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Placental dysregulation of mitochondrial morphology and dynamics as a hallmark of maternal age–related adaptation

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ABSTRACT

Aims: Advanced maternal age (AMA), increasingly prevalent worldwide, is linked to obstetric risk even in clinically uncomplicated pregnancies. Mitochondria are essential for trophoblast metabolism and stress adaptation, and their alteration is associated with gestational pathologies. However, it remains unclear whether maternal age alone alters placental mitochondrial homeostasis.

Materials and methods: Human placentas from AMA and control pregnancies were analysed by transmission electron microscopy (TEM) to assess mitochondrial ultrastructure and mitochondria–endoplasmic reticulum contacts (MERCs). Western blotting was used to evaluate regulators of mitochondrial fusion, fission, and mitophagy.

Key findings: placentas from AMA pregnancies showed a significant increase in mitochondrial number in both syncytiotrophoblast and cytotrophoblast cells, with regional variation between maternal and foetal sides. Despite increased abundance, mitochondria were smaller (reduced area and perimeter), indicating a fragmented phenotype, while circularity was unchanged. MERCs exhibited decreased distance and increased ER coverage, suggesting enhanced stress signaling and fission. Western blotting revealed decreased MFN1 with increased OPA1 and DRP1 expression, whereas MFN2, FIS1, and DNM2 remained unchanged. Mitophagy markers were dysregulated, with reduced OPTN and BNIP3 but elevated FUNDC1.

Significance: AMA is associated with fragmented and stress-adapted placental mitochondria, showing structural imbalance in dynamics and altered quality control even in the absence of clinical pathology. These features may reflect reduced placental capacity to buffer metabolic and stress challenges and contribute to increased vulnerability in pregnancies of older mothers, positioning mitochondria as a potential target for monitoring and improving outcomes in this population.

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1. Introduction

Advanced maternal age is defined as an age ≥ 35 years old in the moment of delivery and has been associated with adverse neonatal and obstetric outcomes, as well as with a high prevalence of a number of gestational complications, including gestational diabetes mellitus (GDM), preeclampsia (PE), preterm delivery, low birthweight, high rate of Neonatal Intensive Care Unit admission, and worse Apgar score [1,2]. Many sociodemographic factors of current societies have driven a gradual increase of the age of first motherhood over the years, pushing the mean age of pregnancy in Europe to 31.2 years old, according to Eurostat [3]. This situation is even more extended in Spain, since according to the National Institute of Statistics, the age of first motherhood is already near 33 years old [4]. Therefore, understanding the biological mechanisms linking maternal aging to placental dysfunction is becoming increasingly relevant.

The most vital organ for the development of pregnancy is the placenta, as it constitutes the active interface between maternal and foetal blood circulations and regulates physiological changes [5]. Successful pregnancy requires precise placental adaptation to a changing metabolic and oxidative environment, and mitochondria play a central role in this process, as they regulate cellular energy production, redox balance, apoptosis, and stress signaling. Aging is characterised by cumulative mitochondrial alterations, including impaired quality control, increased oxidative stress, and structural remodelling [6,7]. Given the high metabolic demands of the placenta, age-associated mitochondrial vulnerability could represent a plausible mechanistic link between maternal aging and impaired placental adaptation, possibly increasing the incidence of pregnancy complications and pathologies in elderly mothers [8]. Eventually, this could also represent a risk factor for the development of non-communicable diseases (NCDs) in postnatal life, behaving as a further cumulative influence conditioning health and disease throughout life [9].

Mitochondrial structure is not static but dynamically regulated through coordinated processes of fusion, fission, and mitophagy, collectively referred to as mitochondrial dynamics. These processes determine mitochondrial morphology, network organization, turnover, and stress responsiveness, thereby directly influencing cellular bioenergetic efficiency and resilience to environmental challenges [10,11]. Alterations in mitochondrial dynamics have been implicated in pregnancy-related disorders such as preeclampsia and foetal growth restriction [12–14], conditions characterised by placental oxidative stress and maladaptation. However, whether maternal aging per se modifies placental mitochondrial dynamics in humans remains largely unexplored. Importantly, mitochondrial morphology and network configuration are tightly linked to functional performance. Increased fission and fragmentation are commonly associated with oxidative stress and reduced respiratory efficiency, whereas fusion supports metabolic flexibility and stress compensation. Therefore, assessing structural and dynamic mitochondrial markers provides biologically meaningful insight into placental adaptive capacity [7,15].

To date, no studies have specifically evaluated the impact of AMA on placental mitochondrial dynamics in human pregnancies. Addressing this gap is essential to determine whether altered mitochondrial remodelling may represent an early mechanistic pathway linking maternal aging to placental stress susceptibility and adverse pregnancy outcomes. The present study was designed to investigate this relationship and to clarify whether AMA is associated with measurable changes in key regulators of mitochondrial dynamics in the human placenta.

2. Methods

2.1. Subjects

The cohort of subjects participating in this study comes from a multidisciplinary research project (GESTAGE) whose aim is to assess the impact of maternal age on various metabolic and physiological pathways in nulliparous mothers. The established inclusion criteria were: freely agree to participate, signed informed consent (by volunteer or parents/legal guardians of the newborn); pregnant woman with normal pregnancy course; body mass index of 18–30 kg/m2 at the start of pregnancy, and singleton gestation. The exclusion criteria were as follows: chronic disease requiring long-term treatment, malnutrition, morbid obesity or underweight, foetal death, chromosomal/congenital malformations, uterine growth retardation, non-acceptance of the informed consent. The recruitment and data collection process involving two hospitals from different Spanish cities has been previously described and published by Puche-Juarez et al., 2024 [16]. Table 1 shows demographic information of AMA and control groups, including anthropometric measurements of mothers and newborns, method of conception, method of delivery, gestational age, and sex of the baby, reporting no statistically significant differences between groups. Participants have the right to withdraw from the study at any point, following the ethical guidelines provided by the Declaration of Helsinki. The study has been approved by the bioethics committee for research with humans: Sistema de Información de los Comités de Ética de la Investigación de Andalucía (approval number SICEIA-2025-000255; date of approval 06/03/2025); and Portal de Ética de la Investigación Biomédica de Andalucía (approval number 27/04/2020/4/2020; date of approval 27/04/2020).

2.2. Placental tissue collection

Placental samples were collected immediately after delivery, being the size, shape, consistency and integrity of the placenta examined to assure the absence of accessory lobes, infarctions, haemorrhages, calcification, tumours and nodules.

To obtain protein extracts, three wedges of 3 × 3 × 2 cm were taken from the same place of placental cotyledons, about 5 cm away from the umbilical cord insertion site, excluding membranes. They were separately subjected to multiple washes with a cold 0.9% NaCl solution with 0.1% butylated hydroxytoluene, being the processing time less than 15 min and keeping them frozen at −80 °C until analysis.

Tissue collected for transmission electron microscopy (TEM) examination was taken from the exact same place, by cutting longitudinally, from the maternal to the foetal side, a 1.5 cm square-shaped segment through the entire thickness of the placental disk. This tissue was

Table 1
Demographic information of AMA and control groups.

