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FACTORS CONTRIBUTING TO THE EFFICIENCY OF SUCKLING**

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# **NIPPLE-SWITCH AND NIPPLE-SEARCH BEHAVIOUR: FACTORS CONTRIBUTING TO THE EFFICIENCY OF SUCKLING**

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## **ABSTRACT**

Despite of their pup's low maturity at birth, wild and domestic rabbits show extremely limited maternal care. Although nursed only once a day rabbit pups grow rapidly since special feeding strategies are used. Pups try to maximise milk intake by efficient nipple search behaviour and nipple switching. We were interested in the age dependency of these behaviours. We measured the impact of disturbing the mother-young interaction on milk intake by the pups and the effect of ageing on the learning associated with locating the nipples. The nipple search time decreased significantly with age while the nipple-switching showed an increasing trend, as the pups grow. Compared to control mothers with free access to their litter, disturbing the doe either by sedation or anaesthesia reduced the amount of milk available and increased the competition among the pups.

## **INTRODUCTION**

Both wild and domestic rabbit show extremely limited maternal care. During the one month of lactation they nurse their pups only once each day (Broekhuizen and Mulder, 1983). These nursing visits are extremely short, and last for only 3-5 minutes. Meanwhile the pups are closed into the nest, so they have to suckle as much milk as possible (Hudson et al, 1996). As the daily behavioural pattern of the mother and the pups are well synchronised (Hudson & Distel, 1986) and there is a so called nipple search pheromone, present on the mother's belly which acts as a start signal and successfully assists them in finding the nipples in 2-3 seconds (Drewett et al, 1982; Hudson and Distel, 1989). Additional odours on the mother's belly are also learnt and release the same behaviour (Bilkó et al, 1997).

It is interesting that pups do not remain on the same nipple, but switch them frequently, which apparently reduces the suckling time and thus possibly milk intake. Previous studies have shown that nipple-switching is very common and frequent (3/minute) (Hudson & Distel, 1989) and its frequency changes with the age of pups (Drewett et al, 1982). However, in those experiments the mother was sedated or placed on her back and only one pup was allowed to suckle at a time. From our experience with wild rabbits (Hudson et al, 1996) we assumed that this high frequency of nipple-switching might have been due to the artificial situation that disturbs both the mother and the pups and thus influence milk letdown (Cross, 1953).

Therefore the aim of the first experiment was to analysed the change of reactivity to the nipple. The aim of the second experiment was to examine how the conditions of nursing affect on the milk intake in domestic rabbits and how it changes with the age of pups.

## **MATERIALS AND METHODS**

### Animals

We used 18 multiparous domestic chinchilla-breed does (Chbb, Tomae, Biberach, Germany) and their litters. Does were kept singly in standard metal rabbit cages (45x75x35 cm

are widthxdepth) from there weaning at  $20 \pm 2$  °C and on a 14L/10D light-dark cycle, with lab chow (Altromin®) and water available ad libitum. Several days before parturition they were also provided with plastic nest boxes (40x30x30 cm), attached to the home cage, and hay for nest building. On the day of birth (day 0) pups were weighted, individually colour-marked on the ears, and the litters were culled to 8 pups per litter in the first experiment, and to 4 pups per litter in the second experiment. We used independent litters in the 2 experiments. Mothers were allowed in to nurse the pups between 9.00-11.00 each morning.

### Experiment 1

In this experiment six litters of seven or eight pups in each were used (46 pups). They were randomly divided into three experimental groups. One third was tested on the second, one third on the fourth and one third on the sixth day postpartum. In the nipple search test pups were put one by one for 90 seconds on a heated (36 °C) rabbit fur surrounded by a 25x25x20 cm plexy -glass arena. The duration of nipple search behaviour within this period was recorded. Each pup participated in two tests in random order (one on milk scented and one on unscented fur). For the milk scent, two drops were put in the middle of the arena for each test session. The milk was taken just before use from the pup's mother, as it has been shown that in room temperature the nipple-search pheromone loses its effectiveness in 20 min (von Strahlendorff and Hudson, 1990).

