Reprod. Nutr. Dev. 40 (2000) 19–32

Original article

Immediate postnatal sucking in the rabbit: Its influence on pup survival and growth

Gérard COUREAUD[a,b], Benoist SCHAAL[a]*, Pierre COUDERT[c], Patricia RIDEAUD[b], Laurence FORTUN-LAMOTHE[d], Robyn HUDSON[e], Pierre ORGEUR[a]

[a] Laboratoire de Comportement Animal, INRA / CNRS, URA 1291, Station de Physiologie de la Reproduction, 37380 Nouzilly, France
[b] Unité Expérimentale de Pathologie Animale, Domaine du Magneraud, INRA, 17700 Surgères, France
[c] Laboratoire de Pathologie du Lapin, Station de Pathologie Aviaire et Parasitologie, INRA, 37380 Nouzilly, France
[d] Station de Recherches Cunicoles, INRA, Castanet-Tolosan, France
[e] Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México

(Received 13 September 1999; accepted 15 November 1999)

Abstract — This study was aimed at characterising the impact of immediate postnatal sucking on pup survival and development. The interactive effects of postnatal success with the day 0 weight of pups, the nest-access regimen (controlled or free) or parity of females was investigated. Pups (n = 900) were categorised according to their initial ingestion of colostrum. In primiparous does: (1) pup mortality between d0-d10 was higher for unsuccessful than for successful early suckers; (2) lighter d0-weight reduced survival for unsuccessful but not for successful pups; (3) free nest-access of females annihilated the survival advantage fostered by the initial sucking success. In secondiparous does, these impacts waned. Finally, whatever the does' parity, only d0-weight influenced pup weight-gain between d0-21. Thus, pup survival seemed to depend (at least in primiparae) on their ability to suck right after birth, and to display a pattern of energy saving without being disturbed by the females' nest entries.

rabbit (*Oryctolagus cuniculus*) / newborn / sucking / colostrum / survival / growth

Résumé — **La tétée initiale du lapereau : influence sur la survie et la croissance**. L'impact de la première tétée sur la survie et la croissance des lapereaux (L ; n = 900) a été étudié en considérant les effets interactifs du poids néonatal, du régime d'accès au nid (contrôlé ou libre) et de la parité des femelles. Chez les femelles primipares : (1) la mortalité entre J0-J10 est plus élevée pour les L qui échouent à la 1re tétée ; (2) un faible poids à J0 réduit les chances de survie uniquement des L qui ne

* Correspondence and reprints
E-mail: schaal@tours.inra.fr

tètent pas initialement ; (3) le libre accès au nid des femelles estompe l'avantage sur la survie conféré par un succès à la 1re tétée. Chez les femelles secondipares, ces conséquences disparaissent. Finalement, et indépendamment de la parité, seul le poids à J0 influence le gain de poids des L entre J0-J21. Au total, la survie des L semble dépendre (au moins dans les portées de primipares) de leurs capacités à téter immédiatement après la naissance et à limiter leurs pertes énergétiques.

lapin (*Oryctolagus cuniculus*) / nouveau-né / tétée / colostrum / survie / croissance

INTRODUCTION

The initial behavioural task of a newborn mammal is to locate its mother, find the mammary region on her body, seize a nipple or teat, and express coordinated sucking behaviour. Mammalian mothers stand or lie still, adopting a special posture that facilitates the investigative activities of their offspring. This complementary interchange normally ends with a first successful bout of nutritive sucking within a short delay after birth in both precocial newborns (for example, wildebeest calves: 8.2 min, range: 2–20 min [12]; piglets: 24 min, range: 5–66 min [19]; lambs: 28 min, range: 18–46 min [41]; kids: 49 min, range: 14–185 min [29]) and altricial newborns (for example, rat pups: 60 min [36]; kittens: 65 min, range: 10–114 min [14]).

These initial successful sucking episodes have major physiological and behavioural importance. They favour the ingestion of colostrum, which is present in the mammary gland in the last days before term and in the postpartum days preceding lactogenesis. The intake of colostrum promotes a variety of adapted responses in mammalian neonates: (1) in doing so, the pup exercises the first coordinated articulation of sucking with respiration and swallowing; (2) it elicits high arousal states in the neonate that favours its first postnatal learning experiences about the social and physical environment [18]; and due to its special nutritional and immunoprotective properties; (3) it conditions the initial endocrine responses to milk intake [35]; and (4) it limits and selects the microflora colonising the neonate's gut after its intensive contact with the mother's skin surface [43]. Missing the first sucking episodes is thus presumably maladaptive for the newborn. For example, in pigs and sheep, species in which females are frequent nursers, neonatal inability to gain colostrum in the first days is linked to an increased mortality [1, 8, 10, 11]. However, in such species, the young may compensate for initial failure to suck by taking advantage of nursing episodes soon after (for example, the average frequency of suckling/hour during the first week after birth: pig, 1/h [24]; sheep, 1.2/h [13]; foal, 4/h [7]; kids, 5.6/h [4]).

The situation is strikingly different in several other taxa, such as Lagomorpha or Scandentia, among which some species have evolved parsimonious mother-infant interactions in terms of both frequency and duration [31, 34, 44]. In rabbits and hares, females leave their offspring in a hide or in a nest after delivery, and only return once/day to nurse them for less than 5 min [3, 22]. During this extremely short period, pups have to quickly find a nipple and suck effectively in a context of severe sibling competition [9]. In the domestic rabbit, pups display nipple search behaviour immediately after being expelled, and they often succeed in gaining colostrum within minutes or even seconds after birth, while the female is consuming the placentae and parturition is still in progress [23]. As pups are born sequentially, first-borns may have greater chances to find and attach to a nipple and ingest colostrum than last-borns. Furthermore, pups that eventually miss initial sucking have to wait a long time until the next opportunity to obtain milk. Indeed, since the peak times for parturition and for the first return of the doe are in the morning hours and in the hours just before sunrise the next day, the period elapsing between the first two sucking opportunities is about 16–20 h (with a 4–32 h range [39]; or a 16–26 h range [25]). Thus, the rabbit offers an interesting opportunity to examine the impact of the initial colostrum intake on individual differences in neonatal adaptation.

