Search for sufficient superovulation and optimal embryo cryopreservation in inbred rat strains in HRDP

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The Hybrid Rat Diversity Program (HRDP) includes a carefully chosen panel of 96 inbred rat strains, selected for power to detect specific genetic loci associated with a complex trait while maximizing the genetic diversity among strains. Key to the success of this resource, as well the continued availability of unique rat models for the scientific community, are cryopreservation and rederivation methods carefully tailored to improve the efficiency and success of embryo collection, storage, and thawing. HRDP rederivation success rates varied by strain and protocol used during the original cryopreservation and rederivation processes. In this study, we evaluated the normality of embryos before and after cryopreservation, to search for efficient methods for collecting embryos by hormone treatment and cryopreservation protocols for the four parental strains used to develop the two panels of recombinant inbred (RI) strains included in the HRDP. These parental strains (BN-Lx/Cub (BN-Lx), SHR/Olalpcv, LE/Stm, F344/Stm), two commonly used inbred strains (SS/JrHsdMcwi, ACI/EurMcwi) in HRDP, and one outbred strain (WI:Crl) were included in this study.

We investigated superovulation characteristics of these strains using conventional pregnant mare serum (PMS), anti-inhibin serum (AIS), and follicle stimulating hormone (FSH) protocols. Our results demonstrated that superovulation with FSH could be used effectively without implanting an osmotic pump. Collected embryos using this method were cryopreserved by vitrification and developed normally both *in vitro* and *in vivo*. The treatment of superovulation in the BN-Lx strain was successful, however, we encountered challenges with mating and fertilization. The estrous cycle of BN-Lx, using both smear and impedance methods, revealed that proestrus was not consistently identified by both parameters simultaneously. This discrepancy likely contributes to the difficulties in mating and embryo collection for this strain. Additional studies will be instrumental to further optimize the protocols for each strain and make this resource more valuable to the rat research community.