### Experiment 2

In this experiment animals were randomly divided into one of the 1 control and 3 treatment groups each of which consisted of 3 litters of 4 pups. The pups were labelled with coloured ribbons on the belly. On the fifth, tenth and fifteenth days the normal nursing time was video recorded (with Panasonic RX20 camera). In the "normal situation" mothers nursed their young in a glass bottomed nursing box. The equipment was lit from below and nursing was filmed by using a mirror. The record was started, when the doe jumped in the box and continued until she left. In the second group mothers were restrained on their back and 4 pups were placed on the belly to suckle. A square shaped arena (39x11.5x12cm) prevented them from falling down. In the so called sedated mother situation the does were restrained on their back and were sedated with ketamine and xylazine (0.1 mg/ kg body weight). The last group was exactly the same except that only one pup suckled at a time. The data of the first three minutes were used. The number of nipple-switching, the time spent searching and the time spent on nipples were calculated. Pups were weighted before and after the nursing, the difference of which gave the milk intake.

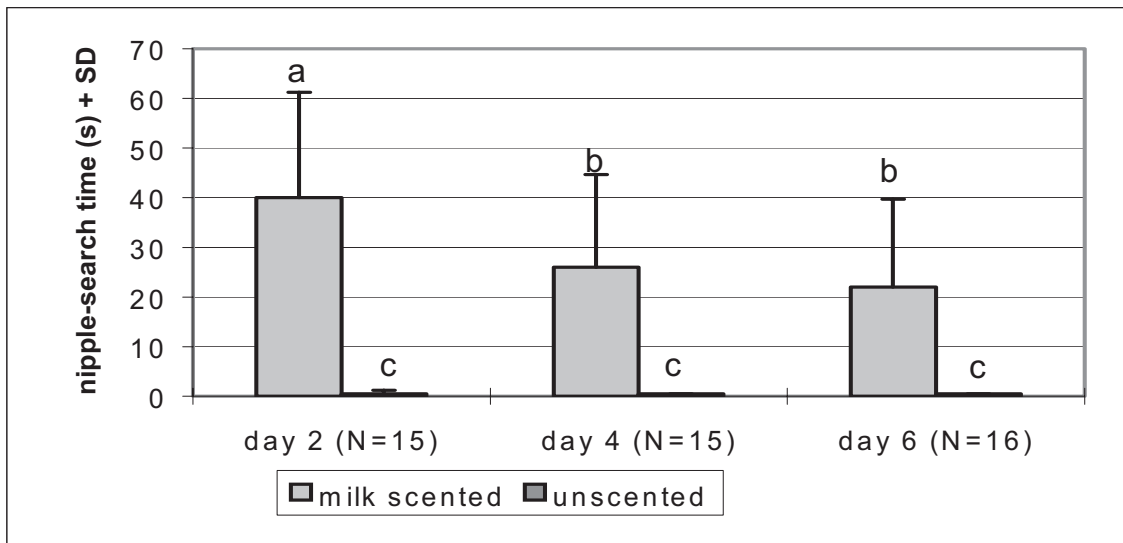
We used One-Way Analysis of Variance (ANOVA) in the first experiment and Two -Way with repeated measures ANOVA in the second experiment. Post hoc Duncan Test was used when significant difference was found between groups.

## **RESULTS**

### Experiment 1

As it can be seen on Fig. 1, the mothers' milk elicits the stereotype nipple-search behaviour at any ages, however, the unscented heated fur was not sufficient to do so (ANOVA,  $F(1, 45)=22.3$ ,  $p<0.001$ ). The nipple search duration on the unscented fur was shorter and this did not change across age (ANOVA,  $F(2,43)=1.035$ ,  $p=0.36$ ). The reaction to the milk however changed with the age of pups (ANOVA,  $F(2,43)=3.69$ ,  $p=0.03$ ). According to the Post hoc Duncan Test the two-days-old litters spent significantly more time with searching than the four and six days old litters however, the latter two was not significantly different from each other.

