work all evaluation results were standardized according to the pattern recommended and utilized presently [1999]. The following characteristics were analyzed: body size, fur colour type, colour purity, fur quality and belly-belt (tab.1); the last feature characterizes belt colour, its breadth, the run of the colour line as well as the contrast of the colour.

Table.1. Factors considered in evaluation of genetic parameters of the population.

Feature	Type	Body size and build	Colour type	Colour purity	Hair quality	Belly-belt
Year of birth x Month of kittening	F	x	x	x	x	x
Sex	F	x	x	x	x	x
The number of the raised litter	C	x	x	x	x	x
Additive genetic effects of animal	A	x	x	x	x	x
Additive genetic effects of animal's mother	M	x	x	x	x	x

The type of factor: F- fixed, C – regression, A and M – random with relationship matrix

The maximal note for body conformation was 30. Individuals receiving 0 (zero) points for any of the traits were disqualified as breeders and excluded from calculations. The evaluations were performed during the whole year on the animals aging 6 months at least. Among the analyzed features, only the chinchilla's body size was analyzed objectively - by weighing the animals; the evaluations of the other traits were subjective.

The components of the co-variance of conformation traits were evaluated by REML method, basing on the multi-traits animal model, using the pack of DMU programs [Madsen & Jensen, 2000]. The factors considered in genetic analyses of particular features are presented in table 1. Owing to the fact, that the examined features have discrete variability, the probit conversions of the received parameters were used in calculations [Żuk, 1989].

Results and Discussion

The values of evaluated traits in studied chinchilla population (tab. 2) were not different from the results presented by other authors [Socha S, Olechno A, 2000].

Table 2. Mean value, heritability (h^2) and maternal effect (m^2) of studied conformation traits.

Trait	**Mean**	**S.D.**	**h^2**	**m^2**
Body size	3.0	0.3	0.1269	0.1310
Colour type	3.7	0.6	0.3887	0.1351
Colour purity	7.6	0.8	0.3625	0.1467
Fur quality	5.0	0.8	0.0709	0.0542
Belly-belt	2.9	0.3	0.2607	0.6722

The heritability coefficients of conformation traits evaluated in the study ranged from 0.071 to 0.389 (tab. 2). In the case of such features as body size and built as well as fur quality, the value of heritability coefficients showed only slight additive effect of the animal on the discussed characteristics. The absence of clearly visible genetic variability may greatly impede the breeding progress, especially when phenotype based selection would be performed

exclusively. In such a situation, the improvement in body size and build may be obtained by influencing environmental factors. However, considering the fact that both body built and fur quality significantly affect the prize of the obtained skins, special attention should be paid to the method of selecting animals in view of such features. Low level of heritability coefficients suggests that the selection of animals based on productive value was encumbered by a significant error. However, on the majority of home farms, this system is most often used. The selection of adequate individuals on the phenotype basis is the quickest and the simplest in farm conditions, however, the received ranging of the animals is conclusive only as for highly heritability factors [Falconer, 1989].

Additive value of an individual was a significant source of variability in the case of the remaining conformation traits in chinchilla. The highest values of the evaluated genetic parameters concerned the coloured type (0.389) as well as the fur colour purity (0.363). These features seem to be significantly susceptible to selection in the case of not only chinchilla but also other fur animals [Lagerkvist et al., 1994; Filistowicz & Żuk, 1995].

The additive maternal effect on the level of the chinchilla's conformation traits is presented in table 2. The additive value of the mother ranged from 0.054 to 0.672 and was a significant source of variability of examined traits. The performed analyses revealed that the maternal effect was important especially in the case of the breadth of the belly-belt (m^2 = 0.627) and body size (m^2= 0.131), exceeding genetic variability resulting from the additive value of a specimen. It shows then, that choosing animals for a selective herd, considering such features, mothers should be paid special attention to. Higher influence of a female, compared with a male, results not only from the indirect genetic and physiologic effects of genes determining the levels of particular traits, but also from the ability to transfer of the mitochondrial DNA genes (mitochondrial heredity) [Charon & Świtoński, 2000].

It seems the body size and build of the animals are determined largely by the maternal effect not only in the case of chinchilla. Similar results determining the influence of this factor on the variability of conformation traits were received in minks [Rozempolska – Rucińska, 2003]. The maternal effect in the case of mink body weight was 0.216, exceeding general genetic variability for this factor. In the studies conducted by Berg [1993a, 1993b], maternal effects ranged from 10 to 40% of the total variability of body weight and the size of mink skins.

Conclusions

Low value of heritability coefficients concerning the quality of fur as well as chinchilla body size and build shows that the selection of the animals based exclusively on their utility value may be subject to error, making it difficult to obtain breeding progress for these economically relevant features.

A significant source of variability of the studied traits appeared to be the additive maternal effect, which, in the case of such traits like belly-belt as well as body size and build of the animals, was considerably more important than genetic variability resulting from additive value of the individual.

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IV – 14 RP

Genetic and phenotypic parameters of animal size and fur traits in common silver fox (*Vulpes vulpes* L.)

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Abstract.
The aim of the work was to evaluate the parameters (heritability and genetic, phenotypic and environmental correlations) of common silver fox.. Coefficients were calculated from dam and sire variance components. Heritability coefficient was the highest for colour type: 0.900 and for other traits was as follows: animal size 0.065, purity of silver colour and colour purity 0.296, fur quality 0.547 and total number of scores 0.443. The genetic correlations ranged from -0.550 (between animal size and fur quality) to 0.900 (between animal size and total score). The phenotypic correlations had lower scope and ranged from -0.160 (between animal size and fur quality) to 0.470 (between animal size and total score). The environmental correlations were on the similar level. Obtained values prove that foxes of larger dimensions were characterised by lower quality of fur.

Introduction

The most important economic traits in carnivorous fur bearing animals are body size and fur quality. They influence decisively the effectiveness of breeding of animals. Genetic and phenotypic parameters of fur quality traits were researched by various authors **(**Maciejowski & Jeżewska, 1981; Kenttämies, 1988; Socha, 1994 and 1996; Filistowicz et al., 1999; Socha et al., 2000**)**. However it should be pointed out that genetic and phenotypic parameters in populations change constantly due to the changes of genetic variability, which are caused by the selection and import of animals. Moreover, the parameters vary among different herds. In view of the mentioned aspects the present work estimates both heritability and genetic, phenotypic and environmental correlations between animal size and fur quality traits in common Silver Fox (*Vulpes vulpes* L.).

Material and methods

The data was obtained from the breeding farm of common silver fox (*Vulpes vulpes* L.) situated in Middle Poland. The data included the evaluations of body dimensions in the autumn (official evaluation period) within 2 years (2000-2001).
The traits analysed were as following: a. body size and conformation, b. colour type, c. purity of silver, colour purity and purity of silver (defined as colour purity), d. fur quality (hair length and fur density) and total score for all the evaluated traits. The animals were estimated according to the new rules (Wzorzec - Norm, 1997), on a point scale. The maximum score that could be obtained is 20 while the minimum 0 (zero) for a trait disqualifies an animal from further breeding. About 800 animals were recorded, 90% obtained positive results. Genetic parameters (heritability and genetic, phenotypic and environmental correlations) were estimated from sire and dam variance components. The mixed model was applied:

$$Y_{ijklm} = \mu + a_i + b_{ij} + r_k + p_l + e_{ijklm},$$

where: μ = overall mean, a_i = random effect of sire, random effect of dam, r_k = fixed effect of birth year, p_l = fixed effect of animal sex, e_{ijklm}= random error.

