Validating Sperm Surface Lectin Targets for Use in the Nanopurification of Bull Semen

Lauren E. Hamilton[1], Abigail L. Zezeski[2], Carolina L. Gonzalez-Berrios[2], Karl C. Kerns[3], Peter Sutovsky[1,4], Thomas W. Geary[2]

[1]Division of Animal Sciences, University of Missouri, Columbia, MO, USA
[2] Fort Keogh Livestock and Range Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Miles City, MT, USA
[3]Department of Animal Science, Iowa State University, Ames, IA, USA
[4] Department of Obstetrics, Gynecology & Women's Health, University of Missouri, Columbia, MO,USA

The spermatozoon glycocalyx is the initial point of contact with the female reproductive tract epithelia and oocyte vestments. Composed of a heterogeneous multi-layered exocellular coating of glycans and glycosylated proteins, this sperm coating has been shown to influence sperm maturation, motility, and fertilization. By targeting the overall composition of the glycocalyx for superior bull sperm selection, our past work demonstrated that nanopurification of pre-processed semen by using lectin PNA-coated magnetic nanoparticles enriched sperm quality and decreased the number of spermatozoa required in an insemination dose by half without compromising pregnancy rates. In the current study, we have evaluated a new set of lectin-conjugated magnetic nanoparticles targeting different surface glycans, both alone and in combination. The lectins PNA, LBA, LCA, and UEA1 were included in our evaluations. We aimed to investigate how selective removal of spermatozoa based on their surface glycan motifs can influence pre- and post-cryopreservation bull sperm parameters. Twelve mature bulls (2.5-4 years old) from the Fort Keogh Livestock and Range Research Laboratory were used for bull semen purification. Semen was collected by electroejaculation, and two ejaculates were pooled per collection for each bull. Semen was assessed for sperm viability, membrane integrity, mitochondrial membrane potential, oxidative potential, and capacitation status (zinc signatures) using conventional flow cytometry at three timepoints (neat semen, post purification and post thaw of cryopreserved samples). Motility and morphology were also assessed at all three timepoints by using an AndroScope computer-assisted semen analysis (CASA) and light microscopy, respectively. Purification using lectin PNA (targets galactose), or a mixture of lectins UEA1 (targets fucose) and LBA (targets *N*-acetylgalactosamine); Mix2decreased ($P < 0.05$) the proportion of bovine spermatozoa with disrupted membranes post thaw when compared to non-sorted control (CON). Purification with Mix2 also increased ($P < 0.01$) the percentage of bovine spermatozoa that were able to effectively respond to an oxidative challenge post thaw when compared to CON, suggesting a greater potential to mitigate oxidative stress. Additionally, purification using a combination of PNA, UEA1 and LBA (Mix1) tended ($P = 0.08$) to improve post thaw motility. We plan to conduct a heterospermic field trial with bull semen from the best performing lectin-coated nanoparticles to assess the impact of the nanopurification on bovine pregnancy rates. Overall, this work will advance our knowledge of sperm surface glycans, their impact on bull sperm survival, fertilization, and potential use as bull sperm fertility biomarkers to improve bovine reproductive efficiency.

This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2022-67015-36300 from the USDA National Institute of Food and Agriculture.