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Periparturient nest building: Implications for parturition, kit survival, maternal stress and behaviour in farmed mink (*Mustela vison*)

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Abstract

Several types of nesting materials with different capacity as substrate for nest building are currently supplied to farmed mink. We investigated whether different nesting resources and possibility of performing periparturient nest-building influenced (a) the parturition, (b) the vitality and survival of neonates, and (c) maternal stress and behaviour. Individually housed female mink (all provided with wood shavings in the nest box) had either (1) restricted possibility of nest building (NON; $n = 60$), (2) restricted possibility of nest building, but an artificial plastic nest available (ART; $n = 60$), (3) full possibility of nest building using straw (STR; $n = 60$), or (4) full possibility of nest building using straw and an artificial plastic nest available (ART + STR; $n = 60$). The experimental period began on average 11 days before the deliveries (range: 5–21 days) and lasted until 7 days after birth of each individual litter. The access to straw for nest building reduced the variation in inter-birth intervals between kits ($P = 0.044$; S.D. STR: 36 min versus NON: 58 min and ART: 53 min), but did not affect the total duration of parturition. The average body weight of kits was significantly reduced in NON litters after 7 days ($P = 0.025$), with no differences on Day 1 after delivery. In addition, the mortality of live-born kits was highest in the NON litters ($P < 0.001$). Female stress hormone metabolites, measured non-invasively in faeces, tended to differ between treatment groups after delivery ($P = 0.064$), with a lower concentration in ART and ART + STR than in NON. In a maternal reactivity test, ART + STR females were quicker than NON females to retrieve their 5-day-old kit to the home nest ($P = 0.027$). In conclusion, an artificial nest alone or in combination with *ad libitum* access to straw tended to reduce maternal stress postpartum, and the combination significantly enhanced maternal kit retrieval. For the kit vitality and survival, an artificial nest appeared as good as a nest of straw created by the dam, due to an

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improved in-nest climate postpartum. However, access to straw for nest building resulted in a less variable parturition, whereas the feedback from an artificial nest had no such effect.

Keywords: Hypothalamic–Pituitary–Adrenal axis; Mortality; *Mustela vison*; Parturition; Reproduction

1. Introduction

In some mammalian species, nest building behaviour is a highly prioritized activity in late pregnancy (e.g. in rats, *Rattus norvegicus*: Kinder, 1927; mice, *Mus musculus*: Bond et al., 2002; rabbits, *Oryctolagus cuiculus*: Gonzalez-Mariscal, 2001; Negatu and McNitt, 2002; pigs, *Sus scrofa*: Arey et al., 1991; Arey, 1992; Castren et al., 1993; Jensen, 1993). Nest building activity is also intensified prior to delivery in the farmed American mink, *Mustela vison* (Malmkvist et al., 2007). In nature, the female mustelid (mink: Dunstone, 1993; weasel, *Mustela nivalis*, stoat, *M. ermine*, longtailed weasel, *M. frenata*: King, 1989) does not necessarily complete a nest from scratch prior to delivery; rather she may locate and take over a nest in dens of suitably sized prey animals, such as rodents, muskrats and rabbits. Presumably, some nest building may then take place, as in weasels that are reported to improve the lining of the nest with fur plucked from dead prey (King, 1989). To our knowledge, the actual timing and on-set of preparturient nest building in mink on farms or in the wild have not been studied in any detail.

Production animals such as mink and pigs are selected for large litters, and the early postnatal period is critical for the survival of the young in both species (e.g. mink: Martino and Villar, 1990; Schneider and Hunter, 1993; Malmkvist et al., 2007, and pigs: Bereskin et al., 1973; English and Smith, 1975; Svendsen et al., 1986). Based on observations of deliveries in yearlings, one recent study concluded that birth problems – as indicated by time spent sniffing/licking their genital region and prolonged duration of parturition – resulted in increased early kit mortality and suboptimal maternal behaviour in farmed mink (Malmkvist et al., 2007). Studies of nest-builders have indicated or hypothesized that lack of substrate for prepartum nest building may affect the level of stress, the course of parturition as well as maternal behaviour. In pig production, a large litter size is related to a high amount of nest building activity prior to the birth of the first piglet (Pedersen et al., 2006), and prenatal stress during late gestation may impair the vitality and survival of the offspring (Otten et al., 2001). Provision of environmental stimuli, which are relevant for nest building, was in one study reported to decrease the duration of the first part of parturition in sows (Thodberg et al., 1999). In addition, provision of nesting material may influence postnatal maternal behaviour. In sows, access to nesting material reduced the proportion of piglet crushing sows, and increased their responsiveness towards piglet distress calls 1–3 days postpartum (Herskin et al., 1998). Likewise, protective maternal behaviour (such as high responsiveness to piglet distress calls) was linked with high nest building activity in one study comparing piglet crushing sows with sows that did not crush any of their piglets (Andersen et al., 2005). Whether a restricted possibility for building a nest has consequences for the parturition, survival of young and maternal behaviour has not been investigated in farmed mink, despite the fact that several types of nesting material (e.g. whole/shredded straw or paper, wood shavings, sawdust, or artificial plastic nests) with very different capacity as substrate for nest building, currently are supplied to mink by the producers.

