configuration, which has been hypothesized to be critical for the ordered exodus of the paternal genome following fertilization. This model describes centromeres clustering in the center (chromocenter), with p- and q-chromosome arms stretching toward the nuclear periphery. However, we recently proposed a refined segmental model of sperm chromatin organization; to further investigate these findings and their relationship to the hairpin-loop model we examined the 3D configurations of chromosomes in human sperm nuclei.

DESIGN: Transversal laboratory study.

MATERIALS AND METHODS: This study was approved by the local IRB, five normozoospermic males were recruited. Three-color fluorescence in-situ hybridization (FISH) was utilized to target the centromeres, and chromosome p- and q-arms of eight chromosomes (2, 3, 6, 8, 10, 12, 16, and 18). Wide-field fluorescence microscopy and 3D modeling was employed to image and visualize sperm cells and FISH probes in 3D. The radial organization of each targeted loci was established by measuring the distance of the geometric center of each loci to the nearest nuclear periphery. Nonrandom organization of was established using the Chi-squared goodness-of-fit test ($p<0.05$). Furthermore, hairpin-loop configurations were determined by the angle created between the p- and q-arms. A minimum of 30 cells per subject, per chromosome were studied.

RESULTS: Distinct reproducible chromosome-specific patterns of organization emerge. All chromosomes were found to possess nonrandom radial organization ($p<0.05$), with the exception of the chromosome 12 centromere. Chromosome arms were found to form discrete hairpin-loop configurations. However, different chromosomes were observed to preferentially form narrower or wider hairpin loops that were largely reproducible between the five subjects enrolled. We did not find evidence to support the existence of a centralized chromocenter(s) with 68.3% of investigated centromeres being more distally localized within the sperm nucleus than one (30.5%) or both (37.8%) of their respective chromosome arms.

CONCLUSIONS: We report reproducible nonrandom hairpin-loop organization of chromosomes that partially supports the proposed hairpin-loop model of organization. However, our findings do not support the existence of a centralized chromocenter. This provides further evidence to support a more segmented chromosome organization in the human sperm nucleus, which could result in specific genomic regions being exposed, remodeled and activated first, following fertilization. The sequential exodus and remodeling could impact patterns of gene activation observed within the early embryo, perturbations in which, could negatively impact fertilization and early embryogenesis. Further research is warranted to evaluate the functional relevance of the nonrandom hairpin-loop organization of chromosomes in sperm observed in this study, and how this may impact spermatogenesis, fertilization and embryogenesis.

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THE UBIQUINONE MITOCHONDRIA-TARGETED ANTIOXIDANT AMENDS THE EFFECT OF MATERNAL AGE ON OOCYTE SPINDLE FORMATION AND DEVELOPMENTAL COMPETENCE.

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OBJECTIVE: To examine the effect of maternal age on mitochondrial function and spindle formation in maturing oocytes, and to investigate whether in vitro treatment with mitochondria-targeted antioxidants (AOs) can reverse the impact of aging on oocyte quality.

DESIGN: Preclinical models of oocyte quality were used for this study. In experiment 1, oocytes were obtained from young (1-month-old) mice and treated with hydrogen peroxide (H2O2) to induced oxidative stress or with H2O2 and mitochondria-targeted AOs. In experiment 2, oocytes were collected from young and old (>12 months) mice and treated with mitochondria-targeted AOs during in vitro maturation (IVM). End point assays in both experiments were stage of maturation reached, mitochondrial function and spindle organisation.

MATERIALS AND METHODS: Cumulus-free oocytes were cultured in vitro for 14 h in M2 or M16 medium, or in the same medium containing H2O2 (25μM), with or without AOs (experiment 1). In a separate experiment, oocytes from young and old mice were matured in vitro in the presence and absence of mitochondria-targeted AOs. At the end of the culture period mitochondrial membrane potential (MMP) was measured by ratioing the fluorescence intensities of Tetramethylrhodamine methyl ester (TMRM), and MitoTracker green (MTG). Oocytes were fixed to label the microtubules and DNA. ImageJ software was used to analyse spindle dimensions and chromosome alignment. Student *t*-test and ANOVA were applied to compare between groups and a P value below 0.05 were considered statistically significant.

RESULTS: We find oxidative stress causes a decrease in MMP ($P< 0.001$) and an increase in the frequency of disrupted spindles and misaligned chromosomes ($P< 0.001$). Co-treatment with H2O2 and AOs reversed the MMP and spindle disruption to control levels. Oocytes from old mice matured to the MII stage in vitro also show decreased MMP and disrupted spindles. These age-related phenotypes were completely reversed by incorporating antioxidants in the culture media. Furthermore, for the first time we have performed live-cell ratiometric imaging of TMRM and MTG for the full time-course of maturation in young and old eggs. This study reveals that MMP increases significantly during IVM in young oocytes ($P< 0.001$) but not in old oocytes.

CONCLUSIONS: Oxidative stress and maternal age are both associated with decreased MMP and spindle disruption and chromosome misalignment. Time-lapse imaging suggests that mitochondria in young oocytes undergo an adaptive increase in MMP during IVM and that this capacity is lost in mitochondria of old oocytes. The compromised mitochondrial function in maternal aging and the ability of the mitochondria-targeted AOs treatment to mitigate against aging and oxidative stress-induced mitochondrial and spindle disruption, suggests that mitochondria may be a useful therapeutic target for improving oocyte quality.

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LOSS OF MITOCHONDRIAL FUSION PROTEIN MFN2 RESULTS IN A REPRODUCTIVE AGING PHENOTYPE WITH TELOMERE SHORTENING, REDUCED FERTILITY, AND ACCELERATED DEPLETION OF FOLLICULAR POOL.

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OBJECTIVE: Mitochondria change their shape through fusion and fission in order to adapt to their metabolic milieu and respond to environmental stress. 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: *Mfn2*$^{flox/flox}$ mice were crossbred with *Zp3-Cre* mice to produce mice with oocyte-specific *Mfn2* deletion (*Mfn2*$^{-/-}$). To evaluate fertility, mature (8-weeks-old) *Mfn2*$^{-/-}$ and wild type (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. RNA sequencing analysis was performed using pooled *Mfn2*$^{-/-}$ and WT GV oocytes and secondary follicle enclosed oocytes (SFOs) (n=3 mice per group). Protein and mRNA expression were assessed using immunofluorescence and qRT-PCR, respectively. Telomere length was assessed using quantitative real-time PCR.

RESULTS: Mature female *Mfn2*$^{-/-}$ mice exhibited reduced fertility compared to WT females (5.21 ± 0.39 vs 7.63 ± 0.31 pups per litter, $p<0.001$). They had decreased number of antral follicles (9.33 ± 2.33 vs 30.67 ± 1.67, $P<0.01$), and generated a significantly lower number of GV oocytes (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, $p<0.001$). RNA-seq analysis revealed 363 and 1041 genes that were differentially regulated in *Mfn2*$^{-/-}$ GV oocytes and SFOs, respectively. Affected pathways included telomere signaling in GV oocytes and cell death (apoptosis) signaling in SFOs ($p<0.01$). Pro-apoptotic protein caspase 6 and ceramide were significantly increased in *Mfn2*$^{-/-}$ SFOs. Telomere length in *Mfn2*$^{-/-}$ GV oocytes was shorter compared to WT with decreased expression of telomere protective protein TRF1. When we assessed changes in follicular pool across mouse reproductive lifespan, we found *Mfn2*$^{-/-}$ ovaries to have significantly lower number of