SEMEN PRODUCTION IN TWO RABBIT LINES DIVERGENTLY SELECTED FOR 63-D BODY WEIGHT

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ABSTRACT

Semen production was analysed in two rabbit lines (low, L and high, H) derived by 5 generations of divergent selection for 63-d body weight from a commercial heavy breed. A total of 31 bucks were collected during 18 weeks, once a week, by 2 solicitations at a 15 min interval (giving the 1st and 2nd rank ejaculate). Performances were recorded only one week out of two, resulting in 9 collection series. Sexual activity did not differ between lines. A total of 256 and 226 ejaculates were observed in the L and H lines, respectively. The percent of efficient ejaculates (= fit for insemination) was markedly higher in the L line (66.5% vs. 44.2% in the H line) due to lower potential elimination for presence of urine, or insufficient volume or insufficient mass motility. Considering only the main effect of the lines, the line influenced mass motility, the volume and the concentration of the ejaculates, as well as 4 parameters from a computer assisted sperm motility analysis (path velocity, VAP, track linearity, LIN, track speed, VCL and beat cross frequency, BCF). Mass motility and the volume of the ejaculates were higher in the L line while the semen concentration was higher in the H line. Overall, the total number of sperms and the number of motile sperms per ejaculate were similar in both lines. The interaction between line and rank of the ejaculate on concentration, percent of motile sperms per ejaculate and LIN was a very interesting feature of the results. For example, in the L line, both ejaculates had the same concentration, while in the H line, the 1st ejaculate was more concentrated than the second one. This pattern was maintained all along the experiment. The L line had higher values of VAP, LIN and VCL but lower value of BCF. The efficient number of sperms by ejaculate, a synthetic criteria taking into account the number of motile sperms per ejaculate and the ability of the ejaculate for insemination was significantly higher in the L line: 229 vs 170 $x10^6$ sperms per ejaculate in the H line.

Key words: rabbit, semen, growth, correlated response.

INTRODUCTION

Information on the genetic relationship between growth traits and reproductive performances in domestic animals is rather scarce. Female reproductive traits may be altered by selection on growth rate (JAAP and MUIR, 1968, DECUYPERE *et al*., 1993, in the

broiler chicken, ARCHER *et al*, 1998 in the beef cattle, for example). Might intensive selection on growth hamper male reproductive performance and particularly semen production? A divergent selection experiment on 63d- body weight in rabbit gave the opportunity to tackle this question. Indeed, this experiment resulted in two lines which differed considerably in growth rate and adult body weight. The objective of this paper is to compare these two lines on their ability for semen collection and on their semen characteristics.

MATERIAL AND METHODS

Animals, management and semen collection protocol

The bucks used belonged to two lines divergently selected on body weight at 63 days of age (H and L lines). The founder population of these lines was formed from a commercial heavy sire line (Grimaud Frères). The founders were introduced in 1996 on the INRA experimental farm (Langlade) after hysterectomy of females. Full selection process was described by LARZUL *et al* (2003). Rabbits were selected on their body weight at 63 days of age on their breeding value, estimated by BLUP applied on an animal model. The two divergent lines were settled for high and low body weight for 5 generations of selection on an intra-group basis in order to limit inbreeding.

At the age of 19 weeks, 18 bucks per line were selected after a 2 wks training period according to their ability to respond to semen collection. The bucks were then solicited for semen collection every week, with 2 solicitations at a 15-min interval, resulting in rank 1 and rank 2 ejaculates. The collection period lasted 18 weeks between October 2002 and March 2003. Semen was recorded only one week out of 2, resulting in 9 weeks of semen evaluation. The rabbits were housed under a continuous photoperiod of 16h light per 24 hours. They were fed 170 g/day a commercial diet containing 175 g/kg protein and 145 g/kg fibre.

Trait measured

Sexual activity was estimated by the time interval between the introduction of the teaser female into the bucks cage and ejaculation. The absence of ejaculation within 2 min was considered as a failing solicitation. Immediately after semen collection, pH, volume and mass motility were estimated according to BOUSSIT (1989). The presence of urine or blood in the ejaculate was also noted. An efficient semen collection was defined as a collection of semen fit for insemination, i.e. without urine or blood, with mass motility ≥ 5 and volume ≥ 0.4mL. The rate of efficient ejaculates was estimated. Sperm motility was analysed by a Computer Assisted Semen Analyses system (HTMA-IVOS, Hamilton-Thorne Research, USA) according to the set-up parameters described by THEAU-CLÉMENT *et al* (1996). The HTMA parameters analysed were: percent motile sperms (PMOT), path velocity (VAP, µm/s), amplitude of lateral head displacements (ALH, µm), linearity of the sperm tracks (LIN, %), track speed (VCL, µm/s) and beat cross frequency (BCF, Hz). Concentration was estimated using a Thoma-Zeiss cell counter. Some additional variables were calculated: TSE (total number of sperms by ejaculate, TSE=volume x concentration), MSE (number of motile sperms by ejaculate, MSE=TSE x

PMOT), ESE (efficient number of sperms by ejaculate, ESE=MSE for an efficient ejaculate or zero else).

