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EFFECT OF DILUENT AND STORAGE TIME OF RABBIT SEMEN ON THE FERTILITY OF DOES REARED UNDER TWO DIFFERENT LIGHTING SCHEDULES

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SUMMARY : Using a 3-factorial design, the effect of two commercial diluents (IMV *vs* Minitüb), two storage times (fresh *vs* 6h storage at ambient temperature) and a lighting schedule was studied on artificial inseminated does. During a 10 month experimental period 140 (nulliparous and multiparous) does were randomly divided in two production groups and submitted to a 16hL:8hD continuously lighting schedule or to a 10hL:14hD schedule. In order to synchronize the oestrus, the lighting period was suddenly increased till 16hL from 5 days before the insemination (alternative lighting). Both production groups were inseminated every 6 weeks with an interval of 3 weeks between them. In total 988 inseminations were performed with an average conception rate (CR) of 56.5 %. The CR with both diluents was comparable, 55.6 % and 57.5 % for IMV and Minitüb, respectively. There was no decrease observed in CR when semen was stored during 6 hours into the insemination straws. CR was respectively 56.6 % (fresh semen) and 56.4 % (stored semen). The difference in CR between lighting schedules was small (2 %) and also not significant. Differences in litter size were not significant and could not be related to the treatments used. The overall low CR (56.5 %) is partly explained by the physiological status (vulva colour) of the does, indicating that 67 % of the does were not receptive (white or rose vulva) at the 1st insemination. However, on does showing a red or purple vulva a CR of 70.3 % was obtained. Litter size of does inseminated with white vulva was significantly (P<0.001) lower, and amounted only 3.9 (nulliparous does) and 5.8 (multiparous does) live youngs instead of 6.9 (nulliparous, rose vulva) to 8.9 (multiparous, purple vulva) when inseminated with coloured vulva.