Results and discussion

The obtained parameters of heritability of traits are presented in Table 1. Heritability coefficient was the lowest for animal size: 0.065, which was lower from the results obtained by Filistowicz et al. (1999) and Socha et al. (2000). The low value proves that animal size is mostly influenced by environmental conditions, such as feeding and only a little by genetic base.
Heritability of other traits was as following: colour type 0.900; colour purity 0.296; fur quality 0.547 and total score 0.443. High heritability of fur colour was also obtained by Pingel et al. (1986) in mink: from 0.53 to 0.86, depending on animal sex and colour type. Borsting & Clousen (1986) estimated it: from 0.10 to 0.49, depending on animal sex and colour type. Heritability of fur quality (fur density and hair length) in this work was higher from the results obtained by both Kenttämies (1986 and 1988) and Borsting & Clausen (1986): from 0.10 to 0.38, depending on animal sex. The higher fur quality in

the present work proves an increase of genetic base on these traits, which should be recognised as very favourable.

Table 1. The heritability coefficients and the correlations coefficients in common silver fox (*Vulpes vulpes* L.)

Traits (number of trait)	Heritability h^2	Number of trait / Genetic correlations / Phenotypic correlations / Environmental correlations			
		(2)	(3)	(4)	(5)
Animal dimension (1)	0.065	0.511	>1	-0.545	0.939
		0.017	-0.066	-0.160	0.472
		-0.848	-0.267	-0.088	0.433
Colour type (2)	0.900		0.081	-0.108	0.230
			0.057	-0.121	0.099
			0.114	-0.456	-0.515
Colour purity (3)	0.296			0.457	1.048
				0.005	0.438
				-0.335	0.094
Fur quality (4)	0.547				0.298
					0.429
					0.561
Total score (5)	0.443				

Table 1 presents correlations between analysed traits. On the whole, all the correlations between total score and the traits evaluated during the license were positive. Increase of value of every trait increased total score (Socha et al., 2000). All the correlations (genetic, phenotypic and environmental) were both positive and negative.

The lowest negative genetic correlation was found between animal size and fur quality (-0.545). Phenotypic correlation between these traits (-0.160) was lowest as well. Negative correlation between animal size and fur quality proves that the foxes of smaller dimensions were characterised by higher fur quality. Pingel et al. (1986) estimated phenotypic correlations between body mass and both fur colour and structure in mink as negative and low. Lohi & Hansen (1990) also obtained negative correlation between body mass and fur quality in mink.

It should be pointed out that genetic correlation between colour purity and fur quality was high and positive (0.457) while phenotypic correlation was low (0.005). The large differentiation of correlation coefficients (positive and negative) between traits in population of foxes makes the selection more difficult. Increase of value of one trait might cause decrease of values of other traits (Socha, 2000).

Conclusions

The estimated heritability was follows: for colour type: 0.900 (the highest), animal size 0.065, colour purity 0.296; fur quality 0.547 and total score 0.443. On the whole, the results were higher than those obtained by other authors. The values of correlations obtained in the present work prove that the foxes of bigger dimensions were characterised by lower quality of fur. From the other side, animal size significantly and considerably more than other traits influenced total score. It should be also pointed out that differentiation of correlations of foxes makes selection difficult, all the more so as in selection all the traits are important. Analysis of prices of skins on the skin auctions indicates that the trait that most significantly influences the price is animal size. Consequently this trait should be taken into special consideration during the selection. However, it is the trait that mostly depends on environmental conditions, such as feeding.

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IV – 15 RP

Genetic parameters of size and fur quality in a mink population (*Mustela vison* Sch.)

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Abstract
The aim of the work was to estimate the heritability and correlations between body size and fur quality, estimated in a breeding-farm of mink. Genetic parameters of the traits were obtained by the REML method with a multitrait animal model. Since the fur traits were evaluated on a discrete scale and distribution of scores differs from a normal distribution, probit transformations of the obtained heritability and phenotypic correlation estimates of traits were performed. Obtained heritability estimates: 0.515 for body size (based on point evaluation of animals) and 0.226 (based on body weight), 0.432 for colour purity, 0.387 for fur quality and 0.499 for the total score. The genetic correlations ranged from –0.125 (between body size and colour purity) to 0.802 (fur quality and total score). The phenotypic and the environmental correlations showed narrower ranges.

Introduction

Minks are the most widespread animals used in fur production. The most important economic traits are body size and fur traits. Heritability, as well as correlations between body size and fur quality of minks were reported by Børsting & Clausen (1986), Pingel et al. (1986), Einarsson (1988), Berg & Lohi (1991), Lagerkvist et al. (1994).

There is a large variation in heritability and correlation estimates among different mink populations. This variability is most probably caused by natural and artificial selection. The grading standard of minks was changed in Poland in 1997. The changes concerned the scale of scores (from 30 to 20 scores) as well as number of evaluated traits and the requirements concerning particular traits. The present paper reports on work done to estimate heritability and correlation between body size and fur quality in a mink farm.

Material and methods

Parameters (heritability and correlations) were estimated on the basis of the data obtained from a mink farm (Standard colour type) in central Poland, in the period of 2 years. The evaluation of body dimensions was done in the autumn (official evaluation period). The traits analysed were: a. body size and conformation, b. colour purity, c. fur quality (hair length and hair density) and total score for all the evaluated traits.

The evaluation of traits was on a point scale. Body size was recorded in grams, even though the norm does not require it (Wzorzec – Norm, 1997). However, it was done for comparison in this work. The maximum score which could be obtained was 20, and the minimum was 0 (zero) for the traits that disqualified animals from further breeding. During the experiment 3877 animals were recorded. Positive results were obtained for 3440 minks (1597 in the first year and 1847 in the second), 2382 females and 1058 males.

The parameters of the traits (heritability and correlations, phenotypic and genetic) were estimated by the REML method with a multitrait animal model. The model took into account the fixed effects: year of evaluation, sex, litter size and random effects: animal and error. The estimates of the variance and covariance components were obtained using SAS/STAT (1998) procedure**.**

Results and discussion

Means and standard deviations of the analysed traits are presented in Table 1. The lowest standard deviation of the scored traits was observed in colour purity (0.71) and the highest in total evaluation (1.19). Heritability, as well as genetic, phenotypic and environmental correlations were estimated (Tables 1 and 2). The heritability found for body size was 0.515, based on the point evaluation or 0.226, when estimated on the basis of animal weights. Heritability estimates of body weight presented by other authors were the following: from 0.22 to 0.78, adjusted for gender and colour type (Pingel et al. 1986); 0.54, while body length was 0.72 (Lohi et al., 1990); between 0.20 and 0.44, depending on the method (Jeżewska & Maciejowski, 1981);

between 0.05 and 0.54, depending on the animal age (Lohi & Hansen, 1989). The high values suggest that selection for increasing body weight of minks should be very effective under suitable conditions, especially suitable feeding.

Table 1. Means, standard deviations and heritability coefficients of conformation traits in mink

Traits	Material		Results	
	Mean	Standard deviation	Herita-bility h^2	Standard errors of heritability – SE_h^2
Body weight; g	1523	476.8	0.226	0.041
Body size and conformation; scores evaluation	5.49	0.666	0.515	0.061
Colour purity	4.44	0.702	0.432	0.050
Fur quality	5.09	0.730	0.387	0.049
Total score	18.02	1.191	0.499	0.053

Estimates of heritability of fur colour purity, fur quality and total score were 0.432, 0.387 and 0.499, respectively. Heritability of fur traits obtained by other authors varied and was the following: between 0.53 and 0.86 for the hue, depending on gender and colour type (Pingel et al., 1986); between 0.10 and 0.49 for fur colour, between 0.10 and 0.38 for fur quality, between 0.32 and 0.79 for body size (estimates related to the gender and colour type, Børsting & Clausen, 1986); between 0.18 and 0.53 for body weight at pelting, between 0.52 and 0.72 for fur colour, between 0.24 and 0.93 for hair length, between 0.33 and 0.49 for hair density and between 0.15 and 0.31 for general fur quality among several mink lines (parameters from the sire variance components, Einarsson, 1988).

In the Table 2 the estimated genetic, phenotypic and environmental correlations between the investigated traits are presented. It should be generally ascertained that all correlation coefficients between total score and the traits particular evaluated during the official evaluation were positive and statistically significant. The increase of the values of each trait influenced the boost of the total score. It was found that the total score (r_G = 0.802) than between total score and colour purity (r_G = 0.584).

