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IMPAIRED FOLLICLE DEVELOPMENTAND SUBFERTILITY IN FEMALE MICE LACKING MFN2 IN OOCYTES.

M. Zhang,a M. B. Bener,a T. Wang,a E. Esencan,a R. Scott, III,a E. Seli.a,b aYale School of Medicine, New Haven, CT; bIVIRMA New Jersey, Basking Ridge, NJ.

OBJECTIVE: Mitochondria change their shape through fusion and fission (collectively referred to as mitochondrial dynamics) in order to adapt to the metabolic milieu. Mitofusin-2 (MFN2) is a key regulatory protein in this process, mediating mitochondrial fusion and interaction with endoplasmic reticulum. The aim of the present study was to determine the role of MFN2 in female reproductive competence using a mouse model with oocyte-specific deletion of Mfn2.

DESIGN: Experimental study.

MATERIALS AND METHODS: Mfn2flox/flox mice were crossbred with Zp3-Cre mice to produce mice with oocyte-specific Mfn2 deletion (Mfn2-/-). Mature (8-weeks-old) Mfn2-/- female mice were compared to wild type (WT). To evaluate fertility, Mfn2-/- and WT female mice were mated with adult WT males of proven fertility for 12 weeks. Follicle development was assessed in serial ovarian sections stained with hematoxylin and eosin. Ability to generate oocytes (germinal vesicle [GV] and metaphase II [MII]), 2-cell embryos and blastocysts was assessed after injection with PMSG (5IU) or PMSG and hCG (5IU) and mating with WT males as indicated. Spindle morphology was determined in in vivo and in vitro matured oocytes by staining with tubulin and DAPI. ATP levels were determined by bioluminescent assay. Mitochondrial DNA (mtDNA) copy number was measured in individual oocytes by cloning of mitochondria specific gene (Cox3) as a standard, followed quantitative real-time PCR (qPCR). ANOVA, student's t-test, and Chi Square analysis were used for statistical analysis as appropriate.

RESULTS: Mature female Mfn2-/- mice exhibited reduced fertility compared to WT females (5.21 _ 0.39 vs 7.63 _ 0.31 pups per litter, P<0.001). Mfn2-/- mice ovaries had similar number of primordial, primary and secondary follicles compared to WT. However, the number of antral follicles numbers in Mfn2-/- mice ovaries was significantly decreased (9.33 _ 2.33 vs 30.67 _ 1.67, P<0.01). Mfn2-/- mice generated a significantly lower number of GVoocytes (16.33 _ 1.20 vs 29.33 _ 0.67, p <0.001), MII oocytes (10 _ 0.58 vs 21.33 _ 1.20, p<0.01), 2-cell embryos (8 _ 0.58 vs 20.33_0.88, p<0.001) and blastocysts (6.33 _ 0.88 vs 13 _ 0.58, p<0.001). A significantly higher number of Mfn2-/- oocytes had abnormal spindles at MII oocyte stage (32.5 _ 2.5% vs 12.5 _ 2.5%, p<0.05). Decreased ATP production (0.82_0.09 vs 1.32_ 0.11, p<0.01) and mtDNA copy number (56,655 _ 20,659 vs 136,268 _ 24,588, p<0.05) were also observed in Mfn2-/-oocytes compared to WT.

CONCLUSIONS: Loss of mitochondrial fusion-regulatory protein MFN2 in oocytes results in female subfertility and abnormal oocyte and embryo development. Future studies are

required to determine how Mfn2 regulates oocyte’s adaptation to ovarian bioenergetic milieu and impacts upon female fertility, and whether MFN2 dysfunction is observed in human females with infertility. OVARIAN RESERVE