

Group housing of farmed silver fox cubs

L Ahola*, J Mononen, T Pyykönen and M Miskala

Institute of Applied Biotechnology, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland

* Contact for correspondence and requests for reprints: Leena.Ahola@uku.fi

Abstract

In the present study, the effects of social environment on the welfare of farmed silver fox cubs were clarified. After weaning, cubs from silver fox litters were housed (1) singly, (2) in litters until the end of September and thereafter singly, or (3) in litters throughout their growing season. Separating the cubs at the onset of the species' natural dispersal time may not be strictly beneficial for the cubs because it may limit the animals' possibilities to fulfil their needs for social behaviour. However, the lower incidence of bite wounds in both the single housed cubs and the cubs from litters that were split in autumn showed some beneficial effects of separating the cubs. The cubs that were group housed in litters for the whole time were focussed on their own social system, were more averse to human presence and showed greater responses to acute stress than the cubs that were single housed for at least part of the time. However, the serum cortisol level following adrenocorticotrophic hormone administration suggested that cubs that were group housed in litters were less stressed over the long-term compared with the cubs that were single housed for at least part of the time; the low incidence of stereotypic behaviour in the cubs raised in litters also supports this hypothesis. Accordingly, and despite some unsolved questions regarding interpretation of the hypothalamic-pituitary-adrenal axis activity, the results from this present study show that social contacts were important for the welfare of silver fox cubs, and suggest that farmed silver fox cubs could possibly be raised in litters without jeopardising their welfare or deteriorating their fur quality.

Keywords: ACTH test, animal welfare, group housing, physiology, silver fox, stereotypies

Introduction

Under farm conditions, cubs of farmed silver foxes (a colour morph of the red fox *Vulpes vulpes*) are raised predominantly in male-female sibling pairs — and only in some cases singly — following weaning, which occurs at the age of approximately eight weeks, and housed in traditional fox cages (105 × 115 × 70 cm, length × width × height). The arguments for the use of the present housing system are based on positive production experiences gathered throughout the history of fox farming. However, raised concern regarding the welfare of farm animals has led to proposals that profitable animal production alone is not sufficient to ensure that the welfare of the animals is assured. Accordingly, the European Convention (1999) has recommended that animals should be provided with housing conditions that take into account their species-specific biological needs, and proposed that research should be carried out on the development of new housing systems, including group housing, for animals raised for fur production.

In the wild, red foxes live in variable social systems (see Sandell 1989), possibly because of their high flexibility in their living habits (see Harris & Lloyd 1991). Accordingly, red foxes have been considered to be both solitary (Lloyd 1975; Cavallini 1996) and social, living in groups often

consisting of individuals that are related to each other (eg Macdonald 1983; see Ahola 2002). Social groups may occur among red foxes when food resources are rich (eg von Schantz 1984); therefore, it could be deduced that on farms, where there is plenty of food available, group housing of silver foxes could be considered to be an alternative, socially enriched way of housing these animals.

However, it is not clear or straightforward to define the best social environment for farmed foxes. Previous studies on farmed silver foxes have shown that there are advantages and disadvantages to both single and group living (see Ahola 2002). For example, silver fox cubs show a higher amount of stereotypic behaviour when housed singly than when two or more cubs are housed together (Ahola *et al* 2002). However, group housed cubs show increased hypothalamic-pituitary-adrenal (HPA) axis activity (Ahola *et al* 2000), a higher occurrence of aggressive behaviour (Ahola & Mononen 2002) and a higher incidence of bite wounds (Ahola *et al* 2001). Based on these previous results and knowledge of the red fox's behaviour in the wild, it has been hypothesised that the welfare of farmed silver foxes could be promoted by altering their social environment according to their developmental stage (Ahola 2002): fox cubs could be housed in

socially-rich sibling groups until their natural dispersal time (for dispersal time in the red fox, see eg Harris & Lloyd 1991), and thereafter singly, in order to alleviate the effects of within-group social tension and aggression.

The previous results of group housing farmed silver fox cubs (Ahola 2002) were based on experiments where the number of individuals and the sex of the cubs within the groups was standardised. This was an artificial situation, distorting the actual situation on fur farms where litters comprise different numbers of male and female cubs within and between the litters. Therefore, the aim of the present study was to clarify the effects of group housing on the welfare and behaviour of farmed silver fox cubs by performing an experiment using non-standardised litters. All the silver fox litters born at the Research Station of the Institute of Applied Biotechnology (University of Kuopio, Finland) during one breeding season were included in the study; there was no selection of the animals. One third of the litters were housed singly after weaning, one third as litters until the end of September and thereafter singly, and one third as litters throughout their growing season. Behavioural, physiological and production-related parameters were monitored from all animals during the experiment, from weaning in July until pelting in January.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee of the University of Kuopio (licence number 02–41).

Animals and housing

Twenty-three farmed silver fox litters, born in April–May (2002), were included in the present study. In total, the litters included 45 male and 45 female cubs; mean litter size was 3.9 ± 1.2 cubs (median 4). The litters were divided into three experimental groups at weaning, when the cubs were approximately eight weeks old. The experimental groups were: (1) litters in which cubs were housed singly from weaning until pelting, which occurred in January (SIN); (2) litters that were housed as litters from weaning until 30 September and thereafter singly until pelting (LIT–SIN); and (3) litters that were housed as litters from weaning until pelting (LIT). The experimental group SIN contained eight litters, with 14 males and 17 females; LIT–SIN contained seven litters, with 15 males and 12 females; and LIT contained eight litters, with 16 males and 16 females.

