# EFFECT OF A 48 HOURS DOE-LITTER SEPARATION ON RABBIT DOE'S REPRODUCTIVE PERFORMANCE AND OFFSPRING'S GROWTH

VIRÁG GY.<sup>1</sup>, KUSTOS K.<sup>2</sup>, SZABÓ L.<sup>1</sup>

(1) Institute for Small Animal Research, GÖDÖLLÖ, Isaszegi út., Hungary, H-2100

(2) Agricultural University of Gödöllö, GÖDÖLLÖ, Páter K. út. 1, Hungary, H-2100

**ABSTRACT**: Artificial insemination of lactating does at 10-11 days after parturition poses difficulties to achieve high fertility rate. It would be important to overcome this constraint because shortening the interval between two parturitions would have an economic advantage. In this study, 308 inseminations of 90 NZW does over a six-parity period were evaluated to determine the effects of a 48 hours of doe-litter separation (S group) compared to the free suckling (R group) on does reproductive performance and on the growth of the actually nursed litter. All litters were equalised at 8 kits per litter on day 1 post partum. For the separated group, the kindling rate showed an overall significant increase of 20 points (64.7% vs 44.9%). The difference between the groups was the highest (around 40 points) for primiparous does. Improvement decreased thereafter for does inseminated during their 2<sup>nd</sup> and 3<sup>rd</sup>

lactation (less then 10 points) and raised again during last lactations (reaching more than 20 points). Weight gain of the actually nursed litter between the 9<sup>th</sup> and 11<sup>th</sup> days post partum (i. e. when one suckling was omitted in the separated group) was significantly different (P < 0.0001) being 50  $\pm$  11 and 243  $\pm$  11 g in the S and R groups, respectively. On the 21<sup>st</sup> day of lactation, this resulted in a significant (P < 0.001) reduction of litter weight (2548  $\pm$  75 vs 2927  $\pm$  66 g for S and R groups, respectively). The difference was the same from lactations 1-4, however it disappeared for later lactations. The results indicate that doe-litter separation is effective in term of kindling rate improvement mainly for primiparous does, however it has severe consequences on the litter weight at 21 days of age.

RESUME : Effets d'une séparation mère-portée de 48 heures, sur les performances de reproduction de la lapine et la croissance des lapereaux.

L'insémination artificielle, au 10-11 en jour après la mise bas, de lapines allaitantes, augmente les difficultés d'obtention d'un taux de fertilité élevé. Ce problème mérite d'être dépassé car le raccourcissement de l'intervalle entre deux mise bas est un facteur économique important dans la production. Dans cet étude, 308 inséminations pratiquées sur 90 lapines NZW jusqu'à la 6ème parité, ont été examinées afin de déterminer les effets d'une séparation mère-portée de 48 heures (groupe S), comparé à l'allaitement continu (groupe R), sur les performances de reproduction des lapines et la croissance des jeunes séparés. Toutes les portées ont été égalisées à 8 lapereaux par portée au jour 1 post partum. Dans le groupe S, le taux de mise bas montre une augmentation significative de 20 points (64,7 % vs

44,9%). La plus forte différence ( environ 40 points) concerne les lapines primipares. L'amélioration décroît pour les lapines effectuant leur 2<sup>ème</sup> ou 3<sup>ème</sup> lactation (moins de 10 points) et augmente à nouveau durant les dernières lactations (atteignant plus de 20 points). Le gain de poids des lapereaux du groupe S entre le 9<sup>ème</sup> et le 11 ème jour post partum (qui ont été privé d'une tétée), est significativement différent (P<0.0001) : 50 ±11 et 243 ± 11 g pour les groupes S et R respectivement. Ce qui conduit au 21 ème jour de lactation à une diminution significative (P<0.001) du poist de la portée. (2548 ± 75 vs 2927 ±66 g pour les groupes S et R respectivement). Ce suivantes. Les résultats montrent que la séparation mère-portée améliore efficacement le taux de mise bas, surtout pour les primipares, mais qu'il a de sévères conséquences sur le poids de la portée à 21 jours.

