Oocyte and Embryo Manipulation and Epigenetics

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Abstract

Regulation of the epigenome is a mechanism by which the environment influences gene expression and consequently the health of the individual. The advent and refinement of novel assisted reproductive technology (ART) laboratory techniques, including vitrification, dynamic culture systems, oocyte in vitro maturation, laser-assisted hatching, intracytoplasmic sperm injection, and preimplantation genetic testing for aneuploidy have contributed to the success of ART. From fertilization through implantation, the epigenetic profile of the embryo changes dynamically. Concurrently with these changes, embryo development in vitro is dependent on laboratory intervention and manipulation to optimize outcomes. The impact of ART techniques on imprinting errors remains unclear, as the infertile population likely confers an independent risk factor for defects in expected epigenetic patterns. Alternations in epigenetic mechanisms may contribute to the incidence of aneuploidy as well as recurrent implantation failure of euploid embryos. Additional investigative efforts are needed to assess the contribution of oocyte and embryo manipulation on imprinting modifications in this vulnerable population. The development of diagnostic modalities involving the discovery of epigenetic alterations to improve in vitro fertilization outcomes is an exciting and promising area of future study.

Keywords
- oocyte
- embryo manipulation
- epigenetics

The number of assisted reproductive technology (ART) cycles has increased in the United States by 32% between 2006 and 2015, while the number of infants born who were conceived using ART has increased by 76%.[1] Numerous studies have been performed to assess if an association is present between ART cycles and an increased risk of birth defects.[2,3] More recent literature has suggested a relationship between an increased frequency of imprinting disorders and ART.[4,5] This association is difficult to elucidate given that such disorders are rare and there may be an underlying increased risk of imprinting disorders in the offspring of subfertile patients.[6]

Epigenetics is the study of heritable changes in gene expression that occur without a change in DNA sequence.[7] Epigenetic modifications include histone modification, DNA methylation, nucleosome remodeling, chromatin reorganization, and regulation by noncoding RNA.[8] These mechanisms help control access of genetic information in the cell and allow for a specific interface between genes and the environment without changing the DNA sequence.

The most common epigenetic modification in the human genome is DNA methylation, which is considered to be a parental specific genomic imprint. Such imprints are maintained into adulthood in the offspring's differentiating cells.[8] Methylation of the fifth carbon of cytosine is achieved by DNA methyltransferase enzymes (DNMTs) and occurs at cytosine-phosphatidyl-guanine (CpG) sites. DNMTs also maintain methylation marks. Methylation of CpG islands located in cis-regulatory regions of genes results in histone modification and an inactive DNA configuration that can occur either as part of development or pathologically as related to disease processes.[9] Histone modification is another studied mechanism of epigenetic regulation. During oocyte maturation and progression through meiosis I and II, histones in oocyte chromatin are broadly deacetylated. It has

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been shown that decreased expression of histone acetylase genes leads to cessation of oocyte development and growth.[10]

Controlled ovarian hyperstimulation (COH), oocyte retrieval, manipulation of gametes, and the duration of embryo culture have been suggested to influence methylation changes and imprinting disorders.[4] Given that the maternal epigenome is established later in comparison to the male, it is thought to be susceptible to changes during COS as imprints are established just prior to ovulation. Increased hormonal levels have the potential to alter the methylome of the maternally inherited allele.[5] Changes in maternal- or paternal-specific gene expression patterns are also associated with diminished viability and certain disease states.[11] However, the literature to date supporting this relationship has been conflicting. Reports regarding an increased incidence of imprinting disorders among children conceived with ART surfaced in 2002. Since then, research efforts have focused increased attention on such concerns. The advent of both novel and improving ART laboratory techniques beckons the question: Are gamete and embryo handling to blame for the increase in imprinting errors in an in vitro fertilization (IVF) population? The goal of this article is to explore the relationship between ART and genomic imprinting as it relates to laboratory manipulation of both the oocyte and the embryo.