		Control group	AMA group
Mother's age (years)		29.8 ± 4.09	38.61 ± 2.45***
Mother's age range (years)		20–34	35–44
Mother's weight (kg)		64.22 ± 4.6	63.96 ± 12.08
Mother's height (cm)		163.12 ± 3.67	164.05 ± 5.51
Mother's BMI (kg/m^2)		23.93 ± 2.69	23.78 ± 4.39
Method of conception	S (%)	81.8	73.9
	I-OI (%)	4.55	4.3
	IVF-ICSI (%)	9.1	13.1
	OD (%)	4.55	8.7
Delivery method	V (%)	54.5	47.8
	C (%)	27.3	34.8
	VO (%)	18.2	17.4
Baby's weight (kg)		3165.33 ± 46.21	3089.42 ± 501.76
Baby's length (cm)		49.68 ± 13.1	49.32 ± 3.55
Gestational age (weeks)		38.95 ± 7.8	38.22 ± 3.77
Sex	F (%)	45.45	52.2
	M (%)	54.55	47.8

AMA: advanced maternal age; BMI: body mass index; S: spontaneous; I-IO: insemination–ovulation inducer; IVF-ICSI: in vitro fertilization–intracytoplasmic sperm injection; OD: oocyte donation; V: vaginal; C: cesarean section; VO: vaginal operation; F: female; M: male. Data are shown as mean ± SEM (Student's t-test). Significantly different from the control group (***P < 0.001).

divided into two parts: maternal (close to the decidua, includes thin basal plate) and foetal (close to the amnion, includes the chorionic plate). These two sides of the placenta represent distinct microenvironments with different composition, exposure to maternal/foetal circulations, spatial heterogeneity in oxygenation, metabolism, and gene expression, which can influence mitochondrial structure, justifying separate ultrastructural analyses of both compartments [17]. They were collected in ice-cold PBS, cleaned of blood, and immediately cut into five 0.5 × 0.5 × 0.5 cm fragments, which were fixed with proper reagents described below [18].

2.3. Ultrastructural examination by TEM

Properly obtaining and processing placentas for TEM examination during human deliveries is rather complex and challenging, being a critical step in the project. Tissue samples ($n = 10$ in each group) were prefixed in glutaraldehyde-formaldehyde in cacodylate buffer 0.05 M for 2 h and subsequently fixed in 1% OsO_4 and 1% potassium ferrocyanide in darkness. Samples were immersed in tannic acid 0.15% in buffer, and after that, in buffer, deionized water, uranyl acetate 2% in deionized water (darkness), and deionized water again. Dehydration was performed using ethanol before infiltrating samples in Epon:ethanol at increasing Epon concentration. Samples were included in complete Epon for 48 h at 60 °C. Ultrathin sections were cut, mounted on copper grids and contrasted with uranyl acetate and lead citrate. They were systematically examined using a LIBRA 120 PLUS Carl Zeiss SMT transmission electron microscope. A minimum of 10 fields of vision were randomly investigated from each tissue sample (that is, each type of trophoblast cell and each placental face), studying images with TEM Image Platform (Olympus) [18]. Individual measures were averaged, obtaining mean values per field, which were subsequently averaged, obtaining mean values per sample. These final averages per sample (10 in the control group and 10 in the AMA group) were used for the statistical analysis. Ultrastructural findings were observed (number, size, morphology, and mitochondrial-endoplasmic reticulum contacts (MERCs)), selected and quantified using ImageJ software. For MERCs measurement (Fig. 1), the outer membrane of the mitochondrion was traced. To obtain the MERC distance, 3–7 lines were drawn between mitochondria and ER, subsequently calculating the average. To obtain the MERC percent coverage, a line was drawn spanning the length of the contact site, dividing this contact length by the mitochondrial perimeter and multiplying the value by 100 [19].

2.4. Protein expression analysis by western blot (mitochondrial dynamics)

Placenta samples ($n = 23$ for AMA group and $n = 22$ for control group; including those analysed by TEM) were homogenised in a Potter-Elvehjem and lysates were extracted through homogenization in T-PER™ Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA, USA) with protease inhibitor cocktail. After total protein quantification and preparation of mother solutions containing 12 μg of total protein each, these were loaded on 4–20% Criterion TGX (Tris-Glycine extended) gels (Mini-PROTEAN TGX Precast Gels; Bio-Rad, Hercules, CA, USA) for separation by electrophoresis (Mini-PROTEAN System; Bio-Rad, Hercules, CA, USA) at 250 V for 20 min. Proteins were transferred from gels to a PVDF membrane (Bio-Rad, Hercules, CA, USA) via semi-dry transfer at 25 V for 3 min (Trans-Blot Turbo Transfer System, Bio-Rad, Hercules, CA, USA). Membranes were washed and incubated with primary antibodies (Ab) overnight and shaking at 4 °C: mitofusin 1 (MFN1) (Cat#:14739), mitofusin 2 (MFN2) (Cat#:11925), optic atrophy 1 (OPA1) (Cat#:80471), dynamin-related protein 1 (DRP1) (Cat#:8570), PTEN induced kinase 1 (PINK1) (Cat#:6946), parkin RBR E3 ubiquitin protein ligase (PARKIN) (Cat#:32833), optineurin (OPTN) (Cat#:58981) (Cell Signaling Technology, Danvers, MA, USA); dynamin 2 (DNM2) (Cat#MA5–32729), FUN14 domain-containing protein 1 (FUNDC1) (Cat#PA5–48853), BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) (Cat#MA1–24688) (Thermo Fisher Scientific, Waltham, MA, USA); and mitochondrial fission 1 protein (FIS1) (Cat#ab156865) (Abcam, Cambridge, UK), using GAPDH (Cat#2118, Cell Signaling Technology, Danvers, MA, USA) for data normalization. After that, membranes were washed and incubated with the secondary conjugated Ab for 1 h at room temperature. The blots were visualised by chemiluminescence, using Clarity Western ECL Substrate (Bio-Rad, Hercules, CA, USA). The quantification of the signal was carried out with ChemiDoc Imaging System (Bio-Rad, Hercules, CA, USA) and analysed with Image Lab software.

