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ScienceDirect
Livestock Science 104 (2006) 233–243
LIVESTOCK SCIENCE
www.elsevier.com/locate/livsci

Reproductive performance of crossbred and purebred male rabbits

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Received 4 November 2005; received in revised form 29 March 2006; accepted 18 April 2006

Abstract

The effect of buck genetic type and crossbreeding parameters on fertility and prolificacy were estimated using two rabbit sire lines and their reciprocal crosses. The relationship between the reproductive performance of inseminated multiparous does and several semen quality traits was also investigated. The semen characteristics evaluated were: pH (pH), mass and individual motility (MM, IM), percentage of viable spermatozoa (Vi), spermatozoa with normal apical ridge (NAR), normal spermatozoa (NSP), spermatozoa with morphological abnormalities of head (HAP), neck-midpiece (NAP), and tail (TAP), spermatozoa with the presence of proximal (PD) and distal (DD) cytoplasmic droplets.

Fertility was analysed as a continuous trait (kindling rate) or as a binary trait (success or failure of kindling). In the first case, the analysis was performed using GLM procedures of SAS v.8 according to a model that included the fixed factors of buck genetic type, number of ejaculates per pool and week of insemination. In the second case, fertility was analysed using GENMOD procedures of SAS v.8 according to a mixed model including the same fixed factors as before plus the physiological status of the does and the permanent random effect of female. Number of kits born alive and number of stillborn were analysed with MIXED procedures of SAS v.8 with the same model used for the analysis of fertility as a binary trait. Estimates of the estimable functions of crossbreeding genetic parameters of the lines were obtained from the solutions of the corresponding models by generalized least squares using GLM, GENMOD and MIXED procedures. Crossbreeding parameters were estimated according to the model of Dickerson. A linear regression was used to determine the relationship between fertility and litter size and the semen characteristics evaluated.

Significant differences in fertility were observed among buck genetic types, which were favourable to type R. Differences between lines in maternal genetic effects were relevant and favourable to type R for fertility. Individual heterosis was important but unfavourable for fertility.

A slight correlation was obtained between all semen quality traits and fertility and prolificacy. Two multiple models were found for fertility, including NAP, IM, NSP, buck genetic type and Vi in one model or NAR in other model. Individual motility had an important positive effect, while NAP had a small negative effect. When MM, TAP and buck genetic type were included in a multiple model for the number of kits born alive, both MM and TAP had significant small effects.

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1871-1413/$ - see front matter
doi:10.1016/j.livsci.2006.04.006

Individual motility and DD appeared to be related to number of kits stillborn, but only DD had a significant although negligible effect.

Keywords: Crossbreeding parameters; Rabbit; Fertility; Prolificacy; Semen

1. Introduction

Male fertility and prolificacy are highly influenced by the amount of semen produced as well as by its quality. Semen production and quality is usually described by means of a wide range of traits (Ducrocq and Humblot, 1997): (i) qualitative characteristics of the ejaculate (presence or absence of gel, urine or calcium carbonate deposits), (ii) characteristics related to the biochemical composition of the ejaculate (enzyme content, fructose content, etc.), (iii) qualitative characteristics of the spermatozoa (quality of the acrosome, abnormal forms of head, tail or neck-midpiece, different parameters defining their movement, etc.) or, (iv) characteristics of the insemination dose (volume, concentration). The importance of these traits should be established through their relationship with the result of insemination (Braundmeier and Miller, 2001). However this relationship is still not well established, because the evaluation and recording of semen characteristics are not frequently performed, and also because the information available usually comes from experiments with different designs for environmental or genetic factors which can affect fertility and prolificacy and must be taken into account when drawing up reliable conclusions or comparing results.

Vicente et al. (2000) and Brun et al. (2002a) found differences between rabbit lines of selection with respect to fertility and prolificacy, when artificial insemination was applied either with or without restrictive dose concentration. Differences may exist between purebred and crossbred males with respect to reproductive traits; however, there is very little information available concerning the use of crossbred male rabbits. Brun et al. (2002a) reported that fertility and litter size were higher when matings involved both F1 crossbred males and females compared to mating involving purebred males and females, however this study did not separately distinguish between the effect of the crossbred male and crossbred female on those traits. However, Buchanan (1987), in swine, and Thrift and Aaron (1987), in cattle, reported that there were no convincing results regarding the benefits of using crossbred sires.

