

## The use of a cat-flap at the nest entrance to mimic natural conditions in the breeding of fattening rabbits (*Oryctolagus cuniculus*)

P Baumann<sup>†‡</sup>, H Oester<sup>†\*</sup> and M Stauffacher<sup>‡</sup>

<sup>†</sup> Centre for Proper Housing, Swiss Federal Veterinary Office, Burgerweg 21, 3052 Zollikofen, Switzerland

<sup>‡</sup> Swiss Federal Institute of Technology (ETH), Institute of Animal Sciences (INW), Physiology and Animal Husbandry, ETH Zentrum LFW B55.1, 8092 Zurich, Switzerland

\* Contact for correspondence and requests for reprints: Hans.Oester@bvet.admin.ch

### Abstract

Management systems allowing free nest access are widely used in commercial rabbit breeding, but these produce a potential conflict with the doe's behavioural goal of a closed nest entrance. Furthermore, the restricted space in commercial breeding units prevents the doe from achieving a sufficient distance between her and the nest, another highly adaptive behavioural goal. This can lead to behavioural problems and pup mortality higher than 20%, attributable to hypothermia, injuries, weakness caused by the scattering and crushing of pups, or even cannibalism. In this study we tested a type of nest entrance (a metal cat-flap) that visually closed the nest box while still allowing the doe free access during the first 15 days after parturition. The effects of a cat-flap access (group CF) and of a permanently open nest box (group O) on nest-related behaviour, general activity, and plasma corticosterone concentration of the does, and on the mortality and weight of the pups, were compared using 15 ZIKA does in each group. Over 24 h, there was no difference between the groups in the frequency of 'nest controls' (approaches or entries to the nest without nursing of pups). However, outside nursing hours, does in group O showed more 'head contacts' with the nest, whereas does in group CF performed more nose contacts and nesting activities outside the nest. Does in group O performed twice as many potentially disturbing nest contacts in this time than does in group CF and had a higher increase of corticosterone after the administration of exogenous adrenocorticotrophic hormone. Pup mortality from days 16 to 35 was significantly higher in group O, and pups born to does in group O left the nest earlier. There was no significant difference in the weaning weights of pups between the two groups. As does with a cat-flap at the nest entrance still showed repeated nest approaches, the cat-flap possibly did not block all nest stimuli from the does. Alternatively, it is possible that the repeated approaches to the nest box result from its being one of very few attractive or interesting features in an otherwise barren environment. Removal of the nest box from the cage is an effective method to eliminate nest stimuli, but this increases work for the staff without improving the barren environment for the rabbits. A better way of increasing the distance between the doe and the nest, as well as presenting a number of other attractive features, is group-housing of does.

**Keywords:** animal welfare, cat-flap, maternal behaviour, nest access, pup mortality, rabbit

### Introduction

Under natural and semi-natural conditions, wild and domestic does dig a burrow a few days before parturition, which they line with plant material and hair plucked from their chest, into which they give birth to a litter of hairless and blind pups (Myers & Schneider 1964; Stodart & Myers 1964). Immediately after birth the doe leaves the burrow, closes the entrance with soil, and only visits her pups once per day to nurse for three to five minutes (Deutsch 1957; Ross *et al* 1963). During the first five days after birth a circadian synchronisation develops (Jilge 1993); the pups dig out of the nest material shortly before the doe visits the nest and are therefore prepared for suckling. The doe spends the rest of the day away from the nest, and the pups save their energy by staying huddled together deep within the insulating nest material. The behavioural goals of a closed nest entrance and maintaining distance from the nest are

anti-predation measures and are evolutionarily highly adaptive. In addition, the closed entrance protects the pups from potentially harmful climatic effects. Both behavioural patterns are performed by domestic rabbit does under semi-natural conditions, but are also attempted in cages (Deutsch 1957; Kraft 1979; Wieser-Fröhlicher 1984, 1986; Wullschleger 1985; Stauffacher 1988).

