BREEDING PROGRAMS FOR IMPROVING MALE REPRODUCTIVE PERFORMANCE AND EFFICIENCY OF AI DOSE PRODUCTION IN PATERNAL LINES: FEASIBILITY AND LIMITATIONS

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ABSTRACT

This paper aims at reviewing the current genetic knowledge of the issues related to the efficient use of bucks in artificial insemination (AI). Differences between lines have been found relevant in semen production and quality traits not necessarily related to their specialization as maternal or paternal lines. Accurate heritability estimates indicate that genetic selection for increasing semen production by improving male libido and reducing the number of rejected ejaculates may not be effective. However, total sperm produced per ejaculate appears to be as an interesting trait to select for, despite that genetic correlation between ejaculate volume and sperm concentration has not been yet accurately estimated. 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 as convenient to select for. Morphological characteristics of the spermatozoa have revealed as medium to highly heritable. There are evidences 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 favorable direct and maternal heterosis in crosses between maternal lines. However, this has not been confirmed in a cross between two paternal lines. Until now, attempts to find parametric or non-parametric functions to predict ejaculate fertility through seminal characteristics recorded in routinely evaluations have been very unsatisfactory. Hence, it may be necessary to find other semen quality markers, or to evaluate some of the currently used ones 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 improving this trait. However, only the semen pH has been checked for this purpose, and a negative result has been obtained. Other traits can be studied in the future but bearing in mind that the required experiments will need 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 to select 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, but it has been found that, under more restrictive conditions of AI, male contributions to fertility and litter size are low but higher in magnitude than the ones 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, such as traits of the ejaculate and semen or the male contribution to fertility and prolificacy, gain importance. However, there is not a review regarding the genetics of this subject. On the contrary, genetics of rabbit doe reproduction traits as well as growth traits has often been reviewed (Rouvier, 1980; Matheron and Poujardieu, 1984; Rochambeau, 1988; 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 of individual males is vital. Hence, processes of dose production in AI centres aim at maximizing the probability of fertilization 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 differ among AI centres but it can be as high as 40% (Brun *et al.*, 2002a o b; Theau-Clement *et al.*, 2003; Brun *et al.*, 2006; García-Tomas *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 it will be discussed later. Thus, it is possible that some rejected ejaculates could be useful or indeed be even better for fertilization 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 centers. For example, inseminations are performed at high sperm dosage in order 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 ejaculates from several males (heterospermia) is a common practice in order to compensate for the negative effects of possible infertile ejaculates. The use of heterospermic doses prevents individual identification, leading to a reduction of 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 one of these traits would determine their possibilities and strategies of improvement.

The genetic studies of traits that are only expressed in active adult bucks have 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 not enough for the genetic studies requirements. To set up these experiments it is usually necessary to collaborate with one or several AI centers in order to achieve the size required. Another additional requirement for the genetic studies is to have the pedigree of the AI bucks connected to the one of the animals of the nucleus of selection. In rabbits, many AI centers do not record the pedigrees of their bucks whereas the participation of these centers 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 contribution to fertility and prolificacy explain why research has been more focused in the female reproduction traits than in those of the male.

An additional 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. Its join treatment obliges to include both types of effects in the same model, increasing the number of genetic parameters to be estimated. Moreover, if the 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., 2011). 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 along the time. Knowledge of this function can help in understanding the behaviour of the trait and moreover, individual differences in these trajectory patterns could be exploited for genetic selection (Sorensen and Gianola, 2002) but it requires the use of complex models.

Since the majority of the males used in AI came from paternal lines selected for growth, feed efficiency and, sometimes, carcass traits (Rochambeau *et al*., 1988; Larzul and Rochambeau, 2005; Estany *et al.,* 1992; Nagy *et al.,* 2006; Khalil and Al-Saef, 2008), an issue of interest is to know the expected correlated responses of the selection for growth traits on buck reproduction traits and the interest of introducing these traits in the objectives of selection, either jointly with the growth traits or alone.

The purpose of this paper is to review the current genetic knowledge of the issues related to the efficient use of the bucks in AI.. Its content will be structured in six 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 on implications for the future will also be presented.

GENETICS OF SEMEN PRODUCTION AND SEMEN QUALITY TRAITS

The existence of genetic variation can be used to improve production of potentially fertile semen doses in different ways: 1) by using bucks from lines or breeds with the best reproductive performance for the traits of interest, 2) by using crossbred males in order to take advantage of the benefits of the possible heterosis and complementarity between traits in the different lines, and finally, 3) through the use of genetic selection within line for one or several traits related to male reproductive performance or semen production. Thus, the three sources of genetic variation are going to be reviewed.

