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Effect of early development on semen parameters and lifespan of rabbit males selected by high growth rate

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Abstract

Life history theory suggests that different body development dynamics may influence survival and future reproductive performance of organisms. The present work studied how these dynamics could influence seminal traits and lifespan of rabbit males selected for growth rate and intended for AI. To achieve this goal, a total of 550 rabbit males were controlled from birth, evaluated both during the testing phase (four consecutive weeks after reaching 147 days of life) and the productive phase (377 of them from the end of the testing phase until 2 years of life). In order to obtain individuals with different body development dynamics, we pre-selected males based on their live weight (LW) at 0, 28, 63 and 147 days and on their average daily gain (ADG) between each period (0-28, 28-63 and 63-147 days). Libido and main seminal traits (semen volume, motility, concentration, and production, as well as normal apical ridge and abnormalities of spermatozoa) were controlled during the testing phase. Semen volume, motility and concentration were subsequently controlled during the productive phase, as well as the length of the male life, calculated as the number of days a rabbit was present at the farm between age 147 and day of death, culling or censoring; set to 2 years of life). The birth weight, the ADG between 0-28 days and between 28-63 days were positively related to some seminal parameters measured during the testing phase (semen volume, concentration, production and motility; $P<0.05$), while the ADG between 63-147 days was negatively related to the seminal productivity throughout the productive life of the males (an increment of 10 g per day on ADG reduced the number of profitable ejaculates by 4.9%; $P<0.05$). In addition, a higher growth between 0-28 and between 63-147 days increased the risk of death or culling of males during the productive phase ($P<0.05$). In conclusion, an adequate body development early in life seems to have a positive effect on the degree of sexual maturity with which male rabbits

begin their reproductive life, but reaching the reproduction onset with excessive weight can reduce their reproductive performance and lifespan.

Keywords: Oryctolagus cuniculus, body development, growth rate, reproduction, lifespan, semen quality.

1. Introduction

In recent decades, the use of artificial insemination (AI) in rabbits has promoted an organisational improvement in the management of commercial farms, as well as the appearance of specific centres for males destined for AI. These AI centres mainly breed rabbit males from genetic lines selected for growth rate at fattening, which are used as breeding males in the three-way crossing scheme. This scheme allows an effective dissemination of the genetic material to optimise the productivity of commercial farms.

Despite the impact of these males on farm profitability, the scientific information on the proper feeding and raising management of male rabbits is still scarce. Nowadays we have information on the influence of nutritional requirements (both in rearing [1,2] and productive periods [3]) and the physiological development of males [4,5] on their fertility. We also have some estimation of the genetic parameters for seminal traits [6,7], but we lack information on how management practices in the early developmental age may influence the future reproductive life.

Furthermore, rabbit males (especially those selected for growth rate) have some reproductive peculiarities which could be improved on (late reproductive onset, moderate both sexual libido and sperm production, ...), justifying a better understanding of the main factors that could affect their reproductive performance.

Some of the most important milestones concerning the reproductive physiological development of male rabbits happens early in life, especially during fattening and rearing periods [5]. In addition, the higher nutritional requirements of males selected for growth rate during early growth development, until 14th weeks of life, are difficult to cover compared to other animals [8,9]. In this sense, we hypothesise that the reproductive success of rabbit males (especially those from lines selected for growth

rate) are influenced by the different development states achieved at key phases of their developmental life (i.e. birth, weaning, and end of both juvenile and pubescent life).

If we look at other animal species, although selection for growth rate is positively correlated to the size of reproductive organs, this selection criterion is negatively correlated to seminal quality (in mice, Eisen and Johnson [10]; in pigs, Johnson et al. [11]; in bulls, Kealey et al. [12]). In the case of breeding rabbits, there are few studies that refer to the influence of body development of juveniles on the subsequent reproductive performance of individuals as adults. Poigner et al. [13] observed that heavier rabbit females at birth later showed larger litter size at their first parturition (+12.4%), while Rommers et al. [14] reported a better reproductive performance (first cycle) among heavier rabbit females at first mating, probably due to their greater degree of maturity when first mated at a fixed age (14.5 weeks old). Recently, Martínez-Paredes et al. [15] observed that fatter rabbit females at the beginning of their reproductive life had smaller litter sizes and higher risk of being culled compared to the lean ones. In rabbit males, Brun et al. [16], using two divergent lines of males selected for growth rate, observed that some reproductive traits were related to LW. Lavara et al. [7] observed the existence of negative genetic correlations between different seminal traits, such as normal apical ridge (NAR) and motility, and the ADG of males selected for growth rate. Altogether, these results indicate that an adequate body development of rabbit males through lactation and the fattening and rearing periods may affect their future reproductive performance.

For this reason, the present work aims to evaluate the effect that early development, from birth to the end of rearing period, could have on the seminal performance of rabbit males selected for growth rate addressed to AI, both in the training phase and throughout their reproductive life, as well as in their lifespan.

2. Material and methods

All experimental procedures were approved by the Animal Welfare Ethics Committee of the Universitat Politècnica de València (UPV), following the Spanish Royal Decree 1201/2005 on the protection and use of animals for scientific purposes.

2.1. Animals and housing

We checked the LW of 550 male rabbits from the R line of UPV (selected for growth rate from 28 to 63 days of age) at 0, 28, 63 and 147 of life. In the four weeks following the 147d of age, we evaluated the libido and some seminal parameters (once a week) on 550 males, pre-selected based on their LW and ADG variability at each weight control. After this testing phase, we selected 377 males to be followed up as potential reproduction males for 2 years of life.

To obtain the experimental males, 179 reproductive rabbit females from the R line, housed at a selection centre (El Adil Redondo S.L., Carrizo de la Ribera, León, Spain) were used. R females were artificially inseminated during five consecutive reproductive cycles, using the semen from 55 R males from a different genetic origin. All the rabbit females were inseminated at the same time (single batch), with a period between inseminations of 42 days. Inseminations were carried out between February and September.