The aim of the present study was to investigate, whether different opportunities for creating a nest influence (a) the birth, growth and survival of neonate mink, and (b) maternal stress and behaviour.

2. Materials and methods

2.1. Animals, treatment and housing

The experiment used 240 mated female mink of the same brown breeding line, randomly selected to four groups, each consisting of 50 one-year-old primiparous and 10 two-year-old second parity females. All experimental mink had access to a protected nest box with the same amount and type of wood shavings for insulation. The four treatment groups were NON: restricted possibility of nest building ($n = 60$), ART: restricted possibility of nest building, but an artificial nest placed in the nest box ($n = 60$), STR: full possibility of nest building using straw ($n = 60$), and ART + STR: full possibility of nest building using straw and an artificial nest placed in the nest box ($n = 60$). The artificial mink nest was a commercially available type, with the bottom and side made of plastic (Mink Solo, Hedensted-Group, Denmark). The experimental period began on April 18 (on average 11 days before expected deliveries) and lasted until 7 days after birth of each individual litter. Groups with access to straw (STR, ART + STR) were given 70–80 g barley straw (length: 3–28 cm) every day at 8–9 a.m. in the wire cage outside the nest box entrance, if less than approximately half of the original amount of straw (100 g April 18) was present in the wire cage. Group NON and ART had no access to straw for nest building.

All mink were housed individually under identical conditions in wire cages (W: 30 cm, H: 45 cm, L: 90 cm) connected to a covered wooden nest box (W: 28 cm, H: 20 cm, L: 23 cm; Hedensted-Group, Denmark). Standard type and amount of wet feed (on average 160 g per female per day; approximately 33% dry matter, 130 kcal/100 g with protein 50%, fat 39%, and carbohydrate 11% of convertible energy) was given once daily at 11 a.m. $\pm$ 15 min. Water was available *ad libitum*. The animals were exposed to natural light only at the farm of Research Centre Foulum, Denmark. We distributed animals from the four treatment groups evenly and mixed them in the farm unit in order to balance for any local environmental effects. One pregnant ART female died on April 20, with enteritis haemorrhagia diagnosed after autopsy.

2.2. Data collection

The sampling events during the experimental period are summarised in Table 1.

2.2.1. Dam body condition pre- and postpartum

The body condition of the dams was scored from 1 (thin) to 4 (fat), defined as (1) thin with hips obvious and broader than the rest of the body, (2) cylindrical shaped body, hips are not clearly broader than the rest of the body, (3) pear shaped body with bulging abdomen and (4) box shaped body with bulging abdomen and body.

2.2.2. Faeces cortisol metabolites pre- and postpartum

We collected a fresh sample of dam faeces from the wire cage after continuous scanning rounds 0–6 h after feeding, and 0.50 g were stored in closed vials at -21 °C until analysis. After thawing and extracting the faeces with 5 ml methanol (80%), concentrations of faecal cortisol metabolites (FCM) were analysed in an aliquot of the supernatant with two different enzyme-immunoassays (EIAs) previously described in detail (FCM1: 11-oxoaetiocholanolone-EIA: Palme and Möstl, 1997; FCM2: 11β-hydroxyaetiocholanolone-EIA: Frigerio et al., 2004). The interassay coefficient for a high and low quality control was 12.3% versus 14.5% for the FCM1 assay and 10.4% versus 10.6% for the FCM2 assay. The sensitivity was 24.3 and 21.1 nmol/kg, respectively. Animals that did not defecate during the collection hours (before delivery: $n = 31$, after delivery: $n = 30$) and barren females ($n = 19$) were excluded in the further analysis.

2.2.3. Climate in nest pre- and postpartum

Temperature (°C) and relative humidity (% RH) were measured and stored every 15 min for 24 h using loggers (Dickson Temperature & Humidity data logger type TL120, Cole-Parmer Instrument Company, IL, USA), in 15% of the nests from each treatment group. Each logger was fixed inside the nest box, 5 cm above

Table 1
Sampling events (a) on fixed days before/after delivery, (b) relative to the birth, and (c) on a fixed day after the period of delivery

Time of sampling	Sampling event	Comment
(a) Before/after delivery		
April 19, May 9	Dam body condition	Score 1–4. [a]Day −10 and 10.
April 21, May 4	Faeces cortisol metabolites	Non-invasive faeces sample. [a]Day −8 and 5.
April 25–26, May 4–5	Climate in nest	24 h Temperature, humidity. [a]Day −3 and 5 (n = 62).
April 27, May 6	Evaluation of nest building	Score 0–4. [a]Day −2 and 7.
(b) Relative to birth		
Day 0	Time of litter birth	Positive indications of young.
	Time of birth of each kit	Kit free of *rima vulva*. Observed from digital recordings of parturitions (n = 46).
Day 1, Day 7	Body weight, sex of each kit	
Day 0 to Day 7	Collection of dead kits	Autopsy, test for being stillborn or not.
Day 5	Kit-retrieval test	Testing maternal reactivity toward own kit.
(c) After delivery		
May 6	Observation of litter assembly	Scanning observation. [a]Day 7.