In the barren environment of commercial housing conditions, a doe can neither close the nest entrance by her own plugging activity nor move a reasonable distance away from the nest after birth or nursing, and this may be a source of stress. The cage provides a restricted amount of space (0.3–0.7 m<sup>2</sup>, height 0.35–0.60 m) with a solid nest box (30 × 30 × 30 cm, length × width × height). The nest box has a permanently open entrance and is lined with wooden shavings, and is either attached to the outside or put into the cage a few days before parturition. The cage floor is made of

wire or plastic (for hygienic and management reasons) and prevents any successful burrowing. It is likely that, in this situation, olfactory stimuli are the main trigger of a doe's activities at the nest (Wieser-Fröhlicher 1986; Baumann *et al* 2005, in press) and, under the commercial conditions described, does can never avoid these nest stimuli by their own activities (closing the nest and moving away). Instead, they carry out several nursing visits per day (Schulte & Hoy 1997; Seitz *et al* 1998) and frequent 'nest controls', during which they enter the nest with their forelegs or their whole body, but without nursing (Baumann *et al* 2005). As milk pressure is not the cause (Vasquez Martinez *et al* 1999), a permanent perception of nest stimuli seems to be a more plausible explanation for the multiple nursing sessions (Baumann *et al* 2005). According to Szendrő *et al* (1993) and Coureaud *et al* (2000), pups did not gain more weight when the doe had free access compared to having only controlled access. Pups may benefit from a higher milk intake, but they have an increased chance of being injured or crushed (Coureaud *et al* 2000). Pups that attempt to suckle during a short visit by the mother, when she is only checking the nest, also risk being dragged outside. Furthermore, pups that fail to suckle during a short visit stay on top of the nest material and only dig back into it after several hours. Consequently, in both situations, there is a critical loss of body temperature (Hudson & Distel 1989).

In commercial breeding units for fattening rabbits (*Oryctolagus cuniculus*), pup mortality is 20% or more before weaning (Szendrő *et al* 1991; Koehl 1999; Guerder 2002). Infectious diseases are of minor importance at this stage; instead, pups mainly die from hypothermia, weakness, starvation, trauma, or bites from the doe. These can be attributed to poor nest quality, lack of milk, scattering of pups, crushing, and cannibalism, which can be caused by a restrictive environment and stress (Lölinger & Matthes 1976; Verga *et al* 1987; Kusche 1993).

In this study we investigated the effect of positioning a cat-flap at the nest entrance, thereby combining the advantages of free nest access (free choice of nursing time by the doe, and potentially more milk for the pups) and of controlled access (reduced nest stimuli). The effect of cat-flap access compared to free nest access was investigated by recording the does' nest-related behaviour, general activity, and plasma corticosterone concentration (as a physiological stress parameter), and the pups' mortality, weight, and time of leaving the nest. Assuming a lower level of nest stimuli with the use of cat-flaps than with free nest access, we expected a lower frequency and intensity of nest controls, and a lower increase of corticosterone concentration after the administration of exogenous adrenocorticotrophic hormone (ACTH). Assuming fewer disturbances and less energy loss for the pups, this was expected to lead to a higher weaning weight and lower pup mortality. Because of the closed entrance, pups were expected to leave the nest later than with free access.

## Materials and methods

### Animals and housing

Thirty does and four bucks, all ZIKA hybrid rabbits (predominantly Californian × White New Zealand), were

housed in an experimental unit situated at the Centre for Proper Housing (poultry and rabbits), Zollikofen, from January to August 2001. The animals were bought from the appointed ZIKA breeder in Switzerland, where the does were reared in groups. In this study, the does were housed in individual cages (0.6 × 0.7 × 0.6 m, length × width × height), with a plastic grid floor and an elevated area of the same material (0.6 × 0.32 m, length × width), 0.27 m above the grid floor. A nest box (0.34 × 0.34 × 0.29 m, length × width × height) lined with pine shavings was attached to the cage three days before parturition. To drain off the urine from the pups and nest material, a plastic grid (2 cm thick) was inserted under the shavings and holes were drilled in the nest box floor. Ventilation holes along the upper third of two side-walls allowed air to circulate. The laterally positioned rectangular entrance (15.5 × 15.8 cm, width × height) could be closed with a sliding door. The cat-flap consisted of the flap (15.2 × 15.4 cm, width × height) and a frame on the cage side of the entrance, which moved with the flap when the doe left the nest box and sealed the gap between flap and nest box (outer side 16.2 × 17.0 cm, width × height, inner side 12.2 × 12.0 cm, width × height). Flap and frame were made of light metal and weighed 347 g with and 243 g without frame.

