

SEARCH OF CANDIDATE GENES FOR PORCINE PROLIFICACY TRAITS BASED ON GENE EXPRESSION DIFFERENCES IN OVARY TISSUE

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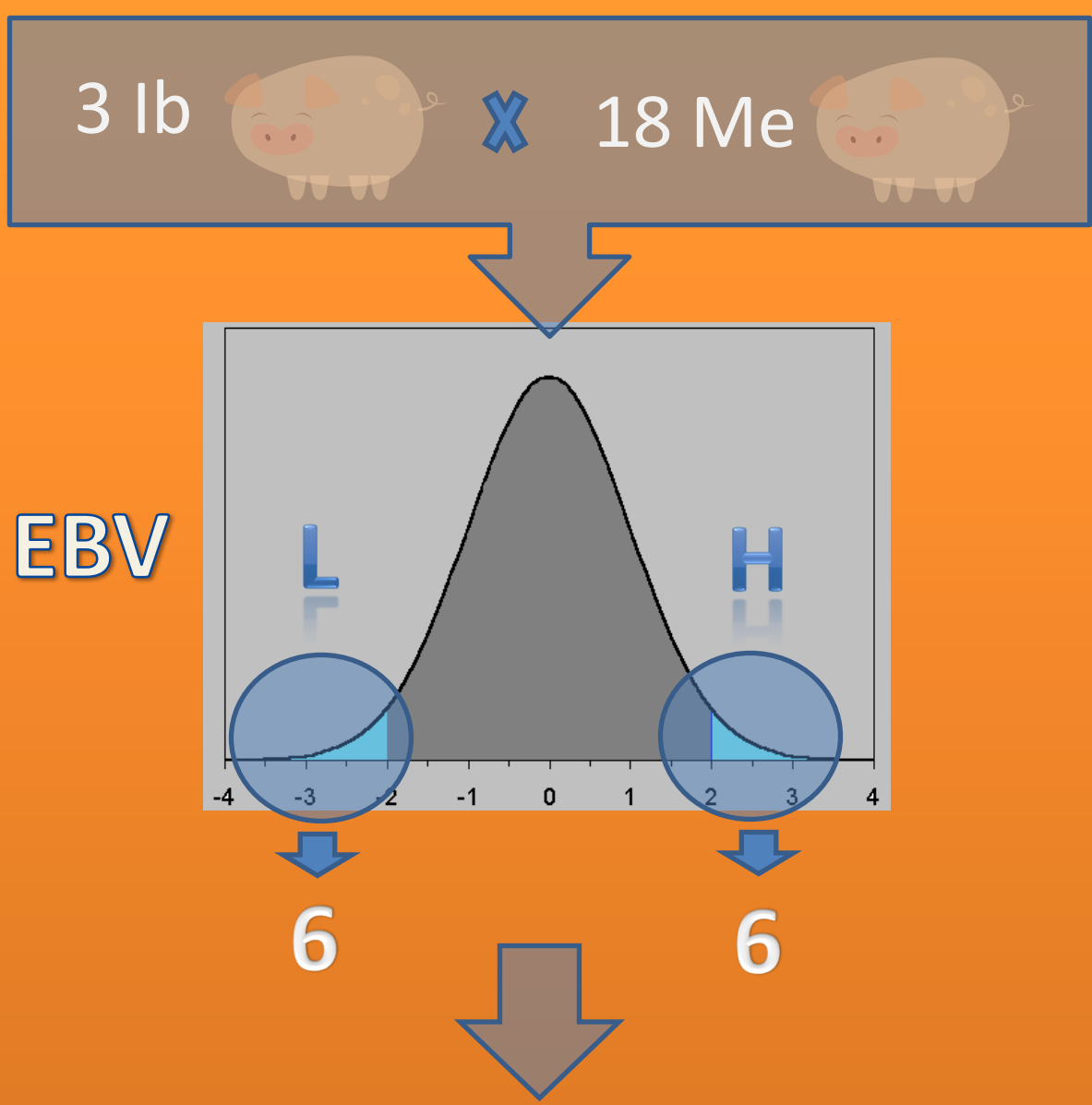
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BACKGROUND

Reproductive traits have an important economical interest in pig breeding. The genetic control of litter size traits has been studied using different approaches (QTL and candidate genes), but few studies have identified the causal mutation.