The experimental period ran from 11 days prior to the average date of expected delivery (April 29) until 7 days post partum. The number (n) is specified when not all 220 females with litters were sampled.
[a] Day relative to the average date of expected delivery. The time relative the actual delivery is calculated for each individual and included in the statistical analysis.

the bottom. The in-nest climate was measured twice (Table 1), with a change of nest between days of measurement. In total 66 different nests were successfully recorded for 24 h. Recordings from nests of barren females (n = 4) were not included in the statistical analysis.

2.2.4. Evaluation of nests pre- and postpartum

The result of nest building activity was scored twice (Table 1) as (0) no signs of substrate manipulation/no hollowing in the nest bottom layer, (1) visible hollowing in the nest bottom layer, (2) open nest with some sidewalls, but no top layer included in the nest, (3) sidewalls and part of top layer included in the nest, but the nest is not completely closed, (4) completely closed nest, with sidewalls and ceiling included.

2.2.5. Parturition and kit growth/survival

During the period April 18 to May 13, we checked all cage units three times daily (at hours 8–9, 14–15, and 19–20) to register time of litter birth (Day 0) and to collect dead kits. Dead kits were categorised as stillborn in case of a negative lung-floating test after autopsy.

We had access to 24 recording units, each with a digital camera and build-in infrared lightning (Monacor TVCCD) fitted to cover one nest box. We recorded parturitions in a subset of the animals only. In order to enhance the power of detecting differences among the NON, ART and STR treatments, we excluded in advance animals of the ART + STR treatment. Each recording unit was placed over a nest box 2–3 days before the first day of expected delivery (calculated as 47 days after last mating), to allow the female to habituate. Approximately 24 h after the birth, the recording unit was transferred to a new pregnant female. Continuous recordings (12 frames per seconds) were stored and analysed from PCs using a digital surveillance system (MSH-Video, M. Shafro & Co., Riga, Latvia). We succeeded to record 46 deliveries (NON: n = 22, ART: n = 15, STR: n = 9), fulfilling the criteria of a period of minimum 3 h before birth of the first kit and 8 h after birth of the last kit. This period was chosen to ensure a high likelihood of sampling the complete delivery (cf. Malmkvist et al., 2007). Time of birth was registered for each individual kit, and the inter-birth interval between succeeding pairs of kits was calculated. For the litter, we define the duration of parturition as the duration from first until last kit born, and S.D. in inter-birth interval as the standard deviation in the inter-birth intervals between the kits. Total number born is 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.

2.2.6. Maternal reactivity: kit-retrieval test on Day 5

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 from the litter, and after restricting the dam in the nest box, placed the kit in the middle of the wire cage, with its head directed toward 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 registered latency to touch kit and latency to retrieve kit back into nest. In case of no kit retrieval within 180 s, the test stopped and the observer returned the test kit to the nest.

2.2.7. Observations of litter assembly

During scanning on May 6, the degree of litter assembly was categorised in three classes, as either (i) assembled: all live kits were together, with a distance of less than one kit breadth between any individual, (ii) mixed: 3/4 or more of the kits in a litter were assembled, but at least one kit was away from the others, i.e. distanced more than one kit breadth away, or (iii) dispersed: less than 3/4 of the litter was assembled.

2.3. Statistical analysis

2.3.1. Parturition

The duration of parturition and the standard deviation (S.D.) in inter-birth intervals ($n = 46$ recorded litters) were analysed using a normal distributed ANOVA model including treatment (NON, ART, STR), female age/parity (1, 2), number of kits delivered (3–12, mean $\pm$ S.E.: 9.0 ± 0.34), body condition prior to delivery (1–4), number of stillborns (0–4), and the interactions between treatment and (i) female age, and (ii) dam body condition. Logarithmic transformation of the response variables was used because it resulted in better residuals in terms of dispersion and variance homogeneity.

2.3.2. Kit body weight and survival

We analysed the mean and S.D. in kit body weight per litter Day 1 ($n = 186$ litters) and Day 7 ($n = 182$ litters) after delivery in a normal distributed ANOVA model including treatment (NON, ART, STR, ART + STR), dam body condition (1–4; score April 19 used for Day 1, and score May 9 used for Day 7 responses), female age/parity (1, 2), number of kits delivered (1–14), duration of gestation (41–63 days, mean $\pm$ S.E.: 47 ± 0.2 days), sex ratio (i.e. proportion of males, 0–100%, Day 1: $49 \pm 2.0\%$, Day 7: $51 \pm 1.7\%$) and the interactions between treatment and (i) dam body condition and (ii) female age. The mean body weight Day 1 was logarithmic transformed as it resulted in better residuals in terms of dispersion and variance homogeneity. The mortality and number of stillborn per litter were analysed in a normal distributed ANOVA model including treatment (NON, ART, STR, ART + STR), female age/parity (1, 2), dam body condition prior to delivery (1–4), duration of gestation (41–63 days) and the interaction between treatment and (i) dam body condition and (ii) female age.

