

EFFECT OF FLUSHING, MOTHER-LITTER SEPARATION AND PMSG ON

THE FERTILITY OF LACTATING DOES AND THE PERFORMANCE OF THEIR LITTER *

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ABSTRACT : Two batches of 80 does each were inseminated (AI) 11 d post parturition during 10 months in order to judge the effect of oestrus synchronisation of lactating does. Three different methods were compared with the not treated control: a separation mother-young during 40 h before AI, a 4 d. flushing prior to the AI with an energetic diet and a PMSG treatment (20 IU, 2 days before AI). A total of 991 inseminations, exclusively of lactating does, were analysed. After a 40 h separation, both receptivity and fertility (78.8 and 78.0%, respectively) were significantly higher ($P < 0.01$) than the control (40.8 and 66.9%) and comparable with the PMSG treatment (75.1 and 76.7%, respectively). Effects were most pronounced for primiparous

and does in their second lactation. However, the separation reduced ($P < 0.01$) weaning weight of the young (40 - 47g). Flushing with the concentrated diet had a negative effect on fertility (54.7 vs 66.9% for controls). This was probably due to the low palatability of the experimental diet. In fact, the daily energy intake during the flushing period was lower (-0.15 MJ ME/day) than in the control group. From this experiment it can be concluded that the bio-stimulation method applied is a real alternative to hormonal treatments for inducing oestrus synchronisation. However, further research is necessary to better define the optimal mother-litter separation time by taking into account also considerations on animal welfare

RÉSUMÉ : Effet d'un flushing, d'une séparation mère-jeunes et d'un traitement avec de la PMSG sur la reproduction des lapines allaitantes et les performances de leurs lapereaux.

Pendant 10 mois, deux groupes de 80 femelles ont été inséminées (IA) 11 jours après la mise bas, pour tester l'efficacité de diverses méthodes de synchronisation de l'oestrus chez des femelles allaitantes. Nous avons comparé à un lot témoin (non traité), les performances de lapines subissant une séparation mères-jeunes (40h avant l'IA), un flushing (aliment énergétique durant 4 jours avant l'IA) et un traitement PMSG (20 UI, 2 jours avant l'IA). Au total, 991 inséminations de femelles allaitantes ont été analysées. La réceptivité et la fertilité des lapines du lot "séparation mères-jeunes" (78,8 et 78,0 %, respectivement) étaient significativement plus élevées ($p < 0,01$) que

celles du lot témoin (40,8 et 66,9%) et comparables avec celles du lot PMSG (75,1 et 76,7 %, respectivement). Toutefois, la séparation mères-jeunes a entraîné une réduction ($p < 0,01$) du poids moyen des lapereaux au sevrage (40 à 47 g/lapereau). L'application du flushing a négativement affecté la fertilité (54,7 vs 66,9 % pour le lot témoin) ; cela semble être une conséquence directe d'une faible appétence pour l'aliment expérimental. En effet, l'ingestion énergétique était moins élevée (-0,15 MJ EM/jour) durant la période de flushing. Cet essai a démontré que la séparation ponctuelle mères-jeunes est une solution alternative à l'induction hormonale de l'oestrus. Toutefois, des expérimentations supplémentaires sont nécessaires pour mieux définir la durée optimale de séparation en liaison avec le bien-être des animaux.

INTRODUCTION

Artificial insemination (AI) has been introduced in large rabbit production units mainly to improve breeding management. However, the irregular alternation of oestrus and anoestrus periods is a disadvantage in view of a regular, synchronised reproduction rhythm. Probably because of the hormonal antagonism between prolactin and gonadotropins, the receptivity of does is a problem during the lactation period (CASTELLINI, 1996, for a review). Especially with lactating, non receptive does low reproductive performances are obtained (THEAU-CLÉMENT & ROUSTAN, 1992). With primiparous does, it has been clearly demonstrated that an important energy loss occurs during the first lactation. The impaired body composition of lactating does explains

the negative consequences on the reproduction performance observed on does inseminated during the lactation period (FORTUN-LAMOTHE and LEBAS, 1995; XICCATO, 1996).