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 three 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 three maternal lines of rabbits and found differences in collection rate, ejaculate volume, sperm concentration, pH and several motility traits. They also concluded that there were also differences in the variability of semen characteristics between and within bucks for some of the seminal traits analysed.

Brun et al. (2006) did not find differences in male libido between two lines divergently selected for body weight at 63 days but reported that males from the lighter line had higher ejaculate volume, sperm 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 fertilizing ability and no differences were found (Theau-Clément *et al.*, 2007).

In another study, Brun *et al.* (2002a) compared sperm production and quality in two maternal lines and they only found differences for ejaculate volume and percentage of motile spermatozoa, probably due to positive maternal effects for those traits. There was not significant difference between strains for mass motility but one of the strains was superior in its maternal effect on this trait.

Garcia-Tomás *et al*. (2006c) found differences in direct genetic effects for some seminal traits in two 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 in general better seminal quality traits (fewer 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 in different ages (García-Tomás *et al.,* 2009).

Summarizing the previous results, it could be said that it is frequent to find relevant differences between lines in semen production and quality traits that are not necessarily related to the specialization 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 the seminal traits can be found in the literature ranging from extremely low to high values (Garcia-Tomás et al., 2006b). The variation in the magnitude of this parameter is due to several factors such as: i) different genetic composition of populations of bucks among experiments; ii) variation in defining the trait, which in some cases consist of means of observations of two consecutive ejaculates or means of several records per male, whereas in other cases 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 time to evaluation. The subjective manner in which some of the seminal traits are evaluated 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 analyzed as classified in 3 categories (no collection, collection after 5 min and intermediate collection) and obtained from data of 158 bucks of 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 other permanent effects, different to 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 they obtained a h^2 estimate of 0.17 from records of 642 bucks obtained during the process of establishing two synthetic new lines from two existing ones. Thus, eleven different genetic types of bucks were jointly analyzed, which could be responsible for a greater estimate than the one expected if only one genetic type is used. However, Tusell *et al*. (2011d) found that male libido recorded as a binary trait (if male successfully or unsuccessfully mounted to 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 carbonates deposits and gel plugs** are considered major criteria for ejaculate rejection in AI centres (Brun *et al*., 2002a; Theau-Clément *et al.,* 2003; Garcia-Tomás *et al*., 2006c). However, they were found to be lowly heritable (Tusell *et al.,* 2011d), which could be attributed in part to the great variability inherent in these traits due to factors involved in semen collection such as i.e. variation in the temperature of the artificial vagina that 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 quality traits was also lowly heritable (h^2 = 0.06). Therefore, genetic selection for increasing semen production by improving male libido and 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 of 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., 2011d).

The estimated h^2 for **ejaculate volume** and **sperm concentration** ranged from 0.06 to 0.13 and from 0.08 to 0.10, respectively for single ejaculates (Brun et al., 2009, 172 bucks of the INRA1001line; 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 two 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; Garcia-Tomás *et al.* (2006b) obtained similar values in a heterogeneous population constituted by purebred and crossbred bucks obtained from two paternal lines (0.38 ±0.03 and 0.39 ±0.03, respectively), whereas Tusell *et al.* (2011d) 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 two reported estimates of the genetic correlation between sperm concentration and ejaculate volume. Brun *et al.* (2009) reported an imprecise value of this parameter which cannot be considered to be different from zero (0.38 ± 0.45) whereas Tusell *et al.* (2011d) 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 two consecutive ejaculates, both values being higher than the corresponding values obtained by Lavara *et al.* (2011) and Brun *et al.* (2009) for individual ejaculates. The r for sperm production was estimated to be 0.33 in a previous research by Garcia-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.* (2011d and 2011c) reported values of 0.11 and 0.18 in two 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 frequency 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, Garcia-Tomás *et al.* (2006b) reported a r of 0.38 in a population of purebred and crossbred bucks, and Tusell *et al.* (2011d) a 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 (≈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 analyzed (mass or individual sperm motility) and on the evaluation procedure employed (a subjective or an 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 in a subjective manner, were similar to the ones obtained for mass

