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Early transfer of mated females into the maternity unit reduces stress and increases maternal care in farm mink

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ABSTRACT

Mated mammals on farms are typically transferred to another housing environment prior to delivery. We investigated whether the timing of this transfer – EARLY (Day −36), INTERMEDIATE (Day −18), or LATE (Day −3) relative to the expected day of birth (Day 0) – affects maternal stress, maternal care and the early kit vitality in farmed mink. We hypothesized that early transfer is beneficial for mink mothers and their offspring in comparison to intermediate or late movement closer to delivery, being the current practice in the commercial production. We used 180 double mated female yearlings in three equally sized groups ($n = 60$): (i) 'EARLY', transfer to maternity unit immediately after the end of the mating period, March 23; (ii) 'INTERMEDIATE', transfer in the middle of the period, April 10; (iii) 'LATE', transfer late in the pregnancy period, April 25. Data collection included weekly determination of faecal cortisol metabolites (FCM) and evaluation of maternal care: nest building, in-nest temperature, plus kit-retrieval behaviour, kit mortality and growth day 0–7 postpartum. We document that mated mink females build and maintain a nest at least 1 month prior to delivery when transferred to an environment with free access to nest building material. During the weeks before delivery, INTERMEDIATE females had 50% higher FCM concentrations than the other two groups ($P = 0.002$), indicative of stress. After delivery, late moved females had, in average, 2.7 °C colder nests compared to early moved females ($P = 0.002$). Additionally, the mortality in group LATE tended to be higher ($P = 0.085$) in affected litters ($N = 92$). Kits from early transferred females displayed less vocalization (17% vs. 40–41% in the two other groups, $P = 0.015$), when tested away from the nest. This indicates enhanced offspring vitality from early moved females. In conclusion, transfer into the maternity unit early after mating, rather than later during the pregnancy period, reduces stress and increases maternal care in farm mink.

1. Introduction

The period after mating and during gestation is regarded as particularly sensitive for stress in the mammalian female (e.g. reviews by Liptrap, 1993; Mulder et al., 2002; Parker and Douglas, 2010; Weinstock, 2001). Therefore, mated females on farms are typically transferred from one housing environment to another – more suitable birth environment – at some point prior to delivery. Likewise, in mink production, females are moved from the mating compartment into a cleaned cage with additional nest building material and nest boxes prepared for delivery. In practice, the timing of transfer varies considerably between

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mink farms, thus from c. 36 to a few days before delivery. The timing of transfer may influence the stress response, the birth process and the maternal behaviour as reported in pregnant laboratory rats (Leng et al., 1987, 1988) and in production sows (Lawrence et al., 1992; Pedersen and Jensen, 2008). However, this has not previously been studied in mink.

The American mink (*Neovison vison*), farmed for the production of fur, has one yearly reproductive season. On average farms, litters of five to six kits per female at weaning are reported, although larger litters are present at birth, based on video recordings of deliveries (Malmkvist et al., 2007). The early period around delivery is critical for survival of mink kits. Birth problems contribute to offspring mortality, and females in intermediate body condition (as opposed to being thin or fat) have quicker deliveries and improved offspring survival, including fewer stillbirths (Malmkvist et al., 2007). Mink are considered altricial as they are born relatively underdeveloped; for example the onset of eye-opening and first signs of hearing begins after 28 days of life (Brandt et al., 2013). In addition, their thermoregulatory and motor abilities are poorly developed during the first weeks of life (Rouvinen-Watt and Harri, 2001; Harjunpää and Rouvinen-Watt, 2004), making younger kits prone to hypothermia when away from the warm nest. Consequently, maternal care – including nest building, nursing, and protection – is crucial for the offspring during the first four to six weeks of life. In line with these results, the importance of giving the dam access to suitable nesting material prior to delivery has been documented. If the mink dam is given the opportunity to nest-build before delivery, this will result in a larger nest, reduced dam stress and fewer birth problems, in combination with increased maternal behaviour and offspring survival during the first week after delivery (Malmkvist and Palme, 2008).

The onset of maternal nest building in mink may occur several weeks before delivery, as indicated in a study reporting lower temperatures in nests of unmated than in nests of mated females already three to four weeks before delivery (Malmkvist and Lund, 2009). Thus movement to the maternity unit three to four weeks prior to delivery could be favourable. However, disturbances around implantation may increase the risk of embryonic loss, reducing the number of kits born. The fertilized eggs implant in the uterus between 16 and 24 days before delivery, concurrent with a peak in progesterone and blastula growth approximately 20 days before delivery in mink (Sundqvist et al., 1989; Stoufflet et al., 1989). Today, we lack knowledge of the optimal time of moving mated female mink to the whelping cage; optimal for both animal welfare and the reproductive output. Therefore, we investigated whether timing of movement before delivery – EARLY (Day −36), INTERMEDIATE (Day −18) or LATE (Day −3) relative to the expected day of birth (Day 0) – affects maternal stress, maternal care, and the early kit vitality (estimated by e.g. growth and calls). We hypothesized that early movement to an environment with extra nest building material is beneficial for mink mothers and their offspring, in comparison to intermediate or later movement closer to the time of delivery.

