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ABSTRACT

The authors conducted two experiments in order to establish how the presence of a male among nursing does influences receptivity, pregnancy rate and litter size. The bucks were placed in empty cages between cages housing females (7 does on either side). Artificial insemination was performed 10 days post partum. The bucks were introduced four days prior to AI (experiment 1) or three days before AI (experiment 2). Based on the results of 383 inseminations in one experiment and 56 in the other the authors concluded that neither the presence of bucks nor the distance of the females from the buck significantly affected receptivity or pregnancy rate in the does or the size of the litters they produced.

INTRODUCTION

Rabbit does that show signs of heat are evidently more reproductive than those which do not (PLÁ *et al*., 1986; THEAU-CLÉMENT and ROUSTAN, 1992). Thus, lactating does are, in practice, given some type of stimulation prior to artificial insemination (AI), for the purpose of inducing better receptivity.

PMSG treatment is the most common means of inducing oestrus. However, regular hormone treatment is to be avoided for two reasons: a) the large molecule of PMSG triggers the formation of antibodies in the doe, which results in poorer fertility, and b) EU standards and animal welfare regulations prohibit regular application of hormones in rabbits (MAERTENS and LUZI, 1995; CASTELLINI, 1996).

One of the aims in establishing the International Rabbit Reproduction Group (IRRG) was to elaborate and evaluate various methods of biostimulation, for the purpose of improving the receptivity, and consequently the reproductive performance, of does, in place of the application of hormone treatments (THEAU-CLÉMENT *et al*., 1998; THEAU-CLÉMENT and BOITI, 1998). Flushing, alteration of the photoperiod, change of cage and mother-litter separation prior to AI have been investigated as such methods of biostimulation.

The objective of these experiments was to determine the effect on receptivity, fertility, kindling rate and litter size at birth of placing bucks in separate cages between cages of lactating does prior to AI.

MATERIAL AND METHODS

This research project consisted of two experiments. One was performed on the rabbit farm of the Institute for Small Animal Research, the other at a private rabbitry. The animals were housed in a closed building (15 to 20 °C, 8D:16L), in wire net flat-deck cages (60x60x30 cm), with *ad libitum* access to a commercial pelleted diet (DM 90%, CP 17.5%, crude fat 3.05%, crude fibre 13.9%, DE 10.3 MJ/kg). Water was also available *ad libitum* from self-drinkers.

Experiment 1 was performed with multiparous New Zealand White lactating does (n=383)~~,~~; *experiment 2* with Pannon White (n=148) nursing females. In both trials insemination was performed 10 days after parturition. The bucks were introduced, four days prior to AI (*experiment 1*) and three days before AI (*experiment 2*), into cages which had been left empty for them when the does were introduced. In *experiment 1* the series of animals in the row of cages was 7 does, 2 bucks, 14 does, 2 bucks, 7 does; thus, there were 7 does on either side of the bucks. In *experiment 2* rabbits were housed in a shorter row of cages with one buck in the first cage and one doe in each of the seven subsequent cages. In *experiment 2* a control consisting of 121 artificial inseminations was performed simultaneously, in another room but under similar circumstances, without the presence of bucks.

Experiment 1

7	male	male	7	7	male	male	7

Experiment 2

male	7

The onset of heat in the does was detected by observation of the vulva (colour and turgidity). Females with a swollen vulva of red or purple colour were regarded as receptive (R^+).

Each doe was evaluated for heat when the bucks were introduced (AI-4 for exp. 1) and on the day of insemination (AI-0) in both trials. In *experiment 1* one third of the females were also checked on each of the three days prior to AI (AI-3, AI-2, AI-1).

The multiparous lactating does were inseminated with individual or pooled fresh semen 10 days post partum. An injection of 1.5 μg GnRH analogue hormone (Ovurelin, Reanal) was used to induce ovulation. In *experiment1* fertility was determined on the result of abdominal palpation (no. of pregnant rabbits per inseminated does, in %).

