

EFFECT OF CHANGE OF CAGE AND/OR 44H MOTHER-LITTER SEPARATION ON PRODUCTIVITY OF NON-RECEPTIVE LACTATING RABBIT DOES. PRELIMINARY INVESTIGATION

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ABSTRACT. The effects of three different bio-stimulation methods, namely change of cage (CC), 44-hour mother-litter separation (MLS) and a combination of the two (CCMLS), for inducing oestrus in non-receptive lactating does, were compared to the performance of receptive lactating does (RD). From March to July, 215 lactating does were taken to males for mating on day 9 after kindling. The 155 RD immediately mated. The does refusing to mate were divided in a balanced manner based body weight and parity and subjected to one of the three bio-stimulations. Receptivity in MLS does (72.7%) was higher than in CC (50.0%) and CCMLS groups (55.5%), and appeared more frequently (45.5%) on day 11, just after the stimulus.

The fertility rate of mated does and the total number of kits born per litter were 75.0, 70.0, 60.0, 88.4% and 7.8, 6.7, 6.6, 8.2, respectively for MLS, CC, CCMLS and RD. In comparison with RD, the 44-hour mother-litter separation reduced the weaning weight of young rabbits (-30 g MLS; -50 g CCMLS, $P < 0.05$), although it affected neither rabbit loss nor the incidence of mastitis. Based on to the few available cases, the use of MLS, as stimulation for inducing receptivity and improving fertility, seems preferable to CC or CCMLS. It needs to be verified whether the reduction of pre-weaning growth of young rabbits is compensated during the fattening phase.

RÉSUMÉ : Effet du changement de cage et/ou de la séparation de la mère et de sa portée, sur la productivité des lapines allaitantes non réceptives.

Afin d'induire l'oestrus, des lapines allaitantes non réceptives ont été soumises à trois méthodes de biostimulation (changement de cage: CC; séparation de la mère et de sa portée pendant 44 heures: MLS; et combinaison des deux: CCMLS) et leurs performances ont été comparées à celles de lapines réceptives (RD). De mars à juillet, 219 lapines ont été présentées au mâle 9 jours après la mise bas. Il y eut 155 lapines réceptives immédiatement saillies. Les lapines ayant refusé la saillie, réparties de façon homogène en fonction du poids vif et de la parité, ont été immédiatement soumises à l'une des trois biostimulations. La réceptivité des lapines MLS (72,7 %) a été plus élevée que celle des groupes CC (50,0 %) et CCMLS (55,5 %)

et observée le plus fréquemment au jour 11, juste après la stimulation. Le taux de fertilité des femelles saillies a été de 75,0 - 70,0 - 60,0 et 88,4 % et le nombre de lapereaux nés par portée de 7,8 - 6,7 - 6,6 et 8,2 pour les groupes MLS, CC, CCMLS et RD respectivement. Comparé à celui du groupe RD, le poids au sevrage des lapereaux du groupe MLS et du groupe CCMLS a été réduit respectivement de 30g et 50g ($P < 0,05$). Par contre, la séparation mère-portée n'affecte ni la perte des lapereaux, ni l'apparition de mammites. Sur la base du faible nombre de cas observés, l'utilisation de la seule séparation mère-portée (MLS) en tant que biostimulant pour induire la réceptivité et améliorer la fertilité, semble préférable aux deux autres méthodes (CC et CCMLS). Il reste à vérifier si la réduction de croissance des lapereaux avant leur sevrage est ensuite compensée au cours de l'engraissement.

INTRODUCTION

In commercial rabbit farms, where cycled production (CP) and artificial insemination (AI) are commonly practised, low reproductive performance is observed on lactating does. Because of hormonal antagonism between prolactin and gonadotropin release, lactation depresses sexual receptivity. As a consequence, artificially inseminated lactating does show both reduced fertility rate and prolificacy (THEAU-CLÉMENT and ROUSTAN, 1992; CASTELLINI, 1996).

The administration of Pregnant Mare Serum Gonadotropin (PMSG) 48-72 hours before AI proved effective in inducing and synchronising oestrus. But several consecutive PMSG injections seem to have negative side-effects, such as immunity response, ovarian dysfunction and increased mortality rate at birth (CASTELLINI, 1996; MAERTENS *et al.*, 1995).

