High fertile boars present a decrease in the mRNA cargo of TRPC1, a key regulator of sperm motility

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ABSTRACT

Subfertile boars largely impact the economic status of artificial insemination (AI) centers and farms. Thus, the primary objective for AI centers is to find suitable biomarkers for early identification of subfertility individuals. Sperm activity is regulated by Calcium (Ca^{2+}), a key molecule that plays an essential role in the modulation of motility, sperm capacitation, and the acrosome reaction. However, whether the modulation of calcium trafficking from sperm cells is linked with the presence of subfertile animals remains to be elucidated. The transport of Ca^{2+} is mainly mediated not only by CatSper channels (sperm-specific) but also by transient potential canonical channels (TRPC). CatSpers facilitates the entrance of cations during sperm capacitation by alterations in membrane potential and pH, via the cAMP-PKA signaling pathway, which plays a pivotal role in sperm egg penetration and its mutation are linked to infertility in humans. On the other hand, TRPCs, mainly located over the membrane of the tail and head of spermatozoa, regulate hyperactivation as well as the acrosome reaction. This study aimed to investigate the mRNA transcript cargo for the β, γ, and δ subunits of CatSper, as well as TRPC channels 1, 4, and 6 in boar spermatozoa from two distinct commercial breeds (Landrace (LD) vs Large White (LW)), with fertility record (high fertility (HF) vs low fertility (LF) (determined based on a farrowing rate of at least 100 inseminations)). For this, the relative abundance of mRNA transcripts for TRPC and CatSper subunits was determined through qPCR analysis in a total of 12 boars (LD n=6 & LW n=6) with fertility record. Total RNA extraction from sperm samples was performed with the miRNeasy mini kit (Qiagen, Germany) following the manufactures protocols. Then, after the synthesis of the first-strand cDNA (High-Capacity RNA-to-cDNA™; Fisher-Scientific) the quantitative polymerase chain reaction (qPCR) was performed by using the SsoAdvanced Universal SYBR-Green Supermix and commercial primers for GAPDH (loading control), TRPC1, TRPC4, TRPC6, CatSper-β, CatSper-δ, and CatSper-γ; (Bio-Rad, USA). Relative mRNA content was calculated using the Pfaffl method. Data were assessed for normal distribution with the Shapiro-Wilk test and parametric data analyzed through a T-test, while non-parametric data through Kolgomorov-Smirnov ($p<0.05$, GraphPad Prim8). qPCR results showed a significant decrease in theTRPC1 mRNA transcript in HF boars(LF: 1.00±0.03 vs. HF: 0.61±0.16, P=0.026), being the rest of transcripts non-significant. TRPC1 plays a crucial role in the acrosome reaction and sperm motility, as it encodes store-operated Ca^{2+} channels (SOCs) associated with these functions, being observed a decrease in transcription during capacitation directly correlated with a reduction in motility. This might suggest a potential connection with interactions in the sperm reservoir for storage within the female's cells. Results from the comparison of the two included breeds (LD & LW), the relative abundance of CATSper-γ mRNA increase in the Large White breed (LD: 1.00±0.17 vs. LW: 1.73±0.18, P=0.027). CATSper-γ is influenced by external signals such as ligands and cell-cell interactions during sperm maturation in the epididymis and/or the female reproductive tract. In

addition, an increase in the relative expression of mRNA has been described in capacitated boar spermatozoa. No significant differences were found in the remaining transcripts. Our preliminary results of breed-and fertility-specific differences pave for further studies in order to find suitable male biomarkers of fertility performance in the boar. Funded by MCIN/AEI /10.13039/501100011033 (Spain) and FEDER funds (EU) (RyC2020-028615-I and PID2022-136561OB-I00).