WORLD RABBIT SCIENCE 1998, VOL. 6 (1), 199-203

EFFECT OF MOTHER-LITTER SEPARATION FOR 24 HOURS, BY CLOSING THE NESTBOX OR CHANGE OF CAGE, ON RABBIT DOE REPRODUCTION PERFORMANCE *

CASTELLINI C. [1], CANALI C. [2], BOITI C. [2]

[1] Ist. di Zootecnica Generale, Facoltà di Agraria, Borgo XX Giugno, 74 - PERUGIA - Italy.
[2] Ist. di Fisiologia Veterinaria, Facoltà di Medicina Veterinaria, Via S. Costanzo, 4, PERUGIA - Italy.

ABSTRACT : The effect of 2 different techniques of mother-litter separation on rabbit doe performance was analysed. In the first experiment, executed in a commercial rabbitry, multiparous lactating hybrid does were successively artificially inseminated (620 AI) while in the second, a flock of 100 nulliparous was followed (524 AI). Does were submitted to one of the following treatments: control no treatment; 24 hours of mother-litter separation, 3 days before A.I, with 2 different procedures: closing the nestbox (Experiment 1) and change of cage (Experiment 2). All the does were inseminated 11 days after kindling. Mother-litter separation did not significantly affect reproductive results while, as expected, sexual receptivity and physiological status strongly influenced it. Probably longer stimuli are necessary to induce oestrus, particularly when does are in lactation. Nulliparous and multiparous non-lactating does showed a significant positive reaction to biostimulation (Experiment 2 - gain of fertility rate: 12.9% and 14.0%, respectively).

RESUME : Effet de la séparation de la mère et de sa portée pendant 24 heures (fermeture de la boîte à nid ou changement de cage), sur les performances de reproduction de la lapine.
Deux techniques différentes de séparation de la mère et de sa portée ont été étudiées. Dans la première expérience menée dans un élevage industriel, des lapines hybrides pluripares allaitantes ont été inséminées artificiellement (620 IA) tandis que dans la deuxième expérimentation un groupe de 100 lapines ont été contrôlées jusqu'à obtenir 524 IA. Les traitements suivants ont été appliqués : groupe contrôle sans traitement, 24 heures de séparation mère-portée trois jours avant l'IA selon deux méthodes - fermeture de la boîte à nid (expérience 1) - changement de cage (expérience 2). Toutes les lapines ont été inséminées 11 jours après la mise bas. La séparation mère-portée n'a pas significativement influencé les performances de reproduction tandis que la réceptivité sexuelle et le statut physiologique ont été fortement influencés. De plus longues stimulations sont sans doute nécessaires pour induire l'oestrus, particulièrement quand les femelles sont allaitantes. Les lapines non allaitantes nullipares ou pluripares montrent une réaction positive significative à la biostimulation (expérience 2 : augmentation du taux de fertilité : 12,9 et 14,0 %, respectivement)

INTRODUCTION

In intensive rabbit farms the females are usually inseminated soon after parturition being able to sustain lactation and pregnancy at the same time. However, lactation induces a physiological depression of the reproductive functions (FORTUN and BOLET, 1995) probably due to an hormonal antagonism between prolactin and gonadotrophins release.

An other main factor affecting reproductive performance is the sexual receptivity of the doe at the moment of artificial insemination (AI): receptive does show higher fertility rate and prolificacy with respect to non receptive females (THEAU-CLÉMENT and ROUSTAN, 1992). In order to improve breeding performance the most common method to induce oestrus is the administration of Pregnant Mare Serum Gonadotrophin (PMSG), sometimes associated with Human Chorionic Gonadotrophin (hCG), 2-3 days before insemination, without any consideration of the receptivity status of does.

However problems experienced with the widespread use of PMSG (ovarian dysfunction, no effect after several administrations) together with the necessity of maintaining a 'natural' image towards the consumers (MAERTENS *et al.*, 1995) suggest that alternative protocols for oestrus synchronisation should be defined (CASTELLINI, 1996).