The efficiency of artificial insemination in animal breeding implies reducing the number of spermatozoa at insemination. An assessment of semen quality is therefore required. In rabbits, most of the studies concerning the relationship between semen quality and fertility have only considered sperm concentration or sperm motility as the main parameters that indirectly define semen quality (Farrell et al., 1993; Alvariño et al., 1996; Viudes-de-Castro and Vicente, 1997; Castellini and Lattaioli, 1999; Brun et al., 2002a; Hagen et al., 2002). However, some other traits or combinations of traits could better explain male fertility. Thus, Ombelet et al. (1995) concluded that most reports emphasize the importance of sperm morphology as a predictor of men's in vivo fertilizing potential; Alm et al. (2001), in dairy bulls, found a correlation between sperm viability and fertility; Gadea et al. (2004), in swine, reported that sperm motility and the presence of proximal cytoplasmic droplets significantly correlated with farrowing rate; and Lavara et al. (2005), in rabbits, observed correlations between kindling rate and percentage of total motile cells, linearity index and the percentage of abnormal sperm.

This study had two aims. The first was to estimate, under commercial conditions, the effect of buck genetic type on male fertility and prolificacy and also the crossbreeding parameters of these traits, by using two sire lines of rabbits and their reciprocal crosses. The second was to estimate the relationship between these traits and several sperm qualitative characteristics.

2. Material and methods

This study was performed from January to March 2003, at the experimental farm of the Institut de

Recerca i Tecnologia Agroalimentàries (Caldes de Montbui, Spain) and at a commercial farm close to this centre. Both farms have isolated roofs and walls, controlled lighting and ventilation, and cooling-systems to prevent high temperatures in summer.

2.1. Animals

Males belonged to 4 groups corresponding to two sire lines of rabbits (C and R) and their reciprocal crossbreds (C × R and R × C). Lines C (Gómez et al., 1999) and R (Estany et al., 1992) were selected for increased post weaning daily gain by individual selection since 1993 and 1980, respectively. Females were obtained from the cross between animals from line Prat (Gómez et al., 1996) and line V (Estany et al., 1989), both being selected on the basis of litter size at weaning. Crossbred females are usually employed in the terminal cross in the current scheme of rabbit production.

Twenty bucks per genetic type were used. After weaning, they were housed in individual cages with a photoperiod of 16-h light/day and at temperatures ranging from 10 to 14 °C. Animals were fed on commercial rabbit pellets ad libitum (15.5% crude protein, 2.3% fat, 17.2% fibber) until 60 days old. They were subsequently restricted to 180 g/day of another commercial diet (16% crude protein, 4.3% fat, 17% fibber). Fresh water was always available.

2.2. Semen collection

Bucks started the training period at 5 months. One ejaculate was collected per male per week, using an artificial vagina (containing water at 50 °C). At 6 months, two ejaculates per male per week were collected, with an interval of 30 min between collections. The ejaculates used in the study were collected when males were 8–10 months old, and for 3 months.

2.3. Semen evaluation

Semen production and quality were evaluated at the experimental farm. All ejaculates were stored in a water bath at 37 °C until evaluation but for no more than 15 min after collection. Ejaculates containing urine and calcium carbonate deposits were discarded, and gel plugs were removed. After that, individual motility (IM) of the ejaculate was measured in aliquots under a microscope with a phase-contrast optic (Nikon) at × 400 according to a subjective scale from 0 to 5 (Roca et al., 2000) where 0 meant 0–10% and 5 meant 90–100% of the motile spermatozoa showing progressive movement. Ejaculates with individual motility lower than 2.5 were discarded.

After this initial evaluation, ejaculates from two to four bucks of each genetic type were pooled to avoid the possible effect associated with using dominant males on the result of the inseminations (Vicente et al., 2004). On average, 5 ejaculates were pooled, with 4 being the minimum and 8 the maximum. The pH (pH) was determined in raw semen by using a 507 Crison pH-metre, mass motility (MM) was assessed according to a subjective scale ranging from 1 to 5, using aliquots (10 μl) of raw semen and a light microscope (Nikon) at × 100. Aliquots (10 μl) of raw semen were fixed using vital nigrosin–eosin staining (Bamba, 1988) to allow posterior measurements of sperm quality traits by examining 200 spermatozoa under a light microscope (Olympus CH-3) at × 1000: percentage of viable spermatozoa (Vi); percentage of spermatozoa with normal apical ridge (NAR); percentage of normal spermatozoa (spermatozoa without abnormalities) (NSP); percentage of spermatozoa with morphological abnormalities of head (HAP), neck-midpiece (NAP), and tail (TAP); percentage spermatozoa with the presence of proximal (PD) and distal (DD) cytoplasmic droplet. After that, pools of ejaculates were diluted (1:5) in a commercial saline extender for rabbit semen (KUBUS m.r.a S.A, Madrid, Spain) and individual motility (IM) was evaluated in the manner explained above. The sperm cell concentration (SCC) was measured in aliquots using fixed spermatozoa (2% glutaraldehyde) in a Thoma-Zeiss counting cell chamber (final dilution 1:50) and a light microscope (Olympus CH-2) at × 400 and adjusting to 30×10^6/ml.