Animals were kept under a constant 14h:10h light:dark cycle (lights on at 0600h) with a stepwise transition of 15 min. During darkness small red light bulbs (25 W) were switched on, allowing video recordings to be made using light-sensitive cameras (PANASONIC WV-BP130), which were placed about 2 m vertically above the cages. Water and pellet food (UFA 856 with 66 mg kg<sup>-1</sup> of the anti-coccidial agent Robenidin) were provided *ad libitum*. The does had free access to straw for feeding and nest construction. Feeding and cleaning of the droppings hull below the cages took place between 1000h and 1100h daily. Temperature and humidity were permanently monitored with HOBO® data loggers at nine different locations; no significant differences were found.

After two weeks of habituation to the new environment, virgin does were mated at the age of 16–18 weeks and then again 11 days after each subsequent parturition. Does with small litters are not able to increase their milk production arbitrarily if pups from larger litters are added to their own litters (uterine induction) (Vasquez Martinez *et al* 1999). Therefore, to prevent any effect on the does' behaviour and pup mortality, litter numbers were not equalised in this study. Pups were weaned at the age of five weeks and given to the same breeder for fattening in groups. After the study, all does and bucks were given to a breeder with group-housing systems.

### Experimental groups

Does were randomly allocated into two groups, each containing 15 does: does that had permanent free access to the nest after parturition (Group O); and does that had permanent free access to the nest, but with the nest closed by a cat-flap from day 0 to day 15 (Group CF). After day 15, the cat-flap was removed. A doe remained in the same group for the whole

study period of up to four litters per doe. Birth monitoring and initial nest closure after birth were carried out at 0800h, 1200h, and 1600h. As soon as a doe had given birth, the litter was examined for stillborn pups, which were removed.

### Data collection

#### *Nest control behaviour, nursing frequency and general activity of does*

For each group, on days 4, 8 and 13 after birth of the second litter, the behaviour of the does was recorded continuously for 24 h by time-lapse video (PANASONIC AG-6730). Because some matings failed to result in pregnancy, the number of does recorded in group O was 14 and in group CF, 10. The transcription of the recordings included continuous sampling for nest-related behaviour and scan sampling for the doe's activity (Martin & Bateson 1993).

For the analysis of a doe's activity, a note was made every 10 min as to whether the doe was active or not. A doe was recorded as being inactive when lying down and performing no additional activities such as grooming. The percentage of scans when activity was recorded was tested for differences between groups and developmental effects (pup mortality, weight, and time the pups left the nest) over the three observational days within groups.

Every approach to the nest entrance by the doe was recorded to analyse nest control behaviour. A bout of nest control behaviour started when the doe's nose came within one head length of the entrance and ended when the doe performed other activities (eg grooming, feeding, drinking, resting, coprophagy) for more than 60 s. However, if the doe resumed nest control behaviour within 1 min, it was recorded as one bout. The nest control bouts were grouped into three different categories according to their duration: short = 1–15 s; medium = 16–60 s; long > 60 s. The frequency of the different categories was tested for differences between the two groups (O and CF), and developmental effects within groups. During a nest control bout, the following behavioural elements were considered:

- Nose — nose at the entrance of the nest box;
- Forehead — head to the ears in the nest;
- Head — ears laid back and the whole head in the nest;
- Forelegs — head and forelegs in the nest;
- Nest visit — whole body in the nest without nursing (leaving the nest again within 10 s);
- Nursing session — whole body inside the nest for more than 10 s;
- Nesting activities — includes scratching movements in front of the entrance (natural behaviour to open the nest burrow) and everywhere on the floor (natural behaviour to collect material for plugging), and tapping in front of the entrance (natural behaviour to plug the nest entrance).

