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EFFECT OF MALE LINE ON PROLIFICACY FROM DOES INSEMINATED WITH LOW SPERM DOSES

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ABSTRACT

The effects of four male genotypes (V, A, H and R) on fertility and prolificacy were evaluated after artificial insemination. After semen evaluation, ejaculates from three to five males of each line with more than 60% motility were pooled and extended. The number of spermatozoa per milliliter was adjusted to 12 million with Tris-citrate-glucose. Multiparous lactating does were inseminated with 0.5 ml of extended semen using a curved plastic pipette (IMV, France). The final number of spermatozoa per doe was 6 million. Seminal characteristics differed among male lines. The growth line (R) showed lower seminal production per ejaculate, motility and acrosomal integrity (140 million, 73% and 85% respectively) than line V, A and H (302, 231 and 327 million, 81%, 82% and 80%, 90%, 90% and 91%, respectively). Significant differences were observed among the male lines in total kits born and kits born alive. Insemination with semen from males of line V resulted in highest total kits born (11.2±0.23) while the inseminations with semen from line A resulted in the lowest total kits born (9.7±0.23).

INTRODUCTION

Many authors have discussed artificial insemination in rabbits relative to low sperm count (Wales and O'Shea, 1968; Williams *et al*. 1990; Farrell et al. 1993, Viudes *et al*., 1997). These studies have presented estimates of minimal sperm levels for optimum fertility in rabbits of 0.05 to 4 million depending on dilution rate and insemination procedure. There are, however, other important factors such as reproductive female status and male and female genotypes that should be considered. Normally, laboratory experiments have not corresponded with real situations in a commercial breeding.

Recently, experiments in industrial rabbitries (Pizzi *et al*. 1996, Viudes *et al.* 1998, Castellini *et al*., 1999) suggested that at least 6 to10 million motile spermatozoa were necessary to obtain optimum fertility. However, in commercial rabbitries, ejaculates are generally not diluted very much (1/4 to 1/10) and sperm count is higher than 16 million (Theau-Clement and Roustan, 1980; Costantini, 1989; Bourdillon *et al*. 1992, Rebollar *et al*. 1992; 1995, Armero *et al*. 1994, Martens and Luzi, 1995).

The objective of this study was to evaluate the effects of male genotype on fertility and prolificacy in artificial insemination with a reduced sperm count.

MATERIAL AND METHODS

Animals

Commercial crossbred does were inseminated with semen of adult males from four selected lines (V, A, H and R):

-Lines V and A have been selected since 1980 and 1982, respectively, for litter size at weaning (Estany *et al.*, 1989).

-Line H has been selected since 1996 for number of kits born alive (Cifre *et al*., 1997)

-Line R has been selected for growth rate from weaning to slaughter (28-63 days of age, Estany *et al.*, 1992).

Inseminated does were assigned randomly to male line.

Semen collection and evaluation

Two ejaculates per male were collected each week using an artificial vagina. Volume of fresh semen was measured in a graduated conical tube. The percentage of motile sperm was evaluated from samples diluted 1:50 in Tris-citrate-glucose (250mM tris-hydroxymethylaminomethane, 83mM citric acid, 50mM glucose, pH 6.8-7.0), placed on a glass slide at 37ºC and observed using a microscope with positive phase-contrast optics at a magnification of x200. The microscope was connected to a video camera and computer and samples were examined with the aid of Sperm Class Analyzer software version 2.0 (SCA, Microptic). Concentration of sperm per milliliter was measured by a Thoma-Zeiss counting cell chamber. To measure acrosomal integrity (NAR) and percentage of abnormal forms, spermatozoa were fixed with 2% glutaraldehyde in Dulbecco's phosphate buffered saline (Pursel and Johnson, 1974) and the proportion of sperm with normal intact acrosome and abnormal forms were estimated using interference contrast optics at a magnification of x750.

Semen extension and insemination

After semen evaluation, ejaculates from three to five males of each line with more than 60% motility were pooled and extended. The number of spermatozoa per millilitre was adjusted to 12 million with Tris-citrate-glucose. The semen was diluted at room temperature (20º-25ºC).