2.3.3. Maternal stress and behaviour

The female faeces cortisol metabolites (FCM1 and FCM2) were analysed for each time of measurement separately, i.e. before and after delivery. For this analysis we used a normal distributed ANOVA model including treatment (NON, ART, STR, ART + STR), female age/parity (1, 2), duration between collection relative to the day of delivery (before delivery: 3–17 days, 8.9 ± 0.18; after delivery: 1–10 days, 4.4 ± 0.16 days), number of kits delivered (1–14), number of stillborns (0–4), duration from faeces sampling to freezing (before delivery: 3–141 min, mean $\pm$ S.E.: 56 ± 2.1 min; after delivery: 20–212 min, mean $\pm$ S.E.: 86 ± 2.3 min) and the interaction between treatment and female age. FCM1 was inverse transformed and FCM2 was logarithmic transformed as these transformations resulted in improved residuals in terms of dispersion and variance homogeneity.

In the kit-retrieval test, latencies to touch and retrieve the kit were analysed with methods for survival analysis, considering censored data (Allison, 1995; Klein and Moeschberger, 2003). Females not retrieving their kit within the test time of 180 s were taken as right censored observations. Plots of the Kaplan-Meier survivor function of each treatment against the test time were used to evaluate whether the hazard rate was proportional between treatments. A Cox proportional hazard (Cph) model was used to test whether survivor functions for latency to react differed between groups. However, in case of no proportional hazard rates (i.e. the survivor function curves of two groups crossed), the non-parametric Wilcoxon test (Wilc) was used instead. There was no difference in the sex of the randomly chosen kit between treatment groups ($\chi^2_3 = 3.6$, $P = 0.31$), and the sex of the kit did not affect the latency to retrieve (Cph: $\chi^2_1 = 1.9$, $P = 0.18$). Preparation and handling prior to the kit-retrieval test did not differ between treatment groups (61 ± 2.1 s per unit; $F_{3,168} = 1.5$, $P = 0.21$).

The scores of litter assembly and nest building were analysed in a generalized linear model for Poisson distributed data (McCullagh and Nelder, 1989), using log as link function in the procedure Genmod in SAS. The model for litter assembly included treatment (NON, ART, STR, ART + STR), female age/parity (1, 2), number of live kit Day 1 (3–13), litter age (0–12 days mean 6.2 ± 0.20 days), and the interaction between treatment and female age. The model for nest building score was equivalent, except that the duration until delivery (1–12 days, mean 3.2 ± 0.2 days) replaced litter age in the analysis of prepartum nest building (i.e. nest building score April 27). Test values for these models are reported with the degrees of freedoms for the chi-square distribution, followed by the degree of freedoms for the deviance.

The in-nest temperature and relative humidity were analysed in a normal distributed ANOVA model including treatment (NON, ART, STR, ART + STR), female age/parity (1, 2), day relative to birth (before delivery: 0–13 days mean ± S.E.: 5.8 ± 0.59 days; after delivery: 1–8 days mean ± S.E.: 4.0 ± 0.33 days), and the interaction between treatment and female age.

All calculations were performed using SAS software (version 9.1, Statistical Analysis Systems Institute, Cary, NC). A probability level (*P*) of 0.05 was chosen as the limit of statistical significance. *P*-values between 0.05 and 0.10 are reported as tendencies, and models were reduced by removing insignificant terms, $P > 0.10$.

3. Results

The dams delivered between April 23 and May 13 2005. The proportion of mated females, which did not give birth was 7.9% and the proportion did not differ between treatment groups ($\chi^2_3 = 4.3$, $P = 0.23$). The barren females ($n = 19$) were excluded from further analysis. Both before and after delivery, the nest building score differed between treatments (April 27: $\chi^2_3 = 130.3$, $P < 0.001$; May 6: $\chi^2_3 = 70.0$, $P < 0.001$). Only females with access to straw were able to build a nest with walls and top included (i.e. score 2–4, Fig. 1). Prior to delivery, the presence of an artificial nest in addition to straw increased the nest score, whereas this effect was non-significant after delivery (Table 2). We found no significant effects of female age/parity, interaction between treatment and female age/parity, duration between scoring day and the day of delivery, or the number of kits. The wooden, covered box was the preferred nest site, regardless of the nesting material available. Only four females before and three females after delivery chose to nest outside in the wire cage. Out of these seven, six were NON females.

