

Prolonged nest building increase the reproductive outcome in American female mink



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ABSTRACT

The timing of the onset of maternal-related nest building in American mink is unknown, and it is expected that farmed mink are highly motivated to perform nest building during times with restricted access to nesting material on mink farms. Not fulfilled motivations due to thwarting can lead to stress response with increased HPA-axis output and performance of abnormal behaviour. We aimed to investigate when maternal nest building have onset and to test how prolonged access to nesting material influences dams stress response and reproductive outcome. We set up two groups that differed in timing of allocation of nesting material in the cage: group ALWAYS from 15 January and group REGULAR from 23 March. On 16 January after allocation of nesting material to ALWAYS, the mink built advanced nests, and their nest scores ($P < 0.001$) and nest temperature ($P < 0.001$) stayed significantly higher through to 23 March. When both groups had access to nesting material, from 23 March through birth, no difference was found in nest score ($P = 0.28$) and temperature ($P = 0.27$). ALWAYS mink females had better reproduction outcome: offspring survival ($P = 0.007$) and litter size (in average +1.2 young) Day 7 after birth ($P = 0.021$). Additionally, access to nesting material during winter/early spring (group ALWAYS) tended to reduce females' faecal cortisol metabolite concentrations (FCM; $P = 0.075$) and the performance of active behaviour including stereotypic behaviour ($P = 0.008$). After mating, maternal nest building was documented as the nest scores increased through the period 24 March to Day 2 after birth. During this period, when both groups had equal access to nesting material, no differences was found in FCM, stereotypic and active behaviour, nest score and nest climate. In conclusion, the current study demonstrated for the first time that adult mink females are motivated for nest building prior to mating, during winter/early spring, thus it is not only a maternal behaviour. Further, prolonged access to nesting material in the cage had a stress-reducing effect and a positive long-term effect on the reproductive outcome in farmed mink.

1. Introduction

In captivity, farm mink (*Neovison vison*) may experience periods where they cannot perform behavioural patterns they are motivated for. Such periods can elicit acute and chronic stress (Jensen and Toates, 1997) and thus decrease welfare. In the current study, we investigated the period where the mink females are expected to develop motivation for performing maternal-related nest building. It is well-known that mated mink females are highly motivated to perform nest building behaviour during most of the gestation period from shortly after second mating through to after birth (Day -36 to Day 7; Day of birth = 0; Malmkvist and Palme, 2008, 2015). However, the timing of the onset of maternal-related nest building is unknown, as pregnant mink females in previous studies built nests within one day after the first allocation of

nesting material during the gestation period, i.e. straw into the cage (studied after mating, from March 23; Malmkvist and Palme, 2015). The instant nest building upon access to straw made us suspect that these mink could be highly motivated for nest building even earlier, due to either thermoregulation or early onset of maternal nest building post-mating as seen in golden hamsters and mice (Richards, 1969; Lynch and Possidente, 1978; Broida and Svare, 1982). Until now, provision of straw or other nesting materials for female mink selected for breeding has only been studied in the reproductive season, more specifically from after second mating until after delivery. The authors concluded that access to nesting material in the cage reduces the mink dams' level of stress hormone (measured as FCM: faecal cortisol metabolites) during the gestation period (Malmkvist and Palme, 2015). The reduction in FCM could be due to either 1) performing highly motivated

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behavioural patterns regarding nest building, 2) having access to a proper nest or 3) a combination of the two factors. Access to straw, an artificial nest or a combination of both reduced postpartum FCM and had a positive effect on the reproduction outcome compared to a group with less nest building possibility and barren nests (Malmkvist and Palme, 2008).

Preventing animals from performing highly motivated behaviour can result in stress responses and/or inclusive abnormal behaviour (Reviewed in, Jensen and Pedersen, 2008). Development of abnormal behaviours due to prevention from performing of highly motivated behaviours is seen in e.g. pigs prior to parturition, when prevented from nesting material and mobilization they start to bar bites (Cronin et al., 1994) and mink prevented from oral manipulation of feed performed more stereotypy and fur-chewing when fed finely ground feed compared to chunky feed (Malmkvist et al., 2013). That the abnormal behaviours were due to frustration stress was supported by both immobilisation in pigs and finely ground feed shown increases HPA-axis output (Cronin et al., 1991; Jarvis et al., 1997, 2002; Oliviero et al., 2008; Malmkvist et al., 2013). These results support the hypothesis that lack of access to nesting material for nest building or lack of having access to a proper nest may act as a stressor for farm mink females shortly after mating.

Prevention of nest building can negatively influence not only pregnant mink females' welfare, by inducing stress and frustration due to not performing motivated behaviour, but also their reproductive success. Generally, mink females with a high level of FCM give birth to litters with fewer young (Malmkvist and Palme, 2008). Further, pregnant mink females not provided with material suited for nest building behaviour had more variable parturitions, i.e. with an increased standard deviation of the inter-birth interval (Malmkvist and Palme, 2008). Higher S.D. of the inter-birth interval is considered indicative of birthing problems and correlates with higher early offspring mortality, including stillbirths in mink as well as pigs (Pedersen et al., 2006; Malmkvist et al., 2007). Likewise, dams awareness towards offspring in need correlated with offspring survival, indicative of the importance of maternal care (Malmkvist and Houbak, 2000). How treatments affect maternal care is tested by difference in offspring retrieval test in current study.

Total offspring mortality on mink farms is hard to estimate in practice; percentages (including stillborn and liveborn offspring) are reported to be 20% (over 6 weeks; Schneider and Hunter, 1993), 28.7% (over 4 weeks; Martino and Villar, 1990) and 28.3% (over 1 week in a study with no man-made intervention; Schou and Malmkvist, 2017). Stillbirths account for around half of the early offspring mortality (proportion of stillborn 37–53%; Schou and Malmkvist, 2017; Malmkvist et al., 2007; Schneider and Hunter, 1993). Stillborn and liveborn mortality seem to be two sides of the same coin as litters with stillborn offspring are in greater risk of experiencing mortalities among the liveborn (Schou and Malmkvist, 2017), which could indicate that the reason for offspring mortality is a dam trait, maybe due to the dams stress response. Stillborn offspring are generally found fully developed (Malmkvist et al., 2007), which indicates that the mortality is occurring close to or during the parturition. Liveborn offspring mortality mainly occurs in the first week after birth (62%; Martino and Villar, 1990) with the first three days accounting for above 90% of the first week mortality (Malmkvist et al., 2007; Schou and Malmkvist, 2017). A majority of the dead offspring have no signs of physical lesions, congenital defects or infection (Martino and Villar, 1990; Schneider and Hunter, 1993). Thus, a relatively high number of apparently vigorous offspring die during the first week after birth. The correlation between stillborn and liveborn offspring mortality indicates that some factors or traits occurring before birth cause offspring mortality. Therefore, we suggest that mink females' behaviour and stress experienced prior to mating and during the gestation period could play a role in determining early offspring survival for both liveborn and stillborn offspring.