The majority of the genetic correlations were positive, negative estimates were found, for instance, between colour purity of fur and body size (-0.030 for body weight and –0.125 of point evaluation for body size). Po sitive correlations ranged from 0.268 (between colour purity and fur quality) to 0.802 (between fur quality and total score).

Table 2. Estimated correlation coefficients between the traits studied in mink

Traits	Correlation estimates			
	Body size and conformation	Colour purity	Fur quality	Total score
Body weight; g	0.693 [a] ** (0.055 [d]) 0.815 [b] ** 0.640 [c] **	-0.030 [a] (0.110 [d]) -0.046 [b] -0.054 [c]	0.371 [a] ** (0.100 [d]) 0.091 [b] -0.019 [c]	0.534 [a] ** (0.083 [d]) 0.408 [b] ** 0.337 [c] **
Body size and conformation (scores evaluation)		-0.125 [a] ** (0.095 [d]) -0.137 [b] ** -0.081 [c]	0.309 [a] ** (0.096 [d]) 0.055 [b] -0.083 [c]	0.589 [a] ** (0.057 [d]) 0.693 [b] ** 0.495 [c] **
Colour purity			0.268 [a] ** (0.093 [d]) 0.011 [b] -0.105 [c] **	0.584 [a] ** (0.057 [d]) 0.669 [b] ** 0.530 [c] **
Fur quality				0.802 [a] ** (0.039 [d]) 0.733 [b] ** 0.542 [c] **

a) genetic correlation, b) phenotypic correlation, c) environmental correlation, d) standard errors of genetic correlation estimates

** - *correlation highly significant* $\alpha \leq 0.01$

Lagerkvist et al. (1994) estimated genetic and phenotypic correlations between certain traits of mink fur. The correlations between colour and density were low and negative (from -0.10 to – 0.05). The correlations between colour and quality were also low but positive (from 0.06 to 0.18). Pingel et al. (1986) estimated negative and low phenotypic correlations between body mass and: fur colour and fur structure. Lohi & Hansen (1990) have found the negative correlations between body weight and fur quality of minks also.

The parameters of conformation traits estimated by REML method indicate that the traits are characterised by additive genetic variability. The achievement of breeding progress might, however, be difficult due to the various factors: the correlation coefficients differ, the right choice of animals for breeding will always be difficult, and the results of the selection will be hard to predict. Large differences between correlation coefficients (from negative to positive) between the conformation traits of minks is a serious problem in their selection. The increase in values of certain traits might cause decrease of others. A selection index might overcome some of the problems.

Conclusions

1. The highest values of heritability were obtained for body size (0.515 of point evaluation), other traits ranged from 0.226 (body weight) to 0.499 (total score). The estimated parameters (except body size) had higher values than those presented by other authors.
2. Negative genetic correlations between body size and other traits (fur purity) were obtained. The correlations between particular traits and total score were high and positive. Thus, selection on total evaluation should be made in order to achieve breeding progress in these traits. The differences between the correlation coefficients might be a serious problem i.e. in the selection of minks' where the detailed estimation of breeding value of animals is essential.

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IV – 16 RP

Comparison of reproduction management intensity of three genetic lines of female chinchillas (*Chinchilla lanigera* M.)

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Abstract
The observations took place on a chinchilla farm located in West Pomerania, Poland, during 1991-2002 and included 359 females assigned to three basic groups that represented separate genetic lines: imports from Sweden (141 females), own-bred (98 females), and imports from Denmark (120 females). The females qualified for the studies had produced at least four litters of offspring during their reproductive life.
The main goal of these investigations was to determine an effect of birth interval on selected reproduction parameters of female chinchillas.
It has been found from the studies that the mean birth interval was 221 days, which demonstrates that the females had been extensively managed in terms of reproduction. birth interval that lasts 8-9 months positively influences litter size and is especially recommended for females that nurse large-size litters. In the Swedish line, a drop in services was observed in the fourth oestrus after delivery, as compared with the remaining genetic lines. The own-bred females achieved the worst nursing success for the young born from services during the post-lactation oestrus, while the Swedish females – for those born from services during the fifth oestrus post-partum.

Introduction
Properly managed reproduction within the herd is the key factors of profitability of each farm. Producing numerous and healthy brood that achieves desired traits is the objective of every farmer, as in fur-bearing animals farming it is immediately associated with the number of produced pelts (Jeżewska et al. 1998, Socha and Szumska 2002).
Chinchillas belong to the animals of relatively low fertility and, which they descend from their wild ancestors, of distinct seasonality of reproduction (Nordholm 1992, Kuroiwa and Imamichi 1977, Seremak and Sulik 2002). Intensive sexual activity, which can be observed on farm, occurs depending on climatic conditions; in Poland this period falls between November and May (Gromadzka-Ostrowska 1998, Jarosz 1993). The periods of intense libido and successful services reach their peak in January and February, which results from longer days. According to Barabasz et al. (2000), seasonality in chinchilla farming begins to diminish, which may demonstrate better and better adaptation of the species to farming environment. Reproduction performance parameters achieved on Polish farms (1.7-2.4 born and 1.5-2.1 weaned young per female per year) should not be considered as fully satisfactory (Sulik and Barabasz 1995), especially as chinchilla reproductive potential is much higher (4-6 follicles maturate in the oestrous cycle).
Improvement of chinchilla herd in Poland is to a large extent based on imported animals, which are supposed to positively influence coat quality of pelts. The import makes an opportunity, however, to also improve reproduction performance. This study is aimed at an analysis and comparison of reproductive careers of females belonging to three genetic lines imported in order to improve performance of domestic lines.

Material and Methods
The data was collected over 11 years, i.e. from 1991 till 2002 and included observations of 359 females assigned into the following genetic groups by their origin:
- own-bred females, 98 animals
- imported from Sweden, 141 females
- imported from Denmark, 120 females

The females qualified for the analysis had produced at least four litters of offspring during their reproductive life.
The females were managed in polygamous sets system with male-to-females ratio like 1:4. Females in each set inhabited their own cages connected with a passage, so the male could access each female of the set. From 48 hours after birth, the passageway of the female' cage was closed. The animals were fed on balanced pellets with water and hay ad libitum. Additionally, each cage was equipped with a dust bath. The animals were housed without bedding, their cages being arranged in a four-storey system.
The analysis was based on farm documentation recordings. The females were within each genetic

group divided into subgroups according to birth interval length:

1. females serviced during the post-partum oestrus (birth interval shorter than 4 months)
2. females serviced during the post-lactation oestrus (birth interval between 5 and 6 months)
3. females with birth interval between 7 and 8 months
4. females with birth interval between 8 and 9 months
5. females with birth interval between 9 and 10 months
6. females with birth interval longer than 10 months

Another criterion of division was the number of litters per female achieved over her reproduction life.

The collected data was subjected to statistical analysis using a spreadsheet and Statistica PL software package.

Results and Discussion

Polish chinchilla farms have been nourished with imported quality animals in order to improve performance parameters, especially in terms of fur quality. Reproduction parameters, however, cannot be ignored in the process of stock improvement, since they significantly affect the profitability of the farm. According to many authors (Felska and Brzozowski 2001, Jarosz and Rewska 1996, Sulik et al. 2001), low fertility of chinchillas is a characteristic of the species reproduction and reaches 2 young per litter in most cases. On the analysed farm, fertility levels were achieved similar to those reported in the literature (2.12 per Polish, 2.01 per Danish, and 2.17 per Swedish female). These results do not show statistically significant differences in relation to the country of origin.

An average number of litters produced per female per year represents a factor that can bring high level of reproduction performance. Considering the gestation length, as well as that females are able to conceive during the post-partum oestrus, it is theoretically possible to obtain 3 litters per year from a single female. The number if litters depends on the interval length between births. Mating during the post-partum oestrus allows obtaining the highest index of female reproduction intensity. In the studied herd (Table 1), from 29.11% matings (own-bred females) to 40.78% (Swedish import) were done during post-partum oestrus, which may demonstrate an intensive utilisation of females; it should be noted that domestic females definitely differ from imported females in this area.

