World Rabbit Science

World Rabbit Sci. 2013, 21: 61-75
doi:10.4995/wrs.2013.1240
© WRSA, UPV, 2003

BREEDING PROGRAMMES TO IMPROVE MALE REPRODUCTIVE PERFORMANCE AND EFFICIENCY OF INSEMINATION DOSE PRODUCTION IN PATERNAL LINES: FEASIBILITY AND LIMITATIONS+

PILES M.*, TUSELL L.*, LAVARA R.†, BASELGA M.†

*Unitat de Cunicultura, IRTA, Torre Marimon s/n, 08140, Caldes de Montbui, Barcelona, Spain.

†Institute for Animal Science and Technology, Universitat Politècnica de València, Camino de Vera s/n , 46022, Valencia, Spain.

Abstract: This paper reviews the current genetic knowledge on issues related with the efficient use of bucks in artificial insemination (AI). Differences between lines have been found to be relevant in semen production and quality traits not necessarily related to their specialisation as maternal or paternal lines. Accurate heritability estimates indicate that genetic selection to increase semen production by improving male libido and/or reducing the number of rejected ejaculates may not be effective. However, total sperm produced per ejaculate appears to be an interesting trait to select for. Semen pH has shown low to medium heritability estimates and a low coefficient of variation, therefore it is not advisable to attempt improvement by direct selection. In general, sperm motility traits have shown low heritabilities, but the rate of motile sperms per ejaculate has been considered convenient to select for. Morphological characteristics of the spermatozoa have been revealed as medium to highly heritable. There is evidence of high genetic correlations between sperm traits before and after freezing-thawing. There are few studies regarding the estimation of heterosis of seminal traits, but results indicate important and favourable direct and maternal heterosis in crosses between maternal lines. However, this has not been confirmed in a cross between 2 paternal lines. Until now, attempts to find parametric or non-parametric functions to predict ejaculate fertility through seminal characteristics routinely recorded in evaluations have been very unsatisfactory. Hence, it may be necessary to find other semen quality markers or assess some of those currently used in a more precise manner, or closer to the AI time, in order to improve the ability to predict ejaculate fertility. Several seminal characteristics phenotypically correlated to male fertility could be considered as potential traits to select for in order to genetically improve this trait. However, only the semen pH has been checked for this purpose, and results show a low genetic correlation of this trait with male fertility. Other traits can be studied in the future, but bearing in mind that the required experiments will need a large number of bucks for an accurate estimation of the genetic correlation of the trait with male fertility. This means that these experiments will be expensive and difficult to set up. The most common criterion for selecting paternal lines, average daily gain, seems not to be genetically correlated to male fertility and seminal traits. Thus, selection for average daily gain has no detrimental consequences on these traits, and a multi-trait selection, including growth rate and seminal traits directly related to an efficient AI semen dose production, is feasible in paternal lines. The male contribution to fertility after natural mating and after AI with semen doses with high concentration is negligible, although it has been found that under more restrictive AI conditions male contributions to fertility and litter size are low, but higher in magnitude than those obtained after natural mating. The genetic correlation between the female and male contributions to fertility has been found to be moderate to high and positive.

Key Words: fertility, genetics, insemination, male, rabbits, semen traits.

INTRODUCTION

The use of artificial insemination (AI) in intensive meat rabbit production is currently common practice and traits related to its efficiency are gaining importance. The production of fertile doses is determined by several components: *i)* male

Correspondence: M. Piles, miriam.piles@irta.es Received *October 2012* - Accepted *March 2013.*
http://dx.doi.org/10.4995/wrs.2013.1240

+This review was presented as a main communication at the *10th World Rabbit Congress, Sharm El-Sheikh (Egypt), September 3rd-6th, 2012.*

libido and characteristics of the ejaculate which form part of the criterion for ejaculate rejection; *ii)* volume and sperm concentration of the ejaculate (determining the amount of doses that can be obtained); and *iii)* the quality of sperm (determining the minimum sperm dosage required to ensure fertilisation). In prolific species, male contributions to fertility and prolificacy can be considered as quality traits because they represent the final expression of the effects of seminal characteristics and the interaction among them and with the female. However, there is no review regarding the genetics of this subject. On the contrary, the genetics of rabbit doe reproduction traits as well as growth traits have often been reviewed (Rouvier, 1980; Matheron and Poujardieu, 1984; Rochambeau, 1989; Blasco, 1996; Baselga, 2004; Garreau *et al.*, 2004; Khalil and Al-Saef, 2008; Khalil and Bolet, 2010; Mocé and Santacreu, 2010).

With AI, the impact of reproductive performance (i.e. male contribution to fertility and prolificacy) of individual males is vital. Hence, dose production processes in AI centres aim to maximise the probability of fertilisation of the oocytes via management decisions on bucks, ejaculates and doses. As a consequence, fertility rate and litter size in commercial farms are usually high (ITAVI, 2008). However, efficient production of potentially fertile doses is suboptimal. First, there is a high pre-selection of ejaculates that are used for preparing the doses. The ejaculate rejection rate differs among AI centres but can be as high as 40% (Brun *et al.*, 2002a, b; Theau-Clément *et al.*, 2003; Brun *et al.*, 2006; García-Tomás *et al.*, 2006c). The criterion to determine the suitability of the ejaculate for AI is based on a subjective combination of several quality traits of the ejaculate and the sperm. However, the ability of these seminal characteristics to predict reproductive performance is very low, as will be discussed later. Thus, it is possible that some rejected ejaculates could be useful or indeed be even better for fertilisation than some of the accepted ones. Second, the type of doses and the storage conditions commonly used limit the production and the distribution area of the AI centres. For example, inseminations are performed at high sperm dosage to overcome the negative effects on fertility of semen with some bad characteristics (Saacke *et al.*, 2000). Additionally, only fresh or refrigerated semen is used in order to avoid the loss of potential fertility during the storage period. These practices reduce the output of AI centres, i.e. only 9 doses at a concentration of 40×10^6 spermatozoa/mL are obtained per ejaculate in the Caldes paternal line. Finally, in rabbits, AI pooling of ejaculates from several males (heterospermia) is a common practice to compensate for the negative effects of possible infertile ejaculates. The use of heterospermic doses prevents individual identification, leading to a reduction in the efficiency of selection for improving male performance. In order to improve the output of the AI centres, it is necessary to know the importance and the roles of the traits involved in fertile dose production and conservation. Knowledge of the different sources of variation that are affecting each of these traits would determine their possibilities and strategies of improvement.

Genetic studies of traits that are only expressed in active adult bucks involve special difficulties in achieving the size needed to obtain accurate estimates of genetic parameters, such as heritabilities or genetic correlations. Bucks commonly used in AI pertain to paternal lines that are selected for growth traits in the nucleus of selection. Only 20 to 50 bucks are active in these nucleuses, numbers that are insufficient for the requirements of genetic studies. To set up these experiments, it is usually necessary to collaborate with one or several AI centres to achieve the size required. Another additional requirement for genetic studies is to have the pedigree of the AI bucks connected to that of the animals from the nucleus of selection. In rabbits, many AI centres do not record the pedigrees of their bucks, whereas the participation of these centres specifically requires inclusion of bucks with known pedigree and traced back to the nucleus pedigree. It is obvious that these problems are much less important for the genetic studies of the female reproduction traits. This fact, and the different magnitude of the buck and doe contributions to fertility and prolificacy at kindling, explains why research has been more focused on female reproduction traits than on those of the male.

A further issue is the methodological complexity derived from the consideration of fertility and prolificacy as traits depending on the buck and doe, commonly analysed as doe traits. Their joint treatment makes it necessary to include both types of effects in the same model, increasing the number of genetic parameters to be estimated. Moreover, if fertility is treated as a threshold trait, determined multiplicatively by the contribution of both sexes, instead of additively, the peculiarities and difficulties of the models to be applied increase (David *et al.*, 2009). On the other hand, the consideration of the longitudinal nature of fertility, prolificacy and seminal characteristics implies that the genetic determinism of these traits could be different at different stages of animal development. Measurements of these traits can be appropriately modelled as a function of the parameters that define their trajectory through time. Knowledge of this function can help understand the behaviour of the trait and, moreover, individual differences in

these trajectory patterns could be exploited for genetic selection (Sorensen and Gianola, 2002), although it requires the use of complex models.