So far, differential expression patterns of genes affecting these traits has been sparsely studied. In this work the study of litter size traits has been raised merging two different approaches: microarrays differential gene expression data and QTL results for litter size (Noguera *et al.* 2006).

EXPERIMENTAL DESIGN & METODOLOGY



Animals from an F2 Iberian x Meishan cross have been ranked by their estimated breeding values (EBV) for litter size.

Animals with EBV extremes were selected to perform the microarray experiment: 6 of low (L) and 6 of high (H) prolificity. 8 animals from each tail were added to perform the qPCR analyses.

-Affymetrix porcine GenechipTM

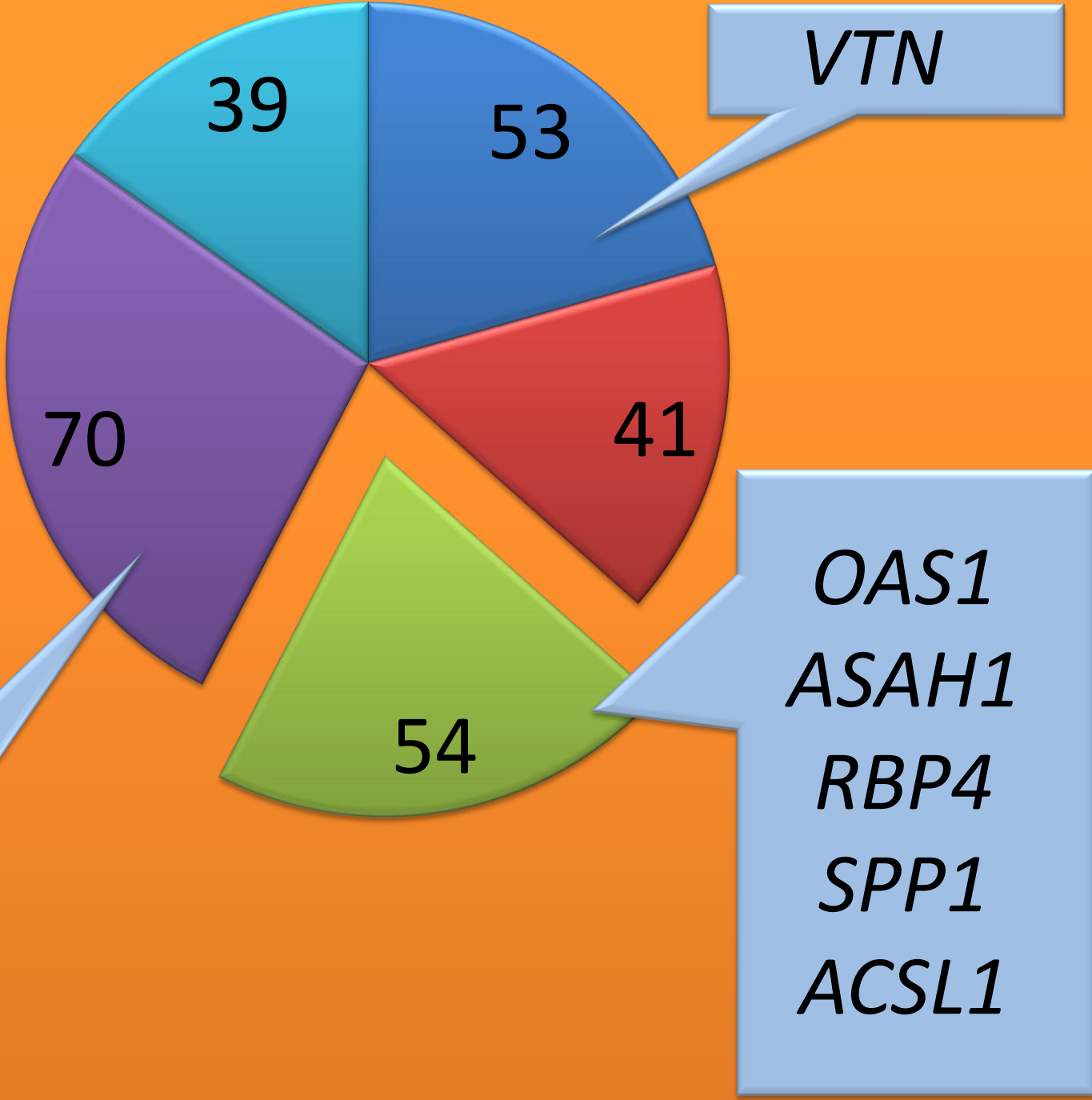
Statistical analysis

- Expression data quality was checked with *simpleaffy* package (Bioconductor) and normalization (GCRMA) was carried out with BRB-Array Tools software.
- Two statistical analysis were carried out:
 - Class comparisson analysis (T-test) with BRB (FDR=0.1)
 - Mixed model methodology by residual maximum likelihood (REML) (Byrne *et al.* 2005) (FDR=0.05)

T-test: 2 probes= 1 unique gene

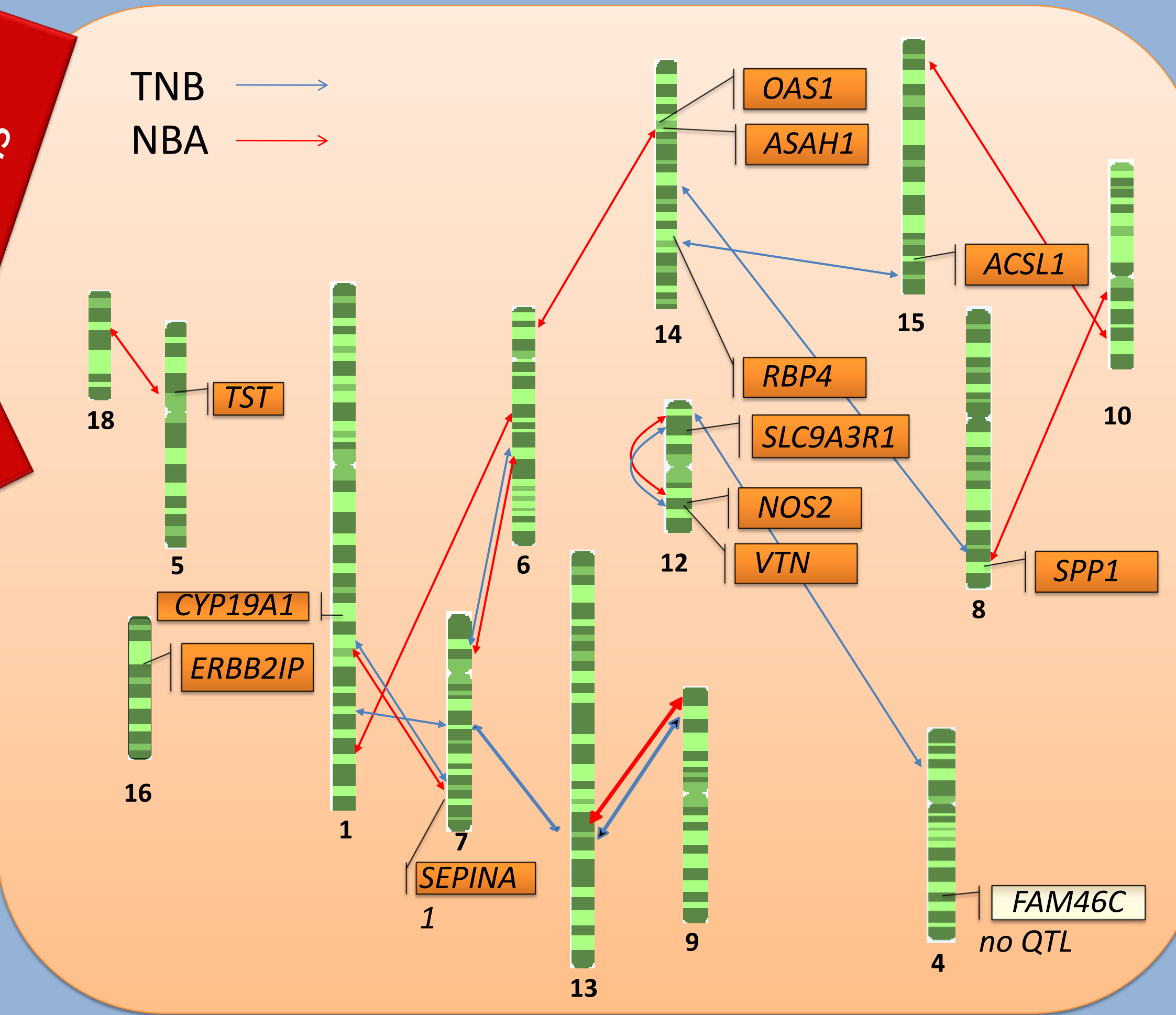
Mixed model (MM): 325 probes = 297 unique genes.