The cubs were housed in an outdoor fur shed with two rows of cages. Because the location of the cage inside a fur shed may induce changes in the behaviour of farmed foxes (blue foxes: Rekilä *et al* 1996; silver foxes: Rekilä *et al* 1998) the litters of the three experimental groups were distributed evenly across the front cages of the shed (the first third of the shed), the middle cages (the second third of the shed) and the rear cages (the last third of the shed).

Foxes that were housed singly (SIN and LIT–SIN from October onwards) were housed in traditional fox cages ($115 \times 105 \times 70$ cm, length \times width \times height; 1.2 m²). Foxes housed as litters (LIT and LIT–SIN from weaning until the

end of September) were housed in cage systems constructed from traditional cages connected together by openings (20×20 cm, width \times height) through the walls between the adjacent cages (see Ahola & Mononen 2002). The number of cages for litters in LIT and LIT–SIN was equal to the number of cubs within the litters; therefore, the space allowance per fox was 1.2 m². Each of the cages was furnished with a resting platform (105×30 cm, length \times width, 25 cm from the cage ceiling) and a feeding tray.

The health of the animals was checked daily. The foxes were fed according to the recommendations given by the Finnish Fur Breeders' Association, and fresh fur-animal feed (Ylä-Karjalan Rehu Oy, Valtimo, Finland) was delivered onto the feeding trays; the daily feed portion per animal was the same for each group.

Measured parameters

The foxes were weighed at weaning and then on 30 September and at pelting, at the beginning of January.

Urinary cortisol was used as a non-invasive method to measure cortisol levels (see Beerda *et al* 1999) in order to assess the HPA axis activity; it was determined twice during the study. A 24 h urine sample was collected from each litter at the end of September (before the litters in LIT–SIN were split) and at the beginning of November, ie 23 urine samples were obtained on each date. Urinary cortisol and creatinine concentrations were determined as described in Ahola *et al* (2002); the content of cortisol in the urine was expressed as the cortisol:creatinine ratio.

The feeding test, developed to measure farmed foxes' fear reactions towards humans (Rekilä *et al* 1999), was performed five times during the growing season: at the end of July, at the beginning of September, at the beginning of October, mid-November and mid-December. During the feeding test, the experimenter stood in front of the fox's cage (for single housed animals) or in front of the middle cage of the cage system (for the litter housed animals) for 60 s and recorded the number of individuals that were eating. Because the animals within the cage systems were not individually identified the results from the feeding tests are expressed as the percentage of animals in each litter eating within the duration of the test.

The behaviour of the foxes was videotaped approximately every six weeks during the experiment: in August, September, October and December. The video system consisted of cameras, wide angle lenses, a camera switcher, a time-lapse video-recorder and lights, as described in Ahola *et al* (2001). The activity level of the foxes (ie the time spent sitting, standing and moving) and the preference for staying in groups (group preference index [GPI]: Gattermann 1990) were recorded from the tapes using instantaneous sampling with a 5 min sampling interval (Martin & Bateson 1993). The occurrence of stereotypic behaviour was recorded from the tapes using one-zero sampling with a 5 min sampling interval (Martin & Bateson 1993). Only locomotor stereotypic behaviour performed without accompaniment by the neighbouring animal was regarded as stereotypy in this study (see Wikman *et al* 1999).

At pelting in January, the foxes were caught and administered with an intramuscular injection of adrenocorticotrophic hormone (ACTH) (0.3 mg synthetic ACTH₁₋₂₄ per animal; Synacthen Depot, Novartis Pharma SA Hünigues, France). The foxes were then placed singly into a smaller cage (70 × 35 × 35 cm, length × width × height), and 2 h after the injection they were euthanased by electrocution according to the methods recommended by the *Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes* (European Convention 1999). Blood samples were immediately drawn using a cardiac puncture. The serum cortisol level, as a maximum response to ACTH administration (see Fraser & Broom 1990; Rekilä *et al* 1999), was analysed using a competitive immunoassay technique (Coat-A-Count Cortisol Assay; Diagnostic Products Corporation, Los Angeles, CA, USA). The plasma growth hormone concentrations (as a stress-related parameter, see Matteri *et al* 2000) were measured with the hGH human growth hormone double antibody kit (Diagnostic Products, Corporation, Los Angeles, CA, USA) at the University of Joensuu, Finland. The assays were validated such that serial dilutions of the silver fox plasma showed linear changes in B B₀⁻¹ values that were parallel with the standard curves produced using human standards. The plasma glucose concentrations (as an indicator of acute stress, see Matteri *et al* 2000) were determined using the liquid reagent hexokinase method with reagents supplied by Randox Laboratories Ltd (Crumlin, UK) using the Technicon RA-XT™ analyser (Technicon Instruments Corporation, Tarrytown, NY, USA) at the University of Joensuu, Finland.

After pelting, the adrenal glands (as an indicator of the HPA axis activity, eg Hemsworth *et al* 1996), heart and gastrocnemius muscle (GAST) from the left hind limb (as an exercise-related indicator, eg Duncan *et al* 1998) were removed, cleaned and weighed. The numbers of bite scars on the fleshed skins were also counted.

Professional fur graders at the Finnish Fur Sales Ltd (Helsinki, Finland) evaluated the quality of the furs using a 10 point scale: 1 = poorest, 10 = best.

Statistical analyses

The cubs within each litter cannot be considered to be independent of each other; therefore, mean values of the cubs within each litter were used for the statistical analyses. The number of cases for statistical analyses in SIN, LIT-SIN and LIT was eight, seven and eight, respectively. Although the statistical analyses were performed using these mean values, sex difference was also analysed for the parameters that were collected from individual animals, ie physiological parameters (except the urinary cortisol:creatinine ratio), body and organ masses, fur quality and the incidence of bite scars.