2.2. Experimental procedure

Litters were sexed at birth to identify the males. A total of 1945 males were individually weighed at birth with a precision balance (±0.01 g). Litter size (total and alive) and the visual presence of milk in the stomach (milk spot) were also recorded. To identify the males born, a 2 × 12 mm glass chip (EI1001, Felixcan S.A., Spain) was injected (subcutaneously) in the back of the animals, between both scapulae. We used a needle disinfected in iodine and a syringe with a plunger. As most of the rabbit offspring had

nursed just after birth, visually checked by the presence of a milk spot, we corrected LW at birth from such animals by applying the following equation:

$$MS\ (g) = -9.497 + 0.239 \cdot OLWB + 0.303 \cdot LSB$$

The estimated milk spot (MS) weight was a function of the offspring's LW at birth (OLWB) with a milk spot and the litter size at birth (LSB). This equation was obtained in a short trial performed on 120 offspring of the same line R, weighing both the mother and their offspring before and after milking at day two after birth.

At 28 days old, the 1159 surviving male rabbits were weighed and re-identified with an ink tattoo on the left ear. Litters were weaned at 36 days, transferring the mother to an adjoining shed and leaving the weaned rabbits in the same cage in which they were born.

Subsequently, the 1025 surviving males were weighed at 63 days of age. At that time, a selection of the males that would be transferred to the AI centres was performed. Selection was made considering the individual LW recorded and the ADG calculated in the period 28-63 days. Males were chosen in each batch to obtain a population (604 males) that covered the maximum possible variability for the LW and ADG (Table 1) in the different growth periods until this moment (at birth, between 0-28 days and between 28-63 days of life; corresponding to the lactation and the fattening period, respectively). Once selected at 63 days of age, males were sent in an equitable manner to two different locations (maintaining the variability profile in both ones). Half of them were sent to AI centre A (Adil Redondo, Carrizo de la Ribera, León, Spain) and the other half to AI centre Z (Zapiños, Abegondo, A Coruña, Spain).

Males were reared until 147 days of age (from 63 to 147 days), and the 550 males that reached this age were weighed for the last time. During the next four weeks (testing phase), the main seminal traits were controlled to determine the potential of each male

to be used as semen producers at the AI centres. For each male, one ejaculate per week was collected with the help of an artificial vagina, and semen volume, motility, concentration and production were determined, as well as the NAR and abnormalities of spermatozoa.

From 175 days up to a maximum of 2 years of life, 377 males were selected from the initial 550 to be maintained at the AI centres. In this period, seminal traits such as volume, motility and concentration were individually recorded twice a week. Productive life represented the number of days a male was present at the AI centre from 175 days old to its death, culling or censoring (set to 2 years).

All the males were fed with the same feeding programme during the trial. During lactation, young males ate the same diets as their dams, with 16.8% crude protein (CP) and 14.1% crude fibre (CF) from 0 to 30 days of age, and with 15.0% CP and 18.4 CF from 30 to 36 days of age (pre-weaning). Once the males were weaned, the litter received a fattening diet with 15.8% CP and 14.7 CF until 63 days of age (including 60 ppm tiamulin fumarate, 450 ppm oxytetracycline and 158 ppm neomycin sulphate to avoid digestive disorders). From this moment until 147 days of age, the males were fed with a rearing diet (15.0% CP and 17.5 CF). Finally, during the productive phase, the diet offered had 16.5% CP and 13.0% CF.

2.3. Semen evaluation

2.3.1. Testing phase (from 147 to 175 days of life)

Collected ejaculates with urine or any irregular aspect were discarded, and the rest (successful attempts) were first observed to determine whether they contained gel (immediately discarded to avoid spermatozoa agglutination). Subsequently, the volume, concentration and sperm production were recorded according to the methods described by Lavara et al. [6]. In brief, they are based on the visual assessment of ejaculation in a

graduated tube followed by the sperm count in a counting chamber (Thoma-Zeiss cell) under a microscope (phase contrast, 40× increase). Visual sperm motility was evaluated under a microscope by a trained technician (percentage of spermatozoa showing motility), after dilution of the complete ejaculate to 1/5 in a tris-citric-acid-glucose buffer. Motility of the ejaculate was presented as a binomial variable: 0 for samples with percentage of motile spermatozoa below 65% or 1, for samples with a percentage of motile spermatozoa above 65%.

NAR and percentage of abnormal spermatozoa were assessed after staining with eosin-nigrosin mix. This procedure was similar to that described by García-Tomás et al. [17], in at least 100 spermatozoa. The evaluation of abnormal forms was assessed according to the criteria described by Barth and Oko [18], including the spermatozoa without flagella.

2.3.2. Production phase (from 175 days to 2 years of life)

First, the aspect of each extracted ejaculate was evaluated, discarding those with a yellowish colour and/or presence of urine, paste, gel, precipitates or blood. After that, the volume of the ejaculate was measured in a graduated tube (± 0.1 mL) and visual motility and concentration in a microscope after a 1:5 dilution with a tris-citric-glucose diluent (250 mM of tris-hydroxymethylaminomethane, 83 mM of citric acid, 50 mM of glucose; pH 6.8-7.0) were evaluated, both by the same trained technician in each AI centre. Total collection attempts in the productive life was defined as the number of times a male rabbit was taken to semen collection. Ejaculates were classified as profitable when they presented adequate traits for use as commercial semen (a normal aspect, an adequate visual motility above 65% and concentration). For each individual, the percentage of profitable ejaculates was calculated in relation to the total collection attempts.

2.4. Statistical analysis

2.4.1. Libido "mating attempts" of young male rabbits during testing phase according to the body development traits

To evaluate the libido of young male rabbits, we implemented a series of multinomial logistic regression models. Models contained two fixed effects, the batch (1 to 5) and the destination farm (A or Z) and a standardised co-variable related to the body development (LW at 0, 28, 63 and 147 days of life, and ADG for the periods 0-28, 28-63 and 63-147). We used the R-software (version 3.5.0) and the "multinom" function of the "nnet" package.