We aimed to enhance the understanding of influences on early

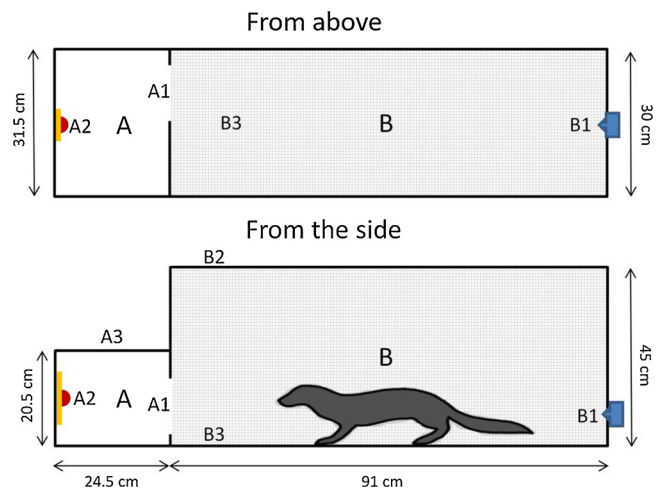


Fig. 1. Illustration of the cage system for a mink female and her litter (originally presented in Schou and Malmkvist, 2017). A: wooden nest box with wire nest insertion and a top lid made of wire; A1: nest box entrance; A2: air climate logger; A3: top layer of straw; B: wire cage; B1: drinking nipple; B2: site for daily fed provision; B3: paper sheet beneath whelping wire net; allocation site for nesting material delivered manually after opening the front cage lid. Each cage was additionally equipped with an elevated platform, according to Danish regulations for the keeping of mink.

offspring survival in farm mink by conducting a study focussing how prolonged access to nesting material affect dams I) nest building (nest score and in-nest climate), II) stress (FCM, abnormal behaviour, offspring-retrieval) and III) reproductive outcome inclusive offspring growth. We hypothesised that female mink are motivated for maternal nest building earlier than 23 March (the earliest time for straw provision in previous studies). Further, we expected that prolonged access to nest building material would reduce mink females' stress response and improve their reproductive outcome.

2. Methods

2.1. Animals and housing

This study was performed on female farm mink first time breeders born the previous year (primiparous) of the colour type brown. The mink were housed individually before and during the study in European standard production cages (Hedensted-Gruppen, Hedensted, Denmark) as illustrated in Fig. 1 and described in Schou and Malmkvist (2017). Each female was mated with the same male twice at an interval of 8 to 9 days (with few exceptions), according to the standard farm procedure (for description see Malmkvist et al., 1997), in the period from 2 to 18 March. Standard commercial wet feed was allocated once a day at around 11:00 h (Hvalpsund mink feed factory, Hvalpsund, Denmark). Danish standard feeding procedure was used; this means they were feed restricted in the pre-mating season from December to flushing from 26 February (*ad libitum* feed = 380 g). They were flushed for 5 days to start of mating and then fed 200–230 g of feed daily through birth. During the rest of the testing period the animals were fed by amounts close to *ad libitum* adjusted to individual consumption. Straw was allocated *ad libitum* on top of the nest box lid (mesh size: 2.5 × 2.5 cm) for all animals during the complete testing period. For use the mink had to pull it through the wired mesh into the nest box. Straw on top of the nest box is standard farm procedure in Denmark, which the animals have experienced from birth. Water was available *ad libitum* via a drinking nipple in the cage. The cage units were placed in a housing facility with natural light and climate conditions at the mink research farm at Aarhus University, Denmark.

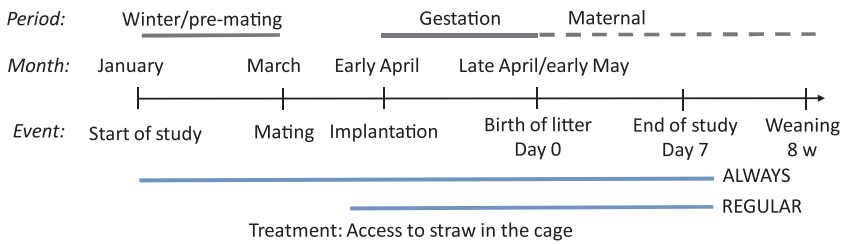


Fig. 2. Time line of the experiment from January until end of study, postnatal day 7. All mink in both treatments had access to straw on top of the nest box through a wired mesh. Group ALWAYS had access to additional straw in a loose pile in the cage during the whole period, whereas group REGULAR had access to additional straw in a loose pile in the cage from after mating (i.e. March 23, equal to best current practice).

2.2. Experimental design and treatments

In November, the mink females were placed in the cages where they stayed through the entire study period. On 15 January the following year, each cage unit was equipped with 1) a whelping net to prevent the offspring from falling through the cage floor, 2) a paper sheet (30 × 30 cm) in the first third of the cage bottom in front of the nest box under the whelping net to prevent nesting material from falling out of the cage and 3) a building brick fitted into the nest box bottom. The neighbouring mink females were moved before parturition to conform the Danish legislation of breeding with an empty cage between every breeding female.