The treatment also affected the nest climate, measured in a representative subset of the animals. Both before ($F_{3,14} = 4.2$, $P = 0.027$) and after delivery ($F_{3,29} = 7.6$, $P < 0.001$), the minimum temperature during 24 h was significantly higher as a mean in ART + STR nests (before: 11.6 °C, after: 22.8 °C) than in any other treatment (before: 6.7–8.2 °C, after: 17.1–18.8 °C). Likewise, ART + STR had higher in-nest average diurnal temperature prepartum than

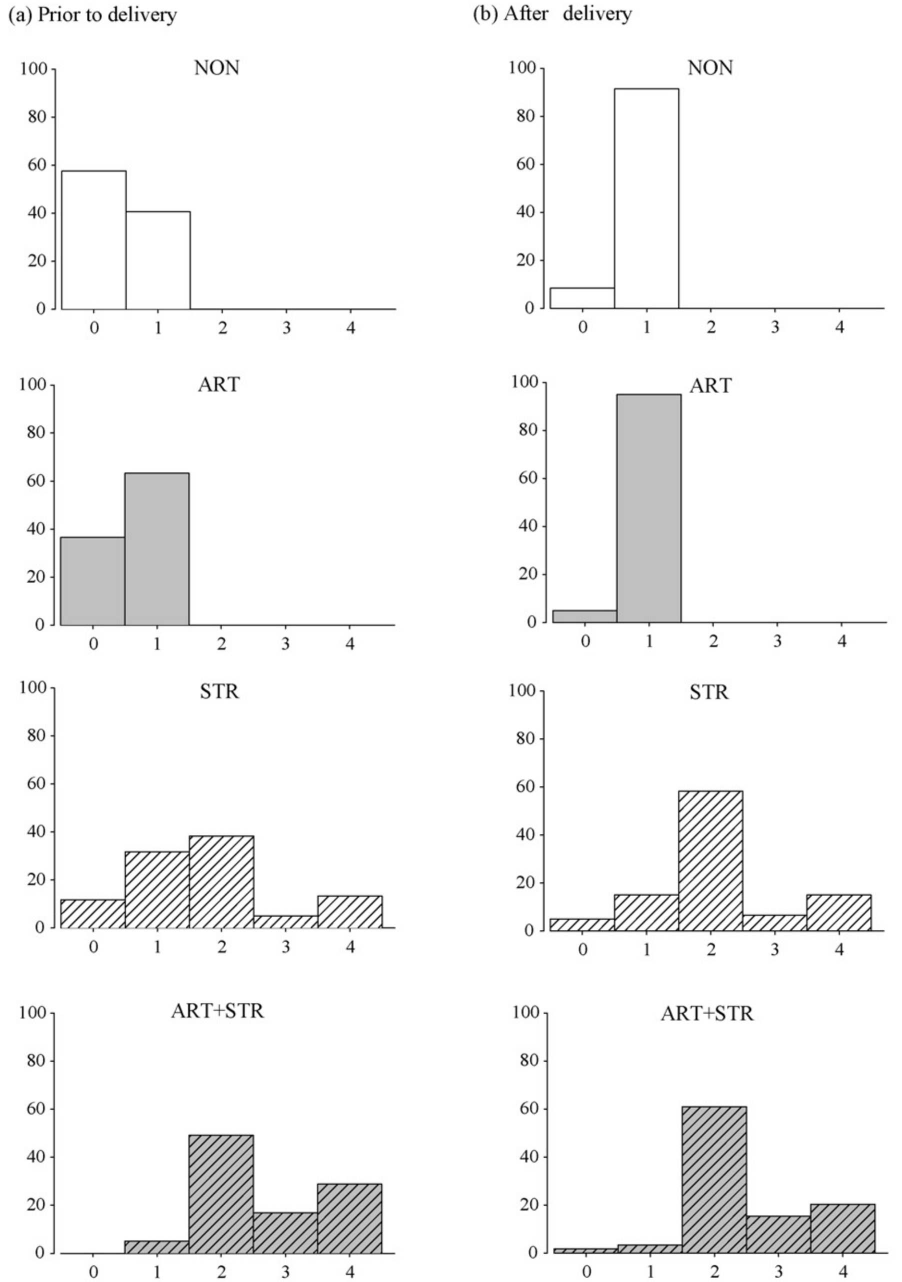

Fig. 1. Distribution of animals per treatment (%) in five classes of nest building scores (a) prior to (April 27) and (b) after (May 6) delivery. Scores are 0: no signs of substrate manipulation, 1: hollowing in the nest bottom layer, 2: open nest with some sidewalls, 3: sidewalls and part of top layer included, and 4: completely closed nest.

Table 2
Nest building score as median (25%; 75% quartiles), nest climate as mean (S.E.) of temperature and relative humidity during a 24-h period and two type of dam faeces cortisol metabolites (FCM) as mean (S.E.), measured (a) before and (b) after delivery

	NON	ART	STR	ART + STR
(a) Before delivery				
Nest building score	0 (0; 1)a	1 (0; 1)a	2 (1; 2)b	2 (2; 4)c
Temperature (°C)	14.2 (0.49)a	16.2 (0.87)a	15.8 (0.60)a	18.4 (0.91)b
Humidity (% RH)	57 (2.3)	59 (2.5)	63 (0.7)	60 (1.2)
FCM1 (nmol/kg)	204 (37.6)	173 (26.7)	192 (32.4)	170 (23.9)
FCM2 (nmol/kg)	366 (32.3)	344 (34.9)	488 (144.8)	445 (47.7)
(b) After delivery				
Nest building score	1 (1; 1)a	1 (1; 1)a	2 (2; 2)b	2 (2; 3)b
Temperature (°C)	20.8 (0.68)a	23.2 (1.22)b	21.4 (0.63)ab	26.6 (0.43)c
Humidity (% RH)	66 (2.2)a	69 (1.3)ab	73 (2.0)b	66 (2.0)a
FCM1 (nmol/kg)	278 (41.7)	296 (46.0)	331 (71.6)	356 (59.1)
FCM2 (nmol/kg)	271 (42.8)a	190 (23.4)b	206 (23.6)ab	176 (26.7)b