The statistical analyses of the data were performed using SPSS® for Windows® (SPSS 1999). The general linear model (GLM) for univariate measures was used to evaluate differences between the three experimental groups for parameters that were normally distributed: the GPI, the activity level of the foxes, body and organ masses, physiological parameters and the percentage of animals with bite scars on

their skin. In order to be able to compare the effects of single living with group living, comparisons between SIN and the groups LIT-SIN and LIT, and between LIT and the groups SIN and LIT-SIN were performed for the parameters measured in July–September and October–January (pelting), respectively. Pair-wise comparisons were carried out using Tukey HSD *post hoc* tests. Differences in the activity level of the foxes and the GPI between the different months of the year were tested using the GLM for repeated measures. A Pearson correlation between the serum cortisol level following ACTH administration and the amount of stereotypic behaviour in December was determined separately for each experimental group. A Pearson correlation between the activity level of the foxes and the amount of stereotypic behaviour was determined separately for each month.

The non-parametric tests, Kruskal-Wallis, Mann-Whitney *U* and Friedman, were used for parameters that were on an ordinal scale, that were not normally distributed or for which the variances between the three experimental groups were unequal: the incidence of stereotypic behaviour, the percentage of animals eating during the feeding tests, fur characters and the number of bite scars per animal. Pair-wise *post hoc* tests for these parameters were performed according to Siegel and Castellan (1988). The results are expressed as mean ± standard deviation (SD); *P* values greater than 0.05 were considered to be non-significant.

Results

Animals and housing

There was no statistically significant difference in the percentage of males of the total number of cubs within the litters (total mean 51 ± 20%), mean litter size (3.9 ± 1.2 cubs) or the age of the cubs (94 ± 13 days on 1 August) between the three experimental groups (*P* > 0.05 for all, GLM for univariate measures). Furthermore, there was no statistically significant difference in the percentage of males of the total number of cubs within the litters, litter size or the age of the cubs between the three sections of the fur shed (*P* > 0.05 for all, GLM for univariate measures).

One fox cub in the SIN group died in mid-November; the cause of death was unknown. In the LIT group, one cub was badly bitten in late September and one had a serious intestinal ailment in early November; these two LIT cubs were euthanased during the experiment.

Measured parameters

There was no statistically significant difference in the plasma growth hormone level, plasma glucose level or serum cortisol level following ACTH administration between the male and female foxes (*P* > 0.05 for all, GLM for repeated measures). Furthermore, there was no difference in the clarity and cover of the fur or in the number of bite wounds between the two sexes (*P* > 0.05 for all). Male foxes were significantly heavier than the females and, accordingly, also had significantly heavier adrenal glands, hearts and GAST than the females (*P* < 0.001 for all body and organ mass parameters). Male foxes also had significantly better density

Table 1 The mean percentage (\pm SD) of foxes in the litters in the three experimental housing systems eating during the five feeding tests, performed at the end of July, at the beginning of September, at the beginning of October, mid-November and mid-December. SIN: cubs housed singly from weaning until January, LIT-SIN: cubs housed as litters from weaning until 30 September and thereafter singly, LIT: cubs housed as litters from weaning until January. P1: SIN versus LIT-SIN versus LIT, P2: SIN versus the groups LIT-SIN and LIT in July-September, the groups SIN and LIT-SIN versus LIT in October-December; ns = not significant, $P > 0.05$.

	SIN	LIT-SIN	LIT	P1 ^a	P2 ^b
July	6 \pm 11	10 \pm 13	29 \pm 39	ns	ns
September	53 \pm 42	34 \pm 19	40 \pm 21	ns	ns
October	44 \pm 37	45 \pm 26	48 \pm 25	ns	ns
November	54 \pm 44	50 \pm 38	42 \pm 20	ns	ns
December	72 \pm 36	72 \pm 34	34 \pm 34	ns	0.035
P ^c	0.015	0.003	ns		

^a Kruskal-Wallis test with *post hoc* tests (see Siegel & Castellan 1988); ^b Mann-Whitney *U* test; ^c Friedman test. No significant differences ($P > 0.05$) in within-month row-means between any of the pair-wise comparisons.

Table 2 The mean percentage (\pm SD) of observations of foxes spent performing active behaviours in the litters in the three experimental housing systems at the beginning of August, mid-September, late-October and mid-December. SIN, LIT-SIN and LIT as described in Table 1. P1: SIN versus LIT-SIN versus LIT, P2: SIN versus the groups LIT-SIN and LIT in August-September, the groups SIN and LIT-SIN versus LIT in October-December; ns = not significant, $P > 0.05$.

	SIN	LIT-SIN	LIT	P1 ^a	P2 ^a
August	33 \pm 2 ^x	32 \pm 3 ^{xy}	29 \pm 3 ^y	0.044	ns
September	33 \pm 6	31 \pm 4	32 \pm 4	ns	ns
October	36 \pm 4	31 \pm 5	33 \pm 5	ns	ns
December	40 \pm 4 ^x	34 \pm 4 ^y	36 \pm 5 ^{xy}	0.028	ns
P ^b	0.000	ns	0.001		

^a GLM for univariate measures with Tukey HSD *post hoc* tests; ^b GLM for repeated measures; ^x, ^y, ^z within-month row-means with different superscripts are significantly different ($P < 0.05$).

of fur and quality of fur than the females ($P < 0.01$ for both). There were no statistically significant interactions between sex and experimental group for any of the measured parameters ($P > 0.05$ for all).