Embryogenesis and Variations in Epigenetic Modification

After oocyte fertilization, development of the embryo is dependent solely on maternal mRNAs and proteins present in the oocyte, which are activated during fertilization and the transition to embryo development.[12] Rates of methylation are different in the zygote between maternally and paternally derived genes. It has been demonstrated that genome-wide cytosine methylation in the sperm neared 90%, while that of the oocyte is closer to 50%.[13] In oocytes, methylation usually occurs within genes, while in sperm it is between genes. The functional asymmetry of maternal or paternal genomes that occurs with imprinting results in parental-specific monoallelic gene expression. Approximately 80 imprinted genes have been identified in humans, some of which play critical roles in placental and embryonic growth. It has been shown that some imprints are acquired in a step-wise and gene-specific order during oocyte growth, while other imprints in the oocyte are not established until after fertilization in humans.[11]

Reprogramming of the epigenome is critical during both gamete maturation and preimplantation development of the embryo[14] (►**Fig. 1**). During both male and female gametogenesis, there is an epigenetic reset, and all previously existing imprints are erased, with the exception of several resistant imprinted regions that are conserved as a result of

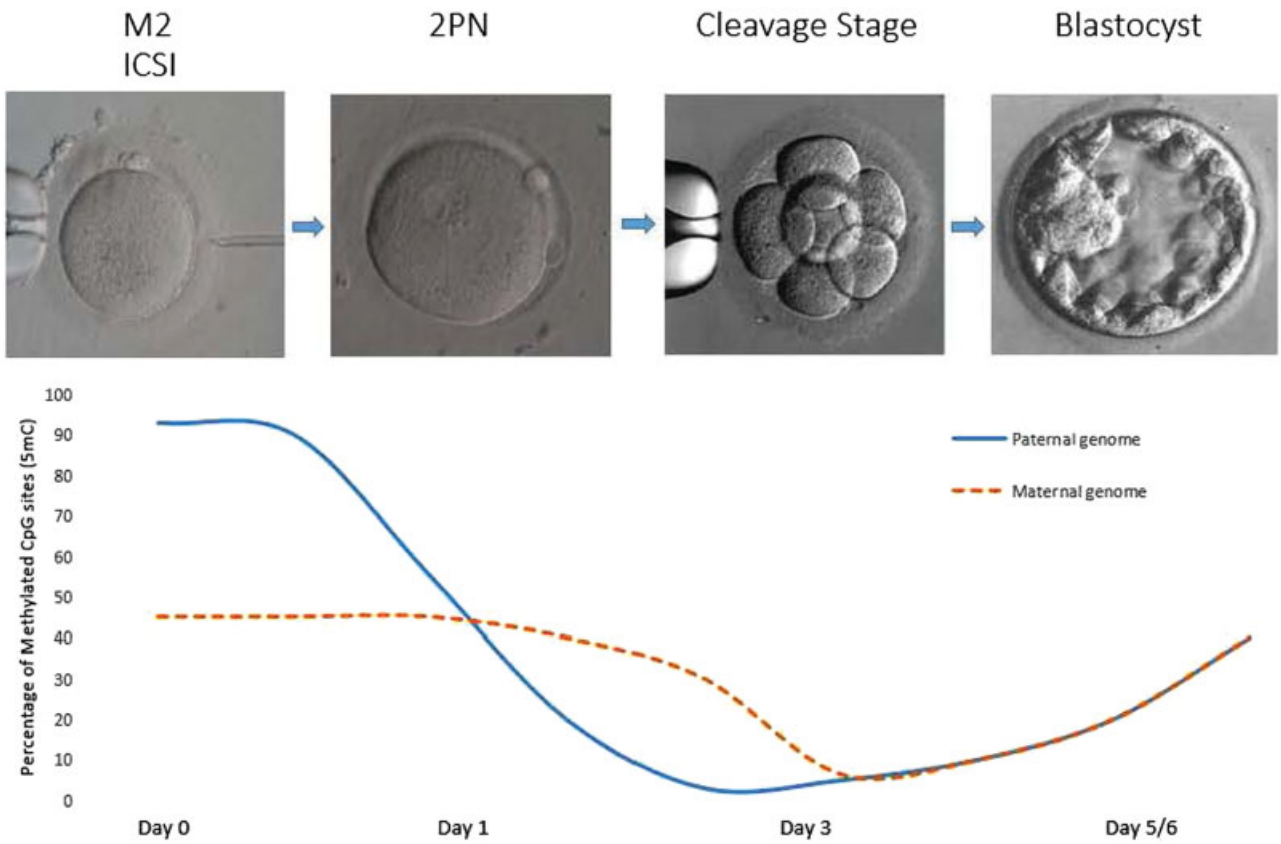

Fig. 1 Methylation patterns in preimplantation embryos. Prior to fertilization, the maternal genome is ~40% methylated, while 90% of the paternal genome is methylated. After fertilization, there is loss of methylation, with active demethylation occurring in the paternal gamete while the maternal genome is passively demethylated. The methylome is reestablished beginning at the cleavage stage and methylation levels increase gradually until after implantation. Methylation patterns are maintained largely by the function of DNMT1. ICSI, intracytoplasmic sperm injection. (Adapted from Messerschmidt et al.[83])

DNMT1.[5] Paternal imprints are established earlier than maternal imprints and therefore it has been suggested that oocytes are more susceptible to epigenetic alterations in comparison to male gametes.[15] In the oocyte, this epigenetic reset whereby all methylation patterns are erased occurs after primordial germ cells enter the gonadal ridge during fetal life. During the 36-hour window when the oocyte matures from a primordial follicle to a Graafian follicle, there is a 15% increase in the methylation of CpG sequences. Different genes become active at progressive stages of oocyte maturation, thus creating germline differentially methylated regions. The pattern of methylation that is established in the oocyte has been proposed to determine the methylation patterns of the maternal genetic content in the embryo. If the correct pattern is not established in the oocyte, embryonic development could potentially be disrupted.[16]

