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The Silent Legacy: Paternal and Maternal Exposure to Microplastics and Differential DNA Methylation in Human Oocytes and Zygotes

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ABSTRACT

The alarming pervasiveness of microplastics in our environment, along with the endocrine-disrupting chemicals (EDCs) they often carry such as phthalates and Bisphenol A BPA) represents a growing and insidious threat to human reproductive health. While epidemiological studies have linked these compounds to negative reproductive outcomes, we still lack aclear picture of their direct molecular impact. Specifically, how does parental exposure shape the epigenetic landscape of the very first stage of human life, the pre-implantation embryo? This study seeks to bridge this gap by investigating whether quantifiable exposure to microplastic-related EDCs in parents correlates with altered DNA methylation patterns in human zygotes. We designed a prospective observational cohort study involving couples undergoing IVF/ICSI treatments. We will measure concentrations of specific EDCs (BPA, phthalates, PFAS) in follicular fluid and seminal plasma using the highly sensitive technique of liquid chromatography-tandem mass spectrometry (LC-MS/MS). On non-viable or supernumerary zygotes (at the 2-Pronuclear stage), we will perform single-cell whole-genome bisulfite sequencing (scWGBS) to map the entire epigenetic landscape. Our analysis will then identify differentially methylated regions (DMRs) by comparing embryo from parents with high versus low EDC exposure, while statistically controlling for potential confounding factors. We anticipate detecting EDCs in a large proportion of the biological samples. Our primary expected outcome is the identification of specific genomic regions in the zygotes where DNA methylation is significantly associated with parental EDC levels. We hypothesize a trend towards hypomethylation a reduction in methylation in the promoter regions of genes critical for development (e.g., HOXA10, DNMT1), with EDC concentration acting as a key predictor. This research aims to provide the first direct human evidence that parental exposure to microplastics and EDCs can leave a targeted epigenetic "signature" on the zygote. We propose this "silent legacy" mechanism, where environmental toxins alter the embryonic epigenome, could fundamentally compromise implantation success and long-term developmental health. These findings would carry profound implications for the fields of assisted reproduction and public health policy.

INTRODUCTION

We are living in an age of escalating environmental contamination, where microplastics (MPs) and their associated chemical additives, like phthalates and Bisphenol A (BPA), have become virtually ubiquitous. These compounds are known as endocrine-disrupting chemicals (EDCs) due to their ability to interfere with the body's delicate hormonal signaling. Disturbingly, they are now routinely detected in human tissues and fluids, including the very niches of reproduction: follicular fluid and semen.

While clinicians have observed correlations between toxin exposure and adverse outcomes such as diminished sperm quality or impaired fetal growth the underlying molecular mechanisms remain largely a black box. This is especially true for the earliest stages of human embryogenesis, a period of critical vulnerability.

Following fertilization, the human zygote undergoes a profound process of epigenetic reprogramming, where DNA methylation patterns are extensively remodeled. These patterns are essential for correct gene expression, genomic stability, and, ultimately, the embryo's ability to successfully implant in the uterus. We contend that aberrant methylation, potentially triggered by environmental stressors like EDCs, can establish what we term a "silent legacy" a transgenerational epigenetic defect that may influence an individual's health trajectory for a lifetime.

A critical knowledge gap persists: we lack direct human data connecting measurable parental exposure to MP/EDCs with specific, site-specific changes in the epigenome of the pre-implantation human embryo. Our study is designed to address this deficit directly. By utilizing discarded biological materials from IVF cycles, we aim to determine if the concentration of MP-related EDCs in follicular fluid and seminal plasma correlates with differential DNA methylation in the resulting zygotes. We hypothesize that higher parental EDC burdens will be associated with statistically significant hypomethylation in the promoter regions of key developmental genes, such as HOXA10 and DNMT1.

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MATERIALS AND METHODS

Study Design and Ethical Considerations

This prospective, observational cohort study will be conducted at the Department of Obstetrics & Gynaecology, Fatima Memorial Hospital, Lahore, Pakistan, between April and June 2025. The study protocol received full ethical approval, and all participating couples will provide written informed consent specifically for the use of their discarded biological samples for this research.

Participant Recruitment and Sample Collection

Inclusion Criteria will involve couples undergoing standard IVF or ICSI treatments where both partners consent to participate. Exclusion Criteria will include patients with known genetic abnormalities, a diagnosis of severe male factor infertility (azoospermia), or those using donated gametes.

The biological samples to be collected are:

- Follicular Fluid (FF): Aspirated during oocyte retrieval and immediately centrifuged. The cell-free supernatant will be stored at -80°C.
- Seminal Plasma (SP): Collected during standard semen preparation for IVF/ICSI.
- Zygotes: Non-viable, morphologically abnormal, or supernumerary zygotes at the 2-Pronuclear (2-PN) stage will be collected after clinical assessment and snap-frozen for analysis.

