Animal Reproduction

Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and "omics"

Are miRNAs related to sperm cryotolerance in boars

Ana Carolina Pedrosa[1], Mariana de Andrade Torres[1], Diego Vilela Alkmin[3], Jorge EliecerPinzon Porras[2], Flávia Vieira de Freitas[1], Simone Maria MassamiKitamura Martins[2], Juliano Coelho da Silveira[2], André Furugen Cesar de Andrade[1]

[1]FMVZ - USP - Faculdade de Medicina Veterinária e Zootecnia - Univesidade de São Paulo (Pirassununga - SP, Brasil); [2]FZEA - USP - Faculdade de Zootecnia e Engenharia de Alimentos - Universidade de São Paulo (Pirassununga - SP, Brasil); [3]DB - Genética Suína - DanBred (Patos de Minas - MG, Brasil).

The present study aimed to evaluate whether the miRNAs profile present in sperm and extracellular vesicles (EVs) from seminal plasma differ between boars ejaculates with high and low freezability, to understand if miRNAs can be biomarkers of sperm cryotolerance. It is known that these molecules have post-transcriptional action in several metabolic processes and are very important during spermatogenesis and sperm maturation. For this purpose, 27 high-quality seminal ejaculates were used (Total motility-TM and plasmatic and acrosomal integrity-MIAI were both higher than 85%). Sperm and seminal plasma samples were separated and centrifuged in aliquots still in the fresh state, both of sperm and seminal plasma. Ejaculates were later cryopreserved and divided after thawing into two groups: High freezability (HF; N=4) and low freezability (LF; N=4), according to the MIAI (HF>40%; LF<25%) and TM (HF>30% and LF<20%), obtained through flow cytometry and computer-assisted sperm analysis, respectively. Once the groups were determined, miRNA was extracted from both sperm and seminal plasma EVs, subsequently cDNA synthesis and real-time polymerase chain reaction (RT-PCR). The data obtained from the RT-PCR were compared by the unpaired Student's T-test considering 10% significance using the JMP8 SAS software. Bioinformatics analyzes were performed using the mirPath v.3 software on the DIANA TOOLS platform with the microT-CDS v5.0 and TarBase v7.0 databases. Among the 383 miRNAs evaluated, only one miRNA was detected differently ($p<0.1$) in spermatozoa cell samples, identified as ssc-miR-503. In seminal plasma EVs, two differently abundant miRNAs ($p<0.1$), identified as ssc-miR-130a and scc-miR-9, were detected. All of the miRNAs were more abundant in LF than the HF group. The miR-503 was, through this study, unprecedentedly detected in sperm. The bioinformatics analyzes performed showed that it could act in different pathways related to the pluripotency of stem cells as TGF-β, PI3K, and WNT signaling pathways that are related which cell proliferation, grown, survival and in their differentiation in various cell types including spermatozoa. MiR-130a and miR-9 have also not been reported in seminal plasma. Our results showed that miR-130a acts inhibiting mainly on fatty acid biosynthesis pathways, it is known that these are essential for the fluidity of the plasma membrane of the sperm, this factor is directly related to the resistance of this structure to cryopreservation. It has also been shown that miR-9 acts on the adhesion complex pathways, modulating the expression of cadherin, a glycoprotein found throughout the entire epididymis, and that has a direct action on fertilization. Based on these results, we were able to conclude that the miRNAs profile present in spermatozoa cell and seminal plasma EVs differ between high and low freezability boars ejaculates, demonstrating that miRNAs can be biomarkers of boars sperm cryotolerance.