motility (0.08 and 0.24 for h^2 and repeatability respectively, Tusell *et al.,* 2011d). During the last decade, 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; Brun *et al.,* 2009; Lavara *et al*. , 2012a). However, these traits seem to be slightly more heritable than the subjective motility traits (0.18 and 0.12; Brun *et al.,* 2009; Lavara *et al.,* 2012a). Most of the sperm movement traits have shown lower h^2 and r estimates than that for individual motility (Brun *et al.,* 2009, Lavara *et al.,* 2012a), only VAP (average path velocity) had higher h^2 and repeatability than motility (0.18 and 0.30, Brun *et al.,* 2009). In general, the sperm motility and movement traits have shown low h^2 but the trait 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 which have an impact on prolificacy of rabbit does after AI. Some of them are the **sperm abnormalities and acrosome status** (Lavara *et al*., 2005; Piles *et al.,* 2012). To our knowledge only Lavara *et al.* (2012a) reported h^2 estimates of these traits. Previously, some authors have 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 been focused on the relationship between **sperm morphology traits** (length, width, area and perimeter of sperm head) and on 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 two 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, Napier, 1961, father-son regression, 47 sires bucks and 127 progeny bucks; h^2: 0.11-0.35 and r^2:0.26-0.46, Lavara *et al.,* 2008; complete pedigree information, 283 bucks of R line).

Nowadays, AI in rabbits is performed with fresh semen or cooled semen (at 16-19ºC) stored for short periods of time (24-48 h) with acceptable results on fertility and prolificacy. Perhaps, in the future, it could be necessary to use frozen semen as it is routinely used in other domestic animals for bio-security reasons. At 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-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 of 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. With this premise, a recursive model was used by Lavara *et al.* (2012b) for the analysys of 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 two maternal lines and their reciprocal crosses. Also Khalil *et al.* (2007) found a favourable direct heterosis effect for ejaculate volume (10.6%), sperm concentration (13.6%), sperm motility (10.5%) and for percentage of spermatozoa with abnormal form and dead spermatozoa (-21.5% and -20.3%, respectively) in the cross scheme of a Spanish maternal line and a Saudi breed performed to get two 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 spermatozoa with abnormal form and dead spermatozoa, respectively). However, the heterotic effects for seminal traits obtained in crosses between two paternal lines of rabbits were of low 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 proved 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 centers. However, this practice would be attenuated if the fertilizing 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 centers. Moreover, this index could be used in order 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 turns, 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 of the 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 centers 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 respect to 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 the 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 modeling some kind 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. Besides, they are very flexible and can learn arbitrarily complex patterns when enough data are available.
There is only one research assessing the predictive ability of male fertility from seminal traits in an independent set of data (Piles et al., 2012). In this experiment AI was performed after a small pre-selection of the ejaculates and 24h of dose storage at 18^oC. 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 of the seminal characteristics. Probably, the percentage of variation in fertility explained by the group of semen characteristics usually recorded is

very low (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 to 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 to the doe fertility, it determines the fertility of the mating as we will 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 direct criteria of selection is difficult, because it requires obtaining information from the result of AI performed with homoespermic 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 fertility, in order to improve male reproductive performance 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 the ones which 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.*, 1992; Theau-Clement *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., 1992; Theau-Clement *et al.,* 2011); pH of the ejaculate (O'Ferral and Meacham, 1968; Vrillon *et al.,* 1979; Bencheikh, 1993; Cofey, 1998, Piles *et al.*, 2012); percentage of total motile cells, some sperm movement characteristics measured with CASA systems (linearity index, amplitude of lateral head deplacement), percentage of abnormal sperm in the sample (Lavara *et al.*, 2005; Theau-Clement *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.*, 2012).

To our knowledge only the paper by Tusell *et al.* (2011c) studies the genetic relationship between male fertility and one of the traits mentioned above: the pH of the ejaculate. Because the semen pH is, in a major part, 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 fertilize. The study involved 243 bucks of the Caldes paternal line that led to obtain 6,613 records of fertility on 2,293 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 two 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 the 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) analyzed with a threshold model. The study reveals again, the negative and linear relationship that exists between the pH and the liability 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 eight 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 to be negative, but the precision of the estimates was poor, despite the relative 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, for checking the same objective for the other seminal traits, referred in the second paragraph of this section, the corresponding large and expensive experiments should be carried out too.

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

As it has been shown before, deciding which set of seminal characteristics should be measured and what levels of those traits are optimal in order to improve the production of fertile doses through some of its 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 implies improving the set of seminal characteristics that are important for obtaining 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 (2000) compared the male reproductive performance of a paternal line selected for growth and three maternal lines. Fertility rate did not differ among lines but prolificacy did, probably due to the selection process of the maternal lines. Garcia-Tomás *et al*. (2006a) found differences in fertility between two 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 fertilizing ability between two lines divergently selected for body weight at 63 days.

