

REPRODUCTIVE RESULTS OF DOES INSEMINATED WITH 5 TIMES OR 10 TIMES DILUTED SEMEN

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Abstract - 514 lactating multiparous does of the Hyplus breed were inseminated 11 days after parturition with diluted fresh sperm in a ratio 1:5 or 1:10. Conception rate (positive abdominal palpations on day 10 or 11 after A.I. [Artificial Insemination]) and fertility rate (number of parturitions after A.I.) did not differ between both dilution rates. For the dilution 1:10, number of total born kits was slightly lower ($p=0.12$) and number of live born kits was significantly lower ($p=0.04$) by 0.62 kits per litter.

INTRODUCTION

Dilution of fresh sperm of bucks has an obvious economic advantage for the artificial insemination (A.I.) centers, because the number of presents males could be reduced. Scientific papers on A.I. (THEAU-CLEMENT and VRILLON, 1989 ; ITAVI, 1991 ; PEREZ-GUTIERREZ, 1995) reported dilution rate from 1:10 to 1:20 in fresh semen with a majority for 1:5 to 1:10.

The quality of rabbit semen varies with males and season (THEAU-CLEMENT *et al.*, 1994). For A.I., it would be useful to know the quantity of semen needed for each doe in term of quantity of spermatozoas in relation with the semen quality. But semen analyser (which evaluate concentration, mortality, speed, linearity of trajectory and morphology of spermatozoa) is not used in farm condition because of its cost and the narrow schedules between collection of sperm in the A.I. center and A.I. of does in the farm. Likewise, the hematimeter (or Thoma cell) is able to count the number of spermatozoa, but it is a time consuming method (BOUSSEAU, 1994).

In 1982, THEAU and ROUSTAN have tested 4 dilution rates (1:10 ; 1:25 ; 1:50 and 1:100) with freezed sperm on 143 inseminated does. There was no effect of dilution rate on conception rate, but the number of kits decreased significantly ($p=0.05$) with dilution.

That's why, to be in farm conditions, we have chosen to compare, with fresh semen, dilution rates of 1:5 and 1:10 with repetitions on several flocks, in the spring and at autumn.

MATERIALS AND METHODS

The experiment was led from March to October 1995 in Sourches (France), the research facility of SANDERS Company.

Animals and housing

Two hundred does of the French hybrid Hyplus Medium were reared in two homogenous groups in term of reproductive stage. The time intervals between the cycle of both groups was 21 days. Does were housed in windowless buildings of 100 places on flat-deck cages for parturition (77.5 x 48 cm) and 16 hours of light per day was provided. A minimum temperature of 16°C was maintained. A dynamic ventilation system with low extraction was used. 20 bucks of the breed Hyplus Heavy were used at each insemination and their semen was pooled. Bucks were kept in a windowless building of 20 places on flat-deck cages (88.5 x 48 cm) and 16 hours of light per day was provided.

Does and bucks were fed with a diet containing 18 % of crude protein and 10.8 MJ DE/kg. Does were fed *ad libitum* and bucks received 150 g per day of the diet.

Insemination of the does

Does were inseminated on the same day for each group (10-11 days after parturition) with pooled fresh semen (polyspermy). Palpation was realised at day 14 after insemination. A.I. was performed in lordose with a pistol equipped with a curved plastic pipette, at 15 centimeters inside the vagina. The insemination took place 9 hours after collection of the fresh sperm. Just after insemination, the females received an intramuscular injection of 0,20 ml of GnRH (Receptal - Distrivet) to induce ovulation.

Semen collection and dilution

Collection of sperm of the 20 bucks was performed in the morning with a specific artificial vagina (IMV). The sperm of each buck was prediluted with the commercial extender Dilap 2000 (IMV) with a factor of 1:3 and mixed gently at room temperature. The 20 samples were then pooled and diluted to obtain final dilution rates of 1:5 and 1:10. 0.6 ml of the semen was then inserted in each pipette and kept at a constant temperature of 20°C until time of A.I.

Treatments

At each A.I., the group of 100 does were split into two groups of 50 females according to the number of previous pregnancies.

One group was inseminated with the pooled semen with the dilution factor 1:5, the other group with the pooled semen with the dilution factor 1:10. The nulliparous and primiparous females were put off as well as non lactating multiparous does. During the experimentation time, we performed 514 inseminations at 6 different times (March 30, April 20, Mai 11, August 24, September 14, October 5).

The following data were recorded on all does : live weight at A.I., number of previous litter, conception rate, birth rate, total litter size and number of alive born rabbits.

Statistical analysis

Statistical analysis have been done on S.A.S. Software 604 (SAS/STAT user's guide, 1988). For the total results, continuous variables were analysed through variance analysis, taking into account sperm dilution rate, number of litter before A.I., live weight class and time of A.I.

Since conception rate and birth rate are discontinuous variables, the statistical analysis was done by a X² test for the 514 results. The test can't be used at each time because there were not enough females.

RESULTS AND DISCUSSION

In overall 256 A.I. were performed in the 1:5 dilution group and 258 A.I. in the 1:10 dilution group.

Effect of semen dilution factor on the conception rate and birth rate

Looking at the results of all A.I. (table 1), dilution factor 1:10 increased not significantly the conception rate (+2.9 points) and the birth rate (+1.4 points) in comparison with the dilution factor 1:5, which confirmed the results of THEAU and ROUSTAN (1982).