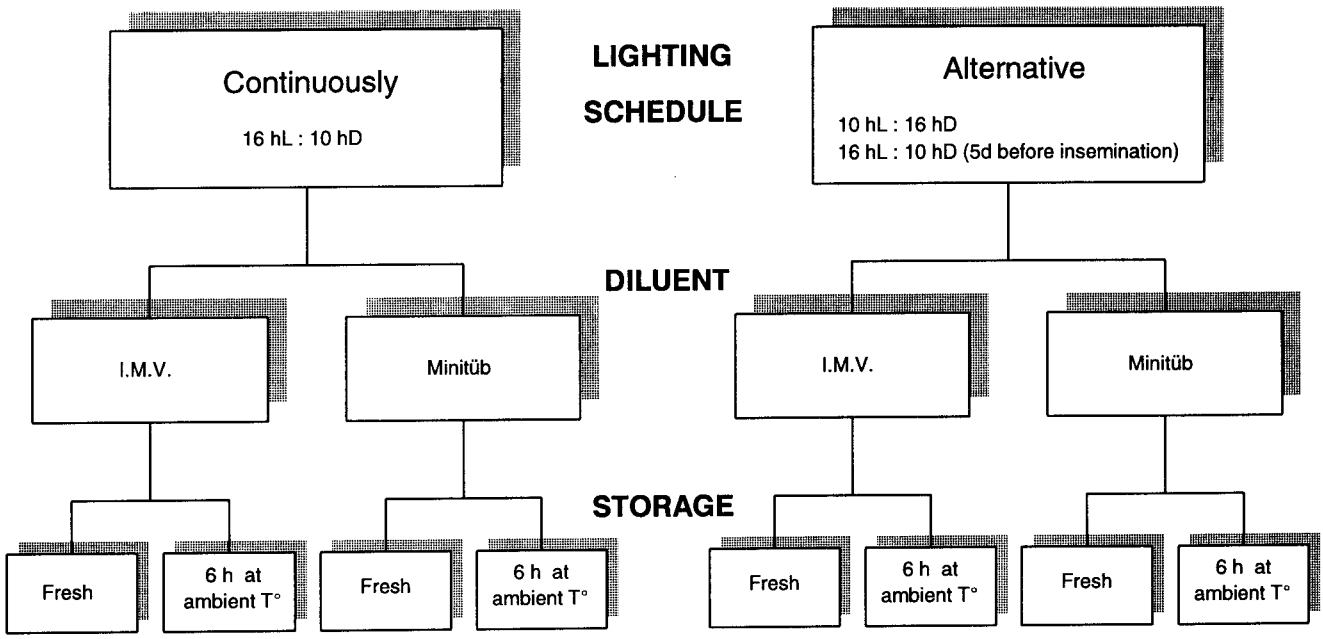
RÉSUMÉ : Effet du diluent et du temps de stockage sur le sperme de lapin et sur la fertilité des lapines soumises à deux schémas d'éclairement différents.
Dans une expérience tri-factorielle, l'effet de 2 dilueurs (IMV vs Minitüb), de 2 temps de conservation (fraîche vs 6h à température ambiante) et d'un traitement lumineux ont été étudiés sur des lapines inséminées artificiellement. 140 lapines (nullipares et multipares) ont été reparties en 2 bandes de reproduction pendant 10 mois et éclairées ou 16 heures par jour ou 10 heures par jour. Cinq jour avant l'insémination, l'éclairement de ce dernier lot était brutalement augmenté à 16 heures pour provoquer la synchronisation de l'oestrus. Les deux bandes étaient inséminées chaque 6 semaines avec une intervalle de 3 semaines entre elles. Au total 988 inséminations ont été réalisées avec une fertilité moyenne de 56,5 %. La fertilité obtenue avec les 2 dilueurs était comparable, respectivement 55,6 % (IMV) et 57,5 % (Minitüb). Nous n'avons pas constaté une chute du taux de fertilité avec le sperme conservé à température ambiante pendant 6 heures. Les taux de fécondité étaient respectivement de 56,6 % pour la sperme frais et 56,4 % pour le sperme stocké. Entre les deux schémas lumineux, l'écart en fertilité était faible (2 %) et pas significatif. Les différences des tailles de portées n'étaient pas significatives et ne peuvent pas être liées aux traitements. Le faible taux de réussite de l'insémination (56,5 %) est expliqué par l'état physiologique (couleur de la vulve) des femelles : 67 % des lapines n'étaient pas réceptives (vulve blanche ou rose) au moment de la première insémination (femelles nullipares et multipares). Au contraire, la fertilité obtenue chez des femelles ayant une vulve rouge ou violette, était 70,3 %. Les femelles inséminées avec une vulve blanche ont donné des portées significativement (P<0,001) plus petites, dont la taille était seulement de 3,9 (nullipare) et 5,8 lapereaux vivants (multipares) au lieu de 6,9 (nullipares, vulve rose) à 8,9 (multipares, vulve violet) chez des femelles inséminés avec une vulve colorée.

INTRODUCTION

In many European countries the use of artificial insemination in rabbit meat production has become widespread in the late eighties. Management reasons as planned production in groups have led to this practice rather than more favourable fertility results.

Artificial insemination is performed only with fresh or shortly stored sperm because of impaired results with frozen sperm yet (CASTELLINI *et al.*, 1992). A storage time of approximately 6 h is very useful for practical application. Such a procedure makes it possible to collect the sperm in the morning and to inseminate the does in the afternoon.

Figure 1 : Experimental design.



The effect of the storage time and the characteristics of many different diluters are described in the literature (BOUSSIT, 1989) but there are few works concerning the use of suitable and controlled diluents with proven efficiency.

Many experiments have clearly demonstrated that the success of artificial insemination is largely depending of the physiological status of the doe (see review THEAU-CLEMENT and VRILLON, 1989, PIZZI *et al.*, 1993). A synchronization of the oestrus on the day of insemination is therefore likely. Studies on nulliparous (LEFEVRE and MORET, 1978) and multiparous does (THEAU-CLEMENT *et al.*, 1990) have shown that artificial light regulation can increase the receptivity of the female.

The purpose of the present 3-factorial experiment was therefore to study simultaneously the effect of diluent, storage time and lighting schedules on the conception rate and litter size of artificial inseminated does.