The frequencies of the different behavioural elements were calculated and statistically tested for their distribution during the 1 h before and after nursing, in comparison to hours outside nursing. As the nest control behaviours 'forehead', 'head', 'forelegs' and 'nest visits' could lead to

contacts with the pups, the frequencies of these are given for each behaviour individually as well as being summed for this group of behaviours. As the nest control behaviours 'nose' and 'nesting activities' were performed outside the nest, there is no potential contact with pups and these behaviours are therefore not included in the above sum, but in the sum of all nest controls.

The percentage composition of nest controls was calculated, for each behavioural element, for both groups. The frequency of 'nursing sessions' was tested for differences between groups, developmental effects within groups, and litter size.

#### *Blood corticosterone concentrations of the does*

Both groups of does were tested for differences with regard to chronic stress. Each doe was subjected to an ACTH challenge test on day 15 to examine pituitary–adrenal capacity as a measure of chronic activation of the hypothalamic–pituitary–adrenal (HPA) axis (Manser 1992). A 0.25 ml sample of blood was taken, in the morning of day 15 after parturition, from the ear artery for the measurement of corticosterone baselines. Synthetic ACTH at a concentration of 0.2 ml kg<sup>-1</sup> (Synacthen®) (Novartis: Switzerland) was then injected intravenously into the ear vein to produce a maximal stimulation of the animal's adrenal cortex. After 30 min, a second blood sample was taken from the ear artery of the other ear. The serum of both blood samples was separated by centrifugation and stored at –20°C until assayed. Serum concentrations of corticosterone were determined by radio-immunoassay (Corticosterone <sup>3</sup>H RIA Kit®) (ICN Biomedicals GmbH: Eschwege, Germany). Does were habituated to the procedure from the beginning of the project.

#### *Pup mortality, pup weights and the time pups left the nest*

All 87 litters, born over four reproductive cycles, were checked for dead pups every morning from birth to weaning. Causes of mortality were identified by systematic autopsy at the Animal Hospital in Zurich by a pathologist blind to the experimental groups. Mortality data were analysed for each doe for the total number of pups born and lost, and tested for differences between the two experimental groups.

All pups were weighed individually after birth and at 0815h on days 15 and 35 (before weaning). The data of pup weights were pooled per doe and category (day 0, 15 and 35) and tested for statistical differences between the groups.

For the 24 second litters, the time the pups left the nest was also investigated. The presence of pups in the cage was assessed by checking the video footage.

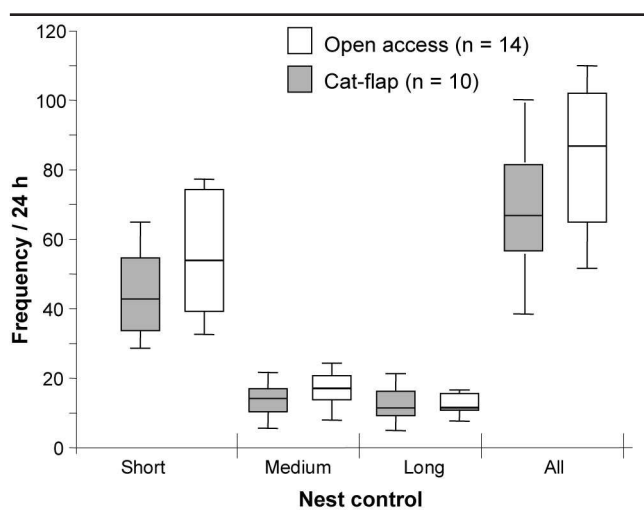
#### Statistical analysis

The effect of nest access on a doe's nest-related behaviour, general activity, plasma corticosterone concentration, and pup mortality was assessed using the Mann-Whitney *U* test (Siegel & Castellan 1988). Tests with inter-dependent data were corrected using the Bonferroni-Holm test procedure. Normally distributed data (pup weights) were tested using the parametric one-way ANOVA test. A Friedman two-way

