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ABSTRACT

A complete diallel cross involving two rabbit sire lines (C and R) was carried out to estimate the crossbreeding genetic parameters (direct additive genetic effects, maternal genetic effects and heterosis) of seminal traits. The analysis concerned 2140 ejaculates from 153 males from the 4 groups of bucks. The recorded trait were: pH, ejaculate volume (V), mass motility (Mm), individual motility (Mi), concentration (Cn), total number of spermatozoa per ejaculate (TSE), percentage of sperm viability (Vi), percentage of sperm with acrosome integrity (NAR), percentage of sperm normalcy (Nr), percentage of sperm morphological abnormalities of head (H), neck-midpiece (Nm) and tail (T) and presence of proximal and distal cytoplasmic droplet (Dp, Dd). Estimates of heterosis, direct additive genetic and maternal effects on seminal traits of the lines were obtained from the solutions of the corresponding mixed model by generalized least squares. Main genetic relationships between individuals were taken into account. Differences in direct genetic effects between lines were significative for Cn, TSE, Vi, NAR, Nm and Dp. Those differences were eminent (about 50% of the crude mean) and favourable to line C for Cn and TSE, and unfavourables for Nm and Dp. Differences in Vi and NAR were favourable to line R but lower in magnitude (about 10%). It seems that line C could be superior in traits related with semen production and inferior in semen quality traits. Differences between lines in the maternal genetic effects were observed for V and Cn at the level of signification of 5%. These differences were eminent (14%) and favourable to line C for V and eminent and favourable to line R for Cn (40%). Favourable and significant individual heterosis was observed in pH and Dp at the level of signification of 5%. Heterosis effect was high for Dp (35%) and negligible for pH (1.6%).

Key words: semen, heterosis, direct genetic effects, maternal effects, rabbit.

INTRODUCTION

Intensive meat rabbit production is based on a three-way crossbreeding scheme in which, the sire used for the terminal cross is a male from a sire line commonly selected for growth rate or weight at a given age. The broad distribution of semen from terminal sires at the lowest possible cost supposes an increase in profitability of AI centres and wider diffusion of genetic progress for important traits in the whole population. To achieve this goal males should be able to produce large number of doses with good fertility. Semen production and quality depends on a great variety of management, environmental and genetic factors: age, sexual preparations, season of collection, number of ejaculates collected and interval between collections (ALVARIÑO, 2000). Concerning genetic factors, VICENTE *et al.* (2000), and THEAU-CLEMENT *et al* (2003) found differences between rabbit lines in seminal characteristics but only BRUN *et al.* (2002) have estimated heterosis effects reporting significant and eminent values for concentration, mass motility and percentage of motile sperm per ejaculate. Results on semen production and quality of crossbred males from sire lines, could lead to a change in meat production scheme with the use of a crossbred male instead of a purebred male in the terminal cross.

The aim of this work was to estimate heterosis, direct additive genetic and maternal genetic effects on seminal traits by using two sire lines of rabbits and their reciprocal cross.

MATERIAL AND METHODS

A complete diallel cross was perfomed involving two rabbit sire lines (C and R) selected for growth rate by individual selection from 1993 and 1980 respectively (ESTANY *et al*., 1992; GÓMEZ *et al*., 1996). The trial was carried out in the experimental farm of IRTA in two different periods corresponding to the warm and cold seasons in Spain. Twenty bucks per genetic type and period were used. After weaning, males were housed in individual cages with a photoperiod of 16 hours light/day and temperatures ranging from 14 to 24.4ºC. Animals are fed *ad libitum* with commercial rabbit pellets until 60 days of age. Then they were restricted to 180g/d of another commercial diet. At five months of age bucks started the training period with artificial vagina. One ejaculated was collected per male and week. At six months of age they started production period. During 7 weeks, two ejaculates per male and week were collected, with an interval of 30 minutes between them.

All ejaculates were stored at 37ºC in a water bath until evaluation, non-later than 15 minutes after collection. Volume (**V**) and **pH** of the ejaculate were determined by using a graduated tube and a pH-metre 507 Crison, respectively. Gel plugs were removed before volume evaluation. Ejaculates containing urine and calcium carbonate sediments were discarded. Mass motility (**Mm**) was assessed in a subjective scale from 1 to 5, using aliquots (10μl) of the raw semen and a light microscope (Nikon) at x10. Percent of sperm viability (**Vi**), percent of sperm with acrosomal integrity (**NAR**), percent of sperm normalcy (**Nr**), percent of sperm morphological abnormalities of head (**H**), neck-midpiece

(**Nm**) and tail (**T**) and presence of proximal and distal cytoplasmic droplet (**Dp**, **Dd**) were determined by counting 200 spermatozoa with a light microscope (Olympus CH-3) at x1000 and using a vital nigrosin-eosin staining (BAMBA,1988). Then ejaculates were diluted (1:5) in a commercial saline extender for rabbit semen (KUBUS m.r.a S.A, Madrid, Spain) and individual motility (**Mi**) was evaluated in a microscope with phase-contrast optic (Nikon) at x400 using a subjective scale from 0 to 5 (ROCA *et al*., 2000). The concentration (**Cn**) was measured using fixed spermatozoa (2% glutaraldehyde) in a Thoma-Zeiss counting cell chamber (final dilution 1:50) and a light microscope (Olympus CH-2) at x400. The synthetic variable total number of spermatozoa per ejaculated (**TSE**) was also evaluated.