2.4. Fertility after artificial insemination

After semen evaluation, pools were stored at 18 °C for a maximum of 24 h prior to artificial insemination at a commercial farm. A total of 595 inseminations were carried out on multiparous lactating and non-lactating does with semen from 112 pools

obtained from 599 ejaculates from 72 bucks. Does were treated with subcutaneous applications of 12–15 IU of eCG (Folligon®, Intervet, Holland) for oestrous induction 48 h before AI. The does were inseminated with 0.5 ml semen from pools with a concentration of 30×10^6 cells/ml. Ovulation of does was immediately induced after AI by an intramuscular injection of 0.8 mg Busereline acetate (Suprefact®, Hoechst-Roussel, Germany). The number of inseminations per group was: 160 C, 159 C × R, 161 R × C and 158 R.

2.5. Statistical analyses

Semen quality traits were subjected to an analysis of variance by using GLM procedures of SAS v.8 (SAS, 2001) according to a model which included the fixed factors of buck genetic type (4 levels; C, C × R, R × C, R), the number of ejaculates per pool (5 levels, 4 to 8), and week of insemination (10 levels), taking into account the small differences in environmental conditions and/or in sample preparation procedures that could exist from week to week.

Fertility was analysed as a continuous trait (kindling rate) or as a binary trait (success or failure of kindling). In the first case, the analysis was performed using GLM procedures of SAS v.8 (SAS, 2001) according to the same model assumed for semen quality traits. In the second case, logistic regression, with logit link function, was used to analyse fertility by using GENMOD procedures of SAS v.8 (SAS, 2001) according to a mixed model including the same fixed factors as before plus the physiological status of the multiparous does (2 levels; lactating, non-lactating) and the permanent random female effect. The random effect associated with the pool was always non-significant and was therefore not included in the analyses.

A mixed linear model was used to analyse the number of kits born alive and number of stillborn kits by using the MIXED procedure of SAS v.8 (SAS, 2001) according to the second model used to analyse fertility.

Estimates of the estimable functions of crossbreeding genetic parameters of the lines were obtained from the solutions to the corresponding models by generalized least squares using GLM, GENMOD and MIXED procedures. Crossbreeding parameters were estimated according to the Dickerson model following the procedure described by Baselga et al. (2003).

Linear regression analyses were used to study the relationships between semen quality traits and fertility and litter size by using REG procedure of SAS v.8 (SAS, 2001). First, a set of bivariate analyses was performed. Then multivariate analyses (stepwise) were carried out including variables whose bivariate test had a p-value lower than or equal to 0.25 (Hosmer and Lemeshow, 1989) and variables considered of biological importance by other authors (Gadea et al., 2004) which were weakly or non-correlated with other variables in the model, in accordance with previous results (García-Tomás et al., in press), to avoid problems due to co-linearity. The factor buck genetic type was included in all regression models for fertility and for number of kits born alive and stillborn.

3. Results

3.1. Effect of buck genetic type on semen characteristics, fertility and prolificacy

Table 1 shows LS means (standard error) for semen characteristics according to buck genetic type. Differences between purebred males were found for pH, NSP, NAP and PD. These differences were always favourable to R males, but were only relevant for NAP and PD (37% and 77% of the mean, respectively). There were also differences between both types of crossbred males: males from the genetic type C × R showed higher NSP (4%) and lower TAP (32%) than R × C males. The C males showed the highest PD and the R × C males showed the highest TAP.