2. Materials and methods

2.1. Animals

We used 180 one-year-old female mink of a brown colour type, each individual mated twice with the same male. The mink were mated according to standard farm procedures, cf. description in Malmkvist et al. (1997), with a ratio of one male to five females. The experimental females were all mated for the first time between March 5 and 9 2012, and for the second time eight days later. The mink were exposed to natural lighting at the farm of Aarhus University, DK-8830 Tjele, Denmark. Breeding mink were housed in wire cages (from Hedensted-Gruppen, DK-8722 Hedensted, Denmark; W: 30 cm, H: 45 cm, L: 91 cm) connected to a wooden nest box with wire ceiling (W: 28 cm, H: 20 cm, L: 23 cm) with access to a layer of chopped barley straw on the top of each nest box. In addition, each cage was equipped with a shelf – one wire tube cylinder (l: 32 cm, diameter: 11 cm) fixed to the cage ceiling – in accordance to the Danish legislation (Ministry of Food, Agriculture and Fisheries of Denmark, 2006). Standard commercial wet feed (Holstebro Minkfodercentral, DK-7500 Holstebro, Denmark; Energy Density 122.7 kcal/g, ME: 50.1% protein, 39.9% carbohydrate, 10.0% fat) and water were available ad libitum.

2.2. Study design and treatment

The 180 mated females were randomly allocated – however with no sisters within each group and distributing half-sisters evenly with 4 females per group – to three equally sized treatment groups ($n = 60$) with different timing of transfer to whelping cages:

(i) 'EARLY', transfer to maternity unit early in the pregnancy period, early after the end of the mating period, March 23.
(ii) 'INTERMEDIATE', transfer to maternity unit in the middle of the pregnancy period, April 10.
(iii) 'LATE', transfer to maternity unit late in the pregnancy period, April 25.

Group EARLY was moved in average on Day −36, INTERMEDIATE on Day −18, and group LATE on Day −3 relative to the day of expected delivery (Day 0), calculated as 45 days after the date of the second mating (cf. study time line, Fig. 1). The timing of the transfer in relation to the actual day of delivery is illustrated in Fig. 2 for all treatment groups.

At the morning of transfer, mink were individually trapped (using wired trapping cages from Hedensted-Gruppen, DK-8722 Hedensted, Denmark) and moved by hand to the maternity unit, in a different shed – larger and more closed, i.e. offering more thermal and wind protection – at the farm, within a distance of 50–200 m. The treatment groups were evenly dispersed within this shed. The size of cages and nest box in the maternity unit was as previously described, but cages were cleaned and nest boxes were additionally prepared for delivery (i.e. lined with barley straw and nest-opening protected by a wind breaker), and the caged bottom equipped with a removable

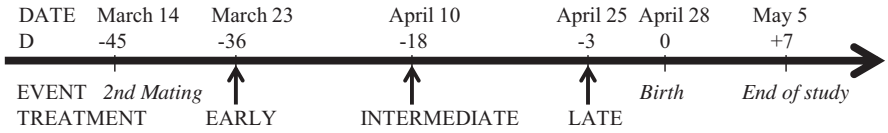

Fig. 1. Time line of the study, with average dates and D for average days relative to birth at Day 0. Treatment is the time for transfer and introduction of dams into the maternity unit. See Table 1 for an overview of the sample events during the study period until D +7.

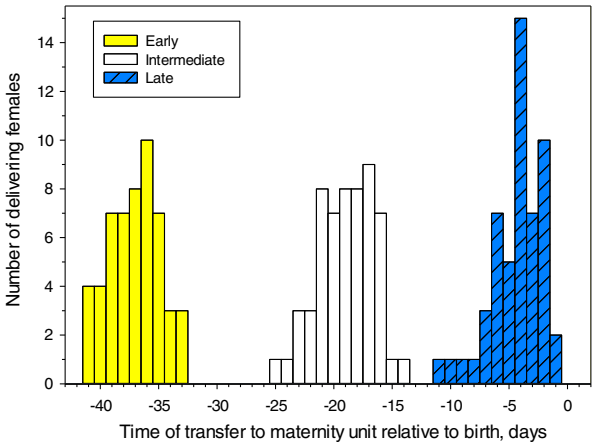

Fig. 2. Distribution of the delivering females ($N = 165$) in each of the three treatment groups, as number of females transferred per day relative to the day of birth. Group EARLY females were introduced in the maternity unit March 23, Group INTERMEDIATE April 10, and Group LATE April 25, 2012.

wire mesh floor insert with a finer mesh in the first two thirds of the cage to avoid straying kits to drop through cage mesh. Besides, every second cage was empty in the maternity unit – in accordance to the legislative requirements (Danish Ministry of Justice, 2006) – to reduce disturbance between mink/litters until delivery, and subsequently during the lactation period until weaning at eight weeks after delivery. The dams in the maternity unit had additional access to barley straw delivered in the cage every Tuesday and Friday.

