

PARITY AND NUMBER OF REPEATED DOE-LITTER-SEPARATION TREATMENTS AFFECT DIFFERENTLY THE REPRODUCTIVE PERFORMANCES OF LACTATING DOES

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ABSTRACT: Using data from three different experiments, the effects of oestrus synchronisation by doe-litter separation (DLS), applied 48 hours before artificial insemination from day 9 to day 11 *post partum*, on productive performance of free nursing rabbit does and their litters in relation to parity and number of successive DLS treatments were evaluated. Initially, 200 New Zealand White does of different parity were homogeneously and definitively distributed in two groups (DLS and Control). On the whole, DLS improved fertility (+23.9%, $P \leq 0.001$), but reduced the growth of young rabbits from day 9 to weaning (-1.7 g/d, $P \leq 0.01$) and their weaning weight (-38 g, $P \leq 0.01$) at day 35. DLS also increased by

19.2%, 32.5% and 35.0% ($P \leq 0.01$) the fertility rate of does from parity 1 to 3, respectively, but was ineffective on does of parity ≥ 4 . When DLS was repeatedly applied to does with more than 3 parity, fertility progressively dropped and, after the first still successful application, it was similar to that of controls. The results suggested that the effect of DLS on fertility of does and growth of suckling rabbit could depend on number of successive treatments rather than on parity. The hypothesis that several DLS treatments could induce a condition of behavioural/psychological and/or physiological adaptation, depressing both fertility and milk yield thereafter, while tempting, remains to be tested.

RÉSUMÉ: La parité et le nombre de séparations mère-jeune successives affectent différemment les performances de reproduction de lapines allaitantes.

En utilisant des données de trois expériences différentes, nous avons évalué l'effet de la synchronisation de l'oestrus par séparation mère-jeune (SMJ), réalisée 48 heures avant insémination artificielle, du 9^{ème} (J9) au 11^{ème} jour (J11) après la mise bas, sur la productivité de lapines en allaitement libre et de leurs portées, en fonction de la parité et du nombre de SMJ successives. Deux cents lapines Néo Zélandais Blanc, de parité variable, ont été réparties de façon homogène et définitive en deux groupes (SMJ et témoin). Globalement, SMJ a amélioré la fertilité (+23,9%, $P < 0.001$), mais a réduit la croissance des lapereaux de J9 au sevrage (-1,7 g/j, $P < 0.01$) et leur poids au sevrage à 35

jours (-38 g, $P < 0.01$). SMJ a augmenté le taux de fertilité des parités 1 à 3 de 19,2%, 32,5% et 35,0% ($P < 0.01$), respectivement, mais a été inefficace à partir de la parité 4. Quand SMJ était appliquée à plusieurs reprises à des femelles ayant eu plus de trois portées, la fertilité diminuait progressivement et, après le premier traitement encore efficace, elle était identique à celle des témoins. Ces résultats suggèrent que l'effet de SMJ sur la fertilité des lapines et la croissance des lapereaux pourrait dépendre du nombre de traitements successifs plutôt que de la parité. L'hypothèse que plusieurs traitements de SMJ pourraient induire un état d'adaptation comportementale/psychologique et/ou physiologique, réduisant à la fois la fertilité et la production laitière reste à vérifier.

INTRODUCTION

In the past few years, the temporary separation of rabbit does from their litter, referred as the doe-litter separation (DLS) technique, has been proposed as an alternative and reliable method for improving the fertility rate in lactating does without the employment of exogenous gonadotropins (THEAU-CLÉMENT, 2000). This technique was particularly effective when applied for 36-48 hours before artificial insemination (AI),

from day 9 to day 11 *post partum*, on free nursing does especially at first lactation (ALVARIÑO *et al.*, 1998; MAERTENS, 1998; SZENDRO *et al.*, 1999; VIRAG *et al.*, 1999; BONANNO *et al.*, 2000). By contrast, BONANNO *et al.*, (1999) reported that DLS lost its efficacy in older controlled nursing does having more than 4 parity.