- Hematological parametres, Immune response
- Metabolism
- Reproductive System
- Tissue and cell development
- Others



Ingenuity Pathway Analysis software

Single and Epistatic QTL detected for TNB and NBA (Tribout *et al.* 2008;Noguera *et al.* 2006)



Search of candidate genes by the coincidence with QTL positions

RESULTS

qPCR confirmation of genes DE () and positional candidate genes ()

Gene	Analysis	Array DE	Ratio	qPCR confirmation	QTL region	Biological function
<i>Ssc.19416.1.A1_at</i>	Both	H<L	1.29X	no	?	?
<i>ERBB2IP</i>	Both	H<L	1.16X	no	SSC16	Embryo development
<i>VTN</i>	MM	H<L	1.21X	H<L	SSC12	Embryo implantation
<i>SPP1</i>	MM	H<L	1.15X	no	SSC8	
<i>RBP4</i>	MM	H<L	1.06X	H<L 3.77X	SSC14 suggestive	Detoxification of embryo toxic molecules
<i>TST</i>	MM	H<L	1.10X	H<L 1.87X	SSC5	
<i>ASAH1</i>	MM	H<L	1.08X	no	SSC14	
<i>ACSL1</i>	MM	H<L	1.05X	H<L 1.87X	SSC15	Lipid biosynthesis and FA degradation
<i>OAS1</i>	MM	H>L	0.81X	H>L 4.64X	SSC14	Ovulation
<i>FAM46C</i>	MM	H<L	1.09X	H<L 1.621X	no	Unknown
<i>NOS2</i>	MM	no DE	—	no	SSC12	Angiogenesis
<i>SLC9A3R1</i>	MM	no DE	—	no	SSC12	Regulated by estrogens

Candidate genes selected in a previous study (Fernández-Rodríguez *et al.* 2009)

CONCLUSIONS

- Different gene expression results are obtained depending on which statistical analysis is used.
- 54 genes implicated in reproductive system were DE.
- 14 genes have been selected to be validated by qPCR but only 6 were confirmed. Except *FAM46C*, all the genes confirmed were located within a QTL region for litter size.
- Combination of gene expression analysis and QTL mapping maximize the successful detection of candidate genes.