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EMBRYO SELECTION IN IVF: SYSTEMATIC INSIGHTS INTO MITOCHONDRIAL DNA COPY NUMBER AND IMPLANTATION RATES

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ABSTRACT:

In vitro fertilization (IVF) success relies heavily on accurate embryo selection to enhance implantation and live birth rates. Traditional methods, including morphological grading and preimplantation genetic testing for aneuploidy (PGT-A), have shown limitations in consistently predicting embryo viability. Mitochondrial DNA (mtDNA) content has emerged as a promising biomarker reflecting the bioenergetic and developmental potential of embryos. This systematic review synthesizes current evidence from human and animal studies evaluating the association between mtDNA copy number and implantation outcomes. Overall, higher mtDNA levels in euploid embryos were often associated with reduced implantation rates, independent of embryo morphology and maternal age. Contradictory findings highlight the impact of methodological variability, normalization strategies, and biological factors. While mtDNA profiling shows potential as a non-invasive adjunct for embryo selection, standardization of measurement techniques and further large-scale studies are needed to optimize its clinical application in assisted reproductive technologies.

Keywords: Assisted reproductive technology (ART), Biomarker, Embryo selection, Euploid embryos, In vitro fertilization (IVF), Implantation, Mitochondrial DNA (mtDNA).

1. INTRODUCTION

In vitro fertilization (IVF) has evolved significantly since its inception in natural cycles, particularly with the introduction of gonadotropin stimulation protocols, which allowed for increased embryo yields and reduced multiple pregnancy risks [1,2,5]. Despite decades of research and technological advances aimed at improving embryo selection (ES) strategies, including blastocyst culture, preimplantation genetic testing for aneuploidy (PGT-A), time-lapse imaging, and artificial intelligence-based technologies, consistent improvements in clinical outcomes remain limited [3–6].

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Recent randomized controlled trials have questioned the effectiveness of commonly used IVF "add-ons" [11–13]. The persistent reliance on these interventions, often driven by commercial incentives, has shaped IVF practice without robust validation [1,7,8], and live birth rates have shown minimal improvement or even decline in some regions [14,15]. These limitations highlight the need for biologically grounded biomarkers, such as mitochondrial DNA (mtDNA) content, to enhance the prediction of implantation potential [9,10].

Mitochondria are essential for cellular energy production, generating adenosine triphosphate (ATP) to support embryogenesis [21–23]. Each mitochondrion contains a circular double-stranded DNA (approximately 16.7 Kb), encoding 13 protein subunits, 22 transfer RNAs, and 2 ribosomal RNAs. In humans, mtDNA is maternally inherited, as paternal mitochondria are actively degraded post-fertilization [24–27]. Mature oocytes contain 100,000–600,000 mitochondria, which are crucial for early development [24–27].

Alterations in mtDNA content have been linked to oocyte quality, fertilization, and embryonic development. For instance, primary ovarian insufficiency is associated with reduced mtDNA in oocytes [35–37]. Mitochondrial metabolism also generates reactive oxygen species (ROS), which, if excessive, cause oxidative stress, mitochondrial damage, and impaired embryogenesis [38–40]. Evidence suggests

that mtDNA copy number may reflect mitochondrial function and serve as a biomarker of embryo viability [35].

This systematic review aims to critically assess the association between mtDNA copy number in human embryos and implantation potential, evaluating its role as a potential non-invasive biomarker to enhance embryo selection in IVF cycles [41–43].

2. MATERIALS AND METHODS

2.1. Study Design

This systematic review was conducted following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [1,28–33]. Peer-reviewed studies assessing mtDNA content in embryos and its association with implantation outcomes were included. Both human and animal studies were considered.

