DNA methylation analysis in sperm from infertile/subfertile boars

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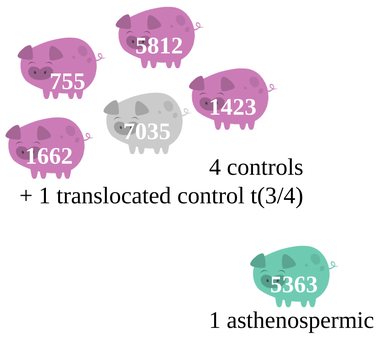
Introduction

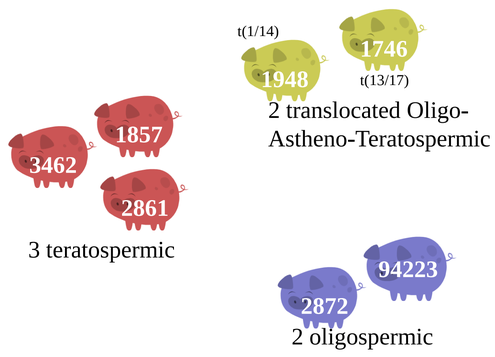
Male infertility is an increasing health challenge for our societies, either for human or livestock's populations. As diffusion of the genetic progress goes through sires, **male infertility consequently slows the improvement of animal selection schemas and farms' productivity**. In the french pig sector, more than 35% of boars selected on agronomic criteria, to be diffused through Artificial Insemination Centers, culled for bad sperm quality.

The epigenetic marks are of major importance in the good development of germ cells and could be the key explaining some phenotypes of infertility. Our aim is to define **specific or common epigenetic signatures of farm animals' infertility**. These epigenetic signatures will be an additional parameter to evaluate the sperm quality and will finally help to lower the number of potentially infertile males introduced in the selection schemas. As some imprinted loci reported to be altered in infertile humans, we focused our analysis on several **imprinted loci** in the pig.

Experiment design



DNA was extracted from sperm and blood of 13 boars with different fertility phenotypes. Five of them are controls, and the others were selected based on their bad sperm quality : defects in spermatozoa morphology (teratospermy), concentration (oligospermy) and/or motility (asthenospermy). Some of them were carriers of chromosomal rearrangements.



27 genes, either pig imprinted genes or genes involved in gamete development, were selected for methylation level analysis in spermatic DNA by Methylated DNA Immunoprecipitation and quantitative PCR.

MeDIP-qPCR analysis

Several genes showed an **increase in methylation level** at specific loci between control animals and some infertile/subfertile boars.

This experiment gave us a qualitative overview on methylation changes in subfertile animals. Thus it enabled us to select some imprinted loci for deeper CpG methylation analysis.

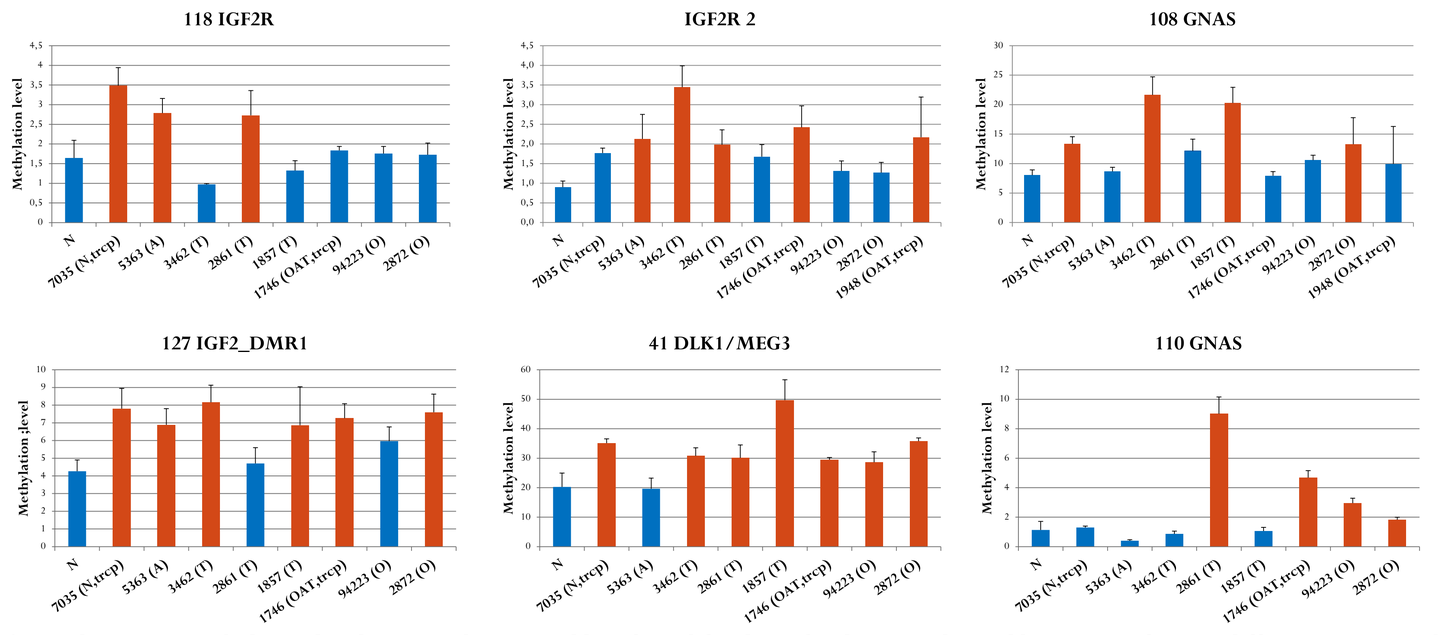


Fig1 : Changes in methylation level at several imprinted loci for subfertiles/infertiles animals. Red bars = significative difference compared to the control group ($p<0.05$, Tukey's test)