2.4.2. Seminal characteristics of young male rabbits during testing phase according to the body development traits

To evaluate the seminal characteristics (volume, concentration, production, motility, NAR, and spermatozoa morphological normality) of young male rabbits, we implemented a series of linear mixed models. All models included the AI centre (A or Z), the batch (1 to 5), and the week of testing (1 to 4) as a fixed effect, the body development traits (LW at 0, 28, 63 and 147 days old, and ADG for the periods 0-28, 28-63 and 63-147) as a covariate, and the permanent effect of each rabbit female (p) and the error term (e) as random effects. Random effects were assumed to have an average of zero and a variance of σ_p^2 for permanent, and σ_e^2 for the error term. We model the variance-covariance among animals by using a compound symmetric structure for the variance-covariance matrix of the residuals. The asymmetrical distribution of the original data led to the logarithmic transformation of data from concentration and production ($\log_{10} 10^6$ spermatozoa per mL or ejaculate, respectively). As motility during the testing phase was recorded as a binomial variable (0, motile sperm below 65%; 1 motile sperm above 65%), data were analysed under the GLIMMIX procedures of SAS

[19] for binomial distributions. Solution provided odds values about the increase of relative probability to be classified as 1 (motile sperm above 65%) per unit of proposed LW or ADG change. Finally, as NAR data presented a Poisson distribution, data were analysed with six GLIMMIX procedures for Poisson distributions. Finally, semen production and motility data during the testing phase were also analysed with the same procedures described above, including the effect of the growth variables as main effect (4 quartile classes) instead of as a covariate, for a better presentation of the obtained results.

2.4.3. Seminal parameters of male rabbits (as adults) during production phase according to the body development traits

For each of the variables average volume, average motility and profitable ejaculates (both number and percentage) controlled during the productive phase, the data were analysed with six GLM procedures of SAS [19]. Each of them included as fixed effects the location, the batch, and as a covariate one of the growth variables to be evaluated. In addition, profitable ejaculates data during the productive phase were also analysed with the same GLM procedures described above. However, for an easy interpretation of results, the body development variables were set as a fixed effect with four levels, representing the four quantiles (quantile limits are in Table 1).

2.4.4. Productive lifespan of rabbit males (as adults) during the productive phase according to the body development traits

Lifespan during the productive phase according to the body development traits (LW at 0, 28, 63 and 147 days old, and ADG for the periods 0-28, 28-63 and 63-147) was analysed using a series of Cox proportional hazard ratio models (one model for each developmental trait). We first tested for the influence of the batch (1 to 5) or AI centre the animal lived in (A or Z) on the productive lifespan. As neither of these variables

229 influence the productive life, they were not included in the models used to assess the
230 influence of the body development traits on the productive lifespan of the adult male
231 rabbits. As for the multinomial model and to avoid the scale effect, each development
232 trait was standardised before being included in the model. Hazard ratios, p-values and
233 the log likelihood ratio test for the model significance of each body developmental trait
234 are shown in Table 5. Kaplan-Meier estimations of survivorship (percentage of live
235 animals) according to the LW at 28 and at 147 days (the developmental traits explaining
236 the variability in the survival of male rabbits during their productive life) are in Figure
237 4.

3. Results

Means, standard deviations, coefficients of variation (CV), quartiles, minimum and maximum values of LW, ADG and main traits controlled during the testing and productive phases are listed in Table 1. Based on this natural variability, we could correctly select individuals with a different dynamic of body development to test the hypothesis that the early body development pattern influences lifespan and the seminal characteristics of male rabbits. Concerning the body development traits, the observed CV varied from 11 to 23%.

For seminal characteristics (production and quality), the CV ranged from 17 to 96% during the testing phase and from 10 to 74% in the productive phase. This variability allows us to correlate the possible differences in the seminal traits to the different growth patterns. The same is applicable for productive lifespan in the productive phase; the productive lifespan ranged from 7 to 550 days on production.

Libido “mating attempts” of young male rabbits during the testing phase according to the body development traits are shown in Table 2. Having a different LW or ADG, at all the controlled times, did not affect the number of successful AI attempts, evaluated as the relative risk of changing from zero to one, two, three or four successful attempts.

The relationships between body development traits and seminal parameters during the testing phase are shown in Table 3. LW at 0, 28 and 63 days old, as well as the ADG between the periods 0-28 and 28-63, influenced the semen characteristics. NAR was not influenced by any developmental traits.

In summary, the increment (per each 10 g) on the LW at 0 days resulted in a higher volume, concentration, production and spermatozoa motility (+0.02 mL, $+1.1\times10^6$ spermatozoa per mL, $+1.1\times10^6$ spermatozoa per ejaculate and an odds ratio of 1.13, the likelihood of observing ejaculates with an adequate motility (>65%) being 13% higher,

263 respectively; P<0.05). In fact, semen production linearly increased with LW at 0 days of
264 male rabbits (Figure 1a) and motility was significantly lower for the males weighing
265 less than 65 g at birth (Figure 2a).

266 An increase in ADG in the period 0-28 days of 10 g represented an improvement in
267 semen concentration, production and motility of $+1.3\times10^6$ spermatozoa per mL,
268 $+1.4\times10^6$ spermatozoa per ejaculate and a likelihood of +56% of having an adequate
269 spermatozoa motility (>65%), respectively; P<0.05. The same pattern was observed for
270 the ADG between 28-63 days and for the measures of LW at 28 and 63 days. In fact,
271 both semen production (Figure 1b) and motility (Figure 2b) linearly increased with the
272 ADG of males in the period 0-28 days; males showing an ADG between 0-28 days
273 below 21 g per day had a diminished semen production and motility in the testing
274 phase.

275 For the ADG in the period 28-63, the improvements on semen concentration,
276 production and motility per each 10 g of increment was $+1.2\times10^6$ spermatozoa per mL,
277 $+1.3\times10^6$ spermatozoa per ejaculate and a 38% greater likelihood of having adequate
278 motility, respectively; P<0.05). In this case, semen production (Figure 1c) and motility
279 (Figure 2c) seem to achieve the highest values for those males showing an ADG in the
280 period 28-63 greater than 63 g/d, although they have no significant differences
281 compared to some other ADG quartiles. At the end of the rearing period (147 day), an
282 increase in LW of 1000 g only improved the semen volume (+0.06 mL, P<0.05),
283 although this was not relevant in practice.

