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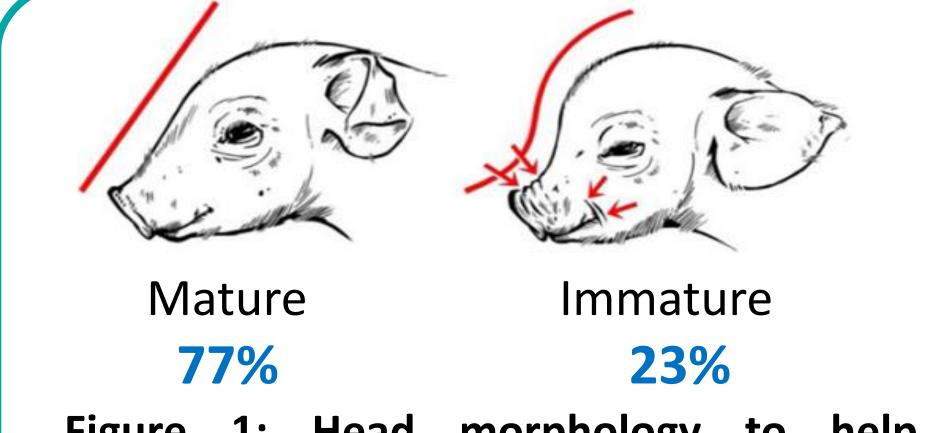
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# Molecular phenotyping to predict neonatal maturity

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**Context.** Improved piglet survival during the suckling period is a strong expectation for breeders and the public alike. This notably involves taking into account the maturity of the piglets at birth. An immature piglet, which has not reached its full development, will have a greater risk of early death. These piglets have a characteristic morphology: a domed head, bulging eyes and head/body asymmetry.

Objectives. Based on image analysis, the Pic'Let project (CASDAR-RT 2019) aims to offer



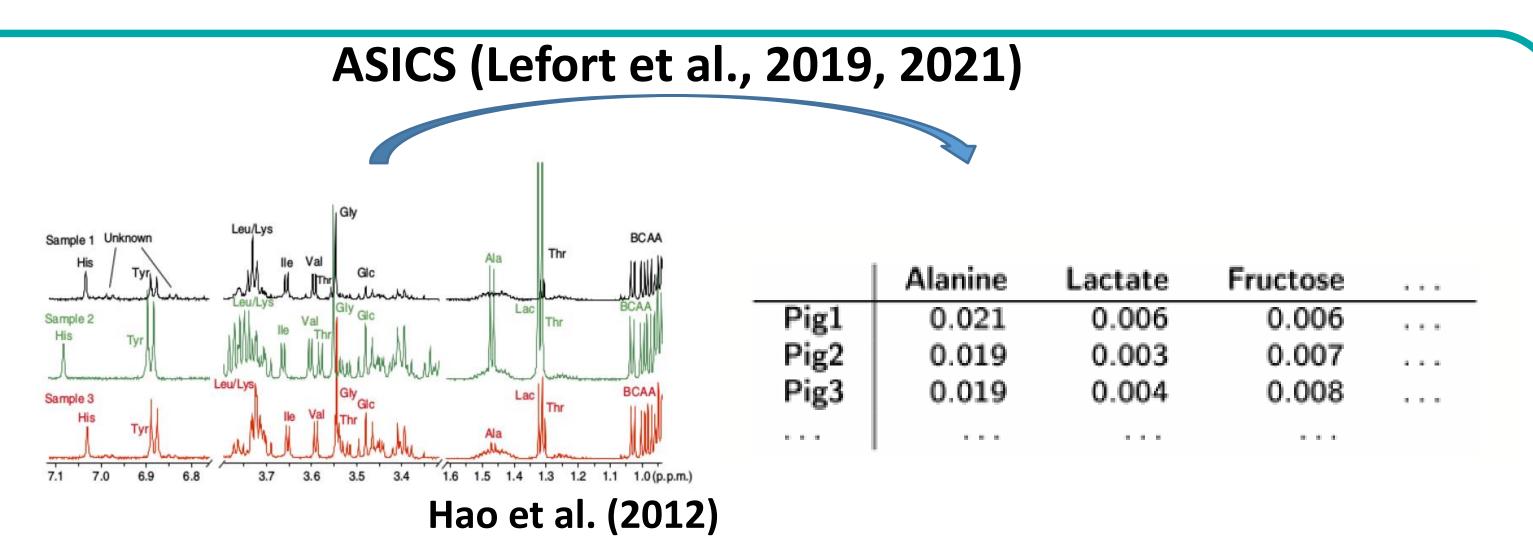
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Figure 1: Head morphology to help identifying immaturity status (Chevaux et al., 2010; Hales et al., 2014). The percentage of mature and immature piglets in this study is given.

breeders an innovative tool for phenotyping piglet maturity (not shown, 3795 piglets). A metabolomics analysis was also performed by <sup>1</sup>H-NMR on blood samples (serum) collected on a subset of 298 newborns.