#### INTRODUCTION

One of the main factors defining effective reproduction is the length of time between two consecutive kindlings. This could be shortened by the insemination of lactating does if combined with high fertility rate. However, high fertility rate is difficult to obtain especially when nonreceptive lactating does are inseminated as reported by THEAU-CLÉMENT and ROUSTAN (1992). This adverse effect of lactation is even much more pronounced when primiparous does are inseminated compared to those of nulli- or multiparous (BOURDILLON et al., 1992). Hormonal treatments to stimulate oestrus are costly, and may cause side effects and consumers aversion. Doe-litter separation for a short period, conversely, is one of the promising methods (THEAU-CLÉMENT et al., 1998) which are defined collectively as biostimulation. Members of the IRRG group studied the effect of 24, 36, 40 and 48 hours of doe-litter separation. Their first results have been summarized in the

report of Theau-Clément and Boiti (1998). Although the outcome of the experiments was fairly different among these studies, their work generally suggests that the method could offer an alternative for hormonal treatments. But separation often resulted an adverse effect on the growth of the actually nursed litter even when it lasted only for 24 hours. Until now, few reports have been published on the long term effect of special treatments applied at the consecutive parities including detailed analysis of multiparous parturition's. However when analyzing results of the hormonal stimulation, several authors have reported cumulative effects of long term application in multiparous females as reviewed by MAERTENS et al. (1995).

In the presented experiment, we have followed the performance of the same does inseminated during their 1<sup>st</sup> - 6<sup>th</sup> lactation. The aim of our study was to evaluate the long term effects of a 48 hour doe-litter separation on fertility and prolificacy of the doe and on her litter which was nursed at the time of the treatment's application.

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Table 1: Number of inseminated lactating does by lactation order

Group / Lactation order	1	2	3	4	5	6
Regular	43	42	34	21	13	5
Separated	39	35	31	23	14	8

## **MATERIAL AND METHODS**

#### Animals and housing

New Zealand White nulliparous females labeled with individual identity number were randomly allocated to one of the two experimental groups: separated (S), where a 48 h doe-litter separation was applied and regular (R), where suckling was non-restricted. Females (N = 100) were divided between the two groups by their body weight so that the average of groups S and R were the same (3994 $\pm$  387 g) when the experiment started. Does were first inseminated at 18 weeks age and their reproductive performance was followed until their 7<sup>th</sup> parities. The experimental treatment involved primiparous does. Number of inseminated lactating does by each lactation is shown in table 1.

The experimental animals were homogeneously distributed in the rabbit house between other non-experimental females inseminated at the same time. Lactating does were placed individually in flat deck cages equipped with an outside feeder and a polypropylene nestbox. They consumed commercial rabbit pellets ad libitum. Light and dark periods were controlled (L: 16h / D: 8h). Temperature was maintained between 10-14°C by heating at winter and kept below 30°C by ventilation at summer.

## Reproduction cycle and artificial insemination

A 42-day cycle was employed in 3 batches, in which does were inseminated with an interval of 14 days. Every batch was inseminated 8 times. Pregnancy was checked by palpation on day 14 and open does were re-inseminated on the 28th day after the previous unsuccessful insemination together with their new batch. Only females without clinical symptoms of any disease were inseminated. Total infertility established after 3 consecutive inseminations, when the doe was culled. Culled does were not replaced in this experiment. At every insemination date, we collected and pooled semen from the same 5 bucks and prepared only one mixed and diluted semen dose for does of each group. Dilution was performed using a freshly prepared extender containing trisodium-citrate, glucose and egg-volk. The quality of the diluted semen and quantity of the sperm cells were evaluated under a microscope according to the method developed by ZÖLDÁGH et al. (1988). One-half a ml of pooled and evaluated mixed sperm was used for insemination of a doe. Does were fixed and inseminated in upright position using heat sterilized glass insemination pipettes. A GnRH analogue was applied i.m. (1.5 µg Ovurelin AUV, Reanal) at the same time.