**Table 1** Frequency of different nest control types and nesting activities at different times in relation to nursing.

Type of nest control	During 1 h before nursing			During 1 h after nursing			Outside nursing times		
	O	CF	P	O	CF	P	O	CF	P
Nest visits	1.67	0.056	ns	0	0	ns	0.015	0	ns
Forelegs	0	0	ns	0	0	ns	0.016	0	ns
Head	0.67	0	ns	0.83	0.083	ns	0.39	0.054	<0.001
Forehead	0.33	0.25	ns	1.0	0.25	ns	0.30	0.29	ns
All contacts	2.67	0.306	ns	1.83	0.333	0.013	0.72	0.34	ns
Nose	2.17	2.86	ns	4.0	4.78	ns	1.85	2.79	0.008
Nesting activities	0.13	0.2	ns	1.83	2.17	ns	0.13	0.33	0.01
All nest controls	4.84	3.17	ns	5.83	5.11	ns	2.46	2.96	ns

Median of 14 does with open access (O) and 10 does with cat-flap access (CF); three 24 h observation periods per doe. ns, no significant difference.

**Figure 1**

Frequency of nest controls with three 24 h observation periods per doe. Short nest control bouts = 1–15 s, medium = 16–60 s, long > 1 min.

ANOVA by ranks test was used to compare the does' behaviour with regard to developmental effects over the three observation days. The Spearman rank correlation was used to test for a relationship between nursing frequency and litter size. All analyses were carried out using NCSS software (Kaysville, USA).

## Results

### Nest control behaviour, nursing frequency and general activity of does

Figure 1 shows the frequency of the different nest control categories (short, medium, long, all). There were no significant differences between groups. From day 4 to day 13, the frequency of all nest controls decreased significantly in group O from 87 to 62 ( $Q = 8.0$ ,  $P < 0.05$ ), whereas there was no significant change in group CF.

The percentages of each behavioural element observed during nest controls, in group O versus group CF

respectively, were as follows: nose 67.3% versus 81.1%; forehead 15.6% versus 14.5%; head 15.0% versus 3.0%; forelegs 0.5% versus 0%; and nest visits without nursing 1.5% versus 1.2%.

The frequency of different nest control behaviours and nesting activities in relation to nursing are shown in Table 1, with data pooled for the three observation days. Does in group O made significantly more head contacts with the nest outside nursing hours than the does in group CF ( $U = 7.5$ ,  $P < 0.001$ ). Outside nursing hours, does in group CF made significantly more nose contacts ( $U = 22$ ,  $P < 0.01$ ) and nesting activities ( $U = 23$ ,  $P < 0.01$ ) than does in group O. There was no significant difference with regard to nest visits, foreleg and forehead contacts. On average, does in group O carried out 0.72 nest controls per hour with potential pup contact outside nursing hours, compared to 0.34 by does in group CF. There was no significant difference between the sum of these contacts (Table 1, 'All contacts').

The nursing frequency over 24 h was significantly lower in group O (1.34) than in group CF (1.9) ( $U = 37$ ,  $P < 0.05$ ). Over the three days of observation, the frequency in group O decreased from 1.6 to 1.1, while it remained the same, at 1.9, in group CF. There was no correlation between litter size and nursing frequency (Spearman rank correlation  $r_s = -0.25$ ).

Over the three days of observation, there was a significant decrease in general activity in group CF (39.9%, 37.2%, and 34.4%,  $Q = 7.4$ ,  $P < 0.05$ ), but not in group O (34.0%, 34.7%, and 29.2%). However, there was no significant difference in the general activity between the two groups of does (group O 32.7% of all video scans, group CF 36.6%,  $n = 144$  scans in 24 h,  $P = 0.0623$ ).

### Blood corticosterone concentrations of the does

The results from the ACTH challenge test on the second litter are presented in Figure 2, which shows the increase of plasma corticosterone concentrations after the administration of Synacthen®. The increase of corticosterone was significantly higher in group O than in group CF (17.1 versus 12.0 ng ml<sup>-1</sup>,  $U = 39$ ,  $P < 0.05$ ).