The administration of exogenous gonadotrophin, Pregnant Mare Serum Gonadotrophin (PMSG), to synchronise the oestrus is widely diffused because of its simplicity and efficacy in organising the reproduction scheme. Numerous favourable results are reported, especially with lactating and primiparous does (MAERTENS *et al.*, 1995, for a review). However, also some disadvantages seems to be linked with the use of PMSG; e.g. increased mortality rate at birth, changed distribution of litter frequencies and immunity response after successive inoculations (MAERTENS *et al.*, 1995; CASTELLINI, 1996). Furthermore, consumers show increasing sensitivity for systematic use of exogenous hormones and for animal welfare. In order to avoid losing the good image of rabbit meat, alternative methods are searched to synchronise the oestrus.

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Quite recently, favourable farm results in synchronising the reproduction were reported when does were separated from their nest for 24 or 36 hours (PAVOIS *et al.*, 1994; DUPERRAY, 1995). Immediately after the controlled suckling, receptivity and conception rate increased about 30% and 10%, respectively.

The purpose of this trial was to compare different methods to synchronise the oestrus: hormonal (PMSG), nutritional (flushing) and through management (temporary mother–litter separation).

MATERIALS AND METHODS

The experiment was performed in one of the experimental stables of the Institute of Small Animal Husbandry between April 1996 and March 1997.

Animals and housing

Initially 160 does, belonging to the Institute's own selected strain (MAERTENS, 1992), were used for the experiment. The nulliparous, primiparous and multiparous does were homogeneously distributed over

the 4 experimental treatments. Blocks of 4 does were housed sequentially on the different rows of cages. Does were not definitively affected to a group, but after each treatment does were randomly assigned to one of the 3 other treatments. Replacement of does was performed only with nulliparous does. Males used for the insemination belonged to a heavy male line of the same strain and were housed in a separate compartment of the same building.

Does were housed in flat-deck cages measuring 600 x 430 x 330 mm high, equipped with an outside placed feeder and nestbox. A minimum inside temperature of 17°C was maintained in the windowless experimental stable during wintertime, using an over–under pressure ventilation system with heated air. No cooling was performed in summertime although temperature exceeded 25°C during 14 days in July and August. Controlled illumination (16 L:8D) was performed throughout the whole experimental period.

Treatments to synchronise the oestrus

Does were submitted to one of the following treatments:

- A. Control: no oestrus synchronisation
- B. PMSG: 20 IU i.m. (0.1 ml Folligon, Intervet) 2 days before the AI
- C. Separation of mother–litter by closing the nestbox during 40h before AI
- D. Flushing : a concentrated diet distributed during 4 days before the AI

No systematic controlled lactation was performed. Mother–litter separation (treatment C) was at 4.00 p.m. and does were allowed to nurse between 8:00 and 9:00 am. The AI was always performed between 9.30 and 11.00 p.m. The composition of the standard reproduction diet and the experimental diet are given in Table 1.

Breeding and management

A 42 days reproduction rhythm was followed using artificial insemination. The total number of does was divided in 2 batches which were inseminated with an interval of 21 days. Both batches were inseminated 7 times. Non–pregnant does changed from batch at weaning and were re–inseminated together with their new batch. Because weaning was performed at day 29 post parturition (PP), the non–pregnant does were re–inseminated 3 days after weaning.

AI was performed with heterospermic semen from 10 males. Semen was collected between 08:30 and 09:00 a.m. using IMV equipment. Immediately after collection each sample was diluted (1:2) with a tris–dilutor (Minitüb). All visually good samples were pooled and thereafter evaluated under a microscope

Table 1: Ingredient and chemical composition of the reproduction diet and the concentrated diet used for flushing.

	Reproduction diet	Concentrated diet
<i>Ingredients</i>		
Alfalfa meal (17% C. Protein)	25.0	14.0
Wheat shorts	16.0	–
Wheat	23.3	25.0
Sunflower meal (29% C.P.)	14.6	–
Soybeans	9.0	17.0
Soybean meal (44)	–	7.0
Corn glutenfeed (20% C.P.)	–	5.0
Casava meal	–	5.0
Whey powder	–	10.0
Meat meal	–	1.0
Flax chaff	5.5	6.0
Cane molasses	4.0	6.0
Vit. + min. Mix	2.5	4.0
L–lysine Hcl	0.1	–
<i>Nutrient composition (g/kg)</i>		
Crude protein	17.4	18.0
Lysine*	0.83	0.96
Ether extract	4.0	5.0
Crude fiber	15.4	10.0
Starch*	18.7	21.1
ME (MJ/kg) *	9.7	11.2
Ca*	1.0	1.4
P*	0.58	0.62