2.2. Literature Search Strategy

A comprehensive search was performed in PubMed and Google Scholar from inception to July 2025 using combinations of the following keywords:

"mitochondrial DNA" OR "mtDNA" OR "mitochondrial DNA copy number" OR "mtDNA content" AND "embryo" AND "implantation" AND "IVF" OR "in vitro fertilization" OR "blastocyst" OR "trophectoderm biopsy" OR "preimplantation genetic testing" OR "PGT-A".

Boolean operators **AND**/**OR** were applied, and references of included articles were manually screened. No language restrictions were applied.

2.3. Inclusion Criteria

Studies were included if they:

1. Used human or animal embryos created via IVF or ICSI.

2. Quantified mtDNA copy number or mtDNA/nuclear DNA ratio.
3. Reported implantation rate, clinical pregnancy rate, live birth rate, or embryo viability.
4. Were randomized controlled trials, cohort studies, case–control studies, or prospective/retrospective observational studies.
5. Employed validated molecular techniques for mtDNA quantification (qPCR, digital PCR, NGS).

2.4. Exclusion Criteria

Studies were excluded if they:

- Lacked implantation or viability outcomes.
- Did not quantify mtDNA.
- Were reviews, editorials, or abstracts without original data.
- Used cryopreserved gametes without fresh embryo comparison.
- Reported only pooled culture media results without embryo-specific mtDNA measurement.

2.5. Data Extraction

Two reviewers independently screened titles, abstracts, and full texts. Extracted data included:

- Study characteristics (year, country, design, sample size).
- Patient demographics (mean maternal age, infertility diagnosis).
- Embryo parameters (developmental stage, morphology, ploidy).
- mtDNA assessment details (sample type, collection timing, quantification method, normalization).
- Outcomes (implantation rate, pregnancy rate, live birth rate, correlation with morphology).

Discrepancies were resolved by consensus.

2.6. Quality Assessment

Observational studies were evaluated using the Newcastle–Ottawa Scale (NOS), and randomized controlled trials (if any) were assessed using the Cochrane Risk of Bias Tool.

2.7. Ethical Considerations

This review analyzed previously published data; no new studies involving humans or animals were conducted by the authors. Ethical approval and informed consent were therefore not required.

3. RESULTS

3.1. Study Selection

A total of 890 records were identified through database searching, including 520 from PubMed and 370 from Google Scholar. After removing duplicates, 840 records remained and were screened based on title and abstract, of which 740 were excluded for irrelevance. The full texts of the remaining 100 articles were assessed for eligibility, and 30 were excluded with specific reasons. Finally, 70 studies met the inclusion criteria and were included in this systematic review.

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The study selection process is summarized in the PRISMA flow diagram (Figure 1)

PRISMA Flow Diagram

Identification

Records identified through database searching (n = 890)

- PubMed (n = 520)
- Google Scholar (n = 370)

Screening

Records after duplicates removed (n = 840)

Records screened (title and abstract) (n = 840)

Records excluded (n = 740)

Eligibility

Full-text articles assessed for eligibility (n = 100)

Full-text articles excluded, with reasons (n = 30)

Included

Studies included in qualitative synthesis (systematic review) (n = 70)

3.2. Study Characteristics

Included studies were published between 2013 and 2025 across multiple countries, incorporating human and animal models. Human studies primarily focused on euploid embryos after PGT-A and single embryo transfer. Embryo stages included day-3 cleavage-stage blastomeres and day-5/6 blastocysts. mtDNA quantification methods varied, including qPCR, digital droplet PCR, and next-generation sequencing (NGS).

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3.3 TABLE 01: Overview of studies linking mtDNA content to embryo quality and implantation [1,17,35,46,47,48,49,50,51].