Multiparous lactating does were inseminated with 0.5 ml of extended semen using a curved plastic pipette (IMV, France). The final number of spermatozoa per doe was 6 million. Does were injected with 0.8 μg buserelin acetate (Hoescht).

Statistical analysis

Effect of male line on seminal parameters (volume, concentration, sperm production, motility and acrosomal integrity), and prolificacy (total number of kits born and number kits born alive) were analysed by GLM procedures (SAS, 1993). Total kits born was were included as a covariate in the analysis of the number of kits born alive. Fertility rates at birth were analysed by a Chi-squared test.

RESULTS AND DISCUSSION

Seminal characteristics differed among the male lines (Table 1). The growth line (R) showed the lowest sperm production, motility and acrosomal integrity (140 million, 73% and 85% respectively,). Seminal differences have been observed by other authors for selected lines (Bencheikh, 1993) or races (Egea and Roy, 1992).

These differences might explain the slight differences observed in fertility rate (line R 75% vs 81%, 80% and 79% for lines H, V and A, P<0.06, Table 2). In previous studies, semen from line R was used to test the effects of reduction of number of spermatozoa in the insemination dose (Viudes and Vicente, 1997) and the results with 6 or 20 million were similar to those obtained in present work . In general, fertility and prolificacy were similar to other studies in artificial insemination of rabbits (Pizzi *et al*., 1996; Alvariño *et al*., 1996).

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Table 1.- Seminal characteristics of 50 ejaculates from each of four lines (Least squared means ±standard error)

Male line	Volume (ml)	Spz/ml[1] (x10^6)	Semen Production spz/ejaculate (x10^6)	Motility (%)	NAR[2] (%)	Abnormal forms (%)
V	0.9 ±0.1^a	338 ±31^a	302 ±29^a	81 ±2^a	90 ±1^a	1 ±0.3^a
A	0.9 ±0.1^a	257 ±26^b	231 ±25^b	82 ±2^a	90 ±2^a	1 ±0.3^a
H	0.9 ±0.1^a	381 ±30^a	327 ±27^a	80 ±2^a	91 ±1^a	4 ±0.3^b
R	0.6 ±0.1^b	230 ±26^b	140 ±25^c	73 ±2^b	85 ±1^b	4 ±0.3^b

[1]Spz/ml: concentration, number of spermatozoa per ml.
[2]NAR: acrosomal integrity.
a, b, c: Values in the same column with different superscripts differ statistically (P<0.05).

Significant differences were observed among the male lines in total kits born and kits born alive. Insemination with semen from males of line V resulted in the highest total kits born while the inseminations with semen from line A males resulted in the lowest total kits born. The differences between these two lines were similar for kits born alive . In addition, when total kits born was included as covariate in the analysis of kits born alive, only significant differences between line R and lines V and A were observed (covariate coefficient: 0.93 ±0.01). This analysis indicated that, with the same litter size, kits from growth line (R) had higher perinatal mortality than lines V and A. This may have been affected by the larger size of the R line kits at birth. Further studies on birth weight and size might confirm or refute this hypothesis.

Table 2.- Fertility and prolificacy from four male lines (Least squared means ± standard error).

Male line	No. Inseminations	Fertility rate (%)	Total kits born	Kits born alive	[1]Kits born alive
V	277	79.1	11.2±0.23^a	10.9±0.23^a	10.1±0.10^a
A	194	80.4	9.7±0.26^c	9.4±0.27^b	10.2±0.01^a
H	216	81.0	10.5±0.25^b	10.4±0.26^a	10.0±0.12^{ab}
R	1500	75.0	10.3±0.10^b	9.8±0.27^b	9.9±0.04^b
Total	2187	76.5	10.4±0.11	10.1±0.11	10.0±0.05

a, b, c Values with different superscripts were statistically different (P<0.05).
[1] Covariance analysis of kit born alive.

In **CONCLUSION**, important differences were observed among male lines in prolificacy when low sperm doses were used to inseminate multiparous lactating does. In later studies, it might be useful to determine the optimal sperm count for each of the lines and to compare the viability of embryos produced with semen from different male lines.

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