Although the basic physiological mechanisms by which the DLS ameliorates the reproductive performances are still unclear, growing evidence suggest that its positive effect are due to an increase of the number of does in oestrous on the day of AI

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(THEAU-CLÉMENT, 1998; UBILLA *et al.*, 2000b). However, even if it is well known that stage and number of lactation affects fertility in rabbits (CASTELLINI, 1996), surprisingly no work has been done to study the long-term effectiveness of DLS on productive performance of rabbit does by analysing separately the individual effects of parity and number of their repeated treatments.

Therefore, this study was aimed to ascertain whether the progressive reduction in differential fertility rate observed in older does (BONANNO *et al.*, 1999) was indeed simply caused by number of parity rather than by the number of successive DLS applications or by the interactions of both factors. Thus, here the basic hypothesis was related to the possibility that several DLS treatments, when applied to the same subjects over a relatively short period of time, may induce a condition of adaptation, capable of depressing the fertility.

MATERIAL AND METHODS

In this study, the data collected from three consecutive experiments were used. The experiments were carried out on the same commercial rabbit farm in Sicily, during the periods: December 1998-August 1999 (experiment 1), February-July 2000 (experiment 2) and February-August 2001 (experiment 3).

Animals and housing

Initially, 200 New Zealand White does (50, 80 and 70 in the experiments 1, 2 and 3, respectively) of different parity were used. Does removed because of mortality, illness, low productivity or three consecutive infertile inseminations, were immediately replaced by primiparous does.

The does were housed in a building ventilated naturally, into flat-deck cages with internal nest-boxes isolated by a wire netting screen, under a light program

of 16 hours per day, and fed *ad libitum* with a commercial pelleted diet (17.2% CP and 15.8% CF).

Reproduction management

A 42 day reproduction rhythm was followed using 2 batches of does inseminated with a 21 day interval. Non-pregnant does were inseminated again 21 days later.

The AI was performed with heterospermic pooled semen collected from several bucks using IMV equipment (L'Aigle, France), assessed for mass motility and diluted 1:5 in a TRIS (experiment 1 and 2) or in a commercial extender (Cortalap, IMV) (experiment 3). The does were inseminated in the lordosis position with 0.6 ml of fresh diluted semen through a single-use plastic pipette. Ovulation was induced at the moment of insemination by i.m. injection of 20 µg of synthetic GnRH (Fertagyl, Intervet, Milan, Italy). At parturition litter size was equalised to 8-9 young within the group, depending on the total available number of young rabbits. Free suckling and weaning at 35 days of age were adopted.

Treatments

Does were homogeneously and definitively distributed in two groups (DLS and Control) on the basis of their physiological status (lactating and non-lactating) and parity at the first AI.

In the experimental group, a 48 hour DLS was performed by closing the nest-box from 10 a.m. of day 9 to 10 a.m. of day 11; the opening of nest-box was immediately followed by litter nursing and, within 15 minutes after nursing, by the AI. In the control group, does had always-free access to nest-box and were similarly inseminated at day 11 post-partum.

Measurements

At insemination, receptivity of does was detected observing the vulva colour and turgidity; does with turgid, red and purple vulva were considered as

receptive. At parturition, the fertility rate (number of parturitions/number of inseminations \times 100), total number born and young born alive were recorded. Size and weight of litters were recorded after equalisation at day 1, immediately before DLS (day 9), and at weaning (day 35).

Statistical analysis

The analysis was performed on records of lactating does, according to a linear model including the effect of experiment (3 levels: 1, 2, 3), treatment (2 levels: DLS, control), parity (4 levels: 1, 2, 3, ≥ 4) and interaction between treatment and parity. The analysis for evaluating the effect of repeated DLS treatments was restricted to only does with more than 3 orders of parity and to the variables affected by parity mainly related to productivity (fertility, litter size and weight at 35 days, daily weight gain from day 9 to 35). The younger does (parity 1-3) were purposely excluded from the model because they responded positively to DLS. In this second model, therefore, the effects of experiment (3 levels: 1, 2, 3), parity (4 levels: 4, 5, 6, ≥ 7), treatment (4 levels: 0, 1, 2 and ≥ 3 repeated DLS), and interaction between treatment and parity were evaluated. In both models, the interactions with the effect of experiment were omitted because they always were not significant. Proportional data were considered as variable of Bernoulli (0-1). The differences between means were tested by Student "t" test. The data were statistically analysed using the GLM procedure of SAS 6.12 (1989).