Table 2 shows LS means (standard error) for fertility, number of kits born alive and number of stillborn kits according to the buck genetic type. When fertility was analysed as a continuous trait, differences between purebred males were important and favourable to R males (about 10% of the mean), and differences between crossbred males were relevant and favourable to C × R males (about 13%). R × C males had the worst fertility ratio; C and C × R males were in an intermediate position, while R males

Table 1
LS means (S.E.) according to the genetic type of bucks for semen 1 characteristics

	pH	MM[1]	IM[1]	Vi[1] (%)	NAR[1] (%)	NSP[1] (%)	HAP[1] (%)	NAP[1] (%)	TAP[1] (%)	PD[1] (%)	DD[1] (%)
Genetic type of bucks											
C	7.8[a] (0.06)	3.2 (0.1)	3.2 (0.07)	84.3[b] (1.3)	85.8[b] (1.2)	85.6[b] (1.22)	0.39 (0.1)	4.5[a] (0.5)	4.1[b] (0.6)	2.3[a] (0.2)	2.9[a] (0.3)
C × R	7.8[a] (0.07)	3.2 (0.1)	3.2 (0.07)	89.7[a] (1.4)	91.1[a] (1.3)	89.2[a] (1.2)	0.49 (0.1)	2.6[b] (0.5)	4.0[b] (0.6)	1.05[b,c] (0.3)	2.2[a,b] (0.4)
R × C	7.8[a] (0.07)	3.3 (0.1)	3.1 (0.07)	88.4[a] (1.4)	89.8[a] (1.3)	85.7[b] (1.2)	0.39 (0.1)	3.5[a,b] (0.5)	5.9[a] (0.6)	1.4[b] (0.3)	1.5[b] (0.4)
R	7.4[b] (0.07)	2.9 (0.1)	3.3 (0.07)	86.1[a,b] (1.3)	87.5[a,b] (1.2)	91.1[a] (1.1)	0.33 (0.1)	2.7[b] (0.5)	3.4[b] (0.6)	0.53[c] (0.2)	2.0[a,b] (0.3)
Week of insemination	ns	*	*	ns	ns	*	ns	*	*	*	ns

ns: non-significant.
[a, b, c] Different letters in the same column indicate significant differences among groups at the 5% level.
[1] MM: mass motility, IM: individual motility, Vi: percentage of viable spermatozoa, NAR: percentage of spermatozoa with normal apical ridge, NSP: percentage of normal spermatozoa, HAP: percentage of spermatozoa with morphological abnormalities of head, NAP: neck-midpiece, TAP: tail, PD: percentage of spermatozoa with presence of proximal cytoplasmic droplet, DD: distal cytoplasmic droplet.
* Significant at the 5% level.

performed the best. When fertility was considered as a binary trait, R × C males also presented the worst percentage for fertility, while there were no significant differences between the other male genetic types. Buck genetic type had no effect on the number of kits born alive or stillborn.

The week of insemination and the physiological status of the female did not have any effect on fertility or on the number of kits born alive or stillborn.

Table 3 shows estimates of estimable functions between direct genetic effects, maternal genetic effects and individual heterosis for fertility and prolificacy. No differences between lines were observed for direct genetic effects with respect to any trait. The only significant differences between lines for maternal genetic effects were found for fertility, defined as a continuous or discrete variable. These differences were relevant (18% of the mean) and favourable to line R. Individual heterosis was significant for fertility considered as a continuous trait and also significantly different from zero at the 10% level when it was considered to be a binary trait. It was relevant in magnitude (10%) but unfavourable to crossbred males. There was no significant heterosis effect on the number of kits stillborn, and although differences between crossbred and purebred males were important (about 20%), and favourable to crossbred males, the values were very small and the differences could also be considered irrelevant.

3.2. Relationship between semen characteristics and fertility and prolificacy traits

Tables 4, 5 and 6 show the results of bivariate and multivariate linear regression analysis for fertility and the number of kits born alive and stillborn on buck genetic type and semen quality traits. Bivariate and multivariate analyses showed small correlations between all semen quality traits and variables that characterize the reproductive performance of does (fertility and prolificacy): R^2 was always less than 0.15.