Fourteen of the 180 females (7.8%) were barren, i.e. with no signs of kits delivered. These barren females are excluded from the data analysis, reducing the experimental mink to 166 dams (EARLY: $n = 54$; INTERMEDIATE: $n = 58$; LATE: $n = 54$). Transferring kits between litters, returning straying kits to the nest, and putting chilled kits in heat incubators (cf. Castella and Malmkvist, 2008) were not done in this study. Dead kits were removed from the females, and autopsied to determine whether they were stillborn or not, using lung flotation in water as indicative of being liveborn. One female from group LATE was excluded, because she delivered in the breeding cage on the day of planned transfer to the maternity unit. The rest (165 dams) was registered to deliver 1351 kits (mean ± SD litter size: 8.2 ± 2.17; min–max: 1–14), of which 216 (16.0%) were stillborn. One female delivered, but had no longer live kits Day 1 postpartum (EARLY: 1 dam). This dam and kits are included in the analysis until Day 1 postpartum (cf. Table 1 for sampling events), but not after this day. Seven kits from six different dams (EARLY: $n = 3$; INTERMEDIATE: $n = 1$; LATE: $n = 2$) were moved to foster dams outside the experiment, as they were found outside the cage, i.e. on the shed floor or in the manure collection tray; these kits are included in the data analysis until the day of movement, and afterwards counted as dead. Eight dams lost all their live kits before D7 (EARLY: $n = 2/53 = 3.8\%$, Intermediate: $n = 2/58 = 3.5\%$, LATE: $n = 4/54 = 7.4\%$), and therefore no data on growth are included for these litters.

2.3. Data collection

For the study time line see Fig. 1. For an overview of the type and timing of data collection see Table 1.

2.3.1. Faecal cortisol metabolites (FCM)

Faeces are the predominating excretory route of cortisol metabolites in mink, and FCM reflect concentrations of circulating cortisol with a time lag of approximately 4 h, as validated in female mink (Malmkvist et al., 2011) and previously measured around parturition (Malmkvist and Palme, 2008). We collected a fresh sample of female faeces from wire nets placed under the cage defecation zone. The collection took place 0–5 h after feeding, on (i) the day before (except for group EARLY), (ii) 6 days after transfer to the maternity unit, and again (iii) May 1st for females not having delivered at this time, and (iv) on Day 3 postpartum (cf. sampling dates in Table 1). The weighed samples (0.50 g) were frozen immediately and stored at −20 °C until analysis. The faecal sample was extracted with 5 ml (80%) methanol (Palme et al., 2013) and FCM measured in an aliquot of the supernatant with an 11ß-hydroxyaetiocholanolone enzyme immunoassay (EIA; Malmkvist et al., 2011). The sensitivity of this method was 6 ng/g, with intra- and inter-assay coefficients of variations of 9.1 and 13.1%, respectively.

2.3.2. Female body condition score

The body condition of each dam was scored March 28, April 11 and seven days postpartum, as 1: very thin, 2: thin, 3: medium, 4: fat, and 5: very fat; similar to the scoring system used for females at commercial mink farms (cf. Bækgaard et al., 2008).

2.3.3. Evaluation of nests and in-nest climate

The result of nest building activity was scored weekly before and once after delivery (Table 1) as (0) no sign of substrate manipulation/no hollowing in the nest bottom layer, (1) minor hollowing with up to 5 cm sidewalls in the nest bottom layer, (2) distinct hollowing with more than 5 cm sidewalls, without any top layer, (3) sidewalls and top layer present, but the nest is not completely closed, (4) the nest with sidewalls and ceiling is completely closed.

Table 1
Sample events for females and litters during the experimental period from after mating to seven days after the birth. The number (n) is specified when not all experimental females were sampled. D: Day relative to delivery (D0).

Time of sampling	Sampling event	Comment
March 29, April 9, 16, 24	Faecal cortisol metabolites	Non-invasive faecal sample.
March 28, April 11, D7	Dam body condition	Score 1–4
April 20 – D7	Climate in nest	Temperature, humidity every 15 min (n = 30)
Once weekly March 28–May 2, D2	Evaluation of nest building	Score 0–4
D0	Time of litter birth	Positive indications of young
D1, D7	Body weight, sex of kits	
D3	Faecal cortisol metabolites	Non-invasive faecal sample
D0–D7	Collection of dead kits	Autopsy, test for being stillborn or not
D5	Kit-retrieval test	Testing maternal reactivity towards own kit

The in-nest climate was measured in a subset of the nests (randomly selected n = 15 EARLY dams, n = 15 LATE dams) from April 20 to seven days postpartum, using thermologgers (model tx120 from Dickson Calibration Services, IL-60101, USA) fixed 5 cm above the nest bottom floor on the sidewall opposite the nest box opening, equidistant from the nest box walls. The loggers registered temperature and relative humidity every 15 min. One additional logger was placed in the nest box of an empty cage to register the climate of the surroundings during the experimental period.

2.3.4. Reproduction, kit mortality and growth

During the period April 24–May 10, all cage units were checked three times daily (at hours 9–10, 15–16, and 19–20) to register time of litter birth (Day 0) and to collect dead kits. The following day (i.e. between 12 and 24 h later) and again on D7, kits were counted, sexed and group-weighed per sex within the litter to the nearest 0.10 g.

The total number of born is defined as all kits delivered (being alive or dead), and mortality is the proportion between the number of kits dead and the total number born in that litter. Dead kits collected within the first seven postpartum days were weighed, sexed, and categorized as stillborn in case of negative lung-floating test.

2.3.5. Maternal kit-retrieval on Day 5 postpartum

The kit-retrieval test is a measure of maternal reactivity towards a 5-day-old progeny placed outside the nest (Malmkvist and Houbak, 2000). The observer randomly selected one kit with the desired sex – alternating between male and female – from the litter, weighing it to the nearest 0.10 g, and after restricting the dam in the nest box, placed the kit in the middle of the wire cage, with its head directed towards the nest box entrance. The test started when the female regained access to the wire cage and stopped when she retrieved the kit back into the nest box. The observer, blind to the treatments, registered latency to touch, retrieve kit back into nest to nearest s, and dam/kit vocalization as one-zero registration. In case of no kit retrieval within 240 s, the test stopped and the observer returned the test kit into the nest.

