Elucidating the Proteome of Seminal Plasma-Derived Extracellular Vesicles from GnRH-II Receptor Knockdown Boars with Poor Semen Quality

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The pig is the only livestock species encoding functional proteins for the second form of gonadotropin-releasing hormone (GnRH-II) and its receptor (GnRHR-II). Previously, we demonstrated that GnRHR-II localizes to the connecting piece of boar sperm, whereas GnRH-II is present within porcine seminal plasma. To examine how the interaction of GnRH-II/GnRHR-II may influence sperm function, we utilized our transgenic swine line with universal knockdown (KD) of GnRHR-II. Compared with littermate controls, GnRHR-II KD boars produce ejaculates containing 39% fewer sperm with diminished total and progressive motility (23% and 31%, respectively), a 2-fold decrease in DNA stability, and reduced energetics (10% increase in mitochondrial depolarization). Thus, GnRHR-II KD boars have fewer, less motile sperm of poor quality. Recently, seminal plasma-derived extracellular vesicles (spEVs) and their bioactive cargos (e.g., proteins) have been implicated in the regulation of sperm function. Therefore, the objective of this study was to evaluate the proteome of spEVs from GnRHR-II KD and littermate control boars, as well as assess GnRH-II concentrations within seminal plasma. Enzyme-linked immunosorbent assays were utilized to quantify GnRH-II concentrations in seminal plasma of transgenic (n = 14) and littermate control (n = 13) boars. Data were analyzed via the MIXED procedure of SAS with a model including genotype (fixed effect) and litter (random effect). A subset of these samples (n = 5 per genotype) was subjected to differential ultracentrifugation to isolate spEVs. After validation of spEVs by nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM), proteomics was performed. Proteome Discoverer (Thermofisher; version 2.4) and Mascot software (Matrix Science) were utilized. Proteins were considered differentially expressed at $P \leq 0.05$ following Benjamini-Hochberg adjustment, and tendencies were considered $0.05 < P \leq 0.10$. Results indicate that concentrations of GnRH-II were significantly reduced ($P = 0.0476$) within seminal plasma from GnRHR-II KD boars compared with littermate controls. Proteomics of spEVs detected 556 proteins. Of these, 73 were differentially expressed proteins (DEPs), and 14 spEV proteins tended to differ between genotypes. Among these, 32 DEPs were upregulated and 55 DEPs were downregulated in spEVs from GnRHR-II KD versus littermate controls. Noteworthy upregulated proteins include: hyaluronidase, zonadhesin, zona pellucida binding protein 2, acrosin binding protein, sperm acrosome associated protein 3, acrosin, lactadherin, amine oxidase, disintegrin and metalloproteinase domain-containing protein 5-like. Downregulated proteins include: pheromaxein A, protein kinase A, glutathione peroxidase 4, inactive ribonuclease-like protein 10, epididymis-specific alpha mannosidase, and ADAM metallopeptidase domain 2. Importantly, these DEPs have been implicated in critical reproductive processes including spermatogenesis, sperm maturation, sperm quality, capacitation, the acrosome reaction, cumulus cell dispersion, hyperactivation, zona pellucida binding, cryopreservation success, oviductal binding, ovulation, farrowing rate, and pheromonal transport. Thus, DEPs within spEVs may be a cause or

consequence of the poor semen quality exhibited by GnRHR-II KD boars. Ultimately, these data elucidate the biology of reproduction in the boar, revealing novel proteins and potential biomarkers or therapeutic targets to enhance boar fertility. Furthermore, these transgenic swine also ubiquitously express the reporter gene ZsGreen1, which was detected within spEVs from GnRHR-II KD boars via proteomics, confocal microscopy, TEM, and fluorescent NTA, thereby representing a novel resource to study the interaction of spEVs with sperm and the trafficking of spEVs within the female reproductive tract. Supported by USDA/NIFA predoctoral fellowship (2017-67011-26036) and AFRI (2017-67015-26508) funds.