**Table 2** Individual weight in g (mean  $\pm$  SD and the number of pups weighed) at days 0, 15 and 35 after birth.

Weight	O	CF
Day 0	60.1 $\pm$ 4.54 (n = 491)	62.5 $\pm$ 6.9 (n = 399)
Day 15	268.2 $\pm$ 30.7 (n = 453)	275.0 $\pm$ 40.9 (n = 378)
Day 35	932.9 $\pm$ 82.5 (n = 433)	957.9 $\pm$ 99.9 (n = 374)
Total litters	46	41

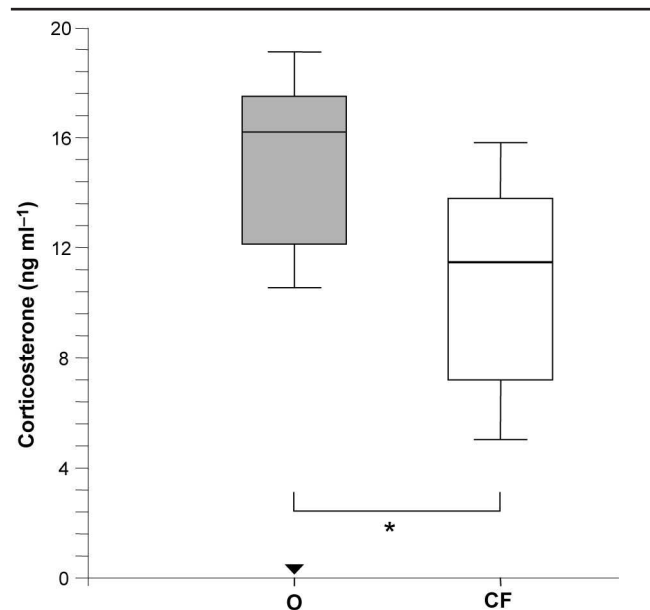
#### Pup mortality, pup weights and the time the pups left the nest

Of the 524 pups born to does in group O, 33 pups were stillborn (3.7%), and of the 491 pups born alive, 58 pups died before day 35 (11.8%). In group CF, of 437 pups, 38 pups were stillborn (8.7%), and of the 399 pups born alive, 25 pups died before day 35 (6.3%). There was no significant difference in the proportion of stillbirths between the two groups. Figure 3 shows pup mortality from day 1 to 15, and from day 16 to 35 over four reproductive cycles. Between days 1 and 15 there was no significant difference in mortality between the two groups. However, between day 16 and weaning at day 35, pup mortality was significantly higher in group O ( $U = 44.5$ ,  $P < 0.01$ ).

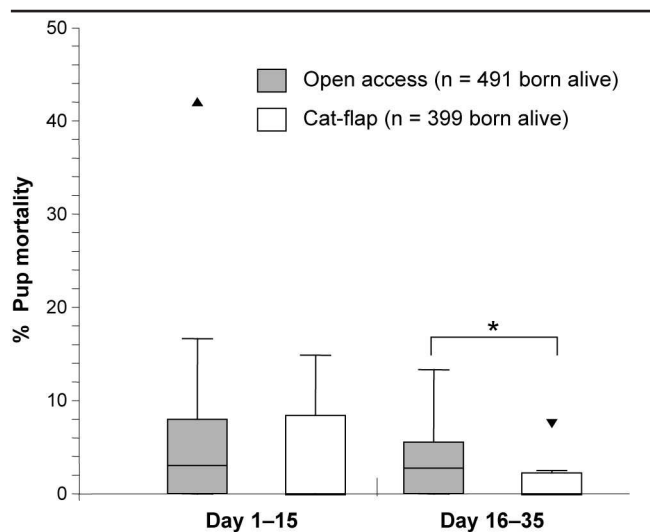
The causes of death in the period between birth and weaning, excluding pups that were stillborn, were similar for both groups. Most pups died from weakness (46.3% versus 38.5%, groups O and CF respectively), unknown causes (25.9% versus 34.6%), infections (13.0% versus 11.5%), doe-related causes (5.6% versus 11.5%), circulatory problems (5.6% versus 3.9%), and digestive disorders (3.7% versus 0%). Doe-related causes led to the death of 3 pups in each group and occurred between the ages of 3 and 18 days. The pups died from being crushed in group O (n = 3), and from bites (n = 1) and nest soiling (n = 2) in group CF. In general, pups died from weakness between the ages of 1 and 28 days, from infection after 7 days, from intestinal problems between days 14 and 19, and from circulatory problems between days 14 and 17. Unknown causes of death, where post mortem examination did not allow a diagnosis, were observed from birth to weaning.