In order to eliminate or greatly reduce gonadotrophin use, two differentiated strategies can be outlined:

- The first one restricts PMSG treatment to few categories of does (e.g. the non-receptive lactating does, primiparous) where the effectiveness of gonadotrophins has been clearly established. In this case, several categories of females, including the nulliparous, the non-lactating and the receptive does (based on the vulva observation), are not treated at all (BONANNO *et al.*, 1995); however this strategy is not well adapted to cycled production;
- The second one turns to manage some environmental factors, such as lighting programs, feed intake, controlled lactation; some of them have been shown to stimulate the sexual receptivity of females (PAVOIS *et al.*, 1994).

* Preliminary results presented at the 2nd IRRG Meeting "Rabbit reproduction: research and practical application" 23-25 October 1997 - Institute For Small Animal Research, Gödöllö - Hungary.

The mechanism by which these stimuli increase the reproductive performance of does are not yet clearly known, but probably they promote sexual receptivity by modulating the endocrine balance of the hypothalamic–pituitary–ovarian axis.

This work has been done to verify the reproductive efficacy of different bio–stimulation techniques based on mother–litter separation. In 2 successive experiments the effect of the nestbox closing (Experiment 1) and the change of cage (Experiment 2) was analysed.

MATERIALS AND METHODS

The 2 trials were conducted in 2 different farms. In the first experiment, executed in a large commercial rabbitry, multiparous lactating does were stimulated only once. In the second, a flock of initially nulliparous does, raised in an experimental stable, was followed during the whole reproductive life, permitting to analyse the long term effects of the stimulus.

Does were divided into 2 homogeneous groups and submitted to one of the following treatments:

- control group had free suckling during the whole lactation.
- mother–litter separation for 24 hours with 2 different procedures : closing the nestbox (Experiment 1) or transfer of the does to another cage during 24h (Experiment 2).

In both experiments the same hybrid does (Provisal) were used. The housing conditions were similar and provided flat deck cages, bucks on the top of the cage lines, fixed light program of 16 hours/day, controlled temperature (min. 17, max. 28° C) and relative humidity 60–80%. The does were fed *ad libitum* a commercial diet.

The 24h mother–litter separation was executed 3 days before AI (from 8:00 of 8th day *post partum* to 8:00 on the following day). All the does were inseminated at 09:00 of 11th day *post partum*, immediately after the injection of 20 μg of GnRH (Fertagyl). All litters were homogenised to 8 kits with adoptions inside the group.

Semen samples : The ejaculates of several bucks showing at least 75% of motility rate and 300 x 10^6 spermatozoa/ml were pooled and gently mixed. The semen extender was a TRIS buffer and the dilution rate 1:10. The semen was inoculated at about 15–18 cm of depth with glass straws with the doe in dorsal position.

Recorded parameters : Vulva colour and turgency was used to classify two categories of does (receptive, non receptive): a doe was considered receptive when the vulva was quite turgescent and the colour was reddish. Fertility rate (number of kindlings/ number of inseminations x 100), born alive, number of weaned rabbits and litter weight at 30 days were recorded.

Just before AI, 185 and 123 does were randomly selected (Experiment 1 and 2, respectively) and blood, were collected into heparinized vacutainers by venopuncture from the marginal ear vein. Plasma was immediately separated by centrifugation and stored at –20°C until assay for progesterone evaluations according to a standardised Radio Immuno Assay procedure (BOITI *et al.*, 1974). On the basis of plasma progesterone concentrations, does showing a value higher than 2 mg/ml were considered abnormal (P+) according to BOITI *et al.* (1996).

Table 1 : Effect of closing the nestbox for 24 hours, 3 days before AI on reproductive performance of does (Experiment 1) Least square means for continuous data and natural mean for discontinuous data.

