



Predicting fertility from seminal traits: Performance of several parametric and non-parametric procedures



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ABSTRACT

This research aimed at assessing the efficacy of non-parametric procedures to improve the classification of the ejaculates in the artificial insemination (**AI**) centers according to their fertility rank predicted from characteristics of the AI doses. A total of 753 ejaculates from 193 bucks were evaluated at three different times from 5 to 9 months of age for 21 seminal variables (related to ejaculate pH and volume, sperm concentration, viability, morphology and acrosome reaction traits, and dose characteristic) and their corresponding fertility score after AI over crossbred females. Fertility rate was categorized into five classes of equal length. Linear Regression (**LR**), Ordinal Logistic Regression (**OLR**), Support Vector Regression (**SVR**), Support Vector Ordinal Regression (**SVOR**), and Non-deterministic Ordinal Regression (**NDOR**) were compared in terms of their predictive ability with two base line algorithms: MEAN and MODE which always predict the mean and mode value of the classes observed in the data set, respectively. Predicting ability was measured in terms of rate of erroneous classifications, linear loss (average of the distance between the predicted and the observed classes), the number of predicted classes and the F_1 statistic (which allows comparing procedures taking into account that they can predict different number of classes). The seminal traits with a bigger influence on fertility were established using stepwise regression and a nondeterministic classifier. MEAN, LR and SVR produced a higher percentage of wrong classified cases than MODE (taken as reference for this statistic), whereas it was 6%, 13% and 39% smaller for SVOR, OLR and NDOR, respectively. However, NDOR predicted an average of 2.04 classes instead of one class predicted by the other procedures. All the procedures except MODE showed a similar smaller linear loss than the reference one (MEAN) SVOR being the one with the best performance. The NDOR showed the highest value of the F_1 statistic. Values of linear loss and F_1 statistics were far from their best value indicating that possibly, the variation in fertility explained by this group of semen characteristics is very low. From the total amount of traits included in the full model, 11, 16, 15, 18 and 3 features were kept after performing variable selection with the LR, OLR, SVR, SVOR and NDOR methods, respectively. For all methods, the reduced models showed almost an irrelevant decrease in their predictive abilities compared to the corresponding values obtained with the full models.

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1. Introduction

Artificial insemination (AI) in rabbit commercial farms is performed with pooled semen from several bucks at a 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 and impedes making right decisions regarding male replacement and management in AI centers. Obtaining an accurate prediction of the fertilizing potential of ejaculates would alleviate those limitations increasing the economical benefits of AI centers.

However, the relationship between the seminal traits and the result of insemination is still not clearly established. Most of the studies have shown that the proportion of the observed variance that is explained by models including the set of traits usually recorded in the AI centers is very low (Brun et al., 2002; Gadea et al., 2004; García-Tomás et al., 2006a). This could be due to: (i) the experimental design. Thus, when AI is performed with semen obtained after a strong pre-selection of the ejaculates, the observed variability is reduced. (ii) the variables used as fertility markers, the way how they are measured and the time when they are recorded with respect to AI time could not be adequate. (iii) The methods used for variable selection and prediction could be too rigid for modeling some kind of relationships. (iv) The use of variables with not relevant or redundant information may mislead the classifiers, leading to dismiss their performance. Finally, (v) It could be possible that, actually, the part of the observed variance of this trait (i.e. fertility at kindling) due to the variation of the characteristics of the ejaculates accepted for AI is very low, being much more important features of the doe and environmental factors. In this case the search of a method, based on features of the ejaculate, to explain a large part of the variation of the AI, would be necessarily unsuccessful.

Objectives of this work were to answer the following: (1) is it possible to improve the accuracy of fertility prediction by using more flexible procedures?; (2) how much the information provided by seminal variables can improve fertility prediction?; (3) among them, which are the ones with highest influence on male fertility?

2. Material and methods

2.1. Animals and data

The research protocol was approved by the animal care and use committee of the Institut de Recerca i Tecnologia Agroalimentàries (IRTA).

2.1.1. Animals

Males belonged to the Caldes line selected for growth rate during the fattening period (Caldes line: Gómez et al., 2002a). Bucks were bred and reared in an experimental farm in Caldes de Montbui (Barcelona, Spain). This farm has insulated walls and roof and the proper cooling equipment to avoid animal exposure to extreme temperatures. After weaning at 32 d, males were housed in collective cages of eight individuals with a photoperiod

of 16 h light/d. Animals were fed a commercial diet of rabbit pellets *ad libitum* (15.5% crude protein, 2.3% fat, and 17.2% fiber) until 60 d. Subsequently, they were housed on the farm of the AI centre under the same environmental conditions as the experimental farm and placed beside it, and they were restricted to 180 g/d of another commercial diet (16% crude protein, 4.3% fat, and 17% fiber). Fresh water was always available.

2.1.2. Semen collection

All males began training to use an artificial vagina at 4.5 month of age. A homemade polyvinyl chloride artificial vagina containing water at a temperature of 50 °C was used. One ejaculate was collected per male each week for the first two weeks. After this period, two ejaculates per male were collected each week, with an interval of 30 min between collections. From 5 to 9 months of age, all males were evaluated at three different times for seminal quality traits and their corresponding fertility score after AI over crossbred females in a commercial farm.

2.1.3. Evaluation of the seminal traits and AI

Ejaculates were stored in a dry bath at 35 °C until evaluation for no more than 15 min after collection. Ejaculates containing urine and calcium carbonate deposits were discarded, and gel plugs were removed. The ejaculate volume was assessed with a micropipette and the pH of the semen was determined using a 507 Crison pH-meter (Crison Instruments, SA, Alella, Barcelona, Spain). Aliquots (25 µl) of ejaculate were diluted 1:4 (vol/vol) in a commercial extender (Galap, IMV Technologies, Saint Ouen sur Iton, France) to assess the individual motility under a microscope with a phase-contrast optic (Nikon, Lewisville, TX) at 400 × magnification, according to a subjective scale from 0 to 5 corresponding to a percentage of sperm showing progressive movement of: 0% to 10%, 11% to 25%, 26% to 50%, 51% to 70%, 71% to 90%, or 91% to 100%, respectively (Roca et al., 2000).

To prepare the AI doses, a small pre-selection of ejaculates was performed, discarding for AI only those with individual motility lower than 2 and a percentage of dead spermatozoa higher than 50%. Semen suitable for AI was immediately prediluted 1:1 (vol/vol) with a commercial semisolid extender (Cunigel, IMV Technologies, Saint Ouen sur Iton, France). After evaluation, the ejaculates obtained per male each day were pooled and cell sperm concentration (**Conc**; millions of spermatozoa per mL) was measured by using a sperm cell counter (NucleoCounter SP-100, Chemometec A/S, Allerød, Denmark). The resultant pool of ejaculates was divided into two parts which were diluted up to 10×10^6 spermatozoa/mL and 40×10^6 spermatozoa/mL, to obtain AI doses at two different sperm concentrations (**DC**). The dilution rate (**Dilu**) was also recorded. Semen doses were stored in straws of 0.5 mL at 18 °C for 24 h until their use.

After 24 h, an AI dose (at each dose concentration) of each male dose was processed to artificially induce the acrosome reaction. The AI dose was tempered at 37 °C for 30 min to allow the liquefaction of the semisolid extender. After tempering, samples were centrifuged and supernatants aspirated. The pellets were then resuspended to

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