Genetic variation has been detected in all the components of fertile dose production at different levels: *i)* Variation among lines or breeds, *ii)* Variation within lines that eventually could be used for selection and, *iii)* Heterosis effects, which could lead to recommending the use of crossbred males. Thus, the purpose of this paper is to review the current genetic knowledge of the 3 sources of genetic variation, as well as the genetic relationships among traits. As the majority of the males used in AI came from paternal lines selected for growth, feed efficiency and, occasionally, carcass traits (Rochambeau *et al.*, 1989; Estany *et al.*, 1992; Larzul and Rochambeau, 2005; Nagy *et al.*, 2006; Khalil and Al-Saef, 2008), it would be of interest to know the expected correlated responses of the selection for growth traits on components of fertile dose production and the utility of introducing these traits in the selection objectives, either jointly with the growth traits or alone.

The paper is structured in 6 main sections: 1) Genetics of semen production and semen quality traits; 2) Prediction of male reproductive performance through ejaculate and semen traits; 3) Genetic relationship between seminal characteristics and male reproductive performance; 4) Male contribution to fertility and prolificacy after natural mating and after artificial insemination; 5) Genetic relationship between seminal traits and male reproductive performance with growth traits; and 6) Models and methods for genetic analysis. Finally, a section containing final remarks and conclusions is also presented.

GENETICS OF SEMEN PRODUCTION AND SEMEN QUALITY TRAITS

Genetic variation between lines

Vicente (2000) found lower sperm production, less motility and more acrosomal defects in a paternal line selected for growth than in 3 maternal ones. In the same study, fertility rate did not differ among lines but prolificacy did, probably due to the selection process of the maternal lines. Theau-Clément *et al.* (2003) compared sperm production and quality in 3 maternal lines of rabbits and found differences in collection rate, ejaculate volume, sperm concentration, pH and several motility traits. They also evidenced differences in the variability of semen characteristics both between and within bucks.

Brun *et al.* (2006) did not find differences in male libido between 2 lines divergently selected for body weight at 63 d, but reported that males from the lighter line had higher ejaculate volume, mass motility and number of ejaculates suitable for AI, but lower sperm concentration than males from the heavier line. In a posterior study, the same lines were compared in fertilising ability and no differences were found (Theau-Clément *et al.*, 2007).

In another study, Brun *et al.* (2002a) compared sperm production and quality in 2 maternal lines and their reciprocal crosses. They only found differences for ejaculate volume and percentage of motile spermatozoa, probably due to positive maternal effects for these traits. There was no significant difference between strains for mass motility, but one of the strains was superior in its maternal effect on this trait.

García-Tomás *et al.* (2006c) found differences in direct genetic effects for some seminal traits in 2 rabbit lines highly selected for growth rate: one of the lines seemed to present better seminal production traits (sperm concentration and total number of sperm in the ejaculate) and the other one had better seminal quality traits in general (lower presence of carbonate deposits in the ejaculate and better sperm morphological traits). In the same study, favourable maternal effects were reported in one of the lines for ejaculate and sperm quality and production traits. The maternal effects in the other line favoured only sperm volume.

Males from rabbit maternal and paternal lines could have different sexual development patterns according to differences found in their percentages of seminiferous tubules with presence of spermatozoa observed at different ages (García-Tomás *et al.*, 2009).

Summarising the previous results, it could be said that it relevant differences are frequently found between lines in semen production and quality traits that are not necessarily related to the specialisation of the lines as maternal or paternal lines.

Genetic variation within lines

In general, a wide range of heritability (h^2) and repeatability estimates for seminal traits can be found in the literature, ranging from extremely low to high values (García-Tomás *et al.*, 2006b). The variation in the magnitude of this parameter is due to several factors, such as: *i)* different genetic composition of buck populations among experiments; *ii)* variation in defining the trait, which in some cases consist of means of observations of 2 consecutive ejaculates or means of several records per male, whereas in other cases it corresponds to individual ejaculates (Ducrocq and Humblot, 1995; Wolft, 2009); and *iii)* the possible effect of collection frequency on the individual variation of seminal traits.

On the other hand, the h^2 estimates are imprecise in most of the reviewed studies. This is partly due to analysing small experimental data sets. Moreover, a large amount of environmental variation is originated during semen manipulation and the time to evaluation. The subjective manner in which some of the seminal traits are assessed is also important in explaining this wide variability of results.

Regarding male libido, Panella *et al.* (1994) reported a h^2 of 0.30 when this trait was analysed as classified in 3 categories (no collection, collection after 5 min and intermediate collection) and obtained from data on 158 bucks from a New Zealand White strain selected to improve semen quality and quantity. However, in that work all the genetic parameter estimates for seminal traits were unusually high, probably because no permanent effects other than the additive value were included in the model. Khalil *et al.* (2007) defined male libido in 5 classes (from 1 for low libido up to 5 for strong libido) and obtained an estimated h^2 of 0.17 from records of 642 bucks obtained during the process of establishing 2 new synthetic lines from 2 existing ones. Thus, 11 different genetic types of bucks were jointly analysed, which could be responsible for a higher estimate than that expected if only one genetic type is used. However, Tusell *et al.* (2011c) found that male libido recorded as a binary trait (if male successfully or unsuccessfully mounted an artificial vagina) was lowly heritable and repeatable (h^2=0.06; repeatability=0.10; 883 bucks) in a line selected for post-weaning growth rate (Caldes line).

The presence of urine, calcium carbonate deposits and gel plugs are considered major criteria for ejaculate rejection in AI centres (Brun *et al.*, 2002a; Theau-Clément *et al.*, 2003; García-Tomás *et al.*, 2006c). However, they were found to be lowly heritable (Tusell *et al.*, 2011c), which could be attributed in part to the great variability inherent in these traits due to factors involved in semen collection, such as variation in the temperature of the artificial vagina, which could lead to a higher presence of urine and calcium carbonate deposits in the ejaculate or unsuccessful mountings (Morrell, 1995).

The suitability for AI of the ejaculate, which involves the subjective combination of several qualitative traits, was also lowly heritable (h^2=0.06). Therefore, genetic selection to increase semen production by improving male libido and/or reducing the number of rejected ejaculates may not be effective. Moreover, the magnitude of the male repeatability (r) for these traits indicates a certain stability of their values over collections from the same male, but it is not high enough to make decisions concerning buck replacement at the beginning of the production period of the male (Tusell *et al.*, 2011c).

The estimated h^2 for ejaculate volume and sperm concentration ranged from 0.05 to 0.13 and from 0.08 to 0.10, respectively, for single ejaculates (Brun *et al.*, 2009, 172 bucks of the INRA1001 line; Lavara *et al.*, 2011, 412 bucks of a paternal line, the R line), whereas they were estimated to be 0.23 and 0.27, respectively, for the pool of 2 consecutive ejaculates (Tusell *et al.*, 2011d). Moderate values of r were found for these traits in different studies indicating the existence of important individual variation. Thus, More O'Ferrall and Meacham (1968) obtained a moderate value of repeatability (0.29) for ejaculate volume in a New Zealand population of bucks; Bencheikh (1995) estimated a repeatability around 0.38 for volume and 0.35 for concentration; García-Tomás *et al.* (2006b) obtained similar values in a heterogeneous population consisting of purebred and crossbred bucks obtained from 2 paternal lines (0.38±0.03 and 0.39±0.03, respectively), whereas Tusell *et al.* (2011c) obtained slightly higher estimates: 0.48 for sperm concentration and 0.46 for ejaculate volume. The value of these parameters could be affected by the collection frequency (Bencheikh, 1995).

In rabbits, there are only 2 reported estimates of the genetic correlation between sperm concentration and ejaculate volume. Brun *et al.* (2009) reported an imprecise estimate which cannot be considered to be different

from zero (0.38±0.45), whereas Tusell *et al.* (2011c) reported a moderate and negative estimate (posterior mean (PM): –0.53; highest posterior density interval at 95% (HPD95%): –0.76, –0.27). Having an accurate estimate of this parameter is important because both traits determine the total amount of sperm produced per ejaculate, which is one of the traits involved in efficient production of AI doses. Tusell *et al.* (2011d) obtained a moderate h^2 (PM [HPD95%]: 0.23 [0.14, 0.31]) and a moderate to high r (0.42 [0.35, 0.49]) for total number of sperm in a pool of 2 consecutive ejaculates, both values being higher than the corresponding values obtained by Brun *et al.* (2009) and Lavara *et al.* (2011) for individual ejaculates. The r for sperm production was estimated to be 0.33 in previous research by García-Tomás *et al.* (2006b) with purebred and crossbred bucks. In summary, the h^2 and r of total sperms produced per ejaculate are high enough to consider this trait for selection.

