Different Ways To Model The Biological Relationship Between Fertility And The pH Of Semen In Rabbits

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Introduction

Fertility (**F**) is one of the most economical important traits in animal breeding. However, due to its low heritability, finding seminal quality traits related with male reproductive performance has been the aim of several studies during years. The hydrogen-ion concentration of the semen (**pH**) is considered an indicator of the general metabolism of the semen because it is determined by the number and the activity of the spermatozoids in the ejaculate. Thus, pH constitutes the expression of several seminal quality traits, and therefore, it is of special interest to determine its relationship with fertility. Fertility and the pH could have a complex biological relationship. The pH could affect the phenotypical expression of fertility but it is not an external effect to the animal and thus, in turn, the pH could also have genetic and permanent effects that modulate its own expression. Recursive models can accommodate this kind of biological relationships. They were introduced in the animal breeding context by Gianola and Sorensen (2004). This work aimed to study the relationship between the semen pH and fertility. Different ways to model fertility and the pH are presented: by ignoring genetic and environmental correlations between both traits; by the classical Multiple-trait Model and, finally, by a Recursive Multiple-trait Model.

Material and methods

Data. Bucks coming from Caldes sire line were used. They were bred and reared on an experimental farm belonging to IRTA in Caldes de Montbui (Barcelona, Spain) with a photoperiod of 16 h light/d. Bucks production period ranged from 5 to 9mo old. Crossbred does Prat x V (Prat line and V line, both lines selected for litter size at weaning) were used. They were reared and housed on a commercial farm (Montmajor, Barcelona, Spain) and followed a semi-intensive reproductive rhythm: first mating at about 4.5 mo old, with subsequent 42 d reproductive cycles. Two ejaculates per male per week were collected with artificial vagina, leaving an interval of 30 min between both collections. Ejaculates containing urine, calcium carbonate deposits and individual motility lower than 2 (subjective scale from 0 to 5) were discarded. The pH of the ejaculate was determined using a pH-meter and the pH of the semen produced per each male in the same day was calculated leading to a total of 430 data. The semen from each buck was pooled, divided in two parts and diluted until 10 and 40 x 10^6 spermatozoa/mL to obtain the AI doses. Semen doses were applied in a

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commercial farm, 24h after its preparation. Ovulation was hormonally induced. Diagnosis of pregnancy was made by palpation, 14 d after AI, confirming the result at parity. The assigned fertility (**F**) score was 1 when the female was diagnosed as pregnant and 0 otherwise. A total of 6,613 data of F was obtained. The experimental work was developed from June 2006 to October 2007 involving 243 males and 2,256 females.

Models and statistical analyses. According to a Recursive Gaussian-Threshold Model notation, the *j*th pair of records for an individual *i* have the following linear relationship:

$$\mathbf{\Lambda}\begin{pmatrix} y_{ij,pH} \\ l_{ij,F} \end{pmatrix} = \begin{pmatrix} \mathbf{x}'_{ij,pH} & 0 \\ 0 & \mathbf{x}'_{ij,F} \end{pmatrix}\begin{pmatrix} \boldsymbol{\beta}_{pH} \\ \boldsymbol{\beta}_{F} \end{pmatrix} + \begin{pmatrix} u_{i,pH} \\ u_{i,F} \end{pmatrix} + \begin{pmatrix} pm_{i,pH} \\ pm_{i,F} \end{pmatrix} + \begin{pmatrix} 0 \\ pf_{l,F} \end{pmatrix} + \begin{pmatrix} pmd_{ij,pH} \\ pmd_{ij,F} \end{pmatrix} + \begin{pmatrix} e_{ij,pH} \\ e_{ij,F} \end{pmatrix}$$

Being y_{pH} the observed pH and l the unobserved liability for fertility. $\mathbf{x}_{ij,k}$ ($k= pH,F$) is the corresponding row of an incidence matrix. $\boldsymbol{\beta}_{pH}$ is the vector of systematic effects for pH which includes day of ejaculate collection and age of the male. $\boldsymbol{\beta}_{F}$ is the vector of systematic effects for F which includes concentration of the AI dose, physiological status of the female, and the combined effects of day-inseminator, and buck age-building. The $u_{i,k}$ is the male genetic additive effect, $pm_{i,k}$ is the male permanent environmental effect, $pf_{l,F}$ is the female genetic plus permanent environmental effect for F, $pmd_{ij,k}$ is the environmental permanent effect resulting from the combination of male and day of AI, and, $e_{ij,k}$ is the residual.

