# *Gm614:* A NOVEL X-LINKED GENE ASSOCIATED WITH MALE INFERTILITY IN MICE

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OBJECTIVE:

Genetic causes of infertility are incompletely understood. Devoting increased study to infertility genes offers an opportunity for targeted fertility treatment. Among the protein-coding genes associated with male infertility, our institution discovered predicted gene 614 (*Gm614*) in the mouse genome. *Gm614* is a novel, X-linked male infertile gene. It has a human ortholog, chromosome X open reading frame 65 (*CXorf65*), and RNA expression data indicates both the mouse and human genes are robustly expressed in the testis. Our research aims to investigate the phenotypic and cellular consequences of *Gm614* deficiency in a knockout mouse model.

MATERIALS AND METHODS:

Adult male C57BL/6N wild-type (WT) and hemizygous *Gm614* knockout (KO) mice were used for all studies. A phenotypic assay of total count, motility and motion kinematics was done with Computer-Assisted Sperm Analysis (CASA). Sections of KO and WT testes were fixed, embedded, stained with Period Acid Schiff, and evaluated for defects in spermatogenesis. Acrosome reactivity was quantified and compared between WT and KO groups using mitochondria localized DsRed2 and acrosome localized GFP transgenes. To assess oocyte binding, in vitro fertilization (IVF) of zona pellucida intact and free oocytes was analyzed. RNA *in situ* hybridization using fluorescent anti-sense RNA probes against *Gm614* and Histone1t (*H1t,* spermatocytes) or Protamine1 (*Prm1*, spermatids) determined the expression of *Gm614* within spermatogenic lineage. Transfections of human embryonic kidney (HEK) cells with GFP-tagged *Gm614* were performed to assess intracellular localization of GM614. Student t-test and Chi square were used to analyze data.

RESULTS:

Histologic staging of spermatogenesis is normal in the KO. All measures of motion kinematics in KO caudal sperm, including progressive motility and velocity, are significantly lower than WT. Other parameters (e.g. total count and motile concentrations) are not significantly different. Acrosome reactivity is normal in KO sperm. In zona-free oocyte IVF, not only do KO and WT groups demonstrate fertilization and blastocyst formation but also produce viable pups. KO sperm has significantly reduced oocyte binding (18.31% vs 100%, $p<0.0001$) and fertilization (0.8% vs 31%, $p<0.0001$) with zona intact. RNA *in situ* hybridization shows overlap between *Gm614* and *Prm1*, not *H1t*, indicating RNA expression specifically in spermatids during spermatogenesis. Furthermore, transfection studies demonstrate GM614 protein localization to cell nuclei and likely association with chromatin.

CONCLUSIONS:

The infertility phenotype of the *Gm614* KO mouse is characterized by abnormal sperm progression, impacting the ability to penetrate and fertilize an oocyte. Otherwise, *Gm614* deficiencies are not evident on traditional semen analysis.

A burst of transcriptional activity in post-meiotic round spermatids is critical to complete sperm maturation. The RNA expression and protein localization data for GM614 suggests a role in regulating genes critical for the final steps of spermatogenesis. Aside from genetic evaluation, no testing exists to identify this defect in patients. An abnormality in *CXorf65*, the human ortholog of *Gm614*, may impact those with undiagnosed male factor infertility and would alter treatment recommendations.

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