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# Mitochondrial dysfunction caused by targeted deletion of *Mfn1* does not result in telomere shortening in oocytes

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**Short Communication**

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## Summary

Telomere shortening during oocyte growth and development is related to reproductive ageing and infertility. The main mechanism involved in the maintenance of telomeres is based on telomerase activity, a specialized enzyme complex, which is capable of adding TTAGGG repeats at the ends of the chromosomes. Mitochondrial dysfunction may cause progressive shortening of telomeres by promoting the generation of reactive oxygen species. Mitofusin-1 is a protein required for mitochondrial fusion. Mice with the mitofusin-1 (*Mfn1*) deletion in the oocyte are characterized by accelerated follicular depletion and infertility, associated with defective oocyte maturation and follicular development. We hypothesized whether mitochondrial dysfunction in oocytes with targeted deletion of *Mfn1* causes telomere shortening. We analyzed telomere length in oocyte and somatic cells in 3-, 6- and 9-month-old $Mfn1^{-/-}$ and wild-type mice. Immunofluorescence in oocyte mice of TRF1 and H2A.X was assessed to evaluate the interplay between the end-protection functions and the response to DNA damage occurring inside the telomeric repeats. Mitochondrial dysfunction due to the deletion of *Mfn1* does not seem to affect telomere length in mouse oocytes.

## Introduction

Telomeres are nucleoprotein complexes that are present at both ends of chromosomes and contain telomeric DNA, which consists of long tandem repeats of the sequence $TTAGGG_n$. Telomeres protect the ends of the chromosomes from sequence erosion and fusion with neighbouring chromosomes. A progressive shortening of telomeres occurs with each cellular division and, when a critical length is reached, cessation of cell division and cellular senescence ensue.

Telomeres, and particularly the 5′-GGG triplet, are very sensitive to oxidative stress-induced DNA damage (Kawanishi and Oikawa, 2004). Mitochondrial dysfunction may cause progressive shortening of telomeres by promoting the generation of reactive oxygen species (ROS). Telomere shortening, in turn, is a key mechanism leading to cell senescence, which has also been associated with decreased oocyte quality through disruption of chromosome alignment and spindle structure during meiosis (Keefe and Liu, 2009). In addition, telomere shortening in mouse and human oocytes triggers apoptosis in embryos.

Mice with oocyte-specific deletion of mitofusin-2 (*Mfn2*, a GTPase localized in the outer mitochondrial membrane and essential for mitochondrial fusion) have subfertility and accelerated follicular depletion (Zhang *et al.*, 2019b). *Mfn2*-deficient oocytes have mitochondrial dysfunction, increased ROS, and shorter telomeres are suggestive of an ageing phenotype. Mitofusin-1 is another protein that is required for mitochondrial fusion. Mice with *Mfn1* deletion in the oocyte are characterized by accelerated follicular depletion and infertility associated with defective oocyte maturation and follicular development (Zhang *et al.*, 2019a).

In the current study, we aimed to determine whether mitochondrial dysfunction in oocytes with targeted deletion of *Mfn1* caused telomere shortening.

## Materials and methods

Germinal vesicle (GV) stage oocytes (collected from six mice for each genotype at each timepoint, and pooled as two mice per sample analyzed) were collected from 3-, 6- and 9-month-old $Mfn1^{-/-}$ mice and compared with wild-type (WT). Granulosa cumulus cells and white blood cells (WBC) were also evaluated as somatic controls.

GV-stage oocytes, oocytes at metaphase of second meiotic division (MII), and cumulus cells were obtained by superovulation of mature 2-, 6-, and 9-month-old $Mfn1^{-/-}$ and WT mice. To obtain oocytes at the GV stage, mice were euthanized by $CO_2$ inhalation 44 h after intraperitoneal injection of 5 IU PMSG. Ovaries were removed and punctured in M2 medium

