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TITOLO **Sperm methylome profiling revealed DNA methylation variations in Piedmontese bulls with different reproductive performances**

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Testo e Riferimenti bibliografici

Artificial insemination (AI) has revolutionized the breeding of livestock species, becoming the most used method in bovine reproduction. Nowadays, early prediction of bull fertility is one of the main challenges for AI-centers as the entire process of bull breeder selection is considered time and money-consuming. It is based on animal selection (genotypic and phenotypic traits), field trial inseminations, and conventional laboratory semen analysis. However, notwithstanding the advance of genomic selection allowed reliable identification of genetically elite bulls, selected bulls with apparently normal semen quality can vary significantly in their field fertility. This suggests that conventional semen evaluation methods are not fully foolproof and other sperm characteristics need to be investigated to explain the remaining variation. Emerging data about abnormal sperm DNA methylation and infertility suggest that epigenetic traits are promising candidates for this purpose [1,2]. Therefore, this study tested the hypothesis that sperm-specific epigenetic characteristics, such as DNA methylation, could discriminate Piedmontese bulls with different reproductive performances. Using a genome-wide approach we have characterized sperm methylome from normal (NF) and high fertility (HF) Piedmontese bulls, by reduced representation bisulfite sequencing (RRBS). Bulls (20) were assigned to either the NF or HF group (10/group) based on an adjusted fertility index provided by the Piedmontese AI center. Reproductive efficiency was predicted by a direct measure of field fertility, calculated as the ability of bulls to make cows pregnant through AI corrected for a wide range of factors (year, season, female genetic, farming). NF fertility index ranged from 0.531 to 0.631 while HF bulls showed values between 0.973 and 0.706. As expected, Computer Assisted Sperm Analysis confirmed that motility parameters were comparable within groups (63,7% NF vs 67,4% HF). RRBS identified a total of 348889 cytosines (10X coverage in all samples) able in part to separate NF or HF animals. We identified 968 differentially methylated cytosine DMCs (FDR<exp-6, delta met 10%, at least 2 near C below 2000bp) between HF and NF bulls. The DMCs were found to be close to 294 differentially methylated genes DMGs. Gene ontology analysis of DMGs identified variations in pathways related to protein glycosylation. Interestingly, the mature coating of glycans on the surface of sperms is a prerequisite to gaining fertilizing capability, and aberrant sperm glycosylation is highly associated with disturbed fertility [3].
Our results suggested that bulls with different fertility exhibited distinct regulation of genes related to sperm glycosylation, making them good candidates to serve as epigenetic markers for bull selection. Overall, this study demonstrated that the sperm methylome of Piedmontese bulls displayed DNA methylation characteristics partially capable of discriminating bulls with different reproductive performances. Additional experiments are still ongoing to explore the interaction between DNA methylation and other epigenetic characteristics, such as micro-RNA, in the regulation of fertility. In conclusion, sperm methylome offers a promising source of information about bull fertility which will complement the genetic data currently employed to enhance reproductive efficiency.

[1] Costes et al. Predicting male fertility from the sperm methylome: application to 120 bulls with hundreds of artificial insemination records, Clinical Epigenetics 14:54, 2022.
[2] Zhang et al. Whole-genome DNA methylation analysis of the sperm in relation to bull fertility. Reproduction, 165:557-568, 2023.
[3] Schröter et al. The glycocalyx of the sperm surface Human Reproduction Update, 5:302-313, 1999.