When fertility was analysed using bivariate regression model, NSP had a small and positive effect on fertility (0.94 ± 0.29), whereas NAP had a small and negative effect (-2.20 ± 0.69). For traits related to prolificacy, only DD had a significant but negligible effect (0.15 ± 0.07) on number of kits stillborn. In all

Table 2
Fertility and prolificacy according to the buck genetic types

	Fertility[1] (%)	Fertility[2] (0, 1)	Kits born alive	Kits stillborn
Buck genetic types				
C	74.8[b] (3.7)	1.2[b] (0.27)	9.2 (0.49)	1.06 (0.27)
C × R	78.7[b,c] (4.1)	1.5[b] (0.31)	9.9 (0.52)	0.78 (0.29)
R × C	65.5[a] (4.0)	0.65[a] (0.29)	10.0 (0.53)	0.86 (0.29)
R	85.3[c] (3.6)	1.93[b] (0.28)	9.9 (0.46)	1.00 (0.25)
Week of insemination	ns	ns	ns	ns
Physiological status		ns	ns	ns

ns: non-significant.
[a, b, c] Different letters in the same column indicate significant differences among groups at the 5% level.
[1] Fertility analysed as a continuous trait.
[2] Fertility analysed as a binary trait. Fertility (%) = $e^{\text{LS-means}}$ / $1+e^{\text{LS-means}}$.

cases, the proportion of the total variance explained by models was less than 0.01.

According to the results of the bivariate analyses and the previous estimates of correlations between semen quality traits, two multivariate regression models for fertility were considered, which included the buck genetic type and seven low or non-correlated variables out of the following ones: IM, Vi, NAR, NSP, NAP, TAP, DP and DD. Table 5 shows the results of the multivariate regression analyses (stepwise) of semen quality traits for fertility. Buck genetic type, IM, NSP, NAP and Vi or NAR appeared to be related to fertility. Individual motility was significant at the 10 % level and it had an important and positive effect (9.71 ± 4.53 in one model and 10.01 ± 4.54 in other model), while NAP appeared as a significant component with a small effect on fertility (-2.01 ± 1.02 in one model and -2.06 ± 1.06 in other model). The percentage of total variance explained by the models was very small ($R^2 = 0.15$).

Table 3
Estimates of the differences between direct genetic effects (d_C, d_R) and maternal genetic effects (m_C, m_R) of the lines C and R, and individual heterosis (h_{CR}) between them for fertility and prolificacy

	Fertility[a] (%)	Fertility[b] (0, 1)	Kits born alive	Kits stillborn
$d_C - d_R$	2.8 (7.5)	0.16 (0.51)	−0.8 (0.9)	−0.03 (0.50)
$m_C - m_R$	−13.2* (4.3)	−0.89* (0.30)	0.13 (0.52)	0.08 (0.28)
h_{CR}	−7.9* (3.9)	−0.46† (0.27)	0.4 (0.48)	−0.21 (0.26)

[a] Fertility analysed as a continuous trait.
[b] Fertility analysed as a binary trait. Fertility (%) = $e^{\text{LS-means}}$ / $1+e^{\text{LS-means}}$.
* Significant at the 5% level.
† Significant at the 10% level.

Also, according to the bivariate analysis, buck genetic type, MM, IM, NSP, NAP and TAP were chosen to construct a multivariate model for number of kits born alive, while buck genetic type, IM, PD and DD were selected for number of kits stillborn. Table 6 shows the results of the multivariate regression analyses for the number of kits born alive and the number stillborn. Of the selected variables, buck genetic type, MM and TAP were finally included in the multivariate model for the number of kits born alive, but only MM and TAP had significant effects (-0.98 ± 0.39 and 0.17 ± 0.07, respectively). Of the variables considered to construct the multivariate model for the number of kits stillborn, DD appeared as a significant component but with a negligible effect (0.17 ± 0.07).

4. Discussion

The values for fertility and prolificacy obtained in this study were similar to those reported in previous studies performed with other rabbit lines (Pizzi et al., 1996; Castellini and Lattaioli, 1999; Vicente et al., 2000; Lavara et al., 2005); only Brun et al. (2002a) reported smaller values for fertility.