* Calculated

Table 2: Effect of treatment on fertility and prolificacy of lactating does

	Controls	PMSG	40h separation	Flushing	SEM	Signif.
No of inseminations	245	249	250	247	-	-
Fertility	66.9 Aa	76.7 ABb	78.0 B	54.7 C	12.1	P<0.001
Total born/litter	7.5 A	9.2 B	8.7 BC	8.0 AC	0.8	P<0.001
Born alive /litter	7.1 A	8.5 B	8.2 BC	7.6 AC	0.9	P<0.001

SEM: pooled standard error of the means A≠B: P<0.01 a≠b: P<0.05

(200x). Further dilution was performed based on the sperm concentration but a minimum concentration around 100 million spermatozoa per ml was respected. Pooled heterosperm was aspirated in IMV straws and introduced in plastic, single use insemination pipettes (0.5 ml or about 50 million sperm cells). All does were inseminated in lordosis position between 09:30 and 11.30 am. First the lactating does were inseminated, followed by the re-inseminations and the nulliparous does. Induction of ovulation was done at the moment of AI with GnRH (0.2 ml i.m. Receptal).

Cross fostering was systematically performed in order to equalise initial litter size without consideration for the experimental groups. Primiparous does received only 7 young, while multiparous does received 7, 8 or 9 young depending on the total available number. Induced parturition was systematically performed, if necessary, on day 32 post insemination (in the afternoon) using oxytocin (0.1 ml i.m.).

Discarded does (mortality, illness, sore hocks, 2 consecutive infertile inseminations or low productivity) were immediately replaced by nulliparous ones.

Registered parameters

The vulva colour and turgency was judged before each insemination. Parity number and type of insemination (1st or re-insemination) were registered. Palpation was performed 14d post insemination.

However, fertility rate was determined on does having partus including females which were pregnant at autopsy. Litter size was checked within maximum 14 h post parturition. Does and their litter were weighed at parturition and also at 7,11, 21 and 29 days PP. Day 31 post insemination was considered as fixed parturition day for all does. Feed intake per doe was measured in 4 periods (0-7, 8-11, 12-21 and 22-29 days PP).

Statistical analyses

Only the inseminations performed on lactating does were considered for the reproductive performance. In total 991 inseminations were analysed using the GLM procedure (SAS, STAT, 1990). The linear model included the treatment and the effects of parity, insemination group, previous treatment (n-1) and the interaction treatment x parity. Because does were not definitively affected to a fixed experimental group, the effect of the previous treatment (n-1) was taken into account. Proportional data were judged using the PROC FREQ/CHISQ procedure.

RESULTS

Fertility and litter size

Table 2 shows the main results of fertility and litter size according to treatment. The technique used to synchronise the oestrus had a significant effect (P<0.001) both on fertility and prolificacy as well. The systematic treatment of does with PMSG on day 9 PP increased (P<0.05) the reproductive results. Also the mother litter separation for 40h was very successful as the results were comparable with the PMSG treatment both for fertility (78.0 and 76.7 %) and litter size (8.7 and 9.2 total born/litter, respectively). In contrast, distribution

Figure 1 : Effect of the treatments on the vulva colour at insemination

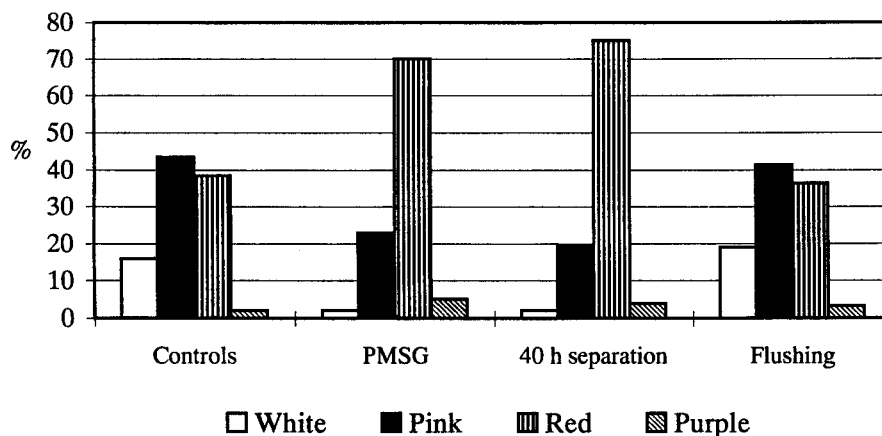


Table 3: Fertility rate (%) of lactating does according to treatment and lactation number