2.5. Statistical analysis

Results were subjected to the Levene and Kolmogorov-Smirnoff tests for homogeneous variance and normality respectively. The parametric

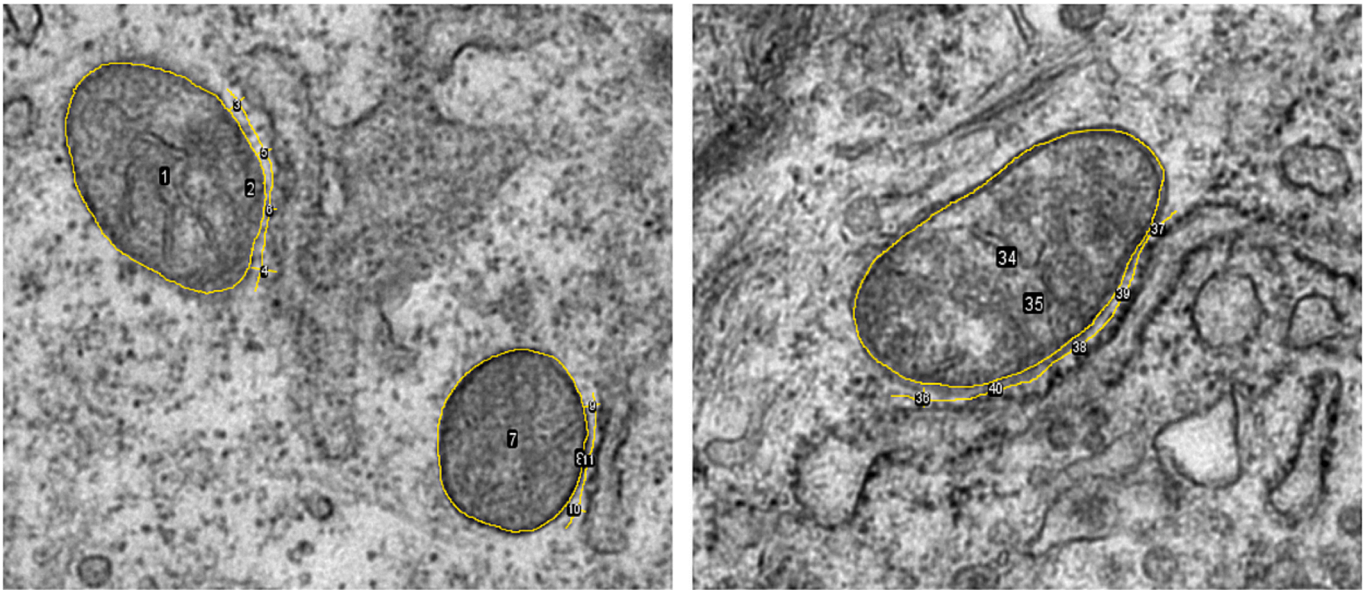

Fig. 1. MERCs measurement process using ImageJ software. Images show the selection of mitochondrial perimeter, endoplasmic reticulum length contact, and distance between the two organelles (taking more measures of the distance in the event the contact length was longer).

Student's *t*-test was used for those data following a normal distribution, using the non-parametric Mann-Whitney *U* test if the data were not normally distributed so as to measure the statistical significance between AMA and control groups. Statistical significance was established when $P < 0.05$. For all comparisons, GraphPad Prism version 9.4.1 statistical software was used (GraphPad Software, Inc., California, USA).

3. Results

3.1. Mitochondrial amount increases in AMA

The number of mitochondria (Fig. 2) measured in both syncytiotrophoblast and cytotrophoblast cells of the whole placenta was increased ($P < 0.05$ and $P < 0.05$) in the AMA group in comparison with the control group. When placental sides were assessed separately, syncytiotrophoblast did not show statistically significant differences in the number of mitochondria within the foetal side, whereas in the maternal side, an increase was reported in the AMA group ($P < 0.05$). With regard to cytotrophoblasts, the differences were reported in the foetal side, with an augmented number of mitochondria in the AMA group ($P < 0.01$), and no differences shown in the maternal side of the placenta.

3.2. Mitochondrial size increases in AMA while morphology does not change

Fig. 3 shows representative TEM images of syncytiotrophoblast and cytotrophoblast cells from both control and AMA groups, as well as frequency distribution histograms of size, morphology and circularity parameters. Qualitative, the images show an increase in number and a reduction in size in the AMA group of both type of cells (Fig. 3A). Histograms manifest the differences among mitochondria from syncytiotrophoblast and cytotrophoblast cells (these last having a bigger area and perimeter), as well as their similarities regarding circularity (Fig. 3B).

Fig. 4 shows the differences observed in mitochondrial size and morphology between groups. In the whole placenta (Fig. 4A), syncytiotrophoblast reported a decrease in the area ($P < 0.01$) and perimeter ($P < 0.01$) of their mitochondria in the AMA group compared with the controls. Cytotrophoblasts exhibited similar results in terms of mitochondrial area and perimeter, both being reduced in AMA pregnancies ($P < 0.001$ and $P < 0.01$ respectively). However, the circularity of mitochondria in both type of cells showed no statistically significant differences.

Evaluating each side of the placenta, syncytiotrophoblast (Fig. 4B) kept the same differences in the foetal side that appeared in the whole placenta, that is, reduced mitochondrial area ($P < 0.05$) and perimeter ($P < 0.05$) in the AMA group compared to the control group. These cells in the maternal side also reported reduced area ($P < 0.05$) and perimeter ($P < 0.05$) of their mitochondria. No differences appeared in circularity. As for cytotrophoblasts (Fig. 4C), their mitochondria also showed a decrease in area in the foetal side ($P < 0.05$), and a decrease in both area and perimeter in the maternal side ($P < 0.01$ and $P < 0.01$). The lack of significant differences in circularity was also observed in these cells.

3.3. Mitochondrial-endoplasmic reticulum contacts (MERCs) are larger and tighter in AMA

With regard to MERCs (Fig. 5), cytotrophoblast's mitochondria of the whole placenta showed reduced distance to the ER in the AMA samples ($P < 0.001$). The percentage of coverage of these organelles by ER did