Table 1 : Effect of semen dilution factor on conception and birth rates

Dilution factor	1:5	1:10	X ²
Conception rate (%)	78.5	81.4	ns
Birth rate (%)	74.6	76.0	ns

ns = difference not significant

The data of conception rate and birth rate for each group (different time of insemination ; Table 2) showed no significant difference. The variability of the results from one insemination group to the other was high.

Table 2 : Effect of semen dilution factor for each A.I. group on conception and birth rates

Dilution factor	1:5	1:10	1:5	1:10
Number of A.I.	Conception rate		Birth rate	
week 13	87	75.0	81.4	81.4
week 16	75	65.8	81.1	78.4
week 19	103	82.4	82.7	76.9
week 34	69	85.3	80.0	71.4
week 37	94	78.3	85.4	77.1
week 40	86	83.7	76.7	69.8

Effect of semen dilution factor on the litter size

Total rabbits born per litter (Table 3) tended to be lower by 0.4 kits with dilution factor 1:10 ($p=0.12$). Rabbits born alive per litter was decreased significantly by 0.6 rabbits with dilution factor 1:10 ($p=0.04$). That fits with the results of THEAU and ROUSTAN (1982) who reported a fall of litter size with an increase of the dilution rate. But, the dilution rates tested were 1:10, 1:25, 1:50 and 1:100 and the dilution rate of 1:5 usually used in A.I. was not tested.

Table 3 : Effect of semen dilution factor on the litter size

Dilution factor	1:5	1:10	Effect of dilution rate	Effect of weight	Effect of number of litter before	Effect of A.I. group
Total rabbits born/litter	11.05 ± 3.48	10.62 ± 3.51	$p=0.12$	**	**	$p = 0.14$
Rabbits born alive/litter	10.44 ± 3.67	9.82 ± 3.84	$p = 0.04$	**	**	$p = 0.14$

** $p < 0.01$

Variance analysis on the data per week of A.I. (Table 4) pointed out better results for the dilution factor 1:5 for weeks 13, 34, 37, 40.

A.I. results of week 16 were globally bad for the dilution rate 1:5, with a birth rate of 63.2 % and a number of born alive per litter of 8.75.

Table 4 : Effect of semen dilution rate for each A.I. group on litter size

	Total rabbits born/litter				
	Dilution rate 1:5	Dilution rate 1:10	Effect of dilution rate	Effect of live weight	Effect of number of litter before
week 13	11.25 \pm 3.11	10.14 \pm 3.68	$p=0.08$	ns	$p=0.006$
week 16	9.08 \pm 3.57	10.24 \pm 3.62	ns	ns	ns
week 19	11.29 \pm 3.39	10.87 \pm 3.42	ns	$p=0.09$	ns
week 34	11.22 \pm 2.78	9.48 \pm 3.46	$p=0.022$	ns	ns
week 37	12.48 \pm 2.78	11.43 \pm 3.18	$p=0.127$	ns	ns
week 40	10.39 \pm 3.93	11.17 \pm 3.63	$p=0.162$	ns	$p=0.10$

	Rabbits born alive/litter				
	Dilution rate 1:5	Dilution rate 1:10	Effect of dilution rate	Effect of live weight	Effect of number of litter before
week 13	10.87 \pm 3.34	9.25 \pm 4.00	$p=0.021$	ns	$p=0.05$
week 16	8.75 \pm 3.43	9.93 \pm 3.60	$p=0.148$	ns	$p=0.12$
week 19	10.30 \pm 3.74	9.90 \pm 3.95	ns	$p=0.13$	ns
week 34	10.40 \pm 3.89	8.40 \pm 3.79	$p=0.038$	ns	ns
week 37	12.15 \pm 2.86	10.67 \pm 3.31	$p=0.056$	ns	ns
week 40	9.76 \pm 4.04	10.37 \pm 4.22	ns	ns	$p=0.06$

Standard deviations for total born and born alive per litter are higher for dilution factor 1:10. This could be explained by a greater variability in the fecondation of ovula when there is fewer spermatozoa in the uterus.

CONCLUSION

Semen dilution factor 1:10 induced in average lower litter size without affecting birth rate in comparison with dilution 1:5. The results reported in this study include only A.I. with multiparous does and it would be necessary to check these data on primiparous and nulliparous females.

No general recommendations can be put forward because of variability of results between the weeks. Actually, there are sometimes more kits with dilution rate 1:10 with dilution rate 1:5.

That's why one should link these reproduction results with the number of spermatozoa inseminated, all the more so that the male spermatozoa production vary very much throughout the year. But, the current spermatozoa counting methods are time consuming and expensive and are therefore hard to use in the commercial farms. We tried to reduce the spread in the number of spermatozoa by choosing groups inseminated in spring and autumn.

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Résultats de reproduction chez des lapines inséminées avec de la semence diluée 5 ou 10 fois - 519 femelles multipares allaitantes de race Hyplus ont été inséminées 11 jours après la mise bas avec du sperme frais dilué 5 ou 10 fois.

Le taux de conception (palpation positive 10 à 11 jours après l'insémination artificielle (I.A.)) et le taux de fertilité ne diffèrent pas entre les deux dilutions.

Pour la dilution au 1:10ème, le nombre de nés totaux était légèrement inférieur ($P=0,12$) et le nombre de nés vivants était significativement inférieur ($P=0,04$) avec 0,62 lapereaux en moins par portée.