	Controls	PMSG	40h separation	Flushing	Signif.
Primiparous	47.5 (40) a	79.5 (39) b	77.8 (45) b	30.2 (43) a	P<0.001
2 nd lactation	57.1 (28) ab	74.4 (43) b	69.4 (36) ab	46.4 (28) a	P=0.015
3 rd lactation	65.5 (29)	66.7 (21)	69.2 (26)	50.0 (34)	P=0.09
4 th and following	74.3 (148) a	78.1 (146) a	81.8 (143) a	64.8 (142) b	P=0.009

(): number of inseminations; a≠b: P<0.05

of the concentrated diet to the does failed and a significantly decreased fertility (P<0.01) was obtained when compared with controls (54.7 vs 66.9%, respectively). Flushing had not any significant effect on prolificacy.

Does parity has a significant effect on fertility. Control does in their 1st lactation had only a fertility rate of 47.5% (Table 3). Both PMSG treatment and the separation technique increased significantly (P<0.05) the fertility of primiparous does (79.5 and 77.8%, respectively). The same tendency was obtained with does in their second lactation. However, results of multiparous does (3rd and following lactations) were comparable with the exception of the does fed the concentrated diet. This last experimental treatment resulted in the lowest fertility rate in each parity group.

A significant (P<0.01) higher number of does were detected with a red vulva at the insemination after PMSG treatment but also when separated during 40 h from their litter compared with the control and the flushing group (Figure 1). A very low percentage (2.0%) of does showed a white vulva when treated with PMSG or after a 40 h separation from their young. When we consider does with a red and purple vulva as receptive, in the PMSG and biostimulated group, respectively 75.1 and 78.8% showed oestrus

signs (Figure1) while this was only 40.8% and 39.6% for control and flushed does, respectively.

Litter performance

Young separated from their mother had a significant lower weight (P<0.001) at the age of 11 days than those of the other treatments (Table 4). This reduced weight (7–8%) sustained further during the lactation period and the young's weaning weight from these mothers was on average 40 to 47g lower than the 3 other treatments. Differences in litter size, at the 5 different ages considered, were small and not significant.

Feed intake

Does fed the concentrated diet had a significant (P<0.01) lower feed intake between days 8 and 11 compared to controls and PMSG treated does (Table 5). The daily energy intake of these last does was on average 3.30 MJ ME between days 8 and 11 PP, when fed the standard diet. However, does failed to have a higher energy intake when fed the concentrated diet (3.15 MJ/d) during this flushing period. Furthermore, a large difference of intake between does was observed when fed the concentrated diet (variation coefficient : 29% instead of 18 – 20 %

Table 4 : Litter performance according to the treatments (least-square means)

	Controls	PMSG	40h separation	Flushing	SEM	Signif.
No of litters	248	249	246	249	–	–
Weight at						
7 d	148	148	150	144	6.8	P>0.1
11 d	211 A	207 A	193 B	206 A	9.6	P<0.001
21 d	359 A	357 A	326 B	355 A	16.5	P<0.001
28 d	670 A	668 A	623 B	663 A	27.7	P<0.001
Litter size at ⁽¹⁾						
1 d	8.11	8.12	8.11	8.05	0.19	P>0.1
7 d	7.85	7.81	7.92	7.92	0.27	P>0.1
11 d	7.80	7.72	7.84	7.89	0.37	P>0.1
21 d	7.68	7.63	7.70	7.80	0.38	P>0.1
29 d	7.63	7.60	7.64	7.74	0.41	P>0.1

⁽¹⁾ after cross fostering on day 1 ; A≠B: P<0.01

Table 5 : Feed intake of does (g/day) during the lactation period

	Controls Signif.	PMSG	40h separation	Flushing	SEM	
Period						
0 – 7 days	295	292	299	288	19	P>0.1
8 – 11 days	341 A	335 A	282 B	281 B	22	P<0.001
12 – 21 days	369	371	368	364	16	P>0.1
21 – 29 days*	520 A	522 A	495 B	508 AB	25	P<0.01

*per cage: including the intake of the young A≠B: P<0.01

for control and PMSG treatment). Between day 12 and 21 after parturition, feed intake was comparable on the different treatments (between 364 and 371 g/d/doe).