In the two groups SIN and LIT-SIN, the percentage of animals eating during the feeding test increased with time; in the LIT group, this percentage remained unchanged throughout the duration of the experiment (Table 1). There was no significant difference between the three experimental groups in the percentage of animals eating during the five feeding tests. In December, however, the animals in the groups SIN and LIT-SIN ate more frequently during the feeding test than the animals in LIT.

In SIN and LIT, the activity level of the foxes increased with the advance of autumn; in LIT-SIN, the activity level remained the same throughout the growing season (Table 2). In August, the percentage of observations of foxes spent performing active behaviours was lowest in LIT. In September-October, there was no significant difference in the activity level of the foxes between the three experimental groups. In December, the litters in SIN spent the highest amount of time performing active behaviours. Single living versus group living had no effects on the activity level of the cubs.

The percentage of observations of foxes performing stereotypic behaviour increased as autumn advanced in both the SIN and LIT-SIN groups; there was no change in the amount of stereotypic behaviour performed by the foxes in the LIT group (Table 3). In August, stereotypic behaviour was observed only in the SIN group. However, from October onwards, stereotypic behaviour increased in the LIT-SIN group, ie in the group where the litters were split at the end of September. The time spent performing stereotypic behaviour increased in the LIT-SIN group so that there was no longer any statistically significant difference in this parameter between SIN and LIT-SIN in October or December. The amount of stereotypic behaviour was higher in the cubs that were housed singly compared with the cubs housed in litters throughout the experiment. No significant correlation was found between the amount of stereotypic behaviour in December and the serum cortisol level following ACTH administration at pelting in any of the experimental groups (SIN: $r = 0.400$; LIT-SIN: $r = -0.648$; LIT: $r = 0.156$; $P > 0.05$ for all groups). However, there was a significant correlation between the activity level of the foxes and the amount of stereotypic behaviour in August, September and December ($r = 0.539$, $r = 0.424$ and $r = 0.503$, respectively, $P < 0.05$ for all months), whereas in October this correlation was non-significant ($r = 0.293$, $P > 0.05$).

Table 3 The mean percentage (\pm SD) of observations of foxes performing stereotypic behaviour in the litters in the three experimental housing systems at the beginning of August, mid-September, late-October and mid-December. SIN, LIT-SIN and LIT as described in Table 1. P1: SIN versus LIT-SIN versus LIT, P2: SIN versus the groups LIT-SIN and LIT in August-September, the groups SIN and LIT-SIN versus LIT in October-December; ns = not significant, $P > 0.05$.

	SIN	LIT-SIN	LIT	P1 ^a	P2 ^b
August	1.8 \pm 2.2 ^x	0.0 \pm 0.0 ^y	0.0 \pm 0.0 ^y	0.001	0.002
September	3.9 \pm 4.6 ^x	0.1 \pm 0.1 ^y	0.0 \pm 0.0 ^y	0.000	0.000
October	4.1 \pm 4.1 ^x	0.5 \pm 0.9 ^{xy}	0.0 \pm 0.0 ^y	0.000	0.000
December	5.4 \pm 4.6 ^x	1.0 \pm 1.1 ^{xy}	0.1 \pm 0.1 ^y	0.001	0.000
P ^c	0.001	0.009	ns		

^a Kruskal-Wallis test with *post hoc* tests (see Siegel & Castellan 1988); ^b Mann-Whitney *U* test; ^c Friedman test; ^x, ^y, ^z within-month row-means with different superscripts are significantly different ($P < 0.05$).

Table 4 Mean values for body mass (BM [kg]) at weaning in July, in late-September and at pelting in January; urinary cortisol:creatinine ratio (C:C $\times 10^{-3}$) in late-September and at the beginning of November; plasma growth hormone (GH [ng ml⁻¹]), plasma glucose (GLU [mmol⁻¹]), serum cortisol (nmol⁻¹) and mass (g) of the adrenal glands, gastrocnemius muscle (GAST) and heart in January in the fox litters in three experimental housing systems. SIN, LIT-SIN and LIT as described in Table 1. P1: SIN versus LIT-SIN versus LIT, P2: SIN versus the groups LIT-SIN and LIT in July-September, the groups SIN and LIT-SIN versus LIT in October-January (pelting); ns = not significant, $P > 0.05$.

	SIN	LIT-SIN	LIT	P1 ^a	P2 ^a
BM: July	2.0 \pm 0.2	2.1 \pm 0.2	1.9 \pm 0.2	ns	ns
BM: September	6.4 \pm 0.7	6.3 \pm 0.6	6.4 \pm 0.7	ns	ns
BM: January	8.0 \pm 0.9	8.2 \pm 0.3	7.9 \pm 0.8	ns	ns
C:C: September	5.3 \pm 1.4	4.7 \pm 0.8	4.4 \pm 1.5	ns	ns
C:C: November	4.0 \pm 2.2	3.3 \pm 0.7	5.3 \pm 6.2	ns	ns
GH: January	1.9 \pm 0.2	1.9 \pm 0.2	1.9 \pm 0.1	ns	ns
GLU: January	7.8 \pm 0.9	7.8 \pm 1.0	8.8 \pm 0.6	0.049	0.013
Serum cortisol: January	520 \pm 75	515 \pm 102	446 \pm 34	ns	0.033
Adrenal glands: January	573 \pm 71	559 \pm 52	546 \pm 44	ns	ns
GAST: January	34 \pm 4	34 \pm 1	33 \pm 3	ns	ns
Heart: January	47 \pm 3	50 \pm 3	48 \pm 3	ns	ns

^a GLM for univariate measures with Tukey HSD *post hoc* tests. No significant differences ($P > 0.05$) in row-means between any of the pair-wise comparisons.

