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EFFECT OF A 48H DELAYED INSEMINATION WITH OR WITHOUT A 48H DOE-LITTER SEPARATION ON PERFORMANCE OF NON-RECEPTIVE RABBIT DOES *

BONANNO A., ALABISO M., DI GRIGOLI A., ALICATA M.L., LETO G.

Istituto di Zootecnica Generale, Facoltà di Agraria, Università di Palermo, Viale delle Scienze, 90128 PALERMO, Italy

ABSTRACT : The effectiveness of delayed artificial insemination (DAI) of non-receptive (R-) does, with or without oestrus synchronisation by doe-litter separation (MLS), was studied and compared with receptive (R+) does. During 10 months, 105 NZW does, in three homogeneous groups (A, B, C), were artificially inseminated (AI) according to a 42-day reproduction rhythm in two batches. Every 21 days, two AI were effected, the second 48 hours after the first. The R+ does of all groups were inseminated at the first AI, while the R- does were treated, in relation to their group, as follows: inseminated at the first AI (A); 48-hour DAI (B); 48-hour MLS by closing the nestbox before AI, when lactating, and 48-hour DAI (C). The lactating does were subjected to systematic controlled nursing from littering until AI. The R+ does had a significantly higher fertility rate than the R- does of all groups. Receptivity was improved by DAI, especially on the non-lactating does (+60.6%), while an important increase was caused by MLS in the lactating does (+15.7%, P<0.05). A different effect on fertility was seen according to the lactation order of the does. A DAI improved fertility of does of lactation order ≥5 in comparison with the A group (+18.2%, P<0.05) and MLS improved fertility of the R- of lactation order 1-4 (+25.0%, P<0.01). MLS did not affect either rabbit loss or the incidence of mastitis, but reduced the weaning weight of rabbits (-52 g equal to -7.7%, P<0.01), especially when their dams were of lactation order ≥5 (-76 g, P<0.01). The weight recorded on day 74, at the end of the fattening period, was significantly lower in rabbits separated from their dams (-75 g, P<0.01), although the relative difference was reduced (-3.3% : 2270 g *vs* 2345 g).

RESUME : Effet d'une insémination différée de 48 heures, avec ou sans séparation mère-portée, sur les performances de lapines non réceptives.
L'efficacité d'une insémination différée (DAI) de lapines non réceptives (R-) avec ou sans synchronisation de l'oestrus par séparation mère-portée (MLS) a été étudiée en comparaison avec l'insémination immédiate des lapines réceptives (R+). Pendant 10 mois, 105 lapines NZW, reparties en 3 groupes homogènes (A, B, C) ont été inséminées artificiellement (AI) selon un rythme de reproduction de 42 jours en deux groupes. Chaque 21 jours, deux séries d'inséminations ont été effectuées, la seconde 48 heures après la première. Les lapines R+ de tous les groupes ont été inséminées lors de la première série, tandis que les lapines R- l'ont été, selon le groupe dont elles dépendent, comme suit : A : insémination immédiate, B : DAI de 48 heures, C : MLS de 48 heures avant l'insémination, par fermeture du nid en période de lactation et DAI à 48 heures. Les lapines allaitantes ont été soumises à un contrôle systématique de l'allaitement de la mise bas jusqu'à l'insémination. Les femelles R+ ont eu un taux de fertilité significativement supérieure, dans tous les groupes, à celui des femelles R- lors de l'IA effective. La réceptivité a été améliorée par le report de l'IA spécialement chez les lapines non allaitantes (+ 60,6%), et la séparation mère-portée à permis une amélioration chez les lapines allaitantes (+ 15,7% ; P<0.05). Un autre effet sur la fertilité a été montré, lié au numéro d'ordre de la lactation : DAI a amélioré la fertilité des femelles ayant effectué ≥5 lactations, comparé au groupe A (+ 18,2%, P<0.05). MLS a amélioré la fertilité des lapines R-, ayant effectué 1 à 4 lactations (+ 25%, P<0.01), et n'a pas affecté la mortalité des lapereaux ou le taux de mammites, mais a réduit le poids des lapins au sevrage (-52g = 7,7%, P<0.01) particulièrement quand les mères effectuaient les lactations de rang 5 et plus (-76g, P<0.01). Le poids vif enregistré à l'âge de 74 jours était significativement inférieur chez les lapereaux ayant été séparées 48 heures de leur mère (- 75g ; P<0.01) bien que la différence relative soit faible (- 3,3% : 2270 g *vs* 2345g).