The general purpose of this study is to assess the role of initial colostrum intake on neonatal adaptation in the rabbit. The main goals are: (1) to provide descriptive data on the ability of newborn pups to gain colostrum immediately after birth; (2) to assess the extent to which missing the initial sucking episode results in compromised chances of survival or in an altered development between birth and day 21 (that is the developmental window of pups' complete dependence on the mother's milk); (3) to examine the interactional effect on pup survival and development of perinatal biomass and success in initial sucking.

This study is part of a larger investigation [5] examining whether a regimen of female nest access that matches or not the species-specific rhythm of a once/day nursing visit differentially affects the survival and quality of offspring. Thus, a fourth aim of the present study consisted in examining the interactive effect on pup survival of the early ability of pups to gain colostrum and of the nest access regimen imposed on the doe. Two hypothetical outcomes were anticipated that may shed light on how the initial stage of the species-specific nursing rhythm develops in the rabbit doe: (1) Females may visit the nest box several times during the first postpartum day before the once/24 h nursing rhythm is completely established [17], providing several additional opportunities for their offspring to gain colostrum. In this case, leaving the nest entrance free of access would result in lower pup mortality as compared to the situation in which females are kept separate from their litters; (2) Alternatively, multiple and/or longer visits of the doe to the nest between birth and the first full nursing episode may interfere with the presumed energy saving strategy of the pups [20], and hence may compromise their survival regardless of their sucking success immediately after birth.

A final aim of this study is to examine whether the success of initial sucking and the parity of the doe have an interactive effect on pup survival and development. The rate of success of initial sucking is thus compared in pups born to primi- or to multiparous females.

2. ANIMALS AND METHODS

2.1. Animals and housing conditions

The litters followed in the present study were a subsample of those used in an earlier study [5]. They originated from the breeding colony of the experimental Inra station (Le Magneraud) as progeny of crosses between New Zealand does and Californian males (Inra strains 1077 and 2066). Experimental females and their litters were housed in 3 separate rooms, and in individual cages ($0.52 \times 0.40 \times 0.31$ m) to which an open-topped nest box ($0.35 \times 0.25 \times 0.15$ m) was attached 2 days before parturitional term. The nest box was lined with pine shavings. It opened to the female's cage by a lateral circular hole (diameter: 0.17 m) which could be closed by a sliding door. Animals were kept under a constant 16 h/ 8 h light/dark cycle (light on at 5.00 h) and at an ambient temperature of 20.3 ± 3.1 °C. Water and pelleted food ('Lapin Elevage INRA', Saint-Fulgent, France) were provided ad libitum. An anti-coccidial agent (Robenidine, 66 ppm·kg^{-1}) was added to the pellets.

Virgin females were artificially inseminated at 120 days of age, and then 10 days after delivery when primi- or secondiparous (namely first and second parity does). The treatment (see below) to which a doe was allocated was the same after the 1st and 2nd parturition.

Females and their offspring were studied between birth (d0) and the beginning of the transition to solid food consumption (d21) across two consecutive reproductive cycles (*n* = 45 for primiparous does and *n* = 45 for secondiparous does). As soon as births were recorded, litters were culled to 10. If necessary, adoptions were randomly effected to reach this litter size. No attempt was made to equalise between-litter weight when foster pups were introduced; mean litter size before fostering was 10.2 ± 1.4 and 10.8 ± 2.04 for primiparous and secondiparous does, respectively.

2.2. Treatments

Females and litters were randomly allocated to one of 3 treatment groups: (1) free access to the nest during d0-21 (abbreviated as F; 15 litters of primi- then secondiparae); (2) controlled nest access on d0-3, then free nest access on d4-21 (15 litters of primi- then secondiparae); and (3) controlled nest access on d0-5, then free nest access on d6-21 (15 litters for primi- then secondiparae). Both of the controlled nest access conditions consisted in denying females access to the nest by closing the nest except for 15 min/day between 8.00 and 9.00 h. In the case of deliveries that could be attended, the initial nest closure was made immediately after delivery, and otherwise within 12 h of parturition. In all cases the nest was closed after the female had spontaneously left it. Each pup was then individually marked with an ear tag. The day after the last controlled nest access, the nest was left permanently open after the daily check of pup mortality. Based on statistical analyses (see below and [5]), the data derived from both the regimens of controlled nest access were pooled (and abbreviated to C in the following).

The proportion of fostered pups was similar between treatments for the litters of primiparous (C: 5.6%; F: 5.3%; $\chi^2 < 1$, $P > 0.05$) and secondiparous does (C: 7%, F: 6.6%; $\chi^2 < 1$, $P > 0.05$). Thus, in all subsequent analyses the data of biological and fostered pups were pooled.

In order not to interfere with the normal course of initial interactions between pups and does, parturitions were not induced.

2.3. Independent and dependent measures

Colostrum intake. The initial ingestion of colostrum was temporally defined as resulting from sucking episode(s) that occurred within the first 12 postpartum hours (e.g., [26]). Colostrum intake was visually estimated by systematic screening of gastric content through the transparent abdominal skin. This task was conducted by the same trained observer, blind to the experimental conditions, who gently stretched the pups' abdominal skin to increase its transparency. According to the size, color, and consistency of the stomach, three groups of pups were defined: (a) the complete inability to visually locate the stomach was interpreted as no or negligible colostrum ingestion (NI); (b) the ability to visually locate the stomach and the absence of or weak gastric distension was interpreted as medium colostrum ingestion (MI); (c) a visually well-defined, strongly distended stomach was interpreted as high colostrum ingestion (HI).