The GPI, calculated for LIT-SIN in August-September and for LIT in August-December, did not change from month to month in either of the groups (LIT-SIN: 24 \pm 8% in August, 28 \pm 8% in September, $P > 0.05$; LIT: 27 \pm 12% in August, 24 \pm 6% in September, 23 \pm 13% in October, 22 \pm 14% in December, $P > 0.05$). Furthermore, there were no differences in GPI between these two groups in August and September ($P > 0.05$ for both months).

In general, the foxes in this experiment grew in a uniform manner and no significant differences in the body or organ masses were found between the three experimental groups (Table 4). In the welfare-related physiological parameters measured at pelting, statistically significant differences were observed only in the plasma glucose level and in the serum cortisol level following ACTH administration (Table 4): in the LIT group the glucose level was significantly higher and the cortisol level significantly lower

than in the cubs housed singly for at least a part of the time, ie SIN and LIT-SIN.

No significant differences were found in any of the fur characteristics (ie density, clarity, cover and quality) between the three different housing systems (Table 5). The percentage of foxes with bite scars on their skins was highest in LIT (Table 5); in line with this result, the number of bite scars was also highest in LIT.

Discussion

The previous results on farmed silver foxes (Ahola *et al* 2000; 2001; 2002; Ahola & Mononen 2002), combined with the knowledge of this species' behaviour in the wild (eg Macdonald 1983), indicate that the welfare of farmed silver foxes could be improved by changing the foxes' social housing conditions according to their developmental stage. In other words, silver fox cubs should possibly be kept in litters

Table 5 Mean (\pm SD) density, clarity, cover and quality of furs (10 point scale: 1 = poorest, 10 = best), mean percentage (\pm SD) of animals with bite scars on their skin and mean (\pm SD) number of bite scars per animal in the fox litters in the three experimental housing systems. SIN, LIT-SIN and LIT as described in Table 1. P1: SIN versus LIT-SIN versus LIT, P2: the groups SIN and LIT-SIN versus LIT; ns = not significant, $P > 0.05$.

Fur characters	SIN	LIT-SIN	LIT	P1 ^a	P2 ^b
Density	6.4 \pm 0.9	7.0 \pm 0.0	6.3 \pm 1.3	ns	ns
Clarity	5.4 \pm 0.5	5.7 \pm 0.4	5.5 \pm 0.9	ns	ns
Cover	6.5 \pm 1.2	7.0 \pm 0.8	5.8 \pm 1.0	ns	ns
Quality	7.0 \pm 0.7	7.4 \pm 0.6	6.3 \pm 1.6	ns	ns
% with bite scars	38 \pm 25 ^x	49 \pm 37 ^{xy}	82 \pm 25 ^y	0.018 ^c	0.006 ^c
Number of bite scars	1.1 \pm 1.2 ^x	2.4 \pm 2.5 ^{xy}	6.3 \pm 3.8 ^y	0.013	0.002

^a Kruskal-Wallis test with *post hoc* tests (see Siegel & Castellan 1988); ^b Mann-Whitney *U* test; ^c GLM for univariate measures with Tukey HSD *post hoc* tests; ^x, ^y, ^z row-means with different superscripts are significantly different ($P < 0.05$).

longer than the present farming practice recommends, and the litters should only be separated after the onset of the species' natural dispersal time, ie from September–October onwards. In this way, cubs could enjoy a socially enriched environment when they are young but avoid increased within-group aggression and social tension as they grow older.

However, the results of this study revealed only minimal differences in the measured parameters between the cubs housed singly from weaning onwards and the cubs that were initially group housed in litters and then housed singly from October onwards. In LIT-SIN the percentage of observations of foxes performing active behaviours did not increase during the experiment, whereas in SIN the activity level increased from August until December. The results also revealed that, in general, the higher the activity level, the higher the level of stereotypic behaviour. Accordingly, it is understandable that in SIN, where stereotypic behaviours were common, the activity level was also high; however, this was not the case in the LIT-SIN group. Therefore, separating the litters, ie unstable social conditions, appears to affect the activity of the animals; however, the importance of this result remains unresolved. The only significant difference between SIN and LIT-SIN was observed in the percentage of observations where stereotypic behaviours were detected. In August, the cubs in SIN already performed stereotypic behaviours, whereas no stereotypic behaviours were observed in LIT-SIN. In SIN, the incidence of stereotypic behaviour increased steadily from the beginning of the experiment until its termination. In LIT-SIN, separating the litters induced an increase in stereotypic behaviours such that by late-October, when the cubs in both SIN and LIT-SIN were living on their own, there was no difference in the amount of stereotypic behaviours between these two groups. The correlations between the level of stereotypy in December and the serum cortisol level showed that, unlike in SIN (and in LIT), this correlation was negative in LIT-SIN. This result could suggest that stereotypic behaviour could have helped the cubs in LIT-SIN to cope with the changes in their living environment (ie a change from group housing to single housing) (see Mason [1991]

for a discussion of stereotypes and coping). Nonetheless, it does appear that the cubs in SIN and LIT-SIN suffered from the absence of cage mates, whereas the cubs in LIT appeared to be better off in this sense showing practically no stereotypic behaviours. Therefore, the present study disagrees with the earlier hypothesis of strictly beneficial effects of separating the litters in late autumn (Ahola *et al* 2002), at least in the non-standardised litters used in the present study. The present results are, however, analogous to the earlier results indicating that social stimuli are important for fox cubs (Ahola *et al* 2002).

