#22: Zinc deficiency impairs fertility and oogenesis in *C. elegans*

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Zinc is an essential micronutrient that is vital for successful reproduction. Zinc plays a role in successful spermatogenesis, oogenesis, fertilization, embryo development, and epigenetic programming. Given the ubiquitous role of zinc in reproduction, investigation into the cellular and molecular dynamics of zinc in reproductive tissues is an important area of research in order to understand infertility, epigenetic inheritance, and improve artificial reproductive technologies. New techniques and models are necessary to fully elucidate zinc's role in this context. The nematode *C. elegans* offers distinct advantages for reproductive research. The majority of *C. elegans* are self-fertilizing hermaphrodites, in which sperm and oocyte arise from a common gonad. This physiological arrangement allows researchers to observe and analyze oocytes, sperm, and developing embryos from the same subject. The short generation time, established genetic sequence, and conserved molecular mechanisms between *C. elegans* and vertebrates make this an intriguing model for reproductive research. We therefore sought to evaluate *C. elegans* as a model for zinc deficiency and test the hypotheses that zinc restriction would 1) lower fertility in *C. elegans* hermaphrodites; and 2) impair oocyte development as seen in mammalian models. We therefore placed developing worms onto culture plates treated with the zinc chelator N,N,N',N'-tetrakis(2-pyridylmethyl)ethane-1,2-diamine (TPEN) in order to restrict zinc. Fertility was then quantified by counting the number of offspring produced by control (n=5) or TPEN-treated (n=4) hermaphrodites throughout their reproductive lifespan. Zinc restriction significantly reduced the average number of progeny produced from 171.8 offspring per control worm to 41.75 progeny in TPEN treated worms ($p<0.001$). Subsequent imaging studies showed fewer maturing oocytes (control=8.4, TPEN=5.3; $p<0.001$) and fewer developing embryos (control=11.4, TPEN=7.5; $p<0.001$) in the reproductive tracts of TPEN treated subjects. DAPI staining also revealed altered chromosomal dynamics in developing oocytes, characterized by an expanded region of pachytene-stage oocytes (control=0.29 pachytene oocytes, TPEN=6.25; $p<0.001$). Supplementing TPEN-treated worms with zinc returned the number of developing oocytes and embryos to control levels and restored chromosomal dynamics. A lower number of unfertilized, developing oocytes in zinc-deficient worms confirms the hypothesis that at least a portion of impaired fertility resulting from zinc restriction is due to impaired oogenesis. Altered chromosomal dynamics also point to oocyte meiosis as a process vulnerable to perturbations in zinc levels. Possible deficits in sperm production, fertilization rate, or embryo development resulting from zinc deficiency remain to be investigated and quantified in this model. These results indicate that *C. elegans* are vulnerable to reproductive deficits as a result of zinc restriction, similar to vertebrate species. Our group has characterized a novel *C. elegans* phenotype that will be a valuable tool in investigating the role of zinc in reproduction. This research was supported in part by NIH Grant T32GM108563 and by the Huck Institutes of the Life Sciences through a J. Lloyd Huck Dissertation Research Grant.