There was no significant difference between the groups in the mean weight of the pups at birth, at day 15 and at weaning over four reproductive cycles (Table 2). As the weight of an individual decreases with increasing litter size resulting in smaller and weaker pups, the litter size at birth among the two groups was tested for differences; no significant difference was found (O = 11.0, CF = 9.9).

The time at which the pups left the nest was investigated for both groups, using data collected from the second litter of pups, with 14 cages in group O and 10 cages in group CF. On day 4, pups in group O were observed outside the nest box in 7% of O cages, whereas no pups had left the nest in group CF by day 4. On day 8, pups from group O were observed outside the nest in 78.6% of all O cages, compared with 40% in group CF. On day 13, pups from group O were observed outside the nest in 85.7% of all O cages compared with 90% in group CF.

**Figure 2**

Increase of corticosterone 30 min after administration of Synacthen® in does with open access (O, n = 14) and cat-flap access (CF, n = 10). \* Significant difference ( $P < 0.05$ ).

**Figure 3**

Percentage pup mortality. \* Significant difference ( $P < 0.05$ ). Triangles are outliers.

#### Discussion

Contrary to our expectations, there was no significant difference in the frequency of nest controls between does in group O and does in group CF. Although nest stimuli were reduced by the use of a cat-flap, the flap itself may have acted as a stimulus; however, playful contact with the door was rarely observed. As expected, does in group O performed significantly more head contacts, potentially disturbing the pups by direct contact with the doe, than does in group CF. In contrast, does in group CF performed significantly more nose contacts and nesting activities outside

nursing times than does in group O. However, pups were not disturbed by the nose contacts and nesting activities as these behaviours were performed outside the nest box.

In both groups, nest closing was the only nesting activity observed. Therefore the cat-flap was not perceived as an obstacle by group CF. Nest closing behaviour was performed everywhere in the cage, as well as in front of the entrance to the nest box, and included the whole range of behavioural elements found under semi-natural conditions, such as scratching, ploughing, tapping, and throwing material backwards between the hind legs using the forelegs. These patterns have not been lost from the behavioural repertoire, despite domestication and being bred in cages for hundreds of generations, which indicates the importance of these patterns. Indeed, nest closing is still fully expressed when domestic does are returned to semi-natural conditions, where adequate material is available (Deutsch 1957; Kraft 1979; Wullschleger 1985; Wieser-Fröhlicher 1986; Stauffacher 1988). Although the cat-flap may have reduced the amount of stimuli being emitted from the nest, it was not sufficient to concentrate nest closing behaviour to the period of time after nursing, as observed with nest removal (Baumann *et al* 2005). The presence of small ventilation holes may have prolonged the nest closing behaviour by the doe. Previous studies have shown that does even try to close ventilation openings (Wullschleger 1985). Thus, the repeated closing behaviour may be a consequence of the doe's inability to remove the nest stimuli.

As expected, the increase of corticosterone after the ACTH challenge was higher in does in group O than those in group CF. A higher production of corticosterone is linked to a thicker adrenal cortex, which is developed when the HPA axis is activated chronically (Manser 1992; Drescher & Breig 1993). Thus, does using a nest box with an open entrance appear to be more stressed than does using a nest box with a cat-flap entrance. Social isolation (from adult social partners), insufficient space and a barren environment (Manser 1992) are possible causes of stress that were present in both groups. These causes, combined with a higher level of olfactory nest cues, the visually open entrance, acoustic stimuli from the pups and the earlier time at which the pups leave the nest, may be responsible for this chronic activation. However, interpretation of the results of this adrenal function test seems to be controversial (Rushen 1991). The test should be applied over several consecutive litters, as variations between individual litters in terms of internal and external factors may result in differences in corticosterone concentration (Manser 1992). In addition, natural individual difference can be high, both for peak corticosterone concentration and time to peak after ACTH challenge (Faulhorn 1979). This test may be more suitable for laboratory animals used to handling to avoid any influence of the handling itself. More research is needed to study the exact influence of each of these factors.

