Transcriptomic Prediction of Piglet Vitality from Umbilical Cord Blood of Purebreds and Crossbreds born in the Same Litter – Comparison of Meishan and Large White Sows

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**ABSTRACT:** Crossbred piglets from Large White sows showed faster early development as compared to their purebred counterparts (P<0.01). In Meishan sows, crossbred females were heavier than purebred females from d1 on, but no difference in relative growth rate was found between purebreds and crossbreds. Piglet growth and behaviour traits related to vitality were loosely correlated. However, both trait categories were involved in differences between the 8 genetic groups defined by the combination of sex and genotype. From this experimental design, 2,247 differentially expressed genes were identified with a significance threshold of 1%, including 39 genes related to discrepancies between genetic groups, and 45 and 1962 genes differentially expressed according to sex and genotype respectively. The co-variation between the umbilical cord transcriptome profiles and phenotypes was measured. Clusters of genes that co-vary with growth rate in early lactation were identified.

**Keywords:** piglet; vitality; crossbred effects; transcriptomics

**Introduction**

Genetic improvement of prolificacy is effective in many pig maternal lines, but is associated with high piglet losses during the first days after birth. These losses have a considerable impact on the profitability of pig production. Previous experimental research estimating genetic trends in the French Large White population showed that despite higher body weight, modern piglets suffer from increased difficulties at birth related to a deterioration of their maturity as compared to old-type piglets (Canario et al. (2007)). In selected lines, piglets that survive the first days exhibit a high potential for growth, which suggests that the biological dysfunction underlying mortality is likely to occur during the perinatal period. Although piglets with high genetic merit for survival are more mature at birth (Leenhouwers et al. (2002)), survival traits are loosely improved by direct selection. The genetic determinant of losses during lactation is different from that of stillbirth (Huby et al. (2003)). Alternatively, selecting for piglet maturity looks like an attractive strategy.

Global gene expression profiling of whole blood was recently validated in human to identify molecular signatures of development at birth (Cohen et al. (2007)). Whole blood is a tissue of interest for biomarker detection due to its key role in biological processes in almost all tissues and organs in the body. Liew et al. (2006) observed that 80% of genes expressed in peripheral organs are detected in whole blood. Furthermore, the simple and non invasive sample collection makes its use attractive. We formulate the hypothesis that expression profiling of umbilical cord blood collected at birth highlights the metabolisms involved in piglet vitality. The objective of the present study was to identify predictors of piglet vitality. The parental genetic influences on early life development were analyzed from the comparison of purebred and crossbred piglets born together from a Meishan or a Large White maternal environment.

**Materials and Methods**

**Genetic design and animal management.** The experiment took place in 2010-2011 in the experimental herd of Le Magnéraud and was designed to take advantage of two extreme breeds for piglet maturity and survival: the Large White (LW) and the Meishan (MS) breeds (Le Dividich et al. (1991)). A total of 24 LW and 24 MS second parity sows were inseminated with mixed semen from the 2 breeds. Three mixtures of semen were made (3x8=24). Sows were managed in a batch system, with a 3-week interval between successive batches. During gestation, sows were kept in groups of females from the same breed. During lactation, sows were housed in individual pens. Farrowing was not induced.

**Data.** The current study was based on the litters from 8 LW sows and 9 MS sows producing a total of 245 piglets. Transcriptome analyses were carried out on 3 to 8 newborn piglets from each litter, resulting in a total of 21 LWLW, 19 MSLW, 22 LWMS and 19 MSMS samples, with similar numbers of males and females in each group (N=9 to 12). Eight genetic groups (GG) according to sex and genotype were thus created. At birth, piglets were weighted, sexed and individually identified. The crown-rump length was measured. Blood samples from the umbilical cord were collected in PAXgene Blood RNA Tube, (PreAnalytiX). After cutting, the remaining part of the umbilical cord was ligatured with a surgical silk. Samples were stored at -80°C. Piglet genotype was confirmed from a tail sample. Piglets were weighed again on d1 (24h after birth of the last piglet born in the litter), on d3 and d7. Piglet mobility (MOBIL) in the weighing box was recorded according to a scale of 0 (motionless), 1 (moving in half surface of the box) and 2 (moving in the all surface). The body mass index (BMI) and ponderal index (PI) were calculated at birth: BMI = body weight / crown-rump length\textsuperscript{2}; PI = body weight / crown-rump length\textsuperscript{3}. Weight gain relative to birth weight was calculated on d1 (WGR1), d3 (WGR3) and d7 (WGR7).

**Statistical analyses.** Analyses were performed with the R software (R Development Core Team, 2008). Phenotypic traits between the 4 genotypes (245 piglets)
were compared with use of a mixed linear model including the fixed effects of the interaction between genotype (GEN) and sex (8 GG), the batch effect (4 levels), the semen mixture (3 levels) and the random effect of the litter of birth, using the REML methodology in the lmer package:
\[ Y = \text{sex} \times \text{GEN} + \text{batch} + \text{mixture} + \text{litter} \]

Least square means and differences were estimated. Multivariate analyses (ade4 package) were used to analyse correlation patterns between phenotypic traits. Principal component analyses (PCA) were carried out following the methodology described in Canario et al (2009) to estimate within-GG and between-GG variability on residuals obtained from the following linear model: \( y = \text{batch + semen mixture + error} \) where \( y \) is the observation. The statistical significance of the between-GG variability was checked using a Monte-Carlo rank test.