INTRODUCTION

In previous investigations (ALABISO *et al.*, 1994; BONANNO *et al.*, 1996), a strategy that limited the PMSG treatment to non-receptive (R-) does was efficiently applied in order to avoid systematic use of the hormone to induce oestrus. Since receptivity has to be detected in advance and two artificial insemination (AI) carried out, the second delayed by 48-72 hours, this method is not very well suited to cycled production. However its application might be appropriate to big rabbitries where a 42-day cycled production is practised (BONANNO and ALABISO, 1996). In the same experiments, it was also noticed that by inseminating the R- does 72 hours later, the global receptivity improved, also without PMSG treatment. Therefore it could be supposed that a 48-72-hour delayed AI (DAI) of R- does might improve their reproductive performance.

Moreover, in a study conducted to compare bio-stimulation methods, a 44-hour doe-litter separation (MLS) seemed to be more efficient in inducing mating receptivity and improving fertility rate and prolificacy of R- lactating does, than a change of cage, or a combination of the two methods (BONANNO *et al.*, 1999). Furthermore, both ALVARIÑO *et al.* (1998) and MAERTENS (1998) observed that a MLS, lasting from 36 to 48 hours, resulted in a similarly efficient response to PMSG in synchronising oestrus and achieving a better fertility rate. They suggested it might be an effective alternative to hormonal methods.

Nevertheless, a 44-hour MLS brought about a reduction in weaning weight, while it did not affect the pre-weaning survival of young rabbits or the incidence of mastitis (BONANNO *et al.*, 1999), in accordance with the

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results of ALVARIÑO *et al.* (1998) and MAERTENS (1998). In order to limit the negative effect on weaning weight to fewer litters, the temporary separation of the doe from the litter could be performed only on R- does. In this way, the R- lactating does undergo a double effect of both DAI and MLS.

This experiment was carried out to evaluate the effect of a 48-hour DAI, with or without a 48-hour MLS, on the reproductive performance of R- does, which were subjected to systematic controlled nursing from kindling until AI. The influence of MLS on the litter growth and viability is considered during both the suckling and fattening periods.

Table 1 : Effect of receptivity, delayed AI and doe-litter separation on the reproductive performance of lactating does (LSM).

	First AI	Delayed AI			RSD
	Receptive does	Non-receptive does			
	A, B, C	A	B Control	C Doe-litter separation	
Inseminations (no)	261	85	82	105	
Receptivity at DAI (%)			39.5 a	55.2 b	49.8
Fertility (%)	80.2 Aa	50.5 Bb	56.7 Bbc	65.1 Bc	44.5
Total born/litter (no)	8.9 Aa	6.6 Bb	7.8 ABbc	8.3 Aac	3.1
Born alive/litter (no)	7.7 A	5.5 B	6.9 AB	7.4 A	3.5
Still-born/litter (no)	0.9	1.1	0.9	0.6	1.9

RSD = Residual standard deviation. A, B: P≤0.01; a, b, c: P≤0.05.

MATERIALS AND METHODS

The experiment was carried out on a small commercial rabbit farm (200 does) in Sicily, between January and October 1998. The rabbitry was ventilated naturally and lacked other environmental conditioning systems.

Animals and housing

Initially 105 primiparous and multiparous New Zealand White does were homogeneously and definitively distributed in three experimental groups (A, B and C). Does culled because of mortality, illness, low productivity, or three consecutive infertile inseminations, were immediately replaced by primiparous does. Eight hybrid males (Grimaud) were used for artificial insemination (AI).

The does were housed in flat-deck cages with internal, isolable nestboxes under a light program of 16 hours per day, and fed *ad libitum* with a commercial diet (18.9% CP and 15.5% CF on a d.m. basis).

Reproduction rhythm and treatments

A 42-day reproduction rhythm was followed using 2 batches. Two AI, the second delayed by 48 hours from the first, were effected every 21 days.

At each AI series, the receptive (R+) does of all three groups were immediately inseminated (the first AI); consequently, for analysis of their performance, they were considered all together (A,B,C), without any distinction between the groups. The non-receptive (R-) does were subjected to one of the following treatments, in relation to their initial group:

A) First AI;
B) 48-hour delayed AI (DAI);
C) 48-hour doe-litter separation (MLS) by closing the nestbox before AI, and 48-hour DAI.