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 the quality of sperm at mating time of most of the males excess the threshold needed to reach fertility (Amann and Hammerstedt, 2002). Thus, although differences among males that are independent of 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 observance 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 genotype and environmental conditions on the farm could also lead to an interaction with the male genotype. By using the Character State model, Tusell *et al.* (2010) demonstrated that male contributions to fertility and litter size after AI were low but higher in magnitude than the ones obtained after natural mating. They found that there could be an interaction between the male genotype and AI conditions, and they postulate that it would be possible to find the conditions that give the maximum genetic progress to optimize the breeding program for male fertility and prolificacy under given conditions of semen utilization. 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 higher response under limited AI conditions than under AI conditions of semen utilization (*e.g.,* the commercial conditions), the superiority of the selected individuals with respect to the average population in the current conditions of semen utilization would be still reduced due to a scale effect, which might not compensate the investment required for selection (Kolmodin, 2003). Still, a favorable 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 centers.

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 parity are greatly conditioned by fetal survival which is only determined by the female. Thus, Piles *et al.* (2012) 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 parity (male genetic plus permanent environmental effects ≤ 0.01; Piles *et al.*, 2006), despite that natural mating was practiced and that 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 fertilized 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 and in a paternal line of rabbits (Piles *et al.*, 2005; David *et al.*, 2010; Tusell *et al.*, 2010; Piles and Tusell, 2011), which indicates that selection for the contribution to fertility of one of the sexes, if successful, could have a favorable correlated response in the contribution to the same trait of the other sex. This correlated response could be responsible, at least in part, to 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 crossbreed males and females from two 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 crossbreed females. Using paternal lines, García-Tomás *et al.* (2006a) found unfavorable 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 crossbreed male for improving the production of fertile doses did result more advantageous than the use of a purebred one and suggested the use of specialized lines to improve dose production in AI centers.

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 was found by Piles and Tusell (2011). 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.* (2011d) found that ADG had a slightly favorable correlation with sperm concentration (0.21, HPD95%= -0.03, 0.48) and a slightly unfavorable genetic correlation with ejaculate volume (-0.19, HPD95%=-0.47, 0.08). Moreover, ADG was genetically uncorrelated with all libido and seminal traits which 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 among semen production and quality with ADG. Some of their estimates had the opposite sign to the ones obtained by Tusell *et al.* (2011d) but they 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 favoring 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 two lines divergently selected for body weight at 63 days, 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. In a posterior study, same lines were compared by their fertilizing ability, and no differences were encountered between them (Theau-Clement *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 almost not genetically correlated. Two interesting consequences derive from this result. First is that selection for increasing ADG is not expected to have detrimental correlated effects on seminal traits involved in AI dose production. Second is that a multi-trait selection, including ADG and others seminal traits directly related to efficient dose production, is feasible. Nevertheless, the decision of defining the objectives of a program of selection has genetic and economic components. Consequently, the economic weighs of growth rate and the more interesting seminal traits need to be previously evaluated for correctly defining the criteria of selection.

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 residuals in the ejaculate and the suitability of the ejaculate for being used 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 it 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 to 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 been, in general, separately analysed, but as the outcome of an AI event depends on both sexes, the two contributions to the final expression of an AI outcome should be jointly analysed. The additive and product threshold

models are two different approaches for the analysis of fertility defined as a binary trait. Both types of models allow estimating 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 two 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 two conditionally independent unobserved variables corresponding to the fertility of the two individuals involved in the mating. This approach could better reflect the biology of the fertility than the additive model. Within the product model, a success in AI can only be achieved when both members of the mating are fertile, whereas with the additive model it would be possible to fit a successful mating of a highly fertile female, that makes liability to exceed the fertility threshold, with an infertile male, or vice versa, which is not biologically possible. Besides, the product threshold model allows extracting more information from the data than the additive threshold model because it provides different estimates of the effect of factors affecting each unobserved phenotype as well as obtaining the probabilities of fertility success for each sex, which allows evaluating 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 coming 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 AI occurs in a sequential order (*i.e*., an observation of a certain value of the trait requires to 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 a success or a failure to AI is observed. This approach has the advantage that it allows including 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, thus they did not have the chance for expressing the trait of interest). However, the assumption of noninformative censoring is probably not correct in most of the data, because females are commonly culled after several unsuccessful matings. Therefore, unexpected results and misleading interpretation can arise (Kalbfleisch and Prentice, 1980). González-Recio *et al*. (2005) adapted three 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 in augmenting the data by sampling from a left truncated distribution every time that a censored record falls into to 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 for taking into account censored records. Finally, another approach to handle censored records of a sequential trait is to use a particular type of proportional hazard models, 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 parity. In 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 three 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 done by the usage of multiple trait models or some extension of them, which are the recursive models. The interest in using this last approach is that these models allow for considering the effect of seminal traits on the phenotypic expression of fertility but they 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 excursiveness are two types of relationships. The first one indicates that changes in one trait affect a second trait and, in turn, the second trait affects the first trait. The second one refers to a situation where one trait affects the other but the last does not affect the first one. 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.* (2011c) 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 on this subject is recent and therefore, its knowledge is in an early stage of development in this species.