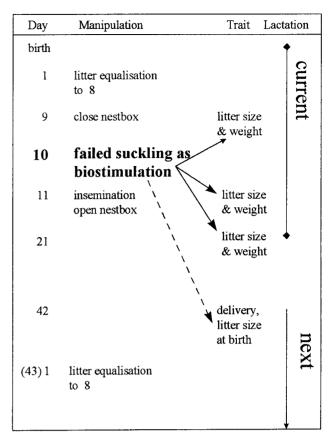


Figure 1: Schematic representation of one cycle which was repeatedly performed using the same does.

## **Biostimulation method**

To eliminate the effect of differences in litter size, currently nursed litters were standardized to 8 kits by involving within group fostering at the  $1^{\rm st}$  day after kindling. Day 32, post insemination was considered as fixed parturition day. Does in group S were separated from their litters on day 9 postpartum after suckling. They were not allowed to suckle on day 10. On day 11, does were inseminated between 10-11 am. Immediately after insemination, nest boxes were opened and the does could nurse their kits. Except for this time in group S, and during the whole experimental period, group R nest boxes were open continuously.

## **Recorded parameters**

When fertility rate was calculated (number of kindlings/ number of inseminations × 100), only present and healthy does at the date of expected parturition were considered. Litter size (total and born alive) was recorded on the day of kindling. Litter size and weight of the currently nursed litters were measured on days 9, 11 and 21 postpartum after the daily suckling was completed and the first value was considered as an initial litter weight (figure 1). Litter size was also measured at weaning (on day 35 post partum).

#### Statistical analysis

Only the inseminations performed on lactating does were considered for this evaluation. In total, 308

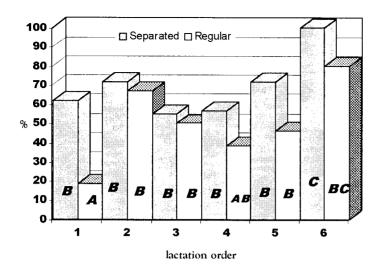


Figure 2: Effect of a 48 h doe-litter separation and of parity on fertility. A,B,C Values displayed by columns lacking a common letter differ at P<0.05 level.

inseminations and performances of litters nursed were analyzed using the GLM procedure (SAS, STAT, 1990). The linear model included treatment, individual effect of the randomly assigned does within treatment, effect of parity and the treatment  $\times$  parity interaction. Proportional data were judged using the PROC FREQ/CHISQ procedure.

#### **RESULTS**

#### **Fertility**

Doe-litter separation had an important effect on fertility of the does inseminated at day 11 postpartum. The proportion of does delivering was different for groups R and S, 44.9 vs. 64.7 %, respectively, showing a 20 % improvement between groups ( $\chi^2 = 12.08$ ; P < 0.001).

Overall kindling rate was also influenced by parity of does at insemination. Kindling rate of the primiparous does was 32.8 % and this was followed by 68.4, 54.6, 47.7, 59.3

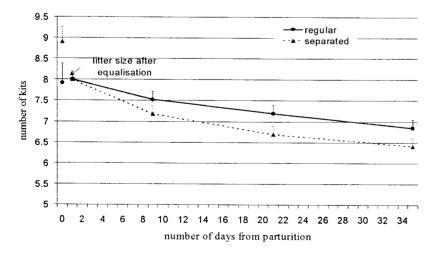


Figure 3 : Mean litter size (LSM  $\pm$  SE) in the regular and separated group.

and 92.3 % when does were inseminated in their 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> lactation, respectively. Kindling rate of primiparous does was statistically different (P< 0.005) from kindling rate of does inseminated in their 3<sup>rd</sup> and 6<sup>th</sup> lactations.