Relevant differences between buck genetic types have been found for fertility. These differences could be explained by differences in maternal genetic effects and the existence of heterosis for this trait. In the present work, maternal genetic effects were high in magnitude and favourable to line R, whereas the effect of individual heterosis was negative and moderate. Thus, due to unfavourable maternal genetic effects, C

Table 4
Bivariate linear regression for kindling rate, kits born alive and kits stillborn on buck genetic type and different semen quality traits

	% Fertility				Kits born alive				Kits stillborn			
	β[a]	S.E.[b]	p-value	R^2	β[a]	S.E.[b]	p-value	R^2	β[a]	S.E.[b]	p-value	R^2
pH	5.16	7.22	0.48	0.006	−0.09	0.87	0.91	0.002	0.28	0.49	0.57	0.004
MM[c]	−1.05	3.35	0.75	0.001	−0.71	0.39	0.08	0.05	0.08	0.23	0.74	0.001
IM[c]	4.22	4.58	0.36	0.008	0.84	0.52	0.11	0.04	−0.50	0.33	0.13	0.02
Vi[c]	0.32	0.28	0.25	0.013	−0.009	0.03	0.79	0.02	−0.008	0.02	0.68	0.002
NAR[c]	0.42	0.29	0.16	0.02	−0.02	0.03	0.62	0.02	−0.009	0.02	0.67	0.002
NSP[c]	0.94	0.29	0.002	0.093	−0.06	0.03	0.07	0.05	−0.015	0.02	0.49	0.005
HAP[c]	0.36	3.77	0.92	0.002	0.37	0.43	0.39	0.03	−0.29	0.27	0.29	0.01
NAP[c]	−2.20	0.69	0.002	0.091	0.13	0.08	0.11	0.05	0.04	0.05	0.43	0.007
TAP[c]	−0.63	0.58	0.28	0.01	0.09	0.06	0.16	0.04	−0.009	0.04	0.84	0.0008
PD[c]	−1.72	1.51	0.26	0.013	−0.0007	0.17	0.99	0.02	0.20	0.11	0.07	0.03
DD[c]	−0.89	1.03	0.39	0.007	−0.11	0.12	0.37	0.03	0.15	0.07	0.04	0.04

[a] β: coefficients of regression.
[b] S.E.: standard error.
[c] MM: mass motility, IM: individual motility, Vi: percentage of viable spermatozoa, NAR: percentage of spermatozoa with normal apical ridge, NSP: percentage of normal spermatozoa, HAP: percentage of spermatozoa with morphological abnormalities of head, NAP: neck-midpiece, TAP: tail, PD: percentage of spermatozoa with presence of proximal cytoplasmic droplet, DD: distal cytoplasmic droplet.

and R × C bucks exhibited lower reproductive performance than C × R and R bucks, but only the fertility of R × C males was significantly different from that of C × R and R males due to the addition of the negative heterotic effect for this group. We cannot offer a hypothesis about the origin of the observed maternal genetic effects on fertility. García-Tomás et al. (in press-b), in individual ejaculates from the same genetic types, and Brun et al. (2002b), in other rabbit lines, found maternal genetic effects for IM that could explain the observed maternal genetic effect on fertility since this trait seems to be related to fertility, as will later be discussed. From our results, the use of a crossbred male instead of a purebred male does not seem to be particularly advisable. Brun et al. (2002a) reported differences in fertility between matings involved crossbred males and females and purebred males and females from lines selected for litter size. However, it was not possible to know from that study whether those differences were due to the crossbred

Table 5
Multivariate linear regression for kindling rate on buck genetic type and semen quality traits

	Multivariate linear models							
	Model 1				Model 2			
	β[a]	S.E.[b]	Partial R^2	p-value	β[a]	S.E.[b]	Partial R^2	p-value
Constant	36.48	53.9		0.50	39.39	56.5		0.48
NAP[c]	−2.01	1.02	0.083	0.004	−2.06	1.06	0.083	0.004
IM[c]	9.71	4.53	0.03	0.073	10.01	4.54	0.03	0.073
Vi[c]	−0.41	0.34	0.015	0.20				
NAR[c]					−0.43	0.37	0.15	0.20
NSP[c]	0.65	0.42	0.013	0.23	0.64	0.42	0.012	0.24
Buck genetic type	−1.85	1.47	0.014	0.21	−1.83	1.47	0.014	0.21
p-value	0.006				0.007			
R^2	0.15				0.15			

[a] β: coefficients of regression.
[b] S.E.: standard error.
[c] NAP: percentage of spermatozoa with morphological abnormalities of neck-midpiece, IM: individual motility, Vi: percentage of viable spermatozoa, NAR: percentage of spermatozoa with normal apical ridge, NSP: percentage of normal spermatozoa.