Beginning with fertilization and completion of the second meiotic division and ending at the late cleavage stage of development, the embryo will become transcriptionally active again through wide-spread demethylation. This is known as the maternal-to-zygotic transition,[17] when control of gene expression shifts from the gametes to the embryo. Just as specific methylation patterns regulate phenotypic and developmental capacity in the developing embryo, demethylation and gene activation is of equal importance. Not until the morula or blastocyst stage of embryonic development does passive demethylation take place. Demethylation is deemed passive because the maternal allele does not depend on the DNMT1 enzyme CpG methylation, unlike the paternal allele.[18] This is of particular importance because the transcriptional activity of each allele varies even at early stages of embryo life. Rates of DNA methylation decline with each cell division, although methylated genes maintain their imprint.[19]

The length of time that passive demethylation continues prior to maintenance of CpG methylation during embryo development is unknown. It has been proposed that after the maintenance phase, the hemimethylated regions of specific gene then become fully methylated and are fixed as such. This transition has been said to occur at six to seven rounds of cell division.[20] A proportion of imprinted genes are not reprogrammed after fertilization and instead maintain their germline methylation patterns throughout development. These imprinted genes are critical for fetal and placental growth as well as neurocognitive development and function after birth.[21] Maintenance of genome methylation through preimplantation requires expression of the methyltransferase DNMT1. It has been reported that the methylome is sustained until the embryo is transferred into the uterus during an ART cycle.[22] However, studies of bovine and murine embryos have suggested a contribution of IVF technique and culture to aberrancies in methylation patterns in both bovine[23] and murine models.[24]

Maternal age has also been reported to have an association with changes in gene expression, potentially contributing to euploid transfer failure in women of advanced maternal age. Kawai et al performed a transcriptome analysis utilizing single-embryo RNA of human blastocysts, comparing women less than and greater than 35 years old.[25] There was reduced expression in over 800 genes in women older than 35 years compared with younger controls, concluding that maternal age impacted regulation of gene expression in human blastocysts. Advancing maternal age due to variations in gene expression caused by aberrant epigenetic mechanisms may contribute to delays in embryonic development. Ploidy status of these embryos was unknown, and thus the effects of age cannot be sufficiently filtered from alterations in methylation related to aneuploidy. A summary of technologies used to profile both genome-wide and gene-specific methylation patterns is listed in ►**Table 1**.