The h^2 for semen pH has been reported to be low. Brun *et al.* (2009) obtained a value for this parameter of 0.06 in a paternal line of rabbits (INRA1011), Khalil *et al.* (2007) reported a value of 0.12 in a set of different genetic types of bucks, and Tusell *et al.* (2011b and 2011c) reported values of 0.18 and 0.11 in 2 subsets of data from the same paternal line (for the pH of individual ejaculates and the pH corresponding to the pooled semen obtained from each male on the day of collection, respectively). There is a wide range of published r estimates for this trait. Bencheikh (1995) compared seminal characteristics in males under different collection frequencies and obtained estimates that ranged from 0.07 to 0.24, whereas Brun *et al.* (2009) obtained a value for this parameter of 0.17 in purebred bucks, García-Tomás *et al.* (2006b) reported an r of 0.38 in a population of purebred and crossbred bucks, and Tusell *et al.* (2011c) an r of 0.33 in a paternal line, all of them under a extensive collection frequency. At first, the low to medium h^2 of semen pH and its low coefficient of variation (approx. 6%) does not advise its direct consideration for selection.

Regarding h^2 values for sperm motility traits, the estimates depend on the type of motility analysed (mass or individual sperm motility) and the evaluation procedure employed (a subjective or objective procedure with adequate computer and software). The h^2 of mass motility was estimated to be 0.05 (Brun *et al.*, 2009) and the r ranged from 0.24 to 0.37 in data sets of only purebred bucks or mixed crossbreed and purebred (García-Tomás *et al.*, 2006b; Brun *et al.*, 2009). Estimates of heritability and repeatability for individual sperm motility, evaluated subjectively, were similar to those obtained for mass motility (0.08 and 0.24 for h^2 and repeatability, respectively; Tusell *et al.*, 2011c). In the last decade, computer assisted sperm analysis (CASA) systems have been used to improve the accuracy of sperm motility records in domestic animals. In rabbits, h^2 and r of individual sperm motility and of some sperm movement characteristics estimated with CASA systems are available in the literature (Brun *et al.*, 2009; Lavara *et al.*, 2012a, 412 bucks of the R line). Estimates of r for mass and individual motility were similar to the corresponding estimates obtained for the same traits but recorded subjectively (0.28 and 0.27; Lavara *et al.*, 2012a). However, these traits seem to be slightly more heritable than the subjective motility traits (0.12; Lavara *et al.*, 2012a). Most of the sperm movement traits have shown lower h^2 and r estimates (Brun *et al.*, 2009; Lavara *et al.*, 2012a) than those for individual motility; only average path velocity had higher h^2 and repeatability than motility (0.30; Lavara *et al.*, 2012a). In general, the sperm motility and movement traits have shown low h^2, but the rate of motile sperms per ejaculate has been considered as convenient for selection (Brun *et al.*, 2009; h^2=0.18).

There are many factors that have an impact on fertility and prolificacy of rabbit does after AI, such as sperm abnormalities and acrosome status (Lavara *et al.*, 2005; Piles *et al.*, 2013a). To our knowledge, only Lavara *et al.* (2012a) have reported h^2 estimates for these traits. Previously, other authors had estimated the r of acrosome status (0.40 and 0.33), indicating that an important part of its phenotypic variance was due to male-related sources of variation (Bencheikh 1995; García-Tomás *et al.*, 2006b). Recent studies in several domestic animals have focused on the relationship between sperm morphology traits (length, width, area and perimeter of sperm head) and either the success of the freezing-thawing process or the results of AI (Hidalgo *et al.*, 2007; Al-Makhzoomi *et al.*, 2008; Marco-Jiménez *et al.*, 2010). Only 2 studies, until now, have actually examined quantitative variation in the morphology of spermatozoa in rabbits. The available data suggests that h^2 and r of sperm morphology traits are medium to high and depend on the estimation method employed (h^2: 0.71-0.74, father-son regression, 47 sire bucks and 127 progeny bucks, Napier, 1961; h^2: 0.11-0.35 and r^2: 0.26-0.46, complete pedigree information, 283 bucks of R line, Lavara *et al.*, 2008).

Nowadays, AI in rabbits is performed with fresh semen or cooled semen (at 16-19°C) stored for short periods of time (24-48 h), with acceptable results on fertility and prolificacy. Perhaps, in the future, it may be necessary to use frozen semen, as it is routinely used in other domestic animals for bio-security reasons. For the moment, few

studies have been performed on this topic (for review: Mocé and Vicente, 2009), and only Lavara *et al.* (2009) reported h^2 and r estimates of sperm traits after the freezing-thawing process, studied in 315 bucks of R line. After the freezing-thawing process, sperm traits showed low to medium h^2 (0.06, 0.07 and 0.21 for individual sperm motility, normal acrosome status and viability) and r (0.19, 0.14 and 0.46 for individual sperm motility, normal acrosome status and viability). Obviously, the value of post-thawing traits will depend on the value of traits before freezing (value of the trait recorded in fresh semen), plus other permanent and additive effects derived from the process of freezing-thawing. On this premise, a recursive model was used by Lavara *et al.* (2012b) to analyse the environmental and total male effects that could have an influence on sperm freezability. The high male correlations found in this study between fresh and frozen-thawed traits suggested that these traits should be genetically related.

Crossbreeding parameters

An improvement in the production of potentially fertile doses could be achieved through the use of crossbreed males, thanks to a possible positive heterosis as well as complementarity between parental lines. Brun *et al.* (2002a) reported high variability in the estimates of direct heterosis for different seminal traits. It was positive for sperm concentration (37.5%), total number of sperm per ejaculate (37.6%), mass motility (6.8%) and percentage of motile spermatozoa (4.1%) when they analysed semen characteristics in 2 maternal lines and their reciprocal crosses. Khalil *et al.* (2007) also found a favourable direct heterosis effect for ejaculate volume (10.6%), sperm concentration (13.6%), sperm motility (10.5%) and for percentage of abnormally shaped spermatozoa and dead spermatozoa (–21.5% and –20.3%, respectively) in the cross scheme of a Spanish maternal line and a Saudi breed performed to achieve 2 new synthetic maternal lines. Moreover, they found favourable maternal heterosis for the same traits (24.0% for ejaculate volume, 10.3% for sperm concentration, 21.8% for sperm motility and –9.6 and –14.7% for percentage of abnormally shaped spermatozoa and dead spermatozoa, respectively). However, the heterotic effects for seminal traits obtained in crosses between 2 paternal lines of rabbits had little relevance and only favourable to the presence of sperm with cytoplasmic droplets (57 and 30% for proximal and distal cytoplasmic droplets, respectively; García-Tomás *et al.*, 2006c), which do not have a clear relationship with fertility. Therefore, the superiority of crossbred bucks was not proven for those lines and traits.

PREDICTION OF MALE REPRODUCTIVE PERFORMANCE THROUGH EJACULATE AND SEMEN TRAITS

Artificial insemination is performed in commercial farms with pooled semen from several bucks at high sperm dosage in order to overcome the negative effects on fertility of semen with suboptimal characteristics. This practice reduces the output of AI centres. However, this would be attenuated if the fertilising potential of ejaculates was accurately predicted by a function index of their seminal characteristics, or if some seminal characteristic was good enough itself to ensure a high reproductive performance, even at low sperm dosage. Predicting male fertility from seminal traits is also necessary to make decisions regarding male replacement and management in AI centres. Moreover, this index could be used to genetically improve male contribution to fertility by indirect selection. This would be the case if this index had at least a moderate heritability and an important genetic correlation with male reproductive performance. Selection for this index, in turn, could improve the relevant seminal traits used to construct the index.

However, the relationship between the characteristics of the ejaculate and the result of insemination is still not clearly established, and most studies have shown that the proportion of the observed variance that is explained by models including the set of seminal traits which are usually recorded in the AI centres is very low. This could be due to: *i)* The experimental design regarding AI conditions related to ejaculate selection and dose preparation. Thus, in most of the researches AI is performed with semen obtained after a strong pre-selection of the ejaculates, which reduces the observed variability. *ii)* The variables used as descriptors of semen quality, the way in which they are measured and the time of recording regarding the time when the AI is performed. In other words, the seminal evaluation is usually performed in a subjective manner and far from the AI time. Thus, seminal traits could change during the storage period and, moreover, these changes could be different depending on the characteristics of the ejaculate. *iii)* The procedures used for selection of the seminal variables to be considered to predict fertility and, also, for constructing the index. Regression analysis has been the method of choice for this kind of studies. Classical regression methods require the assumption of a specific parametric function (e.g. linear, quadratic, etc.) to construct the index, which

could be too rigid for modelling some kinds of relationships. However, non parametric methods (Wasserman, 2006), such as machine learning algorithms, do not require prior knowledge and can accommodate complex relationships between dependent and independent variables and intricate dependencies among explanatory variables. Moreover, they are very flexible and can learn arbitrarily complex patterns when enough data are available.