Results from several AI centers 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 specialized 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 could not be effective. Although, the existence of an interaction between male genotype and the AI conditions suggest that it could be possible to find the AI conditions that give the maximum genetic progress in a breeding program for male reproductive performance under given conditions of semen utilization. However, despite of obtaining higher response under optimal AI conditions than under AI conditions of semen utilization (e.g., the standard commercial conditions), the superiority of the selected individuals with respect to the average population in the current conditions of semen utilization 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 in order to confirm this result requiring coordinated participation of nucleus of selection, AI centers, laboratories and research centers because the amount of information needed to get precise estimates of genetic parameters is great.

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 the current 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 selecting for increasing the total number of sperm in the ejaculate, which is a trait moderately heritable and therefore, it 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 established yet. Again, further research is needed in order to ascertain the correlated effect of selection for increasing semen production on semen quality or male reproductive performance, once a clear definition of semen quality is known.

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

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REFERENCES

Al-Makhzoomi A., Lundeheim N., Haard M., Rodríguez-Martínez H. 2008. Sperm morphology and fertility of progeny-tested AI dairy bulls in Sweden. *Theriogenology,* 70: 682-691.

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

Albert J.H., Chib S. 2001. Sequential ordinal modelling with applications to survival data. *Biometrics 57:829-836.*

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, 2004 September, Puebla, Mexico.*

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

Bencheikh N. 1995. The effect of frequency of ejaculation on semen characteristics and sperm output in the rabbit. *Ann. Zootech., 44:263-279.*

Blasco A. 1996. Genetics of litter size and does fertility in the rabbit. *In: 6th World Rabbit Congress, 1996 July, 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: 13èmes Journ. Rech. Cunicole, 2009 November, Le Mans, France. 11:17-18.*

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

Brun J. M., Theau-Clement M., Bolet G. 2002b. The relationship between rabbit semen characteristics and reproductive performance after artificial insemination. *Anim. Reprod. Sci., 70:139-149.*

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.*

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, 1990 July, Edinburgh, Scotland, 16:366-369.*

Coffey D. S. 1988. Androgen action and the sex accessory tissues. *In: Knobil E., Neill J. D. (Eds.). Physiology of Reproduction. Raven Press, New York, USA, 1081-1109.*

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

David I., Carabano M. J., Tusell L., Diaz C., Gonzalez-Recio O., Lopez 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.*

Ducrocq V., Humblot P. 1995. Genetic characteristics and evolution of semen production of young normande bulls. *Livest. Prod. Sci., 41:1-10.*

Estany J., Camacho J., Baselga M., Blasco A. 1992. Selection response of growht rate in rabbit for meat production. *Genet. Sel. Evol., 24:527-537.*

Foote, R. H. 2003. Fertility estimation: A review of past experience and future prospects. *Anim. Reprod. Sci., 75:119-139.*

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.*

Garcia-Tomás M., Sanchez J., Rafel O., Ramon J., Piles M. 2006a. Reproductive performance of crossbred and purebred male rabbits. *Livest. Sci., 104:233-243.*

Garcia-Tomás M., Sanchez 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.*

Garcia-Tomás M., Sanchez J., Rafel O., Ramon J., Plies M.. 2006c. Heterosis, direct and maternal genetic effects on semen quality traits of rabbits. *Livest. Scie., 100:111-120.*

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

Garreau H, Szendro ZS, Larzul C, 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, 2010 July, Valencia, Spain.*

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

Gianola D., Foulley L. 1983. Sire evaluation for ordered categorical data with a threshold model. *Genet. Sel. Evol. 15:201-224.*