The frequency of nest controls decreased significantly in group O over the three observation days. This may have been a result of the increasing number of pups leaving the

nest box. Under semi-natural conditions, pups leave the nest at the age of about 20 days (Hudson & Distel 1982; Bigler 1986). In our study, pups were observed to leave the nest as early as four days after birth. Eight days after birth, pups were found outside the nest box in 79% of the cages in group O, which is comparable to when pups leave the nest in battery cages (Bigler 1986). Pups from group O left the nest earlier than pups from group CF. This may have been caused by the potentially higher rate of disturbances experienced by pups in group O.

Once pups had left the nest, they were observed to repeatedly approach the doe to suckle, and the doe always reacted by immediately moving away. Does in group O were molested by these suckling attempts sooner after birth than does in group CF because the pups left the nest box earlier. This is supported by the fact that, with an open entrance, the significant decrease in nest controls that occurred between birth and day 15 was not linked with a significant decrease of activity. This could indicate a shift from nest-related to other pup-related activities in the cage, such as escaping from pups after suckling attempts. The cat-flap prevented this by retaining the pups in the nest box for longer, but also complicated the process of the pups returning to the nest when they ended up in the cage involuntarily (eg when pups were dragged out of the nest while suckling, or when they left the nest box through the open cat-flap while the doe was entering or leaving the box).

As expected, pup mortality was significantly higher in group O, but only from day 16 to weaning (day 35), which is after the opening of the entrance in both groups. The main cause of death was weakness: pups were nursed significantly less during the first 15 days; had potentially more direct contacts with the doe outside nursing time, leading to doe-related losses and to weaker and more vulnerable pups; and they were disturbed in their energy-saving strategy of staying huddled together in the insulating nest material (Hudson & Distel 1989).

Weaning weights of litters in group CF were expected to be significantly higher than those of litters in group O because of the combined advantages of free nest access (free choice of nursing time for the doe with potentially more milk for the pups) and reduced nest stimuli (resulting in a lower frequency and intensity of nest controls and fewer disturbances and less energy loss for the pups). However, no significant difference in pup weights was found between the two groups. It is possible that two weeks of different nest access conditions were not sufficient to produce a long-lasting effect on the pups' weights.

#### Animal welfare implications

This is the first study to assess a system of nest access that closes up the nest entrance while still ensuring a free nursing rhythm for the doe. While the installation of a cat-flap at the nest entrance was of some advantage to the pups, the does benefited less than expected.

The installation of a cat-flap at the nest entrance resulted in a lower pup mortality compared to nest boxes with open

entrances. With a cat-flap entrance, most of the nest controls were performed outside the nest box, without leading to pup contact, disturbance and injuries. Therefore, these controls had no aversive effect on the pups' survival and development. The cat-flap seemed to work as a barrier for the pups, as pups with open entrances left the nest earlier, molesting the doe with repeated attempts to suckle.

Nevertheless, the high number of nest controls carried out by does with a cat-flap at the nest entrance indicates that, as long as there is a nest box attached to the cage emitting olfactory nest stimuli, these does still encounter a problem. Because of the odour escaping, eg through the ventilation openings, into the restricted cage space, the does can never eliminate or avoid the olfactory stimuli of the litter and the nest. As long as pup odour is perceivable by the doe, the situation has to be regarded as inadequate and potentially overtaxing the does' adaptability. The problem can be solved by removing the nest box from the cage after nursing at a set time once per day or by increasing the distance between doe and nest box. This can be achieved by increasing the cage size or by group housing in pens.

Removal of the nest box after nursing improved the does' situation, but this is problematic for commercial units because of the increased amount of work (Baumann *et al* 2005). Group housing in pens requires non-aggressive hybrids or races and increased monitoring. This type of breeding has been successfully practised in Switzerland and the Netherlands (Bigler & Oester 2003), and group housing is considered to be an acceptable and recommendable alternative to single housing.

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