RESULTS

The DLS, applied for 48 hours at day 9 *post partum* on free nursing does (Table 1), improved the fertility (+23.9%) and the kits viability at parturition (-0.5 still-born per litter). Nevertheless, the DLS did not significantly affect the receptivity of does at insemination. Moreover, the DLS had a beneficial effect on litter size (+0.3) recorded at 35 days of age,

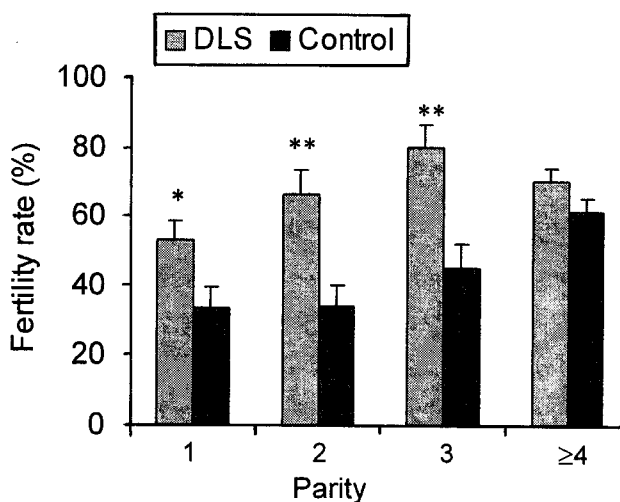


Figure 1: Effect of parity on the fertility rate obtained in lactating does submitted to DLS on day 9 *post partum* and inseminated 48 hours later, independently of the number of treatments received since their second parity. Results represent the mean \pm standard error of 64, 49, 46 and 189 inseminations for DLS does and 64, 61, 40 and 146 for untreated control does, in parity 1, 2, 3 and ≥ 4 respectively. Asterisks above bars indicate significantly different value: * $P \leq 0.05$; ** $P \leq 0.01$.

due to the lower losses of rabbits from day 9 to 35 (-3.5%), as reported in Table 1. However, the DLS caused a reduction on the growth of young rabbits (-1.7 g/d) from day 9 to weaning. Therefore, the young rabbits belonging to the treated group showed a lower weaning weight (-38 g) than the control young rabbits (Table 1).

The efficacy of DLS in improving fertility of does was greatly influenced by parity, as revealed by the significant interaction between treatment and parity (Table 1). In fact, as shown in Figure 1, the DLS significantly increased the fertility rate by 19.2%, 32.5% and 35.0% on does of parity 1, 2 and 3 respectively, whereas no effect was found on does having more than 3 parity.

When DLS was applied repeatedly to does having more than 3 orders of parity, the fertility rate progressively dropped in relation to number of successive treatments (Figure 2). Thus, the improvement margin plummeted from first to third

treatments in respect to the multiparous does, which never underwent DLS. In fact, at the 1st DLS, the gap was rather consistent (+34.3%), at the 2nd, the difference was almost halved (+18.2%) and by the third or more DLS treatments it disappeared.

The parity did not differently affect the litter size and the growth rate of mother-deprived young rabbits. Nevertheless, the size at 35 days of DLS litters was markedly higher than control only for does of parity 2 (7.6 vs 6.8). Instead, the weight gain of young rabbits, from day 9 until weaning, was significantly lower compared to that of control rabbits in correspondence of all lactation orders (Figure 3). However, the reduction in weaning weight was greater in litters of

DLS does of parity ≥ 4 (-51 g than in litters of does of lactation 1, 2 and 3 (-32, -31 and -37 g, respectively).

Although not significantly influenced by the number of repeated DLS, the size of litters at weaning showed a reduction after 3 or more treatments (7.3, 7.6, 7.5 and 6.9 after 0, 1, 2 and ≥ 3 DLS, respectively). Moreover, the young rabbits of litters nursed by does repeatedly treated 3 or more times showed a daily growth from day 9 to 35 lower by 2.6, 2.2 and 1.2 g in comparison with the 0, 1 and 2 DLS treated litters, respectively (Figure 4). As a consequence, their weaning weight was the lowest (823, 814, 804, 743 g for 0, 1, 2 and ≥ 3 DLS, respectively).