2.4. Statistical analysis

We used the software SAS (version 9.2, Statistical Analysis Systems Institute, Cary, NC) for calculation. A probability level (P) of 0.05 was chosen as the limit of statistical significance, and only two tailed tests were used. P-values between 0.05 and 0.10 are reported as tendencies, and models were reduced by stepwise removing insignificant terms ($P > 0.10$) starting with the highest order of interactions, however, keeping as minimum the principal treatment (group EARLY, INTERMEDIATE, LATE) in the model. Time structure was modelled using compound symmetry in ANOVA models with repeated measures. The demand for dispersion and variance homogeneity was evaluated from plots of the final model residuals. Results are reported as mean ± standard error of mean, unless otherwise stated.

FCM concentrations were analyzed using a normal model with repeated measures for dams over the weekly sampling times (procedure 'mixed' in SAS) including the treatment and the interaction between treatment and sampling week. Logarithmic transformation was used as it resulted in better residuals in terms of normal distribution and variance homogeneity. There was a significant interaction between the treatment and whether the females had delivered or not ($F_{2,159} = 15.8$, $P < 0.001$), and the data subsequently analyzed (i) before delivery (sampled: March 29, April 9, 16, and 24) and (ii) after delivery (sampled Day 3 postpartum), with the number of kits born per dam included as covariate in the initial model of postpartum FCM.

For the dam body condition score, the repeated measure analysis (generalized mixed linear model using the procedure 'glimmix' in SAS for Possion distributed data with dam as random factor) did not converge. Therefore, this variable was analyzed per sampling week, using the procedure 'genmod' in SAS for Poisson distributed data.

The nest score developed differently for the groups over time (Fig. 4; treatment * sampling date interaction: $P < 0.001$). Consequently, the nest score was analyzed for each sampling time, with the number of kits born and the mortality of liveborn kits day 0–7 included as covariates.

The in-nest climate data consisted of measurement of temperature and relative humidity every 15 min. The mean, minimum, maximum, and range per 24 h were calculated from the raw data for each nest and used as response variables in the further analysis. For brevity, only statistical results on mean temperature and mean relative humidity are presented; the other calculated variables correlate with those means, and they added nothing additional to the results. Besides, the error bars in Fig. 4 illustrate the distribution around the mean values. A few 24 h periods

Table 2
Influence of different timing of transfer to the maternity unit (EARLY, INTERMEDIATE, LATE) on dam and litter. Data on faecal cortisol metabolites (FCM), reproductive output, within-litter sex-ratio, kit weight/growth, in-nest climate given as mean (S.E.). Latency to retrieve kits given as median [25%; 75% quartiles] for retrieving dams only, and vocalizing kits as the proportion of tested kits. D indicates days relative to the day of birth (Day 0).

	EARLY	INTERMEDIATE	LATE	Test statistics	*P*
Pre-delivery FCM (ng/g)	40.5 (5.62)[a]	59.9 (5.33)[b]	43.0 (5.60)[a]	$F_{2,489} = 6.5$[c]	0.002
Post-delivery (D3) FCM (ng/g)	76.4 (14.15)	47.5 (13.76)	75.3 (14.15)	$F_{2,159} = 2.9$	0.054
Gestation duration (days)	45.1 (0.28)	45.1 (0.27)	45.4 (0.28)	$F_{2,162} = 0.4$	0.68
Number of kits per litter	8.4 (0.30)	7.9 (0.29)	8.3 (0.30)	$F_{2,162} = 0.9$	0.39
Stillborn (%)	15.9 (2.96)	16.7 (2.83)	15.2 (2.93)	$F_{2,162} = 0.1$	0.93
Mortality (D0–D7) of live-born in affected litters[d]	28.9 (4.91)[a]	28.5 (4.83)[a]	42.7 (5.13)[b]	$F_{2,73} = 2.6$	0.085
% males among stillborn	42.7 (7.86)[a]	43.7 (7.72)[a]	71.5 (8.84)[b]	$F_{2,67} = 3.7$	0.031
Among live kits on D1	50.2 (7.62)[a]	40.4 (7.58)[b]	50.2 (7.25)[a]	$F_{2,159} = 3.7$	0.027
Among live kits on D7	54.0 (3.24)	49.1 (3.07)	53.9 (3.24)	$F_{2,151} = 0.8$	0.45
Kit weight (g) on D1	11.2 (0.25)	11.1 (0.24)	11.5 (0.25)	$F_{2,159} = 1.0$	0.38
Kit growth (g) D1–D7	20.9 (0.66)	20.0 (0.63)	20.3 (0.66)	$F_{2,151} = 0.5$	0.62
In-nest temperature (°C) D0–D7	23.4 (0.54)[a]	–	20.7 (0.52)[b]	$F_{1,25} = 12.7$	0.002
Latency (sec) to retrieve kit on D5	33 [19; 51]	24 [17; 36]	27.5 [19; 55]	Surv$\chi^2 = 2.1$	0.34
Proportion (%) of not retrieved kits	2.1	3.9	6.7		
Vocalizing kits (%) as tested on D5	16.7[a]	41.2[b]	40.0[b]	$\chi^2 = 8.2$, $df = 2$	0.015

[a,b] Different letters indicate significant difference ($P < 0.05$) between treatments.
[c] Interaction between treatment group and time, see Fig. 3 and text for details.
[d] Excluding the litters with no mortality among live-born kits, reducing the number of litters to 92.

were discarded from individuals in this data set, in case the logger was displaced from the nest position; data were excluded until 3 h after the logger was fixed again to allow for habituation. Only data from delivering mink were used, reducing the number of nest registrations to 13 for group EARLY and 14 for group LATE. Based upon graphical representation of data (Fig. 4), we divided the data into two periods, (1) the pre-delivery period after transfer (h 0:00 April 26–h 23:59 Day −1 relative to delivery) and (2) the post-delivery period (h 0:00 Day 0–h 23:59 postpartum Day 7), with repeated measure per nest. The number of kits born and the mortality of liveborn kits until Day 7 were included as covariates.

