

NEW ZEALAND WHITE RABBITS SHOW NON-SELECTIVE NURSING IN VARIOUS TYPES OF NESTS

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ABSTRACT: We investigated the capacity of New Zealand White female rabbits to nurse their litter in a nest different from their own (i.e., from another female, in a box containing synthetic or male hair, or in a new box containing part of the original nest material). In females that nursed in their own nest across lactation days 1-3 (one nest-condition) the mere addition of any of the above boxes (without pups) across days 4, 5, 6, 7, or 8 provoked an increase in the latency to enter their own nest for nursing (0.05 ± 0 vs. 2 ± 1 min; $P < 0.01$; Group 1A). In the two-nest condition females took a longer time to enter any box for nursing than they did to enter their own. These differences were significant only for the synthetic nest (4 ± 1.5 min; $P < 0.05$). When the two-nest condition began on lactation day 1 (Group 1B) the latency to enter significantly increased only with the male nest (8 ± 4 vs. 1 ± 0 min, own nest; $P < 0.05$). The time inside the nest box devoted to nursing was practically the same under all conditions and within the normal duration (ca. 3 min). Milk production rose steadily across the days of observation, regardless of the type of nest-box the litter was placed in. We also explored the effect of using alien kits, rather than the female's own, on the rabbit's capacity to nurse them in any of the above nest types (Group 2). The only differences between Group 2 and Group 1B were: a) on day 1 females nursing an alien litter produced less milk (48 ± 5 g) than the two groups nursing their own (69 ± 5 , 75 ± 8 g; $P < 0.02$); b) the latency to enter the male nest was smaller in Group 2 (2 ± 2 vs. 8 ± 4 min; $P < 0.05$). These results show that, although able to distinguish among different nest types, rabbits can nurse their own or alien kits in several environments. These findings can be useful in small farm rabbit husbandry practices for facing the problems of kit death or nest deterioration in early lactation.

Key words: Nest, nursing, maternal behavior, selectivity, milk production, rabbit husbandry

INTRODUCTION

The display of maternal behavior and nursing among mammals can be selective or not. In the former case, parturient females will suckle only a specific individual, which they can reliably identify (usually the one they gave birth to), while in the latter case they will accept any newborn (of the same species) for nursing (for reviews see: Nowak *et al.*, 2000; González-Mariscal and Poindron, 2002; Numan *et al.*, 2006). Examples of selective nursing are sheep (Poindron and Le Neindre, 1980), goats (Poindron *et al.*, 2006), and cattle (Hudson and Mullord, 1977) while non-selective nursing is observed in pigs (Jensen, 1986; Algers, 1993), rodents (Rosenblatt and Lehrman, 1963; Ebensperger *et al.*, 2006), and cats (Rosenblatt *et al.*, 1985, 1962). Regarding rabbits we know, from everyday breeding practices in the laboratory (González-Mariscal *et al.*, 2000) and on the farm (Lebas, 1984; Lukefahr, 1992; 2004), that mothers will readily nurse kits from litters other than their own. In contrast, studies of wild rabbits living in natural or semi-natural conditions have shown that a mother rabbit will nurse only her own kits, i.e., those found in the maternal nest she built before delivery (Hoy and Selzer, 2002; von Holst *et al.*, 2002). These apparently contradictory findings can be interpreted to suggest that lactating rabbits can identify their own nest (rather than their own litter) and will nurse the

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individuals found inside it. To test this possibility in the present work we investigated the effect of placing a lactating rabbit's own litter in a nest different from her own on the display of nursing (Experiment 1). We also explored if maternal behavior and nursing were modified by placing kits from another female, either in the mother's own nest or in an alien one (Experiment 2).

MATERIALS AND METHODS

Animals

New Zealand White nulliparous rabbit females (body weight 3.5-4.0 Kg), kept under controlled light (14L:10D; lights on at 07:00 am) and natural temperature conditions were used. They were housed in individual wire mesh cages (62 cm length \times 42 cm width \times 40 cm height), fed 350 g of Purina rabbit chow/day, and provided with water *ad libitum*. Females were mated with males of the same strain inside a round wire mesh arena (1 m diameter), after which they were returned to their home cages.