Estimates of the estimable crossbreeding genetic parameters of the lines were obtained from the solutions of the corresponding mixed model by generalized least squares using the PEST software package (GROENEVELD, 1990). The model used for this analysis included: type of the male (four leves; C, CxR, RxC, RxR), order of the ejaculate (first and second), day of collection, genetic additive value and permanent non-additive value of the male. Main genetic relationships between individuals were taken into account.

RESULTS AND DISCUSSION

Summary statistics of the recorded traits are shown in table 1. Number of data differs between traits because evaluation of some of them has not still been finished for all the ejaculates. The most remarkable is the high variability of the percentages of sperm morphological abnormalities Cn and TSE. Similar values of the overall means were reported in other experiments (KUZMINSKY *et al.* 1996, ALVARIÑO, 2000), but V was, in this experiment, almost double the value reported by VICENTE *et al.* (1996) in line R.

Table 1 also shows the estimates of estimable functions between order of the ejaculate, direct genetic effects, maternal genetic effects and individual heterosis. Differences in direct genetic effects between lines were significative for Cn, TSE, Vi, NAR, Nm and Dp. Those differences were of high magnitude (about 50% of the crude mean) and favourable to line C for Cn and TSE, and unfavourables for Nm and Dp. Differences in Vi and NAR were favourable to line R but lower in magnitude (about 10%). It seems that line C could be superior in traits related with semen production and inferior in semen quality traits. Differences between strains in seminal characteristics have also been observed by VICENTE *et al.* (1996) or BRUN *et al.* (2002).

Differences between lines in the maternal genetic effects were observed for V and Cn at the level of signification of 5% and also for TSE, pH and Mi at the level of 10%. These differences were eminent (14%) and favourable to line C for V and eminent and favourable to line R for Cn (40%), Mi (17.8%) and TSE (23%). The differences between reciprocal crosses for the traits related to energy metabolism (individual motility) have been explained by BRUN *et al.* (2002) as sex-linked or imprinting effects due to the

Table I: Sumary statistics. Estimable functions between order of the ejaculate, direct genetic effects (d_C, d_R), maternal genetic effects (m_C, m_R) and individual heterosis.

	pH	V [1] (ml)	Mm[1]	Mi[1]	Cn[1] (10^6/ml)	TSE[1] (10^6)	Vi[1] (%)	NAR[1] (%)	Nr[1] (%)	H[1] (%)	Nm[1] (%)	T[1] (%)	Dp[1] (%)	Dd[1] (%)
N[2]	1917	1546	1809	1805	1783	1783	572	555	567	567	561	560	558	558
Mean	7.63	1.19	2.76	2.58	245.35	297.23	78.64	82.41	83.83	0.64	3.89	7.38	1.27	1.84
SD[2]	0.59	0.43	0.90	1.64	227.08	280.39	15.37	11.14	9.09	0.72	3.06	5.56	1.53	2.05
CV[2]	7.73	36.13	32.61	63.56	92.55	94.33	19.54	13.52	10.84	112.5	78.66	75.33	120.47	111.41
Ej1 – E2[3]	**	**	**	ns	**	ns	ns	ns	**	**	**	ns	*	**
	0.049	0.16	-0.38	-	-27.69	0.43	0.38	-0.34	-1.86	0.13	0.48	0.30	0.18	0.56
	(0.02)	(0.017)	(0.032)	0.055	(7.32)	(9.06)	(1.06)	(0.78)	(0.59)	(0.052)	(0.22)	(0.36)	(0.10)	(0.15)
				(0.06)										
$d_C – d_R$[4]	ns	ns	ns	ns	**	**	**	**	ns	ns	**	ns	*	ns
	0.025	-0.074	-0.008	-0.14	122.6	122.2	-11.22	-9.42	-1.28	-0.12	2.18	0.96	0.70	0.11
	(0.13)	(0.10)	(0.21)	(0.39)	(46.7)	(57.7)	(4.36)	(3.14)	(2.41)	(0.21)	(0.88)	(1.47)	(0.41)	(0.60)
$m_C – m_R$[4]	*	**	ns	*	**	*	ns	ns	ns	ns	ns	ns	ns	ns
	0.15	0.17	-0.22	-0.46	-99.60	-69.4	2.12	2.90	2.09	0.04	-0.65	-0.97	0.07	-0.48
	(0.09)	(0.07)	(0.14)	(0.25)	(30.6)	(37.7)	(2.81)	(2.03)	(1.56)	(0.14)	(0.58)	(0.95)	(0.27)	(0.39)
h_{IJ}[4]	**		*	ns	ns	ns	*	ns	ns	ns	ns	ns	**	ns
	-0.125	0.065	0.162	0.268	13.6	34.8	3.60	2.23	1.81	0.04	-0.21	0.39	-0.50	-0.20
	(0.06)	(0.047)	(0.098)	(0.179)	(21.7)	(26.9)	(2.07)	(1.49)	(1.15)	(0.10)	(0.42)	(0.70)	(0.20)	(0.29)