In practice, because the suckling was controlled from kindling to AI, MLS involved the suppression of a daily suckling the day before AI.

Because the does of both B and C groups were submitted to first AI or DAI, the kindling-AI interval of R+ does was from 9 to 11 days, that of R- does was from 11 to 13 days. As a result, on the day when suckling was omitted, the age of litters varied from 8 to 12 days.

Management

The AI was performed with heterospermic semen collected using IMV equipment, assessed for mass motility, and extended (1:5) in a TRIS dilutor. The does were inseminated in the lordosis position with 0.6 ml of fresh diluted semen injected through a sterilised glass pipette. Ovulation was induced at the moment of insemination with 20 μg of synthetic GnRH (Fertagyl, Intervet).

Equalisation of the initial litter size to 7-9 young rabbits was effected within the group, depending on the total number of young rabbits available.

Suckling was controlled from kindling to AI. Nursing was allowed for 15 minutes a day. The does were inseminated within 15 minutes after nursing their young. The litters were weaned 31 days after birth.

Measurements

Vulva colour and turgidity were used to distinguish R+ does (turgid vulva of red or purple colour) from R- does. The fertility rate (number of kindlings/ number of inseminations x 100), number of total born and young born alive were recorded.

For DAI does, the size and weight of suckling litters was recorded after litter equalisation (day 1), on the day before suckling suppression (day 9, on average) and at weaning (day 31). Litters were also weighed at 74 days, at the end of the fattening period. The feed intake of the DAI does was measured for three days, from the day before to the day after suckling suppression.

Statistical analysis

The lactating and non-lactating does were considered separately in this work.

Data were statistically analysed using the GLM procedure of SAS 6.12 software (1989). Proportional data were considered as Bernoulli (0-1) variables.

The analysis was performed according to a linear model including the effect of the treatment [4 levels: R+(A,B,C), R-(A), R-(B), R-(C)], the parity [2 levels: 1-4 and ≥ 5] and their interaction. The treatment effect included in the linear model had 3 levels for non-lactating does [R+(A,B,C), R-(A), R-(B,C)], and 2 levels for the feed intake and the litter performance of DAI does [B, C]. For the individual weight of young rabbits, litter size, or both litter size and age, were used as covariates. The differences between means were tested by Student "t" test.

Table 2 : Effect of receptivity, delayed AI and doe-litter separation on the fertility of lactating does according to lactation order. (1)

	First AI	Delayed AI		
	Receptive does	Non-receptive does		
Lactation order	**A, B, C**	**A**	**B Control**	**C Doe-litter separation**
1-4	81.6 Aa *(152)*	47.6 BCb *(42)*	41.7 Bb *(36)*	66.7 ACa *(39)*
≥ 5	78.9 Aa *(109)*	53.5 Bb *(43)*	71.7 ABac *(46)*	63.6 ABbc *(66)*

(1) Sample size is within brackets. A, B, C: P≤0.01; a, b, c: P≤0.05.

RESULTS

Reproductive parameters of lactating does.

As was to be expected, the fertility rate was higher in the receptive (R+) does than the non-receptive (R-) does of all groups (P<0.01) (table 1).

A 48-hour delayed artificial insemination (DAI) of R- lactating does of B group, without stimulation, did not significantly improve fertility and the number of total and live rabbits born compared to the A group. However these parameters were higher by +6.2 %, +1.2 % and +1.4 %, respectively, than in the A group, in which R- does were immediately inseminated.

Comparing the B and C groups, it can be seen that a 48-hour doe-litter separation (MLS) had a certain efficacy in stimulating sexual receptivity (+15.7%, P<0.05), whereas it did not significantly influence fertility and prolificacy. Nevertheless fertility was higher by +8.4% in does separated from their litters (table 1).

Fertility was influenced (P<0.05) by lactation order (table 2). In R- does of lactation order 1-4, the DAI did not show any efficacy, whereas MLS significantly improved fertility in comparison with the B group (+25.0%, P<0.01). In the higher lactation orders (≥5), the effect of the DAI was evident (+18.2%, P<0.05), but MLS did not produce any improvement.

Reproductive parameters of non-lactating does.

The DAI of R- non-lactating does of B and C groups resulted in a 60.6% increase in their receptivity, but did not significantly affect their fertility and litter size at birth in comparison with the A group (table 3).