Analysis of litter size in relation to birth interval reveals a high level of variability of females' responses to the examined factor. Danish-bred females gave larger litters from post-lactation matings, while domestic and Swedish females produced larger sizes after an about 8-month break. From the economic point of view it seems better when a female have shorter breaks between parturitions. Although a female usually delivers fewer offspring per such a litter, the overall number of born young over a year is higher.

Table 2 presents intensity of female reproduction in relation to the number of litters attained. Gestation intervals in the females decrease with their age, irrespective of their origin. The mean birth interval was from 206 days in won-bred females to 238 days in Danish females. As it has been shown by Neir et al. (1989), who studied the chinchilla herd in Chile, an average gestation interval there was 212 days, which is similar to those achieved on the studied farm. This level allows obtaining 1.5 litters from a female per year on average. Considering the weaning success at the level of 1.88 to 1.97 (Table 1), 2.82 to 2.96 offspring, depending on the origin, were achieved from a female per year on the studied farm. This result is relatively high, as a number of authors state that obtaining 2-2.5 young chinchillas per female per year is considered a satisfactory achievement (Sulik 2001, Sulik and Barabasz 1995, Sulik et al. 2001). The above mentioned data demonstrates that gestation interval, which depends on the resting period length, represents an unusually important index of female reproduction management intensity.

In all the genetic lines, birth intervals were found to become shorter after each consecutive litter, with the eight litter as the limit of this effect (Table 2). Similar is the size of litters, which grows slightly until the eight or ninth litter to drop again by 0.15 to 0.54 young on average in the subsequent litter. However, as long as litter size exceeds two, a result can be considered as very good. The highest pre-weaning mortality rate was found among the Danish females (0.2 young per litter).

Conclusions

1. Birth interval lasting about 8 months positively influences litter size, however on the whole it results in reduced number of offspring obtained per year.
2. The birth interval of 208 to 238 days recorded on the studied farm shows that the females were extensively utilised in terms of reproduction.

3. On the studied farm, a female was on average utilised for 1270 days (3.5 years). This period looks to have been too short, since reproductive intensity was found to increase with the length of their reproductive life.

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Table 1. Distribution of matings in relation to origin and birth interval

Mean birth interval	Number of litters						Litter size									Weaned young per litter								
	Polish		Swedish		Danish		Polish			Swedish			Danish			Polish			Swedish			Danish		
	Ind.	%	ind.	%	ind.	%	M	SD	V	M	SD	V	M	SD	V	M	SD	V	M	SD	V	M	SD	V
Until 5 months (post-partum oestrus)	122	29.11	219	40.78	202	37.33	2.19	0.97	44.3	2.03	0.84	41.4	2.13[b]	0.82	38.5	1.97	0.94	47.7	1.86	0.85	45.7	1.92	0.84	43.6
5-6 months (post-lactation oestrus)	12	2.86	8	1.49	9	1.66	1.91	0.9	47.1	2	0.53	26.5	2.33	1	42.9	1.66	0.98	58.7	2	0.53	26.5	2.11	0.78	36.9
6-7 months	34	8.11	30	5.58	37	6.83	1.97	0.63	32	2	0.87	43.5	2.27	0.73	32.2	1.97	0.67	34	1.86	0.93	50	2	0.94	47
7-8 months	97	23.15	12	2.23	109	20.14	2.14	0.86	40.2	2.06	0.7	34	2.05	0.84	40.8	1.98	0.84	42.4	1.92	0.71	52.1	1.85	0.81	42.9
8-9 months	37	8.83	72	13.4	48	8.87	2.32	0.88	37.9	1.91[a]	0.78	40.8	2.43[ab]	0.79	32.1	2.08	0.72	34.6	1.79	0.71	39.7	2.14	0.74	34.4
9-10 months	33	7.87	41	7.63	33	6.09	2.18	0.88	36.7	2.14	0.76	35.5	2.03	0.98	42.6	2.03	0.8	39.4	1.97	0.68	34.5	2	0.93	46.5
10 and more	84	20.04	155	28.86	103	19.03	2.13	0.67	31.5	1.94	0.69	35.6	2.01	0.76	37.8	2.02	0.72	35.6	1.81	0.68	37.6	1.82	0.78	42.6
Total	419	100	537	100	541	100	2.12	0.82	38.7	2.01	0.73	36.3	2.17	0.85	39.2	1.95	0.87	44.6	1.88	0.72	38.3	1.97	0.83	42.13

Table 2. Intensity of reproductive management of female chinchillas in relation to origin

Origin	No. of litter	No. of females		Mean length of reproductive life (months)			Mean birth interval (days)	Mean litter size (indiv.)			Mean number of weaned young per litter (indiv.)		
				M	min	max	M	M	S	V	M	S	V
Polish	4	40	40.81	667	340	1217	222	2.19	0.8	36.5	2.02	0.81	40.1
	5	27	27.55	959	451	1856	239	1.99	0.75	37.7	1.85	0.71	38.4
	6	7	7.14	1277	731	2465	255	2.09	0.84	40.2	1.95	0.88	45.1
	7	16	16.32	1203	927	2035	200	2.17	0.93	42.9	2	0.88	44
	8	5	5.1	1198	996	1569	171	2.3	1.01	43.9	2.12	0.93	43.9
	9	2	2.04	1397	1241	1553	174	2.44	0.92	37.7	2.11	0.75	35.5
	10 and more	1	1.02	1623	1623	1623	180	1.9	0.73	38.4	1.7	0.67	39.4
	Total	98	100	1189	340	2465	206	2.15	0.85	39.5	1.96	0.8	40.8
Swedish	4	50	35.46	672	340	1456	224	1.96	0.77	39.3	1.8	0.76	42.2
	5	40	28.37	967	522	1835	241	2.04	0.78	38.2	1.9	0.78	41
	6	13	9.22	1198	769	1883	239	2.01	0.81	40.3	1.85	0.78	42.2
	7	19	13.47	1348	829	1868	224	2.03	0.76	36.4	1.86	0.84	50.6
	8	8	5.67	1489	1137	2491	212	2.09	0.79	37.8	1.95	0.74	37.9
	9	4	2.83	1653	1207	2265	206	1.94	0.58	29.9	1.86	0.72	38.7
	10 and more	7	4.96	1819	1322	2105	181	2.07	0.72	34.8	1.92	0.7	36.5
	Total	141	100	1307	340	2491	218	2.02	0.74	36.6	1.88	0.76	40.4
Danish	4	39	32.5	706	342	2404	239	2.05	0.8	38.8	1.78	0.79	43.6
	5	43	35.8	790	436	2331	197	2.19	0.81	37	1.92	0.85	44.3
	6	15	12.5	1146	626	2822	382	2	0.7	35	1.83	0.81	44.3
	7	9	7.5	1379	664	3189	229	2.25	0.76	33.8	2.03	0.71	34.9
	8	3	2.5	1405	956	1968	200	1.79	0.77	43	1.7	0.75	44.1
	9	4	3.3	1685	1370	1901	210	2.52	1.1	43.7	2.19	1.03	47
	10 and more	7	5.83	2117	1581	3100	211	2.01	0.8	39.8	1.96	0.81	41.3
	Total	120	100	1318	342	3189	238	2.12	0.82	38.7	1.92	0.82	42.7

IV – 17 RP

Morphological changes of spermatozoa in breeding raccoon dogs semen during cryopreservation.

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Abstract
Process of dilution and semen freezing provides in a smaller or bigger degree damage of cell membrane of spermatozoa, thereby decreasing its fertilization ability. We provide investigations which aim was to evaluate the damage degree of raccoon dog spermatozoa during freezing process, after administration of different extenders. Investigations were carried out on semen collected manually from 16 raccoon dog males. After evaluation, semen was diluted with EDTA extender with 4, 6 and 10 % glycerol addition. Morphology of spermatozoa was evaluated on thin smears on a slide stained with 5% eosin and 10% nigrosin (1:4 v/v). Spermatozoa normal and not damaged in fresh semen were 63%. Among those with secondary changes a majority of 34.3% were spermatozoa witch proximal droplets. In frozen-thawed semen smears, the observed highly significant decrease of intact spermatozoa was dependent on glycerol addition. Up to 52-54% highly significant increase of spermatozoa with coiled tail and "hair pin" was observed.