Quantifying Environmental Exposure

To assess exposure, FF and SP samples will be prepared using stringent protocols to prevent contamination. We will quantify a panel of target EDCs (BPA, key phthalate metabolites, and PFAS) using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS), with concentrations recorded in ng/mL. Any samples showing excessive levels of common laboratory plasticizers will be excluded to ensure data integrity.

Assessing Epigenetic Outcomes

DNA Extraction will be performed on single zygotes using kits designed for ultra-low DNA input. For the epigenetic analysis, extracted DNA will undergo sodium bisulfite conversion, followed by library preparation and whole-genome bisulfite sequencing (WGBS) on an Illumina platform. This technique allows for precise, single-nucleotide resolution mapping of DNA methylation.

Bioinformatic Analysis will involve aligning sequencing reads to the human reference genome (hg38) and calculating methylation levels (β-values) for each CpG site. To identify Differentially Methylated Regions (DMRs), we will use the DSS R package, comparing the top and bottom quartiles of parental EDC exposure. We will control for covariates like maternal age, and a False Discovery Rate (FDR) corrected p-value of < 0.05 will be considered statistically significant.

Statistical Approach

All analyses will be conducted in R. To test our primary hypothesis, we will employ multiple linear regression models, treating the methylation level of target genes as the dependent variable and parental EDC concentrations as independent variables, while adjusting for confounders. For EDC concentration data that is not normally distributed, we will use Spearman's rank correlation.

RESULTS AND DISCUSSION

Quantifying Parental Exposure

We expect to detect at least one type of target EDC in the vast majority (>75%) of both follicular fluid and seminal plasma samples. The concentration distributions will likely be right-skewed, which we will account for with non-parametric statistics. We also anticipate a weak-to-moderate correlation between EDC levels in FF and SP, pointing to shared environmental sources for the couple.

Patterns of DNA Methylation

While we do not predict major shifts in the overall, global CpG methylation between high and low exposure groups, we do expect to find highly specific Differentially Methylated Regions (DMRs). Our central prediction is a pattern of hypomethylation in the promoter regions of genes vital for DNA repair (e.g., DNMT1) and embryo implantation (e.g., HOXA10) in zygotes from the highest-exposure parents. Conversely, we may see hypermethylation in promoters of other gene classes, such as those involved in inflammation.

Correlation Analysis using multiple linear regression is expected to show that the concentration of a specific EDC in follicular fluid is a significant independent predictor of the methylation level at the identified DMRs, even after accounting for maternal age and BMI.

Discussion

Interpreting the Expected Outcomes

If our hypotheses are confirmed, this study would offer the first direct human evidence that measurable parental exposure to microplastic-related EDCs is associated with targeted epigenetic alterations at the very beginning of life. Discovering hypomethylation in key developmental genes would suggest a plausible mechanism through which environmental toxins could undermine genomic integrity and embryonic competence even before implantation. For instance, hypomethylation of DNMT1 could have cascading effects, disrupting the maintenance of crucial methylation patterns established during gametogenesis.

Contextualizing the Results

The expectation of finding localized DMRs without a global methylation shift is consistent with a newer understanding of EDCs: their effects are likely precise and targeted, not blunt and genome-wide. Identifying which specific EDC metabolites are the strongest predictors will help move the field beyond general toxicity studies and toward pinpointing the high-risk environmental agents in human populations.

Potential Implications

The implications of such findings are significant:

• For ART: The identified DMRs could potentially serve as novel biomarkers for assessing embryo quality and implantation potential, offering a new tool for clinicians, particularly for couples with known high environmental exposure.

• For Public Health: This research would highlight a potential pathway for transgenerational inheritance of environmental damage, strengthening the argument for more rigorous regulation of ubiquitous EDCs.

Limitations and Future Avenues

We acknowledge the limitations of our design. As an observational study, it can reveal correlation but not definitively prove causation. Furthermore, using non-viable zygotes, while necessary, means our findings may not perfectly represent the epigenetic state of viable embryos. We are also focusing solely on DNA methylation, leaving other epigenetic marks like histone modifications unexplored.

Future research should build on these findings by:

• Conducting longitudinal studies to see if these early epigenetic marks correlate with clinical outcomes like miscarriage, live birth rates, and child health.

• Undertaking mechanistic studies in human embryonic stem cells to elucidate how specific EDCs directly affect the enzymes responsible for setting DNA methylation.

CONCLUSION

This research proposes to demonstrate a significant correlation between parental exposure to microplastic-related EDCs and specific alterations in DNA methylation in human zygotes. By establishing this plausible molecular pathway the "silent legacy" we aim to show how pervasive environmental contaminants may be silently compromising human reproductive and developmental health from the very moment of conception.

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