The proportion of barren females and the proportion of litters without dead liveborn kits were Chi-square tested for difference between treatment groups. The other reproductive variables (cf. Table 2) were analyzed in normal ANOVA with treatment as the main explanatory factor. The gestation duration in days was calculated as the date of delivery minus the date of second mating for each individual dam. The number of kits was defined as the maximum number, living or death, registered per litter. The proportion of stillborn was calculated as the number of kits with negative lung-flotation test divided with the number of kits per litter. Due to the distribution of the full data set – with about half of the litters having no loss of live kits – the original statistical model on offspring mortality did not fit well, even after transformations. Consequently, the statistical analyses were performed on (1) the proportion of litters without and with dead liveborn kits (Chi-square test, all dams), (2) the mortality among liveborn until Day 7, for litters affected by mortality only ($n = 92$; reported in Table 2). The offspring sex ratio was calculated as the number of males out of the total number of sexed kits per litter on the day of measurement (Day 1 and Day 7 postpartum), for liveborn and stillborn separately. The initial models of kit weight and growth, the proportion of stillborn, and kit mortality additionally included the dam gestation length, body condition score April 11, the prepartum change in body condition score, the in-litter sex ratio, and number of kits born as covariates.

In the kit-retrieval test, latencies for the dam to touch, retrieve the kit and for the onset of kit vocalization were analyzed with methods for survival analysis, considering censored data (Allison, 1995; Klein and Moeschberger, 2003), i.e. no dam reaction within the test time of 240 s was taken as right censored. A cox proportional hazard model was used to test whether latency to react differed between treatment groups. The model additionally included the test kit body weight as covariate and the sex as explanatory class variable. For brevity, results on latency for the dam touching kit are not presented, as highly correlated with retrieval, adding nothing additional to the results.

3. Results

3.1. Faecal cortisol metabolites (FCM)

The female delivery status influenced FCM concentrations differently in the treatment groups (group × delivery status interaction; $F_{2,159} = 15.8$, $P < 0.001$). FCM levels increased after delivery in two of the treatment groups (EARLY: 36.6 ± 6.43 vs. 92.4 ± 15.20 ng/g; post test $P < 0.001$; LATE: 41.0 ± 6.41 vs. 83.6 ± 15.27 ng/g; post test $P = 0.017$), but decreased for group INTERMEDIATE females (59.8 ± 6.09 vs. 47.3 ± 14.60; post test $P = 0.037$). In the pre-delivery period, group INTERMEDIATE had an overall higher FCM concentration than group EARLY or LATE, whereas FCM at Day 3 postpartum only tended to differ between treatments (Table 2).

The timing of transfer influenced the development in FCM (Fig. 3), with exception of the first sampling week March 29 (post test $P > 0.80$) and the last sampling Day 3 postpartum ($P = 0.054$). On April 9, group EARLY had

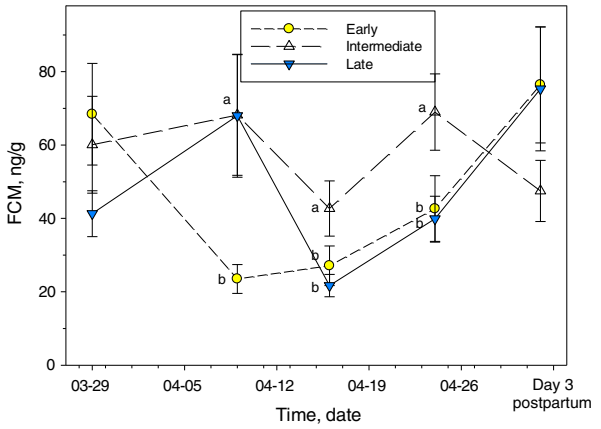

Fig. 3. Changes in faecal cortisol metabolites (FCM) concentration (mean ± SE; ng/g) after mating (sampled weekly 29 March–24 April) and at Day 3 after delivery. Different letters (a, b) indicate significant difference ($P < 0.05$) between treatments within each sampling time. See text and Table 2 for further details.