General experimental procedure

On pregnancy day 21 rabbits were transferred to large, wire mesh, maternal cages (180 cm length \times 42 cm width \times 42 cm height; Figure 1). A wooden nest box (50 cm length \times 29 cm width \times 27 cm height), with a round (24 cm diameter) opening in the front, was introduced and left inside the maternal cage until the day of parturition. This type of nest box, used throughout the experiment, does not have a door that limits the female's entrances into it. By being permanently open this box allowed the females to construct the maternal nest inside it with straw (which we provided daily until parturition) and their own body hair. Starting on pregnancy day 30, females were spot-checked across the day to determine the approximate time of delivery. At parturition mothers were left undisturbed for 5-8 hours after which kits were removed, litter size was adjusted to eight and placed, in a different room, inside a box containing paper shavings, under a mild heat source. On the following days we performed the maternal behavior tests between 11:00 and 13:00 hours. We began each test by weighing the litters and introducing the kits into the corresponding nest box (see below). We then quantified three parameters, characteristic of maternal behavior, as described earlier (González-Mariscal *et al.*, 1994, 2000): a) latency to enter the nest box (time elapsed since the kits were introduced into the nest box until the mother jumped inside it); b) duration of nursing (time elapsed since the mother entered the nest box until she jumped out of it); c) milk output (kits' body weight after nursing minus the one shown before nursing). The test ended when the female jumped out of the nest box. At this time the nest boxes were removed from the female's cage. They were returned on the following day only for the duration of the test.

Experiment 1: own litter in own vs. unfamiliar nests

To determine whether the mere introduction of a second nest box could have an impact on any of the parameters above described, we determined in Group 1A (n=34) the display of maternal behavior and nursing across postpartum days 1 to 3, with only the female's own nest box present. Across days 4 to 8 we determined maternal behavior and nursing with two nest boxes present: the female's own plus one of the following: a) clean wooden box containing straw plus synthetic hair ("synthetic nest"); b)



Figure 1: Large cage used to accommodate two nest boxes for experiments 1 and 2.

clean wooden box containing straw plus male hair (obtained by shaving adult males from our colony; “male nest”); c) female nest (i.e., wooden box where another female from the experiment had built her nest with straw and her body hair; “alien female nest”); d) clean wooden box containing part of the material from the female’s own nest, which we removed on the day of the experiment (“new own nest”). The female’s own litter was introduced in either her own nest box or in any of the “unfamiliar” ones. Immediately afterwards we quantified the latency to enter the nest box that contained the kits, the duration of nursing, and milk output, as described above. The order in which females were exposed to the different “unfamiliar” nest boxes (simultaneously with their own nest box) was counterbalanced among animals. Additionally, the sites where the female’s own nest box and the “unfamiliar” one were placed inside the maternal cage were changed randomly across experimental trials. In Group 1B (n=12) the difference was that the simultaneous presentation of the two nest boxes, one of which contained the kits, was initiated on lactation day 1 and continued into day 5. Again, the order of presentation of the different types of unfamiliar nest boxes (together with the female’s own) was counterbalanced among females and the location of the two nests within the maternal cage was changed across experimental trials.

Experiment 2: alien litter in own vs. unfamiliar nests

We followed the same design as for Group 1B, except that the kits used across lactation days 1 to 5 were not the female’s own but, rather, came from a different mother (“alien litter”): Group 2 (n=12).

Statistical analysis

A two-way ANOVA for repeated samples was used to compare across experimental days the latencies to enter the nest box, the time inside the nest box, and milk output. When pertinent, i.e., when the ANOVA showed a value of $P < 0.05$, Mann-Whitney’s U test was used to compare a specific parameter between two groups on a given day. Wilcoxon’s test was used to compare a specific parameter within a group under two experimental conditions (e.g., latency to enter its own vs. the male nest; Siegel and Castellan, 1974).

RESULTS

Experiment 1

All females in this group showed an interest in the kits as they were placed in the nest box: they sniffed them from outside and, after a variable time, they entered and adopted the crouching posture over the litter characteristic of nursing. Yet, the latency to jump into the nest box varied significantly according to the experimental condition. Rabbits in Group 1A (which had been nursing for three days in their own nest box before an additional one was introduced) took a longer time to enter any of the nest boxes introduced across lactation days 4-8 (including their own), with respect to the value shown on day 3 of the one-nest condition ($P < 0.01$; Figure 2). When comparisons of latencies were made within the two-nest condition, the only significant differences were between the synthetic nest and the female’s own ($P < 0.05$). By contrast, females in Group 1B (which were exposed to two nest boxes from day 1 onwards) showed significantly longer latencies to enter when their litter was placed in the male nest ($P < 0.05$; Figure 3). Once inside the corresponding nest box females from both groups (i.e., 1A and 1B) showed across all test days an almost invariable duration of nursing, i.e., ca. 3 min, which is characteristic of normal rabbits (Table 1). Milk output steadily increased in all females as lactation progressed; the only significant difference in this parameter was observed on day 4, when Group 1A showed lower values than Group 1B (Table 2).

