Genetics and Epigenetics of Infertility and Treatments on Outcomes

Margareta D. Pisarska,[1,2] Jessica L. Chan,[1] Kate Lawrenson,[1] Tania L. Gonzalez,[1] and Erica T. Wang[1,2]

[1]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, California 90048; and [2]David Geffen School of Medicine at UCLA, Los Angeles, California 90095

ORCiD numbers: 0000-0003-1096-6506 (M. D. Pisarska); 0000-0002-9423-6027 (J. L. Chan); 0000-0002-6469-2515 (K. Lawrenson); 0000-0003-3825-8544 (T. L. Gonzalez).

Context: Infertility affects 10% of the reproductive-age population. Even the most successful treatments such as assisted reproductive technologies still result in failed implantation. In addition, adverse pregnancy outcomes associated with infertility have been attributed to these fertility treatments owing to the presumed epigenetic modifications of *in vitro* fertilization and *in vitro* embryo development. However, the diagnosis of infertility has been associated with adverse outcomes, and the etiologies leading to infertility have been associated with adverse pregnancy and long-term outcomes.

Evidence Acquisition: We have comprehensively summarized the data available through observational, experimental, cohort, and randomized studies to better define the effect of the underlying infertility diagnosis vs the epigenetics of infertility treatments on treatment success and overall outcomes.

Evidence Synthesis: Most female infertility results from polycystic ovary syndrome, endometriosis, and unexplained infertility, with some cases resulting from a polycystic ovary syndrome phenotype or underlying endometriosis. In addition to failed implantation, defective implantation can lead to problems with placentation that leads to adverse pregnancy outcomes, affecting both mother and fetus.

Conclusion: Current research, although limited, has suggested that genetics and epigenetics of infertility diagnosis affects disease and overall outcomes. In addition, other fertility treatments, which also lead to adverse outcomes, are aiding in the identification of factors, including the supraphysiologic hormonal environment, that might affect the overall success and healthy outcomes for mother and child. Further studies, including genome-wide association studies, epigenomics studies, and experimental studies, are needed to better identify the factors leading to these outcomes. **(*J Clin Endocrinol Metab* 104: 1871–1886, 2019)**

Infertility affects ~6.1 million people in the United States, equivalent to 10% of the reproductive-age population (1). The use of assisted reproductive technologies (ARTs) includes *in vitro* fertilization (IVF) and contributes to 1.5% of live births in the United States. Other non-IVF fertility treatments (NIFTs), which include ovulation induction and controlled ovarian stimulation with or without intrauterine insemination, contribute to 4.6% of live births (2, 3). Adverse pregnancy outcomes have been associated with the use of ARTs (2, 4–11). A recent meta-analysis of 50 cohort studies comparing singleton pregnancies from ART and

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 6 September 2018. Accepted 12 December 2018.
First Published Online 17 December 2018

Abbreviations: ART, assisted reproductive technology; BMI, body mass index; DEG, differentially expressed gene; EAOC, endometriosis-associated ovarian cancer; GWAS, genome-wide association study; IVF, *in vitro* fertilization; NIFT, non–*in vitro* fertilization fertility treatment; PCOS, polycystic ovary syndrome; RR, relative risk; SNP, single-nucleotide polymorphism.

naturally conceived pregnancies found a significantly increased risk of adverse outcomes, including pregnancy-induced hypertension [relative risk (RR), 1.30; 95% CI, 1.04 to 1.62], gestational diabetes mellitus (RR, 1.31; 95% CI, 1.13 to 1.53), placenta previa (RR, 3.71; 95% CI, 2.67 to 5.16), placental abruption (RR, 1.83; 95% CI, 1.49 to 2.24), postpartum hemorrhage (RR, 1.29; 95% CI, 1.06 to 1.57), preterm birth (RR, 1.71; 95% CI, 1.59 to 1.83), low birthweight (RR, 1.61; 95% CI, 1.49 to 1.75), small for gestational age (RR, 1.35; 95% CI, 1.20 to 1.52), and perinatal mortality (RR, 1.64; 95% CI, 1.41 to 1.90) (12). However, pregnancies conceived by couples using other types of fertility treatment (*i.e.*, NIFTs), including ovulation induction, also have an increased risk of adverse outcomes. For example, preeclampsia affects 3.3% of NIFT conceptions and 4.7% of IVF conceptions compared with 2.4% of spontaneously conceived gestations. The gestational diabetes risk has also been shown to be greater with NIFT conceptions at 5.9% compared with 2.7% for IVF conceptions and 3.4% for spontaneously conceived gestations.

Placental abruption was found to occur in 1.4% of NIFT conceptions and 2.2% of IVF conceptions compared with 0.7% in spontaneously conceived gestations, and placenta previa was greater in IVF conceptions at 3.6% compared with 0.5% in NIFT conceptions and 0.6% in spontaneously conceived gestations. Fetal development will also be affected, with a low birthweight present in 7.4% of NIFT conceptions compared with 5.9% in IVF and 5.1% in spontaneous conceptions. In addition, the risk of fetal growth restriction was 2.1% for NIFT conceptions compared with 1.1% for spontaneous conceptions and 0.9% for IVF conceptions (13). However, it is likely that the underlying infertility contributed largely to these risks. Pregnancies conceived by couples with infertility, regardless of treatment, have an increased risk of adverse outcomes that affect both the fetus and the mother, including maternal morbidity, with a crude prevalence reported of 1.44% in the subfertile population and 3.14% in IVF pregnancies compared with 1.09% in the fertile population, with blood transfusion accounting for the most common maternal morbidity (11, 14–19). Despite the increased risk of adverse outcomes, the overall incidence and RR of these outcomes has been low. However, collectively, the common link to these adverse outcomes associated with infertility and fertility treatments has been that they are associated with placental defects, which likely develop early in gestation during implantation and placentation (20). Additionally, many of these studies did not control for the underlying infertility and the specific treatments used. Thus, the underlying etiology for infertility, resulting from the underlying genetics and/or epigenetics and the potential epigenetic modifications linked to specific fertility treatments, likely affects the outcomes that lead to these defects in implantation and placentation.

Female infertility results from multiple etiologies, some that affect implantation and subsequent placentation, leading to placental dysfunction and adverse outcomes. These etiologies include polycystic ovary syndrome (PCOS), endometriosis, and unexplained infertility. PCOS has been responsible for >70% of cases of ovulatory dysfunction, which accounts for >27% of the cases of infertility. PCOS is a common endocrine metabolic disorder affecting 5% to 15% of women (21, 22). PCOS should be considered present when two of three criteria have been met: ovulatory dysfunction, hyperandrogenism, and/or polycystic-appearing ovarian morphology on pelvic ultrasonography (23). Even after ovulation has been restored, women with PCOS have had decreased cumulative pregnancy rates compared with other subfertile populations (24, 25). Endometriosis is a condition characterized by endometrial glands and stroma outside the uterine cavity, causing pelvic pain, dysmenorrhea, and infertility. It affects ~10% of reproductive-age women but might account for a greater number of cases of infertility, because studies have suggested that 25% to 50% of infertile women will have endometriosis and that 30% to 50% of women with endometriosis will be infertile (26–29). Unexplained infertility, which accounts for 17% of cases of infertility, has no defined pathology. However, it might be due to unrecognized abnormalities that result in subtle genetic and/or epigenetic changes of an underlying subtle phenotype of PCOS and/or endometriosis, which could be attributed to changes in the diagnostic criteria. Because the diagnostic criteria for PCOS have changed, encompassing different phenotypes for the syndrome, a subset of patients with unexplained infertility and a subtle PCOS phenotype, such as ovulatory PCOS, might remain undiagnosed (3, 30). Likewise, although endometriosis has been reported in ≤50% of women with infertility (31), many women might have undiagnosed endometriosis, because laparoscopy, which currently is the only method to definitely diagnose endometriosis, is no longer warranted for the diagnostic workup of infertility (32). These findings might contribute individually or collectively to a proportion of women with unexplained infertility. However, further studies defining a potential genetic link are necessary. These three etiologies, which account for most cases of female infertility, all affect implantation. This has potential implications for normal placentation, subsequent placental function, and the overall short- and long-term outcomes associated with infertility and the treatments used (Fig. 1).