Litter performance of does submitted to DAI with or without MLS.

On day 1 and day 9 after birth, the size and individual weight of suckling litters of DAI does, were the same (table 4). On day 31, the size of the litters was also the same in the two groups, therefore a 48-hour MLS did not affect the survival of young rabbits.

On the other hand, the individual weaning weight was 52 g lower, equal to -7.7% (P<0.01) when young rabbits had a suckling suppression, as a consequence of the lower daily gain (P<0.01) recorded from 9 day to weaning (table 4).

Statistical analysis of the data according to lactation order of dams showed that MLS negatively influenced only the growth performance of litters nursed by does of high lactation order (table 5). In fact, the weaning weight and the daily gain from day 9 to day 31 were not significantly different between groups when dams were younger (with 1-4 lactation order). They differed by 76 g and 3 g, respectively, (P<0.01) when litters were nursed by older does. The weight reduction at weaning was not offset by compensatory growth during the fattening period. In fact, in the C group the weight recorded on day 74 was also 75 g lower (P<0.01). However the daily gain from day 31 to day 74 was analogous to that of the control group. Thus, the relative difference between the weights of the two

Table 3 : Effect of receptivity and delayed AI on the reproductive performance of non-lactating does (LSM).

	First AI		Delayed AI	RSD
	Receptive does	Non-receptive does		
	A, B, C	**A**	**B, C**	
Inseminations (no)	221	42	61	
Receptivity at 2nd AI (%)			60.6	
Fertility (%)	78.8 a	61.8 b	66.7 b	42.7
Total born/litter (no)	8.0	6.7	7.5	3.2
Born alive/litter (no)	6.9	6.0	6.7	3.5
Still-born/litter (no)	0.8	0.5	0.8	1.9

RSD = Residual standard deviation. a, b: P≤0.05.

Table 4 : Effect of doe-litter separation on the litter performance and daily intake of does submitted to a delayed AI (LSM).

		B Control	C Doe-litter separation	RSD
Litters (no)		67	96	
Litter size (no)	day 1 (1)	8.3	8.3	0.7
	day 9 (2)	7.8	7.8	1.1
	day 31	7.1	7.1	1.8
Rabbit losses (%)	9-31 days	8.0	9.5	2.8
	31-74 days	9.5	12.0	3.1
Weight (g)	day 1 (1)	67	65	9.3
	day 9 (2) (3)	157	150	20.9
	day 31 (4)	722 A	670 B	86.5
	day 74	2345 A	2270 B	301.5
Daily gain (g)	9-31 days	25.8 A	23.5 B	3.5
	31-74 days	37.7	37.4	6.8
Daily intake (g d.m./kg $W^{0.75}$)	day before SS	101.5	106.4	42.5
	day 10 (SS) (2)	104.9 A	66.5 B	30.6
	day after SS (AI day)	107.7	109.8	39.4

(1) After litter equalisation. (2) The day number is the average of the days after kindling.
(3) Litter size and age as covariates. (4) Litter size as covariate.
SS = suckling suppression. RSD = Residual standard deviation. A, B: P≤0.01.

groups was reduced during the post-weaning phase, reaching a value of -3.3% at 74 days (table 4).

There was higher mortality in the treated group during the fattening period, probably as a consequence of the lower weight, but the difference was not significant.

Daily feed intake of does submitted to DAI with or without MLS.

The feed intake of does differed between the groups (P<0.01) only on the day when suckling was omitted (table 4). The does that had not been allowed to nurse their young showed a lower daily intake in comparison with both their intake on the day before MLS and that of the control does on day 10. The day after suckling suppression, the feed intake of the C group attained values approaching those of the day before suppression.

Table 5 : Effect of doe-litter separation on the litter growth according to lactation order (1)

	Lactation order	B Control	C Doe-litter separation
Weight on day 31 (g)	1-4	717 *(25)*	690 *(31)*
	≥ 5	727 A *(42)*	651 B *(64)*
Daily gain 9-31 days (g)	1-4	25.8	24.2
	≥ 5	25.8 A	22.8 B

(1) Sample size is within brackets. A, B: P≤0.01.

Culled does

During the experiment, 44.1%, 49,1% and 42.4% of does were replaced in groups A, B and C, respectively. The percentage of does culled because of mastitis was 8.5% in the group in which MLS was performed, which was lower than the values of the A (10.2%) and B (10.5%) groups.