Pup mortality. Pup mortality was checked every morning. This excluded stillborns (for C and F, % stillborns was 7.6 and 7.3% for the primiparous, and 7.3 and 5.3% for the secondiparous does, respectively). Five categories of lethal causes were identified by systematic autopsy of the dead pups. These autopsies were conducted by a highly trained rabbit pathologist (P.R.), blind to the experimental conditions. Three lethal causes were inherent to the pups: (a) starvation (extreme leanness associated with no or a minute amount of milk in the stomach); (b) digestive dysfunctions (enteritis, hepatitis, peritonitis); (c) circulatory or respiratory dysfunctions (lung congestion, pneumonia). Two etiologies were directly associated with the doe's presence in the nest: (d) wounds (skin perforations of the pup's head and/or body) and (e) soiling of the pups and nest with excrements. A final category of losses, unknown causes, was recorded. These represented 7.1 and 4.2% of the total pup losses in primiparous females, and 14.2 and 0% in secondiparous females, for the C and F regimens, respectively. These data were excluded from the cause of death analyses. Mortality data were analysed for each treatment in terms of total number of pups lost.

Pup weight and growth rate. The newborn pups in each category of sucking success (NI, MI and HI) were individually weighed during the period of exclusive dependence on the doe at d0, and again at d21 (at the beginning of the transition to consumption of solid food). It should be noted that to avoid disturbance of doe-pup interactions, pups were not weighed immediately after expulsion, so that d0-weight data did not represent birthweight but birthweight plus the eventual weight of ingested colostrum. Pups were weighed in the morning between 9.00 and 11.00 h. The mean daily weight gain was calculated between d0 and d21 by taking the difference in weight between the two ages, defining the developmental window investigated, divided by the number of days minus one. Longitudinal weight data were only computed for pups which were still alive at d21 (in primiparous does: n = 67, 185, 155 and in secondiparous does: n = 39, 155, 228, for NI, MI and HI groups, respectively).

2.4. Data analysis

Pup mortality was analysed using the two-tailed χ^2 test (with the Yates correction when appropriate; when not otherwise stated $df = 1$). The effect of immediate postnatal sucking by pups on survival was assessed by comparing the differential survival rate of the 3 categories of pups (NI, MI and HI). The effect of the nest access regimen imposed on the females was assessed by comparing the mortality rate of the NI, MI and HI pups in the 2 nest access regimens. The proportion of pups sucking immediately after birth was also compared according to the females' parity. The relative effect of d0 weight and initial sucking success on pup survival in the d0-d10 period was assessed by comparing the mortality rates of the 5 lightest or the 5 heaviest pups in their litter (the cut-off value being the mean d0 weight of the entire litter) in NI, MI and HI pups.

Finally, to assess the effect of d0 weight and initial sucking success on pup weight gain between d0 and d21, 1-way analyses of variance (ANOVA) were run. Pups were first grouped as either the lightest or heaviest (d0 weight) animals within each litter. The relationship between d0 weight and d0-d21 weight gain was then compared for both the lightest and heaviest categories of pups. Lastly, to further analyse the developmental contribution of success in first sucking, we compared the d0-d21 weight gain within the NI, MI and HI pup categories. Statistical analyses were conducted using Systat Software (Evanston, USA).

3. RESULTS

The cumulative mortality of the tagged pups from this study was representative of pup mortality recorded between d0-10 in the whole sample studied by Coureaud et al. [5] for either primiparous (8.7%, n/N = 39/450, to be compared with 9.3% in the whole sample, n/N = 83/890; $\chi^2 < 1$; $P > 0.05$; only the total N will be given below) and secondiparous does (2.2%, N = 450, to be compared with 2.3%, N = 780; $\chi^2 < 1$, $P > 0.05$). Results will be presented separately for primiparous and secondiparous does, and the effect of parity on postnatal sucking success, mortality and weight gain will be presented in the section dealing with secondiparous females.

3.1. Primiparous females

3.1.1. Immediate postnatal sucking: frequency and relation to pup survival

Irrespective of the nest access regimen, 82% of the 450 pups followed individually in all treatments were able to gain colostrum within the first 12 h following birth (Fig. 1) The remaining 81 pups that were unsuccessful (NI pups) were distributed across 68.9% of the litters (*N* = 45) as follows: 1/litter in 24.4%, 2/litter in 22.2%, 3/litter in 6.7%, and > 3/litter in 15.6% of the litters.

Because mortality was similar in MI and HI pups [5.1% (*N* = 197) and 8.7% (*N* = 172), respectively; $\chi^2 = 1.9$, $P > 0.05$], their data were combined in the following analyses. Sixteen per cent (*N* = 81) of the pups which died, belonged to the category that was not successful in obtaining colostrum, whereas only 6.8% (*N* = 369) belonged to the category of pups that were successful suckers. This mortality difference according to colostral intake was statistically significant ($\chi^2 = 7.4$, $P < 0.05$).

The time course and causes of mortality in both categories of pups were heterogeneous: while NI pups died between d2-d6, with a peak at d3, MI+HI pup losses were more evenly spread throughout the whole d1-10 period (Fig. 2). Starvation explained 69.2% (*N* = 13) of the NI pup losses and only 8.7% (*N* = 23 pups for which causes of death were identified among 25 dead pups) of the MI + HI pup losses ($\chi^2 = 11.2$, $P < 0.01$). The doe-related causes of pup death (wounds and soiling of the nest) represented 7.7% (*N* = 13) of the causes implicated in the NI pups' death versus 30.4% (*N* = 23) for MI+HI pups ($\chi^2 = 1.3$, $P > 0.05$). The remaining identified causes were