284 Concerning the influence of LW and ADG on the volume, motility and the number and
285 percentage of profitable ejaculates (Table 4), only the number and percentage of
286 profitable ejaculates were influenced. In this sense, an increment of LW at 147 days of 1
287 kg represented a reduction of 6 percentage points in the percentage of profitable

ejaculates (P<0.05), while animals having gained, on average, 10 g per day more in the period 63-147 had a lower number and percentage of profitable ejaculates (–10.1 and –4.9%, respectively; P<0.05) throughout their productive life. In fact, rabbit males that showed a growth rate above 29 g/d had the smallest number of profitable ejaculates during the productive phase (Figure 3).

Finally, Figures 4 and Table 5 show the influence of early development traits on the hazard ratio of death or culling during the productive phase. We observed that an increase in one standard deviation unit on LW at 28 or at 147 days, as well as for the ADG in the periods 0-28 and 63-147, all increased the risk of leaving the herd by +14.6, +17.5, +14.6 and +16.2 %, respectively (P<0.05). These values return a median survival life for theoretical males weighing 650, 750 and 850 g at 28 days old of 65, 56 and 51 weeks of age, respectively (Figure 4a), and for those weighing 4.6, 4.9 and 5.2 Kg at rearing end would be 60, 53, and 48 weeks of age, respectively (Figure 4b).

4. Discussion

For an adequate assessment of the possible effect of the different degrees of development of the males at different times in their early life, it was essential to build a population of rabbit males that had a wide variability in their growth traits (Table 1). CV values obtained in this work show a wide range for the different growth variables controlled (from 11 to 23%). In fact, the standard deviation observed with 550 males (6.9 g/d) for ADG during the fattening period was similar to that obtained for a population of 12,908 rabbit males of this same genetic line (6.9 g/d; [7]). In addition, mean values of growth traits were very close to the median values and there was, in general, a good equidistance between quartiles on both sides, denoting symmetry in data distribution. These results seem to confirm that the needed population structure was achieved. On the other hand, the average LW and ADG values obtained in this work were slightly higher than those reported in other studies with selected males from this same genetic line [1,7,20]. This fact could be related to different climatic and environmental conditions, as well as differences in the generation of selection in the animals in the different trials.

Regarding the semen traits, data variability was very high both in the testing and production phases (with CV values up to 96%). The same general conclusion was reached by Lavara et al. [7] for the semen traits in rabbit males of R line (with CV values up to 78%). These authors, using R rabbit males after the testing phase, presented similar values to those obtained in our work for NAR during the testing phase and semen motility during the production phase, but lower for the percentage of abnormal spermatozoa (17.0 vs. 34.5 %) and higher motility (65.0 vs. 42.0 %) than our males in the testing phase. Moreover, Pascual et al. [1] observed similar results on motility and NAR values, but a lower number of ejaculates and abnormal spermatozoa, as well as

higher semen volume, concentration and production during testing phase (–1.3, –27,8%, +0.31mL, +113 × 10^6 spermatozoa per mL and +144 ×10^6 spermatozoa per ejaculate, respectively). Differences between trials could be due to the different environmental conditions, maturity degree of the rabbit males used and the use of different assessment techniques in the case of motility (CASA vs. visual).

The testing phase is commonly used by AI centres to determine which rabbit males will be chosen for semen production. Libido is a key behavioural trait in the choice of rabbit males to be destined to AI. The results of the present work have showed that early growth traits did not significantly affect the number of realised attempts of males during the testing phase. However, higher ADG during the fattening period seems to be associated with increased probability of a higher number of attempts ($P<0.10$). Pascual et al. [1] observed that the R rabbit males that adequately cover their nutritional requirements during the different phases of early development, also showed a high number of ejaculates during their testing phase (+0.40 and +0.28 ejaculates for males reared on autumn and spring seasons, respectively). These results could indicate that a proper growth during the first steps of the life could contribute to achieve sooner an adequate maturity degree, with a better libido, at the beginning of the reproductive life, although further studies would be needed to confirm it.

Corroboration of existence of a relationship among body development traits and the seminal parameters, both in the short and long term, would allow to anticipate and improve the choice of the most adequate rabbit males to be destined to AI. To have a higher LW at birth had a slightly positive effect on some seminal parameters at the testing phase (volume, concentration, production and motility), especially for those weighing over 65 g. Fortun [21] observed that the development and multiplication of the primordial germ cells and of the primordial follicles already occurs during own

gestation. In rabbit females, Poigner et al. [13] observed that females born with a heavier weight produced more offspring as adults than lighter ones. However, the positive linear effect of a heavier weight at birth at early reproductive live of rabbit males seem to disappear after during the production period. Therefore, an adequate foetal nutrition and development could contribute to a better semen performance at early reproductive life, but without positive effects in long terms, so it does not seem to be one of the most key development traits in defining future male performance.

Growth during the first two months of life had a strong influence on some seminal traits (motility and semen concentration and production) during testing phase. Rabbit males with patterns of low growth (up to 21 and 53 g per day during lactation and fattening periods, respectively) showed clearly lower semen production with a worse motility at early reproductive life. These results may be due to the fact that important changes in physiological [22] and reproductive (seminiferous tubules; [5]) development take place during lactation and fattening, and lighter males probably did not reach the testing phase with a sufficiently mature reproductive status. Pascual and Pla [23], comparing R rabbits differing in 16 generations of selection for growth rate during fattening, observed that less selected animals (with lower growth rate both during lactation and fattening period) showed a lower physiological development at the end of the fattening period (–13% liver, –8% kidneys and –7% dissectible fat). In addition, rabbit males showing low growth until 63 days of life may be due to their requirements not being properly covered. Pascual et al. [1] observed worse semen traits during the testing phase in those rabbit males that did not correctly cover their nutritional needs from 9 to 14 weeks of life. However, the negative effects of lower growth in the first 63 days of age on semen performance disappeared in the production phase. These results seem to indicate that an early underdevelopment could be delaying the reproductive capacity of

young males, so management programmes (standardised litter size during lactation, specific diets during fattening…) that will ensure the necessary provision of nutrients to the males during the first two months of life would be advisable.