Experiment 2

Presenting the females with an alien litter for nursing did not significantly modify their maternal responsiveness in relation to the group provided with its own kits (i.e., Group 1B; see Figure 3). Thus, the latencies to enter the female’s own nest or any of the other ones that contained the litter

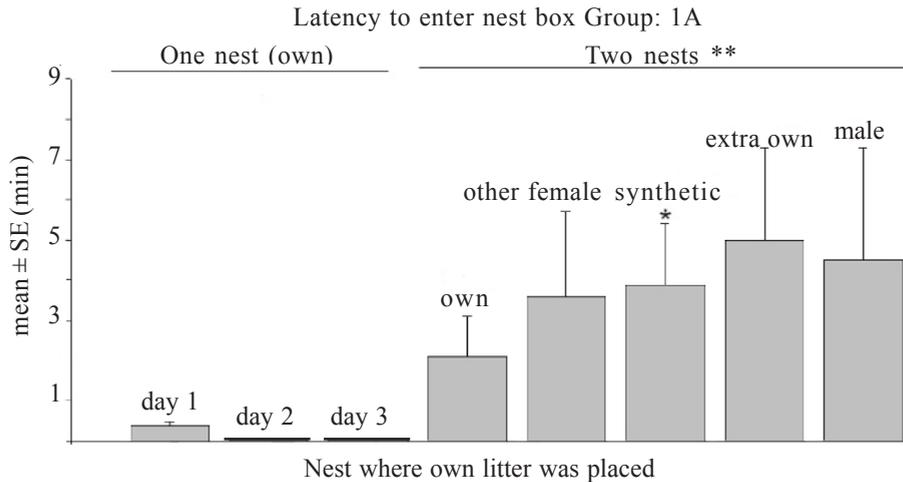


Figure 2: Latency to enter the different types of nest boxes provided to females of Group 1A: across lactation days 1-3 these animals were given only their own nest box. Across days 4-8 a different type of nest box was added daily, randomizing the order of presentation among experimental animals. * $P < 0.05$ vs. own nest, during the two-nest period; ** $P < 0.01$ vs. own nest, day 3 of the one-nest period.

were not significantly different between Group 1B and Group 2, except for the male nest condition. In this case the latencies shown by Group 2 were significantly smaller than the ones observed in Group 1B. By contrast, the time inside the nest box was practically the same as the one observed in the other two groups (ca. 3 min; Table 1). Milk output, however, was significantly lower in mothers given an alien litter (Group 2), specifically on lactation day 1 (Table 2). No significant differences with respect to the other two groups were observed in this parameter on any other day.

DISCUSSION AND CONCLUSIONS

To our knowledge, this is the first work to formally explore the impact of the identity of the kits (i.e., own or alien) and the nature of the maternal nest on the characteristics of rabbit maternal behavior. Our results indicate that rabbits do not show selective nursing, i.e., they effectively suckle their own and alien kits, regardless of the nest they are placed in. Yet, small alterations in maternal behavior were noted, for instance: the increased latency to enter the nest box under specific conditions (i.e., synthetic nest in Group 1A and male nest in Group 1B) suggests that females are able to recognize the lack of a “rabbit cue” in the former case and the presence of an “inadequate” one in the latter.

Table 1: Time inside nest box (min; mean \pm standard error).

Litter type	Nest where litter was placed				
	own	other female	extra own	synthetic	male
Own Group 1A (n=34) ¹	3.7 \pm 0.3, days 1-3 3.2 \pm 0.1, days 4-8	3.3 \pm 0.12	3.1 \pm 0.1	3.0 \pm 0.2	2.9 \pm 0.1 ²
Own Group 1B (n=12)	3.0 \pm 1.0	3.0 \pm 1.0	4.0 \pm 1.0	4.0 \pm 1.0	3.0 \pm 1.0
Alien Group 2 (n=12)	3.0 \pm 0.1	4.0 \pm 0.3	3.0 \pm 0.3	4.0 \pm 0.4	4.0 \pm 0.3

¹Across days 1-3 Group 1A was given its own litter in its own nest without any additional nests present; across days 4-8 its own litter was placed in any of the nest-boxes described, in the presence of its own (empty) nest box. Group 1B and Group 2 received the two nest boxes (i.e., their own and the “unfamiliar” one) across days 1-5; the corresponding litter placed in only one of the two nest boxes. ²Only half of the females in this group (n=17) were tested with a “male nest”.