In general, stereotypic behaviour, often associated with inappropriate housing environments (eg Broom & Johnson 1993), develops with time and may at some time-point become independent of the stimulus that originally elicited its performance (see Mason 1991). In August–September, the single housed cubs were observed (from the videotapes) to perform movements along their cage walls that resembled play-soliciting movements typical for this species (Fox 1987); in these cases, there always was a neighbouring cub performing the same movements in its own cage. Over time, single housed cubs were observed to perform these movements more and more often without a neighbouring animal, and therefore they were assessed as performing stereotypic behaviours. Accordingly, stereotypic behaviour in the single housed fox cubs may have emerged as a consequence of an unfulfilled need for social behaviour in these animals and as a way to cope with this situation.

The presence or the absence of cage mates also affected the cubs' attitudes towards humans. In SIN and LIT-SIN, the percentage of animals coming to eat during the feeding test increased with time and was at its highest in December; in the LIT group the percentage did not change significantly during the experiment. Furthermore, the percentage of animals coming to eat while a human was present was almost 40% lower in LIT than in the other two groups in December. These results reveal that the single housed cubs were, possibly unintentionally, better conditioned to human presence than the cubs in LIT. This may have happened either because the singly living cubs were housed in a

smaller area (1.2 m² in SIN from August onwards and in LIT–SIN from October onwards versus 1.2 m² × the number of animals within the litter in LIT from August onwards) with less space to avoid human presence, or in the absence of cage mates these cubs may have sought social stimuli from humans. In this way, these cubs may have become more confident towards humans and therefore showed fewer fear reactions towards humans. However, the cubs in LIT were more closely bonded to their own within-species social group and were not habituated to humans to such an extent compared with the cubs in SIN and LIT–SIN. Therefore, as also previously discussed by Ahola *et al* (2002), the cubs housed in larger groups (in a larger living area) are possibly either just ignoring humans or are more afraid of humans.

The urinary cortisol:creatinine ratios revealed that there were no differences in the HPA axis activity, ie in the stress status, between the litters housed in the three different housing systems in September and at the beginning of November. Furthermore, the other results, ie the growth of the cubs, the organ masses and plasma growth hormone level, indicated that the stress status of all animals was similar. However, the results also revealed that at pelting in January, the cubs in LIT had a higher plasma glucose level and a lower serum cortisol level than the cubs in SIN and LIT–SIN.

When an animal is confronted with a stressor, its sympatho-adrenal-medullary (SAM) system is activated and catecholamines, such as adrenaline, are released from the adrenal medulla into the blood within a few seconds. Adrenaline is associated with experiences of anxiety and fear (Matteri *et al* 2000), and it facilitates the fight or flight response of the animal by promoting, for example, gluconeogenesis and glycogenolysis (eg Matteri *et al* 2000; Greco & Stabenfeldt 2002), ie adrenaline increases blood glucose concentrations. Therefore, the present result suggests that the cubs in LIT, showing higher plasma glucose levels, possibly experienced more severe acute stress than the cubs in SIN and LIT–SIN. This increased stress level in LIT may be merely attributable to human handling, which preceded the blood sampling at pelting. The concept of acute stress sensitivity in LIT is supported by the result of the feeding test behaviour indicating that the LIT cubs were possibly more afraid of humans than the other cubs. However, Fernandez *et al* (1994) found a positive relationship between aggressive behaviour and plasma levels of glucose in domestic pigs. Therefore, the higher plasma glucose level in LIT may also be attributable to a higher incidence of aggressiveness in LIT than in SIN and LIT–SIN, as indicated by the number of animals being bitten and the number of wounds per animal.

The biological effects of activation of the SAM system are short in duration and, therefore, the SAM system has possibly no significant impact on an animal's long-term welfare (Broom & Johnson 1993; Moberg 2000); this was also seen in the present study. The ACTH administration test is based on the hypothesis that animals that have experienced more long-term stress exhibit higher adrenal cortex

enzyme activities and, therefore, they are better prepared to produce more cortisol following ACTH administration than animals that have experienced less stress (eg Fraser & Broom 1990; Broom & Johnson 1993). Therefore, in this light, the present results on the cortisol levels following ACTH administration indicate that the cubs in LIT, although being more sensitive to acute stress, had been experiencing less long-term stress than the animals in the other two groups. This explanation appears reasonable because the occurrence of stereotypic behaviour was significantly lower in LIT than in SIN and LIT–SIN, and the prevalence of stereotypic behaviour has been regarded as one of the most important long-term stress indicators (Broom and Johnson 1993). Therefore, it appears that social enrichment of the environment facilitated coping with stress-inducing situations in the LIT cubs (see Carlstead & Shepherdson 2000).

However, it is worthwhile noting that, despite the generally accepted view that stereotypic behaviour in animals derives from unsatisfactory living conditions (eg Fraser & Broom 1990; Broom & Johnson 1993), there is no unambiguous view of the relationship between stereotypies and physiological stress parameters (eg Ödberg 1989; Ladewig *et al* 1993). For example, there was no statistically significant positive (or negative) correlation between the level of stereotypic behaviour in December and serum cortisol level in any of the three experimental groups in the present study. Furthermore, the meaning of the higher serum cortisol level and the lower plasma glucose level in SIN and LIT–SIN requires critical assessment. The present results on the ACTH test suggest that the cubs in SIN and LIT–SIN were more stressed and more ready to secrete cortisol than the cubs in LIT. It is also general knowledge that the effects of glucocorticoids, like cortisol, on glucose levels are parallel to those of catecholamines, ie that both cortisol and adrenaline promote gluconeogenesis and there is a synergy in their hyperglycaemic activities (eg Matteri *et al* 2000; Greco & Stabenfeldt 2002). Accordingly, Barnett *et al* (1983) found elevated levels of plasma glucose in conjunction with elevated corticosteroid levels as a chronic stress response in gilts (juvenile female pigs) in an unpleasant handling treatment, and concluded that plasma glucose may be a useful addition to corticosteroid measurements in the assessment of chronic stress. However, why did the increased adrenal cortex activity in SIN and LIT–SIN not increase the plasma glucose levels in these groups? One would have anticipated that these cubs especially should have had higher glucose levels as a result of long-term secretion of cortisol (see Matteri *et al* 2000). One has to remember that the activity of the HPA axis, and its outcome as a measurable physiological stress response, is controlled by several mechanisms, for example, by down-regulation of hormone receptors (Greco & Stabenfeldt 2002; Heidemann 2002). One could hypothesise that, as a consequence of a repeated or long-term exposure to stress-induced ACTH, there may have occurred down-regulation of ACTH-receptors in the adrenal cortex in the LIT cubs. This would then indicate that the cubs in LIT were experiencing more long-term stress than the single housed