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Introduction

The process of semen dilution and freezing provides in a smaller or bigger degree to damaging of cellular membrane of spermatozoa, thus decrease their ability to fertility [Aquirre et al. 1987]. Considerable step during semen freezing is not only testing semen vitality after freezing, but also to estimate the degree of spermatozoa changes after cryopreservation [Bittmar et Kosiniak 1992]. During cryopreservation some structural changes of spermatozoa can be observed. In bulls' semen, vitality test is based on semen mobility [Olar et al 989]. In the boar semen, the acrosome is more susceptible for damage, but not mitochondria in interstitial lamella. After boar semen freezing, spermatozoa show mobility but without biological value due to acrosome damage [Strzezek 1995].] We undertook investigations which aim was evaluation of raccoon dog spermatozoa damage during freezing process with different addition of cryoprotector.

Material and Methods

Investigations were carried out on semen collected after digital manipulation from 16 breeding males of raccoon dogs. Semen was collected and transported in containers at temperature 37ºC. After micro and macroscopic semen evaluation, selected ejaculates were diluted using EDTA extenders of composition given in Table 1. Semen was diluted gradually in glass tubes to obtain final concentration of spermatozoa $150x10^6$ per ml. The diluted semen was cooled and equlibrated at 5^0C. Semen was freezed using MINITUB system using marked 0.5 ml PVC straws which were filled up using syringes cooled down to 4 ºC. To freeze the semen, a polystyrene box with 4.5 cm thick walls and internal dimensions of container 17.5 x 17.5 x 19 cm was used. Straw hanger was placed in the container to keep them in N_2 vapor 4 cm above the liquid nitrogen; after 15 minutes, frozen semen was sunk and stored in GT-14 container (L'air Liquide, France). Frozen samples were thawed in a water bath ST-1 (OSTA Electrinics, Piastów, Poland) at 37ºC during 30 seconds immediately before examination. Spermatozoa morphology was evaluated on a stained 5% eosin and 10% nigrosin (1:4 v/v) slides under immerse magnification using light microscope Bioval. On each slide, at least 200 spermatozoa were evaluated. Number of intact spermatozoa and morphologically abnormal spermatozoa with secondary changes, with cytoplasmatic droplet, with twisted tail, damaged, with a changed cap (acrosome) and aggregated, was evaluated [Blom, 1981]. Statistical analyses of the results were conducted using SAS version 6.03.1987. Distribution of variables was tested. The significance of difference between extenders for variables with regular distribution was tested using analysis of variance and the method of the least squares. Significance of differences between groups for variables with non-regular distribution was tested using non-parametric test of Kolmogrov-Smirnov. Also, correlation between variables using

Spearman method for non-regular distribution variables and Person's method for variables with regular distribution was calculated.

Results and Discussion

The most important problem during semen freezing is keeping progressive motility of spermatozoa as well as enzymatic proteins of acrosome and interstitial lamella. Protein denaturation caused by low temperatures corresponds with decreasing activity of a few cellular enzymes responsible for fertilization ability of spermatozoa [Strzeżek 1998]. The process of dilution and conservation of raccoon dog semen significantly influenced the spermatozoa morphology. After semen thawing, regardless of applied diluents, a highly significant lower number of normal spermatozoa in all samples were observed. The highest decline from 62.75% to 51.76% was observed when diluent's RII with 6% glycerol addition was tested. The presence of normal non-damaged spermatozoa in diluents with 4% (RI) and 10% (RIII) of glycerol addition was slightly higher - 53.75% and 54.97% respectively (Table 1).

Table 1. Composition of EDTA extenders

Components	Extender R-I	Extender R-II	Extender R-III
EDTA (g)	0,37	0,37	0,37
Citric acid (g)	0,375	0,375	0,375
Acid sodium carbonate (g)	0,12	0,12	0,12
Glucose (g)	6,0	6,0	6,0
Distilled water (ml)	Ad 100	Ad 100	Ad 100
Glycerol (ml)	4,0	6,0	10,0
Egg yolk (%)	20	20	20
Osmolarity mOsm	445	446	460

Strzeżek et al. [1984] concluded that during cryoconservation, morphological changes can be observed in the spermatozoa structure causing release of enzymatic proteins. This process is responsible for considerable lowering of semen biological value. In present studies, we investigated glycerol addition for freezing and thawing of raccoon dog semen. Glycerol is generally considered to be the best cryoprotector. Optimal level of glycerol in diluent's is a compromise between its protective and toxic functions [Olar et al. 1989]. The level of toxic influence of glycerol on spermatozoa depends on its concentration and the time of contact with cell, and refers to changes in plasmolemma transmission as a result of denaturation influence of this compound on glycoprotein complexes of cellular membrane. On smears, a highly significant lowering of spermatozoa with a protoplasmatic droplet was observed. The highest decline from 7.94 % in the fresh semen to 0.56 % post-thaw was observed in R-III; the lowest decline in R-II (Table 2).

During freezing and thawing, a highly significant increase of spermatozoa with a coiled tail was observed (from 14.13 % in fresh semen to 23 % after thawing). Differences between applied diluents were not significant (Table 3). Number of spermatozoa with helically coiled tail increased from 5.75 % in fresh spermatozoa to about 16 % after thawing. The least damages were observed in R-I. In the complex cryobiochemical investigations of bull and boar semen it was stated that in the first minutes of spermatozoa contact with diluent's containing glycerol, there can be observed a glycerol activation effect or „dilution effect", which appeared as an intensive degradation of enzymatic proteins related to plasmolemma or presented in acrosome and tail of spermatozoa (Strzeżek, 1987). After thawing, a decreased number of damaged spermatozoa on smears can be observed; the highest (mean by 39 %) was present in samples of semen diluted with R-III and R-I.

Table 2. Morphology of spermatology in raccoon dogs semen during freezing/thawing

Semen	Items	W1	W2	W3	W4	W5	W6	W7
Fresh	Mean	62,75	7,94	14,13	5,75	4,50	0,44	1,44
	SE	1,66	0,90	1,37	0,62	0,53	0,20	0,29
	SD	6,65	3,61	5,49	2,49	2,13	0,81	1,15
	V%	10,60	45,42	38,85	43,30	47,32	186,04	80,20
Diluted with Extender R-I	Mean	53,76	0,95	23,14	16,26	3,51	1,38	1,00
	SE	2,52	0,23	1,29	1,92	0,58	0,39	0,27
	SD	10,10	0,93	5,15	7,68	2,31	1,54	1,10
	V%	18,78	99,06	22,27	47,27	65,98	112,28	109,55
Diluted with Extender R-II	Mean	51,76	1,07	23,32	16,32	4,14	1,32	2,07
	SE	2,32	0,56	1,30	1,74	0,86	0,38	0,57
	SD	9,27	2,24	5,19	6,95	3,44	1,54	2,29
	V%	17,91	210,37	22,25	42,57	83,45	117,11	111,23
Diluted with Extender R-III	Mean	54,57	0,57	23,70	16,69	2,76	0,95	0,76
	SE	1,86	0,20	1,29	1,84	0,53	0,35	0,23
	SD	7,42	0,81	5,17	7,36	2,11	1,39	0,93
	V%	13,60	144,70	21,84	44,13	76,85	148,15	124,13

W1- Intact spermatozooa, W2- With a protoplasmatic drop W3 - Bent tail W4 - Coiled tail W5 – Disintegrated W6 - Acrosomal defect W7 – Agglutinations

Table 3. Test of extender effect on the sperm morhology in fresh and freezing/thawing semen