We set up two groups that differed in timing of allocation nesting material consisting of a loose pile of 80 g straw in the cage, for time line see Fig. 2. Group ALWAYS has access to straw from 15 January until Day 7 after birth (with Day of birth = 0). Group REGULAR had access to a loose pile of straw in the cage from 23 March (REGULAR, proposed best known practice by Malmkvist and Palme, 2015). As mentioned above, both groups had access to straw on top of the nest box through wired mesh during the complete experimental period, the treatments consisted on the timing of allocation of a loose pile of straw into the cage. In January, a group size of 63 animals was chosen to secure a minimum group size of 40 double-mated females for both groups (successfully double mated, ALWAYS $n = 45$, REGULAR $n = 44$), which has previously been found sufficient to test effects on FCM, reproduction and fur-chewing between experimental treatments (Malmkvist et al., 2013). Random selection into groups was done by use of the function 'Randbetween' (Microsoft excel 2010) by numbering each place within each group.

On the first day with free access to nesting material in the cage, a loose pile of 80 g of shredded barley straw was placed in the cage, close to the nest box entrance. Afterwards, access to nesting material was observed three times a week (Monday, Wednesday and Friday). If approx. 75% of the straw was removed from the cage by the mink, an extra 80 g of straw was added to the cages, thereby ensuring that mink permanently had free access to straw. Easy-strø (from Easy-AgriCare; chopped heat-treated straw, 300 g) was added 23 March to all nest boxes as bedding material.

2.3. Sampling and observation procedure

2.3.1. Nest score

Nest building in mink was first scored and reported by Malmkvist and Palme (2008). In the current study, we used a more detailed version with six primary scores: 0) No hollowing in the nest bottom layer, 1) Hollowing without built side walls, 2) Hollowing with built side walls < 5 cm, 3) Hollowing with built side walls ≥ 5 cm, 4) Side walls and top layer present in two thirds of the nest, 5) Nest with side walls and completely closed ceiling. Each of the primary scores was additionally given a secondary level of low (0) or high (+0.5) based on evaluation of whether it was in the lower or higher end of the primary scores. The nests were scored the day before and the day after providing free access to nesting material and thereafter weekly through to Day 2 after parturition. If allocation and nest score occurred on the same day nest score was performed prior to allocation of nesting material.

Depending on the Day of parturition the number of nest scores for each animal was: 17 nest scores for 100% animals, 18 nest scores for 98.8% animals, 19 for 40.5% animals and 20 nest scores for 2.4% animals.

2.3.2. In-nest box air climate

The in-nest box air climate and shed climate were measured every 15 min using iButton temperature and humidity loggers (Resolution: 0.6% RH; ≤ 0.5 °C; Maxim Integrated, San Jose, CA, USA), placed as described previously (cf. Fig. 1, Schou and Malmkvist, 2017). Loggers dislodged by the mink were re-installed, and data from the finding day and 24 h before were excluded from the data before analysis.

2.3.3. Faecal cortisol metabolites (FCM)

Faeces was collected 27 January, 24 February (mid and end of feed restriction) and additionally for the double-mated females during the gestation period on 9, 16, 23 April plus Day 3 after birth. Collection of fresh faecal samples was performed continuously for three hours following feeding. Collected samples were stored in cool boxes for maximum one hour during sample collection and transport to a freezer. Subsequent analysis of FCM were performed using the methodology previously validated for mink (Malmkvist et al., 2011).

2.3.4. Behavioural observation

Direct observations were carried out by scan samples with the observer facing the cage from the feeding aisle 1 m in front of the cage units, with a maximum of six mink at each sample interval of 1 min (Martin and Bateson, 2007). Within the first 15 s of an observation, the animals habituated to the observer. To exclude behaviour performed as a reaction to the observer, active behaviour within the first 15 s was not registered. Each animal was observed for nine sample intervals on 29 January and 23 February (mid and end of feed restriction) and five sample intervals on 17 April between 09:00 h and 11:00 h (before feeding). Behaviour was registered by one-zero sampling with (Martin and Bateson, 2007) with only one type of behaviour registered for each observational sample interval as stereotypic overruled active behaviour, which in turn overruled being passive/in nest (Table 1). No animals performed both stationary and locomotory stereotypy within the same observation. Stereotypic behaviour was defined as a monotonous repeating movement pattern (minimum three times of repetitions) without any apparent function or goal.

2.3.5. Fur-chewing

Fur damage was scored on 6 February, 7 April and 21 May as an indicator of abnormal fur-chewing behaviour. The damage on the fur was observed in the home cage without capturing the mink.

Placement of fur damage was scored as 1) on tail or 2) other position. The score had four levels: No fur-chewing (score = 0.0), Short hair (1.0), Naked skin (2.0), Wound or scab (3.0). Subsequently, the size of the damage was added to the score as + 0 in case of less or equal to 1 cm and + 0.5 in case of > 1 cm of the tail affected.

2.3.6. Offspring retrieval test

Mink dams were tested on Day 5 for their reactivity towards a offspring out in the cage as described in Malmkvist and Palme (2008). Offspring selection alternated between and females within treatments.

Table 1

Ethogram for behavioural observations. Stereotypic behaviour is observed with minimum three non-interrupted repeats. Only one behaviour was scored for each animal for each observation of 1 min, as stereotypic overruled active behaviour, which in turn overruled being passive/in nest.

In nest box	All legs are inside the nest box, or only the two forepaws and the head are out of the nest box, leaning on the bottom
Passive	Lying still out in the cage during the whole observation. Can raise the head, but cannot drink, eat or groom
Active	Active movements of the legs or manipulations by the head of e.g. straw or food out in the cage
Locomotory stereotypy	All stereotypy that include horizontal movement of the whole body involving either walking, running or jumping
Stationary	Stationary stereotypic behaviour not including horizontal locomotion. E.g. nodding (vertical up-and-down movement of the anterior body), circling of the anterior parts on the body and horizontal side-to-side movement of the anterior body.
Biting/Licking	Continuously biting or licking towards the wire mesh, but not directed against the feeding site
Scratching	Scratching on the wire mesh

The offspring was placed in the middle of the cage. Mink dams' reaction was measured as the latency to touch the offspring and latency to retrieve the offspring to the nest. If the dam did not retrieve the offspring within 240 s, the test stopped, and the observer returned the offspring back into the nest.

