GENETICS

Inheritance of epigenetic dysregulation from male factor infertility has a direct impact on reproductive potential

Michelle M. Denomme, Ph.D.,[a] Blair R. McCallie, B.Sc.,[a] Jason C. Parks, B.Sc.,[a] Keith Booher, Ph.D.,[b] William B. Schoolcraft, M.D.,[c] and Mandy G. Katz-Jaffe, Ph.D.[a,c]

[a] Fertility Labs of Colorado, Lone Tree, Colorado; [b] Zymo Research Corp., Irvine, California; and [c] Colorado Center for Reproductive Medicine, Lone Tree, Colorado

Objective: To evaluate the epigenetic consequence on the methylome and subsequent transcriptome in euploid blastocysts of male-factor (MF) infertility patients.
Design: Methylome and transcriptome analysis on individual oligoasthenoteratozoospermia (OAT [MF]) blastocysts.
Setting: Infertility clinic.
Patient(s): Clinical data from 128 couples presenting with OAT (MF) and 118 maternal age–matched control (no MF) subjects undergoing infertility treatment from 2010 to 2014, along with 72 surplus cryopreserved blastocysts donated from 33 couples with their informed consents.
Intervention(s): None.
Main Outcome Measure(s): Methyl Maxi-Seq (Zymo Research) was used to determine genome-wide DNA methylation, and small cell number RNA-Seq was used to examine the global transcriptome. Validation experiments were performed with the use of pyrosequencing or quantitative real-time polymerase chain reaction. Statistical analysis used Student *t* test, analysis of variance in R, Fisher exact test, and pairwise fixed reallocation randomization test where appropriate, with significance at $P<.05$.
Result(s): Clinical pregnancy rates were similar between OAT (MF) patients and control (no MF) subjects after euploid embryo transfer. However, the miscarriage rate for OAT (MF) patients was significantly higher (14.7% vs. 2.2%; $P<.05$). Methylome and transcriptome analyses of individual blastocysts revealed significant alterations in 1,111 CpG sites and 469 transcripts, respectively ($P<.05$). Pathway analysis elucidated genes involved in "regulation of cellular metabolic process" as universally affected. Validation of the genome-wide approaches was performed for *SBF1* and *SLC6A9* ($P<.05$).
Conclusion(s): Methylation and transcription aberrations in individual OAT (MF) blastocysts illustrate an epigenetic consequence of MF infertility on embryogenesis, significantly altering key developmental genes and affecting embryonic competence. This epigenetic dysregulation provides an explanation for the reduced reproductive potential in OAT (MF) patients despite euploid blastocyst transfers. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Male factor, PGT-A, methylome, transcriptome, epigenetic dysregulation

Male-factor (MF) infertility contributes to one-half of all infertility cases worldwide (1, 2). It is typically identified during assisted reproductive technology (ART) procedures by means of standardized semen parameter analyses assessing sperm concentration, motility, and morphology (3). This conventional measurement continues to be the only routine test to diagnose this condition, even though it is known that such descriptive assessments cannot account for all aspects of sperm quality, such as chromatin organization and integrity. Compromised quality of the male gamete is known to affect fertilization potential, cleavage divisions, and embryo grade (4, 5), and is associated with an increase in aneuploidy (6), miscarriage (7), and negative pregnancy outcomes.

Recent evidence suggests that the sperm epigenetic status could also play a role in the etiology of miscarriage (8, 9). Epigenetic reprogramming events occur during gametogenesis and early embryogenesis, including DNA methylation erasure, acquisition, and maintenance (10). Some regions, such as imprinted genes and repetitive elements, can preserve their methylation signature from the parental

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Reprint requests: Mandy G. Katz-Jaffe, Ph.D., Colorado Center for Reproductive Medicine, 10290 Ridgegate Circle, Lone Tree, Colorado 20124 (E-mail: mkatz-jaffe@colocrm.com).

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gametes through to the developing embryo (11). Epigenetic inheritance from the sperm DNA methylation landscape is essential for both proper fertilization of the oocyte and viability of the early embryo, whereby disruption could largely affect embryonic development. A number of studies in both humans and nonhuman animal models have highlighted the importance of a "paternal factor" in miscarriage (9,12–15), but the causality between sperm DNA integrity and embryo development after implantation remains unexplained. There is a wide array of evidence linking MF infertility and aberrant sperm epigenetic signatures (16–21), but little is known about the inherited effects after fertilization (22, 23). Nevertheless, evidence suggests that aneuploidy affects epigenetic regulation, resulting in hypomethylation and genetic instability in blastocysts (24).