Different letters indicate significant difference between treatments.

the other groups ($F_{3,25} = 5.9$, $P = 0.003$) prepartum. After delivery (4–5 May) both in-nest temperature ($F_{3,29} = 10.7$, $P < 0.001$) and humidity ($F_{3,27} = 3.3$, $P = 0.035$) differed between treatment groups (Table 2). There was no effect of female age/parity, days relative to day of delivery and no interaction between female age and treatment group.

3.1. The parturition

The duration of parturition was not influenced by the treatment ($F_{2,42} = 0.02$, $P = 0.98$), and averaged 6 h 00 ± 28.3 min per litter. However, the standard deviation in inter-birth interval differed between treatments ($F_{2,39} = 3.4$, $P = 0.044$). STR females had less variation in the inter-birth intervals during delivery (35.7 ± 6.1 min) than both NON (57.7 ± 16.0 min; $F_{1,39} = 5.8$, $P = 0.021$) and ART (52.9 ± 9.5 min; $F_{1,39} = 6.3$, $P = 0.016$) females. The number of kits delivered by the recorded females did not differ between the treatments (NON: 9.0 (0.45), ART: 8.7 (0.61), STR 9.3 (0.53); $F_{2,44} = 0.48$, $P = 0.62$, NS).

3.2. Kit survival and body weight

Treatment – but not dam body condition, age/parity of females, duration of gestation, and number of stillborn – affected the mortality of live-born until Day 7 ($F_{3,192} = 5.9$, $P < 0.001$). Mortality in NON litters was higher than mortality in the other groups (Table 3). Treatment neither affect the duration of gestation (days from last mating until delivery) ($F_{3,207} = 0.9$, $P = 0.47$), nor the within-litter variation in body weight of live kits the day after birth ($F_{3,180} = 1.3$, $P = 0.28$, based on weighing of 1343 kits from 186 litters Day 1) and 7 days after birth ($F_{3,170} = 1.1$, $P = 0.36$). However, the average body weight of kits was significantly reduced in treatment NON 7 days after birth ($F_{3,176} = 3.2$, $P = 0.025$), whereas no effect was evident on kit weight the first day after delivery ($F_{3,184} = 1.5$, $P = 0.21$) (Table 3). Offspring of second parity females were on average heavier (Day 1: 1.2 g, 10.5%; Day 7: 3.6 g, 12.5%) than offspring of first parity females (Day 1: $F_{1,184} = 8.4$, $P = 0.004$, Day 7: $F_{1,176} = 10.0$, $P = 0.002$). In addition, we found a negative correlation between the number of kits and their average weight (Day 1:

Table 3
Duration of gestation, litter size, kit survival and body weight given as mean (S.E.)

	NON	ART	STR	ART + STR
Duration of gestation, days	47 (0.5)	46 (0.4)	47 (0.5)	47 (0.4)
Litter size	9.0 (0.25)	8.7 (0.28)	9.3 (0.22)	9.0 (0.25)
Stillborn	0.7 (0.14)	0.5 (0.12)	0.6 (0.15)	0.5 (0.18)
Mortality until Day 7 (%)	40 (4.2)a	23 (3.3)b	22 (3.0)b	27 (3.8)b
Kit weight Day 1 (g)	11.3 (0.37)	11.6 (0.33)	12.4 (0.65)	12.3 (0.41)
Kit weight Day 7 (g)	31.0 (0.83)a	34.4 (1.44)b	34.5 (1.10)b	34.9 (1.10)b

Different letters indicate significant difference between treatments.

$F_{1,184} = 4.5$, $P = 0.035$; Day 7: $F_{1,176} = 8.5$, $P = 0.004$) after delivery. Treatment did not affect the number of stillborn kits, but the number of stillborns increased with decreasing duration of gestation ($\chi^2_{1,201} = 9.9$, $P = 0.002$). Females in intermediate body condition (score 2 and 3) delivered significantly fewer stillborn than females being fat (body condition 4; $\chi^2_{3,201} = 14.6$, $P = 0.002$).

3.3. Maternal stress and behaviour

The dam stress level measured as FCM concentrations is reported in Table 2. Prior to delivery, there was no difference between treatments in any of the two types of faeces cortisol metabolites (FCM1: $F_{3,185} = 2.0$, $P = 0.121$; FCM2: $F_{3,185} = 1.4$, $P = 0.25$). However, after delivery the FCM2 concentrations tended to differ between treatments ($F_{1,171} = 2.5$, $P = 0.064$). Post testing indicated that the FCM2 concentration was higher in treatment NON than in ART ($F_{1,171} = 4.8$, $P = 0.030$) and in ART + STR ($F_{1,171} = 6.4$, $P = 0.012$), whereas treatment STR did not differ from the other treatments (NON: $P = 0.163$; ART: $P = 0.48$: ART + STR: $P = 0.30$). In addition, there was a negative correlation between the number of kits delivered and the concentration of FCM2 ($F_{1,171} = 8.5$, $P = 0.004$). In the measurement after delivery, the FCM1 concentrations decreased with time since birth (range 1–10 days). This group of measured faecal cortisol metabolites was thus dependent on day of birth, with highest value observed on the first day after delivery, whereas the FCM2 did not vary significantly relative to the day of parturition.