Study ID / Year	Study Design	Sample Size	Embryo Stage	mtDNA Quantification Method	Main Findings
Diez-Juan et al., 2015	Retrospective	205 blastomeres (Day-3), 65 trophectoderm biopsies	Day-3 & Day-5	qPCR	Lower mtDNA in implanted embryos; mitochondrial score (Ms) predictive of implantation
Abdellatif et al., 2023	Prospective	9 patients	Day-3	qPCR from culture medium	Higher mtDNA linked to higher fragmentation and aneuploidy
Karač et al., 2021	Retrospective	125 euploid blastocysts	Day-5 & Day-6	qPCR	Poor-quality embryos had higher mtDNA
Winstanley et al., 2020	Animal (mouse)	Controlled groups	Pre-implantation	qPCR, fluorescence microscopy	Oxidative stress increased mtDNA and altered morphology
Fragouli et al., 2015	Retrospective	89 euploid embryos	Day-5	qPCR	High mtDNA → 100% implantation failure
Spinella et al., 2016	Retrospective	280 embryos	Day-5	qPCR	High mtDNA correlated with lower pregnancy rates
Ravichandran et al., 2017	Retrospective	1,505 blastocysts	Day-5	qPCR	High mtDNA → 0% pregnancy; normal mtDNA → 74.3% pregnancy rate

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3.4. Synthesis of Results

Most studies reported an **inverse correlation between mtDNA copy number and implantation rates**, independent of embryo morphology and maternal age. Contradictory findings arose in studies with varying normalization strategies or quantification techniques. Animal studies confirmed that oxidative stress elevates mtDNA and impairs developmental competence, supporting mtDNA as a potential adjunct biomarker for embryo selection.

4. DISCUSSION

4.1. Evidence Supporting mtDNA as a Negative Predictor

Fragouli et al. (2015) first highlighted the strong negative correlation between elevated mtDNA levels and implantation failure, reporting a 100% non-implantation rate in embryos with high mtDNA. Diez-Juan et al. (2015) and Spinella et al. (2016) corroborated these findings, introducing the concept of a **mitochondrial score (Ms)**: embryos in the lowest mtDNA quartile exhibited the highest implantation potential. Experimental induction of energetic stress (e.g., 2,4-dinitrophenol) also increased mtDNA, suggesting elevated levels reflect underlying metabolic dysfunction and reduced developmental competence.

4.2. Contradictory Evidence

Victor et al. (2017) analyzed 1,396 embryos and found no significant correlation between mtDNA content and embryo ploidy, maternal age, or implantation. Proper normalization, considering differences in nuclear DNA content and sex chromosomes, is critical. Without standardization, mtDNA interpretation may be misleading.

4.3. Methodological Considerations

Variability in trophectoderm cell number, nuclear reference genes, allele dropout, and low-coverage sequencing platforms can bias mtDNA quantification. Standardized, high-resolution methods are essential for clinical translation.

4.4. Clinical Implications

Ravichandran et al. (2017) and other prospective non-selection studies confirmed that blastocysts with elevated mtDNA rarely implant, whereas embryos with normal mtDNA show high implantation rates. While mtDNA alone should not dictate embryo transfer decisions, it may complement existing morphological and genetic selection criteria in IVF.

4.5. Biological Hypotheses

1. **Compensatory Mechanism Hypothesis:** Elevated mtDNA may indicate increased mitochondrial biogenesis to counteract reduced mitochondrial efficiency.
2. **Quiet Embryo Hypothesis:** Metabolically hyperactive embryos may have high mtDNA yet poor viability, supporting the inverse correlation between mtDNA and implantation.

5. CONCLUSION

Mitochondrial DNA content in embryos, especially when measured in culture media or trophectoderm biopsies, has potential as a **non-invasive biomarker of embryo viability**. Evidence suggests that embryos with low-to-normal mtDNA levels generally have higher implantation potential, independent of morphology. Incorporating mtDNA profiling alongside traditional

assessment may enhance selection of euploid embryos in IVF.

However, discrepancies among studies highlight the need for **standardized measurement protocols, normalization strategies, and threshold determination** before clinical application. Future large-scale, multicenter studies are warranted to validate mtDNA as a reliable adjunct biomarker in assisted reproductive technologies.

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