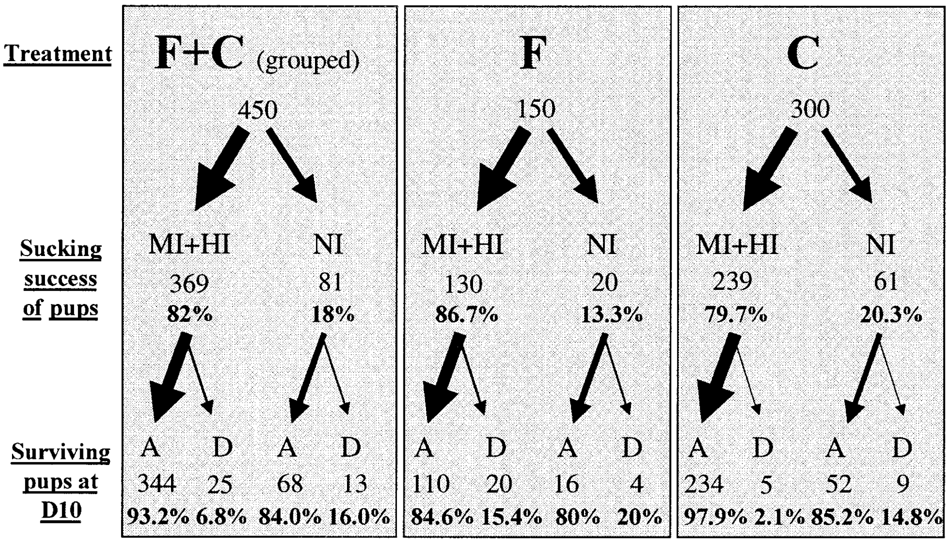

Figure 1. Flow chart of pup survival to d10 as related to the nest access regimen imposed on primiparous females (F: free; C: controlled) and to immediate postnatal sucking success (MI + HI: medium and high colostrum ingestion; NI: absence of ingestion; cf. text for definitions). Normal type numbers indicate the raw effective of pups in each category; bold type indicates the corresponding % (A: alive, D: dead).

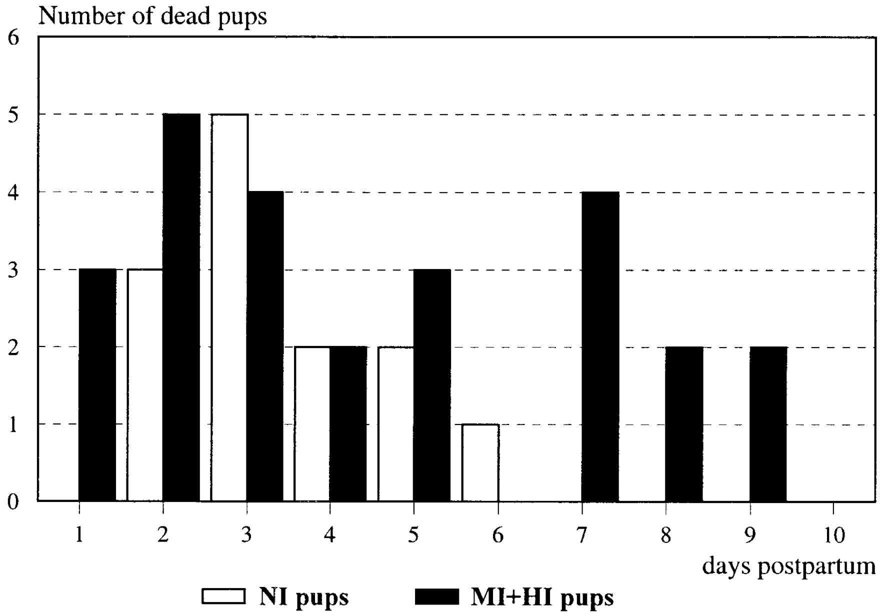

Figure 2. Temporal distribution of pup losses between d0-10 according to immediate postnatal sucking success (MI + HI: medium and high colostrum ingestion; NI: absence of ingestion) in litters from primiparous does.

digestive dysfunctions (23.1 and 47.8% for NI and MI+HI pups, respectively) and circulatory or respiratory dysfunctions (13.1% for MI+HI pups).

Regarding the influence of the nursing regimen imposed on the does, the failure rate of first sucking was similar in the F (13.3%, N = 150) and C regimens (20.3%, N = 300; $\chi^2 = 3.3$, $P > 0.05$) (Fig. 1). However, the interaction of the females' nest access regimen and of the sucking success of the pups significantly influenced the survival of pups within the d0-d10 period in 2 ways: within nest access regimen comparisons indicated that losses of MI + HI pups were higher in the F regimen (15.4%, N = 130) than in the C regimen (2.1%, N = 239; $\chi^2 = 23.5$, $P < 0.001$), while those of NI pups did not differ between the 2 regimens (20 vs. 14.8%; $\chi^2 < 1$, $P > 0.05$); between nest access regimen comparisons indicated that NI pups died significantly more frequently than MI+HI pups in the C regimen (14.8 vs. 2.1%, respectively; χ^2 = 14.8, $P < 0.001$), while both categories (NI vs. MI+HI) died equally frequently in the F regimen (20 vs. 15.4% for NI and MI + HI pups, respectively; $\chi^2 < 1$, $P > 0.05$) (Fig.1).

In summary, in the present conditions, nearly one fifth of the pups (18%) failed to obtain colostrum within 12 h of being born, and these pups were more likely to die than successful suckers regardless of the females' nest-access-regimen. Only in pups which could gain milk immediately after birth was mortality associated with the regimen of nest access; the F regimen inducing a higher mortality rate than the C regimen. Finally, the continuous nest access regimen generated the disappearance of the survival improvement promoted by an initial sucking success.

3.1.2. Immediate postnatal sucking, d0 weight and pup survival

The majority of the NI pups (67.7%, N = 81) were among the 5 lightest pups in their litter on d0 (mean d0 weight: 38 ± 9 g vs. 50 ± 9 g for the lightest vs. heaviest pups, respectively). The weight distribution was not so skewed for the two other categories of pups: 49.2% (N = 197) of the MI and 43% (N = 172) of the HI pups were among the lightest of their litter at d0 (mean weight of the lightest vs. heaviest pups in their litter: 41 ± 8 g vs. 52 ± 10 g and 45 ± 7 g vs. 55 ± 8 g for MI and HI groups, respectively). The d0 weight and initial sucking success were clearly additive in determining the mortality of NI pups; all those which died within the d0-10 period (N = 13) were among the lightest half in their litter. However, d0 weight had no significant impact on survival of either MI or HI pups; mortality was not different whether they belonged to the lightest or heaviest half of their litter (mortality rate for the 5 lightest vs. 5 heaviest pups in MI then HI groups: 7.2 vs. 3%, N = 97 and 100; 8.1 vs. 9.2%, N = 74 and 98; $\chi^2 < 1$, $P > 0.05$ in both cases). In summary, d0 weight had a strong impact on pup survival in pups that did not gain colostrum at initial suckling, in accordance with the expectation that greater weight is protective. Conversely, when pups were successful suckers their weight no longer appeared to have an impact on survival.