DISCUSSION

The results of this work clearly confirm that a 48-hour delayed artificial insemination (DAI) of non receptive (R-) does increases the number of does showing signs of sexual receptivity, such as a turgid red or purple vulva. A 48-hour doe-litter separation (MLS) caused a significant increase in the receptivity of lactating does (+15.7%).

In non-lactating does, a DAI increased the receptivity, but did not significantly influence the fertility. Personal observations (BONANNO AND ALABISO, 1997) revealed that lactating does with turgid red or purple vulvas did not accept the male. Therefore, this absence of correspondence between receptivity and fertility of the non-lactating does could be explained by the inaccuracy of colour and turgidity of vulva as indicators of receptivity. Moreover, it can be supposed that non-lactating does can better respond to GnRH and AI when they are R-, in comparison with the R- does nursing litters.

On the contrary, a DAI, applied without stimulation, significantly improved the fertility of lactating does of higher lactation orders (≥5) (+18.2%).

The does separated from their litters for 48 hours had a significant improvement in fertility (+25.0%) when they were younger (lactation order 1-4), whereas in the higher lactation orders their fertility was below that of non-stimulated R- does. An analogous effect of parity on the fertility of stimulated does was found in other works. THEAU-CLEMENT AND LEBAS (1996) obtained a positive effect of PMSG on fertility of lactating does only in the first four AI. MAERTENS (1998) showed a significant effect of both 40-hour MLS and PMSG treatment in does in the first and second lactation.

As has been reported (XICCATO, 1996), in the first phase of their reproductive career, especially during their first lactation, intensively reared rabbits have high nutritional requirements to sustain simultaneous growth, lactation and pregnancy. Because of their low feed intake, they incur a negative energy balance which contributes to their reduced reproductive performance. In this condition, a DAI is not sufficient to provoke any positive reproductive response. Instead, it can be supposed that a daily suckling suppression, reducing milk production, contributes to limit

the body energy deficit, in this way being able to promote a hormonal change favourable to a fertility improvement.

After their first deliveries, rabbit does increase their feed intake capacity during lactation, produce more milk, and their body energy deficit becomes less important. In this phase, a DAI has positively affected fertility. On the other hand, when milk production is higher, a temporary separation from litter could constitute for the dam a more intense stress. In fact, the combined effect of the reduction of feed intake and the state of udders, full of milk, could negatively act on their hormonal equilibrium and then on their fertility rate.

Therefore, a 48-hour MLS, employed as a bio-stimulation method on controlled suckling does, was efficient in inducing sexual receptivity, and also in improving fertility rate when does were of lactation order 1-4. Moreover it did not increase the cases of does affected by mastitis, nor did affect the viability of young suckling rabbits. However, it caused a reduction in the litter growth and a lower weaning weight, thus confirming previous observations (ALVARIÑO *et al.*, 1998; BONANNO *et al.*, 1999; MAERTENS, 1998).

Considering the long term effect of MLS on the weight of the rabbits, a strategy which restricts MLS only to R- does, submitted to a DAI, may be interesting, because it involves fewer litters.

In reality, in this experiment, the negative effect of MLS on the weaning weight of the young was evident only on litters nursed by does of high lactation order. It is possible that the daily suckling suppression caused a more pronounced decrease in the milk yield of those dams producing more milk in comparison with the younger, less productive does.

Moreover, the reduction of weaning weight of rabbits separated from their dam was not compensated by post-weaning growth, since the weight was lower also at the end of the fattening period. However the relative gap decreased (from –7.7% to –3.3%). It can be concluded that the rabbit does not exhibit compensatory growth.

From the results of this experiment, it can be concluded that the effects of a 48-hour MLS and/or a 48-hour DAI seem to depend on the parity of lactating does. When the dams had a parity of 1-4 litters, MLS improved fertility in comparison with only a DAI, and it did not affect litter weight at weaning. In this way, does of 1-4 parity improved their productivity (as an index deduced from the weight of weaned rabbits per insemination) by 58.3% when separated from their litter, whereas such an improvement, referred to all does, was only by 6.5%. On the other hand, MLS with dams having a parity higher than 4 litters produced no improvement in fertility and reduced the weaning weight. Fertility increased when these same does were submitted to only a DAI.

Further investigations are necessary to confirm and better define the role of parity in relation to the stimulation methods considered here. Of particular importance are doe productivity and performance of their young, especially when a temporary MLS is performed.

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