In order to avoid the negative effects of systematic use of PMSG and to reduce the frequency of injection, only non-receptive does could be treated. This strategy has been shown to improve the sexual receptivity and

fertility of lactating does, but receptivity, based on observation of vulva colour, has to be detected in advance, and moreover, two AI interventions have to be carried out, the second after a gap of 48-72 hours. For this reason it does not really adapt itself to CP, although its application might be suitable for big rabbitries, where a 42-day CP is practised (ALABISO *et al.*, 1994; BONANNO *et al.*, 1996; BONANNO and ALABISO, 1996).

On the other hand, current European Community policy is tending towards reduction in the use of exogenous hormones, in order to protect consumers from meat residues and ensuring animal welfare. Consequently, in order to preserve the natural image of rabbit meat, the so-called «bio-stimulation» techniques could represent alternative methods for inducing and synchronising oestrus. Some of the initial approaches demonstrated the efficacy of change of cage (REBOLLAR *et al.*, 1995) and controlled lactation through mother-litter separation (PAVOIS *et al.*, 1994; DUPERRAY, 1995) for improving sexual receptivity and fertility.

At present, the recently founded IRRG (International Rabbit Reproduction Group) (BOITI, 1998) is conducting a specific research project, which intends to study and develop bio-stimulation methods in order to improve productivity of lactating does (THEAU-CLÉMENT *et al.*, 1998; THEAU-CLÉMENT and BOITI, 1998).

This work represents an initial investigation, planned in conformity with the objectives of the IRRG project. Various bio-stimulation methods, such as change of cage, 44-hour mother-litter separation and a combination of the two were applied on lactating does refusing to mate, the performance of which was compared to that of the lactating does accepting the males. Natural mating was used to better ascertain the sexual receptivity of does before and after bio-stimulations.

The aim of this study is to identify the method which will produce the best results in receptivity and fertility of non-receptive lactating does, verifying also the performance of their suckling litter, in order to test it in a later, larger-scale experiment using A.I..

MATERIAL AND METHODS

Animals and husbandry

The experiment was carried out on a small commercial rabbit farm (200 does) in Sicily, between March and July 1997. The rabbitry was ventilated naturally and lacked other environmental conditioning systems, so during the months from May to July temperatures were often higher than 30°C.

A total of 98 NZW multiparous does were used; they were housed in flat-deck cages with an internal isolable nestbox under a light program of 16 hours per day, fed *ad libitum* with a commercial diet (18.9% CP and 15.5% CF on d.m.), and mated naturally with NZW and Blue Vienna males, according to a semi-intensive reproductive rhythm. The equalisation of litter size at birth to 7-8 young rabbits was implemented, as were controlled suckling for 16 days after kindling and litter weaning at 31 days.

During the experiment, lactating does were grouped in successive batches, according the contemporaneity of their kindling: a total of 215 suckling does in 10 batches was involved in the study. In every batch, on day 9 after kindling within 15 minutes after suckling their litters, does were taken to males for mating. Does refusing to mate were split into three similar groups on the basis of their body weight and parity, and subjected to one of three stimulations, which were performed between day 9 and day 11 after kindling. In this way, does were not definitively attached to a group. Replacement of culled does was not performed.

Treatments

The bio-stimulations were:

- *Change of cage* (CC): on day 9 the doe was permanently transferred with her litter to another cage placed in a different row of the same room.
- *Mother-litter separation* (MLS): the nestbox was closed for 44 hours, from 12.00 noon of day 9 to 8.00 a.m. of day 11 after kindling, suppressing one daily suckling.
- *Change of cage and mother-litter separation* (CCMLS).

Mating of bio-stimulated does was first attempted on day 11, just after bio-stimulation. If non-receptive, the does were tested for mating at 24-hour intervals, always within 15 minutes after litter suckling, until day 14.

Measurements

The following parameters were recorded: day of mating acceptance; size and weight of suckling litters at bio-stimulation (day 9), end of controlled suckling (day 16), and weaning (day 31); fertility rate (kindlings/mated does); total number and number of young born alive.

Statistical analysis

Data were statistically analysed by the GLM procedure of SAS 6.12 software (1989), according to a linear model considering the effects of the group (4 levels = CC, MLS, CCMLS, RD) and the season (2 levels = spring, March 1st - May 15th; summer, May 16th - July 15th). The interaction was omitted because it was always non-significant. For individual weight of young rabbits, litter size was a co-variable. Only the means of the groups are reported in the tables. The differences between means were tested by Student «t» test.