Table 6
Multivariate linear regression for number of kits born alive and kits stillborn on buck genetic type and semen quality traits

	Number of kits born alive			Number of kits stillborn		
	β[a]	S.E.[b]	p-value	β[a]	S.E.[b]	p-value
Constant	11.03	1.35	0.0001	2.53	1.1	0.02
MM[c]	−0.94	0.39	0.05			
IM[c]				−0.62	0.34	0.07
TAP[c]	0.17	0.07	0.03			
DD[c]				0.17	0.07	0.04
Buck genetic type	0.20	0.18	0.26			
p-value		0.03			0.02	
R^2		0.08			0.07	

[a] β: coefficients of regression.
[b] S.E.: standard error.
[c] MM: mass motility, IM: individual motility, TAP: percentage of spermatozoa with morphological abnormalities of tail, DD: percentage of spermatozoa with presence of distal cytoplasmic droplet.

male or to the crossbred female. There is no information available about individual heterosis and direct and maternal genetic effects for male fertility in rabbits. In other species, such as swine and cattle, controversial results have been published about the effect of purebred versus crossbred sires on fertility (see reviews Buchanan, 1987; Thrift and Aaron, 1987). It seems that crossbred males could be more advanced in sexual maturity. However, at the adult stage, differences with respect to purebred males tend to disappear (Wilson et al., 1977; Buchanan and Johnson, 1984). In our experiment, bucks could be considered as adult males and therefore differences in fertility, prolificacy or semen quality were not expected to be due to differences in maturity.

We carried out two different analyses for fertility, considering this variable either as a continuous or a discrete trait. When fertility was defined as a continuous trait, results had a more immediate interpretation but it was not possible to take into account the correlations between data from the same female and/or the effect of other factors related with the female, such as its physiological status, on the frequencies of the different qualitative traits. In the case of experiments, which are not completely equilibrated for these factors, certain results could be masked.

Differences in fertility between buck genetic types were in concordance with the observed differences in semen quality traits. In general, fertility and most semen quality traits were better in R males than in C males, and also were better in C × R males than in R × C males. When ejaculates from the same bucks were evaluated individually (García-Tomás et al., in press-b) similar behaviour of genetic types was obtained for semen quality traits; the use of pools of ejaculates instead of individual ejaculates, the preselection of ejaculates and the environmental factors could explain the differences observed in the results from the two works. Vicente et al. (2000), Theau-Clement et al. (2003) and Brun et al. (2002a, 2004) also observed differences in semen characteristics for males from different lines of selection and from crossbred and purebred males. The relative importance of semen quality traits must be established through their relationship with fertility after insemination or natural mating. It is difficult to draw any general conclusions from the different studies, mainly due to differences in the procedures used to evaluate semen quality and management and preparation conditions associated with the insemination doses.

In rabbits, several authors have studied the relationship between classical semen quality traits (volume, concentration and motility) and fertility (Pizzi et al., 1996; Viudes-de-Castro and Vicente, 1997; Castellini and Lattaioli, 1999; Brun et al., 2002a). Furthermore, variables related with sperm viability and sperm morphological abnormalities have never or hardly ever been considered. In the present study, semen was evaluated for these traits and tested for fertility and prolificacy under commercial conditions at a non-restrictive concentration (15 million spermatozoa per dose) and using ejaculates pre-selected by motility. Motility was not correlated or was only poorly correlated with all the semen quality traits recorded

here (García-Tomás et al., in press); therefore the pre-selection of ejaculates was expected to have no effect on the distribution of these variables. The use of an excessive number of sperm for AI could have a compensatory effect and mask the effect of some semen quality traits on fertility (Tardif et al., 1999).

Under our experimental conditions, only NSP and NAP had small and significant effects on fertility in a bivariate linear regression analysis, but only NAP appeared as a significant component in the multivariate linear regression models considered. The percentage of spermatozoa with morphological abnormalities of neck-midpiece is negatively and moderately correlated with the percentage of sperm normalcy: this relationship explains the positive effect of NSP on fertility and the negative effect of NAP. The effect of these variables on fertility is interesting from a practical point of view, since morphological abnormalities are easily detected under a light microscope at $\times 400$ and this equipment is usually available in artificial insemination centres. Moreover, it is important to bear in mind that NSP is a very repeatable trait (García-Tomás et al., in press) and therefore this trait could facilitate selection of sires for rabbit AI. In rabbits, Lavara et al. (2005) showed a negative correlation between the variable percentage of abnormal sperm and kindling rate. However, when this variable was included in a multiple regression model, it was found to be non-significant. In boars, an inverse relationship has been reported between the number of morphological abnormalities and fertility (Gadea, 2005).