Semen	Spermatozoa	R-I	R-II	R-III
Extender R-II	Intact	0,6994		
	With a protoplasmatic drop	0,9412		
	Bent tail	0,9202		
	Coiled tail	0,9996		
	Disintegrated	0,9996		
	Acrosomal defect	0,9999		
	Agglutinations	0,6994		
Extender R-III	Intact	0,4154	0,0935	
	With a protoplasmatic drop	0,9412	0,9996	
	Bent tail	0,7637	0,8411	
	Coiled tail	0,9999	0,9996	
	Disintegrated	0,9996	0,6994	
	Acrosomal defect	0,6994	0,9412	
	Agglutinations	0,9996	0,6994	
Świeże	Intact	0,0039**	0,0010**	0,0020**
	With a protoplasmatic drop	0,0001**	0,0001**	0,0001**
	Bent tail	0,0001**	0,0001**	0,0001**
	Coiled tail	0,0010**	0,0002**	0,0002**
	Disintegrated	0,0366*	0,0935	0,0366*
	Acrosomal defect	0,4154	0,4154	0,6994
	Agglutinations	0,6994	0,6994	0,2106

** Statistically significant at P < 0.05*
*** Statistically significant at P< 0.01*

Conclusion

Based on our preliminary observations, it can be stated that raccoon dogs semen is unusually sensitive to thermal shock. Insufficient protection is responsible for spermatozoa structure damage, in particular of acrosome and tail.

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IV – 18 RP

Induction of estrus and ovulation in breeding chinchilla by GnRH analogues

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Abstract
Compared to other multiparous rodents the chinchilla have relatively low fecundity. During the heat in ovaries of sexual mature chinchilla we can observe up to 16 ovary follicles, of which only 4 ovulated. The possibility to increase fertility and fecundity of breeding chinchilla by using hormonal specimens containing busereline – a synthetic analogue of hypothalamus hormone – GnRH was investigated. First and second stage of the experiment was conducted on 48 females, which were randomly divided into 3 groups: 2 experimental and 1 control. Each experimental female was inoculated intramuscularly with 0,2 ml of Receptal Intervet – Group I; Bioreline Biochef – Group II, what correspond to 0,85 μg of busereline. Control group – Group III was inoculated intramuscularly with 0,2 ml of *Aqua pro injection* (Polfa). In the first stage of experiment, the effect of busereline administrations was evaluated by structural changes observed in ovaries of 24 females. In a second stage the remaining 24 females were housed for 60 days together with males. Results from the first stage showed that both GnRH analogues caused ovulation, and the female's ovaries showed presence of corpus luteum. Confirmation of this fact was kittening of females from groups: II and I in the second stage. Application of *placebo* in the control group did not induce folliculogenesis or ovulation.

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Introduction

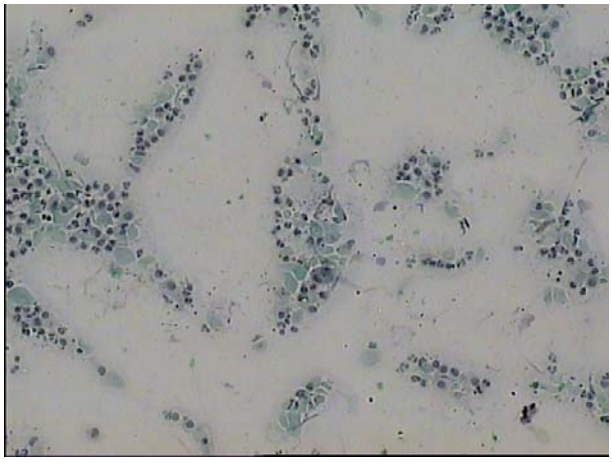
Chinchilla (*Chinchilla lanigera*) is a member of the rodent suborder *Hystricomorha* and bears one of the most valuable pelt in the world. The chinchilla, having great biological potential, show relatively low fertility and fecundity rates in comparison with other multiparous rodents [Jarosz & Rzewska, 1996]. During the sexual cycle up to 16 mature follicles which only 4 ovulate have been observe of in ovaries of mature chinchilla females, so theoretically a female can bear 4 kittens in each litter [Jarosz, 1969]. However, actual data are not in line with this theory. According to available information collected from chinchilla breeders, they have 2-4 kittens from one reproduction female per year [Jarosz, 1969]. Although chinchilla female can have two or even three litters a year from, however such a breeding system is not recommended [Wilk, 1989]. The difference between reproductive potential illustrated by the number of ovulating follicles and the low fecundity of chinchilla makes to introduction biotechnological methods into the practice. Such similar methods that have been successfully used for stimulation of estrus and ovulation in other farming animals [Bielanskii & Tischner, 1996; Kramer 1980]. The authors conducted experiment in order to induce estrus and ovulation in chinchilla females by a synthetic analogue of hypothalamus hormone GnRH.

Material and method

The experiment was conducted in two stages at the Experimental Farm Agricultural Academy of Cracow and at the RABA chinchilla farm in Myslenice near Cracow. In each stages, 24 adult and sexually mature females of standard variety were used. All of them were in good health condition and were fed with pellets applied according to their age and breeding phase. The first stage of experiment started in February. A group of 24 one-year old females were randomly divided into three groups at 8 females each: two experimental and one control. Each female from Group I was intramuscularly inoculated with 0,2 ml Receptal Intervet, which correspond to 0,84 μg busereline synthetic analogue gonadotrophin realizing FSH and LH. Females from Group II were intramuscularly inoculated with 0,2 ml Bioreline Biochef containing 0.90 μg busereline synthetic analogue hypothalamic GnRH. The third Group was given 0,2 ml *Aqua pro injectione* (by Polfa, Cracow,). To estimate morphological and structural changes stimulated by used hormonal preparations, females from Group I and from Group

III were anesthetized in the eleventh day and females of Group II– in the eighth day after inoculation. The number of mature and maturing follicles as well as of post-ovulation corpus luteum on ovaries were recorded. In stage two of the experiment, another 24 females were inoculated with the same preparations (as those of stage one) and then housed with a male in a polygamous system, in ratio 1 male to 4 females. This stage was conducted for 6 months. The effect of hormones applying was measured by detection of the number of barren females and the number of kittens born. Fecundity might happen in the first or/and second estrus cycle.

Photo 1 Diestrus phase. Vaginal smear consists mainly of the leukocytes (10x10)



Before hormonal stimulation, the state and phase of reproductive system of females were determined - vaginal smears were collected with the use of sterile slightly curette moistened with distilled water. The smears were then quickly transferred to a slide, fixed in 96% alcohol and ether solution (1:1 v/v) over at least 30 minutes, and then stained with Papanicolau differential method. The share of cells of each epithelium layer, of estrus cornified cells as well as the presence of leucocytes were later determined (PHOTO 1). The number of leucocytes was evaluated under a microscope by a 5-grade range as follows: ++++ mass number of leucocytes, +++ great number of leucocytes, ++ numerous leucocytes, + single leucocytes, - no leukocytes in the field of vision.

Results

The ovary in sexually mature females chinchilla undergo periodical morphological and functional changes. The main function of this progressive process is to release egg cells (ovulation) together with hormonal substances, which alternately generate estrus and stimulate uterine epithelium to receive fertilized oocytes. These changes depend on hormonal activity of hypophysial-ovary axis which itself is a negative feedback [Szoltys, 1992].

Experimental stage one

Both, growing and mature follicles, as well as corpus luteum, were found in ovaries of experimental chinchilla females in the both experimental groups of animals. The number of follicles corresponded to the hormonal preparation. In females from Group I great number of maturing follicles, 6-10 mature follicles on each ovary in particular females and *corpus luteum* in 5 females only were found (TABLE 1). In Group II also large number of maturing follicles, 2-10 mature follicles and *corpus luteum* in all females were found (TABLE 1).In the control Group III, there great number of maturing follicles and 1-3 mature follicles in particular females were observed. No *corpus luteum* were found (TABLE 1).