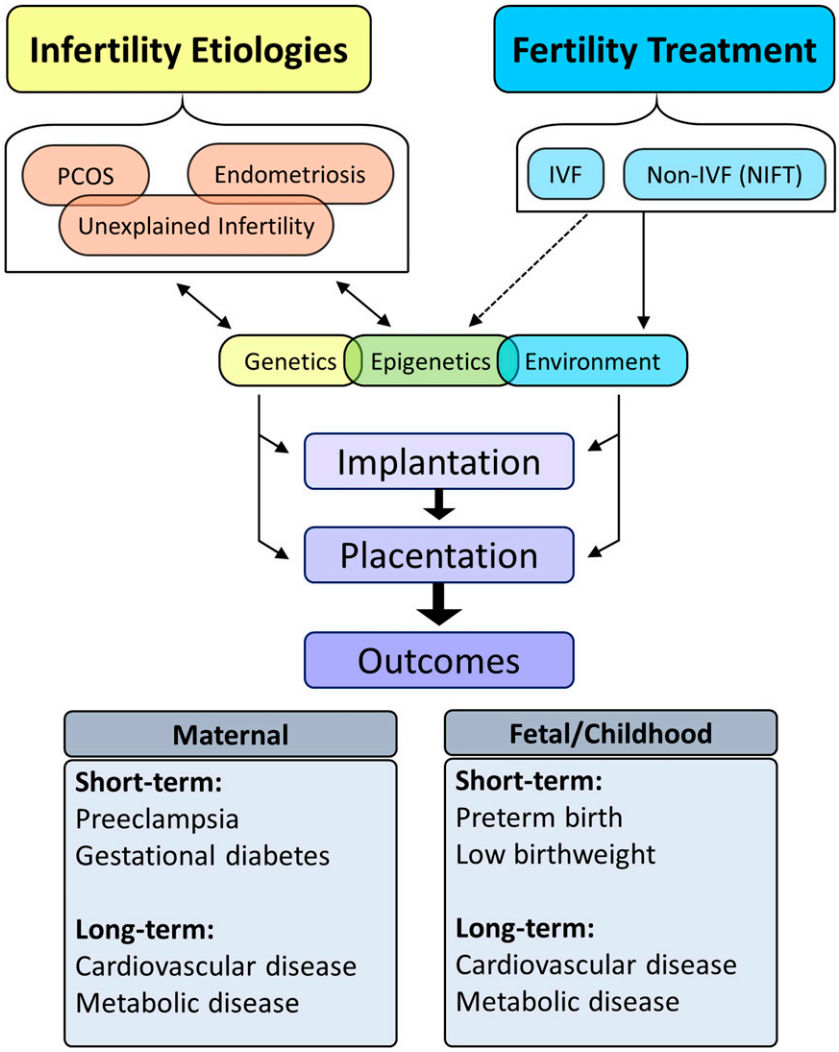

Figure 1. The genetic and epigenetic influences of infertility etiologies and the environmental effect of fertility treatments on epigenetics influence implantation and placentation, which leads to short- and long-term adverse maternal and fetal/childhood outcomes.

Implantation (Consequences of Infertility)

Implantation occurs after apposition of the blastocyst at the uterine epithelium (adhesion), where the trophoblast cells of the blastocyst penetrate the endometrium (implantation) and, ultimately, invade into the uterine stroma to become completely embedded. Cellular adhesion molecules (*i.e.*, selectins, integrins, cadherins, and immunoglobulins) are necessary to mediate cell-to-cell adhesion. Cytokines, leukemia inhibiting factor, and interleukins are also crucial modulators of implantation. Through a well-orchestrated sequence of events, appropriate implantation occurs (33–36). Normal implantation and subsequent placentation leading to a healthy gestation require a receptive endometrium and healthy embryo (37–41). Despite substantial advances in ARTs, 20% to 40% of euploid embryos will fail to implant, 8% to 15% will end in a biochemical pregnancy, and 12% will end in early miscarriage, resulting in failed outcomes (live birth) in >30% of cycles (42–46). These success rates per ART cycle have differed according to the diagnosis: 26.4% for women with ovulatory dysfunction, 24.6% for women with endometriosis, and 28.3% for women with unexplained infertility (47). Assays to study the window of implantation to adjust the hormonal milieu (progesterone) for optimal timing of embryo transfer (personalized embryo transfer) have improved the outcomes (48, 49). However, the implantation rates have only been 33.9%, with ongoing pregnancy rates of 39% to 51.7% after adjustment of the hormonal milieu to create a receptive endometrium. Thus, other factors might be contributing to the failed outcomes and require further investigation to improve the precision of these assays and optimize personalized embryo transfer (49).

Infertility Etiology and Implantation

Both PCOS and endometriosis affect implantation (24, 25, 50). Unexplained infertility could also result from implantation defects, potentially due to a subtle phenotype of PCOS or endometriosis. Similar causes have been implicated in implantation failure among these etiologies of infertility. These include abnormal alpha V beta 3 integrin and glycodelin expression, upregulation of estrogen receptors, and progesterone resistance (24, 25, 51–60). Women with PCOS, even after ovulation has been restored, will have an increased risk of spontaneous miscarriage, with lower cumulative pregnancy rates compared with other subfertile populations (24, 25). In addition, some studies have suggested an increased rate of spontaneous abortion for women with endometriosis compared with women without (61, 62). Furthermore, in a cohort of women with unexplained infertility, a low midluteal progesterone level was associated with a lower probability of obtaining a live birth despite the use of NIFT (ovarian stimulation with intrauterine insemination), suggesting the presence of hormonal dysfunction leading to failed outcomes in select women with unexplained infertility (63). It remains to be determined how the different etiologies of infertility as a

result of the underlying genetics and epigenetics contribute to the endometrial dysfunction that leads to implantation failure, miscarriage, and adverse outcomes resulting from placentation defects and contributing to the short- and long-term outcomes associated with infertility.

Genetics of Infertility Etiology

Single-gene disorders leading to disease and primary infertility have been identified (64). However, these specific disorders have contributed to a small percentage of infertility etiologies and have not accounted for most of the causes, which are likely multifactorial (64). More recently, the most common type of genetic variation among populations, single-nucleotide polymorphisms (SNPs) (65, 66), has been found to be associated with disease leading to infertility. Because these variants are common and have mild effects on risk (OR, ~1.2), they can only be identified using these large population-based genome-wide association studies (GWASs).

Genetics of PCOS

Several large GWASs have been performed using PCOS case-control cohorts and have identified multiple risk associations close to the genes associated with hormone regulation and potential implications in fertility and implantation. The *LHCGR* gene encodes the luteinizing hormone and human chorionic gonadotropin receptors, is critical for normal menstrual function, and is located at locus 2p16.3 (67–70). Additional loci have been identified at genome-wide levels of significance ($P < 5 \times 10^{-8}$), including 9q22.32, 11q22.1, 12q13.2, 12q14.3, 16q12.1, 19p13.3, 20q13.2, 2p16.3, 11p14.1, and 8p23.1 (68, 71). Several of the candidate genes (*FSHR*, *FSHB*, *DENND1A*, *INSR*, and *THADA*) are also related to reproductive hormone function and have been associated with metabolic syndrome and insulin resistance, common in PCOS and a potential cause for an increased risk of miscarriage and gestational diabetes in pregnancy (24, 72).

Genetics of Endometriosis

A number of GWASs have been performed for endometriosis, with some SNPs identified that might play a role in the endometrial dysfunction that leads to implantation failure. This includes SNPs in association with genes such as *CDKN2B-AS*, which encodes cyclin-dependent kinase inhibitor 2B antisense RNA (73). *CDKN2B-AS* is expressed in the uterus and regulates expression of *CDKN2A*, which regulates cell growth in endometrial tissue and has been implicated in the development of endometriosis (74). Other important regions contain the *WNT4* gene, which plays a role in the development of the female genital tract (75); *HOXA10*, which plays a role in uterine development (76); and *GREB1*, which encodes a gene in the estrogen regulation pathway and has been implicated in estrogen-dependent growth in peritoneal endometriosis (77). Additional candidate genes include *VEZT*, which encodes a transmembrane protein component of the E-cadherin–catenin complex at adherens junctions (78). *VEZT* might play a role in the regulation of cell migration and invasion, key processes in the pathogenesis of endometriosis and regulators of trophoblast migration and invasion, which are important for implantation through maternal/embryonic (fetal) cross-talk (79).

Epigenetics of Infertility Etiology

Epigenetic modifications are heritable alterations that do not result from changes in the DNA sequence. They are affected by the genetic variability and environmental influences in disease (80–82). These noncoding mechanisms of gene regulation include DNA methylation and noncoding regulatory elements (*e.g.*, enhancers, promoters, and insulators). They alter DNA accessibility and chromatin structure, thereby regulating patterns of gene expression (83). The noncoding transcriptome, which includes long noncoding RNAs and miRNAs, also affects overall gene expression. Long noncoding RNAs are single-stranded noncoding RNAs with >200 nucleotides. miRNAs are 20 to 24 nucleotides that modulate gene expression through post-transcriptional effects (84, 85). More than 2000 miRNAs have been discovered in humans and consist of one third of genes in the genome (84, 85).

Epigenetics in PCOS

The epigenomics knowledge of PCOS has been evolving and might have implications in fertility. Overall, the DNA methylation profiles in the pathophysiology of PCOS have not been well studied. The human studies have been small. Also, despite no difference in global methylation patterns found in subjects with and without PCOS, the results from animal studies have suggested a role for these epigenetic modifications in PCOS. Studies of PCOS rat models have demonstrated that *PPAR-γ1* hypermethylated and *NCOR1* hypomethylated promoters are associated with decreased *PPAR-γ1* and increased *NCOR1* gene transcription during ovarian maturation and development. Functional studies, performed after GWASs and considering the potential heritability of gene expression, have demonstrated hypomethylation at eight important promoter sites in the *LHCGR* gene. miRNAs have also been implicated in the pathogenesis of PCOS. Some of these include miRNA-21, miR-27b, miR-103, and

miR-155, which appear to function through pathways such as inflammation, hormone metabolism, adipogenesis, and insulin signaling, which not only affect disease but are also involved in implantation (80–82).

