Bull sperm sncRNAs: A new source for potential fertility biomarkers?

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300

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Application Ensuring an optimal quality of semen is a major concern for breeding companies, yet semen field fertility prediction remains challenging. Several quality parameters have been proposed to guarantee semen fertility, but they fail to reach high predictive value and high correlation levels. Our work aims to explore the small non coding RNA (sncRNA) content of sperm as a new source of potential semen fertility biomarkers.

Introduction With the advent of Genomic Selection, marketed semen is now produced by younger or barely mature bulls, without any prior data on their field fertility or progeny performance as was the case previously with progeny testing. Efficient quality control procedures are thus crucial for the cattle breeding sector since sub-fertile bulls lead to delayed conception, a prolonged calving season, and increased culling rates, thereby resulting in economic losses. Several quality control procedures and biomarkers have been proposed in the last decades to guarantee semen fertility including flow cytometry to assess functional key parameters. However, the relevance of most of these biomarkers in routine QC procedure has yet to be ascertained and their predictive value is usually too low (Sellem *et al.*, 2015). Parameters included in these analyses may represent necessary but not sufficient factors to ensure pregnancy and some other essential sperm attributes should probably be added to the procedure to improve the prediction accuracy. In this respect, attention has been paid in recent years to sncRNAs, especially miRNA and piRNA, which have been shown to play a role in fertilization as well as zygotes and 2 cells-embryo development (Hosken and Hodgson 2014; Yuan *et al.* 2016). Our study aimed to characterise the sncRNA content of frozen bull sperm cells and identify miRNA associated with field fertility.

Material and Methods Total RNA was extracted from 30 Montbeliard ejaculates with contrasting fertility (3 adjusted fertility groups based on NNR282 days: in mean +4.6 / +1.8 / -13; N=10 per groups; balanced according to age at production), using an improved protocol. Quality control was ensured by total RNA quantification (Qubit technology) and quantitation of a reference miRNA (miR125) by RT-qPCR (miRCURY assay, Exiqon). NGS sequencing libraries were prepared using small RNA (<200 nucleotides) and sequenced at 40 million 50bp single reads (Illumina HiSeq; Exiqon). Identification and quantification of miRNAs was performed using the miRDeep2 software and miRNA associated with fertility were identified using both differential analysis using the DESeq2 package and discriminant analyses (BCA and Ro-PLS).

Results 67% of reads were annotated as miRNA (16%), rRNA (13%), tRNA (7.5%), long noncoding RNA (7.2%), mitochondrial RNA (6.5%) and mRNA (17%). The lack of *Bos Taurus* databases referencing piRNA hampered identification of these sncRNA in our dataset. A total of 3196 miRNAs (583 known and 2613 putative miRNAs) were identified. The 20 most abundant miRNAs account for the majority of miRNAs reads (75%). The most expressed miRNA, bta-miR-100, accounted for nearly 33% of all reads. An in depth study of miR sequence content showed a great diversity of Isomirs sequences (85±143 Isomirs per miR, min=2 / max=1678). Surprisingly, the canonical sequence as defined by cross-species comparative genomics were frequently not the most expressed (49%) or even not expressed at all (8%). Differential and discriminant analyses were thus performed at the Isomir level. Deseq2 highlighted 91 significant Isomirs (adjusted p value <0.05) between fertility groups, while BCA/Ro-PLS identified a signature effect of 72 isomirs discriminating between the three fertility groups. 16 isomirs are in common between the two statistical approaches, including isomirs from bta-miR-100, bta-miR-148, bta-miR-21, bta-miR-26, bta-miR-335 and bta-miR-130.

Conclusion Work is ongoing to confirm the relevance of miRNA signatures on a larger experimental design comprising 300 bulls. In the meantime, longitudinal studies will explore whether the sperm sncRNA pattern evolves along a bull's lifetime. The final objective is to develop fertility prediction tools based on miRNA expression that could be used as routine quality control by the breeding industry.

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