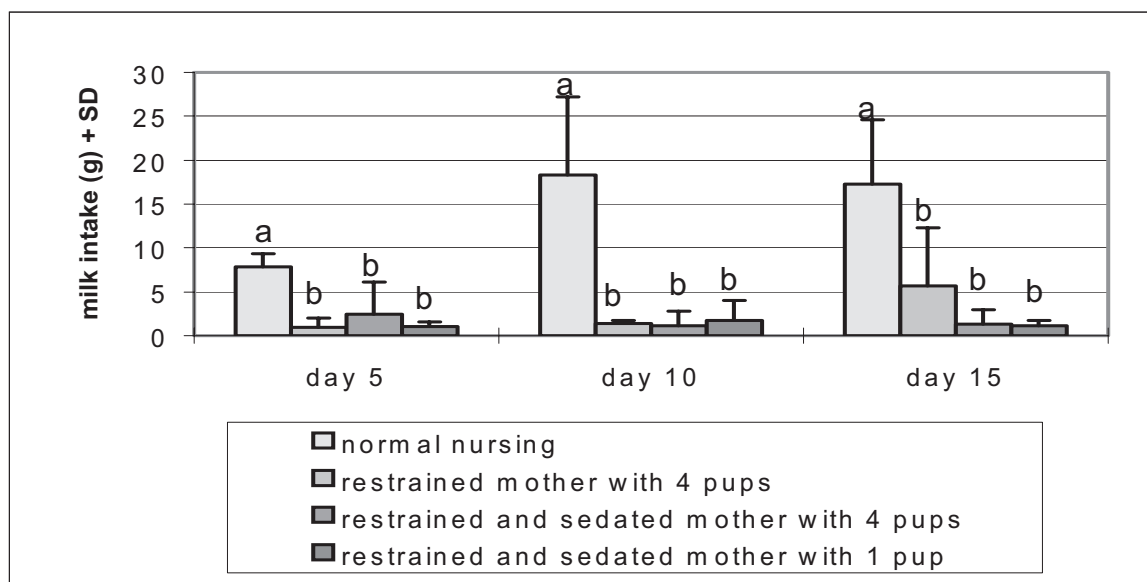
**Figure 1** The time (mean +SD) spent with nipple-search at 3 different ages on milk scented and unscented fur. Significant differences among the experimental groups (One-Way ANOVA followed by Post hoc Duncan Test) are indicated with different letters.



Experiment 2

As Fig. 2 shows, milk intake was significantly higher in the normal nursing situation than in any of the three other test treatments at all investigated age groups (5, 10 and 15 days), (Two-way ANOVA;  $F(3,8)=11.72$ ,  $p=0.003$  for treatment;  $F(2,16)=3.52$ ,  $p=0.054$  for days and  $F(6,16)=2.8$ ,  $p=0.047$  for interaction). However the interaction between the two independent variables showed that milk intake in the experimental group was a function of age.

**Figure 2.** Milk intake of pups (mean +SD) at 3 different ages in the different treatments. Significant differences (Two-Way ANOVA) among the experimental groups are indicated with different letters.



**GENERAL DISCUSSION**

In the present study we examined two phenomena: (1) Do the maternal pheromone induced nipple-search behaviour change with pups' age and (2) How do different nursing conditions affect milk intake.

In the normal nursing situation milk intake changed significantly across the 3 age groups. The lower milk intake of the younger (five days old) pups can be explained by the changing milk production of the doe (Kustos et al., 1996). To maximise milk intake pups can rapidly localise nipples, guided by a so called nipple-search pheromone (Hudson and Distel, 1989). The duration of nipple search activity triggered by milk has changed with age. The activity of the two days old pups was twice as high as the older pups, probably due to their higher reactivity. This explanation seems to be supported by previous findings showing that pups are able to associate any biologically relevant odour with suckling on the first few days postpartum (Hudson and Distel, 1986). The pups extreme motivational state at that age is also supported by the occurrence of searching behaviour even on unscented fur. In this case the poor motor coordination and lack of experience of younger pups is compensated by their much higher reactivity and therefore more robust nipple search behaviour.

Our present results revealed that the experimental manipulation of the nursing (disturbed the mother and /or the pups) greatly influenced milk intake. Both restrain and sedation of the doe led to a significantly lower milk letdown compared to a situation when nursing was undisturbed (normal nursing situation). It was shown earlier that, as an exception among mammals, sedatives could block milk-ejection in the mother rabbit (Cross, 1953) and that this effect is dose-dependent (Neve et al, 1982). The present study showed that the restrain of the mother on her back alone is stressful enough to block milk-letdown and this might reduce the pup's growth.

In conclusion, spontaneous nursing behaviour and learning processes interact in the rabbit pups enabling them to obtain full maternal service during the very short nursing visits.

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