The statistical analysis was performed with the STATGRAPHICS software programme, version 6.0 (Statgraphics, 1992). The significance of receptivity, fertility and kindling rate was checked by chi-square test. In evaluating litter size (total and alive) by means of analysis of variance, the effect of distance from the male (from the first cage to the seventh), manipulation of the doe (AI-3, AI-2, AI-1), and receptivity were investigated. Receptivity at AI was considered a fixed effect (R+ or R-). The following model was used:

$Y_{ijkl} = \mu + D_i + M_j + R_k + e_{ijkl}$

where Y_{ijkl} the individual animal in question
μ overall mean
D_i effect of distance from the male's cage (i=1,2,3,4,5,6,7)
M_j effect of manipulation of the doe (j= AI-3, AI-2, AI-1)
R_k effect of receptivity at AI (k=R^+, R^-)
e_{ijkl} random error

RESULTS AND DISCUSSION

Receptivity

In *experiment 1* the distance between the cage housing the buck and that of the doe did not influence receptivity in the females on any day (Table 1). Fifty eight percent of the does were in heat when the males were introduced (AI-4). On the following days (AI-3, AI-2, AI-1) receptivity decreased and the ratio of does in heat at the time of insemination (AI-0) was 12% lower ($P<0.001$) than four days earlier. It would probably be incorrect to draw the conclusion that placing males close to lactating females has a disadvantageous influence on receptivity on the subsequent days. The operation of heat detection may have exerted an unfavourable effect through the manipulation of the doe, but this hypothesis requires verification in further, targeted experiments.

Table 1: Effect of distance from males (1st to 7th cage) and interval in time from male presence (0 to 4 days) on receptivity in lactating does (Experiment 1)

Days		Cage distance from males							Total
		1	2	3	4	5	6	7	
0 (AI-4)	n	55	55	55	55	55	55	53	383
	R^+	**61.8^{a}**	**52.7**	**50.9**	**60.0**	**67.3^{ab}**	**54.5**	**60.4^{A}**	**58.2^{A}**
1 (AI-3)	n	25	7	23	23	7	26	21	132
	R^+	**40.0^{ab}**	**71.4**	**39.1**	**52.2**	**85.7^{a}**	**46.2**	**23.8^{B}**	**44.7^{ab}**
2 (AI-2)	n	16	23	15	17	22	15	17	125
	R^+	**50.0^{ab}**	**43.5**	**40.0**	**47.1**	**31.8^{b}**	**26.7**	**41.2^{ab}**	**40.0^{ab}**
3 (AI-1)	n	14	25	17	15	26	14	15	126
	R^+	**28.6^{b}**	**44.0**	**23.5**	**40.0**	**34.6^{b}**	**35.7**	**40.0^{ab}**	**35.7^{ab}**
4 (AI-0)	n	54	53	55	55	55	53	50	375
	R^+	**48.1^{ab}**	**39.6**	**45.5**	**50.9**	**54.5^{ab}**	**41.5**	**40.0^{ab}**	**45.9^{B}**

Values indexed with different letters by column indicate significant differences (a, b: $P<0.05$; A, B: $P<0.001$)
R^+: percentage of receptive does

The results of *experiment 2* seem to support the assumption which had been made. Here the bucks were placed close to the lactating females three days prior to AI. The data given in Table 2 indicate that 55% of the does were in heat on day AI-3. Then the receptivity increased non-significantly by 7 per cent to 62% until the day of AI, regardless of the distance from the buck.

Table 2: Effect on receptivity, pregnancy rate and birth litter size of placing males close to lactating does three days prior to AI (Experiment 2)

Trait	Distance between cage and males							Total
	1	2	3	4	5	6	7	
No. of inseminations	21	19	23	22	22	21	20	156
Receptivity, %								
at introduction of buck (AI-3)	**57.1**	**57.9**	**52.2**	**54.5**	**50.0**	**57.1**	**60.0**	55.1
at insemination (AI-0)	**61.9**	**63.1**	**65.2**	**63.6**	**63.6**	**61.9**	**60.0**	62.3
Kindling rate, %	**61.9**	**68.4**	**73.9**	**72.7**	**68.2**	**61.9**	**65.0**	66.7
Total litter size at birth	**8.2**	**9.0**	**8.4**	**9.2**	**9.0**	**9.0**	**9.2**	8.75

In an experiment by LEFÈVRE *et al*. (1976) the presence of males contributed to the induction of oestrus, since the frequency of placings increased the ratio of does in heat among 5-month-old nulliparous rabbits. DUPERRAY *et al*. (1999) observed higher receptivity and 6% better fertility compared to an untreated control when keeping does together in groups of 8 animals

per cage for 15 minutes prior to AI. The females used in these French experiments were nulliparous, and the does kept in the same cage prior to AI had been directly exposed to each other. Thus, both methods differed from that applied in this study, and so direct comparison of the findings obtained is not possible.