2.3.7. Reproduction, weight and mortality

The nest boxes were checked during three daily rounds, i.e. in the morning between 08:00 h and 12:00 h, in the afternoon between 12:00 h and 16:00 h, and in the evening between 19:00 h and 20:00 h. Sound and other signs of offspring were used as indications of birth (Day 0). To determine whether dead offspring were stillborn or liveborn lung tissues was cut out and tested if it float (=liveborn) or sink (=stillborn, [Malmkvist et al., 2007](#)). Litter size, offspring sex and offspring weight for each sex within a litter were registered on Days 1 and 7.

2.4. Statistical analysis

SAS software (version 9.4; SAS Institute Inc. Cary, NC, US) was used for statistical analyses and JMP software (version 13; SAS institute Inc.) was used to create the figures. The significance level was set to 0.05 and *P* values between 0.05 and 0.10 are reported as tendencies. In the models tested, covariates with no tendency to significance ($P > 0.10$) were excluded. The demands for dispersion and variance homogeneity were for ANOVA evaluated from plots of the model residuals. Results are reported as mean \pm standard error of the mean unless otherwise stated.

The statistical testing was grouped in relation of the main hypothesis of our study of prolonged vs. regular straw provision to adult female mink, i.e. regarding the influence on (i) nest building, (ii) stress responses, and (iii) reproductive outcome, including offspring growth.

2.4.1. Nest building

Nest score. Development of nest scores was tested in the two separate periods: (1) 16 January to 23 March (winter through mating season) and (2) 24 March to Day 2 after birth (post mating through birth season).

In-nest climate. The daily air temperature parameters mean, maximum, minimum and standard deviation (temperature fluctuation) were tested in the two separate periods: (1) 15 January to 22 March (winter through mating season) and (2) 23 March to Day 7 after birth (post mating through birth season).

For both indicators (nest scores, in-nest climate), ANOVA models with repeated measures (per animal ID) were performed using the Mixed procedure in SAS ([Littell et al., 1996](#)), testing for main treatment effects (always, regular straw access), time and the treatment*time interaction. The time structure was analysed using an autoregressive first-order covariance matrix [ar(1) in SAS], as this resulted in the best fit according to Bayesian and Akaike information criteria (BIC and AIC in SAS). Real dates were used as observation time in the statistical models for period 1. In period 2, day relative to birth was used instead to take the influence of gestation and birth into account. The standard deviation in daily temperature in period 1 was log transformed to

obtain better residuals.

2.4.2. Stress responses

Cortisol. Treatment effects on the FCM (ng cortisol metabolites/g faeces) were analysed by repeated measures model of the same type as used for the nest building responses nest score and in-nest climate, in two periods: (1) pre-mating during winter with feed restriction (27 January and 24 February) and during gestation (9, 16 and 23 April). FCM data sampled Day 3 after birth were tested separately. The FCM response was in all cases log transformed to obtain a better residual distribution.

Abnormal behaviour. For each behaviour observed (normal and abnormal forms), the percentage of scans in which the animals performed the behaviour was calculated and treatment effects tested in an ANOVA per observation day (January 29, February 23, April 17). Repeated measures were not preferred, due to only three observation days spread out during the pre-mating period with feed restriction (29 January and 23 February), and gestation (April 17) which may influence the biological state of mink. Another reason was the lower number of mink at the last observation time. For the observation during the gestation period (April 17) day relative to birth (-4 to -20) and its interaction with treatment were additionally included in the start model. Data for the variables 'In nest box' and 'locomotory stereotypic behaviour' was square root transformed as it resulted in better residuals. In case the transformation did not result in satisfactory residuals (true for four less common forms of stereotypic behaviour, but not for locomotory and total SB) data was analysed as binomial distributed (using the procedure Genmod in SAS), i.e. based on whether the behaviour was observed or not per individual mink.

Fur-chewing. Data was tested by ANOVA between treatments for each observation day.

Dam offspring retrieval response. In the offspring retrieval test, latencies for the dam to touch and retrieve offspring were analysed with methods for survival analysis (procedure Phreg in SAS) considering censored data ([Klein and Moeschberger, 2003](#)), as some animals did not touch or retrieve the offspring within the test time. The assumption behind using the used cox proportional hazard model was confirmed by approximate parallel lines between the treatment groups in plots of survival function versus the survival time and in the graph of the log (-log[survival]) versus log of survival time (using procedure Lifetest in SAS). Latencies to touch and to retrieve the offspring are presented as medians with 25% and 75% quartiles, and the proportion of non-responders is given in the results.

2.4.3. Reproductive outcome and offspring growth

Litter size and offspring mortalities. The total litter size, 'totborn', was calculated as the total number of offspring born within a litter including both liveborn and stillborn offspring. The number of liveborn offspring dead within a litter is defined by the sum of collected dead offspring tested as liveborn post mortem plus any missing offspring on Day 7 which previously had been counted as alive. The number of liveborn offspring that died was tested for treatment effect using a Poisson distribution with the natural log of number of original liveborn offspring as the offset value (using the procedure Genmod in SAS). A similar

statistical test was performed for the number of stillborn offspring in each litter, however, for which the natural log of total number of offspring born served as an offset value. The total litter size, and the litter size of liveborn D1, D7 were tested in as normal distributed in ANOVA with treatments (ALWAYS, REGULAR) as main effect.

Early offspring growth. Mean offspring weight was tested Day 1 and Day 7 by ANOVA (procedure Mixed in SAS). The mean offspring weight was calculated as the total weight of the litter divided by the number of offspring alive on that day. The total litter weight Day 7 was used as measure of total reproduction outcome of the dams. In order to truly include all litters of our study, we set the total litter weight to 0 g in litters where all offspring died. The sex ratio (ratio of males per litter, from 0 to 1) and treatment was included in the models. To include all litters in the statistical analysis, a mean sex ratio of all recorded litters Day 7 was assigned to litters with total offspring weight of 0 g at Day 7 after birth.