$\mathbf{\Lambda} = \begin{bmatrix} 1 & 0 \\ -\lambda_{F\leftarrow pH} & 1 \end{bmatrix}$ is the matrix of structural coefficients, and $\lambda_{F\leftarrow pH}$, describes and measures the strength of the phenotypical rate of change of the liability of F with respect to a change in pH. $\lambda_{\mathrm{F\leftarrow pH}}$ in the models were always estimated as systematic effects in the equation for F (López de Maturana et al., 2007). Different types of $\lambda_{\mathrm{F\leftarrow pH}}$ were assumed (null, covariate or cross-classified effect) in order to accommodate null, linear and non-linear recursiveness (López de Maturana et al., 2009) of pH on F, respectively. The Gaussian-Threshold models used to describe pH and F relationship were: 1) Model **UMM**$_{\mathbf{cov}}$: which ignored the genetic and environmental correlations and had $\lambda_{\mathrm{F\leftarrow pH}}$ as a covariate; 2) Model **BMM**$_{\mathbf{0}}$: a Bivariate Mixed Model with $\lambda_{\mathrm{F\leftarrow pH}}=0$; 3) Model **RMM**$_{\mathbf{cov}}$: a Recursive Bivariate Mixed Model with $\lambda_{\mathrm{F\leftarrow pH}}$ as a covariate and the residual covariance fixed to 0 to achieve identification of the marginal likelihood (Varona et al., 2007) and, 4) Model **RMM**$_{\mathbf{cross}}$: Recursive Bivariate Mixed Model and $\lambda_{\mathrm{F\leftarrow pH}}$ as a cross classified effect. Inference on the parameters of interest was performed under a Bayesian approach via Gibbs sampling. Single chains of 500,000 iterations were run for all the models, with 50,000 iterations of burn-in.

Prediction ability of fertility. Posterior predictive distribution of fertility data was calculated in the different models. A correct prediction of fertility was assumed when the difference in magnitude between the observed value and the mean of the posterior distribution of the data was lower than 0.5

Results and discussion

Prediction ability of fertility data was equal in all the models: the 85% of success fertility data and the 71% of failure fertility data were well predicted. Thus, although models differed in complexity, no differences between them were found in their prediction ability. This is probably due to the imprecision of estimates of the parameters and also due to the fact that after fitting the models the estimates were such that models were finally very similar.

The estimated values of $\lambda_{F\leftarrow pH}$ as a covariate or as cross-classified effect are consistent across all the models indicating that an increase in the pH of the semen leads to a decrease in fertility and this relation seems to be almost lineal (see Figure 1). This is in accordance with previous studies that evidenced negative correlation between the pH of the ejaculate and fertility (Brun et al., 2002).The higher the concentration and the motility of the spermatozoids in the ejaculates, the higher the production of lactic acid due to the greater metabolic activity and the lower the pH (More O'Ferrall and Meacham, 1968). This relationship has been previously reported by Garcia-Tomás et al., 2006.

Table 1 shows the estimates of the marginal posterior distributions of the heritability (**h^2**) and genetic correlation (**rg**). The h^2 and repeatability (**r**; around 0.19) of male F were similar across all the models and higher than the obtained values after natural mating in the same line (h^2=0.013, r=0.044; Piles et al., 2005). The h^2 for the pH was equal in all the models and r was around 0.21. These results are in accordance with the corresponding values obtained before in rabbits (r= 0.38, Garcia-Tomás et al., 2006; h^2=0.05, r=0.17 Brun, 2009). Although very imprecise, there is some evidence of a negative rg between F and the pH because the P(rg<0) was 0.74 in model BMM_0, 0.67 in RMM_{cov} and 0.68 in RMM_{cross}. To our knowledge, there are no previous published estimates of genetic relationship between fertility and pH of the semen. However, pH has been found genetically correlated with several motility parameters which, in turn, are related to fertility (Brun et al., 2002; 2009).

Table 1: Heritabilities (h^2) and genetic correlation (rg) of male fertility (F) and pH (highest posterior density intervals at 95% in brackets) obtained in the different models.

Model	Trait	h^2 (diagonal), r_g (above diagonal)	
UMM_{cov}	pH	0.13 [0.01, 0.25]	-
	F		0.10 [0.02, 0.20]
BMM_0	pH	0.12 [0.004, 0.24]	-0.25 [-0.99, 0.58]
	F		0.11 [0.02, 0.19]
RMM_{cov}	pH	0.13 [0.001, 0.25]	-0.22 [-0.99, 0.60]
	F		0.10 [0.01, 0.19]
RMM_{cross}	pH	0.11 [0.005, 0.23]	-0.25 [-0.99, 0.65]
	F		0.11 [0.03, 0.20]

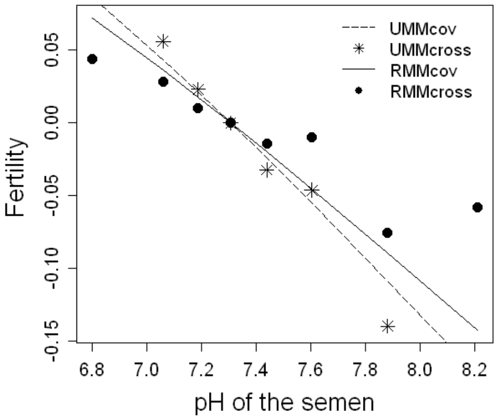

Figure 1: Effect of pH on fertility ($\lambda_{F\leftarrow pH}$) in the observed scale in the different models.

Conclusion

There exists a negative effect of the pH of the semen on fertility and this effect is almost linear. All the models predicted fertility data reasonably well, showing similar ability to do it. To take into account recursiveness with a cross-classified effect in a bivariate model, no constraint is needed because the covariance between traits is no linear any more. No differences in results were obtained between considering or not genetic and environmental correlations between the pH and fertility and assuming or not recursiveness between traits. This is because the magnitude of the effect of the pH on F is not big enough and same results are obtained despite of models differed in complexity. However, due to the low accuracy of the covariance estimates, more research is needed to confirm this result.

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