

## Semen production in two rabbit lines divergently selected for 63-d body weight

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Received 22 November 2005; received in revised form 29 June 2006; accepted 8 July 2006

### Abstract

Thirty-one bucks from two lines divergently selected for 63-d body weight (low, L and high, H) were solicited every week (twice at a 15 min interval) during 18 weeks resulting in 482 ejaculates. While differing markedly on adult body weight (L: 4650 g versus H: 5925 g), both lines had the same testis weight. Libido did not differ between the lines. The proportion of ejaculates suitable for insemination was markedly higher in the L line (66.5% versus 44.2%). Mass motility and the volume of the ejaculates were higher in the L line while the sperm concentration was higher in the H line. Overall, the total number of spermatozoa per ejaculate was similar in both lines but the efficient number of spermatozoa per ejaculate, a synthetic criterion taking into account the ability of the ejaculate for insemination was higher in the L line (229 versus  $170 \times 10^6$ ). The L line had higher values of average path velocity, linearity and curvilinear velocity but a lower value of beat cross frequency. In the L line, both ejaculates had the same concentration, while in the H line, the first ejaculate was more concentrated than the second one. Some male reproductive traits are therefore genetically related to body weight.

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**Keywords:** Rabbit; Semen traits; Growth; Divergent selection; Correlated response

### 1. Introduction

Information on the genetic relationship between growth traits and reproductive performances in laboratory or domestic animals has concentrated on female fertility and prolificacy. In mice, a review of selection experiments [1] reported a decrease in female fertility, through increased pre-natal losses, in lines selected for increased body weight. In domestic animals, female reproductive traits may be hampered by selection on

growth rate (in the broiler chicken [2,3] and in beef cattle [4], for example). In the rabbit, the current results point to a low but positive genetic correlation between prolificacy and growth traits [5]. Little effort has been directed towards studying changes in male fertility concomitant with selection for increased body size. In mice, selection on growth traits has positive correlated responses on reproductive organ weights [6,7] but a negative response on the efficiency of sperm production [6]. In chickens, several experiments have reported negative genetic relationship between body weight and semen quality parameters [8–12]. In the rabbit, there is so far no information on the relationship between weight traits and male reproductive performances. A divergent selection experiment on 63-d body weight

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[13] 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 was to compare these two lines for some male reproductive traits: testis and epididymis weight, ability for semen collection, semen production and sperm motility characteristics.

## 2. Materials and methods

### 2.1. Animals, management and semen collection protocol

The bucks used belonged to two lines divergently selected on body weight at 63-d of age. The base population 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. The full selection process was described by Larzul et al. [13]. Rabbits were selected on their breeding value for body weight at 63-d of age, estimated by BLUP applied on an animal model. The two divergent lines were selected for high (H line) and low (L line) body weight for five generations of selection on an intra-group basis in order to limit inbreeding increase.

At the age of 19 weeks, 17 bucks per line were selected after a 2-week training period according to their ability to respond to semen collection. The bucks were then solicited for semen collection every week, with two solicitations at a 15 min-interval, resulting in ranks 1 and 2 ejaculates. The collection period lasted 18 weeks between October 2002 and March 2003. Semen analyses were performed only 1 week out of 2, resulting in 9 weeks of semen evaluation. The rabbits were housed under a continuous photoperiod of 16 h light and 8 h darkness. They were fed a commercial diet containing 175 g/kg protein and 145 g/kg fibre ad libitum. At the end of the experiment, the remaining bucks were slaughtered in order to weigh the reproductive organs.

### 2.2. Traits 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 (coded from 0 to 9) were estimated according to Brun [14]. The presence of urine or blood in the ejaculate was noted. An efficient semen collection was defined as a collection of semen suitable for insemination, i.e., without urine or blood, with mass motility  $\geq 5$  and

volume  $\geq 0.4$  mL. The rate of efficient ejaculates was estimated. Sperm cell motility was analysed from a 2 mL sample after dilution (1:40) in Galap (IMV France) by a computer-assisted sperm analysis system (HTMA-IVOS, Version 10, Hamilton-Thorne Research, USA) according to the set-up parameters of Table 1. This dilution rate is routinely used with the present CASA system in order to decrease the frequency of refusal of analysis due to too high concentration. This precaution makes it possible to limit the handling of the semen before the analysis. The minimum cell number counted was 50. For each ejaculate, two drops of 10  $\mu$ L each and three fields per drop were evaluated. The HTMA variables analysed [15] were the following: proportion motile spermatozoa (PMOT), proportion progressive spermatozoa (PPRO), average path velocity (VAP,  $\mu$ m/s), amplitude of lateral head displacement (ALH,  $\mu$ m), linearity (LIN, %), curvilinear velocity (VCL,  $\mu$ m/s) and beat cross frequency (BCF, Hz). Concentration was estimated from 20  $\mu$ L of pure semen diluted 1:200 in sodium citrate, using a Thoma-Zeiss cell counter. Some additional variables were calculated: TSE (total number of spermatozoa per ejaculate, TSE = volume  $\times$  concentration), MSE (number of motile spermatozoa per ejaculate, MSE = TSE  $\times$  PMOT), ESE (efficient number of spermatozoa per ejaculate, ESE = MSE for an efficient ejaculate or zero else).

After slaughter and dissection of the genital tract, the right and left testis and epididymis were weighed. The ratio of total testes weight to body weight at slaughter was calculated.

### 2.3. 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 linear 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 (four levels) and all of the two-way and three-way interactions between each other. The four semen collection

Table 1  
Set-up parameters of the CASA system

Frame acquired	20
Frame rate (Hz)	60
Minimum contrast (units of brightness)	40
Minimum cell size (pixels)	3
Threshold straightness (%)	80.0
Low VAP cut-off ( $\mu$ /s)	20
Medium VAP cut-off ( $\mu$ /s)	40
Non-motile head size	11
Non-motile intensity	115

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