3.1.3. Immediate postnatal sucking, d0-weight and subsequent weight gain in pups

Prior to examining the influence of initial sucking on pup development, we defined two categories of pups by separating individuals which survived to d21 into the lightest half and heaviest half in each litter. As expected, the mean d0 weight of these pups was significantly different [42.5 ± 7.9 g vs. 52.8 ± 9.0 g; F (1,405) = 148.9, $P < 0.001$]. To assess whether the d0 weight of pups in these 2 groups influenced their subsequent development between d0 and d21, their weight gain was compared: the lightest pups demonstrated a significantly lower mean weight gain than the heaviest pups [10.0 ± 2.2 g/d vs. 11.3 ± 2.1 g/d; one-way ANOVA: F (1,405) = 32.4, $P < 0.001$]. To examine whether the initial sucking success further influenced pup weight gain over the same period, we separately compared the growth of NI, MI and HI pups in the 2 weight categories. One-way ANOVAs indicated that sucking success had no additional effect on weight gain in either weight category (Tab. I). In sum, d0 weight was clearly more important than the first sucking success as a predictor of weight gain during the d0-d21 period.

3.2. Secondiparous females

3.2.1. Immediate postnatal sucking: frequency and relation with pup survival

Irrespective of the females' nest access regimen, the majority of pups were successful in obtaining colostrum immediately after birth (91.1%, N = 450; Fig. 3). The 40 unsuccessful pups were distributed in 42.2% of the litters (N = 45) as follows: 1/litter in 17.7% of the litters, 2/litter in 11.1%, 3/litter in 6.7%, > 3/litter in 6.7%.

Interestingly, the proportion of successful pups was significantly higher in secondiparous than in primiparous does (91% vs. 82%, N = 450 in both cases; $\chi^2 = 16.0$, $P < 0.001$).

Because mortality was similar in the MI and HI categories [2.9% (N = 171) and 1.7% (N = 239) respectively; $\chi^2 < 1$, $P > 0.05$], these data were pooled. The rate of pup mortality was similar in NI (2.5%, N = 40) and in MI+HI pups (2.2%, N = 410). The only dead pup in the NI category died at d3 (from wounds), whereas mortality of MI+HI pups occurred throughout the d2-d8 period. In

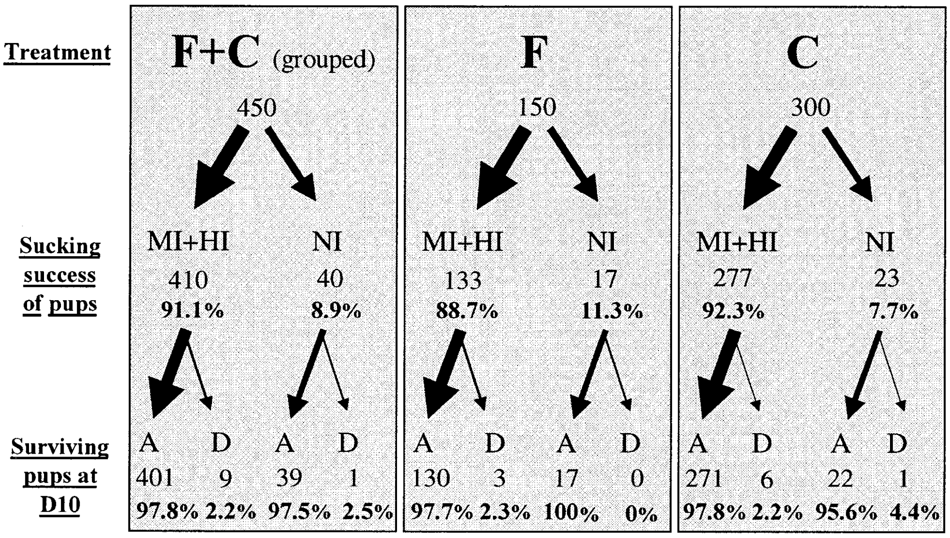

Figure 3. Numbers and proportions of pups surviving to d10 as related to the nest access regimen imposed on secondiparous females and to immediate postnatal sucking success (cf. Fig. 1 for key to abbreviations).

the latter pups, the identified causes of death were equally represented (starvation and digestive/circulatory dysfunctions: 43% and 43%, respectively; wounds: 14%; $N = 7$).

As in primiparous does, the proportion of NI pups was similar in litters assigned to either the F (11.3%, $N = 150$) or C nest-access regimens (7.7%, $N = 300$; $\chi^2 = 1.7$, $P > 0.05$). Among MI+HI pups, d0-d10 mortality was similar in both the F (2.3%) and C regimens (2.2%; $\chi^2 < 1$, $P > 0.05$); among NI pups (8.9% of the pups), 0/17 and 1/23 died in the F and C regimens, respectively. Likewise, the degree of mortality was not significantly different in NI and MI + HI pups in either the F or C regimens (NI vs. MI+HI: 2.3 vs. 0% and 2.2 vs. 4.4%, for the F and C regimens, respectively; $\chi^2 < 1$, $P > 0.05$ in both cases) (Fig. 3). These results indicate that after the first partum the nest access regimen has little if any impact on pup mortality.

3.2.2. Immediate postnatal sucking, d0 weight and subsequent weight gain in pups

As for primiparous does, we first compared the mean d0 weight of individuals in the lightest half and heaviest half of their litter which survived to d21 [48.4 ± 7.5 g vs. 61.3 ± 7.8 g; F (1,420) = 296.7, $P < 0.001$]. To evaluate the association between d0 weight and the subsequent development of these 2 categories of pups during the d0-d21 period, their individual weight gain was compared; the lightest pups showed a reliably lower mean weight gain/day than the heaviest pups [10.5 ± 1.9 g/d vs. 12.0 ± 1.9 g/d; one-way ANOVA: F (1,420) = 67.6, $P <$ 0.001]. To assess if the initial sucking success had some additional impact on daily weight gain over the same period, NI, MI and HI pups were compared in the lightest and heaviest groups. Separate 1-way ANOVAs revealed that sucking success did not enhance the daily weight gain of either

Table I. Means (SD) of daily weight gain between d0-21 ($g \cdot d^{-1}$) of pups which were unsuccessful (NI group), moderately successful (MI group) or highly successful (HI group) in gaining colostrum at birth, and which belong to the 5 lightest (L) or 5 heaviest (H) pups of their litter after the first suckling (P– S: pups born to primiparous – secondiparous does).