MATERIALS AND METHODS

Animals and housing

One hundred and forty does, belonging to the Institute's own selected strain (MAERTENS, 1992), were initially used for the 10–months experimental period. The nulliparous and multiparous does were randomly divided in two identical environmentally controlled rooms of a windowless experimental house. All does were housed in flat deck cages measuring 600 x 430 x 330 mm high. A minimum inside temperature of 16°C was maintained in winter, using an over–under pressure ventilation system with heated air. Does who died during the experiment or were discarded for different reasons (illness, 3 consecutive infertile inseminations or low productivity) were immediately replaced by nulliparous does.

The 10 males used for this experiment belonged to the male line of the same strain than the does. Prior to the experiment, they were selected on their ability for artificial insemination and on their reproductive capacities (sperm production and quality) utilizing the procedures described in literature (BATTAGLINI, 1986). They were housed in a separate compartment of the same building. Their flat deck cages measured 600 x 600 x 330 mm high and half of the bottom was of plastic laths. A cycle of 15 hours dark and 9 hours light was used throughout the year for them.

Semen collection, dilution and storage

Semen was collected using the IMV equipment between 08:00 and 09:00 h. Immediately after collection, motility and concentration were judged

under the microscope (20x). A limited number of ejaculates were excluded because of a too low motility and concentration. Semen graded less than 2 on a scale from 0 to 5 (JEQUIR and CRICH, 1986) was not used for the experiment. The remainder samples were divided in two equal parts within 5 min. after collection, diluted with the respective diluent (1/5) and all pooled. Care was taken to prevent temperature shocks.

Pooled heterosperm was aspirated in IMV straws and introduced in the plastic insemination pipettes. Storage of the insemination straws was performed at ambient temperature (20 ± 2°C) in the laboratory. The quantity of spermatozoa in one 0.5 ml staw content was arround 50 millions

Husbandry and insemination

Both production groups were inseminated every 6 weeks with an interval of 3 weeks between them. Non pregnant does changed from production group after negative palpation (14 days PI). Non lactating does were immediately moved while lactating does were moved to the other production group at weaning, which corresponds with 2 days prior to the next insemination. In this way, all non pregnant does were remated 3 weeks after the negative insemination. Weaning was performed at the age of 30 days.

Equipment of IMV was used to inseminate the does. All inseminations and microscope judgement at a given day were done by one person. Immediately after the insemination, does were induced to ovulate by injecting intramuscularly 0.2 ml of GnRH analog (Receptal ®) into the hind leg muscles. Does were not treated hormonally to synchronize the oestrus. Does received a standard reproduction diet in accordance with the recommendations of LEBAS (1989). The diet was offered always *ad libitum*.

Treatments

A 3–factorial (diluter, storage time, lighting schedule) experimental design was used (Figure 1). Two commercial diluters were compared. The Dilap 2000 which is commercialized (IMV) as a rabbit semen diluter in liquid form. The second diluter, M III (Minitüb) is a tris–diluter used for pig semen. The dry powder has to be dissolved in distilled water before use. Diluted sperm was inseminated within one hour after dilution or stored at ambient temperature for 6 hours.

The following lighting schedules were compared: one production group of does (n = 70) received always 16hL:8hD (continuously schedule). The second group (n = 70) received a 10hL:14hD photoperiod but five days before the insemination, they were shuttled suddenly to 16hL:8hD (alternative schedule). After the insemination, they received again 10hL:14hD. Light was provided using fluorescence bulbs. The light intensity in the room was around 120 lux.

Statistical analysis

Data were analyzed by a three–factorial analysis of variance with interactions, using the STATGRAPHICS ® Package version 5 (1991). The model included the main effects of diluent solutions, storage time and lighting schedules and their interactions, as follows:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \Sigma_{ijkl}$$

where:

Y_{ijkl} = depending variable
μ = overall mean
α_i = fixed effect of diluent (i=1,2)
β_j = fixed effect of storage time (j=1,2)
γ_k = fixed effect of lighting schedule (k=1,2)
$(\alpha\beta)_{ij}$ = interaction
$(\alpha\gamma)_{ik}$ = interaction
$(\beta\gamma)_{jk}$ = interaction
Σ_{ijkl} = random effect of error.