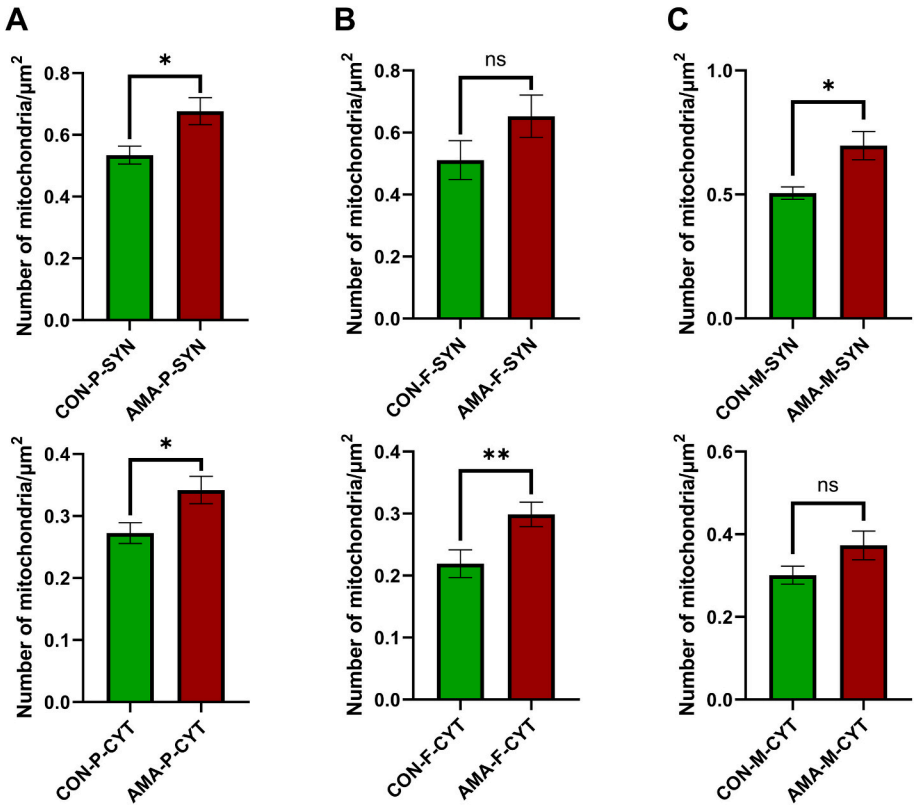

Fig. 2. Mitochondrial density (number of mitochondria per μm^2 of cytoplasmic area) quantified in syncytiotrophoblast (SYN, upper panels) and cytotrophoblast (CYT, lower panels) from placental samples of control (CON) and advanced maternal age (AMA) pregnancies. An increase is shown in SYN and CYT of the whole placenta, as well as in SYN of the maternal side and CYT of the foetal side. Bars represent mean ± SEM (Student's *t*-test). Statistical significance: $*P < 0.05$; $**P < 0.01$; ns = not significant. $n = 10$ placentae per group (CON n = 10; AMA n = 10). (A) Whole-placenta analysis (CON-P vs. AMA—P). (B) Fetal-side analysis (CON-F vs. AMA—F). (C) Maternal-side analysis (CON-M vs. AMA-M).

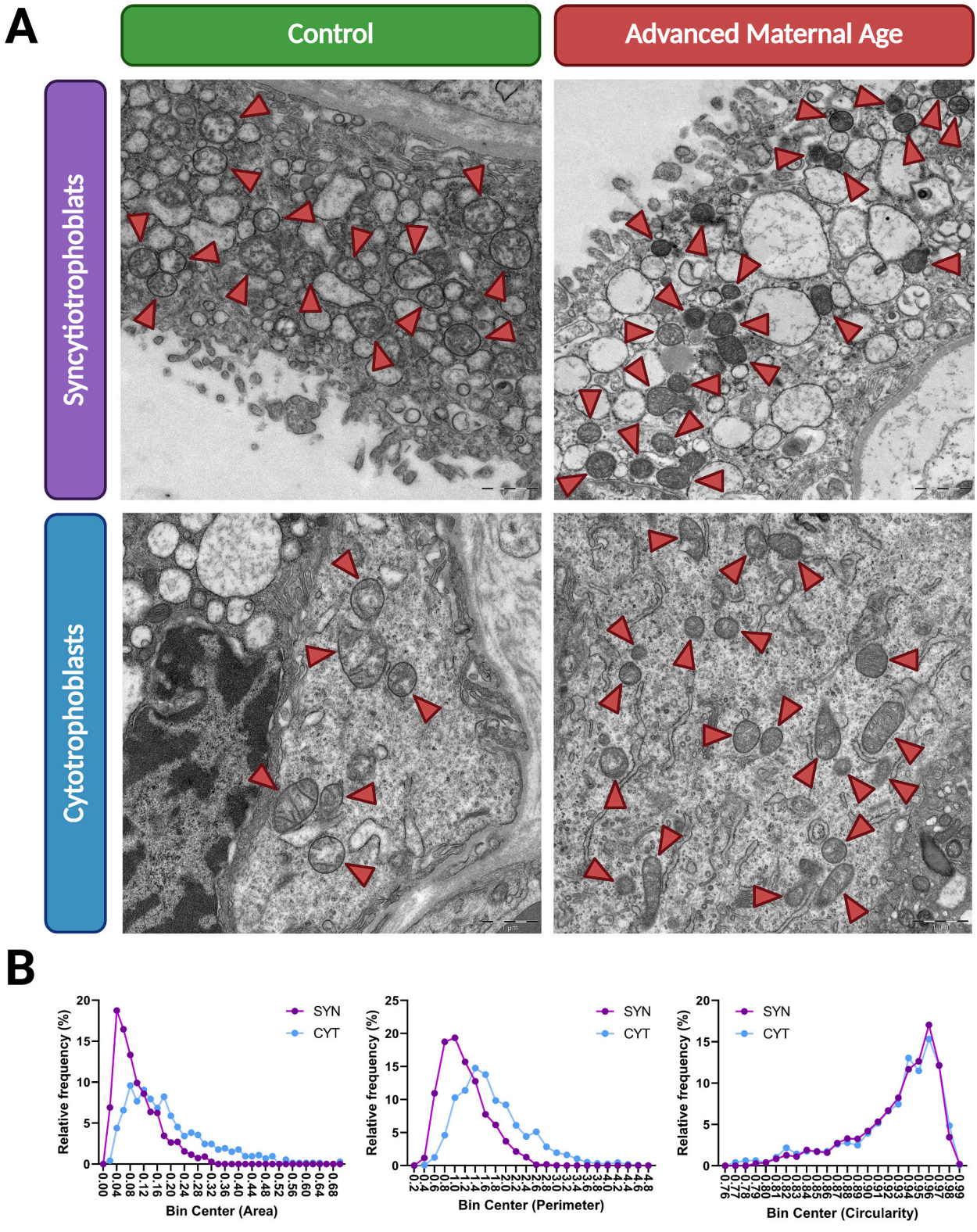

Fig. 3. (A) Representative transmission electron microscopy images of syncytiotrophoblast and cytotrophoblast from control pregnancies and pregnancies of advanced maternal age. Red arrowheads indicate individual mitochondria, highlighting changes in the advanced maternal age samples. Increased number of mitochondria and reduced size can be appreciated in AMA images from both STN and CYT. (B) Relative frequency distribution histograms of mitochondria-derived morphometric parameters from TEM images: mitochondrial area (left), perimeter (middle), and circularity (right), comparing syncytiotrophoblast (SYN) and cytotrophoblast (CYT). Bigger area and perimeter are shown in CYT with respect to SYN, as well as similar circularity.