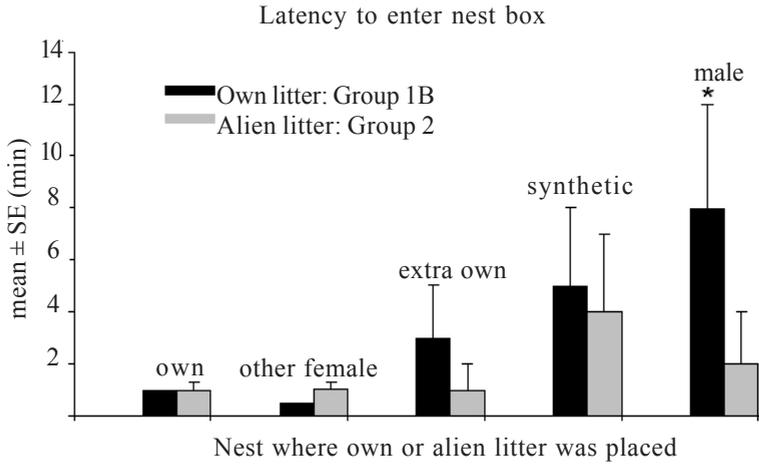


Figure 3: Latency to enter the different types of nest boxes, provided across lactation days 1-5, to females in Group 1B (tested with their own litter) and in Group 2 (tested with an alien litter). * $P < 0.05$ vs. own nest, with own litter and vs. Group 2, male nest.

Although not specifically tested, it is likely that such cues are olfactory as the visual characteristics of all nests were very similar. These observations agree with previous work from our laboratory in which shaved pre-parturient females were provided with several types of hair for nest-building (González-Mariscal *et al.*, 1998). The latencies to collect hair were very short for the female’s own and for synthetic hair and were longer for male hair. Yet, despite such long latencies rabbits did collect even the male hair and used it to line the straw nest previously constructed inside the wooden box. These observations emphasize the importance of olfactory signals in the regulation of rabbit behavior, as revealed by many studies performed in kits and in adults (for review see Schaal *et al.*, 2006).

Taken together, our results indicate that, although able to distinguish among different types of hair pre-parturient rabbits can overcome a presumed aversion for a specific one and use it to perform a critical maternal activity (nest-building). In the present work although the male nest may have been perceived as “inadequate” by some animals all the rabbits overcame a possible dislike for it, entered the box, and nursed the kits normally. Indeed, the time devoted to nursing was remarkably homogeneous across experimental conditions (around 3 min). This finding agrees with the numerous reports on the brief and constant duration of nursing in rabbits kept in semi-natural conditions (Hoy and Selzer, 2002), in the farm (Matics *et al.*, 2004), or in the laboratory (Cross, 1952; Zarrow *et al.*, 1965; Lincoln, 1974; Drewett *et al.*, 1982; González-Mariscal *et al.*, 1994; Hudson *et al.*, 2000). Taken together, the above findings indicate that female rabbits can: a) distinguish among different types of nesting materials, b) prioritize maternal activities over specific aversions, and c) display normal nursing episodes once such aversions are overcome.

The addition of an extra nest *per se* (i.e., independent of its type) altered the females’ behavior as their latency to enter even their own nest was increased with respect to the value observed on day 3 in the one-nest period. Moreover, during the two-nest period the latencies to enter were even larger for one type of nest (synthetic), compared with the female’s own. By contrast, in Group 1 B, that

Table 2: Milk output (g; mean ± standard error).

Litter type	Days of lactation							
	1	2	3	4	5	6	7	8
Own Group 1A (n=34) ¹	69±5	69±5	90±5	87±5 ⁴	102±4	111±5	129±6	154±9
Own Group 1B (n=12)	75±8	78±5	99±14	118±14	120±10			
Alien Group 2 (n=12)	48±5 ^{2,3}	72±7	89±8	94±9	108±8			

¹See notes in Table 1. ² $P < 0.02$ vs. Group 1B. ³ $P < 0.01$ vs. Group 1A. ⁴ $P < 0.03$ vs. Group 1B

received the two nests from lactation day 1 onwards the latencies were significantly larger only towards the male nest. These results show that alterations in the female's immediate environment, though perceived by the animal, modify her maternal responsiveness only slightly.

The findings from Experiment 2 indicate that lactating rabbits do not discriminate between their own and alien kits for nursing. Although we cannot know from our results whether they are able or not to distinguish between these two types of litters it is clear that they will suckle any kits placed in any type of nest box. These results can be valuable for raising rabbits in the laboratory and on the farm as it is not infrequent for kits to die, especially in early lactation. Under such conditions it may be more efficient to remove the few remaining kits from a given female and place them with another lactating animal. Similarly, nest deterioration (provoked by management practices, humidity, heat, etc.) can be easily overcome by using a variety of nesting materials. These strategies may be especially adequate in small production systems where much of the activities of rabbit husbandry are done by hand (Lukefahr, 2004).

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