Both ADG during rearing period and LW at rearing end had no relevant effects on semen traits during the testing phase. However, greater growth during the rearing period and heavier weight at the beginning of their reproductive life had negative effects on the number and percentage of profitable ejaculates obtained throughout the productive life of the males. These negative linear effects associated with the rearing growth could be related to an inappropriate feeding adjustment of programmes during this period. During the rearing period, when using commercial diets provided ad libitum, rabbit males from parental lines tend to over-consume [1], which may lead them to a level of fatness higher than that desirable for the onset of the reproductive phase. Recently, Pascual et al. [24,2] found that when fitting the nutritional requirements and providing a constant daily supply of nutrients to rabbit males selected for growth rate, improvements in some sperm morphological characteristics and the fertility rate of their semen are achievable. In males, Du Plessis et al. [25] also observed that being overweight could sometimes be associated with an increase in abnormal spermatozoa and a high risk of fertility problems.

Finally, lifespan improvement of breeding rabbits should be one of the objectives of rabbit production, as it is usually associated with an improvement in both farm efficiency and the welfare and health of the animals. In the rabbit males used for AI, reaching a higher life expectancy is mainly due to their better adaptability to the farm environmental conditions and to their adequate semen production. In our work, higher growth rates during lactation and rearing periods were associated with a higher risk of death or culling during the production phase of rabbit males. A hypothesis for this

401 higher risk could be related to getting and sustaining an excessive level of fatness in
402 rabbit males during the production phase. During lactation, the total number and size of
403 adipocytes are defined, favoured by energy intake coming from fat [26]. Rabbit milk
404 has a high fat content compared to that of other mammals [27], and young rabbit males
405 showing higher ADG would consequently suckle higher amounts of fat from their
406 mother's milk. A higher number of adipocytes would help the excessive deposition of
407 fat when the nutrition requirements are not fitted in the long term. In fact, the energy
408 requirements for growth and maintenance during the rearing period are scarce, and
409 excessive energy intake could easily occur, which would increase the risk of rabbit
410 males reaching the beginning of their reproductive life with a level of body reserves
411 above that recommended at this age. In rabbits, Maertens [28] proposed fitting the
412 feeding level to daily requirements to reduce the troubles associated to fatness.
413 Recently, some works [29,15] have confirmed that non-adequate body condition around
414 the first mating had clear negative effects on the future reproduction performance and
415 lifespan of young rabbit females. Therefore, it seems that higher level of body reserves
416 at the onset of reproductive life could lead rabbit males to an increased risk of suffering
417 health troubles and/or reducing their libido or semen production or quality, which could
418 increase their risk of death and culling.

5. Conclusions

From these results, it could be concluded that early development of the rabbit males, selected by growth rate during fattening and intended for AI, influences their semen production and quality in the short and long term as well as their lifespan. A greater growth development of the rabbit males until the end of their fattening period seems to have a positive effect on the degree of sexual maturity with which they begin their reproductive life, improving aspects such as libido, spermatozoa motility and seminal production, but not in the long term. However, the rearing period seems to be key to achieving a long and productive reproductive life. Reaching the reproduction onset with an excessive weight seems to reduce the reproductive performance and lifespan of rabbit males. Therefore, fitting the feeding requirements during the final phase of growth, when the energy needs for growth are reduced, could be an adequate management recommendation for the young reproductive rabbit males from paternal lines.

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Table 1. Main descriptive values of population used in the experiment (550 males until testing phase and 377 males for production phase).

Variables	Mean	SD	Min	Lower Q	Median	Upper Q	Max	CV
LW and ADG (g)								
LW 0 days	65.0	11.1	34.3	57.8	64.9	72.1	102.0	17.15
LW 28 days	765	157	318	662	764	870	1240	20.53
LW 63 days	2785	319	1696	2586	2800	3012	3640	11.45
LW 147 days	4909	530	3095	4580	4905	5260	6495	10.79
ADG 0-28 days	25.01	5.43	9.75	21.45	25.00	28.62	41.16	21.72
ADG 28-63 days	57.69	6.92	30.51	53.09	58.17	62.91	73.89	11.99
ADG 63-147 days	25.28	5.74	8.60	21.64	25.15	28.76	42.85	22.69
Semen traits during testing phase[1]								
Ejaculates (n)	2.68	1.43	0.00	2.00	3.00	4.00	4.00	53.29
Average volume (mL)	0.60	0.28	0.00	0.40	0.58	0.75	1.77	46.49
Average motility (%)[1]	42.01	26.42	0.00	23.17	45.00	67.50	82.00	62.89
Average concentration (spz×10^6/mL)	123.8	100.9	0.0	50.9	102.0	165.8	817.5	81.51
Average production (spz×10^6/ejaculate)	79.4	76.2	0.0	22.2	57.5	110.7	530.4	96.04
Average NAR (%)	89.5	15.5	19.9	90.1	96.0	98.0	100.0	17.25
Average spz abnormalities (%)	34.50	20.20	1.56	17.80	30.01	48.14	90.10	58.56
Traits during production phase[2]								
Productive life (days)	351.2	198.2	7.0	155.0	395.0	546.0	550.0	56.42
Collection attempts (n)	88.1	53.1	2.0	38.0	88.0	144.0	182.0	60.25
Extracted ejaculate (n)	83.6	52.3	1.0	36.0	83.0	138.0	159.0	62.62
Average volume (mL)	0.87	0.20	0.00	0.76	0.86	1.00	1.38	22.60
Average motility (%)[3]	76.00	7.57	0.00	75.00	77.00	78.00	82.00	9.96
Profitable ejaculates (n)	67.8	50.1	0.0	22.0	58.0	113.0	155.0	73.92
Profitable ejaculates (%)	69.9	22.5	0.0	54.7	74.6	90.3	100.0	32.16

SD: standard deviation; Min: minimum value; Q: quartile; Max: maximum value; CV: coefficient of variation; LW: live weight; ADG: average daily gain; spz: spermatozoa; NAR: normal apical ridge; Profitable ejaculates: ejaculates of normal appearance with an adequate visual motility and concentration (percentage of the total collection attempts).