Table 1 Ovary size and the stage of folliculogenesis in experimental and control chinchillas

Group	Number of females	Ovary length		Ovary dimension		Maturing follicles	Mature follicles	Corpus luteum
		Left	Right	Left	Right			
I	8	6.14 ± 0.42	5.79 ± 0.63	2.95 ± 0.11	3.65 ± 0.66	Numerous	6 - 10	0 - 4
II	8	6,99 ± 1.63	6.21 ± 0.82	4.00 ± 0.35	3.30 ± 0.28	Numerous	2 - 10	1 – 5
III	8	5.27 ± 0.97	5.01 ± 0.34	2.22 ± 0,32	2.56 ± 0.60	Numerous	1 - 3	0

Experimental stage two
Ability of females to fecundation was evaluated based on conception ratio and the number of newborn kits.

Table 2 The results of reproduction of female chinchillas after hormonal stimulation

Group	Number of females	Whelped females	Litter size
I	8	5	1.25 (0-2)
II	8	8	1.6 (1 – 3)
III	8	0	0

In Group I (the animals with Receptol acting on their ovaries), the 5 females were successfully mated and the most of them gave 2 kittens in a litter. In Group II (Bioreline inoculated), all females had kittens: 4 of them gave 1 kitten and the rest had 2 or 3 kittens in a litter. In the control Group, no kittens were born during the trial (Table 2).

Discussion

The GnRH hormone, being synthesized in subthalamic nucleus and released from there, modulates the secretion of gonadotrophines from secretion cells of gland part of hypophysis. The influence of hypothalamus realized by release of LH-RF and FSH-RF [Szoltys, 1990]. There is a high correlation between GnRH pulses and release of LH and FSH, which depends on light [Bielanski & Tischner, 1996]. Primary and secondary follicles on ovaries are stimulated to grow by FSH hormone and, when passing into more complex phase, they secrete estrogens. The follicle phase, intensified by physiological growth of estradiol in blood, stimulates the synthesis process of GnRH receptors and its releasing as well. The GnRH wave must grow rapidly to start generation of LH wave from hypophysis. Additionally, stimulation of GnRH is necessary during the whole period of LH wave [Karsch et al., 1997].

Conclusions

According to morphological and reproduction results, both hormonal substances used in experiment: Bioreline and Receptal stimulate the estrus and ovulation processes in experimental chinchillas ovaries. Both preparations generated rapid growth of growing follicles, (of) ovulation process and production of corpus luteum. However, Bioreline gave better results than Receptol. The litters obtained in the trial also confirm the expectations of application of GnRH analogues. However, Bioreline gave better results than Receptol (more kids were born)

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IV – 19 RP

Growth parameters and organ size of American marten (*Martes americana*) born in captivity

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Abstract
This research examined the seasonal growth parameters and organ size of juvenile and adult male and female marten (*Martes americana*) born in captivity. The body weight data was collected from 25 male and 20 female marten from the Nova Scotia Agricultural College marten colony during 1999-2003 and the organ weight data from 19 males and 13 females in January 2003 and 2004. At 7 days of age the body weight of the male kits was 61.7±2.1 g and the female kits 55.1±1.7 g (P=0.019), while in December the juvenile males weighed 924.4±17.5 g and the females 633.9±8.1 g (P<0.001). The body weights of the juvenile female marten 631.2±7.9 g were significantly different in January from those of the mature female marten 667.6±11.8g (P=0.013), and the weights of the juvenile males 909.3±16.2 g differed significantly from those of adult males in January 1057.2±29.7 g (P=0.001). The marten exhibit pronounced seasonal fluctuation in their body condition throughout the year with both males and females being the heaviest in April, males being the smallest in August and the females being the smallest in July. Significant sex differences were observed in the weights of most internal organs. These results are valuable for the characterization of growth and seasonal changes in body condition in the American marten.

Introduction
The American (pine) marten (*Martes americana*) is a small fur bearing animal approximately the size of a small house cat with a long and slender body, and is a member of the Mustelidae family (Strickland *et al.*, 1982). The American marten shows sexual dimorphism, with the males being larger and heavier than the females. In wild populations, in Ontario, winter body weights of adult male marten are on average 821 g, and the winter body weights of juvenile male marten 734 g (Strickland & Douglas, 1999). Winter body weights of adult female marten average 482 g, and the winter body weights of juvenile female marten 488 g (Strickland & Douglas, 1999). The body length of a mature male marten is about 50-63 cm, which is longer than the body length of a mature female marten (46-56 cm) (Marshall, 2001). Marten also show visible seasonal variations in their pelt colour and quality. The winter pelt is dark brownish in colour with a distinctive orange-buff patch on the throat (Cornish, 2002).
The American marten is an arboreal species, which prefers dense coniferous forests with sufficient ground litter such as dead trees, branches and leaves to support and provide cover to various rodents, their principal food source (Snyder, 1991). In the wild, marten are opportunistic feeders that hunt mainly on the ground and their diet consists of a variety of items depending on what is available, including mice, voles, snowshoe hares, shrews, red squirrels, birds, fish, eggs, carrion, insects, fruit or nuts (Wydeven, 2000).
Female marten have induced ovulation and delayed implantation of the blastocyst for seven to eight months. The gestation period is 27 days with parturition occurring in late March to early April. There are typically 2-3 kits per litter, each weighing 28 g on average. They are born altricial and thus depend entirely on their mother for food and protection. The kits grow rapidly and by four weeks of age males weigh about 200 g and females about 173 g (Strickland *et al.*, 1982). The kits are weaned at 6 weeks of age, reach full growth by three months and are sexually mature at 15 months of age, but typically do not successfully reproduce until 24 months of age (Strickland *et al.*, 1982). According to a study on the European pine marten (*Martes martes*) both males and females show seasonal fluctuations in body weight,

where the weights are highest in the summer and lowest in the winter (Korhonen *et al.*, 1995). Korhonen *et al.* (1995) also reported that the European pine marten shows sexual dimorphism in some organ weights and sizes.

Since 1990, the Nova Scotia Agricultural College has developed a captive breeding program for the American pine marten (Rouvinen-Watt *et al.*, 1999) with successful matings and whelpings since 1995. The wild species status of the American pine marten has shown a decline in population in the Atlantic Provinces due to excessive trapping for their valuable fur along with accidental trapping (Boss, 1987). In Nova Scotia, the American marten is now a "species at risk", defined as any indigenous species, variety, or geographically defined population of wild fauna or flora that is at risk of becoming any of the following: extinct, extirpated, endangered, threatened or special concern (COSEWIC, 2002).

Growth parameters such as developmental and seasonal growth curves and organ sizes of the American pine marten (*Martes americana*) born in captivity are not well characterized. The objectives of this research were to create growth curves for captive-born juvenile male and female marten, to establish seasonal body weight curves for adult male and female marten and to measure organ size. This information would be valuable for the evaluation of animal performance in captive breeding and species conservation programs.

Material and Methods

The daily and seasonal care of the American pine marten colony at the Nova Scotia Agricultural College followed the practices outlined in the Standard Operating Procedure for the American marten (Rouvinen *et al.*, 1999). Body weights of 25 male and 20 female captive-born marten were recorded monthly during 1999-2003. The marten were considered juvenile if they were less than one year old and adult thereafter. Marten selected for pelting and subsequent organ scaling data collection consisted of 19 males and 13 females. These marten were euthanised by means of carbon monoxide. Each marten was weighed (g), body length measured (cm), pelted and carefully dissected removing mesentery tissue and fat. The following organs were individually weighed: heart, liver, stomach (full and empty), spleen, pancreas, intestine (full and empty), kidneys, adrenal glands, testes from the male marten and the reproductive tract of the female marten.

For statistical analyses, the marten were grouped into categories of juvenile males, juvenile females, adult males and adult females. For each category, the mean monthly weights (±SD) were calculated. A 2-sample t-test was used to test differences between male and female marten as well as juvenile and adult animals (Minitab Statistical Software). The means (±SD) were calculated for the weight, body length and organ weight data from the euthanised marten. A 2-sample t-test was used to compare if significant differences existed between the male and female marten.