		Groups			*F*-values[a]
		NI	MI	HI	
P	L	9.7 (2.4) *n* = 41	10.2 (2.2) *n* = 89	11.3 (2.4) *n* = 66	0.8 ns
P	H	11.3 (1.7) *n* = 26	11.2 (2.0) *n* = 96	10.6 (1.8) *n* = 89	0.1 ns
S	L	9.7 (1.6) *n* = 21	10.6 (2.1) *n* = 92	10.6 (1.8) *n* = 95	2.1 ns
S	H	11.2 (1.8) *n* = 18	12.3 (1.5) *n* = 63	12.0 (2.0) *n* = 133	2.6 ns

[a]: one-way ANOVA, df = 2 in all tests.
ns: non significant.

weight category (Tab. I). Regardless of nest access regimen, the pups from second litters were an average 7 g heavier at birth than pups from first litters (54 ± 10 g vs. 47 ± 10 g; F (1,898) = 105, $P < 0.001$). This weight difference was stable throughout the follow-up period; at d21, pups from the second litters weighed an average 20 g more than pups from the first litters [292 ± 48 g vs. 272 ± 51 g; F (1,827) = 34, $P < 0.001$]. Thus, pups born to secondiparous females were heavier than those from primiparous does; in addition, and regardless of parity, d0 weight was the better predictor of weight gain of pups during the first 3 weeks of life.

4. DISCUSSION

This study offers a preliminary analysis of the influence of immediate postnatal intake of colostrum on the survival and growth of rabbit pups. Firstly, it provides a descriptive account of the proportion of pups that were unsuccessful in reaching a nipple or in obtaining first milk in the hours following birth. Lack of initial sucking success involved a non-negligible fraction of the 450 pups investigated, and was distributed across 2 thirds of the 45 litters studied. It differed significantly between primiparous and secondiparous does (18% vs. 9%), reflecting parity-related differences in either pup sensori-motor abilities, or maternal behavioural, communicative, parturitional or lactational properties that will be discussed at more length below.

Secondly, this study underlines the immediate and longer term adverse effects of missing the first sucking opportunity. Significantly more pups (from primiparous does) which did not suck within 12 h following birth died between d0 and d10 than pups which were successful suckers. The bulk of these unsuccessful pups died at d3 postpartum, and their total loss was registered by d6. During this period, they died mainly from starvation, indicating that they were unable to make up for the missed feed during the nursing episodes following the initial one. This result is consistent with previous data which have demonstrated the vital need for pups to obtain milk during the very first sucking opportunities. Venge [39] showed that pups that were unsuccessful feeders during the first 3 days of life did not survive beyond d4. McNitt and Moody [32] observed that 7 of 20 pups which missed one or more sucklings between d0-d6 died within 6 days of birth (in the same period, no successful pup died). Thus, the immediate postnatal ability of pups to gain colostrum appears critical for early survival as unsuccessful individuals die more than successful ones. However, this interpretation must be qualified by the confounding impact of the birthweight variable (or d0 weight, since we took the option not to interfere with doe-pup interactions in weighing pups right after birth). Did pups die or fail to thrive because of failure to gain colostrum or because they were too small and weak to face competition under the mother with stronger littermates? This issue was approached by comparing d0-d10 losses in lighter and heavier pups that were either (1) both unsuccessful suckers or (2) both successful suckers. While initial weight was the limiting factor for survival in unsuccessful suckers, it was not the case in successful suckers. In other words, when colostrum can be ingested, initial weight looses its critical character. By weighing pups immediately after expulsion and reweighing them after the initial sucking bout(s), one might separate the relative impact of natal biomass and biomass gained by sucking on survival. Such an intrusion would, however, entail a disturbance to the female and the pups that is probably not justified.

While early colostrum intake promotes survival, our data indicate that it does not confer any significant long term qualitative advantage on surviving pups. Indeed, in females of either parity, early successful suckers did not necessarily demonstrate subsequent higher weight gain. However, weight at d0 was strongly associated with pups' weight gain; after the initial sucking, the daily weight gain of the heaviest pups was higher than that of the lightest pups.

Thirdly, the influence of early ingestion of colostrum on pup survival waned by the second reproductive cycle of the doe; pups born to secondiparous females died equally frequently whether they were successful or unsuccessful early suckers. This interactive influence of parity and initial sucking success on pup mortality could be explained by various factors: (a) By the influence of birthweight. The increase in the average birthweight of offspring from multiparous does (see [5, 16] and the present study) may be protective in 2 ways: (i) an increased adipose mass may enable pups to miss early sucking opportunities without adverse effects, and (ii) an increased muscular mass may favour a vigorous response to the intense behavioural challenge represented by the competitive nursing situation. Verga et al. [40] revealed indeed that the locomotor ability of rabbit pups born to multiparous does was greater than that of pups born to primiparous does. (b) Compared to experienced females, primiparous does may react more aversively to the stimuli emitted by the pups. This may affect their readiness to position themselves adequately over the pups (according to a process observed in the rat, [15]), and promote multiple, temporally inadequate nursing visits to the litter. (c) Undergoing a first complete maternal cycle may stimulate function of the chemo-emission system involved in the production and emission of the pheromonal cues that direct nipple search behaviour and suckling in the offspring [21, 22]. Finally, (d) for optimal pup survival and vigor, parturition must proceed very rapidly in the rabbit [23]. Thus, greater parturitional efficiency in multiparous does may result in the birth of more vigorous pups, better able to overcome the adverse effects of missing initial sucking or of nest disturbance than those born to primiparous does.