[1] Testing phase: from 147 to 175 days old; [2] Production phase: from 175 to 2 years old (maximum);

[3] Visual motilities done by the same technician at each centre.

Table 2. Relative risks ratio, of changing from zero mating attempts to one, two, three or four successful attempts, per each unit of increase in the standardised variables related to the early development.

Standardised variable[1]	Number of attempts made					P-values			
	Zero[2]	One	Two	Three	Four	One	Two	Three	Four
LW 0 days	1.00	0.852	0.979	0.840	0.927	0.431	0.905	0.221	0.584
LW 28 days	1.00	1.182	1.277	1.140	1.180	0.417	0.184	0.386	0.274
LW 63 days	1.00	1.184	1.444	1.266	1.195	0.417	0.052	0.119	0.232
LW 147 days	1.00	1.349	1.394	1.354	1.184	0.238	0.131	0.103	0.356
ADG 0-28 days	1.00	1.207	1.294	1.165	1.198	0.360	0.161	0.314	0.235
ADG 28-63 days	1.00	1.125	1.437	1.281	1.146	0.590	0.074	0.117	0.371
ADG 63-147 days	1.00	1.227	1.101	1.109	1.023	0.398	0.634	0.547	0.894

[1]LW = live weight and ADG = average daily gain. [2] Zero represents the reference level to which the pairwise comparisons were performed.

Table 3. Linear effect of early growth traits[1] on semen parameters during the testing phase (estimate ± standard error).

		LW at birth[2] (×10g)	LW at weaning (×100g)	LW at rearing onset (×1000g)	LW at rearing end (×1000g)	ADG lactation (×10g)	ADG fattening (×10g)	ADG rearing (×10g)
Volume (mL)		0.019 ± 0.009*	0.013 ± 0.007	0.069 ±0.036	0.057 ±0.024*	0.037 ±0.021	0.024 ±0.017	0.020 ±0.023
Concentration	($\log_{10}$ 10^6spz/mL)	0.038 ± 0.015*	0.043 ± 0.012***	0.210 ±0.061***	0.046 ±0.042	0.123 ±0.036***	0.071 ±0.030*	-0.048 ±0.039
	(10^6spz/mL)	1.091	1.104	1.622	1.112	1.327	1.178	0.895
Production	($\log_{10}$ 10^6spz/ejac)	0.051 ± 0.018**	0.055 ± 0.015***	0.286 ±0.072***	0.092 ±0.050	0.158 ±0.043***	0.102 ±0.036**	-0.038 ±0.046
	(10^6spz/ejac)	1.125	1.135	1.932	1.236	1.439	1.265	0.916
Motility	(ln odds)[3]	0.123 ± 0.059*	0.155 ± 0.049**	0.870 ±0.248***	0.238 ±0.157	0.447 ±0.142**	0.321 ±0.122**	0.000 ±0.146
	(odds)[3]	1.131	1.168	2.387	1.269	1.564	1.379	1.000
NAR (%)		0.000 ± 0.023	-0.001 ± 0.019	0.003 ±0.095	-0.003 ±0.063	-0.002 ±0.056	0.003 ±0.046	-0.006 ±0.058
Spz abnormalities (%)		-0.003 ± 0.004	-0.003 ± 0.004	-0.024 ±0.018	0.001 ±0.012	-0.007 ±0.011	-0.012 ±0.009	0.010 ±0.011

[1] The change in each growth trait needed to obtain the estimated change for the different semen parameter is shown in brackets.
[2] Standardised weight at birth (corrected by milk spot presence).
[3] Motility of the ejaculate presented as a binomial variable: 0, motile sperm below 65%; 1, motile sperm above 65%. Odds: increase of relative probability to be classified as 1 (motile sperm above 65%) per unit of proposed LW or ADG change (see [a]).
LW: live weight; ADG: average daily gain; NAR: normal apical ridge; spz: spermatozoa; ejac: ejaculate.
Weaning (28 days old); Rearing onset (63 days old); Rearing end (147 days old).
*P<0.05; ** P<0.01; *** P<0.001.

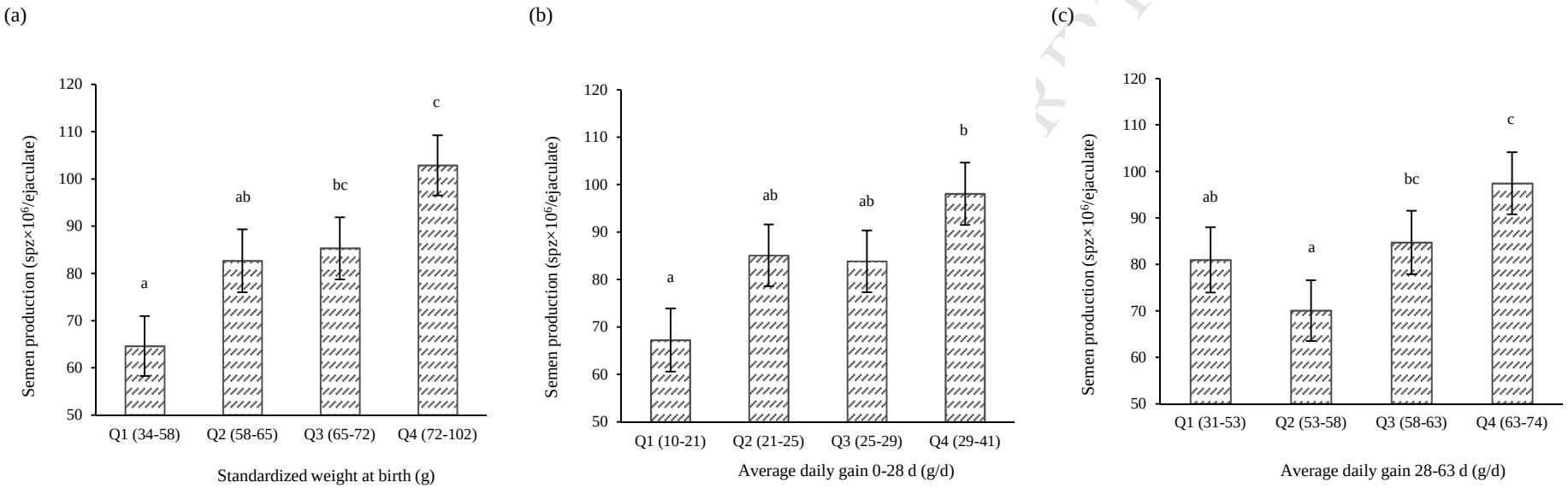

Figure 1. Average semen production (spermatozoon×10^6 per ejaculate) according to the classification by quartiles of the population for: (a) standardised weight at birth (corrected according to milk spot presence), (b) average daily gain from 0 to 28 days of age and (c) average daily gain from 28 to 63 days of age.