3. Results

3.1. Nest score

The mean nest score was low and equal for both groups (ALWAYS = 0.3 ± 0.04 ; REGULAR = 0.3 ± 0.03) at 14 January when they only had access to nesting material through a wire mesh on top of the nest boxes (Fig. 3). However, on the first day after allocation of nesting material in the cages, ALWAYS mink had already built advanced nests (mean = 1.5 ± 0.15). The nest score stayed significantly higher for group ALWAYS (2.1 ± 0.05) v. REGULAR (0.4 ± 0.02) through the period 16 January to 23 March ($P < 0.001$), i.e. during which only ALWAYS mink had access to nesting material inside the cage. Through the same period, there was no significant effect over time ($F_{1,516} = 0.1, P = 0.72$). REGULAR mink females also built nests within one day after allocation of nesting material in the cages 23 March (Fig. 3). Through the period 24 March to Day 2 after birth, the nest score increased significantly over time for both groups ($F_{1,341} = 26.8, P < 0.001$; Fig. 4) but with no significant difference between the groups ($F_{1,179} = 1.2, P = 0.28$).

3.2. In-nest box air climate

ALWAYS females had a significantly higher daily nest air temperature during the first period from 15 January to 22 March (winter and pre-mating period) measured as mean, minimum and maximum temperature (Table 2). The in-nest box climate also had higher daily fluctuation and higher relative humidity for ALWAYS than for REGULAR mink. All in-nest climate parameters were positively affected by date,

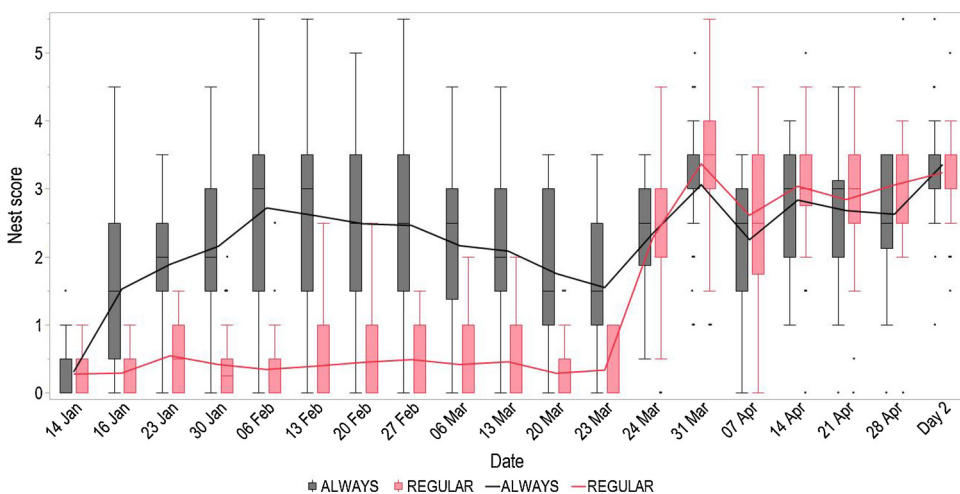


Fig. 3. Daily nest score for ALWAYS and REGULAR from 14 January to Day 2 after birth. The lines present the mean nest score. Boxplot present the median and distribution of the nest score data and outliers are showed by dots. Group ALWAYS with permanent access to straw in the cage had significant higher nest scores during 16 January to 23 March, indicative of non-maternal nest building. The nest score increase from 24 March (after mating) through to after birth due to maternal nest building in both groups see Fig. 4.

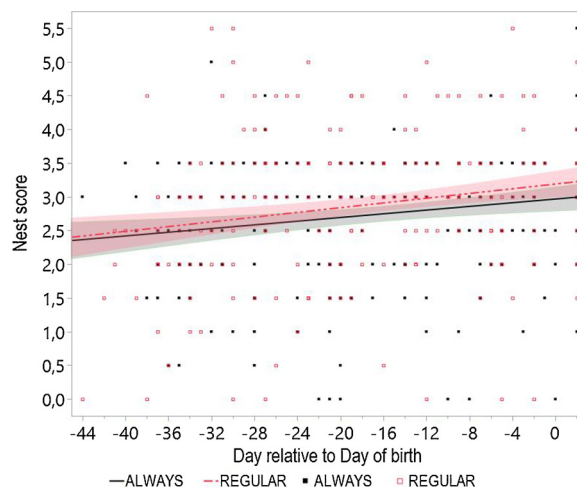


Fig. 4. Maternal nest building. Line of fit for daily mean nest score relative to day of birth (Day 0), during the period from 23 March to Day 2 after birth.

and there was a significant interaction between date and treatment for the daily temperature fluctuation parameter. When both groups had access to nesting material (23 March to Day 7), daily (relative to day of birth) nest temperature did not differ between ALWAYS and REGULAR (Table 2). All the daily temperature parameters were significantly affected by relative date of birth (increasing mean, minimum, maximum; decreasing standard deviation). Humidity was significantly higher for ALWAYS, and the in-nest humidity decreased towards the day of birth.

3.3. Faecal cortisol metabolites (FCM)

Female mink of group ALWAYS (302.4 ± 50.78 ng/g) had lower FCM concentrations than group REGULAR (362.8 ± 52.90 ng/g) during January and February, when only ALWAYS had access to nesting material in the cages (tendency, $F_{1,121} = 3.2, P = 0.075$). After both groups had equal access to nesting material in the cages, no statistical difference was found between their FCM concentrations (9, 16 and 23 April; overall mean: 56.0 ± 6.46 v. 65.7 ± 8.36 ng/g; $F_{1,81} = 157, P = 0.45$). Likewise, on Day 3 after birth, FCM did not differ between the two groups (61.3 ± 7.95 v. 65.2 ± 7.21 ng/g; $F_{1,78} = 0.4, P = 0.55$).

3.4. Behavioural observation

Only minor difference was evident in behaviour. In the late pre-mating period, February 23, group ALWAYS mink spent less time in the

Table 2

Nest box air climate for group ALWAYS and REGULAR presented as daily means (± SE) for the two periods: (1) 20 January to 22 March, winter and mating season and (2) 23 March to day 7 after birth.

