Differential expression of oestrogen and androgen receptors in spermatozoa from different boar breeds and fertility levels

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ABSTRACT

Boar spermatozoa undergo maturation and acquire motility upon reaching the epididymis. Evidence among species suggests that this maturation is mediated by steroid hormones acting on the epididymis. Regulation of steroid hormones is crucial in livestock such as the porcine species. The epididymial function relies on androgens and estrogens, modulating the expression and secretion in the pig epididymis. Androgens (testosterone, androsterone and androstenedione) exert effects via the androgen receptor (AR), at the cellular level, influencing epididymal segmentation and luminal fluid composition. Androgen-regulated proteins are likely involved in sperm membrane remodeling, facilitating zona pellucida attachment. Estrogens play a vital role in regulating accessory sex glands and influence epididymal contractility through the RhoA/Rho-kinase pathway, potentially regulating the ejaculation process. Estrogen receptors (ER) are crucial for epididymal function and fertility maintenance. Previous studies suggest estrogen-regulated proteins contribute sperm membrane remodeling for proper sperm-oocyte interaction. Sperm storage in the epididymis cauda may also depend on estrogen-regulated proteins. Expression of AR and ER in spermatozoa varies among and within species, with limited research regarding AR and ER presence in adult boar sperm. The purpose of this study was to identify the presence of estrogen and androgen receptors mRNA in pig spermatozoa from two different commercial hybrid breeds (Landrace (LD) vs Large White (LW)). Moreover, a comparative analysis was carried out between two levels of fertility (high fertility (HF) vs low fertility (LF)). Briefly, a total of 12 boars (LD n=6 & LW n=6) with fertility record (determined based on a farrowing rate of at least 100 inseminations) were used. Total RNA extraction was isolated from sperm samples by using the miRNeasy mini kit (Qiagen, Germany) following the manufacturer's instructions. Quantitative polymerase chain reaction (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 (porcine-specific) were used: GAPDH (loading control), ER and AR (all purchased from Bio-Rad, USA). Calculations of the relative mRNA content were performed by using the Pfaffl method. After normal distribution and homoscedasticity control, a T-test (parametric) and Kolgomorov-Smirnov (non-parametric) were carried out (GraphPad Prism 8). Results in our qPCR showed a significant increase in the expression of AR mRNA transcripts in Large White vs Landrace boars (LD: 1.00±0.77 vs. LW: 13.33±19.23, P=0.0022). However, under our experimental conditions, there was no expression of ER mRNA transcripts. Moreover, no significant differences were found between HF and LF boars in any of the mRNA transcripts analyzed. Androgens play a key role in maintaining epithelial cell morphology and preventing cell death. The expression of AR in the cells of the epididymis can determine changes in the organ's ability to carry out its important functions of maturation, transport and protection of spermatozoa. Moreover, it is known that in the low presence of

androgens, spermatozoa present altered motility, lose their fertilizing capacity and even die, suggesting that the presence of androgens is essential for both the development and maintenance of epididymal functions. Further studies are needed to evaluate the potential reason behind the differences in the expression of these receptors among groups of different breeds. Supported by RyC2020-028615-I and PID2022-136561OB-I00, funded by MCIN/AEI /10.13039/501100011033 (Spain) and FEDER funds (EU).