The results of the kit-retrieval test are summarised in Table 4, with the cumulative probability of kit retrieval illustrated in Fig. 2. ART + STR females were quicker than NON females to touch

Table 4
Maternal behaviour evaluated in (a) kit-retrieval test Day 5 and as (b) litter assembly score

	NON	ART	STR	ART + STR
(a) Kit-retrieval test				
Latency to touch kit (s)	39 (27; 57)a	21 (10; 75)ab	25 (13; 54)ab	27 (12; 68)b
% Censored, touch	31.3%	30.6%	21.7%	12.2%
Latency to retrieve kit (s)	54 (37; 88)a	40 (26; 79)ab	47 (35; 80)ab	37 (10; 75)b
% Censored, retrieval	32.7%	34.7%	28.3%	16.3%
(b) Litter assembly				
Assembled/mixed/dispersed (%)	91.1/6.7/2.2	87.8/10.2/2.0	85.4/9.8/4.9	91.8/6.1/2.0

Latencies to touch and retrieve kit are given as median (25%; 75% quartiles). The proportions of non-reacting females within the 3-min test are given as censored, with $n = 43$–49 per treatment.
Different letters indicate significant difference between treatments. Survival analysis considers censored data.

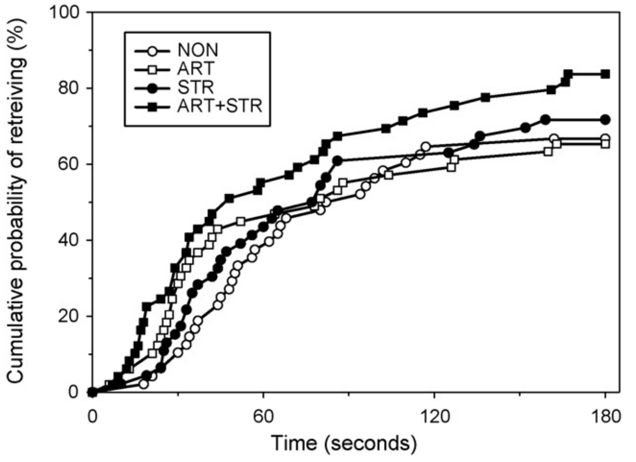

Fig. 2. Cumulative probability of kit retrieval (%) during the 3-min test Day 5. Group ART + STR were more reactive and had a lower latency to retrieve a kit than group NON ($P = 0.027$).

and to retrieve their kit (touch, Cph: $\chi^2_1 = 6.1$, $P = 0.014$; retrieve, Cph: $\chi^2_1 = 4.9$, $P = 0.027$). In addition, group ART + STR tended to touch (Cph: $\chi^2_1 = 3.6$, $P = 0.056$) and retrieve (Cph: $\chi^2_1 = 3.2$, $P = 0.070$) quicker than group ART females. All other comparisons resulted in P-values greater than 0.10 (Cph, Wilc). The degree of litter assembly was not significantly different between treatments (Table 4; $\chi^2_{3,181} = 0.18$, $P = 0.98$).

4. Discussion

The nesting resources affected the course of parturition in farmed mink females. Access to straw significantly reduced the variation in inter-birth intervals during the delivery. Our results indicate that access to straw for nest building is beneficial for the progress of parturition, whereas the feedback from an artificial nest alone had no such effect. A high variation in inter-birth intervals indicates long periods between the births of kits combined with short intervals between the births of other kits within the same litter. Birth problems and prolonged parturition have previously been found to be characteristics of farmed mink females loosing a high proportion of their litter (Malmkvist et al., 2007).

Stress can arise both as a consequence of adverse external stimulation and as a consequence of internal causal factors driving the animal to attempt to carry out species–specific behaviour, such as nest building, in case of no or insufficient feedback (Jensen and Toates, 1997). Thus, prepartum stress due to lack of possibility to create a nest could be expected in our study, and this may explain the higher standard deviation in inter-birth interval in the groups without access to straw. Stressors may influence the labour controlling hormone oxytocin and prolong the delivery in other polytocous species, such as rats (*R. norvegicus*) and pigs (*S. scrofa*). For example, moving to a new environment during mid-parturition prolonged the birth process in rats (Leng et al., 1987, 1988). Similarly, moving of sows to a farrowing crate during mid-parturition resulted in depressed oxytocin release through opioid inhibition and prolonged the birth process (Lawrence et al., 1992). In our study, we did not detect any treatment effects in the basal cortisol level prior to delivery. Thus, our HPA-axis indicator did not indicate that straw could have a stress reducing capacity in mink, which may be in line with reports of no effect of nesting substrate on