Oocyte Maturation, Manipulation, and Epigenetic Events

It has been theorized that COH and oocyte retrieval and manipulation have the potential to interfere with normal patterns of maternal methylation during oocyte maturation. Ovarian stimulation using exogenous hormones may disrupt the establishment of imprints in the developing oocyte.[26] It has also been suggested that exogenous gonadotropins used to drive multifollicular development may force growth of all oocytes that otherwise would be destroyed but instead may have incompletely imprinted genes. There has been a lack of human studies linking DNA methylation defects to medications used during IVF directly. The impact of gonadotropins on methylation patterns of the oocyte and embryo cannot be filtered from other confounders such as parental age, underlying infertility, or laboratory manipulation of gametes.[27]

During an IVF cycle, oocytes are matured in vivo during the course of COH. In vitro maturation (IVM) refers to the process whereby oocytes are retrieved from antral follicles in the germinal vesicle stage of metaphase I and then are cultured to maturity. Gonadotropins are typically added to culture media to assist in maturation. IVM was originally developed as an ART technique to avoid COH and has been adapted for use in fertility preservation efforts. The American Society of Reproductive Medicine (ASRM) has stated that IVM should only be performed as an experimental procedure given the low maturity rates of oocytes, decreased blastulation rates, clinical pregnancy rates, and live births.[28] Experimental data have drawn attention to epigenetic differences between human IVM and in vivo matured oocytes. According to one review, there are no clearly reported epigenetic differences between IVM and in vivo murine and bovine oocytes, although there is currently a lack of well-designed studies.[29]

A particular gene of interest related to epigenetic changes associated with ART is the imprinted gene insulin-like growth factor 2 (*IGF2*)/*H19* locus on chromosome 11p15.5. *IGF2* is one of the genes responsible for fetal growth. Epigenetic alterations in *IGF2*/*H19* may contribute to the low birth weight associated with pregnancies resulting from IVF.[30] The *IGF2*/*H19* locus is inherited in parent-of-origin manner as *IGF2* is inherited from paternal allele and *H19* is the associated noncoding controlled region of *IGF2*, which is from the maternal allele.[31] *H19* serves as a suppressor of *IGF2*, and thus when inherited paternally is methylated, resulting in

Table 1 Available technologies for the assessment of genome-wide and gene-specific methylation patterns

Method	Genome treatment	CpG coverage	Amount of starting material	Advantages	Limitations
WGBS[76]	Bisulfite	>90–100% of the genome	50–100 ng	• Comprehensive assessment of nearly all CpG sites, including low-density areas. • Determines sequence context and absolute methylation level	• Cost • Sequencing and alignment difficulty
RRBS[77,78]	Restriction enzyme and bisulfite	85% of CpG islands	1 μg	• Lower cost compared with WGBS	• Lack of coverage at intergenic and distal regulatory elements
MeDIP-Seq[79]	Affinity enrichment	70–85% of genome	5 ng–5 μg	• Cost-effective • Increased sensitivity with low CpG density	• No investigation of single CpG sites • Biased toward hypermethylated areas
Infinium BeadChip 450K[80,81]	Site-specific probes + bisulfite	96% of CpG islands	500 ng	• Cost-effective • Does not require large DNA input • High sample throughput	• Human samples only • Considerable degradation of DNA after bisulfite treatment
Targeted bisulfite sequencing[82]	Site-specific probes + bisulfite	>68–100% of targeted of CpG islands	100 ng–5 μg	• Highly reproducible • Require lower amount of input DNA	• Complex probe design • Expensive probes

Abbreviations: CpG, cytosine-phosphatidyl-guanine; MeDIP-Seq, methylation analysis by immunoprecipitation sequencing; RRBS, reduced representation bisulfite sequencing; WGBS, whole-genome bisulfite sequencing.