	ALWAYS	REGULAR	Statistical test value	P value
Period 1: 29 January to 22 March				
Mean temperature, °C	9.6 ± 0.06	7.5 ± 0.04	$F_{1,364} = 118.6$ $F_{1,520} = 200.4$	< 0.001 Date < 0.0001
SD temperature, °C	2.5 ± 0.02	1.7 ± 0.01	$F_{1,1371} = 26.2$ $F_{1,1373} = 423.2$	< 0.001 Date < 0.001
Minimum temperature, °C	4.9 ± 0.04	4.4 ± 0.04	$F_{1,1371} = 25.9$ $F_{1,805} = 25.0$	Date*treatment < 0.001 < 0.001
Maximum temperature, °C	14.6 ± 0.08	11.1 ± 0.06	$F_{1,929} = 436.0$ $F_{1,566} = 298.9$	Date < 0.001 < 0.001
Mean relative humidity, %	85.8 ± 0.15%	78.6 ± 0.11%	$F_{1,710} = 493.3$ $F_{1,253} = 111.9$ $F_{1,424} = 202.5$	Date < 0.001 < 0.001 Date < 0.001
Period: 23 March to Day 7, tested relative to day of birth				
Mean temperature, °C	16.6 ± 0.09	17 ± 0.08	$F_{1,227} = 1.2$ $F_{1,345} = 707.5$	0.27 Relative date < 0.001
SD temperature, °C	2.9 ± 0.02	2.9 ± 0.02	$F_{1,596} = 0.1$ $F_{1,683} = 65.7$	0.76 Relative date < 0.001
Minimum temperature, °C	10.6 ± 0.09	10.8 ± 0.09	$F_{1,374} = 1.5$ $F_{1,471} = 1086.2$	0.22 Relative date < 0.001
Maximum temperature, °C	22.2 ± 0.09	22.6 ± 0.08	$F_{1,314} = 1.8$ $F_{1,415} = 397.6$	0.18 Relative date < 0.001
Mean relative humidity, %	73.1 ± 0.23%	70.4 ± 0.19%	$F_{1,201} = 9.3$ $F_{1,344} = 9.8$	0.003 Relative date < 0.002

Table 3

Behaviour of mink females with ALWAYS or REGULAR access to straw in the cage presented as mean (± SE) scans during three observations days, during the pre-mating (29 January, 23 February) and the gestation period (April 17). Total SB (Stereotypic Behaviour) consists of several forms, see Fig. 5 and text for details. Difference between treatment groups (P < 0.05) is marked with shading.

	January 29		February 23		April 17 599	
	ALWAYS	REGULAR	ALWAYS	REGULAR	ALWAYS	REGULAR
In nest box, %	5 ±1.2	4 ±1.6	6 ±1.6	3 ±0.8	46 ±5.0	52 ±5.4
<i>statistics</i>	$F_{1,122} = 0.0, P = 0.99$		$F_{1,122} = 4.5, P = 0.035$		$F_{1,87} = 0.8, P = 0.39$	
Active cage, %	61 ±3.2	64 ±3.9	55 ±4.1	63 ±4.2	37 ±3.6	34 ±4.2
<i>statistics</i>	$F_{1,122} = 0.4, P = 0.55$		$F_{1,122} = 1.7, P = 0.19$		$F_{1,87} = 0.3, P = 0.62$	
Total SB, %	30 ±3.6	28 ±4.0	33 ±4.2	32 ±4.3	7 ±2.7	6 ±2.1
<i>statistics</i>	$F_{1,122} = 0.1, P = 0.74$		$F_{1,122} = 0.1, P = 0.81$		$F_{1,87} = 0.1, P = 0.75$	
Total active ¹ , %	91 ±1.6	92 ±1.8	88 ±1.6	94 ±1.2	44 ±4.5	40 ±4.7
<i>statistics</i>	$F_{1,122} = 0.3, P = 0.60$		$F_{1,122} = 7.22, P = 0.008$		$F_{1,87} = 0.3, P = 0.59$	

1: Total active = Active cage + Total SB.

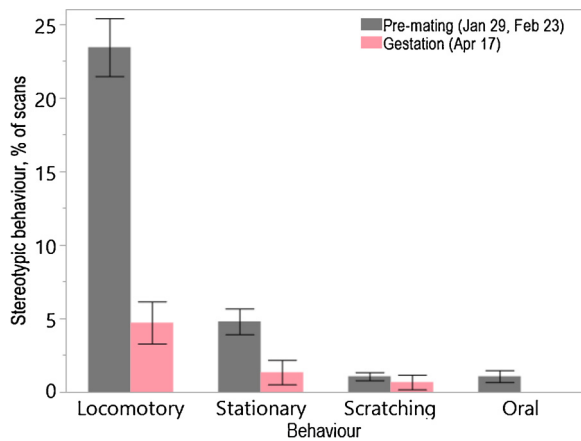


Fig. 5. Distribution of forms of stereotypic behaviour observed. The percentage of observations each animal was observed performing each of the stereotypic forms was calculated for each observation day (29 January, 23 February and 17 April). Data is presented as the mean performance of each stereotypic form pre-mating (29 January and 23 February) and post-mating/gestation (17 April).

nest box and were more active in total out in the cage than group REGULAR females (Table 3).

Total activity in the cage is the sum of active and stereotypic behaviour (SB) in the cage. We find this variable relevant as (i) SB is a part of the total activity, and (ii) mink can perform activities appearing similar to SB, however, not reaching the criterion of three repetitions as used for the lower limit for SB categorisation. We suggest that a higher level of activity due to arousal or restlessness would increase the total activity of mink out in the cage.

There was no difference in fully developed stereotypic behaviour between group ALWAYS and REGULAR dams (Table 3). The stereotypic behaviour consisted of several different forms displayed by the mink, during the observation divided into five categories as locomotory, stationary, scratching and oral (biting and licking; for distribution of forms see Fig. 5). We did not find any difference in the occurrence of these forms of SB between females from the two treatment groups ($P > 0.10$).

3.5. Fur-chewing

Fur-chewing, another type of abnormal behaviour recorded as a stress response, did not differ between ALWAYS and REGULAR on any of the three observation days: 6 February (0.7 ± 0.12 v. 0.5 ± 0.11 ; $F_{1,121} = 0.5$, $P = 0.50$), 7 April (0.8 ± 0.13 v. 0.6 ± 0.12 ; $F_{1,87} = 1.2$, $P = 0.27$) and 21 May (0.1 ± 0.05 v. 0.2 ± 0.08 ; $F_{1,87} = 2.4$, $P = 0.12$) when tested by ANOVA. Only fur-chewing on the tail was observed – there were no signs of fur damage on other parts of the mink body.