Statistical analyses
Except ESE (analysed on all the observed ejaculates), semen characteristics were analysed on ejaculates without urine and blood, by analysis of variance using a mixed model, with the GLM procedure of SAS. The fixed effects were the line (L and H), the rank of the ejaculate (1 or 2), the collection batch (4 levels) and all 2 ways and the 3-way interactions between each other. The 4 semen collection batches were defined by regrouping the 9 initial weeks of semen evaluation as follow: [1,2], [3,4,5], [6,7], [8,9]. The individual buck was put as a random effect in the model.

RESULTS

Figure 1 illustrates the contrasting body weights of both lines along with their parallel evolution following the collection week number. The adult body weight was reached on the 6th batch in both lines, with average values at 4650 g and 5925 g in the line L and H respectively.

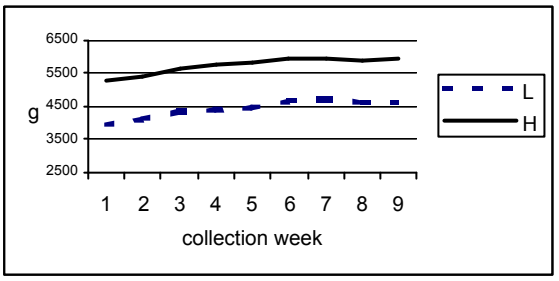

Figure 1. Body weight of the bucks following the collection week number.

Table 1 gives some production statistics, the components of the ability of semen for insemination and the semen characteristics of the ejaculates without urine and blood.

The response to solicitation and sexual activity was similar in both lines.

Ability for insemination of the observed ejaculates: the percent of efficient ejaculates was significantly higher in the L line, which showed a lower percentage of ejaculates with urine, with an eliminatory volume and mass motility.

Semen characteristics of the ejaculates without blood and urine:
Overall differences between lines (significant effect of the line) were observed for mass motility, volume and concentration of the ejaculates. Mass motility was higher in the L line. The L line had ejaculates with higher volume but lower concentration, so that their product, the total number of spermatozoa per ejaculate (TSE) was similar in both lines. However, the synthetic criteria ESE was higher in the L line, 229 $x10^6$ sperms per ejaculate vs.170 in the H line. Except PMOT and ALH, all sperm movement criteria showed significant differences between lines.

Table 1. Semen production traits in lines L and H

	Line L	Line H	Statistical significance
Sample size and response to solicitation			
N bucks studied	16	15	
N solicitations	284	260	
N observed ejaculates	256	226	
Response to solicitation (%)	90.1	86.9	ns
Sexual activity (time lag, s)	28.3	27.5	ns
Ability for insemination of the observed ejaculates			
With urine (%)	4.7	13.9	***
With blood (%)	1.6	0	ns
Eliminatory volume (%)	23.8	43.7	***
Eliminatory mass motility (%)	7.2	13.4	*
Efficient ejaculate (%)	66.5	44.2	***
ESE [A] ($x10^6$ spz)	229	170	*
Semen characteristics of ejaculates without blood and urine			
Mass motility	6.78±0.07	6.46±0.09	**
pH	6.94±0.02	6.93±0.02	ns
Volume ($x10^{-2}$mL)	60.1±1.6	46.2±2.1	***
Concentration ($x10^6$ spz mL^{-1})	634±20	738±27	**
TSE [A] ($x10^6$ spz)	368±16	336±21	ns
MSE [A] ($x\ 10^6$ spz)	302±15	290±19	ns
Motile sperms (PMOT, %)	76.3±0.7	75.8±0.9	ns
Path velocity (VAP, µm/s)	108.7±0.7	99.3±0.9	***
Lateral head mvt (ALH, µm)	7.00±0.04	6.91±0.05	ns
Track linearity (LIN, %)	41.4±0.4	40.1±0.5	*
Track speed (VCL, µm/s)	202.8±1.1	194.8±1.4	***
Beat frequency (BCF, Hz)	38.8±0.2	39.7±0.3	*

[A] ESE=efficient number of sperms per ejaculate, TSE=total number of sperms per ejaculate, MSE=number of motile sperms per ejaculate.

To better analyse the influence of the line on semen traits, we distinguished several cases according to the joint statistical significance of the line effect as main effect and of the 2- and 3- ways interactions involving the line and the batch (table 2).

Line effect without interaction with batch: this was the case for mass motility of sperms (higher in the L line) and for concentration. Concentration was higher in the H line, but this superiority was restrained to the 1st rank ejaculate.