Epigenetics in endometriosis

Epigenetic modifications that affect endometriosis also have consequences in implantation and the subsequent outcomes. DNA methylation, through DNA methyltransferases, have differential expression patterns in endometriotic tissue compared with normal endometrium. More than 40,000 CpGs have been identified to be differentially methylated in endometriosis. In addition, global histone acetylation profiles have shown certain histones to be hypoacetylated in endometriotic stromal cells compared with normal endometrium, suggesting a role in the disease. Overall, these endometrial DNA methylation patterns in endometriosis have been identified and associated with altered gene expression in cell proliferation, the inflammation/immune response, angiogenesis, and steroid hormone response (86, 87). Certain miRNA expression patterns have been identified in patients with endometriosis compared with controls. More than 50 different miRNAs have been shown in various studies to be differentially expressed in endometriotic cells; however, the studies have varied in the miRNAs included, the differential expression patterns, and their potential role in implantation (80–82).

Implantation—Genetics and Epigenetics

Implantation requires a functional and receptive endometrium. A small number of studies have identified SNPs within individual genes previously reported to affect endometrial function. A statistically significant difference was observed in the allelic and genotypic frequencies of the rs28362491 promoter in the *NF-kB* gene, important in embryo implantation among patients with repeated implantation failure (88). SNPs in the *VEGF*, *TNF-α*, and *LIF* genes have also been identified and implicated in implantation failure (88–90). Some of these genes, potentially important in endometrial function will also likely play an important role in implantation.

Alterations in the epigenome through global methylation in the endometrium have been identified throughout the menstrual cycle in small studies, with the greatest variability in the late secretory and menstrual phases (91, 92). This suggests that the epigenome plays a substantial role in endometrial function. Epigenomic changes throughout the menstrual cycle likely result from variations in the hormonal milieu, which can affect implantation because greater progesterone levels have been associated with increased levels of 5-mC, H3K9me2, H3K9ac, and H3K27me3 methylation in the peri-implantation period. miRNAs also likely affect implantation. Thirteen differentially expressed miRNAs were identified in a small cohort of women with implantation failure (93). Three of these miRNAs (miR-145, miR-23b, and miR-99a) were also associated with endometriosis resulting from progesterone resistance, suggesting a critical role of the infertility etiology due to epigenetics on successful implantation (94, 95).

Overall, substantial advances have occurred in identifying a receptive transcriptome during the window of implantation. A recent meta-analysis identified 57 mRNA putative receptivity markers, with 39 confirmed in a separate cohort (96). Bioinformatic prediction analysis identified 348 miRNAs that could regulate 30 endometrial receptivity-associated genes. Finally, 19 miRNAs with 11 corresponding upregulated meta-signature genes were confirmed (96). However, the underlying etiology of infertility was not considered. The epigenetics of etiology influences the outcomes, because altered endometrial DNA methylation in endometriosis alters the gene expression in pathways critical for implantation (86, 87). Also, some miRNAs, both in endometriosis and PCOS, function through pathways such as inflammation and hormone metabolism, which play substantial roles in implantation (80–82, 86, 87, 94, 95).

Placentation—Effect of Infertility Etiology

After implantation, the process of placentation with trophoblast differentiation and migration occurs throughout the first trimester of pregnancy and entails a finely orchestrated cellular and molecular interplay of maternal and fetal tissues. Invasion and remodeling of maternal uterine vasculature, immunomodulation at the maternal–fetal interface, and expression of cytokines, growth factors, and autocrine, paracrine, and juxtacrine mediators are pivotal for healthy gestation. Aberrations leading to abnormal placentation and failure of first trimester trophoblast invasion have been associated with adverse pregnancy outcomes (97–107). Pregnancies conceived in couples with infertility have an increased risk of adverse outcomes that affect both mother and fetus. Many are related to placental dysfunction, which occurs as a result of abnormal placentation. Compared with pregnancies conceived spontaneously, pregnancies conceived by couples with infertility, regardless of the type of treatment, are more likely to deliver at an earlier gestational age (2, 4, 10, 11, 108). Also, such infants are more likely to have increased neonatal morbidity compared with spontaneously conceived infants at the same gestational age (16). These outcomes have largely been attributed to placental dysfunction, which leads to low

birthweight, preeclampsia, placental abruption, placenta previa, preterm labor and delivery, retained placenta, and placenta accreta and contributes to delivery at an earlier gestational age and increased neonatal morbidity (2, 4–10, 13, 15, 109–111). Although increased maternal morbidity has been demonstrated in women undergoing ARTs (112), this has also been demonstrated in women undergoing any type of fertility treatment (14). In one study, the most common and serious maternal morbidities were attributed in large part to placental dysfunction, including obstetrical hemorrhage, cardiovascular disease, hypertensive disorders, and placental hemorrhage (*i.e.,* bleeding from placenta previa or placenta accreta) (14). In addition, fertility treatment on multivariate analyses was associated with an increased risk of serious maternal morbidity compared with spontaneous conception (14).

To develop a better understanding of the potential genes affecting placentation, during fertility treatment, RNA-sequencing analyses was performed on the first-trimester chronic villi of 141 pregnancies conceived spontaneously or with NIFT or IVF and resulting in the delivery of a viable offspring. Only one protein-coding differentially expressed gene (DEG), *CACNA1I*, was identified between the spontaneous and infertility groups that was also identified between the spontaneous and IVF groups. However, five protein-coding DEGs were identified between the NIFT and IVF groups, *SLC18A2*, *CCL21*, *FXYD2*, *PAEP*, and *DNER*, with a number of DEGs having greater expression levels in NIFT pregnancies, and IVF pregnancies segregating more with the spontaneous pregnancies. This might reflect differences in the underlying etiology of infertility and not the IVF treatment itself. This is also consistent with the characteristics of the cohort, because the NIFT group had a higher body mass index (BMI), which might indicate a different etiology for infertility, including PCOS, which has been implicated in placentation defects leading to adverse outcomes (113).

PCOS and placentation

Women with PCOS have an increased risk of pregnancy and neonatal complications that is independent of the fertility treatment used (24, 72, 114). These complications include hypertension and gestational diabetes, which have been associated with dysfunctional placentation (24, 72, 114). The genetic and epigenetic factors associated with PCOS, which lead to hyperandrogenism, elevated lipid concentrations, reactive oxygen species, and inflammatory markers, also likely contribute to aberrant placentation. For example, hyperandrogenism in PCOS might directly act on trophoblast invasion and placenta function (115–117). In addition, endothelium dysfunction due to elevated lipid concentrations, reactive oxygen species, and inflammatory markers leads to remodeling of spiral vessels with reduction in uterine artery impedance, a reduced depth of endovascular trophoblast invasion, and, ultimately, abnormal placentation (117, 118). Histologic studies evaluating both macroscopic and microscopic changes in the placenta of women with PCOS found a substantial reduction in the placental weight, thickness, density, and volume in patients with PCOS (115). Because macroscopic and microscopic alterations in the placenta, including the weight, thickness, and density, are present in women with PCOS, according to the PCOS phenotype, genetic and epigenetic influences likely play a critical role (116, 119).

Endometriosis and placentation

Women with endometriosis have a significantly greater risk of complications, including preeclampsia and placental complications, such as placenta previa, leading to greater rates of preterm birth, small-for-gestational-age infants, and neonatal death (50, 120–123). The factors contributing to disease, influenced by genetics and epigenetics, could also be associated with potential mechanisms that lead to defective placentation, including an imbalance of endocrine and inflammatory markers and molecular and functional abnormalities of the eutopic endometrium (124–127).

Unexplained infertility and placentation

Women with unexplained infertility despite treatment have shown differences in outcomes when stratified by progesterone levels, with a lower probability of a live birth with lower progesterone levels (63). In addition, variability has been found in pregnancy outcomes associated with placentation in women with unexplained infertility based on the allostatic load (a measure of chronic physiologic stress). The allostatic load, determined *a priori* from the BMI, waist/hip ratio, systolic blood pressure, diastolic blood pressure, dehydroepiandrosterone sulfate, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, C-reactive protein, and homeostatic model assessment score, was associated with an increased risk of preeclampsia, preterm birth, and low birthweight. Specifically, a 1-unit increase in the two-tailed allostatic load score was associated with a 62% increased risk of preeclampsia (OR, 1.62; 95% CI, 1.14 to 2.38), 44% increased risk of preterm birth (OR, 1.44; 95% CI, 1.02 to 2.08) mediated by preeclampsia, and 39% increased risk of low birthweight (OR, 1.39; 95% CI, 0.99 to 1.97) (128). Thus, as we are defining the potential etiologies that might be genetically or epigenetically affecting the variability in placentation and outcomes in those with unexplained

infertility, and those with PCOS or endometriosis, further studies are needed to determine the genetic and epigenetic influences of infertility etiology on implantation that lead to aberrant placentation and subsequent adverse outcomes.

Epigenetic Influence of Fertility Treatments on Placentation

A number of imprinted genes are expressed during the preimplantation period and are potentially vulnerable to disruption during fertility treatment that involves ARTs, where embryos are developed *in vitro* (129, 130). Embryos developed *in vitro* and animal models have demonstrated that the embryo culture conditions affect gene imprinting (131–133). However, in humans, the reported data have been conflicting. Some studies found that the use of ARTs is associated with abnormal DNA methylation in human gametes, embryos, placentas, and umbilical cord samples (134–139). However, other studies concluded that this does not occur (140–142). Likewise, specific procedures have also been associated with methylation differences in term placenta, suggesting specific fertility treatments affect the placental epigenome, which might have implications for placental function. For example, differential DNA methylation levels at repeated sequences (*LINE1* elements) have been identified in placenta, with fresh embryo transfers having substantially different *LINE1* methylation compared with spontaneous conceptions and frozen embryo transfers. This suggests that the hormonal differences between fresh and frozen embryo transfers might affect placental methylation (143).