There is only one research work assessing the predictive ability of male fertility from seminal traits in an independent set of data (i.e., in a data set not used to obtain the predictive function, which allows us to know the ability to predict fertility of future semen samples from a set of explanatory variables measured on them instead of having just an indicator of the goodness of fit of the predictive function to the data used to obtain it; Piles *et al.*, 2013a). In this experiment, AI was performed after a small pre-selection of the ejaculates and 24 h of dose storage at 18°C. This study uses non-parametric procedures, such as Support Vector Ordinal Regression and Non-Deterministic Ordinal Regression, to predict the fertility rank of an ejaculate from the selected characteristics of the artificial insemination doses. These procedures, compared to the classical regression procedures, seem to improve the success in the fertility classification of the ejaculates, but the improvement is minimal and, in fact, it is not very different from the prediction obtained without information on the seminal characteristics. The percentage of variation in fertility is probably explained by the fact that the group of semen characteristics usually recorded is very low in number (Brun *et al.*, 2002b; Gadea *et al.*, 2004; García-Tomás *et al.*, 2006a) and it may be necessary to find other semen quality markers, or evaluate some of the currently used ones in a more precise manner or closer to the AI time.

GENETIC RELATIONSHIP BETWEEN SEMINAL CHARACTERISTICS AND MALE REPRODUCTIVE PERFORMANCE

Male fertility is an interesting trait in rabbit breeding because, together with doe fertility, it determines the fertility of the mating, as we shall discuss in the next section. The economic importance of the contribution of a buck to fertility is increasing with the use of AI (Alvariño, 2000), but its consideration as a direct criterion for selection is difficult, because it requires obtaining information from the result of AI performed with homospermic doses with semen from bucks of the nucleus of selection or their close relatives. In this context, it would be of interest to find some semen trait, early and easy to record, with high h^2 and genetically correlated to male reproductive performance (i.e. male contribution to fertility and prolificacy) in order to improve this trait by indirect selection.

In order to evaluate what can be expected from this selection strategy, first it is necessary to know the genetic correlations between the traits. Promising traits are those that have been reported to have a relevant phenotypic correlation with male reproductive performance: mass motility and number of motile sperm per ejaculate (Bencheikh, 1993; Brun *et al.*, 2002; Theau-Clément *et al.*, 2011); percentage of motile spermatozoa; concentration of spermatozoa in the ejaculate and variables related to it, correlated to the male prolificacy (Brun *et al.*, 2002; Theau-Clement *et al.*, 2011); pH of the ejaculate (O'Ferral and Meacham, 1968; Vrillon *et al.*, 1979; Bencheikh, 1993; Cofey, 1998, Tusell *et al.*, 2011b); percentage of total motile cells, some sperm movement characteristics measured with CASA systems (linearity index, amplitude of lateral head displacement), percentage of abnormal sperm in the sample (Lavara *et al.*, 2005; Theau-Clément *et al.*, 2011); rate of spermatozoa with presence of cytoplasmatic droplet and rate of reacted spermatozoa during the process of acrosome reaction induction (Piles *et al.*, 2013a).

To our knowledge, only the paper by Tusell *et al.* (2011b) studies the genetic relationship between male fertility and one of the traits mentioned above: the pH of the ejaculate. As the semen pH is mainly a consequence of the number and activity of the spermatozoa present in the ejaculate, it has been considered as an interesting indicator of the ejaculate capability to fertilise. The study involved 243 bucks of the Caldes paternal line and obtained 6613 records of fertility on 2293 crossbred females. Two-trait models, non recursive or recursive (including the pH as a covariate or as a cross-classified effect in the fertility model), were considered to estimate the genetic correlation between the 2 traits. The fertility was also studied with a one-trait model, including the pH in the same form as previously explained for the two-trait approach, which allowed an estimation of the phenotypic effect of pH on fertility. The pH was considered a Gaussian trait and the fertility a binary trait (success or failure of the mating to achieve a pregnancy), analysed with a threshold model. The study reveals again the negative and linear relationship that exists between the pH and the likelihood of fertility at both the phenotypic and environmental level. A regression coefficient of -0.6 ± 0.11 in the one-trait model (phenotypic level) and -0.15 ± 0.07 in the two-trait recursive model (environmental level) were

estimated and the linearity of this relationship was checked through the obtained estimates of the effects of the 8 pH classes in the alternative models. The estimates of the genetic correlations, depending on the model, had a PM between –017 and –0.44 and HPD95% between [–0.99, 0.48] and [–0.99, 0.10], which indicates a high probability of the correlation being negative, but the precision of the estimates was poor, despite the relatively high number of bucks and inseminations involved in the experiment. Thus, to confirm or discard the interest of the pH as a useful trait to indirectly improve male fertility, it would be necessary to perform more experiments involving a larger number of bucks to allow for more accurate estimates of the genetic correlation. Similarly, to check the same objective for the other seminal traits referred in the second paragraph of this section, the corresponding large and expensive experiments should also be carried out.

MALE CONTRIBUTION TO FERTILITY AND PROLIFICACY AFTER NATURAL MATING AND AFTER ARTIFICIAL INSEMINATION

As has been shown before, deciding which set of seminal characteristics should be measured and what levels of these traits are optimal to improve the production of fertile doses through some of the components is difficult. The alternative could be direct genetic improvement of male reproductive performance, after overcoming the problems associated with collecting such data. Improving male contribution to fertility and prolificacy also involves improving the set of seminal characteristics that are important for obtaining potentially fertile doses. Note that male reproduction performance can be considered to be the final expression of the effects of semen quality traits and the interaction among them and with the female (Koops *et al.*, 1995; Foote, 2003).

Genetic variation between lines

Vicente *et al.* (2000) compared the male reproductive performance of a paternal line selected for growth and 3 maternal lines. Fertility rate did not differ among lines but prolificacy did, probably due to the selection process of the maternal lines. García-Tomás *et al.* (2006a) found differences in fertility between 2 rabbit lines highly selected for growth rate; however, no relevant differences were found either for number of kids born alive or stillborn. Theau-Clément *et al.* (2007) did not find differences in fertilising ability between 2 lines divergently selected for body weight at 63 d.

Genetic variation within lines

Few works have been performed to investigate the possibilities of selection for male reproductive performance in rabbit. The first studies showed that fertility and prolificacy after natural mating had an almost null male contribution (Piles *et al.*, 2005; Piles *et al.*, 2006). Results from following studies confirmed a similar effect when AI is performed at high sperm dosage (Tusell *et al.*, 2010).

Tusell *et al.* (2010) indicated that these conditions of AI are not optimal for detecting individual variation among males, probably because the number and quality of sperm at mating time of most of the males exceeds the threshold needed to reach fertility (Amann and Hammerstedt, 2002). Thus, although differences among males that are independent on sperm dosage are maintained, differences among males that can, at least in part, be overcome by increasing the amount of sperm are not detected (Saacke *et al.*, 2000). Reducing the number of sperm in the dose could lead to more accurate assessment of differences in reproductive performance among males. This would be a specific case of the existence of an interaction between the male genotype and the sperm dosage. Other factors involved in the AI process as a whole, e.g. conditions and duration of dose storage, female physiological status and environmental conditions on the farm, could also lead to an interaction with the male genotype. By using the character state model (Falconer, 1952), Tusell *et al.* (2010) demonstrated that male contributions to fertility and litter size after AI were low, but higher in magnitude than those obtained after natural mating. They found that there could be an interaction between the male genotype and AI conditions, and postulate that it would be possible to find the conditions that give the maximum genetic progress to optimise the breeding programme for male fertility and prolificacy under given conditions of semen utilisation. In this way, the response to selection for male fertility could be improved by including in the selection criteria the male additive effect predicted from information obtained from AI performed under limited AI conditions. However, despite obtaining a higher response under limited AI conditions than under the commercial

conditions of semen utilisation, the superiority of the selected individuals compared to the average population in the current conditions of semen utilisation would still be reduced due to a scale effect, which might not compensate the investment required for selection (Kolmodin, 2003). Still, a favourable correlated response could be obtained in semen quality traits, leading to a higher production of fertile doses per ejaculate if selected males are used in the AI centres.