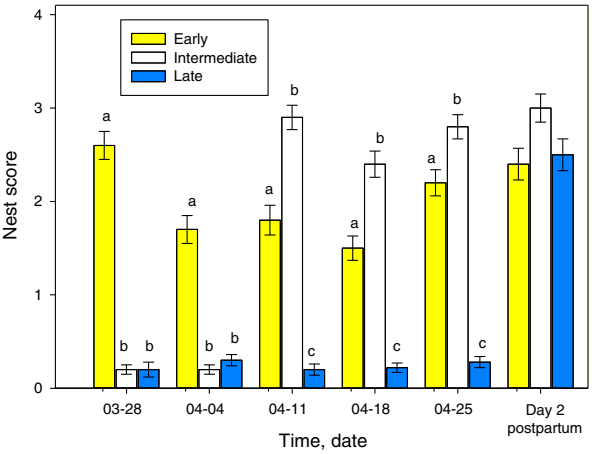

Fig. 4. Nest building (mean ± SE) scored weekly from March 28 to April 25, and on Day 2 after delivery for the three treatment groups: EARLY, INTERMEDIATE, LATE introduction of the dam to maternity unit. Scores are 0: no signs of substrate manipulation, 1: hollowing in nest bottom layer with up to 5 cm sidewalls, 2: distinct hollowing with more than 5 cm sidewalls without any top layer, 3: sidewalls and top layer present, but the nest is not completely closed, 4: completely closed nest. Different letters (a, b, c) indicate significant difference ($P < 0.05$) between treatments within each sampling time.

lower FCM than the two not transferred groups (post test $P < 0.003$). This indicates that the maternity unit is a more favourable environment around this period. In the rest of the experimental period (April 16 to Day 3 postpartum), however, group EARLY and LATE (not transferred until April 25) females had the same FCM concentration, whereas group INTERMEDIATE differed with higher concentrations in each sampling up to delivery (post test $P < 0.001$).

To investigate the link between FCM and kit number/survival, we performed correlations between the number of kits born, the mortality until Day 7 and the FCM concentration (i) last sample day before delivery (April 24) and (ii) D3. There was a negative correlation between the number of kits born and the concentration of FCM at Day 3 postpartum (Pearson correlation coefficient $r = -0.21$; $P = 0.007$, $N = 162$). Thus, FCM after delivery were generally higher in dams with fewer kits, ranging from 1 to 14 kits per litter.

3.2. Female body condition score (BCS)

The median BCS was 3 [3; 4] at the three sampling times; only two females scored 5 in the pre-parturient period, and no females were thin (i.e. score 1–2). There was no treatment difference in female BCS, evaluated just after the mating, March 28 ($ChiSq_{2,163} = 0.4$, $P = 0.84$), just after implantation period, April 11 ($ChiSq_{2,163} = 1.1$, $P = 0.59$), and 7 days after delivery ($ChiSq_{2,163} = 0.0$, $P = 0.99$).

3.3. Nest score and in-nest climate

The majority of dams nested within the nest box (98.8%), and only two females had a nest in the wire cage at Day 2 after delivery. Fig. 4 illustrates development in the nest scores for the three treatment groups. Group EARLY dams maintained a relatively constant nest score throughout the study period. The females moved April 10 (group INTERMEDIATE) managed to build even more elaborate nests ($F_{2,162} = 10.8$, $P < 0.001$) already the day after transfer to the maternity unit and for the rest of the period until delivery (post tests $P < 0.001$). On Day 2 postpartum there was no longer statistical difference in nest scores between the treatment groups ($F_{2,162} = 2.0$, $P = 0.14$; Fig. 4). At the last date of nest scoring (April 25) before delivery there was a positive link between the nest score and the number of kits born ($F_{2,161} = 4.0$, $P = 0.047$); based on model estimates, one additional kit was born per 0.03 unit increase in the average nest score.

Time in relation to delivery affected both the in-nest temperature and the humidity (Fig. 5). Additionally, EARLY moved females had in average 2.7 °C warmer nests Days 0–7 (23.4 ± 0.54 vs. LATE: 20.7 ± 0.52 °C, $F_{1,25} = 12.7$, $P = 0.002$), whereas the average relative humidity (RH) per 24 h did not differ between the two groups (EARLY: 62.8 ± 2.78 vs. LATE: 63.3 ± 2.68%, $F_{1,25} = 0.0$, $P = 0.91$).

There was no visible difference between the quality of the nests equipped with in-nest thermologgers, scored Day 2 (Median nest score, EARLY: 3, LATE: 3, $F_{4,21} = 0.5$, $P = 0.75$). However, the 24 h temperature at Day 2 was significantly ($F_{1,25} = 6.7$, $P = 0.016$) higher in EARLY (23.7 ± 0.67 °C) than in LATE (21.3 ± 0.64 °C) females; this can reflect a better thermal quality of nest – not evident in the nest scoring system used. The number of live kits did not differ significantly between the nests with climate measurement ($F_{1,25} = 1.9$, $P = 0.18$). In addition, both the nest building score and the number of liveborn kits were insignificant ($P > 0.75$) in statistical models of in-nest temperature on Day 2, affected only by whether the female was moved EARLY or LATE prior to delivery.

There was a significant correlation between the mortality of liveborn kits Day 0–7 and the minimum in-nest temperature measured Day 2 (Pearson correlation coefficient $r = -0.41$, $P = 0.034$, $n = 27$); thus the highest early kit mortality is linked to the lowest in-nest temperature. The