**Strategy.** A metabolomics analysis was also performed by <sup>1</sup>H-NMR on serum samples collected on 298 newborns (99 LR, 98 LW, 98 LRxLW, Figure 1). Metabolites were identified with the R package ASICS and 55 with non-zero variance have been used.

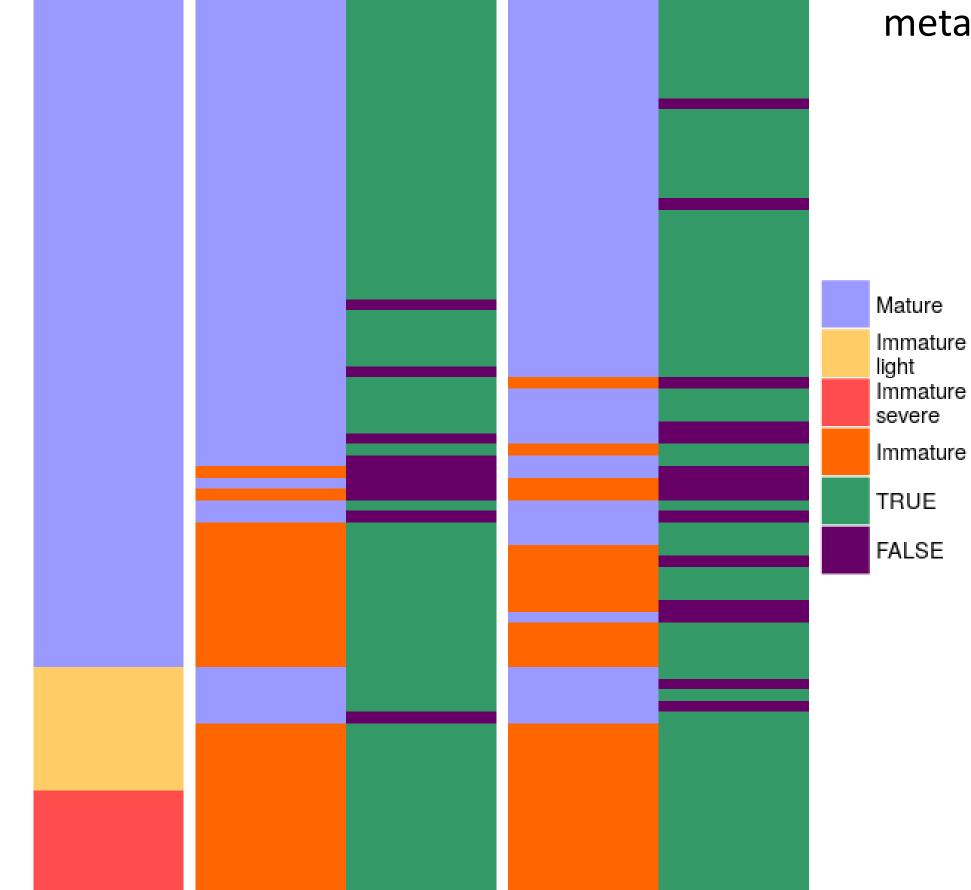
Two predictive models based on all variables or with a selection of variables. Maturity status has two levels: mature (77% of the piglets) vs. light or severe immaturity (23%). The imbalance characteristic of the dataset was adjusted by downsampling and model aggregation of two complementary methods: Random Forests and GLM/Lasso.