Pyrosequencing analysis

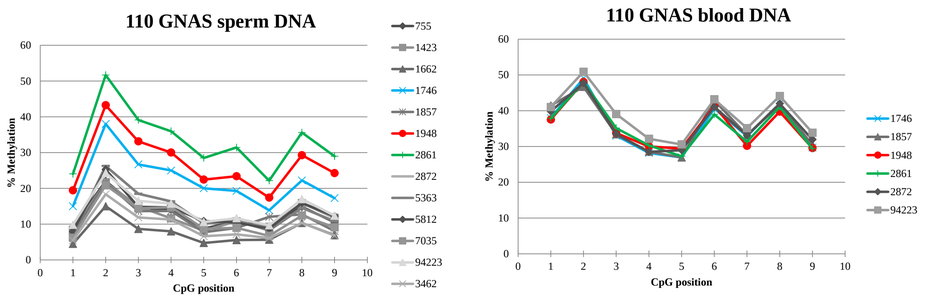


Fig2 : Methylation percentage of 9 CpGs in the GNAS region in sperm DNA and blood DNA

After bisulfite conversion, sperm DNA was analysed using the Pyromark Q24. One region of 9 CpGs in the **GNAS complex locus** appeared to be **hypermethylated** for 3 animals, thus confirming the MedIP-qPCR experiment. The hypermethylation was sperm specific, as no change was observed in blood DNA, where the basal methylation level is higher than in sperm DNA.

The concerned animals are a teratospermic boar and the two OAT boars 1948 and 1746 carrying respectively the 1/14 reciprocal translocation and the 13/17 reciprocal translocation. This last rearrangement could have an impact on GNAS expression or imprinting status as its breakpoint is situated 10kb from GNAS on chromosome 17.

Perspectives

To go deeper in the analysis of the GNAS complex locus, we designed several **pyrosequencing** primers in the CpG islands covering this region. The results will be compared to future experiments of sperm DNA methylation analysis in the GNAS region of subfertile cows (coll. Hélène Jammes) and humans.

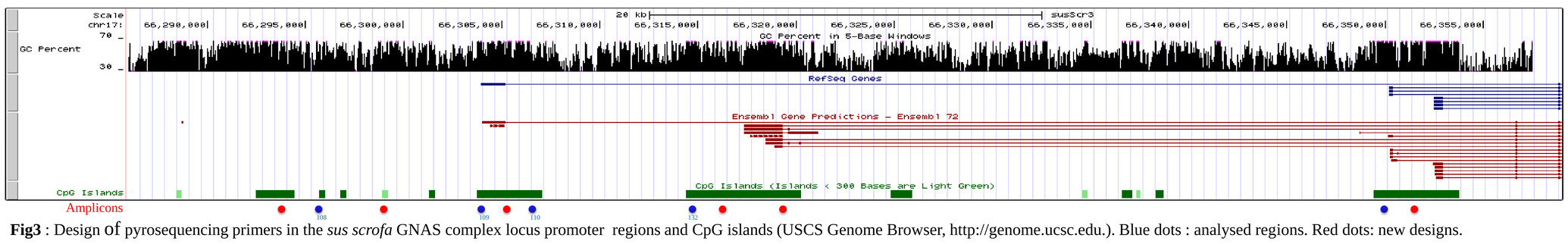


Fig3 : Design of pyrosequencing primers in the *sus scrofa* GNAS complex locus promoter regions and CpG islands (USCS Genome Browser, http://genome.ucsc.edu.). Blue dots : analysed regions. Red dots: new designs.

GNAS (guanine nucleotide-binding protein, α stimulating) is a complex imprinted locus that code for several proteins, including NESP55, Gsα (4 isoforms), Xlas and ALEX (predicted only in pig), as well as several non coding transcripts. Some of these transcript variants are **maternally expressed**, like NESP55, **paternally expressed**, like XLas, while others are **biallelic**, depending on their exon composition and on the tissue. GNAS is located on the telomeric part of chromosome 17, where several QTL for body mass and piglet growth have been identified (Thomsen *et al*, 2004). Moreover, in humans and mice several studies show a important role of GNAS imprinting for **fetal development** and **growth** (Richard *et al*, 2013, Eaton *et al*, 2013).

Other studies have correlated loss of methylation at the IGF2/H19 locus with infertile phenotypes. Here, we describe another genomic region, usually involved in fetal growth, that could be a novel actor of pig fertility mechanisms. This project gives us a first insight in the complex role of imprinting through DNA methylation in germ cells and its consequences on pig fertility.

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