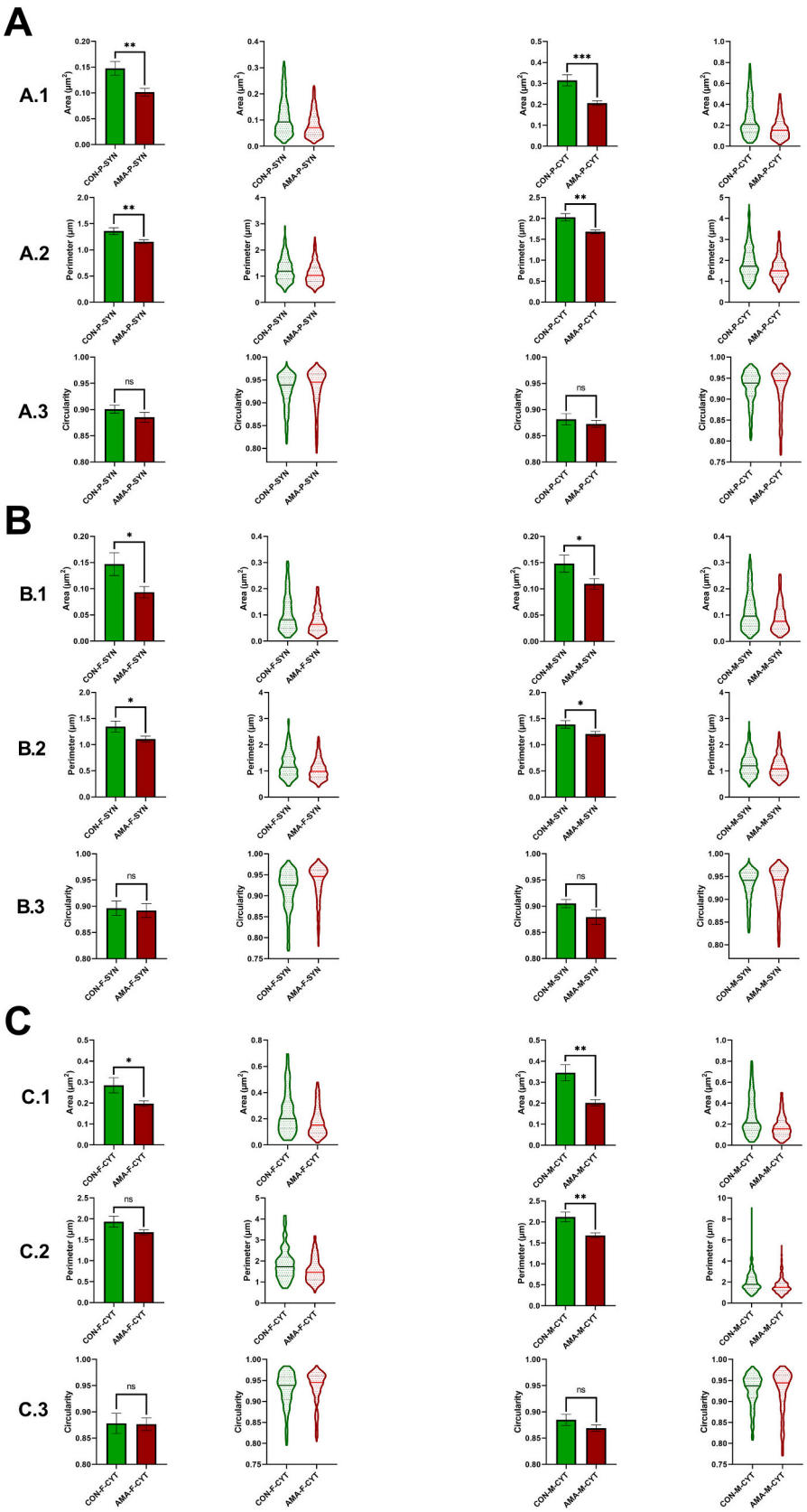

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Fig. 4. Mitochondrial morphometric parameters quantified from transmission electron microscopy (TEM) images from control (CON) and advanced maternal age (AMA) placentas. The whole placenta shows decreased mitochondrial area and perimeter in both SYN and CYT of AMA samples. With regard to placental sides, both SYN and CYT keep these same differences, except for a lack of significant differences in the perimeter of CYT in the foetal side of the placenta. No differences were reported regarding mitochondrial circularity. Bars represent mean ± SEM (Student's *t*-test), and individual mitochondrial distributions are displayed as violin plots showing median (IQR) (Mann–Whitney *U* test). Statistical significance: *P < 0.05; **P < 0.01; ***P < 0.001; ns = not significant. n = 10 placentae per group (CON n = 10; AMA n = 10) (A) Whole placenta (CON-P vs. AMA—P) analysis: (A.1) area, (A.2) perimeter, (A.3) circularity. (B) Syncytiotrophoblast analysis of foetal side (CON-F vs. AMA—F) and maternal side (CON-M vs. AMA-M) analysis: (B.1) area, (B.2) perimeter, (B.3) circularity. (C) Cytotrophoblasts analysis of foetal side (CON-F vs. AMA—F) and maternal side (CON-M vs. AMA-M): (C.1) area, (C.2) perimeter, (C.3) circularity.