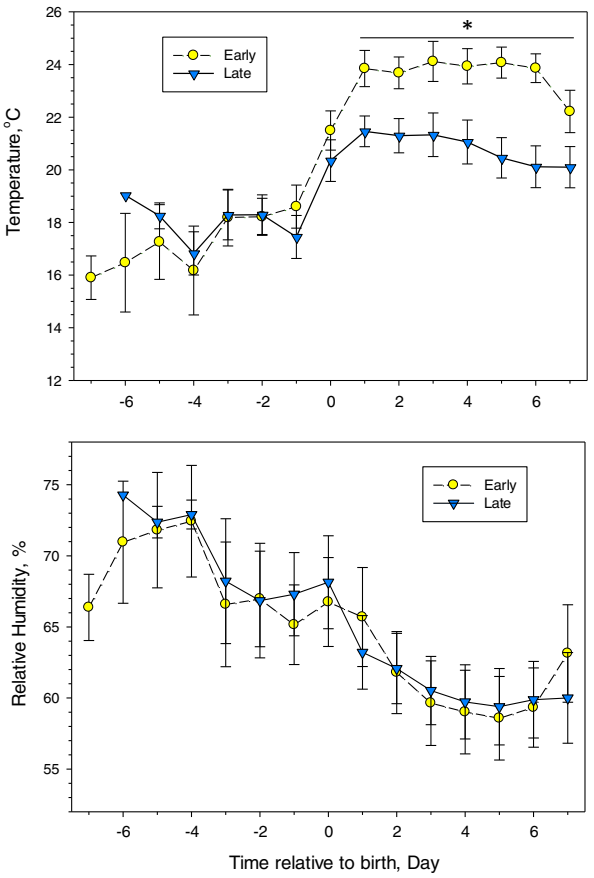

Fig. 5. In-nest (a) temperature and (b) relative humidity (mean ± SE per 24 h) in relation to time of birth Day 0, measured for group EARLY ($n = 13$) and group LATE ($n = 14$). After delivery, group EARLY had higher temperature ($P = 0.002$) in the nest with no treatment effect on the relative humidity ($P = 0.91$).

in-nest temperature was uncorrelated with the temperature of the surroundings (24 h mean temperature measured in the nest of an empty cage: $r = -0.06$, $P = 0.76$; minimum temperature $r = 0.02$, $P = 0.92$) within the temperature range of the study period: 5.5–22.5 °C.

3.4. Reproduction and kit mortality

The proportion of barren females was not significantly different between treatments (EARLY: $n = 6$; INTERMEDIATE: $n = 2$, LATE: $n = 6$; $\chi^2 = 2.5$, $df = 2$, $P = 0.29$). For females delivering (dams), timing of transfer did not affect the duration of gestation (Table 2; range 40–51 days after the second mating), nor the number of kits born in total (Table 2; range EARLY: 4–12, INTERMEDIATE: 1–14, LATE: 2–13).

The mortality among liveborn until D7 was 14.5 (3.41)% for EARLY, 13.9 (3.36)% for INTERMEDIATE and 18.0 (3.48)% for LATE transferred dams. The treatment tended to influence the mortality among liveborn until D7 for litters affected by mortality ($n = 92$; Table 2). The proportion of litters without and with dead liveborn kits was not significantly different between the treatment groups (EARLY: 50.9%, INTERMEDIATE: 49.1%, LATE: 55.6% of litters with no liveborn mortality, $\chi^2 = 0.5$, $df = 2$, $P = 0.79$). Eight dams lost all their live kits before D7 (EARLY: $n = 2/53 = 3.8\%$, INTERMEDIATE: $n = 2/58 = 3.5\%$, LATE: $n = 4/54 = 7.4\%$).

The experimental treatment affected the sex ratio of kits at different times; there were a higher proportion of males among stillborn kits collected from the LATE dams, and in live kits D1 the sex ratio was favoured towards females in the INTERMEDIATE dams (Table 2). Besides, the proportion of males increased with the number of kits born ($F_{1,159} = 19.1$, $P < 0.001$). There was a tendency ($F_{1,159} = 2.4$, $P = 0.092$) that females in an intermediate body condition (score 3 prior to delivery; $n = 61$) had more male kits (50.6%) vs. females being fatter (score 4: 42.8%, post-test $P = 0.029$; $n = 103$). Further pairwise comparisons between females in different body condition categories are infeasible as only two females scored 5 in pre-parturient body condition, and no experimental females were thin (score 1–2).

3.5. Kit weight and growth Day 1–7

Live male kits were marginally heavier (in average 3.6%) than female kits at D1 (male range: 6.5–19.5 g, female range: 6.0–16.0 g), but as the within-litter sex ratio was insignificant for kit weights ($F_{1,158} = 0.4$, $P = 0.53$), a common average for males and females is presented in Table 2. The D1 kit weight was lower with increasing number of kits born ($F_{1,159} = 26.8$, $P < 0.001$) and increased slightly with the duration of gestation ($F_{1,159} = 4.8$, $P = 0.030$). Based upon model estimates, the average kit weight is reduced with 0.35 g per extra kit born in the litter and increased with 0.16 g per extra day of gestation within the range of kits born (1–14) and gestation duration (40–51 days) in the current study.

The early growth is high in mink offspring (Table 2); the first week of life, kits grew in average to 180 (S.E. 3.4) % of their initial weight. In our study – weighing kits of the same sex together D1 and D7 – the growth of male and female kits did not differ, and the within litter sex-ratio did not affect the weight increase ($F_{1,150} = 1.5$, $P = 0.22$). The increase in kit weight was positively correlated with the D1 kit weight ($F_{1,151} = 70.5$, $P < 0.001$), with no effect of treatment (Table 2).