Line by batch interaction, but no 3-way interaction: this means that the lines evolved differently with batch number, whatever the rank of the ejaculate. This was the case for PMOT and LIN (figure 2). In line L specifically, PMOT showed a significantly lower value

at the 1st batch, as compared to the other three batches, as if maturity was not yet reached in that line. LIN was plateauing in the L line but decreased with batch number in the H line. Moreover, both traits showed a significant interaction between line and ejaculate rank. In the L line, PMOT and LIN were higher in the 2nd rank ejaculate, but lower or similar in the H line.

Table 2. Statistical significance of the effects on semen traits

	N	R^2	Line (L)	Batch (B)	Rank ej.(R)	Inter LxB	Inter LxR	Inter BxR	Inter LxBxR
Mass motility	424	0.19	**	ns	ns	ns	ns	ns	ns
pH	410	0.40	ns	ns	***	ns	ns	ns	***
Volume	424	0.39	***	*	***	ns	ns	ns	***
Concentration	422	0.45	**	***	ns	ns	*	*	ns
TSE	422	0.31	ns	***	*	ns	ns	ns	**
MSE	366	0.36	ns	***	*	*	ns	ns	*
Motile spz %	368	0.52	ns	***	ns	***	*	ns	ns
VAP	368	0.66	***	***	***	**	ns	**	**
ALH	368	0.61	ns	***	***	ns	ns	ns	*
LIN	368	0.46	*	Ns	*	ns	**	ns	ns
VCL	368	0.69	***	***	***	***	ns	*	*
BCF	368	0.43	*	***	ns	ns	ns	ns	ns

See table 1 for the meaning of the abbreviations

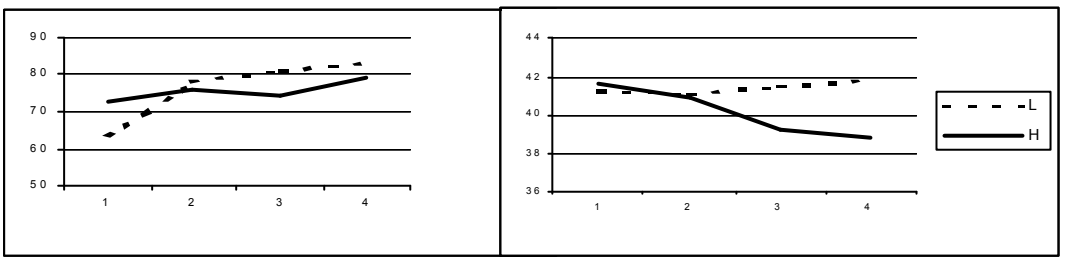

Figure 2. Evolution with batch number of the percent of motile sperms (PMOT, left side) and of the linearity of sperm tracks (LIN, right side).

For traits showing a significant 3-way interaction, the evolution with batch number depended both on the line and on the ejaculate rank. This was the case for pH, volume, TSE, MSE, VAP, ALH and VCL. As an example, figure 3 shows the situation for MSE. It illustrates that the H line had a very different evolution pattern for the 1st and the 2nd ejaculate whereas both ejaculates had similar evolutions in the L line.

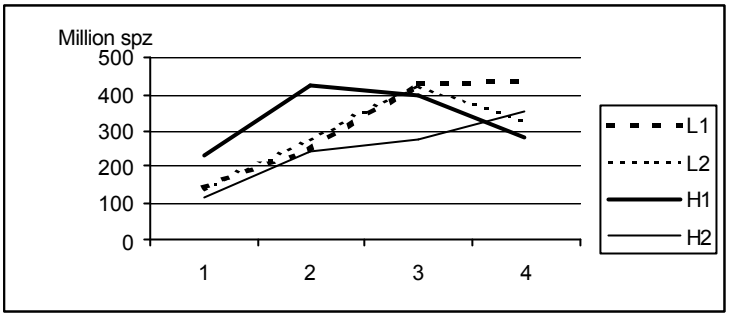

Figure 3. Evolution with batch number of the number of motile sperms per ejaculate (MSE) depending on line and rank of the ejaculate.

CONCLUSION

Semen production was evaluated in two lines divergently selected for 63-d body weight, with bucks differing by as much as 1.3 kg in the adult body weight. No difference in sexual activity was evidenced. However, relatively to the L line, upward selection resulted in a decrease of 26% of efficient sperms per ejaculate. Indeed, the percent of ejaculates fit for insemination was markedly lower in the H line, due to higher percentages of ejaculates with urine or with insufficient volume or insufficient mass motility. Analysed on the sample of ejaculates without urine and blood, semen volume and mass motility were higher in the L line, while concentration was higher in the H line, resulting in similar number of sperms per ejaculate. Concentration showed an interesting feature, with similar values for the 2 ejaculates collected at a 15 min interval in the L line, but n°2 lower than n°1 in the H line. Several motility parameters also showed line differences. At this stage, it is still difficult to conclude whether semen quality was hampered by intense selection on growth. The next step is to analyse the fertilising ability of the semen from both lines after insemination and try to relate the eventual differences to the semen differences observed in this experiment. This will enable to better assess the potential consequences in terms of semen quality of intense selection on growth rate.

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