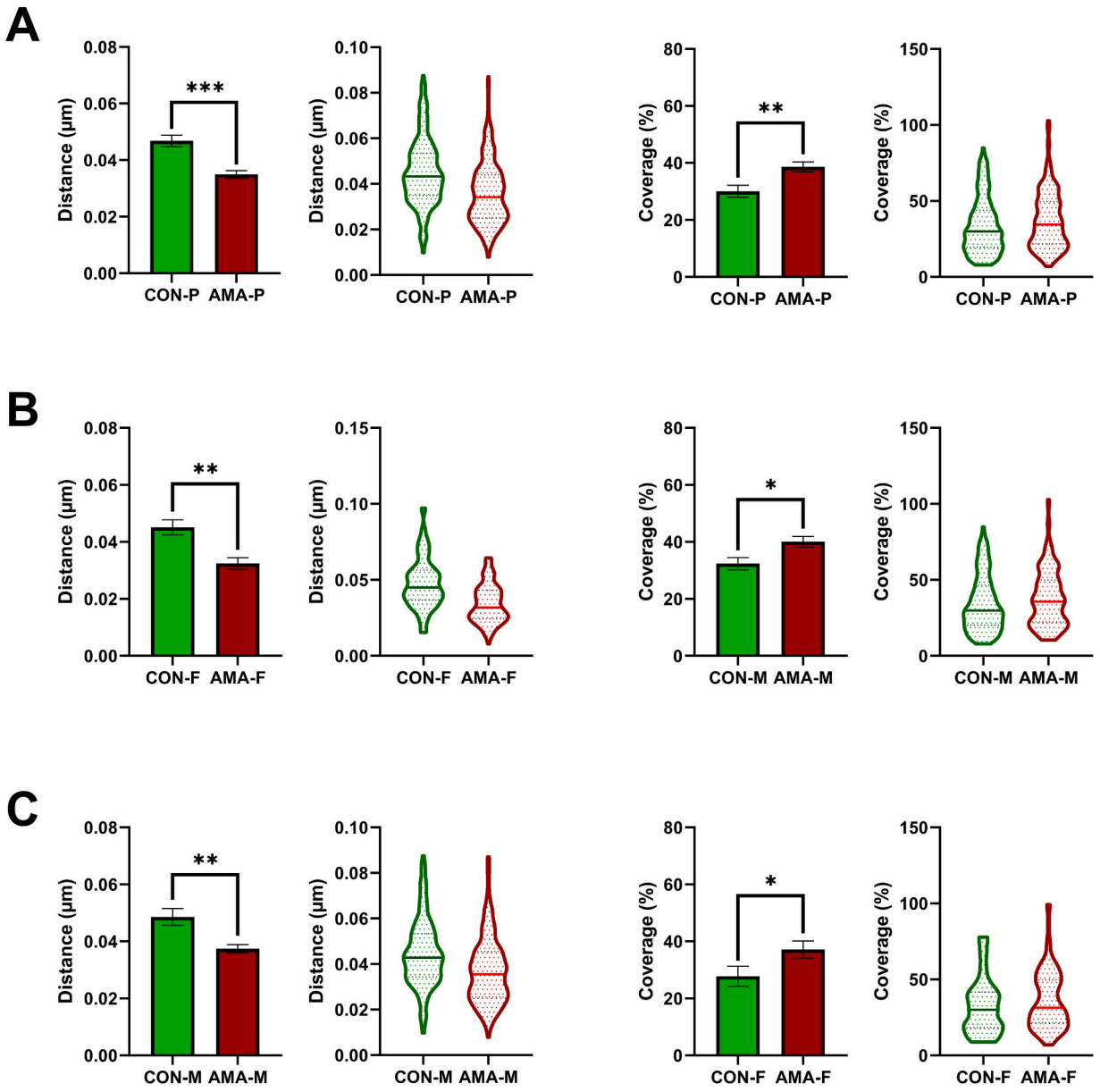

Fig. 5. Quantification of MERCs expressed as mitochondria–ER distance (µm) and ER coverage of the mitochondrial perimeter (%). MERCs in AMA placentas are characterised by reduced distance and augmented coverage. Bars represent mean ± SEM (Student's t-test), and full distributions are depicted as violin plots showing median (IQR) (Mann–Whitney U test). Statistical significance: *P < 0.05; **P < 0.01; ***P < 0.001. n = 10 placentae per group (CON n = 10; AMA n = 10). (A) Whole placental analysis (CON-P vs. AMA—P). (B) Foetal side analysis (CON-F vs. AMA—F). (C) Maternal side analysis (CON-M vs. AMA-M).

show an increase in the AMA group (P < 0.01) in comparison with the control group. Addressing placental sides separately, there is a decrease in distance (P < 0.01) and an increase in coverage (*P* < 0.05) in the foetal side of the placenta. Results in the maternal side are similar, again showing with decreased distance (*P* < 0.01) and increased percentage of coverage (*P* < 0.05).

3.4. Mitochondrial dynamics is unbalanced towards a fragmentation pattern in AMA

To determine whether these ultrastructural changes were associated with altered regulatory pathways, protein expression of mitochondrial dynamics markers was assessed (Fig. 6). With regard to fusion, protein levels of MFN1 were reduced in the AMA group (P < 0.001) compared to

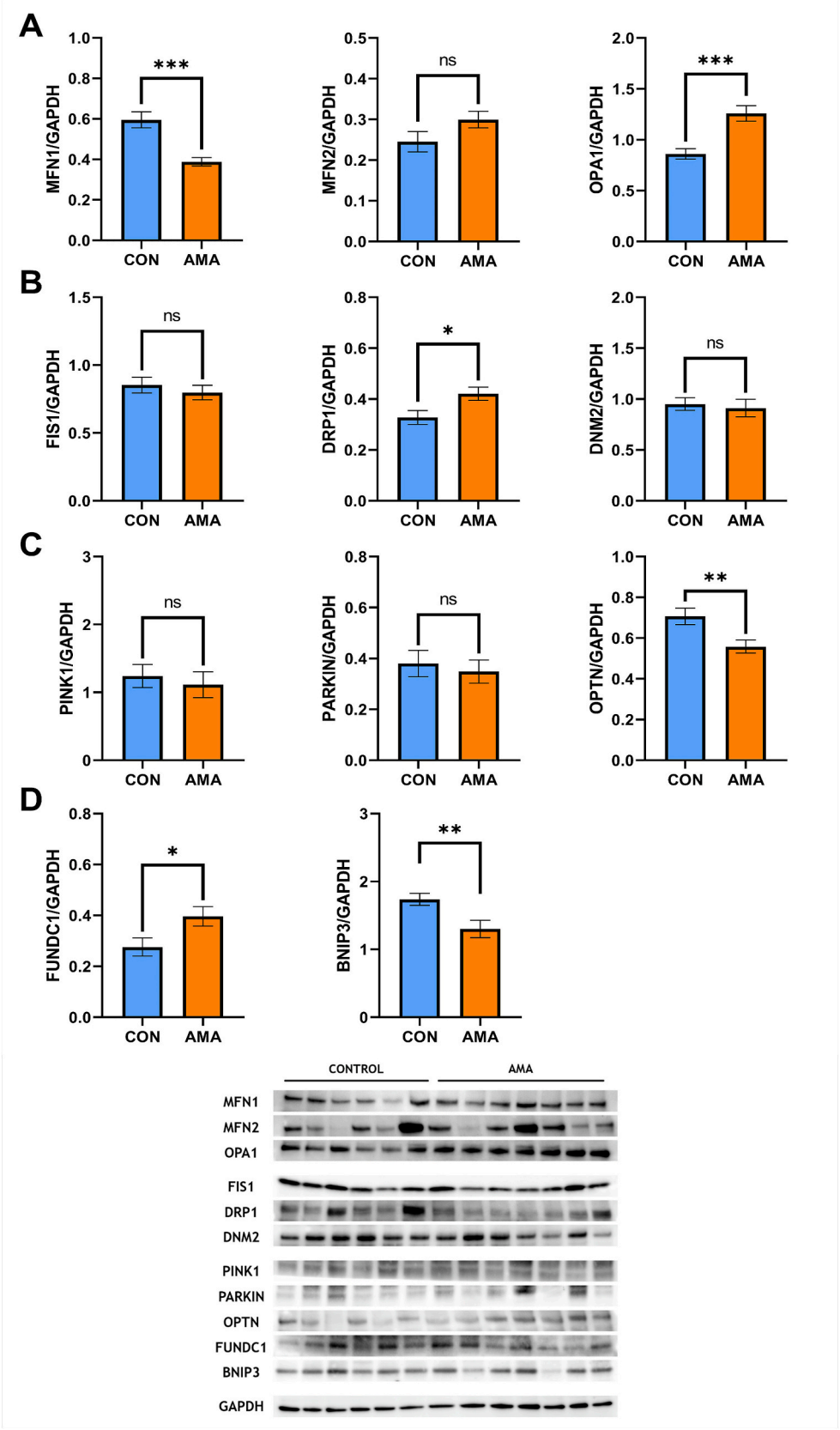

Fig. 6. Western blot quantification of key regulators of mitochondrial dynamics in placental tissue from control (CON) and advanced maternal age (AMA) pregnancies. AMA samples show decreased levels of MFN1, OPTN and BNIP3, as well as increased levels of OPA1, DRP1 and FUNDC1. Protein expression was normalized to GAPDH. Bars represent mean ± SEM (Student's t-test). Statistical significance: *P < 0.05; **P < 0.01; ***P < 0.001; ns = not significant. CON $n = 22$; AMA $n = 23$. (A) Fusion-related proteins: MFN1, MFN2, and OPA1. (B) Fission-related proteins: FIS1, DRP1, and DNM2. (C) PINK1–PARKIN mitophagy axis: PINK1, PARKIN, and OPTN. (D) Receptor-dependent mitophagy: FUNDC1 and BNIP3.

the control group, while MFN2 showed no statistically significant differences. On the contrary, OPA1 reported an increase in the placentas of AMA mothers ($P < 0.001$). A for mitochondrial fission, protein expression of DRP1 showed to be augmented in the AMA group ($P < 0.05$), whereas FIS1 nor DNM2 reported significant differences between groups.

