PROTAMINE 2 DEFICIENT SPERM CAUSE ABNORMAL EMBRYOGENESIS IN MICE

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Background
Little is known regarding the molecular causes of male infertility, including how defects in sperm DNA organization may impact embryogenesis. Protamines are critical organizers of the sperm genome and are replaced by maternally-provided histones after fertilization. Protamine 2 knock-out (PRM2KO) male mice are infertile with immotile sperm, but few studies have tested the downstream effects on fertility if motility-defects are bypassed by intracytoplasmic sperm injection (ICSI) [1, 2].
Objective
To assess the developmental progression of embryos fertilized with PRM2KO sperm and identify molecular features that differ in comparison to normal fertilization.
Materials and Methods
ICSI was performed on oocytes from superovulated B6D2F1 mice using sperm extracted from C57BL/6 (WT) or PRM2KO mice. Embryos were monitored until controls reached the blastocyst stage at 84-96 hours post fertilization (hpf). We assessed DNA damage using the TUNEL assay in fixed early PN-stage zygotes. Later PN-stage zygotes were fixed and stained for DNA and Lamin A/C. FIJI and Imaris were used to assess fluorescence intensity signals as well as pronuclear volume and surface area. Two-sample t-tests were used for statistical analyses.
Result(s)
59% ± 26% of WT zygotes (n=72) developed to the 2-cell stage (24 hpf) and 66% ± 19% developed to the 4-cell stage (48 hpf). 29% ± 27% of PRM2KO-fertilized zygotes (n=82) developed to the 2-cell stage, and 0% ± 0% developed to the 4-cell stage. 57% ± 27% of the PRM2KO-derived embryos appeared fragmented at 24 hpf, before resolving to a 2-cell-like or fragmented stage by 48 hpf (80% ± 16%). Immunofluorescence of 0.5, 1, and 2 hpf fixed embryos revealed that paternal DNA signal is lost within the first 2 hpf, while maternal chromosomes proceed through anaphase II and pronuclear formation. TUNEL assay revealed that the PRM2KO sperm used in ICSI were positive for DNA breaks until the sperm's disappearance. Lamin A/C and Hoechst staining showed that the maternal pronucleus of 6 hpf PRM2KO-derived zygotes is abnormal, including significantly increased surface area as well as diminished Lamin A/C intensity.
Conclusion(s)
PRM2KO sperm-derived DNA is rapidly lost following ICSI-based fertilization into WT oocytes, but does not appear to impact oocyte activation and formation of a parthenogenic zygote. Maternal pronuclear morphology is defective and development does not proceed past the 2-cell stage, possibly due to the activation of an inappropriately-timed DNA damage response pathway.

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References:

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2. Schneider S, et al., *Protamine-2 Deficiency Initiates a Reactive Oxygen Species (ROS)-Mediated Destruction Cascade during Epididymal Sperm Maturation in Mice. Cells*. 2020 Jul 27;9(8):1789.