**Figure 2: Joint automatic metabolite identification and quantification of a set of** <sup>1</sup>**H-NMR spectra.** Raw spectra were analyzed with ASICS allowing the identification of 120 metabolites.

| M | Morphological<br>score | 55 metabolites |                      | 14 metabolites |                      |
|---|------------------------|----------------|----------------------|----------------|----------------------|
|   |                        | Predicted      | Methods<br>concensus | Predicted      | Methods<br>concensus |
|   |                        |                |                      |                |                      |

**Figure 3: Summary of prediction results on test samples (30% of the complete dataset).** Morphological score was the preliminary phenotype information to help to develop the methodology. Predictive score was given with a meta-analysis (meta) combining glm/lasso and random forest with all the 55



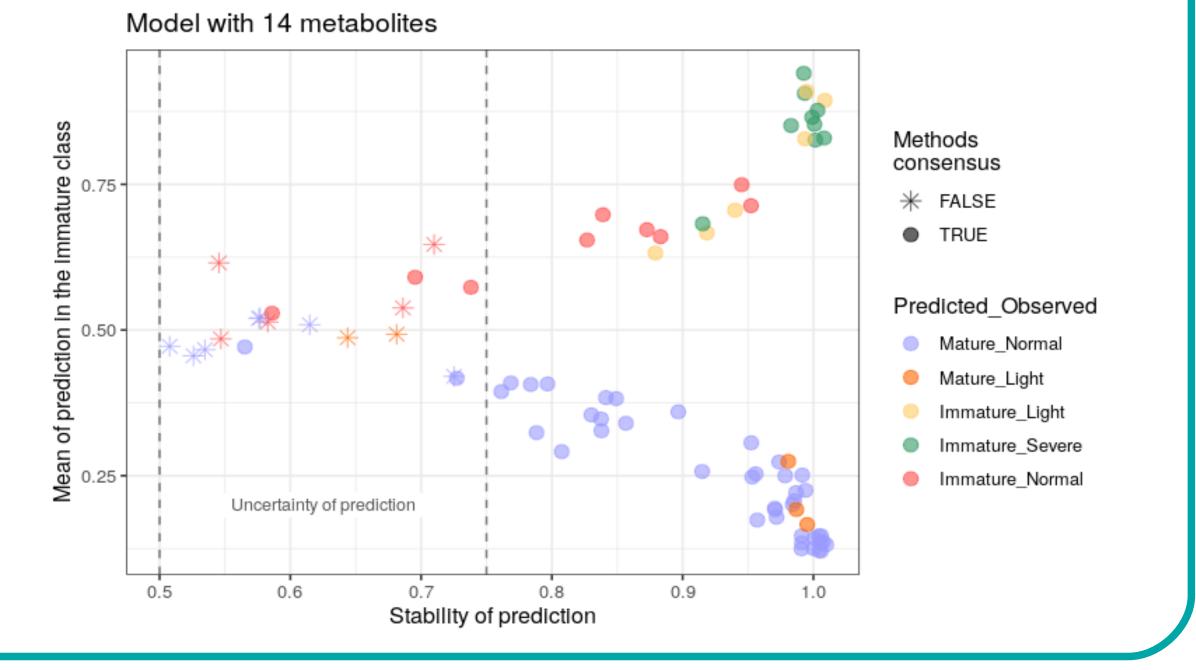
metabolites or a subset of optimized 14 metabolites.

**Results.** The two models predict 100% of the severe immaturity status in the training and the test samples. Some piglets morphologically determined as mature are expected to be immature with a strong confidence [80-100%] according to metabolic data.

•92% of the test samples are predicted in a same manner with all the 55 metabolites or with a subset of 14 selected metabolites.

- 100% of piglets with severe immaturity are classified immatures.
- 90% of piglets with light morphological immaturity are classified immatures.
- 74% of piglets with normal morphological maturity are effectively classified matures.
- 27% of piglets with normal morphological maturity are classified immatures.

**Figure 4: Indicators for helping prediction output.** Morphological indicator of maturity will not be available in future datasets. The proposed model not only give a maturity score based on the aggregation of two algorithms (Methods consensus) but also two indicators. The first one, the stability of the prediction is based on how many times the predicted score



(Mature or Immature) is given on the 200 small models. The second, the mean of prediction is the average probability of belonging to the immature class over the 200 models. The plot may help to decide to keep the maturity score as proposed by the model or to not classify some piglets.

**Conclusion and Perspectives.** Altogether, we identified a molecular signature based on metabolic data able to predict the neonatal maturity status. Next steps will be 1/ to apply this on the complete set of other 600 newborns phenotyping in different context (several genotypes, different farms), 2/ to develop a similar predictive model with available gene expression datasets on same piglets, 3/ to develop similar predictive models on other available traits (mortality, vitality, growth).



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