However, it remains unclear when these changes occur, because differences in methylation profiles have been found in the human placenta across gestation (144) and even in different tissues of term placenta (145). These findings suggest that changes might be occurring during gestation and could be influenced by other environmental cues, including the hormonal milieu during placentation and even later in gestation, affecting placentation. The hormonal milieu at conception and during early pregnancy will be elevated by fertility treatment, mostly during IVF. Because superovulation has led to altered expression of endometrial genes critical to tissue remodeling and placentation and resulted in low birthweight in mouse models, this hyperstimulated hormone milieu has been implicated in an increased risk for low birthweight and pregnancy complications related to abnormal placentation in human studies (146–149). Therefore, the hormonal milieu could be affecting outcomes because global methylation was similar among the IVF, NIFT, and spontaneous conceptions early during placentation in the first trimester in a small study. However, differential methylation has been identified in multiple loci in three genes, *ANAPC2*, *CXCL14*, and *RIMS1*, between IVF and NIFT pregnancies but not when compared with spontaneous conceptions. This suggests that differences are present in the infertile population that might be due to the underlying etiology or specific treatments, including the hormonally hyperstimulated environment during implantation and placentation (150).

Hormonal exposure in the intrauterine environment also influences placental development. The hormonal milieu at conception and in early pregnancy becomes elevated during fertility treatment. This, in part, results from the treatments themselves, including elevated estradiol levels from multifollicular development and exogenous supplemental estradiol and progesterone hormone treatment. Even after hormone supplementation has been discontinued and ovarian hormone production has declined, the estradiol and progesterone levels have remained persistently elevated in mothers of pregnancies conceived with fertility treatment, both IVF and NIFT, compared with those who conceived spontaneously, at a time when the placenta becomes the source of hormone production (151, 152). Metabolomics studies have suggested that the supraphysiologic hormonal state during fertility treatment and hormone supplementation causes the increased phospholipid and glycerol lipid production, providing increased cholesterol to the placenta for hormone production, to maintain these pregnancies in a high hormonal state. These early elevations of estrogen from fertility treatment and supplementation might also increase cholesterol uptake in trophoblast cells (153), stimulate cholesterol production in the fetal liver (154), and increase placental P450 enzyme activity (155), all of which enhance the production of progesterone. Thus, the placenta might be reprogrammed after implantation, ultimately leading to increased hormone production from syncytiotrophoblasts (152).

A sustained high hormone environment creates an altered hormonal milieu, in which trophoblast function can be altered and affect placentation. Baboon studies have found that elevated estradiol levels in the first trimester lead to decreased extravillous trophoblast invasion of the uterine spiral arteries (156). Recent studies have suggested that impairment in placentation can occur via estradiol-induced differential expression of the *GATA3* transcription factor (157) and the *GRB10* gene (158). Elevated progesterone, similar to elevated estradiol, also affects placentation. Elevated progesterone, in conjunction with estrogen, induces first-trimester trophoblast tubulogenesis through the lysophosphatidic acid pathway (159). Because the elevation in steroids

leads to dysfunction in trophoblast cells, these elevated states might be contributing to the adverse outcomes associated with infertility and the treatments used. Further studies are necessary to determine the exact mechanisms leading to these outcomes.

Infertility and Maternal Outcomes

Infertility affects both short- and long-term maternal health. Infertility is often physiologically or genetically linked to other long-term disease states and conditions. Some disease states and conditions are associated with infertility in general, and others result from the specific etiology of the infertility, which is affected by the coding genome and the epigenome. In general, infertility increases the risk of pregnancy complications that can have long-term health implications for the mother, some of which are associated with future chronic comorbidities. This includes preeclampsia and gestational diabetes and more severe maternal morbidities, including hemorrhage, embolism, stroke, and acute myocardial infarction (160, 161). Some of these pregnancy complications might result from the increased maternal age in the infertility population (160, 161). However, this increased risk has persisted even after adjusting for maternal age (14). Most of these complications have been associated with placentation abnormalities, which might be more pronounced in women with infertility, leading primarily to long-term complications such as cardiovascular and metabolic disease (10).

Infertility etiology and pregnancy outcomes

The underlying infertility etiology likely plays a role in short-term maternal complications. Women with endometriosis have a significantly greater risk of preeclampsia and placental complications, leading to greater rates of preterm birth, small-for-gestational-age infants, and neonatal death (50, 120–123). In addition, women with PCOS have an increased risk of adverse pregnancy outcomes due to a placental etiology, such as gestational diabetes, hypertension, and preterm delivery (24, 72). Although gestational diabetes has been commonly associated with PCOS, women with unexplained infertility have a 45% greater risk of developing gestational diabetes compared with women without infertility, an increase in risk similar to that (52%) for women with infertility due to ovulation disorders (including PCOS) (162–167). These maternal complications have long-term health implications and can be used as a marker of overall health.

PCOS and endometriosis, independently and in association with infertility, are also known to be associated with long-term comorbidities, such as metabolic dysfunction, vascular dysfunction, cardiovascular disease, and cancer. PCOS has been associated with a fourfold increase risk of endometrial cancer (168, 169) and metabolic dysfunction, including type 2 diabetes, metabolic syndrome, hypertension, and, possibly, cardiovascular disease (170–173). Endometriosis, a condition linked to systemic inflammation, has been associated with oxidative stress (174), abnormal lipid profiles (175), coronary heart disease (176), and autoimmune conditions (177). Although endometriosis is a benign condition, it has been shown to be a risk factor for clear cell cancer and endometrioid ovarian cancer (178, 179). Recent data have also linked unexplained infertility with an increased cardiovascular risk based on an atherogenic lipid profile and elevated high-sensitivity C-reactive protein levels (180).

The underlying genetics of infertility etiology likely cause the pregnancy complications, marked through placental dysfunction, and long-term comorbidities. These include preeclampsia, preterm delivery, and gestational diabetes. Preeclampsia increases the risk of developing hypertension (RR, 3.70; 95% CI, 2.70 to 5.05), which, on average, occurs 7.7 years earlier than in woman without a history of preeclampsia (181, 182). These women also have a nearly twofold increased risk of ischemic heart disease, stroke, and venous thromboembolism (181). The risk of death from cardiovascular disease increases 2- to 10-fold in women with preeclampsia, depending on when the preeclampsia manifested in the pregnancy, with an increased risk of death when preeclampsia occurs early in gestation (183).

Preterm delivery from any cause also results in an increased risk of developing cardiovascular disease. A linear association between the number of preterm deliveries and the future risk of cardiovascular-related hospitalizations was found in a population-based study (184). Another cohort study found that maternal cardiovascular disease mortality was inversely related to the birthweight of the offspring and that women with premature deliveries also had an increased cardiovascular disease risk (185).

A history of gestational diabetes predisposes women to metabolic syndrome. These women will have atherogenic lipid profiles and signs of early vascular dysfunction at $\geq$3 months postpartum (186–188). A history of gestational diabetes will also predispose women to type 2 diabetes compared with women with normoglycemic pregnancies (RR, 7.43; 95% CI, 4.79 to 11.51). Also, this risk doubles from an RR of 4.69 in the first 5 years after delivery to an RR of 9.34 at $>$5 years after delivery (189). Women with gestational diabetes will also have an elevated risk of developing cardiovascular disease, and at a younger age, than women without a history of gestational diabetes (184, 190–192).

Epigenetics of infertility etiology and outcomes

In addition to the potential genetic link with infertility etiology, unique long noncoding RNAs and miRNAs have been identified in PCOS, endometriosis, and the associated chronic conditions related to these underlying diseases. miRNAs, including circulating miRNAs, are associated with PCOS and might explain the epigenetic contribution to insulin resistance associated with PCOS. One study demonstrated that the expression levels of miR-320 were increased 50-fold in adipocytes owing to insulin resistance. Insulin sensitivity was restored with the addition of anti-miR-320 oligos, with resulting upregulation of GLUT4, an insulin-dependent glucose transporter, leading to increased insulin-mediated glucose uptake (193). A number of studies also identified miRNAs as key contributors to endometriosis (194–197). Furthermore, functional studies linking miRNAs to endometriosis and inflammation have been performed. One such study found a link with miR-20a, which resulted in decreased expression of phosphatase-2 and, subsequently, prolonged extracellular signal-regulated kinase activation through hypoxia inducible factor, which contributes to the inflammatory process in endometriosis (198). miRNAs have also been identified linking clear cell adenocarcinoma of the ovary [or endometriosis-associated ovarian cancer (EAOC)] to endometriosis. These miRNAs might act as a potential precursor for EAOC. One study identified 23 candidate miRNAs that were differentially expressed among healthy controls, patients with endometriosis, and patients with EAOC (199), with three distinct miRNA signatures identified, including miR-16, miR-21, and miR-191, that could differentiate between healthy and endometriosis cases, including some overlap with miRNAs expressed in cases of EAOC. Further studies are needed to identify the underlying genetics and epigenetic modifications that contribute to the infertility etiology and long-term chronic morbidity.