Fertility, kindling rate and litter size

In *experiment 1* distance from the males did not have an impact on the fertility in the does, or on kindling rate or litter size at birth. With the exception of positions 6 and 7, kindling rate varied between 50 to 56% (Table 3). Neither did the position of the doe influence litter size, although females housed in the cage next to the bucks produced the largest litters, those in the second cage significantly the smallest, and those in the 7th position larger litters.

In *experiment 2* distance from the male had no significant effect either on kindling rate or on litter size at birth (Table 2). Here no significant difference between the treated room and the control room (without male presence) was detected: receptivity proved to be 62.3 and 57.0%, pregnancy rate 66.7 and 62.8% and birth litter size 8.75 and 8.40, respectively. BEREPUBO *et al*. (1993) investigated the individual and combined effect on doe sexual maturity at first AI of the presence of bucks and light supplementation. By the use of two illumination patterns the presence of bucks increased kindling rates by 5 and 10% as compared to the untreated control group. These results refer to nulliparous does, so it can be assumed that while male presence exerts an advantageous effect on receptivity and kindling rate at the first breeding, it does not affect the performance of multiparous lactating does.

Table 3: Fertility, kindling rate and litter size in relation to distance from males (1st to 7th cage), doe manipulation and receptivity at AI (Experiment 1)

		Fertility rate		Kindling rate		Litter size					
						Total			Alive		
		n	%	n	%	n	LSM	se	n	LSM	se
Overall mean		368	61.4	337	54.0	182	7.83	0.24	179	7.67	0.24
Doe Position	1	53	**60.4**	47	**53.2^{ab}**	25	**8.82^{a}**	0.65	23	**8.71^{a}**	0.67
	2	52	**57.7**	50	**56.0^{ab}**	28	**6.85^{b}**	0.61	28	**6.73^{b}**	0.62
	3	53	**62.3**	49	**53.1^{ab}**	26	**7.07^{ab}**	0.63	26	**6.82^{b}**	0.64
	4	54	**57.4**	51	**51.0^{ab}**	26	**7.92^{ab}**	0.63	25	**7.83^{ab}**	0.65
	5	54	**59.3**	48	**50.0^{ab}**	24	**7.40^{ab}**	0.67	24	**7.35^{ab}**	0.67
	6	53	**73.6**	48	**68.8^{a}**	33	**7.98^{ab}**	0.56	33	**7.95^{ab}**	0.57
	7	49	**59.2**	44	**45.5^{b}**	20	**8.75^{a}**	0.72	20	**8.29^{ab}**	0.73
Doe manipulation	AI-3	125	**62.4**	110	**57.9**	66	**7.45**	0.41	64	**7.38**	0.42
	AI-2	122	**62.3**	112	**55.4**	62	**7.90**	0.41	62	**7.75**	0.41
	AI-1	121	**59.5**	111	**48.6**	54	**8.14**	0.44	53	**7.88**	0.45
Receptivity at AI	R+	171	**69.0^{A}**	153	**62.7^{A}**	96	**8.02**	0.33	95	**7.86**	0.33
	R-	197	**54.8^{B}**	184	**46.7^{B}**	86	**7.63**	0.36	84	**7.48**	0.36

Values indexed with different letters by column indicate significant differences (a, b: P<0.05; A, B: P<0.001)

In *experiment 1* all the females were examined for signs of heat on days AI-4 and AI-0 and a third of them each day after the introduction of the males (AI-3, AI-2 and AI-1). From the results obtained it seems that the manipulation involved in checking the state of the vulva for receptivity could affect the performance of does, since kindling rate decreased slightly while birth litter size increased in the order of the groups (Table 3). The differences were not significant, but this finding highlights the necessity of further consideration and investigations.

CONCLUSIONS

According to the results obtained, receptivity, kindling rate and litter size were not influenced either by placing males close to lactating does three or four days prior to AI or by keeping different distances between bucks and females. Literature data suggest that the presence of males has a favourable effect on the reproductive performance of nulliparous rabbits. This effect, however, seems not to be the case for lactating does inseminated 10 days after parturition.

On the basis of the findings made in this study it is not possible to exclude the possibility that the manipulation of the doe for heat detection (i.e. picking up the female and manually examining the vulva) may have an influence on receptivity, pregnancy rate and birth litter size. However, verification of this hypothesis claims further investigations.

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