There was a significant interaction on fertility between groups of does (S and R) and parity (Figure 2). The number of does delivering was a slightly higher in S group consistently throughout the whole experiment, but the effect of the biostimulation on fertility for a specific lactation number was significant only for primiparous does (P<0.001).

For S primiparous does, kindling rate was 61.5%, but at the same time it was only 18.6% for R primiparous does, the lowest kindling rate observed during the whole experiment. Kindling rate increased to approximately 70 % in each of the two groups when does were inseminated during their  $2^{\rm nd}$  lactation, later the kindling rate decreased

again. Does in group S and R inseminated at their  $5^{th}$  and  $6^{th}$  lactation period met the earlier level of kindling rate, although the raise was slower for regularly nursing does.

#### Litter size at birth

The total litter size at birth was  $8.4 \pm 2.2$ , on average. There was a one pup difference per litter between group S and R ( $8.9 \pm 0.4$  and  $7.9 \pm 0.5$ , respectively, figure 3) which was nearly significant (P < 0.1). The parameter values were the same in each lactation and they did not show any tendency of change. No interaction was observed on the litter size between the group and the parity of the does.

## Changes of litter size and weight of the currently nursed litter

The litter size was balanced to 8 on the 1<sup>st</sup> day postpartum by within group fostering (except primiparous

does when pups from other does were used). The overall mean was  $7.5 \pm 1.0$  on day 9 post partum, before separation was performed in the S group. This value slightly dropped subsequently, being  $7.2 \pm 1.2$  and  $6.7 \pm 1.6$  on days 21 and 35 postpartum, respectively. Despite the slight difference between group R and S (figure 3), there was no significant effect of the doe-litter separation on the size of nursed litter, the lack of milk had no deleterious effect on kits.

Mean litter weight (figure 4) on day 9 post partum after suckling was  $1310 \pm 202$  g without difference between the groups of separated and freely suckling does. Lactation order had no influence on the value distribution between the two groups.

Litter weight between 9<sup>th</sup> and 11<sup>th</sup> day of lactation and litter weight on the 21<sup>st</sup> day of age was significantly and negatively influenced by the biostimulation (figure 4).

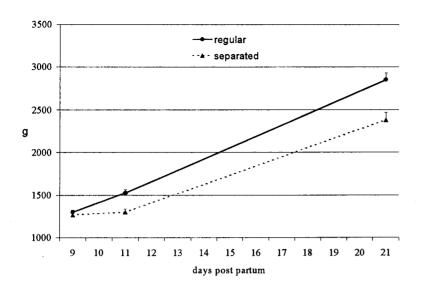
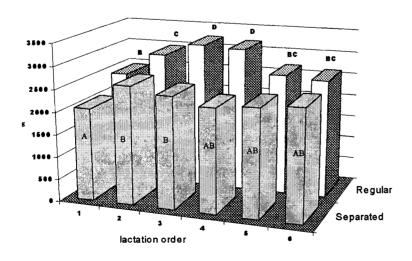


Figure 4: Litter weight (average  $\pm$  SE) on days 9, 11 and 21 post-partum

In the S group, the litter weight increase since day 9 was only  $50 \pm 11$  g after the suckling allowed on day 11. This low weight gain was the consequence of the lack of one suckling. On the contrary, in group R, where kits received their milk ration also on day 10, the litter weight difference between days 9 and 11 (weighing after suckling) was  $243 \pm 11$  g (P<0,0001).