expression of the IGF2 gene. Hypomethylation of *H19* results in its overexpression and downregulation of *IGF2* results, leading to growth restriction disorder known as Silver-Russell syndrome (SRS).[32] In contrast, hypermethylation of *H19* leads to overexpression of *IGF2* and fetal overgrowth in Beckwith-Wiedemann syndrome (BWS).[33] Although these epigenetic events have been studied frequently in animal and human embryo development, studies in oocyte maturation are limited. Borghol et al examined methylation patterns of the *H19* region in the genome of oocytes matured in vitro compared with in vivo.[34] Mentioned previously, when this imprint is maternally inherited, it is normally unmethylated, resulting in decreased IGF-2 expression. Pools of five to thirty oocytes were retrieved at the germinal vesicle or MI stage and were matured in vitro to the MII stage. Bisulfite-treated polymerase chain reaction (PCR) was utilized to assess methylation patterns of oocytes in progressive stages of maturation and compared with those retrieved at the MII stage. Twenty-five percent of in vitro matured oocytes were found to have methylation of the normally unmethylated *H19* region, while 50% of that arrested had altered methylation patterns. This is suggestive of a relationship between epigenetic immaturity and the inability to complete meiosis.

In contrast to this, Kuhtz et al performed single-cell methylation analysis using a bisulfite sequencing technique on 71 oocytes from polycystic ovary syndrome patients matured from the germinal vesicle stage to M2 stage as well as 38 in vivo matured control oocytes.[35] There were no significant differences in methylation patterns of maternally or paternally imprinted genes.

A recent study noted that oocytes matured in vivo had increased numbers of upregulated genes involved in control of transcription and translation, histone acetylation, fatty acid oxidation, and cytoskeleton organization compared with oocytes matured in vitro. Interestingly, the addition of granulosa cells to the culture media of IVM oocytes led to a gene expression profile that was similar to in vivo matured oocytes.[36] These oocytes were retrieved from women without underlying infertility during natural cycles, making these results less applicable. It appears that histone modification between IVM oocytes and in vivo matured bovine oocytes are comparable, although this facet of epigenetic modification is much less studied.[37] Based on animal and human studies, it can be inferred that the incidence of imprinting defects is higher in oocytes matured in vitro as compared with in vivo. However, it remains unclear whether these aberrancies are due to intrinsic deficiencies within the immature oocyte or effects of embryo manipulation during ART treatment. In addition, studies of epigenetic errors in oocytes are potentially confounded by contamination by DNA from somatic cells derived from the cumulus which could mimic abnormal methylation.[38]

Laboratory Manipulation and Variations in Epigenetic Patterns

Given the compelling data on the incidence of imprinting defects in murine and bovine embryos, recent attention has been drawn to standard ART laboratory procedure and the potential epigenetic ramifications. The impact of culture media and oxygen concentration—discussed in detail in

the previous volume, "Embryo culture conditions and the epigenome," as well as intracytoplasmic sperm injection (ICSI), laser-assisted hatching (LAH), and cryopreservation effects on differential gene expression in the embryo—is discussed below.

Intracytoplasmic Sperm Injection

Intracytoplasmic sperm injection involves the injection of a single sperm directly into the ooplasm. It has led to increased fertilization rates in patients with male factor infertility and has utility in cases with low oocyte yield.[38] To fertilize the oocyte, the sperm must replace the majority of histones in the chromatin with protamines with simultaneous acetylation of remaining histones.[39] This promotes sperm motility and helps protect from oxidation within the female genital tract. The paternal genome is actively reset and widely hypomethylated after fertilization. Methylation defects have been identified in the sperm of oligozoospermic and azoospermic men[40] and the incidence of imprinting abnormalities has been noted to be much higher than that of the offspring. This suggests that the embryo is able to autocorrect epigenetic error in its process of resetting the methylome profile. Furthermore, the selection of the most morphologically normal sperm for the process of ICSI may also reduce the risk of inheritance of methylation abnormalities.[41]

Hammoud et al performed a genome-wide analysis of both histone retention and methylation patterns at developmental and imprinted gene loci in the sperm of seven infertile male patients, three of who had poor embryogenesis during an IVF cycle and four had abnormal semen parameters and altered protamination as compared with fertile controls.[42] Histone fractions were measured using chromatin immunoprecipitation and Illumina GAIIx sequencing, while methylation profiling was performed using bisulfite sequencing. The majority of men had aberrant and randomly distributed histone retention rather than the expected pattern of protamination. While there were no differences in histone methylation, there was widespread hypomethylation of developmental gene promoters. The clinical significance of these findings remains unknown.