	Receptivity rate %	Fertility rate %	P+ %	Current weaned rabbits n.	Pre-weaning mortality %	Born alive n.	Weaned rabbits n.	Individual weaning weight g
Control	57.2	59.9	14.0	7.7	9.5	8.4	7.6	615
Biostimulation	69.3	66.8	13.7	7.7	12.7	8.6	7.5	640
Receptive	100	75.5 B	14.8	7.9	7.8	8.8	7.8	618
Non receptive	–	52.8 A	12.9	7.5	7.4	8.2	7.4	637
n	620	620	185	620	363	410	363	363
χ^2	5.3	2.1	1.4	–	15.3	–	–	–
SE	–	–	–	2.9	–	2.8	2.5	33.9

For the same effect on the same column : A, B : P < 0.01.

Table 2 : Interaction between treatment and assay (Experiment 1).

Treatment	Assay	Sexual receptivity %	Fertility rate %
Control	1	62.5	57.0 A
	2	66.0	60.5 A
	3	67.3	60.4 A
Biostimulation	1	63.5	58.2 A
	2	67.7	74.8 B
	3	67.8	62.1 A

On the same column : A, B : $P < 0.01$.

Experiment 1 : A total of 620 AI, in 3 successive assays (June, July and August), were performed. Since the does were followed only for one kindling, mortality rate was calculated during the current lactation.

Statistical analyses : Continuous data were analysed according to a linear model (GLM–SAS procedure) comprising the fixed effect of treatment (control, biostimulation) and sexual receptivity (receptive or not). The interaction treatment per assay, when significant, was also added to the model. Other non significant effects, simple or interactive, were omitted. Discontinuous data were analysed with a CATMOD–SAS procedure.

Experiment 2 : 100 nulliparous hybrid does, definitively allocated to the same group during their entire productive life (about 14 months; n = 524 A.I) were analysed.

First insemination of nulliparous occurred at 4.5 months of age. Not pregnant does were re–inseminated 3 weeks later and eliminated after 3 consecutive negative inseminations.

Statistical analyses : Continuous data were analysed with a linear model comprising the effect of treatment (control, biostimulation), sexual receptivity (receptive or not), 5 physiological stages (nulliparous, primiparous lactating (L+) or not (L–), multiparous L+ and multiparous L–) and the interaction between physiological stage and treatment. Non significant interactions were omitted.

RESULTS

Experiment 1

The mother–litter separation for 24 hours, obtained by closing the nestbox 3 days before AI, did not significantly affects overall reproductive performance or the weight of rabbits at weaning (Table 1). The percentage of does with abnormal blood progesterone level was quite similar in the control and biostimulated group.

As expected, fertility rate was significantly higher in sexual receptive does (+22.7%, $P<0.01$) while the other variables were not influenced.

Table 3 : Effect of change of cage for 24 hours 3 days before AI on reproductive performances of rabbit does (Experiment 2) Least square means for continuous data and natural mean for discontinuous data.

		Receptivity rate %	Fertility rate %	P^+ %	Born alive n.	Weaned rabbits n	Pre-weaning mortality %	Individual weaning weight g
Control		66.5	62.4	3.5	8.3	7.1	14.7 a	670
Biostimulation		71.6	68.5	5.8	8.8	6.8	22.6 b	658
Receptive		100	72.3 b	4.3	8.6	7.2 b	15.7 a	668
Non receptive		–	50.0 a	5.0	8.5	6.7 a	21.3 b	660
Nulliparous	L-	78.7 b	85.6 B	2.0	8.2 a	6.5 a	20.7	610 a
Primiparous	L-	70.3 b	85.1 B	4.1	8.8 b	7.2 b	18.2	659 ab
	L+	55.1 a	41.5 A	5.1	8.6 ab	7.0 ab	18.6	675 bc
Multiparous	L-	75.8 b	77.6 B	9.1	8.9 b	7.3 b	17.9	663 b
	L+	63.1 ab	50.9 A	3.2	8.5 ab	7.2 b	15.2	711 c
n		524	524	123	355	303	303	303
χ^2		3.5	2.7	1.1	–	–	26.2	–
SE		–	–	–	2.5	2.2	–	14.3

For the same effect on the same column : A, B : $P < 0.01$; a, c : $P < 0.05$.

The treatment showed a significantly different efficacy in the three successive assays (Table 2): the biostimulated group, in the second assay, showed higher fertility rate (74.8 *vs* 60.5%, P<0.01) without any relevant modification of sexual receptivity.