Another important factor that could limit the amount of observed variation due to male effects is the stage of gestation, because fertility and litter size at birth are greatly conditioned by fetal survival, which is mostly determined by the female. Thus, Piles *et al.* (2012b) found that male individual variation was higher for the number of implanted embryos and embryo survival estimated at day 12 of second gestation by laparoscopy (h^2: 0.05 [0.01, 0.10] and 0.07 [0.02, 0.12] for each trait, respectively) than for litter size at kindling (male genetic plus permanent environmental effects ≤0.01; Piles *et al.*, 2006), despite that fact that natural mating was practiced and records were only taken from pregnant females. In prolific species, these traits could be considered as fertility measurements because they indicate the number and rate of fertilised ova which are able to initiate the embryo development. They also suggest that the male contribution to this trait obtained after limited AI conditions could be used to improve male reproductive performance and seminal characteristics.

The genetic correlation between the female and male contributions to fertility has been found to be moderate to high and positive in a maternal line and a paternal line of rabbits (Piles *et al.,* 2005; Tusell *et al.*, 2010; David *et al.*, 2011; Piles and Tusell, 2012), which indicates that selection for the contribution to fertility of one of the sexes, if successful, could have a favourable correlated response in the contribution to the same trait of the other sex. This correlated response could be responsible, at least in part, for the observed differences in semen quality traits among bucks from maternal and paternal lines (Vicente *et al.*, 2000).

Crossbreeding parameters

Brun *et al.* (2002b) found that crossbred males and females from 2 rabbit maternal lines had better conception rate and prolificacy than the purebred ones. However, it was not possible to ascertain whether those differences were due to the effect of crossbred males or crossbred females. Using paternal lines, García-Tomás *et al.* (2006a) found unfavourable individual heterosis effects for male fertility but not for total number of kids born alive or stillborn. Therefore, they concluded that the use of a crossbred male to improve the production of fertile doses was not clearly more advantageous than the use of a purebred one and suggested the use of specialised lines to improve dose production in AI centres.

GENETIC RELATIONSHIP BETWEEN SEMINAL TRAITS AND MALE REPRODUCTIVE PERFORMANCE WITH GROWTH TRAITS

Selection for average daily gain (ADG) is not expected to have any effect on male contribution to fertility after natural mating or after AI under common commercial conditions of use of the bucks, because both traits seem not to be genetically correlated (PM=0.017, HPD95%=0.24, 0.24) and the genetic variance of male fertility is very low, as found by Piles and Tusell (2012). They also reported that ADG is negatively correlated with the female contribution to fertility, but the magnitude of this correlation (PM=–0.31) is probably not high enough to lead to an important impairment of the reproductive performance of paternal lines of rabbits selected for average daily gain, since the probability of a genetic correlation <0.5 was 0.0001. Moreover, several studies involving rabbit maternal lines indicated that the genetic correlation between growth and the female contribution to litter size was negative, null or positive, but always of low magnitude (Camacho and Baselga, 1990; Gómez *et al.*, 1998; Garreau *et al.*, 2000; García and Baselga 2002). Therefore, it is concluded that growth is not, or is poorly, genetically correlated with the reproductive performance of rabbits.

Although estimates are generally imprecise, there is some evidence of the existence of a genetic relationship between semen production and quality with ADG. Tusell *et al.* (2011c) found that ADG had a slightly favourable correlation with sperm concentration (0.21, HPD95%=–0.03, 0.48) and a slightly unfavourable genetic correlation with ejaculate volume (–0.19, HPD95%=–0.47, 0.08). Moreover, ADG was genetically uncorrelated with all libido and seminal traits that are usually included in the criterion for ejaculate rejection for AI, such as pH, individual motility and the presence

of urine, blood and other elements that preclude the use and/or evaluation of the ejaculate. Lavara *et al.* (2011) also obtained estimates of the genetic correlation between semen production and quality with ADG. Some of their estimates had the opposite sign to those obtained by Tusell *et al.* (2011c) but were very imprecise, making it difficult to draw reliable conclusions. Regarding sperm motility, Lavara *et al.* (2012) obtained a moderate and negative genetic correlation with ADG (–0.53, HPD95%=–0.95, 0.02); nevertheless, sperm movement characteristics measured with CASA systems, such as average path velocity, straight-line velocity, curvilinear velocity, straightness, etc, could have no genetic relationship with growth (the PM of the genetic correlations ranged between 0.03 and –0.14; and the intervals HPD95%, were around –0.50, 0.50). In relation to sperm morphology and acrosome membrane functionality, Lavara *et al.* (2012a) concluded that there is an apparent tendency for genes favouring increased daily gain to slightly decrease normal sperm per ejaculate (less sperm with normal acrosome status and more with abnormal forms), but the magnitude of the genetic correlations does not seem to be high (–0.40, HPD9%=–0.78, –0.02 for normal acrosome status; 0.25, HPD95%=–0.18, 0.66 for sperm abnormalities). On the other hand, Brun *et al.* (2006) did not find differences in male libido between 2 lines divergently selected for body weight at 63 d, but reported that males from the line with the lowest body weight had higher ejaculate volume, sperm motility and number of ejaculates suitable for AI, but lower sperm concentration than males from the line with the highest body weight. Overall, the total number of sperms per ejaculate was not significantly different between the 2 lines. In a posterior study, these lines were compared by their fertilising ability and no significant differences were found between them (Theau-Clément *et al.* 2007). Because of the antagonism between volume and sperm concentration of the ejaculate, the genetic correlation between ADG and total number of sperm in the ejaculate seems to be almost null.

The general conclusion of this section is that the seminal traits and growth rate seem to be null or only minimally genetically correlated. Two interesting consequences derive from this result. The first is that selection to increase ADG is not expected to have detrimental correlated effects on seminal traits involved in AI dose production. The second is that a multi-trait selection, including ADG and other seminal traits directly related to efficient dose production, is feasible. Nevertheless, the decision-making to define the objectives of a selection programme involves genetic as well as economic components. Consequently, the economic weights of growth rate and the most interesting seminal traits need to be evaluated beforehand to correctly define the selection criteria.

MODELS FOR THE GENETIC ANALYSIS OF REPRODUCTIVE PERFORMANCE AND SEMINAL TRAITS

Genetic analysis of discrete traits

The analysis of fertility as well as certain traits involved in the production of seminal doses, such as the presence of certain residues in the ejaculate and the suitability of the ejaculate for use in AI, requires the use of special models which consider the discrete nature of the trait. The threshold model was proposed by Wright (1934) and postulates that a categorical observed response is related to an underlying normally distributed variable, called liability, and to fixed thresholds that divide the continuous liability scale into intervals that delimit the response categories. The main problem associated with the application of the threshold model methodology is the so-called extreme category problem (ECP). This could arise when there are only few observations per level of systematic effect and all the observations fall exclusively into one of the categories. The major consequence of ECP is that biased estimates would be obtained. Several authors have proposed the use of different prior distributions for fixed effects in order to alleviate this problem (Hoeschele and Tier, 1995; Moreno *et al.*, 1997; Rekaya *et al.*, 2011).

In the specific case of fertility traits, male and female contributions to them have, in general, been separately analysed, but as the outcome of an AI event depends on both sexes, the 2 contributions to the final expression of an AI outcome should be analysed jointly. The additive and product threshold models are 2 different approaches for the analysis of fertility defined as a binary trait. Both types of model allow an estimate of the genetic correlation between male and female contributions to fertility.

The additive threshold model proposes that the underlying variable of fertility is the result of the sum of genetic and environmental effects of the 2 individuals involved in the mating (Varona and Noguera, 2001; Piles *et al.*, 2005), whereas the product threshold model proposed by David *et al.* (2009) postulates that the observed reproduction outcome is the result of the product of 2 conditionally independent unobserved variables corresponding to the fertility

of the 2 individuals involved in the mating. This approach could better reflect the biology of the fertility than the additive model. Within the product model, successful AI can only be achieved when both members of the mating are fertile, whereas with the additive model it would be possible to obtain successful mating of a highly fertile female, which makes liability exceed the fertility threshold, with an infertile male, or *vice versa*, which is not biologically possible. Besides, the product threshold model allows us to extract more information from the data than the additive threshold model because it provides estimates of the effect of factors affecting each unobserved phenotype (i.e. male fertility and female fertility, while the observed trait is the result of the mating) obtained from a bi-variated model as well as obtaining the probabilities of fertility success for each sex, which allows us to evaluate which sex is most responsible for an AI failure. Performance of the product and the additive threshold model, in terms of predicting ability, was compared using real data from 3 livestock species: sheep, cattle and rabbits (David *et al.*, 2011).