RESULTS

Mating receptivity, fertility and prolificacy (table 1).

The receptive does (RD) that mated immediately on day 9 numbered 155 (72.1%). Receptivity at 9 days after kindling was 87.4% in the spring and 55.8% in the summer (P<0.01).

In all treated groups the receptivity appeared more frequently on day 11, just after application of bio-stimulation, and it was higher in MLS does than in CC and CCMLS groups.

The fertility percentages of bio-stimulated receptive does were lower than the RD group; the lowest value was realised by the CCMLS group.

Moreover, the fertility rate was higher in the MLS group (54.5%) in comparison with the CC and CCMLS groups (35.0% and 33.3% respectively).

The total number and number of young born alive per litter of MLS does was comparable to that

Table 1 : Effect of treatment on mating receptivity, fertility and prolificacy of lactating does (LSM).

	Bio-stimulated does			Receptive does (RD)	RSD
	Change of cage (CC)	Mother-litter separation (MLS)	Change of cage and mother-litter separation (CCMLS)		
Lactating does (no)	20	22	18	155	
Receptivity after bio-stimulation (%) (1)					
day 11	25.0	45.5	38.9		
day 12	15.0	18.2	5.5		
days 13-14	10.0	9.0	11.0		
Total	50.0	72.7	55.5		49.0
Fertility (%) (1)	70.0 ab	75.0 ab	60.0 a	88.4 b	34.6
Total born/litter (no)	6.7	7.8	6.6	8.2	2.8
Born alive/litter (no)	5.7	7.5	6.4	7.0	3.2
Still-born/litter (no)	1.0	0.3	0.1	1.2	1.9

RSD=Residual standard deviation. (1) Natural means. a, b: P≤0.05.

Table 2. Effect of treatment on litter size, losses and individual weight of suckling rabbits (LSM).

		Bio-stimulated does			Receptive does (RD)	RSD
		Change of cage (CC)	Mother-litter separation (MLS)	Change of cage and mother-litter separation (CCMLS)		
Litter size (no)	day 9	7.7	7.0	6.9	7.2	1.4
	day 16	7.7	6.9	6.9	7.1	1.4
	day 31	7.3	6.7	6.6	6.8	1.5
Rabbit losses/litter (no)	9-16 days	0.03	0.01	0.00	0.10	0.3
	16-31 days	0.42	0.28	0.29	0.23	0.6
Weight (g) (1)	day 9	151	158	158	157	28.5
	day 16	263	243	251	257	41.7
	day 31	651 ab	631 ab	613 a	664 b	93.4

RSD=Residual standard deviation. (1) Litter size as co-variable. a, b: P≤0.05.

of RD, but the latter had higher kit loss at birth. Litter size was larger in MLS does than in CC and CCMLS groups, but the differences were not significant.

Size and weight of suckling litters (table 2).

At 9 days after kindling, does of the CC group had a tendency for a larger litter size than the other bio-stimulated groups, so at 31 days they weaned a slightly larger number of young rabbits. In fact, during

suckling, from day 9 to day 16, the loss of young rabbits did not vary among the bio-stimulated groups and were lower than in RD. It was observed that higher losses of suckling rabbits occurred from day 16 up to weaning, in all does.

On day nine, the individual weight of suckling rabbits was analogous in all groups. Similarly, on day 16, it did not significantly differ between groups. On day 31 the litters separated from their mother for 44 hours showed a lower individual weight at weaning in comparison with RD, especially the CCMLS rabbits (P<0.05). It is evident that mother-litter separation brought about a certain growth reduction in suckling rabbits.

The weight of suckling rabbits was always significantly lower in summer than in spring (591 vs 688g, P<0.01).

Culled does

Culled does were equal to 10% of both bio-stimulated does and RD.

After they weaned their litters, six does previously subjected to bio-stimulation were culled. Three died and the other three were affected by mastitis

(one after CC and two after CCMLS).

Seven RD does died. Two does were eliminated because of infertility and the other six were affected by mastitis. None of the latter had previously been subjected to bio-stimulation, especially MLS.

DISCUSSION

This work was planned as an initial investigation with the aim of obtaining preliminary information

about the efficacy of certain bio-stimulation methods on the receptivity and fertility of lactating does refusing to mate, and also on the consequences on viability and growth of suckling rabbits. These indications were considered important before applying one of the methods studied in a larger successive experiment.