Previous reports have cited an increased incidence of low birth weight, sex chromosome aneuploidy, and birth defects in children conceived by IVF with the use of ICSI.[43] This has been theorized to be due in part to imprinting errors.[44] Advancing paternal age has been linked to decreases in gene regulation by epigenetic factors.[45] Additionally, genome-wide hypermethylation of DNA has been noted in men with poor-quality sperm, suggesting DNA methylation aberrancies during spermatogenesis as a cause.[46]

Epigenetic variations that may be present in the sperm of infertile men have the potential to be exacerbated by ART technology. However, children conceived from ART with low birth weight were not noted to have a difference in methylation patterns in cord blood after delivery compared with normal weight, spontaneously conceived children.[47] Palermo et al demonstrated no difference in malformation rates of 14,211 children conceived with ICSI compared with conventional insemination with IVF.[48]

Cord blood methylation profiles of children conceived with IVF-ICSI were compared with both infertile controls conceived with intrauterine insemination (IUI) and fertile natural conception controls.[49] The Illumina Infinium HumanMethylation 450K BeadChip was utilized to determine whole genome-wide methylation. No extensive or consistent DNA methylation changes across the entire genome were present between groups. However, it was concluded that both infertility and ICSI impact DNA methylation at specific loci. Methylation patterns of DNA from the ICSI-frozen embryo transfer group and the IUI study groups were dissimilar from naturally conceived controls at a particular gene locus. It is difficult to filter the effects of infertility from attributions of ART techniques on the epigenetic landscape in a study such as this, given that IVF with ICSI is a more aggressive treatment typically reserved for couples with poorer prognoses compared with those who receive treatment with IUI. Accordingly, they may be more susceptible to epigenetic alterations compared with couples with less severe causes of infertility.

The clinical translation of the aforementioned findings in embryos conceived with IVF and implications for disease in offspring is still unknown. The majority of human methylation studies utilize leukocytes from cord blood and placental sampling, and the accuracy of gene imprinting profiles in these tissues is difficult to determine. However, several studies have shown stable methylation patterns at specific loci across a variety of tissues.[50]

Laser-Assisted Hatching (LAH)

The use of LAH for disruption of the zona pellucida in preimplantation blastocysts is standard practice in IVF. Although it has been suggested that LAH for use in polar body biopsy has negative effects on embryo development,[51] its use for blastocyst hatching results in higher clinical pregnancy rates in an IVF population.[52] A noncontact diode laser relies on heat for zona pellucida disturbance and the effect of such thermal energy on the gene expression of the embryo is unknown.

Honguntikar et al investigated the epigenetic response of preimplantation embryos exposed to LAH in two, six, and eight cell mouse embryos.[53] RT-qPCR was used to quantify the expression of DNMT3a and DNMT3b genes and bisulfite sequencing with subsequent nested PCR was utilized to detect methylation differences between genomes. Expression of the DNMT levels was reduced in two cell embryos exposed to LAH, while there were no significant differences between DNMT levels in six and eight cell embryos that underwent assisted hatching with the laser as compared with control embryos that were not exposed to laser hatching. There were no differences in the methylome composition between groups. Human studies are needed to further elucidate potential impact of LAH on the differential gene expression in the embryo.