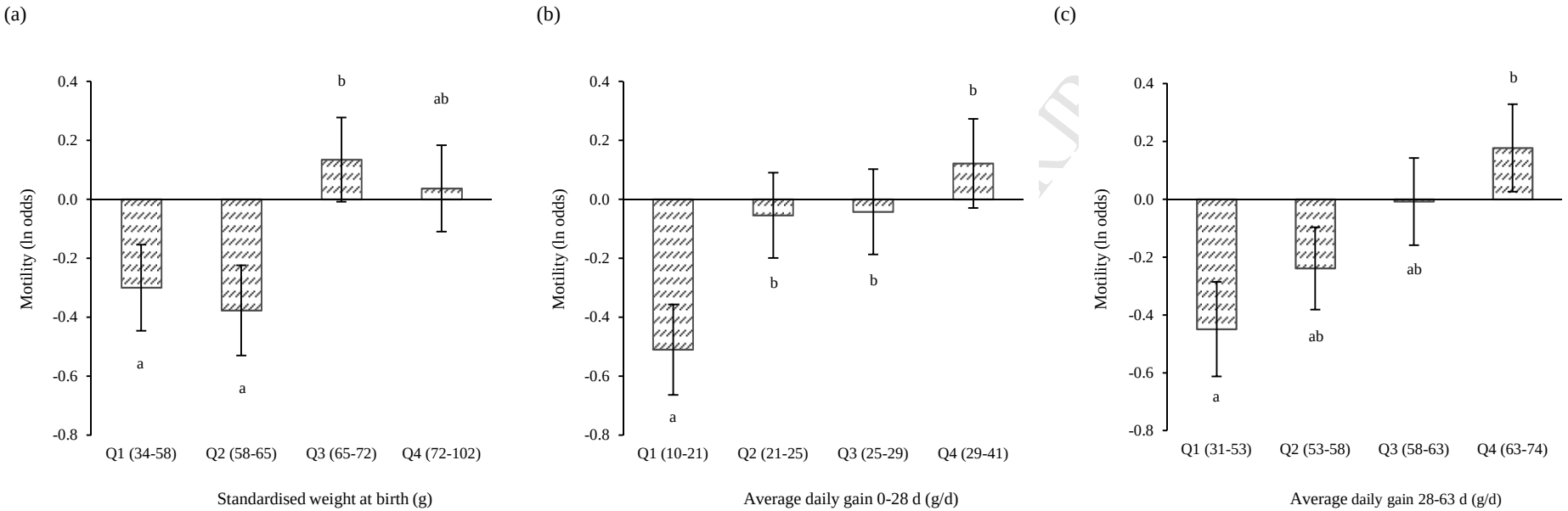

Figure 2. Average motility (ln odds) according to the classification by quartiles of the population for: (a) standardised weight at birth (corrected by milk spot presence), (b) average daily gain from 0 to 28 days of age and (c) average daily gain from 28 to 63 days of age. Motility of the ejaculate was presented as a binomial variable: 0, motile sperm below 65%; 1, motile sperm above 65%. Odds: increase of relative probability to be classified as 1 (motile sperm above 65%).

Table 4. Linear effect of early growth traits[1] on semen parameters during the productive phase (estimate ± standard error).

	LW at birth[2] (×10g)	LW at weaning (×100g)	LW at rearing onset (×1000g)	LW at rearing end (×1000g)	ADG lactation (×10g)	ADG fattening (×10g)	ADG rearing (×10g)
Average volume (mL)	0.000 ±0.007	0.000 ±0.005	-0.008 ±0.026	-0.030 ±0.018	0.000 ±0.015	-0.005 ±0.012	-0.028 ±0.016
Average motility (%)	0.029 ±0.102	0.101 ±0.077	0.492 ±0.370	0.119 ±0.243	0.298 ±0.226	0.168 ±0.177	-0.198 ±0.219
Profitable ejaculates (n)	-2.036 ±2.115	-2.314 ±1.590	-3.261 ±7.709	-10.945 ±5.219	-6.584 ±4.607	1.399 ±3.682	-10.057 ±4.699*
Profitable ejaculates (%)	-0.208 ±0.788	-0.804 ±0.592	-2.911 ±2.867	-5.829 ±1.898**	-2.374 ±1.715	-0.672 ±1.371	-4.856 ±1.713**

[1] The change in each growth trait needed to obtain the estimated change at the different semen parameter is shown in brackets.
[2] Standardised weight at birth (corrected by milk spot presence).
LW: live weight; Weaning (28 days old); Rearing onset (63 days old); Rearing end (147 days old).
Average volume: average value of ejaculates with normal aspect; Average motility: average value of ejaculates with normal aspect.
Profitable ejaculates: ejaculates of normal appearance with an adequate visual motility and concentration (percentage of the total number of attempts to extract semen.).
*P<0.05; ** P<0.01.