The parameters analyzed were: conception rate, total litter size and alive litter size. The percentage of conception rate were transformed to square roots ; however, original data were used for statistical analysis because there were no differences from those based on transformed data. Moreover, the number of total and alive born were analyzed according to the insemination number, vulva colour and parity (nulliparous *vs* multiparous) of does with a factorial analysis of variance with interaction.

RESULTS AND DISCUSSION

Effect of diluent and storage time of the semen

Differences in conception rate (CR) and litter size according to diluent (IMV, Minitüb) or storage time (fresh and 6 hours) were not significant (Table 1). No significant interactions were found and therefore they are not presented in Table 1. The highest CR (58.9 %) was even obtained after a 6h storage with the Minitüb diluent while the 6h stored IMV semen showed the lowest CR (53.7 %) (Figure 2).

Our results are in good agreement with the *in vitro* determinations of BERGONZONI *et al.* (1994) in which they compared five type of diluters (Tris–buffer, IMV, physiological solution, lactose and BF5). After a comparable storage time (6 hours) at 15°C, viability of the semen was somewhat higher when diluted in a Tris diluent compared to IMV or physiological solution. On the contrary, GOTTARDI (1993), with a chemical–physical analysis of 4 diluters (Tris, Tris–buffer,

Tartrate and IMV) obtained the highest viability with the IMV diluent after a storage time of 24 hours at 15 and 25C° ambient temperature. With fresh semen, UZCATEQUI and JOHNSTON (1988) and CASTELLINI (1990) obtained a comparable CR and litter size with physiological saline, sodium citrate or Tris–buffer solutions.

Effect of lighting schedule

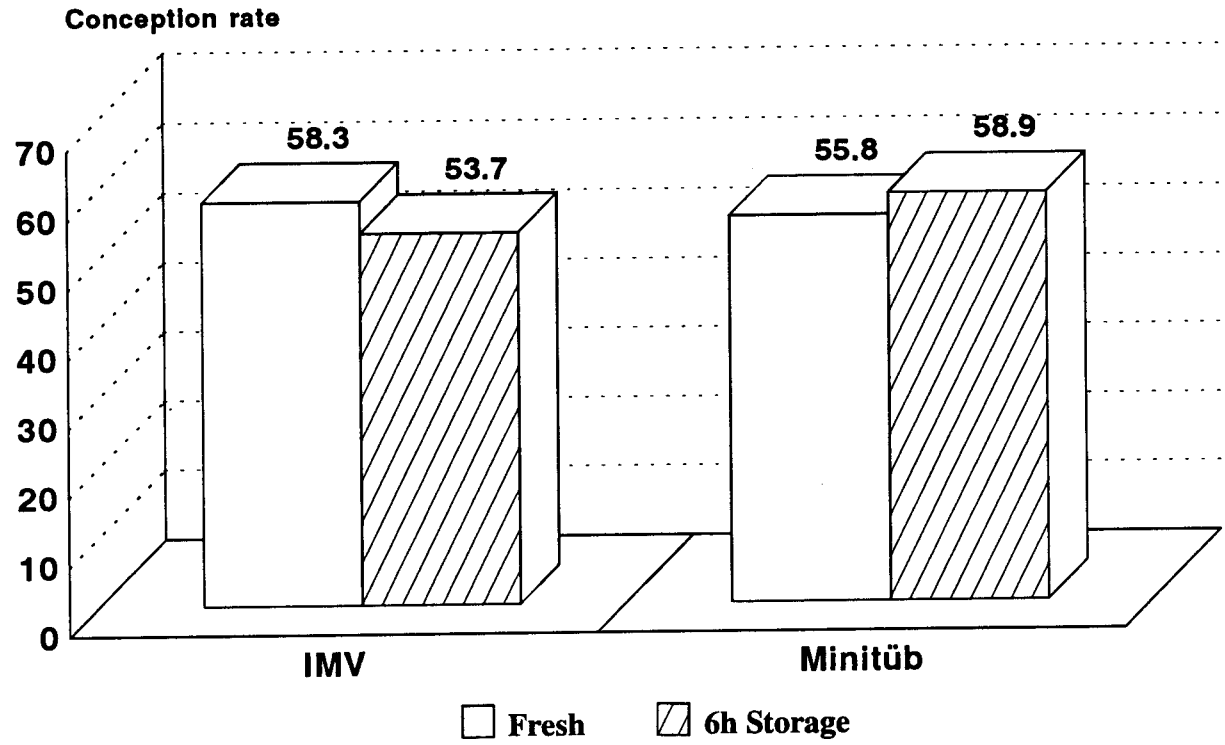
Reproductive performances of the does reared under the constant lighting program *versus* the alternative lighting are given in Table 1. The CR was a little higher with does receiving continuous 16hL photoperiod but not significant yet (57.7 *vs* 55.4 respectively). Also the total and alive litter size were

