Quercetin Affects Boar Sperm Motility During Refrigerated Storage

Felipe Martínez-Pastor[1,2]; Estíbaliz Lacalle[1,3]; Gabriela García-Blanco[1]; Victoria Alonso-Pascual[1]; Beatriz Sánchez de Blas[1]; Estela Fernández-Alegre[1,3]; Ileana Bonilauri[1,3]; Beatriz Martín-Fernández[1,2]

[1]INDEGSAL, Universidad de León, León, Spain; [2]Molecular Biology (Cell Biology), Universidad de León, León, Spain; [3]Bianor Biotech, University of León, León, Spain

Polyphenols are a group of bioactive molecules produced by the secondary metabolism of plants and other organisms. They have many properties, including being antimicrobials. This is a very exciting application since antimicrobial resistance (AMR) is a critical challenge for humankind. Polyphenols (with no risk for promoting AMR on substances of medical importance) could substitute antibiotics in many applications. Animal production, being an area with extensive use of antibiotics, could benefit, including animal reproduction and the production of sperm media. Specifically, the pig industry annually uses a considerable amount of semen extenders containing wide-spectra antibiotics. Whereas the production of pure polyphenols is often expensive and therefore impractical for the requirements of the pig industry, new developments have enabled the production of plant extracts through cost-effective and environmentally friendly procedures. These extracts might allow the reduction or substitution of antibiotics in boar semen doses, improving the industry's sustainability.

However, the effects of polyphenols on sperm physiology and fertility are still little researched, making it necessary to perform preliminary studies to increase our knowledge and refine the application of complex extracts. In this regard, quercetin is a particularly interesting polyphenol from the flavonol class, which is found in appreciable quantities in plant extracts obtained by many methods. It offers considerable promise as a potent antioxidant, anti-inflammatory drug, and modulator of detoxification pathways. Considering the effects on spermatozoa, quercetin could modulate motility by blocking (Ca2++Mg2+) ATPases, and it has been reported in ruminant spermatozoa (e.g., doi:10.1016/0005-2736(83)90063-9). Still, more information on boar spermatozoa must be available, especially during refrigerated storage. Therefore, we designed a panel to test quercetin in a wide range of concentrations (semi-logs from 32 nM to 100 µM), using no supplementation and vehicle (0.1% DMSO) as controls, and the polyphenol gallic acid (GA, usually used as a reference for polyphenol quantification) for comparison. Semen doses were obtained from a stud center, pooled, and split among the treatments. Spermatozoa were stored at 17 °C and assessed by CASA (Computer Assisted Sperm Analysis) at 37 °C on days 0, 2, and 6 (3 and 7 days of storage). The experiment was triplicated, and data were analyzed by linear mixed-effect models and heatmaps (effects relative to the control).

Results indicated that the vehicle did not affect motility. Quercetin did not affect total or progressive motility or improve it compared to the control on days 0 and 2 in the nM-low µM range, decreasing them at 10 µM-100 µM. On day 6, results were neutral at 32 and 316 nM, with lower motility for the other concentrations. Comparatively, GA only showed a negative effect on day 6 by 10 µM-100 µM, being positive in the low range of concentrations.

Nevertheless, on day 2, quercetin was superior to GA by 32–316 nM. Considering the kinematic variables, quercetin showed a trend of lowering sperm velocity dose-dependently on day 0. It was less clearly associated with concentration on days 2 and 6, whereas linearity was promoted overall.

In conclusion, plant extracts for pig sperm diluents with a low quercetin content should be selected. However, future studies must consider the combined effects on plant extracts and the possible benefits of other sperm features.

Supported by NeoGiANT 101036768 (H2020-LC-GD-2020-4) and PID2022-137640OB-I00 (MCIN/AEI/10.13039/501100011033, by "ERDF A way of making Europe"). Thanks to AIM/Topigs-Norsvin and Á. López.