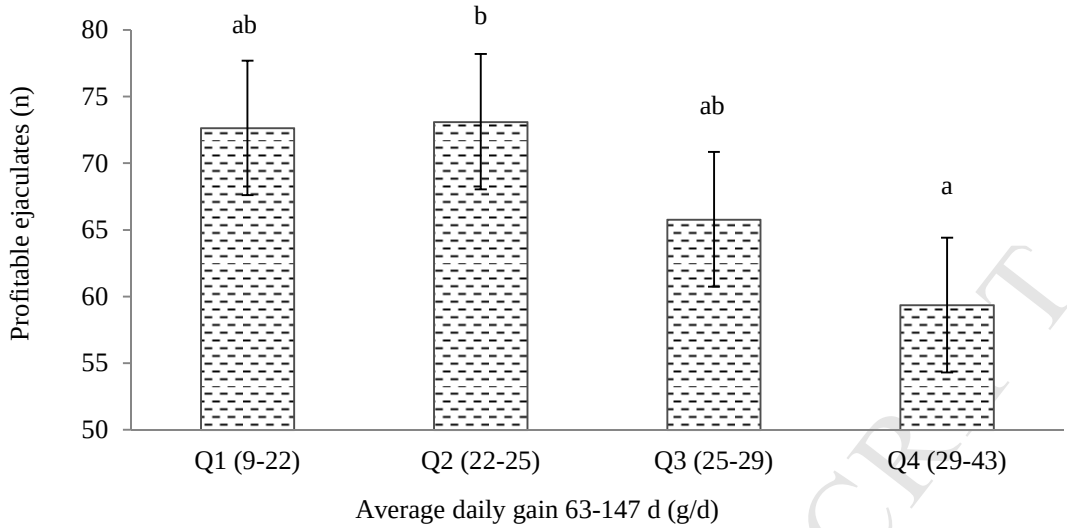

Figure 3. Number of profitable ejaculates (ejaculates of normal appearance with an adequate visual motility and concentration) according to the classification by quartiles of the average daily gain from 63 to 147 days of age.

Table 5. Influence of the early development variables (standardised variables) on the hazard ratio of death or culling of males during its productive life. Values above one represents an increment on the hazard ratio and therefore a reduction on the productive life.

Standardised variable[1]	Hazard ratio	95% Confidence interval	P-value	Likelihood ratio test[2]
LW 0 days	1.067	[0.93-1.22]	0.34	0.30
LW 28 days	1.146	[1.00-1.31]	0.04	0.04
LW 63 days	1.039	[0.91-1.19]	0.57	0.60
LW 147 days	1.175	[1.02-1.35]	0.03	0.03
ADG 0-28 days	1.146	[1.00-1.31]	0.04	0.04
ADG 28-63 days	0.965	[0.84-1.10]	0.59	0.60
ADG 63-147 days	1.162	[1.02-1.33]	0.03	0.03

[1]LW = live weight and ADG = average daily gain. [2] P-values of likelihood ratio test for the null hypothesis that all covariates of the implemented model are equal to zero.

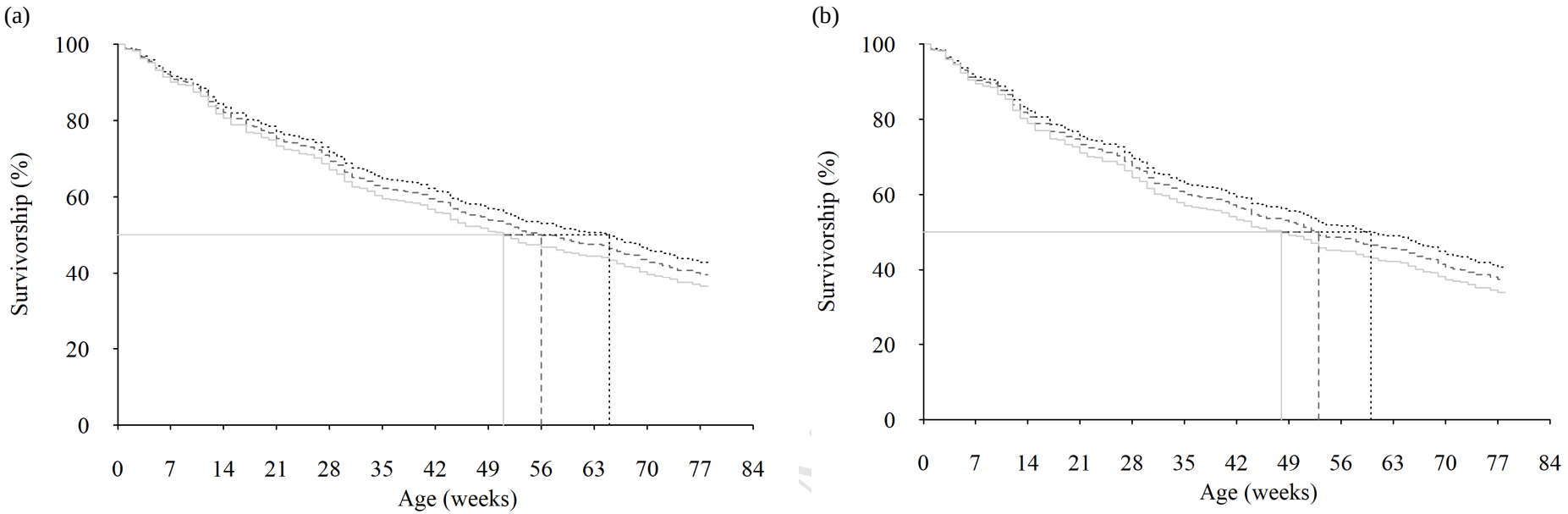

Figure 4. Survivorship estimation (percentage of live animals) across time (age in weeks) according to the live weight at different points of development: a) theoretical males with different live weight at weaning (light grey: 650 g; dark grey: 750 g; black: 850 g) and vertical segments represent the median survival time: 65, 56 and 51 weeks, respectively; b) theoretical males with different live weight at the end of the rearing period (light grey: 4600 g; dark grey: 4900 g; black: 5200 g) and vertical segments represent the median survival time: 60, 53 and 48 weeks, respectively.

Highlights

Body development of young rabbit males influences on their semen performance and lifespan.

Growth until 2-months age had only effects at the beginning of males' reproductive life.

Excessive growth at rearing have negative consequences on males' semen in long-term.

Excessive growth at lactation or rearing could have negative effects on males' lifespan.