Table 1: Effect of a 48-hour doe-litter separation (DLS) on the performance of lactating does and their litters (means \pm standard error). Inseminations at day 11 postpartum.

	Age (d)	Treatment		Significance level				Roc MS
		DLS	Control	Experiment	Treatment (TR)	Parity (PR)	TRxPR	
Inseminations (n)		348	311					
Receptivity (%)		65.5 \pm 3.0	58.2 \pm 3.1					47.4
Fertility (%)		67.2 \pm 3.0	43.3 \pm 3.0		***	***	**	47.4
Total born (n)		8.5 \pm 0.2	8.4 \pm 0.3	*				3.0
Born alive (n)		7.5 \pm 0.3	7.0 \pm 0.4					3.4
Still-born (n)		0.82 \pm 0.1	1.3 \pm 0.2		*			1.6
Litter size (n)	1	8.5 \pm 0.0	8.5 \pm 0.0	***		***		0.5
	9	7.9 \pm 0.1	7.9 \pm 0.1	***		***		1.2
	35	7.2 \pm 0.1	6.9 \pm 0.1	***	*	***		1.6
Rabbit losses (%)	1-9	7.5 \pm 0.6	7.2 \pm 0.6	***			**	26.7
	9-35	8.6 \pm 0.7	12.1 \pm 0.7	***	***	***		29.1
Weight (g)	1	64 \pm 1	64 \pm 1					11.4
	9	171 \pm 2	168 \pm 2	***		***		29.1
	35	781 \pm 7	819 \pm 7	***	**	***		11.4
Daily gain (g)	1-9	13.8 \pm 0.2	13.3 \pm 0.2	***		***		3.0
	9-35	22.7 \pm 0.2	24.3 \pm 0.2	***	***	***		3.8

*P \leq 0.05; **P \leq 0.01; ***P \leq 0.001

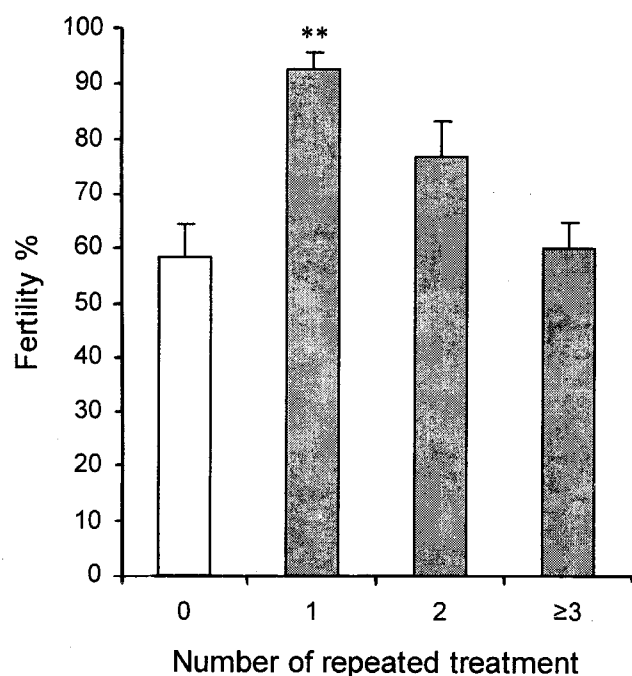


Figure 2: Effect of repeated DLS treatments (from none to ≥ 3) on the fertility rate of does with four or more parities. A total of 335 inseminations were analyzed, of which 149, 39, 52 and 92 after none, 1, 2 and ≥ 3 DLS applications, respectively. Two asterisks above the bars indicate significantly different value from 0 DLS treatment ($P \leq 0.01$).

DISCUSSION

Altogether, the results confirm the efficacy of a 48 hour DLS in improving fertility of free nursing does inseminated at 11 day *post partum*, and the reduction in the weaning weight of the young rabbits (ALVARIÑO *et al.*, 1998; BONANNO *et al.*, 2000; MAERTENS, 1998; VIRAG *et al.*, 1999). In opposition to other works, here sexual receptivity was not significantly improved by the DLS, giving a further demonstration that the subjective observation of the vulva colour and turgidity can not be considered a reliable method of scoring the sexual receptivity of rabbit does.