The litter weight of young rabbits shortly separated from their dam was significantly lower (P < 0.001) at 21 days post partum:  $2548 \pm 75$  and  $2927 \pm 66$  g for group S and R, respectively, (figure 4). Lactation order also had an effect on litter weight as it was lower (P < 0.05) for primiparous does compared to the weight of litters in the  $2^{nd}$  -  $4^{th}$  lactation of multiparous ones,  $2257 \pm 105$  and  $2788 \pm 95$  g, respectively. There was a biostimulation  $\times$  lactation order interaction (figure 5) since the litter weights at 21



**Figure 5 : Mean weight (g) of the actually nursed litter at 21 days age**. A,B,C,D Columns lacking a common letter are significantly different at P<0.05 level.

days post partum were significantly (P<0,05) different in the two groups for the 1<sup>st</sup> to the 4<sup>th</sup> lactation but this difference totally disappeared in the 5<sup>th</sup> and 6<sup>th</sup> lactations.

#### **DISCUSSION**

#### Effect of the biostimulation

Separation resulted in a similar improvement in kindling rate to that obtained in other studies performed by the **IRRG** members involving similar experimental conditions, reviewed by THEAU-CLÉMENT and BOITI (1998). The highest difference in fertility rate (20 %) was found in this experiment. The overall kindling rate in group R was only 45 % which might be explained in that we used purebred does in this experiment instead of crossbred does. However, REBOLLAR et al. (1992) reported also a 46% kindling rate for

crossbred does inseminated on day 11 after parturition. The 64.7% fertility rate in the group S was consistent with the artificial insemination results summarized for one-year periods on the rabbit farm involved in this experiment earlier when a more extensive reproduction rhythm was performed.

Litter size at birth was also influenced by the separation. A one kit difference between groups S and R could be important; however it was statistically verified at only the P < 0,1 level. Each of the observed mean values resulting from AI was appropriate for the New Zealand White breed (Torres *et al.*, 1987). Litter weight gain and litter weight at 21 days were adversely affected (-380 g) by doe-litter separation. Similarly, MAERTENS (1998) also found that litters of separated does were lighter by 310 g.

#### Effect of the lactation order

The lactation order effect significant only for kindling rate and litter weight at 21 days age. A very poor performance, only 18.6 % kindling rate, was recorded for primiparous does which were regularly suckling although this might not be merely explained by the negative effect of lactation. This was counteracted by the doelitter separation so that a 61.5 % kindling rate was detected in this group. The higher kindling rate of does inseminated in the 6th lactation within both experimental groups could be a consequence of that only the best performing does were able to survive to this relatively high lactation number within the given experimental period.

Litter size at birth was not affected by lactation order, however the lightest litter weight was detected in primiparous does. The same difference for primiparous does was also found by BATTAGLINI *et al.* (1986).

#### Interactions between biostimulation and lactation order

An interaction effect was found between kindling rate and litter weight at 21 days. A significant effect of doe-litter separation on kindling rate was statistically verified only when primiparous lactating does were inseminated. In other cases, although insemination of the separated does resulted in consistently higher kindling rate, the difference was only nearly significant. Considering the continuously decreasing number of inseminated lactating does with increasing lactation order, these results should be confirmed with a greater number of AI.

Primiparous does separated before insemination produced the lightest litter weight at 21 days age when compared to the primiparous regularly suckling and to the multiparous does of both groups. At each subsequent lactation, the litter weight was equal in the group of S does. The R does however produced increasingly heavier litters at each of the  $2^{\rm nd}$  to  $4^{\rm th}$  lactations which could be explained by the increasing milk production of the does (McNitt and Lukefahr, 1990).

#### **CONCLUSIONS**

Based on the results presented herein, dam – litter separation, as a biostimulation method, could possibly be adopted by practical breeders. However, our results should be confirmed by further experiments performed with an extended number of does and under conditions where higher overall fertility rate can be attained. For practical reasons, comparison of total weight of rabbits obtained at weaning (during the same period of time at 8 to 12 months) for 100 initial does, would be recommended to study the positive effect of separation on kindling rate (+20 points according to our results). The negative effect on offspring growth (litter weight at 21 days reduced by 13% in our case, but after litter size equalization on day 1) should be considered.

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