ACTH or cortisol levels in the preparturient pig (Jarvis et al., 2002). However, faecal cortisol metabolites could be less sensitive indicators than the S.D. in inter-birth interval. Moreover, the concentration of one group of faecal cortisol metabolites peaked around the day of delivery, introducing individual variation due to the fixed date of sampling, and making it harder to detect treatment differences from our data (cf. also Palme et al., 2005). Finally, the association between stressors, HPA-axis and the progress of parturition are complex as indicated by unequivocal results. For example, one study reported that provision of straw increased the duration of parturition, with no effect on plasma cortisol and oxytocin in sows (Jarvis et al., 2004), whereas another study reported that thermal stress around parturition increased plasma cortisol without any effect on plasma oxytocin and the progress of parturition in sows (Malmkvist et al., 2006, 2008).

We did not attempt to quantify the frequency of nest building behaviour, which normally takes place in the covered and dark nest box, using direct observation. Instead, we used nest scores to evaluate the nests in the four treatment groups (cf. Fig. 1). Thus, we cannot rule out that mink perform nest-building activity using wood shavings as substrate, even though this behaviour cannot result in a structured nest. In a study on sows, no effects of access to long-stemmed straw were found on maternal behaviour (Damm et al., 2005), which the authors suggested to be due to the control group having access to wood shavings/chopped straw and thereby were able to show the same frequency of nest building behaviour. Moreover, the actual timing and on-set of preparturient nest building in mink on farms or in the wild are unknown due to lack of studies.

For the kit vitality and survival, the artificial nest appeared as good as a self-made nest of straw. This result is most likely explained by the improved in-nest climate postpartum, where the combination of straw *ad libitum* and an artificial nest had a higher average and less diurnal variation in temperature. Groups with the artificial nest had also an improved in-nest climate postpartum compared to the groups with a self-made straw nest or wood shavings only. The thermal environment at delivery and during the early postpartum period is particularly important for the altricial mink kit. At birth, their energy reserves are limited with only 1% of body weight as fat (Tauson, 1994), and mink kits are not able to thermoregulate before 22–29 days of age (Harjunpaä and Rouvinen-Watt, 2004; Rouvinen-Watt and Harri, 2001; Tauson et al., 2006). Hypothermia is one of the main reasons of early kit mortality, and may be the reason of the higher mortality and less growth in neonates raised in the nest box with wood shavings only. Thus, in spite of its worldwide use at mink farms, this nesting environment appears insufficient, which the higher basal cortisol level (at $P = 0.064$) of dams raising kits in this environment also indicate.

The nesting environment affected the maternal behaviour of mink, as documented in the kit-retrieval test. Females with access to straw in combination with an artificial nest were more attentive and quicker to retrieve one of their 5-day-old progeny placed away from the safe nest. In other production species, enriched nesting environments have also been found to facilitate maternal behaviour (e.g. sows responsiveness to piglet vocalisations during a test; Cronin and van Amerongen, 1991, and towards playbacks of a piglet distress call; Herskin et al., 1998—although it is questioned whether this test reflects relevant maternal behaviour in sows, cf. Chaloupková et al., 2008). The other participant in the retrieval-test is the kit, which is blind, deaf, poikilothermic and rather immobile, but may vocalise during the test. In mice and rats, vocalisations of infants play a role in retrieval, initiating maternal orientation towards the stimulus source, followed by the retrieval of a pup back to the nest (Ehret, 2005). A recent study on mink found that kits vocalise intensively before being retrieved (Clausen et al., 2008). The mink kits raised in the ART + STR environment were on average heavier than the mink kits raised in the NON environment at Day 7. Thus, the increase in retrieval may result from a combination of dam and kit behaviour, e.g. in case heavier kits are able to vocalise more and better attract maternal attention. It may also be that offspring placed outside the nest has to "fight for priority" against the rest of the litter (as suggested for rodents by Ehret, 2005). This trade-off may consequently enhance the chance of kit-retrieval in females housed in the superior thermal nesting environment over females in nest boxes with wood shavings only, the latter having to allocate more effort into keeping kits warm.

Other significant findings were in our study unrelated to treatment. The negative correlation between the number of kits and their average body weight is to be expected based on earlier findings (e.g. Hoy et al., 1998). More interestingly, our data showed that second parity females had on average 11–13% heavier offspring the first week. This could be a consequence of the older females being better mothers due to experience and/or because they have been selected for reproduction traits, allowing only the most successful as second year breeders. Females with a short period of gestation and fat females (body condition score 4) may be considered at risk, since they independently delivered more stillborn kits in our study.

5. Conclusion

An artificial nest in combination with *ad libitum* access to straw significantly enhanced maternal kit-retrieval, and an artificial nest alone or in combination with straw tended to reduce maternal stress postpartum. For the kit vitality and survival, an artificial nest appeared as good as a nest of straw created by the dam, due to an improved in-nest climate postpartum. However, access to straw for nest building resulted in a less variable parturition, whereas the feedback from an artificial nest had no such effect.

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