3.6. Offspring retrieval test

The majority of dams retrieved their offspring within the test duration; 3 of 39 tested (7.7%) ALWAYS and 5 of 31 tested (16.1%) REGULAR mink dams did not retrieve their offspring. Mink dams in group ALWAYS and REGULAR did not differ in latency to touch (median [25%; 75%]; 12 [5; 18] s v. 10 [5; 33] s; $F_{1,59} = 1.2$, $P = 0.27$) and latency to retrieve (22 [10; 36] s v. 28 [14; 37] s; Chi-sq = 1.0, $P = 0.32$) offspring into the nest box.

3.7. Reproduction, weight and mortality

The results show that access to nesting material in the cages from January resulted in significantly better offspring survival and litter size Day 7 (Table 4). In fact, mink females in group ALWAYS had better

reproduction results for all variables than the REGULAR group, although not all were statistically significant. Three females from each group were barren and did not give birth to a litter. In addition, large total litter size ('totborn') increased the risk of giving birth to stillborn offspring ($F_{1,80} = 9.39$, $P = 0.003$).

4. Discussion

4.1. Access to nesting material in the months prior to mating increases reproduction

Access to straw as a loos pile in the cage from winter/early spring had a positive effect on mink female reproduction; this was evident in spite of both ALWAYS and REGULAR having access to straw during most of the gestation period, i.e. from 23 March. Previously, it has been shown that access to nesting material during the gestation period also increases mink dams' reproduction (Malmkvist and Palme, 2008, 2015), expected to be due better maternal care, inclusive warmer nests and due to lower pre-parturition stress. The ALWAYS mink females had a better reproduction for all nine reproduction parameters measured. The total number of offspring born was higher in ALWAYS dams with prolonged access to straw, although not reaching significance. Higher number of offspring born can be explained by a stress-reducing effect of nest building material on mink females during the time of implantation and through the gestation period. In mammals, stress during gestation is known to induce embryo loss at different stages (e.g. Wilmut et al., 1986; Pratt and Lisk, 1989). The lower occurrence of stillbirths in ALWAYS supports the conclusion that access to straw before mating likewise has a long-term effect on the parturition. In mink, a lack of nesting material around the time of parturition is stressful and increases the standard deviation in inter-birth intervals (Malmkvist and Palme, 2008), which is suggested to be one of the main mechanisms leading to stillbirths in mink as well as pigs (Pedersen et al., 2006; Malmkvist et al., 2007). After parturition, ALWAYS mink females also did better, with higher liveborn offspring survival and thus larger litter size Day 7. These results support previous results indicating that stillborn and liveborn mortality were due to the same problem, as the occurrence of stillbirth in a litter increased the risk of the liveborn offspring dying within the same litter (Schou and Malmkvist, 2017). With no difference in nest score and nest air temperature around parturition, climate around parturition cannot explain the difference in reproduction. This results supports a previous study showing that nest climate after parturition under conditions with plenty of nesting material, like in current study, does not affect reproduction (cf. Schou and Malmkvist, 2017). Rather, we attribute the improved periparturient performance and early offspring survival in ALWAYS females to be an effect from having prolonged and easy access to nesting material, i.e. during the previous months before and during mating.

4.2. Access to nesting material may reduce stress

Access to nesting material showed a tendency for a reduce FCM concentration in group ALWAYS compared to REGULAR pre-mating when only ALWAYS had access to straw. Thus even though the results are not significant it support the hypothesis, that access to nesting material may have a reducing effect on female minks stress response at the onset of maternal nest building. With this in mind together with previous studies may pre-mating nest building have an small impact on mink females' cortisol (FCM) prior to mating (current) like it previously have been found during gestation (Malmkvist and Palme, 2015) and shortly after giving birth (Malmkvist and Palme, 2008). Similar results are found prior to parturition in pigs, where prevention from performance of nest building increases plasma cortisol concentration (Lawrence et al., 1994).

Access to nesting material pre-mating did surprisingly not affect the performance of stereotypy, and thereby indicate that ALWAYS mink

Table 4

Reproduction results presented as group means (\pm SE) for all dams giving birth in groups ALWAYS (N = 42) and REGULAR (N = 41).

Variable	ALWAYS	REGULAR	Statistical test value	P value
Totborn, n	8.6 \pm 0.31	8.2 \pm 0.34	$F_{1,81} = 0.6$	0.45
Alive Day 1, n	7.4 \pm 0.35	6.5 \pm 0.35	$F_{1,83} = 3.1$	0.08
Stillborn, n (% of Totborn)	0.8 \pm 0.17 (8.8%)	1.0 \pm 0.19 (13.5%)	Chi-sq = 2.4	0.12
Litters with stillborn mortality, n	17 of 42	22 of 41	Chi-sq = 2.1 Chi-sq = 7.1	0.14 Totborn: 0.008
Liveborn mortality day 0-7, n (% of Totborn)	1.2 \pm 0.19 (13.7%)	1.8 \pm 0.30 (20.5%)	Chi-sq = 7.2	0.007
Mean offspring weight Day 1, g	11.2 \pm 0.28	11.0 \pm 0.34	$F_{1,76} = 0.2$	0.63
Mean offspring weight Day 7, g	32.5 \pm 1.19	32.3 \pm 0.96	$F_{1,76} = 0.02$	0.88
Total litter weight Day 7, g	219.6 \pm 15.95	174.9 \pm 13.40	$F_{1,80} = 5.77$	0.019
Alive Day 7, n	6.6 \pm 0.38	5.4 \pm 0.37	$F_{1,81} = 5.5$	0.021

females had lower stress response. However, there were some difference in behaviour between the groups that suggest that ALWAYS mink may have experienced a lower stress response. Late pre-mating when only ALWAYS had access to nesting material, did ALWAYS mink use more time inside the nest box and performed less total activity inclusive stereotypy.

