Transition nuclear protein 2 (*TNP2*) mRNA cargo: a putative marker for assessment of breed differences?

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ABSTRACT

Artificial insemination (AI) centres and farm management require fine control of the quality status of the boars. In particular, sperm quality through conventional methods fails to address the presence of sub-lethal characteristics that led to a detrimental effect not only on the sperm itself but also on the final fertility performance. Boar sperm plasma membrane presents a high proportion of polyunsaturated fatty acids, increasing its susceptibility to oxidative stress, such as the induced by *in vitro* handling (e.g. centrifugation, and dilution), causing chromatin alterations. In this sense, DNA integrity is an already established quality marker of sperm fertilizing ability. DNA integrity relies, among other factors, on DNA packaging, being the histones, commonly found in eukaryotic cells, mostly replaced by protamines in mammalian spermatozoa. Protamines are acidic proteins that form very complex structures with DNA, forming disulfide bridges between the cysteine residues of protamines during sperm epididymis transit. There are two main types of protamines: protamine 1 (PRM1) and protamine 2 (PRM2), the latter present only in horses and humans with a known function. In addition, the transition nuclear proteins (TNPs) represent 90 % of the chromatin basic proteins during histone removal. This study **aimed** to investigate the mRNA transcripts content of the PRM1, PRM2, TNP1 and TNP2, in pig spermatozoa from two commercial breeds (Landrace (LD) vs Large White (LW)), with a field fertility record (determined based on a farrowing rate of at least 100 inseminations; high-fertility (HF) vs low-fertility (LF)). For this purpose, the relative abundance of mRNA transcripts for protamines and transition nuclear proteins was analyzed through quantitative polymerase reaction (qPCR) analysis in a total of 12 boars (LD n=6 & LW n=6; HF n=6 & LF n=6) with fertility record . Total RNA isolation from sperm samples was performed by using the miRNeasy mini kit (Qiagen, Germany) following the manufacturer's protocol. qPCR) was performed by using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, USA), after the first-strand cDNA synthesis (High-Capacity RNA-to-cDNA™; Fisher Scientific, Sweden). The following commercial primers (all porcine-specific and from Bio-Rad, USA) were used: GAPDH (loading control), PRM1, PRM2, TPN1, TPN2. Calculations of the relative mRNA content were performed by using the Pfaffl method. After normal distribution and homoscedasticity control, parametric data were analyzed through a T-test and non-parametric data through Kolgomorov-Smirnov using GraphPad Prim 8. Our qPCR **results** showed a significant decrease in the TNP2 mRNA transcripts in the Large White Boars relative to Landrace (LD: 1.00±0.36 vs. HF: 0.57±0.33, P=0.048), being the rest of the transcripts non-significant. TNP2 is closely linked to the two protamine genes, which might suggest their origin by gene duplication and, perhaps, retaining some common functions. In contrast to TNP1, abundantly expressed and conserved in various mammals, TNP2 is poorly expressed and protein abundance varies among species. Therefore, our results might suggest that the differential expression of TNP2 found among breeds could be linked to a non-described yet differential compaction status. Finally, no differences between high and low fertility boars were found in any of the mRNA transcripts analyzed. In **conclusion**, our preliminary results of breed-specific differences pave for further studies to elucidate whether this differential expression could have an impact on sperm *in vitro* handling techniques, in particular paying attention to DNA integrity preservation. Supported by RyC2020-028615-I and PID2022-136561OB-I00, funded by MCIN/AEI /10.13039/501100011033 (Spain) and FEDER funds (EU).