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

Gomez 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,1998 January, Armidale, Australia. 25:552-555.*

Gómez E.A., Rafel O., Ramon J. 2002. The Caldes strain (Spain). *Options Méditerranéennes. Série B, Études et Recherches, 38:193-198.*

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.*

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

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

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

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, 2008 June, Verona, Italy.*

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, 2010 August, Leipzig, Germany.*

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.*

Koops W. J., Grossman M., den Daas J.H.G. 1995. A model for reproductive efficiency in dairy bulls. *J. Dairy Sci., 78(4): 921-928.*

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

Lavara R, David I, Mocé E, Baselga M, Vicente J.S. 2012b. Environmental and male variation factors of freezability in rabbit semen. Submited to *Theriogenology.*

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

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), 2009 May, Zaragoza, Spain, I:96-98.*

Lavara R., Moce 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.*

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.*

Lavara R, Vicente J.S, Baselga M. 2012a. Estimation of genetic parameters for sperm quality traits and growth rate in a paternal rabbit line. Submited to *Theriogenology*.

Marco-Jimenez 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*.

Matheron G., Poujardieu B. 1984. La génétique du lapin : le point, les perspectives. *In : Proc. 3rd World Rabbit Congress, 1984 June, 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, 2010 August, Leipzig, Germany.*

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

Mocé E, Vicente J.S. 2009. Rabbit sperm cryopreservation: A review. *Anim. Reprod. Sci., 110:1-24*.

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.*

Morrell J. M. 1995. Artificial insemination in rabbits. *Br. Vet. J., 151:477-488.*

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.*

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

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

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

Piles M., Garcia 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.*

Piles M., Tusell L. 2011. Genetic correlation between growth and female and male contributions to fertility in rabbit. *J. Anim. Breed. Genet.(in press).*

Piles M., Díez J., del Coz J.J., Montañés E., Quevedo J.R., Ramon J, Rafel O., López-Béjar M., Tusell Ll. 2012. Predicting male fertility from semen quality traits: performance of several parametric and non-parametric procedures. *Submitted to J. Anim. Sci.*

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

Rochambeau H. de R.1988. Genetics of the rabbit for wool and meat production. *In: Proc. 4th World Rabbit Congress, 1988 October, Budapest, Hungary.*

Rochambeau H.de, 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.*

Rouvier R. 1980. Génétique du lapin (*Oryctolagus cuniculus*). *In: Proc. 2nd World Rabbit Congress, 1980 April, Barcelona, Spain.*

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.*

Theau-Clement 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, 2011 November, Le Mans, France*

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

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

Tusell L. 2011a. Exploring the genetics of the efficiency of fertile AI dose production in rabbits. *Phd diss. Dept. of Ciencia Animal. Universitat politècnica de València.Valencia, Spain. Available online at https://www.educacion.gob.es/teseo/mostrarRef.do?ref=944328 and http://www.pantomaquet.com/thesis/*

Tusell, L., David, I., Bodin, L., Legarra, A., Rafel, O., López-Bejar, M., Piles, M. 2011b. Using the product threshold model for estimating separately the effect of temperature on male and female fertility. *J. Anim. Sci., 89*, 3983-3995

Tusell L., García-Tomás M., Baselga M., Rekaya R., Rafel O., Ramon J., López-Bejar M., Piles M. 2010. Genotype x AI conditions interaction for male effect on fertility and prolificacy. *J. Anim. Sci., 88, 3475-3485.*

Tusell L., Legarra A., Garcia-Tomás M., Rafel O., Ramon J., Piles M. 2011c. Different ways to model biological relationships between fertility and pH of the semen in rabbits *J. Anim. Sci., 89,1294-1303*

Tusell L., Legarra A., García-Tomás M., Rafel O., Ramon J., Piles M. 2011d. Genetic basis of semen traits and their relationship with growth rate in rabbits. *J. Anim. Sci. jas.2011-4165; published ahead of print November 18, 2011, 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.*

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, 2000 July, Valencia, Spain A:273-277*

Wasserman L.A. 2006. All of nonparametric statistics. *New York: Springer. ISBN 10 0387251456*

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

Wolf J. 2010. Heritabilities and genetic correlations for litter size and semen traits in Czech large white and Landrace pigs. *J. Anim Sci., 88:2893-2903.*

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. Vol. 28, p. 106.*

Wright S. 1934. An analysis of variability in number of digits in an inbred strain of Guinea pigs. *Genetics 19:0506-0536.*