## Optimization of the Sperm Energy Restriction and Recovery (SER) Treatment in the Bovine Model

<u>Maria G. Gervasi<sup>1</sup></u>; Camila Arroyo-Salvo<sup>2</sup>; Claudia Osycka-Salut<sup>3</sup>; Silvina Perez-Martinez<sup>2</sup>; Rafael Fissore<sup>4</sup>; Pablo Visconti<sup>4</sup>

- 1. Department of Animal Science, University of Connecticut, Storrs, United States
- 2. Centro de Estudios Farmacologicos y Botanicos (CEFYBO, CONICET-UBA), Buenos Aires, Argentina
- 3. Instituto de Investigaciones Biotecnologicas (IIB-INTECH), Universidad Nacional de San Martin, San Martin, Buenos Aires, Argentina
- 4. Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, United States

In recent years, there has been a rise in the utilization of in vitro produced embryos within the global cattle industry. Enhancing embryo production to augment the quantity and quality of embryos capable of producing viable offspring holds significant promise for the agricultural sector. Our recent investigations have shown the efficacy of sperm energy restriction and recovery (SER) treatment in enhancing sperm function and increasing fertilization and early embryo development rates following in vitro fertilization (IVF) in mice. In addition, in the bovine model, SER treatment has shown to enhance the outcome of intracytoplasmic sperm injection (ICSI) without the need for exogenous chemical activation. When applied prior to ICSI, SER-treated sperm led to a 3-fold increase in the percentage of 2-cell embryos compared to non-treated sperm (control, 50% SER vs. 16% control). Notably, 17% of SER-derived embryos progressed to the blastocyst stage while none of the control-derived embryos did. Here, we focused on the optimization of the SER treatment for the bovine model and evaluated its impact on sperm function. Our findings reveal that a onehour incubation under starvation conditions, followed by recovery in a complete media (containing lactate and/or pyruvate), significantly enhance total and progressive sperm motility (N=4), as assessed by computer-assisted sperm analysis (CASA). This improvement in sperm motility is most pronounced under non-capacitating conditions (N=4). Given the nutrient deprivation aspect of SER treatment, we examined the effects of the addition of either pyruvate, lactate, or a combination of both during the recovery phase. Our results indicate that the maximal improvement in sperm motility with SER occurs when sperm are incubated under starving conditions and subsequently recovered in a medium containing both lactate and pyruvate (N=3). In summary, our findings underscore the efficacy of SER in enhancing sperm function and support its potential application for the in vitro production of bovine embryos.

This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2022-67016-36302 from the USDA National Institute of Food and Agriculture.