During the last period (21–29d) before weaning, daily feed intake per cage was significantly lower for does earlier separated from their young (495g instead of 520 and 522 for control and PMSG does, respectively)

DISCUSSION

The 40h separation of mother from young resulted in a significantly increased receptivity and consequently in a favourable conception rate. These results are consistent with the data obtained in a farm experiment (PAVOIS *et al.*, 1994) and the response declared by a group of 33 commercial breeders (DUPERRAY, 1995). Especially the positive results in our experiment with primiparous does and lactating does, known as less fertile (THEAU-CLÉMENT and ROUSTAN, 1992), are very encouraging. The number of receptive does (with red and purple vulva) was nearly twice as high (78.8 vs 40.8) as in the control. The sexual receptivity and the fertility were even higher than in the PMSG treated does, showing that this bio-stimulation method is a real alternative to the hormonal oestrus synchronisation.

An explanation for this effect has to be searched in the hormonal balance of the doe. Both the separation effect as the thereafter suckling probably change drastically the oestrogen, prolactin and oxytocin levels. Studies of the hormonal changes provoked by this bio-stimulation are necessary to explain the positive results. Interesting is to mention that although the decreased feed intake between day 8 and 11 PP in this experimental group, favourable reproduction performances were obtained. The separation of 40 h decreased feed intake probably only the day before the insemination (does were not allowed to nurse). Anyway, this decreased feed intake coincides with an increase of receptivity and fertility. Because these does produced less milk (one nursing was prohibited) a

hypothesis could be that their energy balance was better than in free nursing does.

The negative effect on the weight of the young at 11 days of age, induced by the 40h separation can be explained by the lower milk intake. In fact they missed one nursing compared to the other treatments. Taking into account that the daily milk intake

represents about 15% of the live weight of the young (LEBAS, 1969), it is obvious that a significant decreased weight was observed at 11 days of age. However, the decreased weight (7–8%) sustained during the further lactation period and the young of these mothers had a 40–47 g lower weight at weaning. Due to the large interval between 2 sucklings (40 hours), it is possible that the milk yield of these mothers was decreased during their further lactation and thus partly responsible for the decreased weight of the young. However, the feed intake of mother + young in this group, was significantly lower between day 21 and weaning and could therefore also partly be responsible for the lower weaning weight.

The systematic treatment of does with PMSG to synchronise the oestrus increased both fertility and prolificacy. These results are consistent with the numerous literature reports. In agreement with BOURDILLON *et al.* (1992), the positive response was very pronounced with primiparous does (32.0 % increased fertility than control) but much less (only 3.8 %) for does having a parity of at least 4 litters. Because of the increased feed intake capacity, does are in a less negative energy balance with increasing parity number (XICCATO, 1996, for a review). Consequently the fertility of controls increased from 47.5% (primiparous) to 57.1% (2nd lactation) and 65.5% (3rd lactation) till 74.3% (4 or more lactations).

A real flushing as intended was not obtained with the concentrated diet, explaining the low reproduction results of this treatment. Instead of decreasing the energy deficit, concentrated diet feeding increased the negative nutrient balance during the second week of lactation and consequently does fertility. In fact the does did not appreciate the concentrated diet. A low palatability of this diet induced the lower energy intake and the intended hypothesis could not be verified.

Finally, it has to be stressed that in this experiment the does changed each litter from treatment group. Although the analysis did not reveal significant effects of the treatment before, conclusions concerning

long term effects of the applied treatments can not be drawn with our experimental design.

From this experiment it can be concluded that the bio-stimulation methods are real alternatives for the hormonal induced oestrus synchronisation. Further research is necessary to better define the optimal separation time by taking into account also considerations on animal welfare. Although the first results were discouraging, further efforts to solve the energy deficit of the doe during the lactation could contribute to optimise the reproduction of does in commercial rabbitries.

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