Identification and Characterization of Novel Candidate Post-Fertilization Sperm Mitophagy Determinants

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Maternal inheritance of mitochondria and mtDNA is conserved across most species. Paternal mtDNA leakage and mitochondrial heteroplasmy may have detrimental effects on health and fitness outcomes. To ensure exclusively maternal mitochondrial inheritance and normal preimplantation development, paternal mitochondria in mammals are targeted and selectively degraded shortly after fertilization through a species-specific process called sperm mitophagy. This phenomenon has been extensively studied in recent years and it has been discovered that sperm mitochondrial degradation is mediated by the ubiquitin-proteasome system and autophagic proteins and pathways. Previously, the ubiquitin-binding pro-autophagic receptors SQSTM1 and GABARAP as well as protein dislocase VCP, which mediates the presentation of ubiquitinated sperm mitochondrial proteins to the 26S proteasome, have been reported to support sperm mitophagy. Following these findings, a unique cell-free system recapitulating early post-fertilization events was developed to identify new potential pro-autophagic proteins. This cell-free system combines porcine oocyte extracts and primed boar spermatozoa that have been demembranated and subjected to disulfide bond reduction to mimic processes occurring in vivo during and after sperm/oocyte fusion. The porcine cell-free system has been shown to mimic post-fertilization sperm mitophagy events and has been used to study early fertilization proteomics. The system was used in conjunction with mass spectrometry and 185 proteins with statistically significant changes in abundance between control and cell-free-treated spermatozoa were identified. We hypothesize that not only factors such as SQSTM1, GABARAP, VCP, autophagy, and the ubiquitin-proteasome system (UPS) work together during sperm mitophagy to degrade proteins, but that other autophagic proteins and pathways also play a role. Using the cell-free system, several proteins have been validated as candidate mitochondrial inheritance determinants. The present study focuses on new potential pro-autophagic candidates, including FUN14 domain containing 1 (FUNDC1), a mitophagy receptor involved in mitochondrial quality control following hypoxic stress, optic atrophy 1 protein (OPA1), a regulator of mitochondrial morphology and cristae maintenance, which is also known to regulate mitochondrial fission, dynamin 2 (DYN2), a protein proposed to induce membrane fission to complete mitochondrial division, and mitochondrial fission regulator 1 (MTFR1), a regulator of mitochondrial dynamics that promotes mitochondrial fission and protects against oxidative stress. These proteins are being studied in porcine model using cell imaging, IVF and cell-free system to decipher their role in sperm post-fertilization mitophagy. To further investigate their role in the mitophagy process, the proteins will be inhibited by antibody transfection into zygotes. Identification of novel regulators of post-fertilization mitophagy may help to further unravel the mechanism of mitochondrial inheritance

and provide more insight into early embryonic development, as well as a better understanding of mitochondrial diseases resulting from failed sperm mitophagy. This study is funded by USDA NIFA Grant number 2020-67015-31017.