Individual motility is considered a good indicator of the functionality and integrity of the membranes. In rabbits, Brun et al. (2002a) showed that mass motility, evaluated according to a subjective scale, had a positive influence on the kindling rate but Hagen et al. (2002) found that sperm velocity, measured in μm/s of a linear trajectory, was not significantly correlated with fertility. Farrell et al. (1993) and Lavara et al. (2005) studied the relationship between fertility and sperm motility parameters assessed using a computer-assisted sperm analysis (CASA). Farrell et al. (1993) found that the correlation between fertility and several motility parameters was $r=0.53$. Lavara et al. (2005) concluded that the velocity parameters average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s) and linearity index (LIN, %) had the highest impact in a multiple regression model for fertility, although these variables were statistically non-significant. The high correlation obtained between VAP and VCL (0.95) could lead to problems due to co-linearity and mask results. In swine, Tardif et al. (1999) identified sperm motility (the percentage of motile spermatozoa visually assessed by microscopy) as a useful indicator of sperm fertilizing capacity in vivo when suboptimal sperm numbers were used for insemination and Gadea et al. (2004) found that sperm motility was a major factor in fertility when commercial doses were used for insemination. However, in our experiment there was no clear relationship between individual motility and fertility. In the bivariate analysis, this trait was non-significant, and in the multivariate model it was important but significant only at 10% level. These results were probably due to the small range of variation for this trait after the pre-selection of the ejaculates.

Viability and NAR were non-significant components in the two multivariate models for fertility. The relationship between fertility and viability has been considered by several authors, although there was disagreement in the results. The acrosome integrity also has been judged to be an important factor in male fertility; in rabbits, Courtens et al. (1994) showed a negative and moderate correlation ($r=-0.55$) between fertility and percentage of abnormal acrosomes.

In our experiment, fertility did not vary according to the physiological status of the does at the time of insemination. Castellini and Lattaioli (1999) did not find any significant differences in fertility between receptive multiparous lactating and non-lactating does either, but Piles et al. (2005) reported a negative effect of lactation on fertility in C line and Fortun-Lamothe and Bolet (1995) and Brun et al. (2002a) also observed this effect in other populations.

In the present study, no significant differences were observed among buck genetic types in terms of the number of kits born alive and stillborn. These results do not agree with those presented by Vicente et al. (2000) and Brun et al. (2002a), but the properties of either the genetic lines or of the doses used for AI in our experiment may partly explain this lack of agreement.

Heterosis and differences between lines in direct and maternal genetic effects were found to be non-significant for prolificacy traits. Buchanan (1987) reported that experiments concerning the effect of purebred versus crossbred boars on litter size at birth

showed contradictory results but for litter size at weaning most research indicated a small advantage for purebred boars.

Although no bivariate model was significant for the number of kits born alive, MM and TAP were significantly correlated with this trait in a multivariate model. Similarly, Gadea et al. (2004) found no significant univariate model for piglets born alive but a significant multivariate model was constructed with two variables of motility (percentage of motile spermatozoa and forward progressive motility) and the variable folded tail. For the number of kits stillborn DD appeared as a significant but negligible component, in both the bivariate and multivariate models. Sperm parameters accounted for only a very small part of the percentage of variation in litter size.

Additional methods are needed to better predict the fertilizing capacity of spermatozoa, since the use of a single attribute does not seem to be sufficient (Colenbrander et al., 2003) and because the classical methods of semen evaluation are poor predictors of fertility outcome (Gadea et al., 2004). In field commercial conditions, immediate, simple and non-expensive assays are needed to evaluate the ejaculates and optimise the use of males in AI centres.

5. Conclusions

Crossbred sires tended to express a moderate advantage for various semen quality traits, but when semen from these bucks was used for AI under commercial conditions, a negative heterotic effect was observed on fertility. The differences in fertility found in the present work were mainly explained by differences in maternal genetic effects. Therefore, the use of crossbred males does not provide a major advantage with respect to the use of purebred males from sire lines.

The qualitative characteristics of the spermatozoa evaluated here were poor predictors of the fertility outcome and prolificacy.

Acknowledgements

This research was supported by INIA project SC 00-011 and UB project 56.5.48.000.00 689.05 (MC 066605). Mónica García received a fellowship from the Generalitat de Catalunya. The authors are grateful to Dr. Blasco and Dr. López-Bejar for comments and suggestions.

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