Experiment 2

The change of cage for 24 hours, three days before AI, did not affect sexual receptivity, fertility rate or the percentage of P+ does (Table 3) ; an increase in mortality rate (22.6 *vs* 14.7%; P<0.05) was observed in the biostimulated group, although no significant negative effect was observed in litter size at birth or at weaning and in the individual rabbits weight at weaning.

Receptive does showed a significant gain of fertility rate, number of weaned rabbits and pre-weaning viability (+22.3%, +0.5, +5.6%, respectively).

Physiological status, combining the effect of lactation and kindling order, significantly affected most of the reproductive parameters. Nulliparous does showed the lowest number of born alive, weaned rabbits and the lowest weaning weight (P<0.05) and, in comparison with the lactating does, the highest sexual receptivity and fertility rate. A reduction of sexual receptivity (-15.2%) and fertility rate (-43.6%) was particularly evident in primiparous lactating does. On the contrary non-lactating females showed quite the same results, whatever the kindling order.

The temporary change of cage increased the receptivity and the fertility rate of non lactating does (Table 4 : L- nulliparous *vs* primiparous *vs* multiparous : +22.1 *vs* +15.0% *vs* +10.5 %, and +12.9% *vs* +20.0% *vs* +14.0% respectively); on the contrary, lactating does were not significantly affected by the treatment.

Table 4 : Interaction between physiological stage and treatment (Experiment 2).

	Treatment	Sexual receptivity %	Fertility rate %
Nulliparous L -	Control	67.4 B	79.2 CD
	Biostimulation	89.5 C	92.1 E
Primiparous L -	Control	63.5 AB	80.0 D
	Biostimulation*	78.5	100.0
Primiparous L +	Control	55.3 A	40.2 A
	Biostimulation	54.2 A	49.1 AB
Multiparous L -	Control	67.0 B	70.5 C
	Biostimulation	77.5 BC	84.5 DE
Multiparous L +	Control	67.1 B	46.7 AB
	Biostimulation	58.3 AB	53.4 B

On the same column : A, E : P < 0.01.
* 50% of cells have expected count less than 5 observations; χ^2 may not be a valid test and statistical significance is omitted.

DISCUSSION

PAVOIS *et al.* (1994) avoiding the access to the nest for 24 hours, just before AI, obtained an increase of receptivity (+25.6%) and fertility rate (+13.4%), without any negative consequence on litter weight. However, the extent of these improvements did not appear much repeatable since the same bio-stimulation in successive assays, in which simultaneously two methods of insemination were used, resulted in different fertility gains (+16.3%, P=0.03 *vs* +9.7%, NS).

Also DUPERRAY (1995) in a field experiment, applying a 36 hours doe/litter separation associated with a feed additive, concluded that an increase of fertility rate (+8.5%) can be obtained in about 70% of rabbitries without any negative consequence for the doe and for the litter.

The findings of our first experiment partially agree with the previously reported data : a non significant increase of receptivity and fertility (+11.9 and +6.9%, respectively) although, diversified in the three assays, was observed. In fact, only in the second experiment the fertility rate was greater than in the control group (+14.3 %; P<0.01).

Probably, the effect of biostimulation on reproduction activity is much more dependant on other internal and external factors and on the general status of doe (hormonal balance, healthy condition). While gonadotrophin immediately affects target organs like the ovaries, environmental stimuli are modulated on individual basis by the nervous system via the hypothalamic-pituitary-ovarian axis thus making their action more variable.

As regard to the preweaning mortality rate, in both trials, the biostimulated groups with respect to control ones, showed an increase very close to that reported by the above mentioned authors (+3%); probably the higher born alive obtained in the Experiment 2 could explain the differences (7.9% *vs* 3.2% ; P<0.05)

In the second experiment mother-litter separation, obtained by transfer of the doe to another cage, did not

increase the reproductive response with respect to the nestbox closing ; probably longer period of mother–litter separation (36 hours, PAVOIS *et al.*, 1994) is necessary to induce oestrus, especially if does are lactating. In both experiments the fertility gains of multiparous lactating does were not significant and similar (6.9 % *vs* 6.7 % NS) while non–lactating does showed a significant positive reaction to biostimulation.