Infertility and Long-Term Childhood Outcomes

Research has linked the use of ART with adverse perinatal outcomes, including preeclampsia and low birthweight (2, 200), both of which have been associated with future chronic morbidities. Both preeclampsia and low birthweight result from placental abnormalities that lead to hypoperfusion and oxidative stress. The latter have long-term effects for the neonate and the development of the child into adulthood. Children born to women with preeclampsia and hypertensive disorders of pregnancy have a greater risk of diabetes mellitus and cardiovascular disease in later life (201–203). Endothelial function is significantly reduced after preeclampsia (204). This finding is consistent with the offspring also having increased levels of cardiovascular risk factors such as higher blood pressure (205–207) and BMI (205, 206) and an increased risk of stroke in adulthood (208). Low birthweight has also been associated with chronic disease. It is a marker of *in utero* stress and has been associated with an increased risk of metabolic disease and insulin resistance in adulthood (209, 210).

Epigenetics of long-term disease

Despite the underlying infertility etiology, epigenetic modifications as a result of the infertility treatment have been implicated in chronic long-term disease, including differential methylation of genes important to growth and development, with long-term health implications. Differences in the methylation levels of 23 genes in the placenta and cord blood, including *ANGPT4*, *APOE*, *CDK2*, *GRB10*, *OSBPL5*, and *REG1B*, which have been associated with the growth phenotype, have been identified to explain the variances in birthweight (211). It remains to be determined whether any of these differentially modified genes are associated with infertility or the treatments used. If so, this might explain the role of infertility and fertility treatment on birthweight. Furthermore, it would be ideal if any of the factors associated with chronic disease could be modified at treatment. A 2015 randomized control trial found that antioxidant administration to IVF children improved nitric oxide bioavailability and vascular responsiveness in the systemic and pulmonary circulation (212). These findings suggest that in young individuals, ART-induced vascular dysfunction is subject to redox regulation and is reversible, although this requires further investigation. Thus, it is critical to develop a better understanding of the underlying factors in fertility treatment that might be modified to improve the outcomes.

Conclusion

Overall, the goal of fertility treatment is to overcome infertility and achieve a pregnancy. A second goal is to have a successful outcome, with a healthy mother and child. Despite the risk of adverse outcomes associated with infertility and fertility treatments, these risks are small. However, as we develop a better understanding of the genetics and epigenetics of the infertility etiology and the effect of fertility treatment on epigenetics, modifications could ultimately lead to improvements in pregnancy outcomes and overall long-term health. Further studies are necessary to identify the role of genetic variability and the epigenome on implantation and placentation to foster personalized medicine for fertility

treatment that would lead to optimal short- and long-term outcomes.

Acknowledgments

We gratefully acknowledge the support from the National Institutes of Health National Institute of Child Health and Human Development. The funding agency was not involved in the design, analysis, or interpretation of the data reported.

Financial Support: The present study was supported by the National Institutes of Health National Institute of Child Health and Human Development (Grant R01HD074368 to M.D.P.).

Correspondence and Reprint Requests: Margareta D. Pisarska, MD, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, 8635 West Third Street, Suite 160, Los Angeles, California 90048. E-mail: Margareta.Pisarska@cshs.org.

Disclosure Summary: The authors have nothing to disclose.

References

1. Faddy MJ, Gosden MD, Gosden RG. A demographic projection of the contribution of assisted reproductive technologies to world population growth. *Reprod Biomed Online*. 2018;**36**(4):455–458.
2. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med*. 2002;**346**(10):731–737.
3. Sunderam S, Kissin DM, Crawford SB, Folger SG, Boulet SL, Warner L, Barfield WD. Assisted reproductive technology surveillance—United States, 2015. *MMWR Surveill Summ*. 2018;**67**(3):1–28.
4. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol*. 2004;**103**(3):551–563.
5. Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med*. 2002;**346**(10):725–730.
6. Verlaenen H, Cammu H, Derde MP, Amy JJ. Singleton pregnancy after in vitro fertilization: expectations and outcome. *Obstet Gynecol*. 1995;**86**(6):906–910.
7. Klemetti R, Gissler M, Sevón T, Koivurova S, Ritvanen A, Hemminki E. Children born after assisted fertilization have an increased rate of major congenital anomalies. *Fertil Steril*. 2005;**84**(5):1300–1307.
8. Rimm AA, Katayama AC, Diaz M, Katayama KP. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. *J Assist Reprod Genet*. 2004;**21**(12):437–443.
9. Strömberg B, Dahlquist G, Ericson A, Finnström O, Köster M, Stjernqvist K. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet*. 2002;**359**(9305):461–465.
10. Jackson S, Hong C, Wang ET, Alexander C, Gregory KD, Pisarska MD. Pregnancy outcomes in very advanced maternal age pregnancies: the impact of assisted reproductive technology. *Fertil Steril*. 2015;**103**(1):76–80.
11. Wang ETRL, Ramos L, Vyas N, Bhasin G, Simmons CF, Pisarska MD. Maternal and neonatal outcomes associated with infertility. *J Matern Fetal Neonatal Med*. 2018;**20**:1–4.
12. Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: a meta-analysis of cohort studies. Fertil Steril. 2016;105(1):73–85.e1-6.
13. Shevell T, Malone FD, Vidaver J, Porter TF, Luthy DA, Comstock CH, Hankins GD, Eddleman K, Dolan S, Dugoff L, Craigo S, Timor IE, Carr SR, Wolfe HM, Bianchi DW, D'Alton ME. Assisted reproductive technology and pregnancy outcome. *Obstet Gynecol*. 2005;**106**(5 Pt 1):1039–1045.
14. Wang ET, Ozimek JA, Greene N, Ramos L, Vyas N, Kilpatrick SJ, Pisarska MD. Impact of fertility treatment on severe maternal morbidity. *Fertil Steril*. 2016;**106**(2):423–426.
15. Sundheimer LW, Chan JL, Buttle R, DiPentino R, Muramoto O, Castellano K, Wang ET, Williams J III, Pisarska MD. Mode of conception does not affect fetal or placental growth parameters or ratios in early gestation or at delivery. *J Assist Reprod Genet*. 2018;**35**(6):1039–1046.
16. Wang ET, Sundheimer LW, Spades C, Quant C, Simmons CF, Pisarska MD. Fertility treatment is associated with stay in the neonatal intensive care unit and respiratory support in late preterm infants. *J Pediatr*. 2017;**187**:309–312.
17. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, Haan EA, Chan A. Reproductive technologies and the risk of birth defects. *N Engl J Med*. 2012;**366**(19):1803–1813.
18. Merritt TA, Goldstein M, Philips R, Peverini R, Iwakoshi J, Rodriguez A, Oshiro B. Impact of ART on pregnancies in California: an analysis of maternity outcomes and insights into the added burden of neonatal intensive care. *J Perinatol*. 2014;**34**(5):345–350.
19. Kawwass JF, Badell ML. Maternal and fetal risk associated with assisted reproductive technology. *Obstet Gynecol*. 2018;**132**(3):763–772.
20. Kroener L, Wang ET, Pisarska MD. Predisposing factors to abnormal first trimester placentation and the impact on fetal outcomes. *Semin Reprod Med*. 2016;**34**(1):27–35.
21. Carmina E, Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. *J Clin Endocrinol Metab*. 1999;**84**(6):1897–1899.
22. Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med*. 2005;**352**(12):1223–1236.
23. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK; Endocrine Society. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2013;**98**(12):4565–4592.
24. Schulte MM, Tsai JH, Moley KH. Obesity and PCOS: the effect of metabolic derangements on endometrial receptivity at the time of implantation. *Reprod Sci*. 2015;**22**(1):6–14.
25. Giudice LC. Endometrium in PCOS: implantation and predisposition to endocrine CA. *Best Pract Res Clin Endocrinol Metab*. 2006;**20**(2):235–244.
26. Stratton P, Berkley KJ. Chronic pelvic pain and endometriosis: translational evidence of the relationship and implications. *Hum Reprod Update*. 2011;**17**(3):327–346.
27. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. *Lancet*. 2010;**376**(9742):730–738.
28. Giudice LC, Kao LC. Endometriosis. *Lancet*. 2004;**364**(9447):1789–1799.
29. Hickey M, Ballard K, Farquhar C. Endometriosis. *BMJ*. 2014;**348**(mar19 5):g1752.
30. Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarali H. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. *Hum Reprod*. 2012;**27**(10):3067–3073.
31. Eskenazi B, Warner ML. Epidemiology of endometriosis. *Obstet Gynecol Clin North Am*. 1997;**24**(2):235–258.

32. Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility: a committee opinion. *Fertil Steril*. 2012;**98**(3):591–598.
33. Enders AC, Schlafke S. Cytological aspects of trophoblast-uterine interaction in early implantation. *Am J Anat*. 1969;**125**(1):1–29.
34. Finn CA, Martin L. The control of implantation. *J Reprod Fertil*. 1974;**39**(1):195–206.
35. Simón C, Moreno C, Remohí J, Pellicer A. Cytokines and embryo implantation. *J Reprod Immunol*. 1998;**39**(1-2):117–131.
36. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update*. 2006; **12**(6):731–746.
37. Hess AP, Hamilton AE, Talbi S, Dosiou C, Nyegaard M, Nayak N, Genbecev-Krtolica O, Mavrogianis P, Ferrer K, Kruessel J, Fazleabas AT, Fisher SJ, Giudice LC. Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators. *Biol Reprod*. 2007;**76**(1): 102–117.
38. Cross JC. Formation of the placenta and extraembryonic membranes. *Ann N Y Acad Sci*. 1998;**857**:23–32.
39. Pijnenborg R, Vercruysse L, Hanssens M. The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta*. 2006;**27**(9-10):939–958.
40. Whitley GS, Cartwright JE. Cellular and molecular regulation of spiral artery remodelling: lessons from the cardiovascular field. *Placenta*. 2010;**31**(6):465–474.
41. Weimar CH, Post Uiterweer ED, Teklenburg G, Heijnen CJ, Macklon NS. In-vitro model systems for the study of human embryo-endometrium interactions. *Reprod Biomed Online*. 2013;**27**(5):461–476.
42. Franasiak JM, Scott RT. Contribution of immunology to implantation failure of euploid embryos. *Fertil Steril*. 2017;**107**(6): 1279–1283.
43. Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril*. 2013; **100**(3):697–703.
44. Coates A, Kung A, Mounts E, Hesla J, Bankowski B, Barbieri E, Ata B, Cohen J, Munne S. Optimal euploid embryo transfer strategy, fresh versus frozen, after preimplantation genetic screening with next generation sequencing: a randomized controlled trial. *Fertil Steril*. 2017;**107**(3):723–730.e3.
45. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, Treff NR, Scott RT Jr. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril*. 2013; **100**(1):100–107.e1.
46. Friedenthal J, Maxwell SM, Munné S, Kramer Y, McCulloh DH, McCaffrey C, Grifo JA. Next generation sequencing for preimplantation genetic screening improves pregnancy outcomes compared with array comparative genomic hybridization in single thawed euploid embryo transfer cycles. *Fertil Steril*. 2018;**109**(4): 627–632.
47. Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2016 Assisted Reproductive Technology National Summary Report. Atlanta, GA: US Department of Health and Human Services; 2018:26.
48. Domínguez F, Garrido-Gómez T, López JA, Camafeita E, Quiñonero A, Pellicer A, Simón C. Proteomic analysis of the human receptive versus non-receptive endometrium using differential in-gel electrophoresis and MALDI-MS unveils stathmin 1 and annexin A2 as differentially regulated. *Hum Reprod*. 2009; **24**(10):2607–2617.
49. Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, Gómez E, Fernández-Sánchez M, Carranza F, Carrera J, Vilella F, Pellicer A, Simón C. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertil Steril*. 2013;**100**(3):818–824.
50. Berlac JF, Hartwell D, Skovlund CW, Langhoff-Roos J, Lidegaard Ø. Endometriosis increases the risk of obstetrical and neonatal complications. *Acta Obstet Gynecol Scand*. 2017;**96**(6):751–760.
51. Petousis S, Prapas Y, Margioula-Siarkou C, Ravanos K, Milias S, Mavromatidis G, Kalogiannidis I, Haitoglou C, Athanasiadis A, Prapas N, Rousso D. Unexplained infertility patients present the mostly impaired levels of progesterone receptors: prospective observational study. *Am J Reprod Immunol*. 2018;**79**(6):e12828.
52. Dorostghoal M, Ghaffari HO, Marmazi F, Keikhah N. Overexpression of endometrial estrogen receptor-alpha in the window of implantation in women with unexplained infertility. *Int J Fertil Steril*. 2018;**12**(1):37–42.
53. Elnaggar A, Farag AH, Gaber ME, Hafeez MA, Ali MS, Atef AM. AlphaVBeta3 integrin expression within uterine endometrium in unexplained infertility: a prospective cohort study. *BMC Womens Health*. 2017;**17**(1):90.
54. Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, Marrocchella S, Greco P, Resta L. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Hum Reprod*. 2015; **30**(2):323–330.
55. Cakmak H, Taylor HS. Implantation failure: molecular mechanisms and clinical treatment. *Hum Reprod Update*. 2011;**17**(2): 242–253.
56. Wu M, Yin Y, Zhao M, Hu L, Chen Q. The low expression of leukemia inhibitory factor in endometrium: possible relevant to unexplained infertility with multiple implantation failures. *Cytokine*. 2013;**62**(2):334–339.
57. Talbi S, Hamilton AE, Vo KC, Tulac S, Overgaard MT, Dosiou C, Le Shay N, Nezhat CN, Kempson R, Lessey BA, Nayak NR, Giudice LC. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology*. 2006; **147**(3):1097–1121.
58. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty KS Jr. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J Clin Endocrinol Metab*. 1988;**67**(2):334–340.
59. Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Promoter hypermethylation of progesterone receptor isoform B (PR-B) in endometriosis. *Epigenetics*. 2006;**1**(2):106–111.
60. Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA, Giudice LC. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology*. 2003;**144**(7):2870–2881.
61. Barbosa MA, Teixeira DM, Navarro PA, Ferriani RA, Nastri CO, Martins WP. Impact of endometriosis and its staging on assisted reproduction outcome: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2014;**44**(3):261–278.
62. Leone Roberti Maggiore U, Ferrero S, Mangili G, Bergamini A, Inversetti A, Giorgione V, Viganò P, Candiani M. A systematic review on endometriosis during pregnancy: diagnosis, misdiagnosis, complications and outcomes. *Hum Reprod Update*. 2016;**22**(1):70–103.
63. Hansen KR, Eisenberg E, Baker V, Hill MJ, Chen S, Talken S, Diamond MP, Legro RS, Coutifaris C, Alvero R, Robinson RD, Casson P, Christman GM, Santoro N, Zhang H, Wild RA; NICHD Reproductive Medicine Network. Midluteal progesterone: a marker of treatment outcomes in couples with unexplained infertility. *J Clin Endocrinol Metab*. 2018;**103**(7): 2743–2751.
64. Joshi N, Chan JL. Female genomics: infertility and overall health. *Semin Reprod Med*. 2017;**35**(3):217–224.
65. Telenti A, Pierce LC, Biggs WH, di Iulio J, Wong EH, Fabani MM, Kirkness EF, Moustafa A, Shah N, Xie C, Brewerton SC, Bulsara