Oocyte and Embryo Cryopreservation

Vitrification has increased the success of frozen embryo transfer to a synchronous and more physiologic uterus, accounting for the increase in cryo-thawed cycles worldwide.[54] The effect of vitrification and thawing on the genomic imprint and methylation status is largely unknown. The gene expression

level of the imprinted gene G*RB1*0 was dramatically decreased in vitrified eight-cell mouse embryos versus nonvitrified embryos, although the rate of blastocyst formation was comparable.[55] Wang et al demonstrated loss of methylation of *H19/IGF2* in mouse embryos cultured in vitro as compared with in vivo, with a larger effect in the vitrified group.[56] Mouse metaphase II oocytes that were vitrified and subsequently thawed had decreased methylation levels of *H19*, Peg 3, and *SNPRN* compared with nonvitrified controls.[57]

In the only human study to assess the impact of vitrification on genomic methylation in humans, embryos were cryopreserved at day 3 and subsequently thawed and cultured to day 5. Methylation levels of the *H19/IGF2* DMR were compared between embryos that were cryopreserved and those that were cultured to day 5 without vitrification. No significant difference was found between CpG methylated genes between vitrified and control groups.[58] Although there is an overwhelming trend toward cryopreservation at the blastocyst stage of embryo development, these findings are reassuring.

ART Technology and Diseases of Imprinted Methylation Defects

Environmental stresses and genetic factors associated with a subfertile population can affect epigenetic methylation of imprinted genes both at gametogenesis and after fertilization. ART procedures and embryo manipulation as well as predispositions of a subfertile population may result in epigenetic errors leading to imprinting disorders, namely, loss of CpG site methylation. Many imprinted genes are expressed during embryo development prior to implantation. Environmental stress after laboratory manipulation during embryo culture in vitro potentially could account for this paradigm. Abnormal expression of imprinted genes can either occur as a result of genetic disorders, such as uniparental disomy, or as a result of epigenetic errors in methylation.

A large-scale methylation analysis was performed of 27,578 CpG sites from DNA samples collected from cord blood of live births resulting from both ART and natural births.[59] There was resultant hypomethylation (<30% methylation) of 2.7% in the ART group with 24 genes with two or more CpG sites that were significantly different from the natural birth control group. This study demonstrated greater variability in genome-wide DNA methylation, inferring that pregnancies resulting from ART may have more instability of the epigenome.

The timing of ART in conjunction with epigenetic reprogramming events may contribute to aberrancies in epigenetic events that may confer phenotypic risk. Multiple studies have reported an association between ART and imprinting disorders, such as BWS, the Angelman syndrome (AS), and SRS. BWS is a multigenic disorder that results from abnormal expression of several closely linked genes on chromosome 11p15 associated with the cell cycle and growth control. Genes implicated in these disorders include *H19, IGF2*, and *KCNQ1OT1*.[60] The *H19* gene is found to be hypermethylated in 17% of patients with BWS[61,62] and 92% of those with SRS[63,64] conceived by ART compared with 5% of naturally conceived children with BWS and 40% of those with SRS. Patients with ART AS had SNRPN imprinting defects of 46%, compared with 5% of natural conceptions with AS.[65] Given the rarity of imprinting disorders in the general population and the number of children born as a result of ART, it is difficult to draw definitive conclusions about the effect that ART technology has on the incidence of these diseases. Routine screening for imprinting disorders is not recommended given the infrequency of these diseases.

Retinoblastoma, a malignant tumor of the retina, occurs with loss of maternal and paternal alleles of the tumor suppressor gene *RB1*. Childhood retinoblastoma is influenced by epigenetics, namely, the hypermethylation of CpG islands in the *RB1* promoter region. There have been some case reports of an increased incidence of spontaneous retinoblastoma in children conceived by IVF.[66,67] A recent study utilized methylation-specific multiplex ligation-dependent probe amplification to identify promoter hypermethylation in the *RB1* gene from tumor tissue of affected children with retinoblastoma who were conceived using ART.[68] Specimens from seven patients were studied and none exhibited hypermethylation of the *RB1* promoter, but rather de novo germline mutations, nonsense mutations, frameshift mutations, and loss of heterozygosity. None of these mutagenic alterations occurred as a result of epigenetic changes.

