Proteins of Huntington's Disease Pathway Participate in Pig Sperm Capacitation

Michal Zigo[1], Karl Kerns[1,2], and Peter Sutovsky[1,3]

[1]Division of Animal Sciences, University of Missouri, Columbia, MO 65211
[2]Department of Animal Science, Iowa State University, Ames, IA 50011
[3]Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, MO 65201

We have reported previously that proteins of neurological disorder-associated (NDA) pathways, such as Huntington's disease (HD) and Parkinson's disease, are highly represented in both the human and porcine sperm zincoproteomes. Therefore, it is plausible that NDA pathway proteins may play an important role in sperm physiology, especially in sperm capacitation which can be modulated in artificial reproductive therapies/technologies (ART). With this hypothesis and aim in mind, we used small molecule inhibitors of HD pathway proteins during pig in vitro capacitation (IVC) and monitored changes in sperm motility, velocity, and movement parameters by using computer-assisted semen analysis (CASA); as well as capacitation status – monitored with our zinc signature assay; sperm viability – monitored with nuclear propidium iodide (PI) incorporation; acrosomal integrity probed by fluorescently labeled peanut agglutinin (PNA); and cholesterol efflux assessed with BODIPY-cholesterol. We used the following small molecule inhibitors: Oligomycin A (cat # 41-105, R&D Systems; inhibitor of ATP synthase F0 in mitochondria), Azoxystrobin (cat # NC1774837, Chem Service; inhibitor of cytochrome b-c1 complex in mitochondria), Dynarrestin (cat # HY-121802; Medchemexpress; inhibitor of dynein 1 and 2 in axoneme), and Pitstop 2 (cat # HY- 115604; Medchemexpress; inhibitor of amphiphysin with clathrin interaction in Golgi/acrosome). We report that the addition of Oligomycin A or Azoxystrobin in IVC media had a significant inhibitory effect on sperm motility ($P<0.05$), including total and progressive motility; curvilinear, average path, and straight line velocities; linearity, straightness, and beat cross frequency when compared to vehicle control IVC spermatozoa. Furthermore, both Oligomycin A and Azoxystrobin significantly decreased ($P<0.05$) the capacitation-associated sperm zinc efflux and PI incorporation into the nucleus. Spermatozoa incubated with Azoxystrobin during IVC had a significantly higher ($P<0.05$) percentage of intact acrosomes, while Oligomycin A treatment significantly decreased cholesterol efflux during IVC. Spermatozoa capacitated with Dynarrestin exhibited significantly decreased ($P<0.05$) curvilinear velocity, amplitute of lateral head displacement, and increased cholesterol efflux when compared to IVC vehicle control. Lastly, a significant reduction ($P<0.05$) of zinc efflux was observed in Pitstop 2 IVC spermatozoa. Our results provide the first functional evidence of the participation of NDA pathway proteins in sperm capacitation. The reversible nature of the selected inhibitors enables them to be used as modulators of semen extenders, insemination doses, or in vitro fertilization media for both human and animal ART.

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