3.6. Maternal kit-retrieval Day 5

Female kits were retrieved earlier than male test kits (survival analysis, Surv $\chi^2 = 3.8$, $P = 0.050$), with no difference between the treatment groups (Table 2). The different latency to retrieval is linked to sex and not body weight; the body weight of tested kits (D5) did not differ (male 23.5 (0.66) g vs. female 23.4 (0.68) g; $F_{1,142} = 0.0$, $P = 0.99$). There was no sex difference in the proportion of kits vocalizing during the test (male: 48.6% vs. female: 34.8%, $\chi^2 = 2.3$, $df = 1$, $P = 0.13$). It should be noted that with longer latency to retrieval, the male test kits had longer test duration for calls to be registered. This result does not support that the reason for quicker retrieval of female offspring is due to them having a higher amount of calls. Considering the censored data (58.7% of kits did not vocalize during the test on D5), i.e. taking the test time into account, reveals no sex

difference in the latency or estimated probability for kits to vocalize (Surv $\chi^2 = 1.7$, $df = 1$, $P = 0.20$).

4. Discussion

Mink females moved early to the maternity unit (in average 36 days before delivery) had warmer nests during the first postnatal week than females moved later during pregnancy. The warmer nests of early introduced females could be a result of both (1) improved maternal nest building and (2) the dams staying more with the kits inside the nest box. From the logger data only we cannot distinguish between these two explanations. Nevertheless, the higher degree of maternal care induced by early introduction – whether caused by the dam building denser nests or the dam staying more inside the nest – results in a better protection against hypothermia, being considered as one death cause for young mink kits. The kit vitality was also higher in this group, supported by the markedly less vocalization in kits of the early introduced dams. In addition, the proportion of liveborn kits dying tended to be higher in dams moved late in the pregnancy period. Thus, the results point at an early introduction to the maternity unit as beneficial to mink.

The weekly scoring of nests demonstrated that mated mink females built and maintained a nest at least one month prior to delivery when given free access to nesting material as in the maternity unit. This period of nest building is longer than reported in e.g. production sows; sows begin to build a nest on the last day of gestation, with intensified activity around 8 h before delivery (Thodberg et al., 1999; Malmkvist et al., 2009). In rabbits, nest-building begins in late pregnancy, initiated approximately four days before delivery by digging (Day 25–27 of gestation) followed by straw-carrying and hair-pulling (reviewed in González-Mariscal et al., 2007). In contrast to sows and rabbits, female mice may build maternal nests already four days after mating (reviewed in Weber and Olsson, 2008), i.e. approximately 16 days before expected delivery. Thus, the relatively long period of maternal nest building reported in mink is parallel to findings in another altricial mammal; the mice.

Intermediate introduction, transfer in average 18 days before expected delivery – resulted in nearly 50% higher concentration of glucocorticoid metabolites in faeces (FCM) during the weeks prior to delivery, in comparison to groups of dams transferred either early or late. FCM were measured as a parameter of overall adrenocortical activity (Palme, 2012). The function of cortisol in circulation is to mobilize energy from bodily reserves (Mormede et al., 2007). It can be hypothesized that a high number of kits in uterus would increase the energetic burden and thereby also increase cortisol concentrations in the dam. However, the elevated concentration of cortisol in intermediate transferred females cannot be explained by a higher reproductive output – on the contrary. Firstly, the intermediate transferred dams had a relatively low average litter size at birth compared to the other two groups. Secondly, concentrations of baseline FCM in the dams correlated *negatively* with the number of liveborn mink kits. Therefore, we interpret the higher FCM concentration as indicative of elevated levels of stress, with transportation to the new housing environment around 18 days before expected delivery being more aversive. The timing of this transfer coincides with the time of implantation, peak levels of progesterone, and the period of early blastula growth (Stoufflet et al., 1989). Based on our results, transfer of mated mink around the implantation period should be avoided as linked with increased stress responses.

The effect of timing of transfer on the sex ratio of stillborn (more male stillbirths in litters of dams transferred close to delivery) as well as in liveborn (fewer males born in dams transferred 18 days before expected delivery), points at a sex-dependent difference in the prenatal sensitivity for stressors – with males being the sensitive sex harvested. Further studies are needed to fully understand the causality, why male kits appear more sensitive to transportation stress during the periods around implantation and late in gestation. Generally, it has been suggested that the sex growing fastest in uterus will be the most susceptible to mortality when maternal condition is compromised (Forchhammer, 2000); the growth spurts of male kits postpartum is well known, however, we are not familiar with studies of sex difference in mink foetal growth.

Intermediate moved females built a more elaborate nest, already within 24 h after their introduction to the maternity unit with additional nesting material. This could be indicative of rebound behaviour – observed when animals are highly motivated and after a period get the possibility to perform a previous thwarted task (Mills et al., 2010). Whether mink dams in general display rebound nest-building behaviour after being given additional nesting materials is open for further investigation; our selected timing for scoring nests was less suited to evaluate rebound effects in the dams introduced early or late into the maternity unit. However, the conclusion remains that mated mink dams can build an elaborate nest relatively quickly (within one day) when given free access to nesting material.

We used first year's females in our study to optimize the effective group size/statistical power, and because the majority – typically more than 60% – used for breeding at farms belong to this age group. Besides, all commercial breeding dams have been first year's breeders, as non-breeders are culled before the following breeding season. It is unknown whether the findings on one-year old can be generalized to older females.

5. Conclusion

Overall, transfer to the maternity unit early after mating (around Day −36), rather than later during pregnancy (Day −18/Day −3 relative to expected birth), reduces pre-delivery stress and increases maternal care in farm mink dams. It may be beneficial for farmers to move dams early after mating rather than later during the gestation period – based on results obtained on first years' females only.

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