The role of parity in influencing the fertility of rabbit does and growth of their young rabbits in response to the DLS was here better defined. The DLS positively improved the fertility of young does in lactation 1-3, but it was ineffective on old does of

parity higher than 3, thus confirming previous findings. In fact, all the authors who studied the DLS reported that this technique was much more efficacious, although with different degree, in lower than higher order of parity whether a controlled (BONANNO *et al.*, 1999; SZENDRO *et al.*, 1999) or free nursing (MAERTENS, 1998; VIRAG *et al.*, 1999) management system was adopted.

Regarding the possible causes for this differential age- and/or parity-dependent effect of the DLS on fertility some plausible hypothesis can be suggested.

A sparing effect on energetic fuels, due to reduction of milk secretion by the temporary suppression of suckling associated with DLS, has been advocated by BONANNO *et al.*, (1999) to explain the improvement of fertility found in young treated does. Young does, to sustain both growth and lactation, frequently incur an energy deficit that may partially limit their reproductive activity (XICCATO *et al.*, 1996). Based on the same metabolic hypothesis, the ineffectiveness of the DLS technique in high-parity does can be justified by their better energy balance achieved with ageing. However, those metabolic factors that may regulate the hypothalamus-pituitary-gonad (HPG) system have never been identified in the rabbits.

Also, some hormonal clue can be involved in improving the fertility of lactating does following DLS. Decreased prolactin levels were found 24 hours after DLS by UBILLA *et al.*, (2000) probably due to absence of suckling episodes. The lowered prolactin concentrations may stimulate ovarian follicular growth and steroidogenesis and concur in improving receptivity and fertility (THEAU-CLÉMENT and ROUSTAN, 1992).

According to another hypothesis (THEAU-CLÉMENT *et al.*, 1998), the DLS evokes a mild stress on the doe exerting, via different neural pathways, permissive

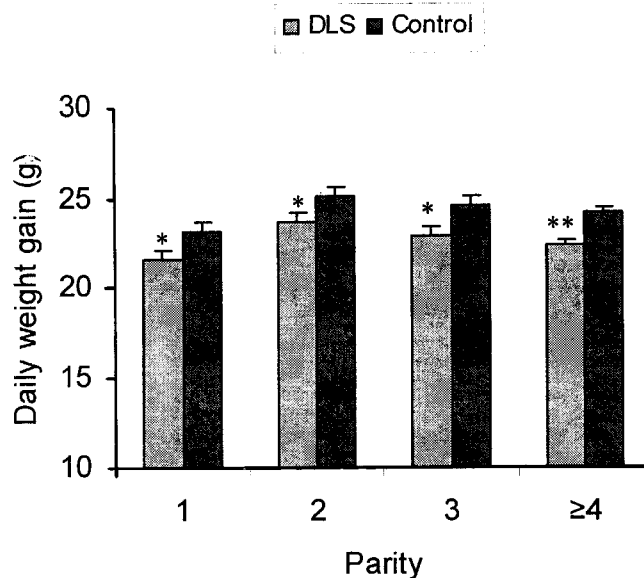


Figure 3: Daily weight gain (g) between day 9 and 35 in DLS and control young rabbits, as a function of parity of mothers. Asterisks above bars indicate significantly different value: * $P \leq 0.05$; ** $P \leq 0.01$.