For blood transcriptome analysis, we used a 60K microarray (Agilent Technology) from the 44K Porcine (V2) Gene Expression Microarray. After quality control and a normalization step, 81 microarray data containing 34505 spots were used to identify differentially expressed genes (DEG) in the 8 GG. We first implemented exploratory analyses (PCA). We tested for the significance of fixed and random effects by comparison of complete model and reduced model (without tested effect) using the ML and REML methodology respectively. The fixed effects of batch and mixture were significant on the expression of some of the spots. Consequently, following the methodology proposed by Voillet et al. (2014), we applied a mixed linear model on each spot of the full model (model (1)) followed by correction for multiple tests with Bonferroni or False Discovery Rate (FDR)). The list of DEG was then partitioned into 4 sub-models using the Bayesian information criterion (BIC): sub-model (1) reveals interaction effects, sub-model (2) = sub-model (1) without interaction, sub-model (3) = sub-model (2) – sex, sub-model (4) = sub-model (2) – GEN. Functional annotation of genes from sub-model 1 was based on Gene Ontology (GO) using web application GeneCodis (Tabas-Madrid et al. (2012)). Finally, we correlated phenotypes (residuals) and gene expression data from the 81 piglets with a sparse Partial Least Square analysis with regression mode to identify parsimoniously the relationships between both data categories, in an attempt to predict piglet vitality from genes expression (Lê Cao et al. (2008)). The co-variation between the umbilical cord transcriptome profiles and phenotypes was measured from Clustered Image maps.

Results and Discussion

Phenotypic Analysis. In LW sows, crossbred females were heavier than purebred females until d3 and next, the difference was significant in both sexes (P<0.01); males were heavier than females in purebreds but not in crossbreds. In MS sows, the only difference found was on crossbred females that were heavier than purebred females from d1. To facilitate GG comparison, we studied relative weight gains (Table 1). The within-GG PCA showed low correlation between growth traits and mobility traits. However, the 2 trait categories were important to discriminate MSxLW females from other GG on the 2\(^{nd}\) axis from the map of canonical weights. Indeed, although most of the variability was observed within-GG, between-GG variation was significant (14% of total variation, \( P<0.0001 \)).

Table 1. Least square means for growth and behaviour of purebred and crossbred piglets from Large White (LW) and Meishan (MS) sows.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Sex</th>
<th>LW⁺</th>
<th>MSxLW⁻</th>
<th>LWxMS⁻</th>
<th>MS⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>M</td>
<td>1.83</td>
<td>1.86</td>
<td>1.22</td>
<td>1.09</td>
</tr>
<tr>
<td>WGR7</td>
<td>M</td>
<td>0.92</td>
<td>1.24</td>
<td>0.92</td>
<td>1.09</td>
</tr>
<tr>
<td>MOBIL7</td>
<td>M</td>
<td>1.86</td>
<td>2.32</td>
<td>1.83</td>
<td>1.48</td>
</tr>
</tbody>
</table>

\( ^{⁺} \text{BW} = \text{birth weight in kg}, \text{WGR7} = \text{Weight gain at d7 relative to BW}, \text{MOBIL7} = \text{score of mobility of piglet in the weighing box at d7} \)

Transcriptome Analysis. A total of 2,247 DEG were identified with a significance threshold of 1% with the Benjamini–Hochberg correction. With the same correction of BH 1%, we found 39 DEG associated with the interaction, 228 DEG for the addition of sex and genotype, 45 DEG for sex alone and 1962 DEG for genotype alone. Accordingly, the PCA on all expressed genes confirmed the main role of genotype (more then GG) on data structure (results not shown). Then, we focused our interest on the DEG for interaction between sex and genotype. The \( XIST \) gene located on chromosome X and essential for the spread of X-inactivation, ranked first in this list (p-value) and was up-regulated in LW females as compared to other female genotypes (figure 1). This difference translates into opposite gradients (down-regulation in LW females) for other DEG located on SSCX (e.g. \( COX7b, \text{cytochrome c oxidase subunit VIIb} \)). More interactions were found for important functional genes (e.g., \( SEPP1 \) (selenoprotein P, plasma, 1) on SSC16, Figure 2). The sparse-PLS indicated clusters of genes that co-vary with growth rate (WGR3 and WGR7, Figure 3) of individuals on the right side of the sample map (Figure 4). Although not accounted for in the analysis, the representation of individuals shows a partitioning according to genotype. These preliminary results will be confirmed and further investigation will indicate the biological links between these genes and phenotypes.

Conclusion

The use of mixed semen on Large White and Meishan sows enabled a fine analysis of genetics of piglet vitality, considering phenotypic and transcriptomic information in purebred and crossbred piglets. Results highlight different gene expression between genotypes, and some relevant genes differentially expressed according to sex x genotype. Additional investigations will be realized to confirm associations with piglet vitality traits.
Figure 1. Expression of gene XIST in the umbilical cord blood of piglet genetic groups defined by sex (F: female, M: male) and genotype (LW: Large White, MS: Meishan). Piglets were produced with use of mixed semen. Expression was normalized on log 2 scale. For crossbreds, the combination indicates boar breed x dam breed.

Figure 2. Expression of gene SEPP1 in the umbilical cord blood of 8 piglet genetic groups defined by sex (F: female, M: male) combined with genotype (LW: Large White, MS: Meishan). Legend: see figure 1.

Figure 3. Correlation between gene expression and phenotypes selected from the sparse regression analysis. Selected genes and phenotypes are represented through their projection on the 2 first components of a correlation circle.

Figure 4. Sample map of piglets obtained from the sparse regression analysis. Females are indicated with circles and males with squares. Colours attributed to each of the 4 genotypes are the same as in Figure 2.

Literature Cited