The introduction of intracytoplasmic sperm injection (ICSI) circumvents conventional MF infertility, thus eliminating the majority of barriers to fertilization. Sperm are selected for ICSI based on the best possible combination of motility and normal gross morphology. However, this does not ensure the genetic or epigenetic integrity of the sperm, and disruptions may play a significant role in impairing the development of the embryo after fertilization. The incorporation of preimplantation genetic testing for aneuploidy (PGT-A) has proved to be successful in improving embryo selection by removing abnormal chromosomal contributions, and randomized controlled trials have shown increases in sustained implantation and delivery rates (25–29).

The present study explored the clinical outcomes of PGT-A for oligoasthenoteratozoospermia (OAT [MF]) patients after a euploid blastocyst transfer. More importantly, it investigated the generational epigenetic effects of severe MF infertility by examining the global methylome and subsequent transcriptome of blastocysts derived from OAT (MF) infertility patients.

MATERIALS AND METHODS

Patient Selection

IVF cycle data and pregnancy outcomes were collected from couples (n = 246) that underwent frozen euploid blastocyst transfers at the Colorado Center for Reproductive Medicine from 2010 to 2014. Surplus cryopreserved blastocysts (n = 72) were donated from couples (n = 33) 5+ years after their infertility treatment with their informed consents and Institutional Review Board approval (WIRB study no. 1145350).

OAT (MF) was defined as motility <40%, morphology <3%, count <20 million/mL, and total motile count (TMC) <13 million/mL. OAT (MF) infertility patients (n = 128) and maternal age–matched control subjects with normozoospermia (no MF; n = 118), underwent routine infertility treatment during a similar time frame in the IVF laboratory. Ovarian stimulation, oocyte retrieval, ICSI, embryo culture, trophectoderm biopsy, PGT-A, and vitrification procedures were routinely performed as previously reported (30). Euploid blastocysts were warmed and transferred in a subsequent frozen embryo transfer. Statistical analysis for ovarian reserve, IVF cycle data, and pregnancy outcomes included Student *t* test with significance at $P<.05$.

PGT-A

Trophectoderm biopsies underwent cell lysis and DNA amplification with the use of Taqman quantitative real-time polymerase chain reaction (qPCR) (31). The copy number for all 22 pairs of autosomes and two sex chromosomes was characterized with the use of standard methods of relative quantification (32). This PCR methodology has demonstrated 98.6% accuracy (31, 33). Only embryos diagnosed as euploid 46XX or 46XY with sufficient confidence were deemed to be suitable for transfer. Limitations of qPCR methodology include the inability to detect mosaicism, chromosome segmental imbalances, and structural rearrangements.

Blastocyst Grading

Blastocysts included in the study were scored based on inner cell mass and trophectoderm quality (34), and were all morphologically graded as high, transferrable quality (grade ≥3BB). Blastocysts were divided into two groups based on semen parameter analysis at the time of oocyte retrieval. Blastocysts (n = 36) were selected for the control group based on the inclusion criteria of motility ≥40%, morphology ≥3%, count 20 million/mL, and TMC ≥13 million/mL, and donor oocyte. Blastocysts (n = 36) were selected for the OAT (MF) group based on the inclusion criteria of motility <40%, morphology <3%, count <20 million/mL, and TMC <13 million/mL.

Whole-Genome Bisulfite Sequencing

Trophectoderm biopsies (~5–10 cells; ~60 pg DNA) from individual blastocysts (n = 6) underwent whole genome bisulfite sequencing with the use of the Methyl Maxi-Seq platform (Zymo Research). Extremely-low-input library preparation of the samples with the use of the Pico Methyl-Seq Library Prep Kit (Zymo Research) was followed by amplification steps to add adapter sequences, PCR purification (DNA Clean & Concentrator 5; Zymo Research), and sequencing on the Illumina HiSeq 2500 platform. Sequence reads were identified with the use of standard Illumina base-calling software with a 5× minimum read count filter and theoretical resolution for detection at 20%, and bisulfite sequence data alignments were performed with the use of the alignment software Bismark (www.bioinformatics.babraham.ac.uk/projects/bismark/), between OAT (MF) and control (no MF) groups. Index files were constructed with the use of the entire reference genome, hg19. The methylation level of each cytosine was estimated as the number of reads reporting a C, divided by the total number of reads reporting a C or T. Quantification of the statistical significance of the methylation difference was determined with the use of the Student *t* test, where $P<.05$ was deemed to be significant. A limitation to the study is that a correction for multiple comparisons was not performed owing to the low starting material in individual blastocysts combined with the small sample size and lack of statistical power.

Whole-Transcriptome RNA-Seq

Individual blastocysts (n = 11) underwent RNA isolation with the use of the Picopure RNA Isolation Kit (Molecular