Natural mating was used in order to score sexual receptivity of lactating does based on acceptance of males. Detection of colour and turgidity of the vulva are subjective, less reliable methods. Natural mating also allows determination of response-time to all bio-stimulations in the manifestation of receptivity in non-receptive lactating does.

This approach greatly limited the available number of lactating does subjected to bio-stimulation, in fact the non-receptive does were only 60, equal to 28% of controlled does.

Therefore the results only refer to a few cases, an aspect that restricts the possibility of reaching definite conclusions. At the same time, the data obtained could give further indications concerning the type and the modality of application of bio-stimulation methods, and they could constitute a starting point for other, more exhaustive studies.

Being limited to very few observations, the comparison of the bio-stimulation techniques tested clearly shows that the best response in mating acceptance and fertility rate and total number and number of young born alive per litter was obtained by separating the mother from her litter for 44 hours, without transfer to another cage.

The efficacy in receptivity, fertility and litter size of a 40-hour mother-litter separation was demonstrated more recently by MAERTENS (1998), while ALVARIÑO *et al.* (1998) observed an improvement in fertility following at least 36 or 48 hours of mother-litter separation in lactating does inseminated 11 days *post partum*.

Nevertheless, 44-hour mother-litter separation resulted in a growth reduction in suckling rabbits from day 9 up to day 31, and consequently a lower weight at weaning of young rabbits than the other treatments, but it did not increase the losses of suckling rabbits and the cases of does affected by mastitis. In practice such a method involves a daily suckling suppression when lactation is controlled, so young rabbits reduce their milk intake and consequently their growth.

MAERTENS (1998) and ALVARIÑO *et al.* (1998) also found that the application of mother-litter separation caused a growth reduction in suckling litters, but did not negatively affect the pre-weaning survival of young rabbits.

Hypotheses to explain the positive effect of mother-litter separation in physiological terms are advanced by THEAU-CLEMENT and BOITI (1998), although they underline the necessity of further

experiments to better define the physiological mechanism and the application modality in relation to productive responses.

LUZI and CRIMELLA (1998) observed an improvement in fertility of multiparous does transferred to another cage two days before AI. On the contrary, in this study, the change of cage, either combined or not with mother-litter separation, resulted in inferior reproductive performance, especially in terms of receptivity and litter size.

Therefore, mother-litter separation did not show a positive effect when it was associated with an environmental modification, such as a cage transfer. The data is consistent with the results of CASTELLINI *et al.* (1998), who observed no improvement in sexual receptivity and fertility of lactating does when separated from their litter, through change of cage, for 24 hours, three days before AI. However, in that case, the interval between stimulation and AI was probably too long.

According to VERITÀ and FINZI (1980), the change of cage is a «macrostressor», because it induces a modification of feeding behaviour and a reduction of feed intake for at least 24 hours. The mother-litter separation combined with a change of cage could act as a more intense stress, strongly reducing the does feed intake for a longer time, in this way contributing to lower reproductive response and milk yield. This hypothesis could also explain the lowest weaning weight recorded in the CCMLS rabbits.

In this work, most of the non-receptive bio-stimulated does accepted the male just after stimulus application. A non-receptive doe can become naturally receptive in the following days, without any stimulation; moreover, also the contact with the male, which here occurred during the mating attempts, can stimulate sexual receptivity. Therefore it has to be noted that the change of receptivity recorded on day 9 is not the effect of bio-stimulation only.

Nevertheless, it has been clearly observed that, in does separated from their litters, mating receptivity was more frequent just after stimulus application, when the nestbox was open. Such a circumstance might indicate the need for further indication defining the time-interval between bio-stimulation and AI.

In conclusion, on the basis of the results of this preliminary experiment, a 44-hour mother-litter separation, employed as stimulation in order to induce receptivity and to improve fertility, appears to be preferable to change of cage or a combination of the two methods. Furthermore, the validity of this bio-stimulation technique is supported by findings of both MAERTENS (1998) and ALVARIÑO *et al.* (1998), who observed an efficacy comparable to PMSG treatment, therefore retaining mother-litter separation as an effective alternative to hormonal methods. It remains to be

determined whether the growth reduction to weaning is compensated during the fattening phase.

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