Threshold model methodology can also be used for the analyses of other fertility measurements, such as number of inseminations to conception, in which the number of AIs occurs in a sequential order (i.e. an observation of a certain value of the trait must have passed through all previous stages). The ordinal threshold model (Gianola, 1982; Gianola and Foulley, 1983) assumes that the several sequential categories of response are the result of the hypothetical existence of several ordered thresholds in the liability. An alternative approach for the analyses of these type of traits is the sequential threshold model (Albert and Chib, 2001), in which the liability represents the individual ability to pass from one stage to the next. Hence, one stage can only be reached after passing the previous ones and, once the stage is reached, either successful or failed AI is observed. The advantage of this approach is that it allows the inclusion of specific factors affecting each stage (e.g. specific effects of each AI).

Another characteristic of number of inseminations to conception, as well as other fertility traits, is the presence of censored records (e.g. records from females that have been culled after AI, so they did not have the chance to express the trait of interest). However, the assumption of no informative censoring is probably not correct in most of the data, because females are commonly culled after several unsuccessful matings. Therefore, unexpected results and misleading interpretations can arise (Kalbfleisch and Prentice, 1980). González-Recio *et al.* (2005) adapted 3 methods to deal with the presence of censored records on the number of inseminations to conception in dairy cows. First, they extended the ordinal threshold model to accommodate censored records to analyse this trait. The ordinal censored threshold model uses a method consisting of augmenting the data by sampling from a left truncated distribution every time that a censored record falls into one of several possible known categories. In that specific case, the truncation point was the threshold corresponding to the last observed insemination of the particular animal. The sequential threshold model was also adapted by these authors to take censored records into account. Finally, another approach to handle censored records of a sequential trait is to use a particular type of proportional hazard model, the grouped survival model (Prentice and Gloeckler, 1978). This approach treats the number of inseminations to conception as time periods until an event of interest, which is birth. In the absence of parity, a censored record in the last insemination is assumed. This model defines the probability of having a pregnancy given that the female was inseminated at a certain time period. González-Recio *et al.* (2005) compared the 3 approaches in terms of prediction ability of the models and concluded that the sequential threshold model had better predictive ability at the first insemination than the other two, but the predictive ability in subsequent AIs was better for the censored threshold model.

Models for the joint analysis of seminal and reproductive performance traits

The joint analyses of seminal traits and male reproductive performance can be performed using multiple trait models or some extension of the same, which are the recursive models. The interest in using this latter approach is that these models allow us to consider the effect of seminal traits on the phenotypic expression of fertility, but also take into account that seminal traits in turn also have genetic and permanent effects contributing to their phenotypic expression.

A recursive multi-trait model is a particular case of a structural equation model, which Gianola and Sorensen (2004) introduced to the field of quantitative genetics. These models are useful for describing biological relationships between traits. For a pair of traits, simultaneity or recursiveness are 2 types of relationships. Simultaneity indicates that changes in one trait affect a second trait and, in turn, the second trait affects the first trait. Recursiveness refers

to a situation where one trait affects the other, but the latter does not affect the former. These authors also pointed out that, in the presence of these relationships, if they are not properly taken into account, biased (co)variance estimates can be obtained. Tusell *et al.* (2011b) implemented this model for the joint analysis of fertility and ejaculate pH, and Lavara *et al.* (2012b) for the analysis of fresh and frozen-thawed sperm.

CONCLUSIONS AND IMPLICATIONS FOR THE FUTURE

Reproductive performance and seminal production and quality traits have gained prominence as a consequence of the expansion in the use of artificial insemination, because with the use of this technique the impact of males on reproduction success is great. The increased interest in this subject is recent and its knowledge is therefore still in an early stage of development in this species.

Results from several AI centres suggest that male reproductive performance and seminal characteristics should be improved in order to increase the efficiency of production of potentially fertile doses. The information currently available indicates that the use of specialised lines rather than crossbreeding is probably the best approach to improve these traits. Direct selection for male reproductive performance after natural mating or after AI with standard doses may not be effective. Nevertheless, the existence of an interaction between male genotype and the AI conditions suggest that it could be possible to find the AI conditions that provide maximum genetic progress in a breeding programme for male reproductive performance under given conditions of semen utilisation. However, despite obtaining a higher response under optimal AI conditions than under AI conditions of semen utilisation (e.g. the standard commercial conditions), the superiority of the selected individuals compared to the average population in the current conditions of semen utilisation would be still reduced due to a scale effect, which might not compensate the investment required for selection.

One recent study in rabbit has suggested that response to selection for male reproductive performance could be greater by using as selection criteria the male contribution to the number of implanted embryos or embryo survival at day 12 after AI measured by laparoscopy, because the genetic determinism of this trait was greater than in later stages. More research is needed to confirm this result, requiring coordinated participation of nucleus of selection, AI centres, laboratories and research centres, as the amount of information needed to achieve precise estimates of genetic parameters is considerable.

Selection for a set of seminal characteristics could have no correlated response in male fertility and prolificacy, at least for the seminal characteristics evaluated to date. It is very important to find new, immediate, inexpensive and easy to measure fertility markers which can be used to improve semen quality and indirectly male reproductive performance. Moreover, it is necessary to know the optimum levels for these traits in the ejaculate. On the other hand, the results from different experiments suggest that it is possible to improve semen production by selection to increase the total number of sperm in the ejaculate, which is a moderately heritable trait and therefore could lead to an increase in the amount of AI dose produced per buck. However, the relationship between semen production and semen quality is not yet clearly established, although there is evidence of a favourable genetic correlation between semen production and sperm motility (Brun *et al.*, 2009). Again, further research is needed to ascertain the correlated effect of selection to increase semen production on semen quality or male reproductive performance, once a clear definition of semen quality is known.

For the future, if selection for male characteristics related to semen, fertility or prolificacy is to be implemented, it will be necessary to estimate the economic weights of these traits, together with those of the common traits currently used for selecting paternal lines.

Acknowledgements: This study was supported by the Generalitat Valenciana Research Programme (Prometeo 2009/125) and Spanish Research Projects (INIA RTA2005-00088-CO2; INIA RTA2008-00070-CO2; CICYT AGL2008-03274). Raquel Lavara was supported by a research grant from the Spanish Ministry of Education (MEC, FPU AP2007-03755) and Llibertat Tusell by a research grant from INIA.

REFERENCES

Al-Makhzoomi A., Lundeheim N., Håård M., Rodríguez-Martínez H. 2008. Sperm morphology and fertility of progeny-tested AI dairy bulls in Sweden. *Theriogenology, 70: 682-691. doi:10.1016/j.theriogenology.2008.04.049*

Alvariño J. M. R. 2000. Reproductive performance of male rabbits. *In Proc.: 7th World Rabbit Congress, 4-7 July, 2000. Valencia, Spain, A: 13-35.*

Albert J.H., Chib S. 2001. Sequential ordinal modelling with applications to survival data. *Biometrics, 57: 829-836. doi:10.1111/j.0006-341X.2001.00829.x*

Amann R.P., Hammerstedt R.H. 2002. Detection of differences in fertility - andrology lab corner. *J. Androl., 23: 317-325.*

Baselga M. 2004. Genetic improvement of meat rabbits. Programmes and diffusion. *In Proc.: 8th World Rabbit Congress, 7-10 September, 2004. Puebla, Mexico, pp. 1-13.*

Bencheikh N. 1993. Production de sperme et fertilité du lapin mâle *Oryctolagus cuniculus*. Effet de la fréquence de collecte et du type génétique. *Thése d'Etat. Ecole Nationale Agronomique de Toulouse.*

Bencheikh N. 1995. Effet de la fréquence de collecte de la semence sur les caractéristiques du sperme et des spermatozoïdes récoltés chez le lapin. *Ann. Zootech., 44: 263-279. doi:10.1051/animres:19950306*

Blasco A. 1996. Genetics of litter size and does fertility in the rabbit. *In Proc.: 6th World Rabbit Congress, 9-12 July, 1996. Toulouse, France, 2: 219-227.*

Brun J.M., Sanchez A., Duzert R., Saleil G., Theau-Clément M. 2009. Paramètres génétiques des caractéristiques de la semence de lapin. *In Proc.: 13èmes Journées de la Recherche Cunicole, 17-18 Novembre, 2009. Le Mans, France, 11: 133-136.*

Brun J.M., Theau-Clément M., Bolet G. 2002a. Evidence for heterosis and maternal effects on rabbit semen characteristics. *Anim. Res., 51: 433-442. doi:10.1051/animres:2002030*

Brun J.M., Theau-Clément M., Bolet G. 2002b. The relationship between rabbit semen characteristics and reproductive performance after artificial insemination. *Anim. Reprod. Sci., 70: 139-149. doi:10.1016/S0378-4320(01)00197-X*