N, Garner C, Metzker G, Sandoval E, Perkins BA, Och FJ, Turpaz Y, Venter JC. Deep sequencing of 10,000 human genomes. *Proc Natl Acad Sci USA*. 2016;**113**(42):11901–11906.
66. McClellan J, King MC. Genetic heterogeneity in human disease. *Cell*. 2010;**141**(2):210–217.
67. Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, Li Z, You L, Zhao J, Liu J, Liang X, Zhao X, Zhao J, Sun Y, Zhang B, Jiang H, Zhao D, Bian Y, Gao X, Geng L, Li Y, Zhu D, Sun X, Xu JE, Hao C, Ren CE, Zhang Y, Chen S, Zhang W, Yang A, Yan J, Li Y, Ma J, Zhao Y. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nat Genet*. 2011;**43**(1):55–59.
68. Shi Y, Zhao H, Shi Y, Cao Y, Yang D, Li Z, Zhang B, Liang X, Li T, Chen J, Shen J, Zhao J, You L, Gao X, Zhu D, Zhao X, Yan Y, Qin Y, Li W, Yan J, Wang Q, Zhao J, Geng L, Ma J, Zhao Y, He G, Zhang A, Zou S, Yang A, Liu J, Li W, Li B, Wan C, Qin Y, Shi J, Yang J, Jiang H, Xu JE, Qi X, Sun Y, Zhang Y, Hao C, Ju X, Zhao D, Ren CE, Li X, Zhang W, Zhang Y, Zhang J, Wu D, Zhang C, He L, Chen ZJ. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. *Nat Genet*. 2012; **44**(9):1020–1025.
69. Mitri F, Bentov Y, Behan LA, Esfandiari N, Casper RF. A novel compound heterozygous mutation of the luteinizing hormone receptor—implications for fertility. *J Assist Reprod Genet*. 2014; **31**(7):787–794.
70. Toledo SP, Brunner HG, Kraaij R, Post M, Dahia PL, Hayashida CY, Kremer H Themmen AP. An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. *J Clin Endocrinol Metab*. 1996;**81**(11):3850–3854.
71. Hayes MG, Urbanek M, Ehrmann DA, Armstrong LL, Lee JY, Sisk R, Karaderi T, Barber TM, McCarthy MI, Franks S, Lindgren CM, Welt CK, Diamanti-Kandarakis E, Panidis D, Goodarzi MO, Azziz R, Zhang Y, James RG, Olivier M, Kissebah AH, Stener-Victorin E, Legro RS, Dunaif A, Dunaif A; Reproductive Medicine Network. Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. *Nat Commun*. 2015;**6**(1):7502.
72. Li YY, Ye SQ, Zhong ZH, Xu Q, Mai WB, Yin CX, Zhu ZQ, He XQ, Xiao Q. Associations between maternal BMI as well as glucose tolerance and adverse pregnancy outcomes in women with polycystic ovary syndrome. *Gynecol Endocrinol*. 2017;**33**(4):328–331.
73. Uno S, Zembutsu H, Hirasawa A, Takahashi A, Kubo M, Akahane T, Aoki D, Kamatani N, Hirata K, Nakamura Y. A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. *Nat Genet*. 2010;**42**(8):707–710.
74. Goumenou AG, Arvanitis DA, Matalliotakis IM, Koumantakis EE, Spandidos DA. Loss of heterozygosity in adenomyosis on hMSH2, hMLH1, p16Ink4 and GALT loci. *Int J Mol Med*. 2000; **6**(6):667–671.
75. Vainio S, Heikkilä M, Kispert A, Chin N, McMahon AP. Female development in mammals is regulated by Wnt-4 signalling. *Nature*. 1999;**397**(6718):405–409.
76. Painter JN, Anderson CA, Nyholt DR, Macgregor S, Lin J, Lee SH, Lambert A, Zhao ZZ, Roseman F, Guo Q, Gordon SD, Wallace L, Henders AK, Visscher PM, Kraft P, Martin NG, Morris AP, Treloar SA, Kennedy SH, Missmer SA, Montgomery GW, Zondervan KT. Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. *Nat Genet*. 2011;**43**(1):51–54.
77. Pellegrini C, Gori I, Achtari C, Hornung D, Chardonnens E, Wunder D, Fiche M, Canny GO. The expression of estrogen receptors as well as GREB1, c-MYC, and cyclin D1, estrogen-regulated genes implicated in proliferation, is increased in peritoneal endometriosis. *Fertil Steril*. 2012;**98**(5):1200–1208.
78. Hyenne V, Harf JC, Latz M, Maro B, Wolfrum U, Simmler MC. Vezatin, a ubiquitous protein of adherens cell-cell junctions, is exclusively expressed in germ cells in mouse testis. *Reproduction*. 2007;**133**(3):563–574.
79. Pagliardini L, Gentilini D, Sanchez AM, Candiani M, Viganò P, Di Blasio AM. Replication and meta-analysis of previous genome-wide association studies confirm vezatin as the locus with the strongest evidence for association with endometriosis. *Hum Reprod*. 2015;**30**(4):987–993.
80. Li S, Zhu D, Duan H, Tan Q. The epigenomics of polycystic ovarian syndrome: from pathogenesis to clinical manifestations. *Gynecol Endocrinol*. 2016;**32**(12):942–946.
81. Ilie IR, Georgescu CE. Polycystic ovary syndrome—epigenetic mechanisms and aberrant microRNA. *Adv Clin Chem*. 2015;**71**: 25–45.
82. Grimstad FW, Decherney A. A review of the epigenetic contributions to endometriosis. *Clin Obstet Gynecol*. 2017;**60**(3): 467–476.
83. Handy DE, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. *Circulation*. 2011; **123**(19):2145–2156.
84. Hammond SM. An overview of microRNAs. *Adv Drug Deliv Rev*. 2015;**87**:3–14.
85. Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. *RNA Biol*. 2013;**10**(6):925–933.
86. Xiong Y, Wang J, Liu L, Chen X, Xu H, Li TC, Wang CC, Zhang S. Effects of high progesterone level on the day of human chorionic gonadotrophin administration in in vitro fertilization cycles on epigenetic modification of endometrium in the peri-implantation period. *Fertil Steril*. 2017;**108**(2):269–276.e1.
87. Houshdaran S, Nezhat CR, Vo KC, Zelenko Z, Irwin JC, Giudice LC. Aberrant endometrial DNA methylome and associated gene expression in women with endometriosis. *Biol Reprod*. 2016; **95**(5):93.
88. Luo L, Li DH, Li XP, Zhang SC, Yan CF, Wu JF, Qi YH, Zhao J. Polymorphisms in the nuclear factor kappa B gene association with recurrent embryo implantation failure. *Genet Mol Res*. 2016;**15**(2).
89. Vagnini LD, Nascimento AM, Canas MC, Renzi A, Oliveira-Pelegrin GR, Petersen CG, Mauri AL, Oliveira JB, Baruffi RL, Cavagna M, Franco JG Jr. The relationship between vascular endothelial growth factor 1154G/A polymorphism and recurrent implantation failure. *Med Princ Pract*. 2015;**24**(6):533–537.
90. Boudjenah R, Molina-Gomes D, Torre A, Boitrelle F, Taieb S, Dos Santos E, Wainer R, de Mazancourt P, Selva J, Vialard F. Associations between individual and combined polymorphisms of the TNF and VEGF genes and the embryo implantation rate in patients undergoing in vitro fertilization (IVF) programs. *PLoS One*. 2014;**9**(9):e108287.
91. Rahmioglu N, Drong AW, Lockstone H, Tapmeier T, Hellner K, Saare M, Laisk-Podar T, Dew C, Tough E, Nicholson G, Peters M, Morris AP, Lindgren CM, Becker CM, Zondervan KT. Variability of genome-wide DNA methylation and mRNA expression profiles in reproductive and endocrine disease related tissues. *Epigenetics*. 2017;**12**(10):897–908.
92. Saare M, Modhukur V, Suhorutshenko M, Rajashekar B, Rekker K, Sõritsa D, Karro H, Soplepmann P, Sõritsa A, Lindgren CM, Rahmioglu N, Drong A, Becker CM, Zondervan KT, Salumets A, Peters M. The influence of menstrual cycle and endometriosis on endometrial methylome. *Clin Epigenetics*. 2016;**8**(1):2.
93. Revel A, Achache H, Stevens J, Smith Y, Reich R. MicroRNAs are associated with human embryo implantation defects. *Hum Reprod*. 2011;**26**(10):2830–2840.
94. Burney RO, Hamilton AE, Aghajanova L, Vo KC, Nezhat CN, Lessey BA, Giudice LC. MicroRNA expression profiling of eutopic secretory endometrium in women with versus without endometriosis. *Mol Hum Reprod*. 2009;**15**(10):625–631.
95. Ohlsson Teague EM, Van der Hoek KH, Van der Hoek MB, Perry N, Wagaarachchi P, Robertson SA, Print CG, Hull LM. MicroRNA-regulated pathways associated with endometriosis. *Mol Endocrinol*. 2009;**23**(2):265–275.
96. Altmäe S, Koel M, Võsa U, Adler P, Suhorutšenko M, Laisk-Podar T, Kukushkina V, Saare M, Velthut-Meikas A, Krjutškov K,