*, ** significant at the level of 10%, 5%.[1]V: ejaculate volume, Mm: mass motility, Mi: individual motility, Cn: concentration, TSE: total number of spermatozoa per ejaculated, Vi: percentage of sperm viability, NAR: percentage of sperm with acrosomal integrity, Nr: percentage of sperm normalcy, H: percentage of sperm morphological abnormalities of heat, Nm: percentage of sperm morphological abnormalities of neck-midpiece, T: percentage of sperm morphological abnormalities of tail, Dp: percentage of sperm with proximal cytoplasmic droplet, Dd: percentage of sperm with distal cytoplasmic droplet. [2]N: Number of data, SD: standard desviation, CV: coefficient of variation, [3]Ej:ejaculate order. [4]d_I: direct genetic effect of line I, m_I: maternal genetic effect of line I, h_{IJ}: heterotic effect between lines I and J.

maternal transmission of the mitochondria (cell organites involved in energy metabolism).
Opposite maternal effects for pH with respect to Cn and Mi, may be due to the antagonist relationship between pH and Mi and Cn found here (results not shown) and previously reported by BRUN *et al.* (2002). They can be explained by the metabolic activity of the spermatozoa, which releases lactic acid and consequently decreases pH. Differences for the other traits were in general small (always lower than 5%) and non-significant, with the exception of Dd and Nm, which present eminent but non-significant differences due to the lower amount of data.

Favourable and significant individual heterosis was observed in pH and Dp at the level of signification of 5% and for Mm and Vi at the level of 10%. Heterosis effect was high for Dp (35%) but low for Mm and Vi (6.1% and 4.7% respectively) and almost negligible for pH (1.6%). BRUN *et al.* (2002) also reported high variability in the estimates of heterosis effects in function of the seminal trait. Thus, they observed a 6.8% of heterosis in Mm, 4.1% in percentage of motile spermatozoa and high values of heterosis in Cn (37.5%), TSE (37.6%) and number of motile spermatozoa per ejaculate (42.3%). In swine, BUCHANAN (1987) in a review of the experiments aimed at comparing crossbred and purebred boars showed that crossbred boars exhibited large ejaculate volume (14%) and had better semen quality (percent motility and abnormal sperm) than purebred boars. In cattle, THRIFT and AARON in their review showed that crossbred bulls had fewer abnormal spermatozoa. One of the possible explanations for the great negative heterosis effect in proximal cytoplasmic droplet and the positive heterosis effect in mass motility and percent sperm viability could be a faster sexual maturation in crossbred males. The results reviewed by BUCHANAN (1987) and THRIFT and AARON (1987) generally supported this concept but It should be confirmed in rabbit.

The order of the ejaculate had a considerable influence (about 15%) in most of semen quality traits. The second ejaculate showed higher Mm and Cn, and fewer V, H, Nm, Dp and Dd than the first one. It seems that the second ejaculate present a better quality. The higher mass motility is in agreement with the results of BRUN *et al.* (2002) and THEAU-CLEMENT *et al.* (2003) and the higher Cn and lower V are in agreement with THEAU-CLEMENT *et al.* (2002) and BONANNO and COSTANZO (1987).
The effect of day of collection was significant for all traits (results not shown).

CONCLUSIONS

Eminent differences have been found in direct genetic effects for some semen production traits (concentration and total number of spermatozoa) and some semen quality traits (percent sperm viability, percent sperm with acrosomal integrity and percent sperm morphological abnormalities of neck-midpiece) between two sire lines highly selected for growth rate. Maternal genetic effects were also found significant for pH, ejaculate volume, individual motility, concentration and total number of spermatozoa per ejaculated. Only two of the studied variables showed a significant heterosis effect. This was high and favourable for proximal cytoplasmic droplet but negligible for pH. Therefore, the superiority of crossbred bucks has not been proved for the lines and traits

evaluated. Further research is needed in order to know performance of crossbred and purebred males for other traits or molecular markers that could be related with male fertility.

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