Brun J.M., Theau-Clément M., Esparbié J., Falières J., Saleil G., Larzul C. 2006. Semen production in two rabbit lines divergently selected for 63-d body weight. *Theriogenology, 66: 2165-2172. doi:10.1016/j.theriogenology.2006.07.004*

Camacho J, Baselga M. 1990. Genetic correlation between reproductive and growth traits in rabbits. *In Proc.: 4th World Congress on Genetics Applied to the Livestock Production, 23-27 July, 1990. Edinburgh, Scotland, 16: 366-369.*

David I., Bodin L., Gianola D., Legarra A., Manfredi E., Robert-Granié C. 2009. Product versus additive threshold models for analysis of reproduction outcomes in animal genetics. *J. Anim. Sci., 87: 2510-2518. doi:10.2527/jas.2008-1586*

David I., Carabaño M.J., Tusell L., Diaz C., González-Recio O., López de Maturana E., Piles M., Ugarte E., Bodin L. 2011. Product versus additive model for studying artificial insemination results in several livestock populations. *J. Anim Sci., 89: 321-328. doi:10.2527/jas.2010-3167*

Ducrocq V., Humblot P. 1995. Genetic characteristics and evolution of semen production of young Normande bulls. *Livest. Prod. Sci., 41: 1-10. doi:10.1016/0301-6226(94)00029-7*

Estany J., Camacho J., Baselga M., Blasco A. 1992. Selection response of growth rate in rabbits for meat production. *Genet. Sel. Evol., 24: 527-537. doi:10.1186/1297-9686-24-6-527*

Falconer D.S. 1952. The problem of environment and selection. *Am. Nat., 86: 293-298. doi:10.1086/281736*

Foote R.H. 2003. Fertility estimation: a review of past experience and future prospects. *Anim. Reprod. Sci., 75: 119-139. doi:10.1016/S0378-4320(02)00233-6*

Gadea J., Sellés E., Marco M.A. 2004. The predictive value of porcine seminal parameters on fertility outcome under commercial conditions. *Reprod. Domest. Anim., 39: 303-308. doi:10.1111/j.1439-0531.2004.00513.x*

García M.L., Baselga M. 2002. Estimation of correlated response on growth traits to selection in litter size of rabbits using a cryopreserved control population and genetic trends. *Livest. Prod. Sci., 78: 91-98. doi:10.1016/S0301-6226(02)00093-3*

García-Tomás M., Sánchez J., Rafel O., Ramon J., Piles M. 2006a. Reproductive performance of crossbred and purebred male rabbits. *Livest. Sci., 104: 233-243. doi:10.1016/j.livsci.2006.04.006*

García-Tomás M., Sánchez J., Rafel O., Ramon J., Piles M. 2006b. Variability, repeatability and phenotypic relationships of several characteristics of production and semen quality in rabbit. *Anim. Reprod. Sci., 93: 88-100. doi:10.1016/j.anireprosci.2005.06.011*

García-Tomás M., Sánchez J., Rafel O., Ramon J., Plies M. 2006c. Heterosis, direct and maternal genetic effects on semen quality traits of rabbits. *Livest. Sci., 100: 111-120. doi:10.1016/j.livprodsci.2005.08.004*

Garreau H., Piles M., Larzul C., Baselga M., de Rochambeau H. 2004. Selection of maternal lines: last results and prospects. *In Proc.: 8th World Rabbit Congress, 7-10 September, 2004. Puebla, Mexico, 14-25.*

Garreau H., Szendrő Zs., Larzul C., de Rochambeau H. 2000 Genetic parameters and genetic trends of growth and litter size traits in the White Pannon breed. *In Proc.: 7th World Rabbit Congress, 4-7 July, 2000. Valencia, Spain, 403-408.*

Gianola D. 1982. Theory and analysis of threshold characters. *J. Anim Sci., 54: 1079-1096.*

Gianola D., Foulley J.L. 1983. Sire evaluation for ordered categorical data with a threshold model. *Genet. Sel. Evol., 15: 201-224. doi:10.1186/1297-9686-15-2-201*

Gianola D., Sorensen D. 2004. Quantitative genetic models for describing simultaneous and recursive relationships between phenotypes. *Genetics, 167: 1407-1424. doi:10.1534/genetics.103.025734*

Gómez E.A., Rafel O., Ramon J. 1998. Genetic relationships between growth and litter size traits at first parity in a specialized dam line. *In Proc.: 6th World Congress on Genetics Applied to Livestock Production, 11-16 January, 1998. Armidale, Australia, 25: 552-555.*

González-Recio O., Chang Y.M., Gianola D., Weigel K.A. 2005. Number of inseminations to conception in Holstein cows using censored records and time-dependent covariates. *J. Dairy Sci., 88: 3655-3662. doi:10.3168/jds.S0022-0302(05)73051-4*

Hidalgo M., Rodríguez I., Dorado J.M. 2007. The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. *Anim. Reprod. Sci., 100: 61-72. doi:10.1016/j.anireprosci.2006.07.003*

Hoeschele I., Tier B. 1995. Estimation of variance components of threshold characters by marginal posterior modes and means *via* Gibbs sampling sampling. *Genet. Sel. Evol., 27: 519-540. doi:10.1186/1297-9686-27-6-519*

ITAVI. 2008. Gestion technico-économique des éleveurs de lapins de chair (programmes RENACEB et RENALAP) résultats 2008. *Comité de pilotage de 10 de septembre 2009. Available at: http://www.Itavi.Asso.Fr/economie/references/lapins.php Accessed October 2009.*

Kalbfleisch J.D., Prentice R.L. 1980. The statistical analysis of failure time data. *John Willey and Sons, New York, USA.*

Khalil M.H., Al-Sobayil K.A., Al-Saef A.M., García M.L., Baselga M. 2007. Genetic evaluation for semen characteristics in a crossbreeding project involving Saudi and Spanish V-line rabbits. *Animal, 1: 923-928. doi:10.1017/S1751731107000341*

Khalil M.H., Al-Saef A.M. 2008. Methods, criteria, techniques and genetic responses for rabbit selection: a review. *In Proc.: 9th World Rabbit Congress, 10-13 June, 2008. Verona, Italy, 1-22.*

Khalil M.H., Bolet G. 2010. Sustainable rabbit breeding and genetic improvement programs achieved in developing countries. In Proc.: 9th World Congress on Genetics Applied to Livestock Production, 1-6 August, 2010. Leipzig, Germany. Communication 962.

Kolmodin R. 2003. Reaction norms for the study of genotype by environment interaction in animal breeding. *Phd diss. Dept. of Animal Breeding and Genetics. Swedish Univ. of Agricultural Sciences. Uppsala, Sweden.*

Koops W.J., Grossman M., den Daas J.H.G. 1995. A model for reproductive efficiency of dairy bulls. *J. Dairy Sci., 78: 921-928. doi:10.3168/jds.S0022-0302(95)76707-8*

Larzul C., de Rochambeau H. 2005. Selection for residual feed consumption in the rabbit. *Livest. Prod. Sci., 95: 66-72. doi:10.1016/j.livprodsci.2004.12.007*

Lavara R., David I., Mocé E., Baselga M., Vicente J.S. 2012b. Environmental and male variation factors of freezability in rabbit semen. *Theriogenology, 79: 582-589. doi:10.1016/j.theriogenology.2012.11.007.*

Lavara R., García M.L., Torres C., Vicente J.S., Baselga M. 2008. Genetic parameters for semen traits of rabbit males: I. Production, morphology, and sperm head morphometry. *In Proc.: 9th World Rabbit Congress, 10-13 June, 2008. Verona, Italy, 153-157.*

Lavara R., Mocé E., García M.L., Vicente J.S., Baselga M. 2009 Parámetros genéticos del semen descongelado de conejo. Resultados preliminares. *In Proc.: XIII Jornadas de Producción Animal (AIDA), 12-13 May, 2009. Zaragoza, Spain, I: 96-98.*

Lavara R., Mocé E., Lavara F., Viudes de Castro M.P., Vicente J.S. 2005. Do parameters of seminal quality correlate with the results of on-farm inseminations in rabbits? *Theriogenology, 64: 1130-1141. doi:10.1016/j.theriogenology.2005.01.009*

Lavara R., Vicente J.S, Baselga M. 2011. Genetic parameter estimates for semen production traits and growth rate of a paternal rabbit line. *J. Anim. Breed. Genet., 128: 44-51. doi:10.1111/j.1439-0388.2010.00889.x*

Lavara R., Vicente J.S., Baselga M. 2012a. Estimation of genetic parameters for sperm quality traits and growth rate in a paternal rabbit line. *Theriogenology, 78: 567-575. doi:10.1016/j.theriogenology.2012.03.002*

Marco-Jiménez F., Vicente J.S., Lavara R., Balasch S., Viudes de Castro M.P. 2010. Poor prediction value of sperm head morphometry for fertility and litter size in rabbit. *Reprod. Domest. Anim., 45: 118-123. doi:10.1111/j.1439-0531.2009.01532.x*

Matheron G., Poujardieu B. 1984. La génétique du lapin : le point, les perspectives. *In Proc.: 3rd World Rabbit Congress, 4-8 April, 1984. Rome, Italy, 1: 3-34.*

Mocé M.L., Santacreu M.A. 2010. Genetic improvement of litter size in rabbits. In Proc.: 9th World Congress on Genetics Applied to Livestock Production, 1-6 August, 2010. Leipzig, Germany. Communication 25.