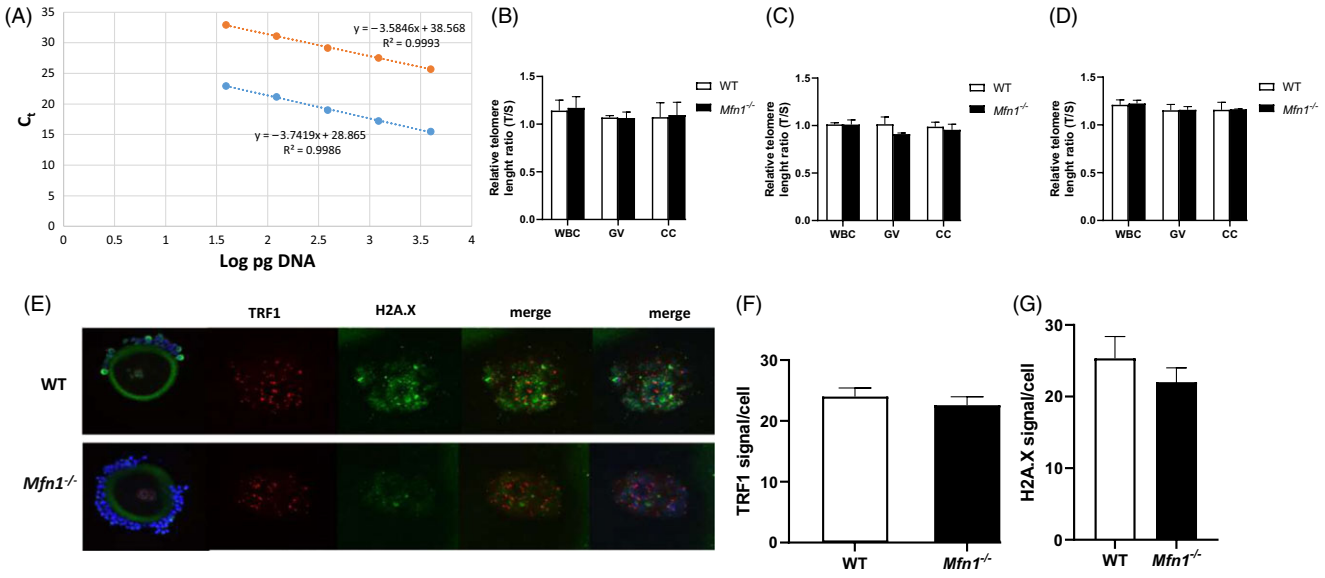


**Figure 1.** Telomere length in *Mfn1*$^{-/-}$ female mice. The standard curve was generated by serial dilution of known amounts of DNA to calculate relative DNA concentrations (log DNA) from $C_t$ values of the qRT-PCR products. Blue dots, telomere gene; orange dots, 36B4 single-copy gene control. The correlation regression equation and coefficients (R2) of $C_t$ versus log DNA are shown (A). The relative telomere length of white blood cells (WBC), GV oocytes and cumulus cells (CC) are represented as ratio of T/S, in 3-, 6- and 9-month-old wild-type (WT) and *Mfn1*$^{-/-}$ female mice respectively (B–D). Immunofluorescence double staining of TRF1 (red) and H2A.X (green) in cumulus–oophorus complexes of *Mfn1*$^{-/-}$ and WT mice. 4′,6-Diamidino-2-phenylindole (DAPI) was used to stain nuclei (blue) (E). Quantitative analysis of TRF1 and H2A.X immunofluorescence in *Mfn1*$^{-/-}$ and WT GV oocytes (F, G). Data are presented as mean ± SEM using a *t*-test.

(Sigma, St. Louis, MO) with 10 μM milrinone (Sigma, St. Louis, MO) under a dissecting microscope (Olympus SZH-ILLK) with a 26½-gauge needle to isolate cumulus–oophorus complexes containing GV-stage oocytes and cumulus cells. Oocytes were stripped from cumulus cells using a mouth pipette and collected in individual tubes. DNA was extracted from oocytes and cumulus cells using the QIAmp DNA Micro Kit (Qiagen, Valencia, CA) and quantified. Approximately 0.5 ml of blood sample was collected from each mouse and blood DNA was extracted using the DNA isolation kit for mammalian blood (Roche, Basel, Switzerland). A standard curve for polymerase chain reaction (PCR) was generated by serial dilutions of known amounts of DNA from somatic (WBCs and granulosa) cells (Fig. 1A). The telomere/single-copy gene ratio (T/S) was used as an indicator of telomere length (Liu *et al.*, 2007). Immunofluorescence for TRF1 and H2A.X was quantified using a rat anti-TRF1 monoclonal antibody (Abcam, Cambridge, UK, cat. no. ab192629) and a mouse anti-H2A.X monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA, cat. no. 05-636-25UG) as the primary Alexa Fluor 568-conjugated goat anti-rat antibody and Alexa Fluor 488-conjugated goat anti-mouse antibody and secondary antibodies, respectively.

## Results and Discussion

A standard curve for PCR was generated by serial dilution of known amounts of DNA from somatic (WBCs and granulosa) cells (Fig. 1A). Telomere length in oocytes from 2-month-old *Mfn1*$^{-/-}$ mice was not different compared with WT. The number of PCR cycles (21.8 ± 1.79 vs 21.4 ± 1.68, $P = 0.84$) and telomere/single-copy gene ratio (T/S), used as an indicator of relative telomere length (1019 ± 0.02 vs 1027 ± 0.06, $P = 0.93$), were also similar (Fig. 1B). Telomere length of granulosa cells and WBCs in 2-month-old *Mfn1*$^{-/-}$ mice were also unchanged compared with WT (Fig. 1B). To determine if there was an age-related effect, 6- and 9-month-old *Mfn1*$^{-/-}$ and WT mice were similarly tested. There was no statistically significant difference in telomere length in oocytes, granulosa cells and WBCs compared with WT at 6 and 9 months (Fig. 1C, D). Also, *Mfn1*$^{-/-}$ oocytes had a similar expression of telomere protective protein TRF1 (Fig. 1E, F). DNA repair through histone H2A.X phosphorylation also did not seem to be activated in *Mfn1*$^{-/-}$ (Fig. 1E, G) as there was no significant increase in the co-localization of TRF1 and H2A.X (Fig. 1E).

In conclusion, mitochondrial dysfunction due to the deletion of *Mfn1* does not seem to affect telomere length in mouse oocytes.

**Author contributions.** MC and ES conceived the idea, wrote the manuscript and provided approval for the version to be published. All authors agreed to be accountable for all aspects of the article.

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**Conflicts of interest.** MC declares no conflict of interest. ES is a consultant for and receives research funding from the Foundation for Embryonic Competence; he is also co-founder and a shareholder of ACIS LLC and co-holds the patent US2019/055906 issued for using electrical resistance measurement for assessing cell viability and cell membrane piercing.

**Ethics approval for animal study.** Mice care, breeding, and experimental procedures were conducted according to Yale University animal research requirements, using protocols approved by Institutional Animal Care and Use Committee (protocol #2020-11207).

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