Finally, the relative impact of pup-related or doe-related explanations of pup mortality was approached by examining interactional effects of the success of initial sucking and the regimen of doe-litter interaction (free vs. controlled nest-access). Based on data from previous studies [5, 27, 33] and the present findings which show that one/day nursing visits by (primiparous) does and early sucking success were both protective to the offspring, it was hypothesised that pups of the 4 groups defined by crossing these 2 factors would demonstrate differential survival. Specifically, it was predicted (1) that successful suckers raised on the species-specific once/day nursing schedule (combining 2 sources of adaptive factors) would be in the most optimal conditions, and (2) that unsuccessful suckers exposed to continuous nest access by the female (2 sources of maladaptive factors) would be in the worst conditions, for survival. A third prediction was that the 2 other pup categories, namely those which could gain colostrum at birth but were raised in free nest access conditions, and those which were unsuccessful suckers but were raised by mothers restricted to a once/day nursing visit (exposed to one adaptive and one maladaptive factor), should demonstrate intermediate levels of losses. Our data clearly support predictions 1 and 2, but only partially support prediction 3. While the degree of mortality was intermediate and similar in both latter groups (that is 15.4 and 14.8%, respectively), it was not significantly different from that of the unsuccessful suckers raised under the free nursing schedule (20%). Therefore, the possibility for females to enter the nest more than once during the first days (as suggested in [38]) places pups that missed the first sucking at a clear disadvantage (that is they fail to make up for the adverse effects of their initial unsuccess). In addition, the continuous nest access regimen appeared to cancel out the initial gain of the first successful colostrum intake. Thus, at least in litters of primiparous does, for optimal survival, pups need to (1) gain colostrum right after birth and then (2) be able to implement their energy saving strategy of circadian, suckling-associated arousal [20], that is not be repeatedly aroused by multiple nest entries by the female. In multiparous does, both of the above factors seem to loose their importance for pup survival.

In conclusion, these results underline the importance of the quality of mother-pup interactions occurring during the first hours after parturition for optimal pup survival and fitness. It is often asserted that rabbit pups do not need colostrum intake because passive immunity is prenatally conferred via the highly permeable haemochorial placenta. The present study suggests, however, that the ability of rabbit mothers to provide, and of the pups to gain, colostrum after parturition contributes to pup survival, at least in first litters. From birth onwards, colostrum is rich in proteic and lipidic compounds, contains more lactose than later in lactation [6, 26], and is rich in immunoglobulins [2, 37, 42]. It is thus well suited to serve as fuel and antigen defense in the newly born rabbit. However, in order to firmly conclude that it is colostrum per se that confers an important protective function, its effects need to be separated from those resulting from the mere experience of sucking and ingestion of mature milk. This may be assessed by comparing the survival and growth curves of normally raised pups and of pups starting their postnatal life without colostrum (that is fostered for their initial sucking onto does which are in their fifth lactation day).

Finally, future investigations should be directed to examining the subtle events and processes that regulate doe-pup interactions in the hours following birth. For example, the expression of the species-typical nursing rhythm appears to be in a phase of transition immediately after birth. Although it is known that females display a short and constant duration of nursing [30, 44], it has been suggested that this is not yet stabilised neither in duration nor in frequency during the first postpartum day, even in multiparous females [17]. In another polytoccous species, the pig, a transition from asynchronous and continuous to synchronous and cyclical nursing has been reported to occur within the first 12 h postpartum [28]. The rabbit female provides an interesting model for the investigation of the initial development of the nursing rhythm, to determine the underlying psycho-neuroendocrinological processes which are initiated after the initial partum, and how these processes can be optimised by breeding practices that match the evolved one/day nursing behaviour and natural nest conditions of the rabbit female.

ACKNOWLEDGMENTS

This work is dedicated to Jean-Louis Vrillon at the occasion of his retirement. It could not have been done without the expert help of Pierre Mercier, Jacqueline Bellereau, Annie Malineau, and Cindy Castaigne at the Experimental Station of Inra at Le Magneraud. Two anonymous referees are thanked for their useful comments and the continued, sympathetic encouragements of Marc-Antoine Driancourt at numerous coffee-breaks is fully appreciated. G.C. was funded by a scholarship from the Poitou-Charentes Région/Inra. The study was supported by the Ministère de l'Agriculture et de la Pêche (AIP INRA P29 'Bien-Etre Animal et Sciences du Comportement').

REFERENCES

[1] Alexander G., Peterson J.E., Neonatal mortality in lambs, Aust. Vet. J. 37 (1961) 371–381.

[2] Asofky R., Small P.A., Colostral immunoglobulin-A: Synthesis in vitro of T-chain by rabbit mammary gland, Science 158 (1967) 932–933.

[3] Broekhuizen S., Bouman E., Went W., Variation in timing of nursing in the Brown Hare (*Lepus europaeus*) and the European Rabbit (*Oryctolagus cuniculus*), Mammal Rev. 16 (1986) 139–144.

[4] Bungo T., Shimojo M., Nakano Y., Okano K., Masuda Y., Goto I., Relationship between nursing and suckling behaviour in Tokara native goats, Appl. Anim. Behav. Sci. 59 (1998) 357–362.

[5] Coureaud G., Schaal B., Coudert P., Hudson R., Rideaud P., Orgeur P., Mimicking natural nursing conditions promotes early pup survival in domestic rabbits, Ethology (in press).

[6] Cowie A.T., Variations in the yield and composition of the milk during lactation in the rabbit and the galactpoietic effect of prolactin, J. Endocr. 44 (1969) 437–450.

[7] Crowell-Davis S.L., Nursing behaviour and maternal aggression among Welsh ponies (*Equus caballus*), Appl. Anim. Behav. Sci. 14 (1985) 11–25.

[8] Dalton D.C., Knight T.W., Johnson D.L., Lamb survival in sheep breeds on New Zealand hill country, N.Z. J. Agric. Res. 23 (1980) 167–173.

[9] Drummond H., Vásquez E., Sánchez-Colón S., Martínez-Gómez M., Hudson R., Sibling competition for milk in the domestic rabbit, Ethology (in press).

[10] Dyck G.W., Swierstra E.E., Causes of piglet death from birth to weaning, Can. J. Anim. Sci. 67 (1987) 543–547.

[11] English P.R., Smith W.J., Some causes of death in neonatal piglets, Vet. Ann. 15 (1975) 95–104.