Finally, some differences were obtained from the study of mitophagy associated proteins. Protein expression PINK1 and PARKIN showed no statistically significant differences between groups, but changes were reported regarding OPTN expression, with reduced protein levels in the AMA group ($P < 0.01$). As for FUNDC1 and BNIP3, both reported significant differences, being the first one increased ($P < 0.05$) and the second one decreased ($P < 0.01$) in AMA pregnancies in comparison with the ones of the control group.

4. Discussion

This research addressed potential changes associated with maternal age in the number, size, and morphology of mitochondria regarding the two types of trophoblast cells: syncytiotrophoblast and cytotrophoblast. These parameters, alongside the actual activity of these organelles gets altered with age, and it has been documented that, despite the strong selection bottleneck in the germline, elderly mothers can transfer aged, damaged mitochondria to oocytes [20]. The mitochondrial content within cells is of vital importance due to their relevance in cellular metabolism, especially when it comes to such an energy-demanding process as pregnancy. Nevertheless, a higher number of mitochondria is not always indicative of a healthy status, being crucial other factors like their quality, location and functionality. The mitochondrial pool can be affected by a wide range of situations, including those related to cellular stress, such as caloric restriction, physical exercise, hypoxia, and exposure to reactive oxygen species (ROS), which frequently trigger a cellular response aimed to either augment the number of mitochondria or adjust the content/activity ratio [21]. Importantly, in the placenta, these adaptive responses may vary depending on oxygen tension, nutrient availability, and maternal metabolic status, factors known to modulate mitochondrial size, morphology, and respiratory performance [22]. Responses include different mitochondria-related routes being modulated, from mitochondrial biogenesis to mitochondrial dynamics and electron transport chain assembly [23]. The coordinated regulation of these processes is essential to preserve mitochondrial quality, as disturbances in fusion–fission balance or turnover efficiency are early events leading to organelle dysfunction during maternal aging [11].

Age is indeed a relevant factor to consider, since the present study shows that the number of mitochondria seems to increase in the placentas of AMA pregnancies. This is in line with a recent rodent-based study, reporting that the increase in mitochondrial content could be a placental physiological adaptation to maintain appropriate bioenergetic capacity in AMA pregnancies [24]. Similar results have been observed when studying the mitochondrial state of the placentas from pregnancies with pathologies like intra-uterine growth restriction (IUGR) and PE, which showed mitochondrial damage together with a rise in the number of these organelles in syncytiotrophoblast and/or cytotrophoblast cells [25,26]. Challenging conditions like hypoxia has also been associated with increased number of mitochondria in trophoblasts cultures [27]. It is important to highlight that the increase in mitochondrial number observed in AMA pregnancies should be interpreted with caution, as it does not necessarily reflect improved bioenergetic function. Accumulation of small or morphologically altered mitochondria has been linked to impaired organelle turnover and mitophagic inefficiency, leading to the persistence of dysfunctional mitochondria [28]. This situation, combined with the chronic oxidative and metabolic stress characteristic of maternal aging, could compromise mitochondrial quality and placental efficiency even in the absence of clinical pathology.

To date, there is a clear lack of studies in the scientific literature comparing any mitochondrial parameter taking into account the side of the placenta. However, the number of mitochondria also seems to be differently modified depending not only on the type of trophoblast cell, but also on the side of the placenta. The present study's results show the need of more research in this regard, since the currently available data on the subject are more related to differences in other pathways like lipid metabolism [29]. Increased number of mitochondria could be related to different pregnancy pathologies and therefore to a possible alteration in their function, and AMA appears to negatively affect, in a more intense way, the syncytiotrophoblast of the maternal side of the placenta, and the cytotrophoblasts of the foetal side [22].

Mitochondrial size is so relevant regarding the capacity of these organelles to produce energy and meet cellular requirements, as well as ROS production [30]. In general terms, reduced mitochondrial size has been associated with damage and apoptotic signals, frequently leading to organ dysfunction [31]. Size changes frequently go hand in hand with morphological changes, which has been related to less bioenergetic capacities, ROS generation, and increased susceptibility to damage [32]. However, these morphological changes were not reported in the present study. In the light of the present study's results, mitochondrial size seems to be importantly modified by age. This suggests a possibly detrimental state of the mitochondrial pool, similarly to what happens under stress conditions. This phenotype is consistent with a shift towards dominant mitochondrial fission, a response commonly observed under chronic oxidative load or calcium dysregulation, which often precedes loss of membrane potential and mitophagic modulation [33]. Thus, research assessing gestational hypoxia has found similar results, describing smaller mitochondria, which seems to be indicative of an imbalance in mitochondrial dynamics [34]. These data reinforce the hypothesis that even dealing with healthy mothers, the risk of obstetric pathologies might be increased due to AMA.

MERCs are specific regions where the endoplasmic reticulum (ER) is physically associated with mitochondria. They are related to a wide range of cellular responses, including oxidative stress and mitochondrial quality control. A potential role for MERCs in cell survival has been proposed, consisting in facilitating the removal of mitochondrial lipid radicals under mitochondrial damage and oxidative stress [35]. MERCs play a pivotal role in mitochondrial fission, since its initial step requires the participation of the ER tubules to contact with mitochondria and wrap around them. In fact, MERCs correlate with localization of DRP1 among other components of the fission machinery, including OPA1 [36]. The results attained by the present study could be indicative of an upregulation of mitochondrial fission events aimed to eliminate defective organelles. In addition, it could be a defensive mechanism whose objective is to enhance lipid transport so as to counter ROS damage derived from increased oxidative stress. Alternatively, sustained MERC tightening has been linked to impaired autophagic flux and reduced mitophagy, a combination that could contribute to the accumulation of damaged mitochondria observed in placentas from AMA pregnancies [28,33]. The link between MERCs disruption and pregnancy has been poorly addressed in the scientific literature to date and it has been mainly focused on complications like PE [37]. The increased MERC formation observed in AMA cytotrophoblasts may therefore reflect a double-edged adaptation: initially protective to sustain metabolism under stress, yet potentially maladaptive if prolonged, contributing to trophoblast vulnerability and placental aging.

With regard to mitochondrial fusion, the present study reports a relevant modification in the expression of key proteins of this process in AMA. MFN1 under-expression (which is in line with results derived from TEM analysis) can compromise the ability of the mitochondrial machinery to get larger organelles with higher energy production capacity and more able to mitigate mitochondrial damage due to excessive oxidative stress [38]. On the other hand, OPA1 expression shows to be increased in AMA pregnancies. Even though the main known activity of OPA1 is to mediate the fusion of the inner mitochondrial membrane (IMM), an upregulation in this sense would be of little use in these

trophoblasts since the machinery developing the OMM is downregulated. Nevertheless, there are other plausible explanations for this increase. One is related to the participation of OPA1 in the maintenance of mitochondrial cristae structure and therefore respiratory chain complexes' activity [39]. This adaptive upregulation of OPA1 has been described as a short-term response to stress that helps preserve cristae integrity and limit cytochrome *c* release [40]. In aged or metabolically challenged cells, however, persistent OPA1 overexpression may instead promote inner membrane remodelling and contribute to altered cristae architecture [41]. In this way, the upregulation of this protein could be a response mechanism trying to enhance the cristae structure and activity of those smaller mitochondria. The other explanation is associated with the existence of two OPA1 isoforms, being the shorter one also known for its role in the promotion of fission events and its location in the MERCs [42]. The augmented number of smaller mitochondria and higher abundance of MERCs in the present study would support this.

