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LETTER TO EDITOR

Reply to: Technical specificities of the study of the mitochondrial genome

Sir,

In response to the letter sent by Boguenet *et al.* (2022) raising concerns about the method used to quantify mitochondrial DNA (mtDNA) in cumulus cells in our article, we would like to clarify two points.

First, the quantification of mtDNA was indeed performed as described by Boguenet *et al.* (2022) and the Materials and methods section in our article (Martínez-Moro *et al.*, 2022): two parallel qPCRs amplified an mtDNA sequence and a nuclear gene on each sample. That procedure is usually named relative quantification (Schmittgen and Livak, 2008)—not absolute—as the copy number of mtDNA is relative to the copy number of the nuclear gene. Transforming those Ct values to copy number/cell as suggested by Boguenet *et al.* will be also a relative method and will not change the lack of significant differences between groups, as $2^{-\Delta Ct}$ (the parameter used) is obviously proportional to copy number/cell (the parameter proposed). In other words, the graphs would be the same but with different units on the *Y*-axes and no relation between mtDNA amount in cumulus cells and oocyte developmental potential will be observed.

Second, we acknowledge that most primers used to quantify mtDNA can amplify sequences inserted on the chromosomal DNA that we can call NUMTs for simplicity. This is not a problem exclusive to our primers, but also to primers used in other CCs studies. For instance, the primers used in the publications where the authors of the letter reported a relation between mtDNA in CC and oocyte competence (Desquiret-Dumas *et al.*, 2017; Taugourdeau *et al.*, 2019) (i.e. F: 5′-TAGACCAAACCTACG CCAAA-3′ and R: 5′-GTAACGTCGGGGCATTCCGG-3′; NC_012 920.1) also amplify a NUMT in chromosome 1 (NC_000001.11) of the same length (107 bp) as the sequence amplified in mtDNA, so the amplification of a NUMT is not a valid reason to explain the differences between those studies and ours. In any case, the amplification of a NUMT does not significantly affect mtDNA quantification in CCs for two reasons: (i) mtDNA copy number greatly outnumbers chromosomal copy number in cumulus cells (∼50 to 100× according to Fig. 2 in Taugourdeau *et al.* (2019)), so most of the quantified DNA will be of mitochondrial origin (98–99 molecules out of 100), and (ii) the copy number of the NUMT is always proportional to the copy number of the chromosomal sequence used to perform the relative analysis, as both are from chromosomal origin.

Given the reasons explained above, we firmly believe that the methods used in our article are solid and, therefore, we recommend not using mtDNA content in cumulus cells as a parameter to infer oocyte developmental competence in humans, based on our results and those obtained by others (Kumar *et al.*, 2021; Liu *et al.*, 2021).

Conflict of interest

The authors have no conflicts of interest to disclose.

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