ABSTRACT OF THESIS

ANALYSIS OF THE MICRORNA EXPRESSION PROFILES OF CHICKEN PRIMORDIAL GERM CELLS BEFORE AND AFTER FREEZING

Author: Afewerki Yosief Alem

JMG9D7

MSc. Animal Biotechnology Department, Institute of Genetics and Biotechnology

Primary supervisor: Dr. Elen Gócza

Scientific advisor

Independent Consultant: Maria Teresa Salinas Aponte

PhD. candidate

Institute/Department

MATE GBI, Animal Biotechnology Department Applied Embryology and Stem cell Biology Group

Gödöllő, Hungary

Primordial germ cells are the precursors of functional gametes. Semen cryopreservation is the most practical method for preserving genetic resources in birds, but it only conserves the male genome. Embryo and oocyte cryopreservation would retain the W chromosome but is impractical due to the nature of the egg (large yolk). Because of all these disadvantages of semen and embryo preservation, PGC storage could become the most important in avian species. PGCs from chickens can be isolated, cultured, and cryopreserved and remain committed to the germ line.

MicroRNAs (miRNAs) are small noncoding RNAs, about 22 nucleotides long, that target mRNAs for translational repression or degradation. Stem cell-specific miRNAs are important in chicken primordial germ cells (PGCs) because they regulate gene expression during PGC development. PGCs are the cells that give rise to the germ cells in the adult chicken, and they undergo a series of developmental changes as they differentiate and migrate to the gonads.

This study aims to determine the expression of CVH, DAZL, miR-92, and miR-302b-5P before and after freezing to test two freezing media for long PGC conservation. PGCs were established from a 3-day-old chicken embryo blood sample by isolating, culturing, and expanding PGCs in vitro. PCR sexing was used to determine the embryo's gender after extracting DNA from the isolated tissue. RNA samples were extracted from male and female PGC cell lines before and after freezing with two different avian-freezing media. cDNA was

synthesized from the RNA samples to examine the expression levels of CVH, DAZL, miR-302b-5p, and miR-92 via qPCR. GenEx software analyzed the Ct (cycle threshold) values obtained from the qPCR machine. By examining gene expression levels, the study aimed to determine the effects of freezing on PGCs' gene expression and identify the most suitable freezing medium for PGC storage.

The study compared the expression of marker genes and miRNAs in male and female samples. The results showed a significant difference (P = **0.043)** in the expression of CVH between males and females, with higher expression in males. Conversely, there were no significant variances in the expression of DAZL, miR-92, and miR-302b-5p between the two genders.

In addition, a Comparison was done on the expression of four genes in three groups of samples without considering gender. The groups were "Before freezing-FAM1", "Before freezing-FAM2", and "FAM1-FAM2". The result showed significant differences in DAZL expression before and after FAM1 treatment (**p = 0.006**) and before and after FAM2 treatment (**p = 0.014**). DAZL expression was significantly decreased after FAM1 and FAM2 treatments. However, there were no significant variances in the expression of CVH, miR-92, and miR-302-5p among the three groups.

Furthermore, the study compared the expression of CVH, DAZL, miR-92, and miR-302-5p among four groups: females before freezing and after freezing with FAM1, females before freezing and after freezing with FAM2, males before freezing and after freezing with FAM1, and males before freezing and after freezing with FAM2. The study found that in males, CVH expression was significantly higher before freezing than after treatment with FAM1 (**P = 0.055**). Meanwhile, DAZL expression was higher before freezing than after treatment with FAM1 (**P = 0.012**) and FAM2 (**P = 0.037**). However, no significant difference was detected in the expression of miR-92 and miR-302-5p before freezing and after treatment with FAM1 and FAM2 in males. In females, no significant differences related to the freezing media treatments were observed in the expression of CVH, DAZL, miR-92, and miR-302-5p.

FAM2, including DMSO and fetal bovine serum, was the optimal medium for PGC freezing. The expression of CVH is higher in males than in females before freezing. DAZL expression was higher before freezing than after FAM1 and FAM2 treatments in males. The study suggests that the FAM2 freezing procedure could be an appealing answer to the problem of maintaining the stemness for conserving PGCs for genetic material in avian species.