Table 1 : Effect of diluent, storage time and lighting schedule on conception rate and litter size of A.I does.

	Nb. of insemination	Conception rate		Nb. born total/litter		Nb. born alive/litter	
		LSM	SEM	LSM	SEM	LSM	SEM
Diluent							
IMV	496	55.57	2.56	9.11	0.23	8.21	0.23
Minitüb	492	57.45	2.56	8.68	0.22	8.02	0.22
Storage time							
Fresh	503	56.59	2.56	8.87	0.23	7.94	0.23
6 hours storage	485	56.42	2.56	8.92	0.23	8.29	0.23
Lighting schedule							
16hL continuously	561	57.65	2.37	8.77	0.25	8.16	0.25
10hL/16hL alternative	427	55.37	2.73	9.02	0.21	8.07	0.21
OVERALL mean	988	56.51	1.81	8.89	0.16	8.13	0.16

Between treatments differences and interactions are not significant (P>0.05)

Figure 2 : Effect of diluent and storage time on conception rate of artificially inseminated does.



similar each other. The receptivity of the does, judged by the colour of the vulva, tended to be a little changed by the photoperiod. A white vulva was found by 20.7 % and 15.9 % of the does on the 16hL or 10hL–16hL lighting schedule, respectively. For red vulva these frequencies were respectively 36.7 % and 42.9 %.

However, the reproductive management used in our trial could have influenced the effect of the lighting schedule. Non pregnant does changed from production group and lighting schedule. If they were lactating, they received the new lighting schedule only two days prior to the insemination. The effect of the lighting schedule has to be judged taking into account these possible interferences.

Our results partly agrees with those of THEAU-CLEMENT *et al.* (1990) who compared a regular 16hL:8hD lighting schedule *versus* a 8hL:16hD photoperiod until a week before the insemination, when the light duration period was increased to 16hL/day. Although the receptivity of the does was significantly higher, the CR was not significantly increased with their alternative lighting schedule. Recently, DEPRES *et al.*, (1994) obtained a significantly increased receptivity to the male and a higher proportion of does with coloured vulva when 6 hours extended light was provided to nulliparous does under subtropical conditions. However the overall breeding performances were not increased in the extended light group.

Previous experiments at our Institute (MAERTENS and OKERMAN, 1987) with nulliparous does, in order to induce the oestrus by increasing the photoperiod, failed also if the does were not transferred towards a more favourable environment. However, recently MIRABITO *et al.* (1994) obtained encouraging results using a sudden increase from 8hL to 16 hL, one week before the insemination. As well the receptivity as the CR of multiparous does tended to be more favourable. The difference in lighting schedule could probably explained the absence of a positive effect of the light stimulation in our experiment. An increase from 10hL to 16hL seems not enough to have a real synchronizing effect on the oestrus. Because of the tendency to more favourable results with an increase from 8hL to 16hL, further research has to be done if even a more drastically stress provokes an oestrus synchronization.