Mocé E, Vicente J.S. 2009. Rabbit sperm cryopreservation: A review. *Anim. Reprod. Sci., 110: 1-24. doi:10.1016/j.anireprosci.2008.08.015*

More O'Ferrall G.J., Meacham T.N. 1968. Relationship between pH, other semen traits and fertility in rabbits. *In Proc.: VI Cong. Intern. Reprod. Anim. Insem. Artif., 1968 July, Paris, 2: 1279-1281.*

Moreno C., Sorensen D., García-Cortés L.A., Varona L., Altarriba J. 1997. On biased inferences about variance components in the binary threshold model. *Genet. Sel. Evol., 29: 145-160. doi:10.1186/1297-9686-29-2-145*

Morrell J.M. 1995. Artificial insemination in rabbits. *Brit. Vet. J., 151: 477-488. doi:10.1016/S0007-1935(05)80022-3*

Nagy I., Ibáñez N., Romvári R., Mekkawy W., Metzger Sz., Horn P., Szendrő Zs. 2006. Genetic parameters of growth and *in vivo* computerized tomography based carcass traits in Pannon White Rabbits. *Livest. Sci., 104: 46-52. doi:10.1016/j.livsci.2006.03.009*

Napier R.A.N. 1961. Fertility in the male rabbit III. Estimation of spermatozoa quality by mixed insemination, and the inheritance of spermatozoan characters. *J. Reprod. Fertil., 2: 273-289. doi:10.1530/jrf.0.0020273*

Panella F., Castellini C., Facchin E. 1994. Heritability of some male reproductive traits in rabbit. *Cahiers Options Méditerranéennes, 8: 279-283.*

Piles M., Rafel O., Ramon J., Varona L. 2005. Genetic parameters of fertility in two lines of rabbits with different reproductive potential. *J. Anim. Sci., 83: 340-343.*

Piles M., García M.L., Rafel O., Ramon J., Baselga M. 2006. Genetics of litter size in three maternal lines of rabbits: Repeatability *versus* multiple-trait models. *J. Anim. Sci., 84: 2309-2315. doi:10.2527/jas.2005-622*

Piles M., Tusell L. 2012. Genetic correlation between growth and female and male contributions to fertility in rabbit. *J. Anim. Breed. Genet., 129: 298-305. doi:10.1111/j.1439-0388.2011.00975.x*

Piles M., Díez J., del Coz J.J., Montañés E., Quevedo J.R., Ramon J, Rafel O., López-Béjar M., Tusell L. 2013a. Predicting fertility from seminal traits: Performance of several parametric and non-parametric procedures. *Livest. Sci., 155: 137-147.*

Piles M., Mocé M.L., Laborda P., Santacreu M.A. 2012b. Male contribution to prolificacy at early stages of gestation. *In Proc.: 10th World Rabbit Congress, 3-6 September, 2012. Sharm El-Sheikh, Egypt, 83-87.*

Prentice R.L., Gloeckler L.A. 1978. Regression analysis of grouped survival data with application to breast cancer data. *Biometrics, 34: 57-67. doi:10.2307/2529588*

Rekaya R., Sapp R.L., Hay El H., Davis R., Bertrand J.K. 2011. Simulation study for analysis of binary responses in the presence of extreme case problems. *Genet. Sel. Evol., 2011, 43: 41-46 doi:10.1186/1297-9686-43-41*

Rochambeau, de H. 1988. Genetics of the rabbit for wool and meat production. *In Proc.: 4th World Rabbit Congress, 10-14 October, 1988. Budapest, Hungary, 2: 1-68.*

Rochambeau, de H., de la Fuente L.F., Rouvier R., Ouhayoun J. 1989. Sélection sur la vitesse de croissance post-sevrage chez le lapin. *Genet. Sel. Evol., 21: 527-546. doi:10.1186/1297-9686-21-4-527*

Rouvier R. 1980. Génétique du lapin (*Oryctolagus cuniculus*). *Ann. Genet. Sel. Anim., 12: 295-296. doi:10.1186/1297-9686-12-3-295*

Saacke R.G., Dalton J.C., Nadir S., Nebel R.L., Bame J.H. 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. *Anim. Reprod. Sci., 60: 663-677. doi:10.1016/S0378-4320(00)00137-8*

Sorensen D., Gianola D. 2002. Likelihood, bayesian, and MCMC methods in quantitative genetics. *Springer Science and Business Media, LLC, New York, NY.*

Theau-Clément M., Ailloud E., Sanchez A., Duzert R., Saleil G., Brun J.M. 2011. Relation entre les caractéristiques de la semence de lapin et sa fécondance. *In Proc.:14èmes Journées de la Recherche Cunicole, 22-23 November, 2011. Le Mans, France, 73-76.*

Theau-Clément M., Brun J.M., Esparbié J., Falières J., Garvanèse J., Lamothe E., Larzul C., Milcent N., Saleil G. 2007. Une sélection pour la vitesse de croissance influence-t-elle la fécondance de la semence de lapin? *In Proc.: 12èmes Journées de la Recherche Cunicole, 27-28 November, 2007. Le Mans, France, 53-56.*

Theau-Clément M., Brun J.M., Sabbioni E., Castellini C., Renieri T., Besenfelder U., Falières J., Esparbié J., Saleil G. 2003. Comparaison de la production spermatique de trois souches de lapins: Moyennes et variabilités. *In Proc.: 10èmes Journées de la Recherche Cunicole, 2003 November, Paris, France, 81-84.*

Tusell L., García-Tomás M., Baselga M., Rekaya R., Rafel O., Ramon J., López-Béjar M., Piles M. 2010. Interaction of genotype × artificial insemination conditions for male effect on fertility and prolificacy. *J. Anim. Sci., 88: 3475-3485. doi:10.2527/jas.2009-2773*

Tusell L., David I., Bodin L., Legarra A., Rafel O., López-Béjar M., Piles M. 2011a. Using the product threshold model for estimating separately the effect of temperature on male and female fertility. *J. Anim. Sci., 89: 3983-3995. doi:10.2527/jas.2011-3924*

Tusell L., Legarra A., García-Tomás M., Rafel O., Ramon J., Piles M. 2011b. Different ways to model biological relationships between fertility and pH of the semen in rabbits. *J. Anim. Sci., 89: 1294-1303. doi:10.2527/jas.2010-3242*

Tusell L., Legarra A., García-Tomás M., Rafel O., Ramon J., Piles M. 2011c. Genetic basis of semen traits and their relationship with growth rate in rabbits. *J. Anim. Sci., 90 :1385-1397, doi:10.2527/jas.2011-4165*

Varona L., Noguera J.L. 2001. Variance components of fertility in Spanish Landrace pigs. *Livest. Prod. Sci., 67: 217-221. doi:10.1016/S0301-6226(00)00205-0*

Vicente J.S., Viudes de Castro M.P., Lavara R., Lavara F. 2000. Effect of male line on prolificacy from does inseminated with low sperm doses. *In Proc.: 7th World Rabbit Congress, 4-7 July, 2000. Valencia, Spain, A: 273-277*

Wasserman L.A. 2006. All of nonparametric statistics. *New York: Springer.*

Wolft J. 2009. Genetic correlations between production and semen traits in pig. *Animal, 3: 1094-1099. doi:10.1017/S1751731109004686*

Vrillon J.L., Donal R., Poujardieu B., Rouvier R., 1979. Sélection et testage des lapins mâles de croisement terminal 1972-1975. *In: Bulletin Technique du Département de Génétique Animale. INRA. 28: 106.*

Wright S. 1934. An analysis of variability in number of digits in an inbred strain of Guinea pigs. *Genetics, 19: 506-536.*