Comparative Application of a Natural, Sustainable Plant Extract with Antimicrobial Properties in Fish, Rooster, Bull, and Boar Spermatozoa

<u>Felipe Martínez-Pastor</u>^{1,2}; Borys Dzyuba³; Estíbaliz Lacalle^{1,4}; Gabriela García-Blanco¹; Julián Santiago-Moreno⁵; Estela Fernández-Alegre^{1,3}; Sonia Martínez-Martínez⁶; Martin Faldyna⁷; Ileana Bonilauri^{1,4}; Raquel Ausejo⁸; Beatriz Martín-Fernández^{1,2}; Úrsula Álvarez⁸; Serhii Boryshpolets³

¹INDEGSAL, Universidad de León, León, Spain; ²Molecular Biology (Cell Biology), Universidad de León, León, Spain; ³University of South Bohemia, České Budějovice, Czech Republic; ⁴Bianor Biotech, University of León, León, Spain; ⁵INIA-CSIC, Madrid, Spain; ⁶Veterinary Research Institute, Brno, Czech Republic; ⁷Animal Health, Universidad de León, León, Spain; ⁸Magapor, Ejea de los Caballeros, Zaragoza, Spain

Antimicrobial resistance (AMR) is one of the challenges for humankind, mainly caused by the overuse of antibiotics. Applying natural plant extracts, rich in polyphenols and with antimicrobial properties, could positively contribute to solving this problem. Semen extenders (used in the millions of liters yearly) usually contain wide-spectra antibiotics, being a target for new formulations using antimicrobial extracts. However, spermatozoa are sensitive to bioactive molecules, and it is necessary to establish compatible concentrations.

This communication presents preliminary results on sperm motility from applying grape marc extracts, obtained by an environmentally friendly method, on spermatozoa from species of commercial interest: sturgeon, carp, trout, chicken, pig, and cattle. This information was obtained in the NeoGiANT H2020 project (<u>https://neogiant.es</u>) for replacing antibiotics in animal production with plant extracts obtained from a sustainable, environmentally friendly, and patented method. Some details are temporarily limited due to intellectual property protection issues.

Sperm doses were collected and diluted using species-specific methods and extenders and evaluated after refrigerated storage. In total, samples from 30 sturgeons, carps, and trouts, 20 roosters, 10 boars, and 10 bulls were used. In all cases, we tested extracts obtained using a glycol and an alcohol (both in the chemical GRAS category), following a progressive experimental design: a first trial was focused on concentrations around 1%, and successive ones focused on higher or lower ranges, depending on results (including control with no extracts). Sperm motility (CASA, computer-assisted sperm analysis) was assessed at day 0 and after 3, 6, and 9 days at 5 °C for fish, 1 and 2 days at 5 °C for chicken, 3 and 7 days at 17 °C, and 24 h at 5 °C for bull. Data were analyzed by linear mixed-effects models.

Results indicated that fish and boar spermatozoa were sensitive to these polyphenol-rich extracts, but low concentrations were compatible and potentially beneficial for sperm storage. On the contrary, bull spermatozoa tolerated the polyphenol-rich extracts well, with an excellent potential for application in sperm preservation. Rooster spermatozoa showed moderate compatibility, but the tolerated concentration was higher than that of fish and pig.

Establishing these ranges will enable the development of suitable extenders with no or reduced antibiotic contents. Further studies should focus on sperm physiology to determine how the extracts modulate it during storage and assess their effects on sperm cryopreservation, which is particularly interesting for the cattle industry.

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