Effect of physiological status of does

As widely demonstrated (MAERTENS *et al.*, 1983 ; COSTANTINI, 1986 ; ZANIRATO, 1989 ; THEAU-CLEMENT *et al.*, 1990), the physiological status of the doe at the time of insemination is very important in view of receptivity, and CR as well. Our results (Table 2) stresses the importance and explain partly the low overall CR (56.7 %). The CR of does showing a red or purple vulva was on average 70.3 %, while only 27.2 % of the does inseminated with a pale vulva were pregnant. Because the effect was quite similar for nulliparous and multiparous does, overall results are presented in Table 2. These results clearly demonstrate that our does were not in optimal oestrus condition for insemination. Sixty-seven % of the does (nulliparous and multiparous) showed a white or rose vulva at the 1st insemination. As a result, average CR of the first insemination was low (51.0 %), and illustrates the antagonism between lactation and ovulation frequency (REBOLLAR *et al*, 1992 ; THEAU and ROUSTAN, 1992).

Table 2 : Effect of the insemination number and vulva colour on the receptivity and conception rate of the overall results (nulliparous + multiparous does).

	Vulva colour				
	White	**Rose**	**Red**	**Purple**	**Total**
1st insemination					
N° or %	173 or 26 %	272 or 41 %	187 or 28 %	35 or 5 %	667
Conception (%)	26.6	51.1	70.6	65.7	51.0
1st remating					
N° or %	8 or 3 %	72 or 28 %	159 or 63 %	15 or 6 %	254
Conception (%)	37.5	70.8	69.8	86.7	70.1
2nd remating					
N° or %	3 or 4 %	17 or 26 %	43 or 64 %	4 or 6 %	67
Conception rate %	33.3	58.8	62.8	100	62.7
Overall results					
N° or %	184 or 19 %	361 or 37 %	389 or 39 %	54 or 5 %	988
Conception (%)	27.2	55.4	69.4	74.1	56.5

At the first remating (after negative palpation), nearly 70 % of the does showed a red or purple vulva with a fertility rate of 71.3 %. This 2nd insemination was executed 2 days after weaning (for multiparous does). Its well known that an oestrus synchronization occurs some days after weaning leading to favourable CR (THEAU and ROUSTAN, 1992 ; SZENDRÖ *et al*, 1992).

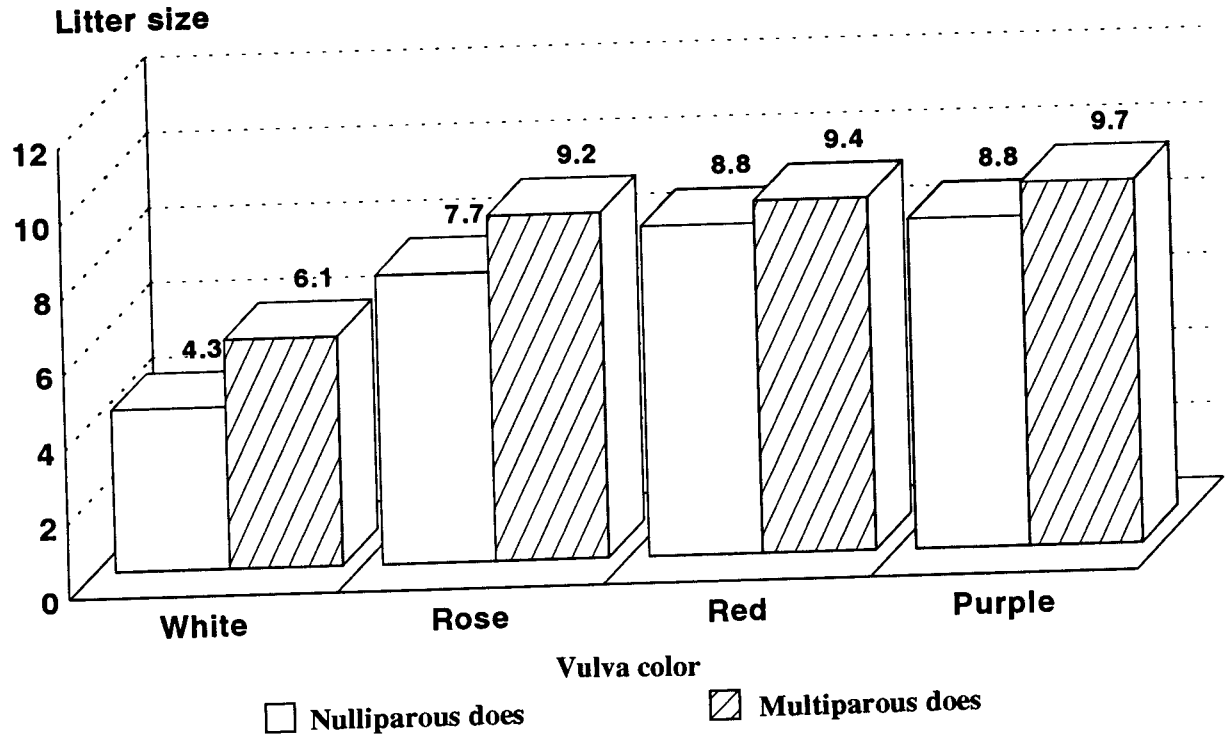
Important is to stress the relationship between the colour of the vulva at the insemination and the litter size (Table 3 and Figure 3). A significant (P<0.001) lower number of youngs were born when