cubs. This hypothesis is supported by the greater sensitivity to acute stress, fear of humans and incidence of bite wounds in the LIT cubs than in the other cubs. Consequently it is recommended that future studies include more detailed analyses of the factors affecting the outcome of the HPA axis activity, for example by measuring both cortisol and ACTH and adrenaline levels or studying receptor down-regulation and hormone feed-back systems.

Conclusions and animal welfare implications

The results of this study reveal that social contacts are important for the welfare of silver fox cubs, as previously shown by Ahola *et al* (2002). Contrary to the previous hypothesis, based on experimental data and the knowledge of the species' behaviour in the wild (see Ahola 2002), separating the litters at the onset of the species' natural dispersal time may not be strictly beneficial for the cubs and may limit the animals' possibilities to fulfil their needs for social behaviour. However, the lower incidence of bite wounds in both the single housed cubs and the cubs from litters that were separated in autumn do show some beneficial effects of separating the litters (Ahola *et al* 2002). With regard to housing silver fox cubs in litters, the results show that the cubs that were group housed in litters were more bound to their own social system and experienced more aversion to human presence than cubs that were single housed for at least part of the time. Furthermore, the cubs housed in litters showed greater responses to acute stress and had more bite wounds than the other cubs. However, the serum cortisol level suggested that the cubs housed in litters were possibly experiencing less long-term stress than the cubs housed either singly or in a socially changing environment. The low incidence of stereotypic behaviour in the cubs housed in litters indicates the same. It may be that the presence of cage mates in litters *per se* overrode other negative effects of group housing, ie the increased incidence of bite wounds, sensitivity to acute stress and fear of humans. Furthermore, the GPI did not significantly decrease in litters with the advance of autumn in the present study, unlike the results of previous studies of farmed silver fox cubs (Ahola & Mononen 2002; Ahola *et al* 2002). Therefore, it appears that the cubs housed in litters somehow adapted, despite some problems, to their group housing system; this has also been observed in farmed mink, a species which is considered to be rather solitary (Mononen *et al* 2000; Hänninen *et al* 2002). The adaptation of farmed silver foxes to group living is not difficult to comprehend because red foxes in the wild may live in groups comprising several adult foxes in rich food environments (eg Macdonald 1983). Accordingly, and despite some unresolved questions on the interpretation of the results on the HPA axis activity, the present results imply that farmed silver fox cubs could possibly be raised also in litters without jeopardising their welfare or damaging their fur quality.

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References

- Ahola L** 2002 *Effects of Social and Physical Housing Environment on the Welfare in Silver Foxes (Vulpes vulpes)*. PhD Thesis, University of Kuopio. Kuopio University Publications C. Natural and Environmental Sciences 145
- Ahola L and Mononen J** 2002 Family break-up in farmed silver foxes (*Vulpes vulpes*) housed in enlarged cage systems as families. *Acta Ethology* 4: 125-127
- Ahola L, Harri M, Kasanen S, Mononen J and Pyykönen T** 2000 Effect of family housing of farmed silver foxes (*Vulpes vulpes*) in outdoor enclosures on some behavioural and physiological parameters. *Canadian Journal of Animal Science* 80: 427-434
- Ahola L, Harri M, Mononen J, Pyykönen T and Kasanen S** 2001 Welfare of farmed silver foxes (*Vulpes vulpes*) housed in sibling groups in large outdoor enclosures. *Canadian Journal of Animal Science* 81: 435-440
- Ahola L, Mononen J, Pyykönen T, Mohaibes M and Rekilä T** 2002 Effects of group size and space allocation on physiological, behavioural and production-related welfare parameters in farmed silver fox cubs. *Agricultural and Food Science in Finland* 11: 185-197
- Barnett JL, Hemsworth PH and Hand AM** 1983 Effects of chronic stress on some blood parameters in the pig. *Applied Animal Ethology* 9: 273-277
- Beerda B, Schilder MBH, Bernadina W, van Hooff JARAM, de Vries HW and Mol JA** 1999 Chronic stress in dogs subjected to social and spatial restriction. II. Hormonal and immunological responses. *Physiology & Behavior* 66: 243-254
- Broom D and Johnson KG** 1993 *Stress and Animal Welfare*. Chapman & Hall: London, UK
- Carlstead K and Shepherdson D** 2000 Alleviating stress in zoo animals with environmental enrichment. In: Moberg GP and Mench JA (eds) *The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare* pp 337-354. CABI Publishing: Wallingford, UK
- Cavallini P** 1996 Variation in the social system of the red fox. *Ethology, Ecology and Evolution* 8: 323-342
- Duncan ND, Williams DA and Lynch GS** 1998 Adaptations in rat skeletal muscle following long-term resistance exercise training. *European Journal of Applied Physiology* 77: 372-378
- European Convention** 1999 *Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes. Recommendation Concerning Fur Animals*. T-AP (96)19. Council of Europe, France. <http://www.mapya.es/ganaderia/pags/bienestar/pdf/recpeleteriaingles.pdf> (accessed 18 October 2005)
- Fernandez X, Meunier-Salaün MC and Mormede P** 1994 Agonistic behavior, plasma stress hormones, and metabolites in response to dyadic encounters in domestic pigs: interrelationships and effect of dominance status. *Physiology & Behaviour* 56: 841-847
- Fox MW** 1987 *Behaviour of Wolves, Dogs and Related Canids*. Robert E Krieger: Florida, USA
- Fraser AF and Broom DM** 1990 *Farm Animal Behaviour and Welfare*. Baillière Tindall: London, UK
- Gattermann R** 1990 *Verhaltensbiologisches Praktikum*. VEB Gustav Fisher Verlag: Jena, Germany [Title translation: *Practices for Behavioural Studies*]
- Greco D and Stabenfeldt GH** 2002 Endocrine glands and their function. In: Cunningham JG (ed) *Textbook of Veterinary Physiology* pp 341-372. WB Saunders Company: Philadelphia, USA
- Hänninen S, Mononen J, Harjunpää S, Ahola L, Pyykönen T, Mohaibes M and Sepponen J** 2002 The effects of family housing on welfare of juvenile farmed mink (*Mustela vison*). In: Koene P and the Scientific Committee of the 36th ISAE Congress (eds) *Proceedings of the 36th International Congress of the International Society for Applied Ethology* p 87. 6-10 August 2002. Egmond aan Zee, The Netherlands