Lactation probably decreased the sensitivity of the doe to environmental stimuli; this depressive effect was particularly negative on primiparous does which adjoin to the ordinary antagonism lactation–reproduction, a considerable energy deficit (PARIGI–BINI and XICCATO, 1993).

In nulliparous does, also REBOLLAR *et al.* (1995) showed that one day of cage changing, 2 days before AI, is very effective as the females showed the same fertility rate than PMSG controls.

Proportion of does with anomalous progesterone profiles in the two experiments (13.8% and 8.5%, respectively) was not affected by mother–litter separation and ranged in the standard limits indicated in a previous paper (from 4.6 to 34.3% of P+, BOITI *et al.*, 1996).

Other stimuli (single or associated) and intervals between stimulation and AI should be investigated. The comprehension of the effect of these stimuli on the endocrine balance of does could clarify many doubts and simplify the choice of a stimulation factor.

Undoubtedly other considerations regarding manpower, management and economical aspects should be considered; for example the transfer of the doe to another cage for only one or some few days is a expensive labour technique.

Acknowledgements : Research supported by a grant from MURST 40%.

Received : February 4th 1998
Accepted : February 26th, 1998

REFERENCES

BOITI C., CECCARELLI P., BEGHELLI V., DANIOTTI P., PENNISI F., 1974. Messa a punto di un metodo per la determinazione radioimmunologica del progesterone plasmatico. *Atti Soc. It. Sci. Vet.*, **28**, 366–371.

BOITI C., CANALI C., MONACI M., STRADAIOLI G., VERINI SUPPLIZI A., VACCA C., CASTELLINI C., FACCHIN E., 1996. Effect of postpartum progesterone levels on receptivity, ovarian response, embryo quality and development in rabbit. In : *Proc. 6th World Rabbit Congress, Toulouse, Vol 2, 45–50.*

BONANNO A., ALABISO M., ALICATA M.L. Leto G., TODARO M., 1995. Effetti del trattamento differenziato con PMSG sull'efficienza riproduttiva di coniglie sottoposte ad inseminazione artificiale. *XI Cong ASPA, Grado, 129–130.*

CASTELLINI C., 1996. Recent advances in rabbit artificial insemination. In : *Proc. 6th World Rabbit Congress, Toulouse, Vol 2, 13–28.*

DUPERRAY J., 1995. Enquête terrain sur la Guystimulation. *Cuniculture*, **22**, 87.

FORTUN L., BOLET G., 1995 Les effets de la lactation sur les performances de reproduction chez la lapine. *INRA Prod. Anim.*, *8, 44–56.*

MAERTENS L., LUZI F., GRILLI G., 1995. Effect of PMSG in induced oestrus on the performances of rabbit does: a review. *World Rabbit Sci., 3, 27–34.*

PAVOIS V., LE NAOUR J., DUCEP O., PERRIN G., DUPERRAY J., 1994. Une méthode naturelle pour améliorer la receptivité des lapines allaittantes en insémination artificielle. *6èmes Journ. Recherche cunicole, La Rochelle, Vol. II, 528–535.*

PARIGI–BINI R., XICCATO A., 1993. Recherches sur l'interaction reproduction et lactation chez la lapine. Une revue. *World Rabbit Sci., 1, 155–161.*

REBOLLAR P.G, ALVARINO J.M.R., ARCO J.A., BUENO A., 1995. Control del celo en conejas nuliparas: manejo y tratamiento con PMSG. *6th Jornada sobre Prod. Anim. Vol. extra n.16, Tomo I.*

SAS/STAT, 1990. Basic and statistics. Version 6. *Ed. SAS Inst. Inc. Cary, NC, USA.*

THEAU–CLÉMENT M., ROUSTAN A., 1992. A study on the relationship between receptivity and lactation in the doe, and their influence on reproductive performance. *5th Congr. World Rabbit Science, July 25–30 Corvallis USA, Vol. A, 412–421.*