Results and Discussion

Figure 1 represents the growth rate of juvenile male versus female marten born in captivity from 7 days of age to 9 months of age. At 7 days of age the body weights of the male kits were 61.7±2.1 g and the female kits 55.1±1.7 g ($P=0.019$). Both sexes reached their peak body weights in October, when the males weighed 968.4±17.7 g and the females 659.8±9.3 g ($P<0.001$). By December both male and female juvenile marten had reached their adult body size, males weighing 924.4±17.5 g and females 633.9±8.1 g ($P<0.001$).

The male and female marten born in captivity have lower body weights at four weeks of age 156.1±3.3 g and 139.5±3.1 g, compared to body weights reported in literature for wild male and female marten at the same age 200 g and 173 g (Strickland *et al.*, 1982). Winter body weights of captive-born juvenile male (924.4±17.5 g) and female (633.9±8.1 g) marten were heavier, compared to the winter body weights reported in literature of wild juvenile male marten (734 g) and female marten (488 g) (Strickland & Douglas, 1999).

Figure 1. Growth curves for juvenile male and female American marten.

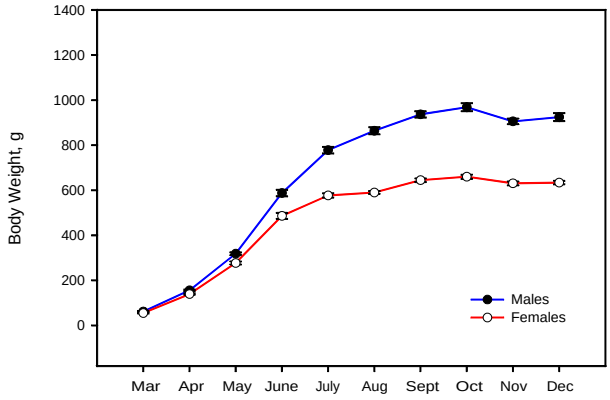

Figure 2. Seasonal fluctuations in body weight of adult and juvenile male and female American marten.

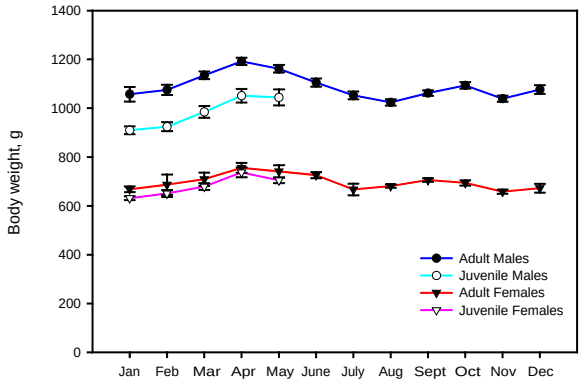

Figure 2 shows the seasonal fluctuations in body weight of adult and juvenile male and female marten. Juvenile male and female marten were included in the seasonal growth curve, but were separate from adults from January to May because they were considered to be fully grown, but not mature. In June, weights of the juvenile male and female marten were incorporated into the adult seasonal growth curves. The body weights of the juvenile female marten (631.2±7.9 g) were significantly different in January from those of the adult female marten (667.6±11.8 g; P=0.013), and the weights of the juvenile males 909.3±16.2 g differed significantly from those of adult males in January (1057.2±29.7 g; P=0.001). Both male and female marten exhibited pronounced seasonal fluctuation in their body condition throughout the year with both juvenile and adult males (1050.9±28.0 and 1191.9±14.8 g) and juvenile and adult females (737.0±19.9 and 755.9±20.6 g) being heaviest in April, adult males being the smallest in August (1023.6±13.2 g) and the adult females being the smallest in July (666.9±23.9 g). It was found that the winter body weights of adult male (1057.2±29.7 g) and female (667.6±11.8 g) captive-born marten were higher than the values reported in the literature for the American marten in the wild with adult males weighing on average 821 g and females weighing 482 g (Strickland & Douglas, 1999). These seasonal fluctuations in body condition for captive-born male and female marten are somewhat different from those reported for the wild European pine marten. Korhonen *et al.* (1995) reported that both male and female European pine marten show seasonal changes in body weight, where the weights are highest in the summer and lowest in the winter due to their limited body fat reserves.

Table 1 shows the comparison of body and organ weights of captive-born male and female marten. All organs, except for the stomach (full) and the spleen (P=0.085), showed a significant difference between the male and female marten. These findings agree with those by Korhonen *et al.* (1995) on organ weights of the wild European pine marten (*Martes martes*) showing large sex differences in the weights of most internal organs. The organ weights of the captive-born American marten appear smaller than the values reported for the European marten (Korhonen *et al.*, 1995) with the exception of the males' spleen and kidneys, and the females' liver and kidneys.

Table 1. Comparison of body and organ weights (mean ± SD) of captive born male and female American marten.

Variable measured	Males	Females	P-value
Number, n	19	13	-
Body weight, g	969±138	651±45	< 0.001
Body length, cm	40.8±1.7	36.0±1.6	< 0.001
Liver, g	44.4±6.3	34.9±2.7	< 0.001
Spleen, g	2.3±1.4	1.6±0.3	0.085
Pancreas, g	2.5±0.3	2.1±0.4	0.002
Stomach (full), g	16.0±11.5	14.2±8.1	0.614
Stomach (empty), g	6.5±0.7	4.7±0.6	< 0.001
Intestine (full), g	30.1±5.9	25.0±5.8	0.023
Intestine (empty), g	18.3±3.8	13.9±2.1	< 0.001
Heart, g	7.2±0.7	5.1±0.5	< 0.001
Kidney (left), g	3.7±0.5	2.9±0.4	< 0.001
Kidney (right), g	3.7±0.5	2.9±0.4	< 0.001
Adrenal gland (left), mg	26.6±3.9	22.4±2.5	0.003
Adrenal gland (right), mg	22.7±3.3	19.8±3.2	0.020
Testicles, g	0.29±0.07	-	-
Reproductive tract, g	-	0.70±0.4	-

Table 2 displays the results from the comparison of organ weights of captive-born male and female marten in relation to body size. Although this method of comparison removes the effect of the large difference in body size due to sexual dimorphism, some significant differences remained between the male and female marten organ weights. In relation to body size, the liver, pancreas, intestinal weight, kidneys and the adrenal glands were relatively larger in the female marten in comparison to the males.

Table 2. Comparison of body and organ weights (mean ± SD) of captive born male and female American marten in relation to body size (% of body weight).

Variable measured	Males	Females	P-value
Number, n	19	13	-
Liver, %	4.6±0.6	5.4±0.3	< 0.001
Spleen, %	0.25±0.17	0.25±0.05	0.970
Pancreas, %	0.26±0.04	0.32±0.05	< 0.001
Stomach (full), %	1.6±1.1	2.2±1.2	0.198
Stomach (empty), %	0.68±0.07	0.71±0.08	0.167
Intestine (full), %	3.2±0.7	3.9±1.0	0.040
Intestine (empty), %	1.9±0.4	2.2±0.3	0.040
Heart, %	0.75±0.07	0.78±0.06	0.188
Kidneys, %	0.78±0.1	0.89±0.07	< 0.001
Adrenal glands, % x 10^{-3}	5.4±0.8	6.5±0.9	0.002
Testicles, %	0.03±0.01	-	-
Reproductive tract, %	-	0.11±0.06	-

Juvenile male and female marten born in captivity show successful developmental growth with a significant difference between sexes. Adult captive-born male and female marten are sexually dimorphic and exhibit pronounced seasonal fluctuations in their body condition throughout the year with males being heavier than the females. Comparisons of adult male and female marten born in captivity showed a

significant difference in body weight, length and the weight of some organs. These results are valuable for the characterization of growth and seasonal changes in body condition in the American marten and can be used to evaluate growth performance of martens in captive breeding and species conservation programs.

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