- Harris S and Lloyd HG** 1991 Fox *Vulpes vulpes*. In: Corbet GB and Harris S (eds) *The Handbook of British Mammals* pp 351-367. Blackwell Scientific Publications: Oxford, UK
- Heidemann SR** 2002 The molecular and cellular bases of physiologic regulation. In: Cunningham JG (ed) *Textbook of Veterinary Physiology* pp 2-29. WB Saunders Company: Philadelphia, USA
- Hemsworth PH, Barnett JL and Campbell RG** 1996 A study of the relative aversiveness of a new daily injection procedure for pigs. *Applied Animal Behaviour Science* 49: 389-401
- Ladewig J, de Pasillé AM, Rushen J, Schouten W, Terlouw EMC and von Borell E** 1993 Stress and the physiological correlates of stereotypic behaviour. In: Lawrence AM, Rushen J (eds) *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare* pp 97-118. CAB International: Wallingford, UK
- Lloyd HG** 1975 The red fox in Britain. In: Fox MW (ed) *The Wild Canids, Their Systematics, Behavioural Ecology and Evolution* pp 207-215. Van Nostrand Reinhold: New York, USA
- Macdonald DW** 1983 The ecology of carnivore social behaviour. *Nature* 301: 379-384
- Martin P and Bateson P** 1993 *Measuring Behaviour: An Introductory Guide*. Cambridge University Press: Cambridge, UK
- Mason GJ** 1991 Stereotypies: a critical review. *Animal Behaviour* 41: 1015-1037
- Matteri RL, Carroll JA and Dyer CJ** 2000 Neuroendocrine responses to stress. In: Moberg GP and Mench JA (eds) *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare* pp 43-76. CABI Publishing: Wallingford, UK
- Moberg GP** 2000 Biological response to stress: implication for animal welfare. In: Moberg GP and Mench JA (eds) *The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare* pp 1-21. CABI Publishing: Wallingford, UK
- Mononen J, Kasanen S, Harjunpää S, Harri M, Pyykönen T and Ahola L** 2000 A family housing experiment in mink. *Scientifur* 24: 114-117
- Ödberg FO** 1989 Behavioural coping in stress conditions. In: Blanchard RJ, Brain PF, Blanchard CD and Parmigiani S (eds) *Ethoexperimental Approaches to the Study of Behaviour* pp 229-238. Kluwer Academic Publishers: Boston, USA
- Rekilä T, Ahola L, Mononen J and Harri M** 1998 Effect of the environment inside and outside the cage on the activity and behaviour test performance of silver foxes. *Agricultural and Food Science in Finland* 7: 13-19
- Rekilä T, Harri M, Jalkanen L and Mononen J** 1999 Relationship between hyponeophagia and adrenal cortex function in farmed foxes. *Physiology & Behaviour* 65: 779-783
- Rekilä T, Mononen J and Harri M** 1996 Effect of inside-cage and outside-cage environment on behaviour test performance of blue foxes (*Alopex lagopus*). *Acta Agriculturae Scandinavica, Section A, Animal Science* 46: 247-252
- Sandell M** 1989 The mating tactics and spacing patterns of solitary carnivores. In: Gittleman JL (ed) *Carnivore Behavior, Ecology, and Evolution* pp 164-182. Cornell University Press: New York, USA
- Siegel S and Castellan NJ** 1988 *Nonparametric Statistics for the Behavioural Sciences*. McGraw-Hill Book Company: New York, USA
- SPSS** 1999 *SPSS® Base 9.0 Applications Guide*. SPSS Inc: Chicago IL, USA
- von Schantz T** 1984 Non-breeders in the red fox *Vulpes vulpes*: a case of resource surplus. *Oikos* 42: 59-65
- Wikman I, Mononen J, Rekilä T and Harri M** 1999 Stereotyped behaviour in juvenile foxes. In: Bøe KE, Bakken M and Braastad BO (eds) *Proceedings of the 33rd International Congress of the International Society for Applied Ethology* p 109. 17-21 August 1999. Lillehammer, Norway