With respect to mitochondrial fission, the increase in DRP1 expression would be translated in an increase in fragmentation, also in line with the TEM results, reduced fusion, and increased OPA1 expression. An excessive activation of DRP1 has been previously linked to trophoblast stress and mitochondrial depolarization in complicated pregnancies, suggesting that hyper-fission might be an early hallmark of placental dysfunction [43]. Taken together, these results would indicate an imbalance in mitochondrial dynamics characterised by a tendency of the mitochondrial pool to fragment more and fuse less.

Fission is often the previous step before the removal of defective mitochondria through mitophagy, in order to maintain a healthy mitochondrial pool [39]. Nevertheless, results from this study do not highlight a corresponding upregulation of mitophagy, but much of a modulation of its pathways in AMA. From the PINK1-PARKIN axis, the only reported change is a decrease in the protein expression of OPTN, indicating a slight downregulation of this pathway, which could however, be compensated by an increased expression of other different proteins with similar roles as OPTN. As for reported changes in receptor-mediated mitophagy, the divergence between decreased BNIP3 and increased FUNDC1 expression mirrors other stress-adaptation models, in which FUNDC1-dependent mitophagy predominates under sustained hypoxia or oxidative load [44]. However, such compensation is often incomplete and may result in decreased mitochondrial clearance and elevated ROS. These results show an undeniable alteration of the different mitophagy pathways in the placentas of AMA mothers, with a possible inability to fully eliminate defective mitochondria, hence the high amount of small mitochondria found in the TEM analysis [45]. Altogether, these findings suggest that AMA disrupts the fine coordination between mitochondrial dynamics and quality control, favouring a state of persistent fragmentation with insufficient removal of damaged organelles—an imbalance that could underlie the premature placental aging phenotype described in recent studies [46].

When it comes to age, scientific research has prominently focused on the study of mitochondrial dynamics modifications in terms of gestational age, showing dysregulations of crucial mitochondrial processes throughout pregnancy [10]. The present study intends to clarify if AMA could also be characterised by mitochondrial alterations that would add to those inherently experimented by the aging placenta. Other maternal features like obesity have already been linked to mitochondrial affectation within the placenta, showing increased fission capacity [47]. Mitochondrial dynamics has also been to some extent studied with regard to pregnancy complications like PE, showing rather contradictory results, even though there is a more common tendency to a fission pattern [12,26]. Reduced fusion and increased fission have also been reported in IUGR and chronic gestational hypoxia [14,34].

Since there are no pathologies in the mothers studied, some changes observed in them can also be approached as a successful compensatory mechanism of placental mitochondria to achieve homeostatic maintenance of the placenta and a safe pregnancy, e.g. mitophagy not meeting the fission increase in order to avoid the removal of defective but still useful organelles. Even though age is not causing pathologies in these mothers, it may cause small changes that increase aggression to the placenta, producing other modulations aimed to compensate for these new challenges. This shows the capability of the placenta to adapt to adversity, also being important to highlight that the functional reserve in AMA mothers might not that of the young ones. AMA pregnancies can be working to the limit up to a certain point, which would mean that the appearance of any new aggression may have the capacity to overwhelm the system and even lead to pregnancy complications [48].

The presence of a pro-oxidative intrauterine environment linked to maternal aging is supported by evidence from animal models of AMA, showing elevated oxidative damage, impaired antioxidant defences, and mitochondrial abnormalities. Independent models of reproductive aging have documented similar changes in redox balance, biogenesis, and mitochondrial structure. Given this, the mitochondrial remodelling seen in the current human population might be an adaptive reaction to long-term metabolic and oxidative stress [24,49,50]. Given that pregnancy increases placenta's oxidative demands, several micronutrients—such as vitamins C and E (direct antioxidants) and selenium, copper, zinc, and manganese (cofactors of antioxidant enzymes)—are crucial for antioxidant defence. Notably, this research group has previously documented that this AMA group has lower dietary intake of antioxidant micronutrients, which may further compromise placental redox equilibrium. All these results point to oxidative stress as a likely upstream cause of the mitochondrial structural and quality-control changes reported in AMA, which may be worsened by inadequate antioxidant micronutrient status [16].

Foetal sex was not considered in the present analyses, although sexual dimorphism in placental mitochondrial biology has been reported. On the other hand, as mitochondrial content was altered in AMA pregnancies, normalization to a mitochondrial-specific loading control (e.g., VDAC) may have provided additional accuracy. These represent the two main limitations of the present study.

5. Conclusion

In summary, this study demonstrates that AMA is associated with significant ultrastructural and molecular alterations in placental mitochondria, characterised by smaller and more fragmented organelles, modified mitochondria–endoplasmic reticulum contacts, and imbalanced expression of proteins regulating fusion, fission, and mitophagy. These findings indicate that even in the absence of clinical complications, AMA pregnancies exhibit signs of mitochondrial stress and disrupted organelle homeostasis, which could compromise trophoblast energy metabolism and overall placental efficiency. Such alterations may underlie the increased vulnerability of aged pregnancies to adverse outcomes. By integrating quantitative ultrastructural analysis with molecular assessment, this study provides one of the first comprehensive evaluations of mitochondrial organization in the placentas from AMA pregnancies. These results highlight the need to further investigate how maternal age interacts with other gestational factors and whether the observed mitochondrial signatures could serve as therapeutic targets to improve pregnancy outcomes in AMA.

CRediT authorship contribution statement

Juan M. Toledano: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Puche-Juarez:** Writing – review & editing, Methodology, Investigation, Data curation. **María Paz Carrillo:** Resources. **Javier Diaz-Castro:** Project administration, Methodology, Funding acquisition. **Javier Sanchez-Romero:** Resources. **Francisco Manuel Ocaña-Peinado:** Software, Data curation. **Catalina de Paco Matallana:** Resources. **Estefania Martín-Alavarez:** Resources. **Jorge Moreno-Fernandez:** Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Julio J. Ochoa:** Visualization, Project administration, Funding acquisition.

Ethics approval and consent to participate

All participants provided written informed consent prior to their inclusion in the study. The study has been approved by the bioethics committee for research with humans: Sistema de Información de los Comités de Ética de la Investigación de Andalucía (approval number SICEIA-2025-000255; date of approval 06/03/2025); and Portal de Ética de la Investigación Biomédica de Andalucía (approval number 27/04/2020/4/2020; date of approval 27/04/2020).

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Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2026.124338.

Data availability

Data will be made available on request.

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