White et al compared methylation profiles of the imprinted genes *SNPRN*, *KCNQ1OT1*, and *H19* of high-quality day 3 embryos and blastocyst stage embryos. Interruptions in methylation patterns of the genes of interest were reported as 76% in day 3 embryos and 50% of blastocysts.[69] Cause of infertility, maternal age, and hormonal dosage levels did not impact frequency of methylation errors. Additionally, embryos utilizing donor sperm also had abnormal methylation patterns in paternally expressed alleles, citing processes involved in ART as a possible contributor to these findings.

Methylation pattern analysis of arrested embryos, high-grade blastocysts, and the corresponding sperm and oocytes with which they were created revealed hypomethylation of the paternal allele of gene *H19* in 50% of arrested embryos, but normal methylation in the parental sperm. This instability was attributed to errors in the demethylation process which occur during preimplantation development.[70]

Identification of a particular ART laboratory technique or point in time during oocyte or embryo maturation in vitro that could contribute to imprinting disorders is difficult. The frequency of methylation errors in murine and bovine embryos cultured in vitro is much higher than that reported in human embryos.[71] The studies of methylation defects in animal embryos are prospective compared with the majority retrospective studies performed with children affected by imprinting disorders after conception with ART technology. Furthermore, there is a lack of naturally conceived controls for comparison in prospective human studies.

Epigenetic Aberrancies and Aneuploidy

Embryonic aneuploidy is a consequence of maternal meiotic error in the oocyte, and to a lesser extent a result of postfertilization mitotic error or sperm meiotic error. Methylation

plays an intricate role in establishing the structure of chromatin, which is critical for chromosomal segregation during meiosis.[72] Deviations in epigenetic norms may potentially contribute to errors of chromosomal segregation. McCallie et al sequenced the global methylation patterns of 316 cryopreserved aneuploid blastocysts, which were compared with control euploid blastocysts utilizing the Infinium HumanMethylation 450K BeadChip.[73] Significant hypomethylation of regulatory genes coding for DNA methyltransferases, chromatin modifying regulators, and posttranslational modifiers were observed in monosomic but not trisomic or euploid embryos. Given the diminutive reproductive potential of monosomic embryos, such findings highlight a potential epigenetic contribution to aneuploidy.

Could errors in epigenetic reprogramming contribute to the failure of euploid embryo transfer? Many mechanisms have been implicated in recurrent implantation failure, including endometrial dyssynchrony, aberrant immunomodulation, and compromised sperm quality. During sperm maturation, the majority of histones are replaced by protamines, both protecting the sperm from oxidative stress and enabling highly efficient chromatin packaging. Newer literature has also implicated deviant DNA methylation patterns of sperm and reduced fecundity in men.[74] The relationship between poor sperm DNA quality and embryo development following implantation is not well understood. Embryos conceived with sperm from men with oligoasthenoteratozoospermia have been shown not to have significantly different implantation rates compared with euploid controls, but do have higher rates of miscarriage. Genome-wide methylation profiling of these trophectoderm biopsies demonstrated significant alterations in methylation of over 1,000 CpG associated with cellular metabolic processes.[75] A causative relationship is difficult to ascertain, and even more difficult is a diagnostic or therapeutic intervention that may be of utility in patients with recurrent implantation failure.

Conclusion

This review sought to investigate the role of ART laboratory technique as well as oocyte and embryo manipulation on the rate of implantation errors and their role in development of disease in an IVF population. Epidemiologic studies have revealed a higher incidence of aberrant methylation patterns in patients with imprinting disorders. Although there is no lack of animal embryo data, there is a paucity of high-quality studies with human blastocysts and the association of infertility with methylation defects independent of treatment is unquantifiable. The translation of imprinting errors in a research environment to development of disease is difficult. Additionally, it is challenging to isolate one particular technique's contribution to errors in genomic imprinting. Future research on human gametes and embryos is needed to assess the incidence of imprinting errors, as there are continued improvements in laboratory technique and optimization of a culture environment in vitro.

Conflict of Interest
None declared.

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