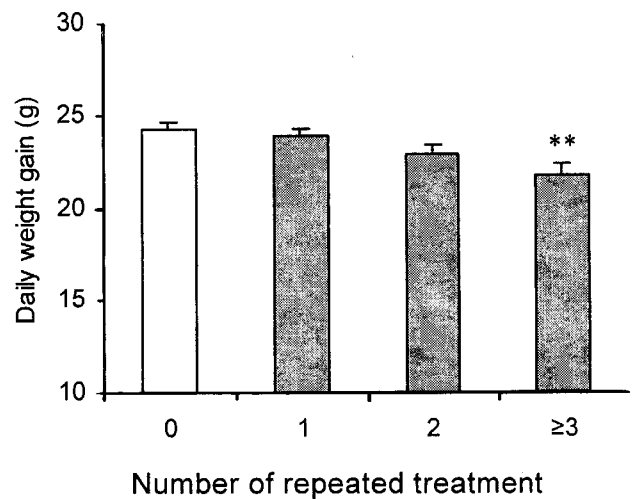


Figure 4: Daily weight gain (g) between day 9 and 35 in young rabbits nursed by does submitted to multiple DLS treatments (from none to ≥ 3). Two asterisks above the bars indicate significantly different value from 0 DLS treatment ($P \leq 0.01$).

actions on the hypothalamic centres that control GnRH-dependent gonatotropins release and, indirectly, both receptivity and fertility. There is an increasing body of evidence confirming that the level of activity of the HPG axis is modulated not only by several internal, but also by many external factors. However, a variety of different transmitter systems including neuropeptide Y, endogenous opiates, catecholamines, dopamine, serotonin corticotropin-releasing hormone as been implied in the regulation of the HPG system (PAU *et al.*, 1986). Therefore, waiting for specific studies to test them in the rabbit, the involvement of several brain regions; such as telencephalic structures, amygdala, and hippocampus, and activation of sympathetic adrenergic systems by extero-sensory inputs triggered by DLS can also be suggested to explain both parity- and treatment-depending effects of DLS (UBILLA *et al.*, 2000; UBILLA *et al.*, 2001). However, the hypothesis that older does may achieve over the time a sort of adaptation to multiple DLS treatments responsible for it reduced efficacy on fertility has never been taken into account before. Therefore, in this study the fertility of does of ≥ 4 lactations was analyzed in relation to the repeated

treatments (0, 1, 2 and ≥ 3), taking into account the effects of their parity (4 to ≥ 7) and their interaction. Interestingly, here it was found that in older rabbits, if 4 or more parity, only the first and partially the second DLS were successful in improving fertility, whereas successive DLS treatments lost their efficacy. Therefore, does having received 3 or more DLS treatments in previous lactation become refractory to this procedure, and her fertility drop to that of untreated control does. On this basis, fertility of DLS multiparous does seems to be more influenced by number of repeated treatments consecutively applied, rather than by their high-parity. This result suggests that rabbit females can habituate themselves to the DLS practise, acquiring a sort of adaptation to this bio-stimulation, the efficacy of which is progressively reduced for yet unknown reasons.

Moreover, the DLS litters nursed by does at lactation ≥ 4 showed a more pronounced reduction of weaning weight, confirming the observation of BONANNO *et al.*, (1999), that found a significant decrease in weaning weight only on young rabbits separated by does of parity higher than 4. In order to

explain these effects, the same authors suggested that the reduction of feed intake and the feeling of fullness in udders, provoked by DLS, could negatively act on milk secretion, and then on litter growth, of more milk producing does.

Growth and weaning weight of rabbits nursed by high-parity does were greatly reduced in correspondence of the 3rd DLS treatment. In older does the milk accumulating in udders due to the 48 hours long DLS treatment, especially when reiterated several times, might contribute to quicken the physiological ageing process of the mammary gland, thus reducing milk yield and litter growth thereafter. In cows, an increase of the amount of residual milk left in the mammary gland due to incomplete milking, when prolonged, causes a reduction of the milk secretory efficiency in the succeeding lactations (WILDE and KNIGHT, 1989). In rabbit does avoided to nurse beyond 24 hours, CALVERT *et al.*, (1985) observed a decline in their milk secretory rate and involuntional changes in milk composition, but they did not refer to any consequence on successive lactation.

CONCLUSION

The effect of DLS on fertility of does and growth of suckling rabbit seems to depend, rather than on parity, on number of successive treatments. When DLS was applied 3 or more times, rabbit does, independently of their parity, showed no improvement on fertility and a more pronounced reduction in growth of their young. Several DLS treatments may induce a condition of behavioural/psychological and/or physiological adaptation, capable of depressing the fertility, and reduce the secretory efficiency of the udder tissue, with negative repercussions on milk yield and litter growth. Nevertheless, both hypotheses need to be tested by specific studies.

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