Table 3 : Effect of the insemination number, parity and vulva colour of the does on the litter size.

	Number of litters	Total born/litter LSM	SEM	Born alive/litter LSM	SEM
Inseminations (1)					
1st insemination	320	8.30	0.26	7.49	0.26
1st remating	168	8.93	0.61	8.56	0.61
2nd remating	40	7.72	1.06	6.73	1.06
Vulva colour					
nulliparous does					
white	7	4.29A (2)	1.47	3.86Aa	1.54
rose	33	7.67B	0.50	6.94ABb	0.60
red	69	8.78B	0.29	8.02B	0.31
purple	6	8.83B	1.42	8.50B	1.36
multiparous does					
White	38	6.11A	0.56	5.82A	0.58
Rose	152	9.18B	0.24	8.56B	0.28
Red	191	9.38B	0.25	8.45B	0.30
Purple	32	9.69B	0.44	0.88B	0.55

(1) : Overall means of nulliparous and multiparous does ;
(2) : Means within the same column with different superscripts differ significantly ; A B : P<0.01 ; a b: P<0.05 ;
(3) : Interactions are not significant (P<0.05).

Figure 3 : Litter size according to the physiological status of does. Total born/litter (528 litters)



does were inseminated with white vulva. Litter size amounted only 3.9 (nulliparous does) and 5.8 (multiparous does) live youngs instead of 6.9 (nulliparous, rose vulva) to 8.9 (multiparous, purple vulva) when inseminated with coloured vulva. This explains why in some comparative studies, litter size with A.I. inseminated does tended to be lower than with natural mating (COSTANTINI, 1986, UZCATEQUI and JOHNSTON, 1988 ; BLOCHER and FRANCHET, 1990).

General conclusions

Six hours stored semen at ambient temperature did not shown any negative effect on the CR or the litter size of artificial inseminated does. Taking into account the results of some *in vitro* experiments, it seems preferable to store the diluted semen at an ambient temperature of 15°C, although in our experiment, a storage temperature of 20 ± 2°C has no depressing effect on CR or litter size. Such an easy storage procedure is usable for rabbitries doing their own semen collection but also for insemination centres when the distance between the delivery station and the rabbitry is limited to some hundreds of km. The results obtained with the pig semen Tris diluent were comparable with the specialized rabbit semen diluent. In order to reduce the costs of artificial insemination, low cost diluents as this Tris buffer may be used to a large extent in practice.

Although our alternative lighting schedule failed to obtain a synchronised oestrus and consequently a more favourable CR, further work seems necessary using, e.g. a stronger light stimulation, intermittent lighting (UZCATEQUI and JOHNSTON, 1992) or special wavelengths in order to avoid the systematically use of hormonal induced oestrus. Finally our results stresses the well-known importance of the physiological status of the doe at the time of insemination. Acceptable results (> 70 % CR and >9 born/litter) can only be obtained when the does are receptive.

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