[12] Estes R.D., Estes R.K., The birth and survival of Wildbeest calves, Z. f. Tierpsychol. 50 (1979) 45–95.

[13] Ewbank R., Nursing and suckling behaviour amongst Clun Forest ewes and lambs, Anim. Behav. 15 (1967). 251–258.

[14] Ewer R.F., Further observations on suckling behaviour in kittens, together with some general considerations of the interrelations of innate and acquired responses, Behaviour 17 (1961) 247–260.

[15] Fleming A.S., Luebke C., Timidity prevents the virgin female rat from being a good mother: Emotionality differences between nulliparous and parturient females, Physiol. Behav. 27 (1981) 863–868.

[16] Fortun-Lamothe L., Effects of pre-mating energy intake on reproductive performance of rabbit does, Anim. Sci. 66 (1998) 236–269.

[17] González-Mariscal G., Díaz-Sánchez V., Melo A.I., Beyer C., Rosenblatt J.S., Maternal behavior in New Zealand white rabbits: Quantification of somatic events, motor patterns, and steroid plasma levels, Physiol. Behav. 55 (1994) 1081–1089.

[18] Goursaud A.P., Nowak, R., Colostrum mediates the development of mother preferences by newborn lambs, Physiol. Behav. 67 (1999) 49–56.

[19] Hemsworth P.H., Winfield C.G., Mullaney P.D., A study of the development of the teat order in piglets, Appl. Anim. Ethol. 2 (1976) 225–233.

[20] Hudson D., Distel H., The pattern of behaviour of rabbit pups in the nest, Behaviour 79 (1982) 255–271.

[21] Hudson R., Distel H., Nipple location by newborn rabbits: Behavioural evidence for pheromonal guidance, Behaviour 85 (1983) 260–275.

[22] Hudson R., Schaal B., Bilkó Á., Altbäcker V., Just three minutes a day: The behaviour of young rabbits viewed in the context of limited maternal care, 6th World Rabbit Congress, Toulouse, Vol. 2, 1996, pp. 395–403.

[23] Hudson R., Cruz Y., Lucio R.A., Ninomiya J., Martínez-Gómez M., Temporal and behavioral patterning of parturition in rabbits and rats, Physiol. Behav. (1999) 599–604.

[24] Jensen P., Stangel G., Algers B., Nursing and suckling behaviour of semi-naturally kept pigs during the first 10 days postpartum, Appl. Anim. Behav. Sci. 31 (1991) 195–209.

[25] Jilge B., The ontogeny of circadian rhythms in the rabbit, J. Biol. Rhythms 8 (1993) 247–260.

[26] Lebas F., Composition chimique du lait de lapine, évolution au cours de la traite, et en fonction du stade de lactation, Ann. Zootech. 20 (1971) 185–191.

[27] Le Normand B., Jego P., Maiche N., Intérêt de l'allaitement contrôlé en élevage cunicole, VI[es] Journées de la Recherche Cunicole, La Rochelle, Vol. 2, 1994, pp. 499–504.

[28] Lewis N.J., Hurnik J.F., The development of nursing behaviour in swine, Appl. Anim. Behav. Sci. 14 (1985) 225–232.

[29] Lickliter R.E., Behavior associated with parturition in the domestic goat, Appl. Anim. Behav. Sci. 13 (1985) 335–345.

[30] Lincoln D.W., Suckling: A time-constant in the nursing behaviour of the rabbit, Physiol. Behav. 13 (1974) 711–714.

[31] Martin R.D., Reproduction and ontogeny in treeshrews (*Tupaia belangeri*), with reference to their general behaviour and taxonomic relationships, Z. f. Tierpsychol. 25 (1968) 409–495.

[32] McNitt J.I., Moody Jr G.L., Nest box behavior of the domestic rabbit ("... and may your nestboxes be full."), J. Appl. Rabbit Res. 10 (1987) 159–162.

[33] Moret B., Dagorne M., Vers une limitation de la mortalité des lapereaux sous la mère, Cuniculture 2 (1975) 85–90.

[34] Mykytowycz R., Territorial marking by rabbits, Sci. Am., 218 (1968) 116–126.

[35] Nowak R., Goursaud A.P., Lévy F., Orgeur P., Schaal B., Picard M., Meunier-Salaün M.C., Belzung C., Alster P., Uvnäs-Moberg K., Cholecystokinin receptors mediate the development of a preference for the mother by newly born lambs, Behav. Neurosci. 111 (1997) 1375–1382.

[36] Rosenblatt J.S., Lehrman D.S., Maternal behavior of the laboratory rat, in: Rheingold H. (Ed.), Maternal Behavior in Mammals, Wiley, New York, 1963, pp. 8–57.

[37] Schley P., Kaninchenmilch – Zusammensetzung und Probenentnahme, Berliner-Münchener Tierärztliche Wochenschrift 88 (1975) 171–173.

[38] Schulte I., Hoy S., Untersuchungen zum Säuge- und Saugverhalten und zur Mutter-Kind-Beziehung bei Hauskaninchen, Berl. Münch. Tierärztl. Wschr. 110 (1997) 134–138.

[39] Venge O., The influence of nursing behaviour and milk production on early growth in rabbits, Anim. Behav. 11 (1963) 500–506.

[40] Verga M., Canali E., Pizzi F., Crimella C., Induced reactions in young rabbits of dams of different parity and reared on two different nursing schedules, Appl. Anim. Behav. Sci. 16 (1986) 285–293.

[41] Vince M.A., The newly born lamb's patterns of movement before, during and after the first sucking bout, Appl. Anim. Behav. Sci. 33 (1992) 27–33.

[42] Wise R.D., Lichter E.A., Dray S., Presence in rabbit colostrum of IgA autoantibodies to a gastric antigen, J. Immun. 107 (1971) 47–55.

[43] Xu R.J., Development of the newborn GI tract and its relation to colostrum/milk intake: a review, Reprod. Fertil. Dev. 8 (1996) 35–48.

[44] Zarrow M.X., Denenberg V.H., Anderson C.O., Rabbit: Frequency of suckling in the pup, Science 150 (1965) 1835–1836.