In the current study, we used first-parity females. Thus, they may not yet have developed fixed stereotypic patterns with three or more repetition, which is supported by observations of activities appearing similar to SB, however, not reaching the criterion of three repetitions as used for the lower limit for SB categorisation. The results are in alignment with one-year-old mink females performing less stereotypy in February and spring compared to older, 2- to 4-year-old, females (Mason, 1993; Malmkvist and Schou, unpublished, 2018). Therefore, high overall activity could be an indicator of arousal or restless behaviour in one-year-old mink females due to thwarting from nest building or access to a self-built nest. This is supported by increased running is occurring during the same time a increased stereotypy (Hansen et al., 2007)

We did not find other indications and evidence of frustration in the abnormal behaviour such as fur-chewing. Fur-chewing is otherwise known to appear from lack of oral stimulation in mink (Malmkvist et al., 2013).

We did not find any clear results showing that ALWAYS mink had lower stress response due to prolonged access to nesting material as hypothesised. However, some minor results indicate that they may experienced a lower stress response, but the difference may require larger sample size or more precise and sensitive measures, compared to FCM and observation of abnormal behaviour. Cortisol plays a role in energy mobilisation (Mormède et al., 2007), and a colder nest may increase the need for energy mobilisation for keeping warm in group REGULAR mink which may also explain the higher stress response (FCM concentration and activity) during the cold month January and February compared to April and May. Thus REGULAR individuals seem to have a higher stress response either due to thwarting nest building, prevention from access to a nest or due to low temperature stress. However the lower temperature within the REGUALR net boxes may be a by product of the less time used within the nest.

4.3. Straw rack principle limits the use of nesting material in farm mink

For the first time we demonstrate that the way nesting material is allocated greatly impacts how mink use the material. For both groups we found a drastic increase in nest score within one day after allocating nesting material as a loos pile in the cages compared to only having nesting material on the top of the nest boxes through wired mesh. Thus, having nesting material only on top of the nest box wire, like in a straw rack, is not sufficient for nest building, even though it is known that mink can retrieve some straw through the mesh (Hansen et al., 2007). This result is clearly illustrated by the difference in nest score between

group ALWAYS (straw in the cage) and group REGULAR (straw on the nest box lid) during the winter/spring before mating (Fig. 1).

4.4. The onset of nest building motivation in breeding mink females

We found that mink females were motivated for nest building from the very beginning of the study period (15 January). This is approximately 1.5 months before the mating, and this early motivation of nest building has not previously been documented in farm mink. The mink in group ALWAYS maintained a relative high nest score (> 1.5), which indicates they were motivated for nest building from January through to birth in late April/early May. Therefore, the nest building was not only a result of novelty value or a rebound effect but due to a relatively constant motivation for nest building throughout the period. The small drop in nest score mid- and end of March is probably either due to the increase in environmental temperature or a change in motivation due to mating. The current findings demonstrate that mink females are motivated for nest building activity in winter/early spring. We suggest that this is not related to maternal behaviour but rather a seasonal or general motivation for nest building in farm mink due to thermoregulation, similar to what is found for both sexes in rats and mice (Denenberg et al., 1969; Lisk et al., 1969). Thermoregulation may be the motivation for mink females' nest building during the cold months, as seen in mice, rats, golden hamsters and gerbils, with increased nest building when exposed to cold environment (Denenberg et al., 1969; Rajendram et al., 1987; Latham and Mason, 2004). This is supported by the daily nest air temperature being significantly higher for ALWAYS through the period 15 January to 23 March compared to REGULAR. The higher temperature for ALWAYS is probably due to both increased insulation capacity by the self-built nests, and the fact that the mink females stayed inside the nest box for longer in the self-built nests. We did see that ALWAYS animals stayed inside the nest box for longer during behavioural observations in February.

We did not see a clear day-to-day increase in nest score during the gestation period related to maternal nest building as expected and found in rats, rabbits and mice (Denenberg et al., 1963, 1969; Lisk et al., 1969; Latham and Mason, 2004). However, after mating, a significantly steady increase in nest score was seen through the period 24 March to Day 2 after mating. The increase in nest score after mating is most likely due to maternal-related nest building, taking into account the seasonal increase in temperature during the same period. A steady maternal nest score increase is similar to what is reported in golden hamsters (Richards, 1966, 1969) and in an unpublished study built mated mink females more advanced nests than non-mated (Lund, 2009). A hormonal manipulation study on mink would help clarify whether the increase in nest building during gestation is controlled by hormones connected to maternal behaviour as in for example mice, rats and rabbits (e.g. Lisk, 1971; Zarrow et al., 1971). More studies on captive farm mink's motivation for nest building at different seasons, temperature, age and sex could be of great interest for further

knowledge of the mink's motivation for nest building and thereby welfare.

4.5. Stillbirths increase with the number of offspring born in total

The risk of stillborn offspring in litters increased with the total litter size at birth for both groups of mink. It has previously been shown that the total number of offspring born has a negative effect on birth interval between offspring during the birth process (Malmkvist et al., 2007), offspring birth weight (Schneider and Hunter, 1993; Hoy et al., 1998; Malmkvist and Palme, 2015), growth and chance of survival (Schou and Malmkvist, 2017). The increased risk of stillborn offspring with increasing numbers of offspring born supports *in utero* competition and/or birth problems (induced by female stress) as main factors for reduced offspring survival in farm mink. This deduction is supported by findings in pigs (Quiniou et al., 2002) for which litter size is of great concern for the offspring size and survival. On farms, it is not normal procedure to count newborn offspring and search for dead offspring as thoroughly as in the current and previous studies (Malmkvist and Palme, 2008, 2015; Schou and Malmkvist, 2017). Therefore, selection for larger litter size on farms, by selecting females with a high number of offspring at a specific time, does not take into account a high number of stillborn or liveborn offspring that die in the first week. Selection for larger litters should therefore be performed with caution, if not stopped, or changed to also consider a reduced early offspring mortality as a breeding goal. Instead, the fur-production should focus on the potentials regarding higher offspring survival, as current study underlined it is possible to reduce offspring survival and thereby litter size Day 7.

5. Conclusion

Mink females are motivated for non-maternal nest building over a long period from winter through to mating, followed by maternal nest building characterised by a steady increase through the gestation period to Day 7 after birth, at least. Prolonged access to straw significantly increased mink dams reproduction outcome, indicative of access to nesting material may have a stress reducing effect on female mink.

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