Aghajanova L, Lalitkumar PG, Gemzell-Danielsson K, Giudice L, Simón C, Salumets A. Meta-signature of human endometrial receptivity: a meta-analysis and validation study of transcriptomic biomarkers. *Sci Rep*. 2017;**7**(1):10077.
97. Brosens IA. Morphological changes in the utero-placental bed in pregnancy hypertension. *Clin Obstet Gynaecol*. 1977;**4**(3): 573–593.
98. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *Br J Obstet Gynaecol*. 1986;**93**(10):1049–1059.
99. Kim YM, Chaiworapongsa T, Gomez R, Bujold E, Yoon BH, Rotmensch S, Thaler HT, Romero R. Failure of physiologic transformation of the spiral arteries in the placental bed in preterm premature rupture of membranes. *Am J Obstet Gynecol*. 2002; **187**(5):1137–1142.
100. Smith GC, Smith MF, McNay MB, Fleming JE. First-trimester growth and the risk of low birth weight. *N Engl J Med*. 1998; **339**(25):1817–1822.
101. DaSilva-Arnold SC, Zamudio S, Al-Khan A, Alvarez-Perez J, Mannion C, Koenig C, Luke D, Perez AM, Petroff M, Alvarez M, Illsley NP. Human trophoblast epithelial-mesenchymal transition in abnormally invasive placenta. *Biol Reprod*. 2018;**99**(2):409–421.
102. Hannibal RL, Cardoso-Moreira M, Chetty SP, Lau J, Qi Z, Gonzalez-Maldonado E, Cherry AM, Yu J, Norton ME, Baker JC. Investigating human placentation and pregnancy using first trimester chorionic villi. *Placenta*. 2018;**65**:65–75.
103. Monteiro LJ, Cubillos S, Sanchez M, Acuña-Gallardo S, Venegas P, Herrera V, Lam EW, Varas-Godoy M, Illanes SE. Reduced FOXM1 expression limits trophoblast migration and angiogenesis and is associated with preeclampsia [published online ahead of print 1 January 2018]. *Reprod Sci*. doi: 10.1177/1911877898.
104. James-Allan LB, Whitley GS, Leslie K, Wallace AE, Cartwright JE. Decidual cell regulation of trophoblast is altered in pregnancies at risk of pre-eclampsia. *J Mol Endocrinol*. 2018;**60**(3):239–246.
105. Hod T, Cerdeira AS, Karumanchi SA. Molecular mechanisms of preeclampsia. *Cold Spring Harb Perspect Med*. 2015;**5**(10): a023473.
106. Canfield J, Arlier S, Mong EF, Lockhart J, VanWye J, Guzeloglu-Kayisli O, Schatz F, Magness RR, Lockwood CJ, Tsibris JCM, Kayisli UA, Totary-Jain H. Decreased LIN28B in preeclampsia impairs human trophoblast differentiation and migration [published online ahead of print 11 October 2018]. *FASEB J*. doi: 10.1096/fj.20181163R.
107. Huang X, Wu L, Zhang G, Tang R, Zhou X. Elevated microRNA-181a-5p contributes to trophoblast dysfunction and preeclampsia [published online ahead of print 11 October 2018]. *Reprod Sci*. doi: 10.1177/19337191188086916.
108. McGovern PG, Llorens AJ, Skurnick JH, Weiss G, Goldsmith LT. Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization-embryo transfer or gamete intrafallopian transfer: a meta-analysis. *Fertil Steril*. 2004;**82**(6): 1514–1520.
109. Esh-Broder E, Ariel I, Abas-Bashir N, Bdolah Y, Celnikier DH. Placenta accreta is associated with IVF pregnancies: a retrospective chart review. *BJOG*. 2011;**118**(9):1084–1089.
110. Fitzpatrick KE, Sellers S, Spark P, Kurinczuk JJ, Brocklehurst P, Knight M. Incidence and risk factors for placenta accreta/increta/percreta in the UK: a national case-control study. *PLoS One*. 2012;**7**(12):e52893.
111. Kaser DJ, Melamed A, Bormann CL, Myers DE, Missmer SA, Walsh BW, Racowsky C, Carusi DA. Cryopreserved embryo transfer is an independent risk factor for placenta accreta. *Fertil Steril*. 2015;**103**(5):1176–84.e2.
112. Martin AS, Monsour M, Kissin DM, Jamieson DJ, Callaghan WM, Boulet SL. Trends in severe maternal morbidity after assisted reproductive technology in the United States, 2008–2012. *Obstet Gynecol*. 2016;**127**(1):59–66.
113. Lee B, Koeppel AF, Wang ET, Gonzalez TL, Sun T, Kroener L, Lin Y, Joshi NV, Ghadiali T, Turner SD, Rich SS, Farber CR, Rotter JI, Ida Chen YD, Goodarzi MO, Guller S, Harwood B, Serna T, Williams J III, Pisarska MD. Differential gene expression during placentation conceived with different fertility treatments compared to spontaneous pregnancies. *Fertil Steril*. 2019;**111**(3): 535–546.
114. Roos N, Kieler H, Sahlin L, Ekman-Ordeberg G, Falconer H, Stephansson O. Risk of adverse pregnancy outcomes in women with polycystic ovary syndrome: population based cohort study. *BMJ*. 2011;**343**(13 1):d6309.
115. Palomba S, Russo T, Falbo A, Di Cello A, Tolino A, Tucci L, La Sala GB, Zullo F. Macroscopic and microscopic findings of the placenta in women with polycystic ovary syndrome. *Hum Reprod*. 2013;**28**(10):2838–2847.
116. Palomba S, Falbo A, Chiossi G, Tolino A, Tucci L, La Sala GB, Zullo F. Early trophoblast invasion and placentation in women with different PCOS phenotypes. *Reprod Biomed Online*. 2014; **29**(3):370–381.
117. Palomba S, Russo T, Falbo A, Di Cello A, Amendola G, Mazza R, Tolino A, Zullo F, Tucci L, La Sala GB. Decidual endovascular trophoblast invasion in women with polycystic ovary syndrome: an experimental case-control study. *J Clin Endocrinol Metab*. 2012;**97**(7):2441–2449.
118. Palomba S, Falbo A, Russo T, Battista L, Tolino A, Orio F, Zullo F. Uterine blood flow in pregnant patients with polycystic ovary syndrome: relationships with clinical outcomes. *BJOG*. 2010; **117**(6):711–721.
119. Palomba S, Santagni S, Falbo A, La Sala GB. Complications and challenges associated with polycystic ovary syndrome: current perspectives. *Int J Womens Health*. 2015;**7**:745–763.
120. Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, Talbot JM, Baker HW. Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria Australia. *Hum Reprod*. 2010;**25**(1): 265–274.
121. Takemura Y, Osuga Y, Fujimoto A, Oi N, Tsutsumi R, Koizumi M, Yano T, Taketani Y. Increased risk of placenta previa is associated with endometriosis and tubal factor infertility in assisted reproductive technology pregnancy. *Gynecol Endocrinol*. 2013; **29**(2):113–115.
122. Igarashi TM, Bruner-Tran KL, Yeaman GR, Lessey BA, Edwards DP, Eisenberg E, Osteen KG. Reduced expression of progesterone receptor-B in the endometrium of women with endometriosis and in cocultures of endometrial cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fertil Steril*. 2005;**84**(1):67–74.
123. Podgaec S, Abrao MS, Dias JA Jr, Rizzo LV, de Oliveira RM, Baracat EC. Endometriosis: an inflammatory disease with a Th2 immune response component. *Hum Reprod*. 2007;**22**(5):1373–1379.
124. Brosens IA, Fusi L, Brosens JJ. Endometriosis is a risk factor for spontaneous hemoperitoneum during pregnancy. *Fertil Steril*. 2009;**92**(4):1243–1245.
125. Brosens I, Brosens JJ, Fusi L, Al-Sabbagh M, Kuroda K, Benagiano G. Risks of adverse pregnancy outcome in endometriosis. *Fertil Steril*. 2012;**98**(1):30–35.
126. Petraglia F, Arcuri F, de Ziegler D, Chapron C. Inflammation: a link between endometriosis and preterm birth. *Fertil Steril*. 2012; **98**(1):36–40.
127. Viganò P, Somigliana E, Panina P, Rabellotti E, Vercellini P, Candiani M. Principles of phenomics in endometriosis. *Hum Reprod Update*. 2012;**18**(3):248–259.
128. Barrett ES, Vitek W, Mbowe O, Thurston SW, Legro RS, Alvero R, Baker V, Bates GW, Casson P, Coutifaris C, Eisenberg E, Hansen K, Krawetz S, Robinson R, Rosen M, Usadi R, Zhang H, Santoro N, Diamond M. Allostatic load, a measure of chronic physiological stress, is associated with pregnancy outcomes, but not fertility, among women with unexplained infertility. *Hum Reprod*. 2018;**33**(9):1757–1766.

129. Monk M, Salpekar A. Expression of imprinted genes in human preimplantation development. *Mol Cell Endocrinol*. 2001; **183**(Suppl 1):S35–S40.
130. Jirtle RL, Sander M, Barrett JC. Genomic imprinting and environmental disease susceptibility. *Environ Health Perspect*. 2000; **108**(3):271–278.
131. Zaitseva I, Zaitsev S, Alenina N, Bader M, Krivokharchenko A. Dynamics of DNA-demethylation in early mouse and rat embryos developed in vivo and in vitro. *Mol Reprod Dev*. 2007;**74**(10): 1255–1261.
132. Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod*. 2000;**62**(6): 1526–1535.
133. Khosla S, Dean W, Brown D, Reik W, Feil R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol Reprod*. 2001;**64**(3): 918–926.
134. El Hajj N, Haaf T. Epigenetic disturbances in in vitro cultured gametes and embryos: implications for human assisted reproduction. *Fertil Steril*. 2013;**99**(3):632–641.
135. Al-Khtib M, Blachère T, Guérin JF, Lefèvre A. Methylation profile of the promoters of Nanog and Oct4 in ICSI human embryos. *Hum Reprod*. 2012;**27**(10):2948–2954.
136. Khoueiry R, Ibala-Romdhane S, Al-Khtib M, Blachère T, Lornage J, Guérin JF, Lefèvre A. Abnormal methylation of KCNQ1OT1 and differential methylation of H19 imprinting control regions in human ICSI embryos. *Zygote*. 2013;**21**(2):129–138.
137. Nelissen EC, Dumoulin JC, Daunay A, Evers JL, Tost J, van Montfoort AP. Placentas from pregnancies conceived by IVF/ICSI have a reduced DNA methylation level at the H19 and MEST differentially methylated regions. *Hum Reprod*. 2013;**28**(4): 1117–1126.
138. Li L, Wang L, Le F, Liu X, Yu P, Sheng J, Huang H, Jin F. Evaluation of DNA methylation status at differentially methylated regions in IVF-conceived newborn twins. *Fertil Steril*. 2011; **95**(6):1975–1979.
139. Katari S, Turan N, Bibikova M, Erinle O, Chalian R, Foster M, Gaughan JP, Coutifaris C, Sapienza C. DNA methylation and gene expression differences in children conceived in vitro or in vivo. *Hum Mol Genet*. 2009;**18**(20):3769–3778.
140. Rancourt RC, Harris HR, Michels KB. Methylation levels at imprinting control regions are not altered with ovulation induction or in vitro fertilization in a birth cohort. *Hum Reprod*. 2012;**27**(7):2208–2216.
141. Oliver VF, Miles HL, Cutfield WS, Hofman PL, Ludgate JL, Morison IM. Defects in imprinting and genome-wide DNA methylation are not common in the in vitro fertilization population. *Fertil Steril*. 2012;**97**(1):147–153.e7.
142. Wong EC, Hatakeyama C, Robinson WP, Ma S. DNA methylation at H19/IGF2 ICR1 in the placenta of pregnancies conceived by in vitro fertilization and intracytoplasmic sperm injection. *Fertil Steril*. 2011;**95**(8):2524–2526.e1–3.
143. Ghosh J, Coutifaris C, Sapienza C, Mainigi M. Global DNA methylation levels are altered by modifiable clinical manipulations in assisted reproductive technologies. *Clin Epigenetics*. 2017;**9**(1):14.
144. Buckberry S, Bianco-Miotto T, Hiendleder S, Roberts CT. Quantitative allele-specific expression and DNA methylation analysis of H19, IGF2 and IGF2R in the human placenta across gestation reveals H19 imprinting plasticity. *PLoS One*. 2012; 7(12):e51210.
145. Herzog E, Galvez J, Roks A, Stolk L, Verbiest M, Eilers P, Cornelissen J, Steegers E, Steegers-Theunissen R. Tissue-specific DNA methylation profiles in newborns. *Clin Epigenetics*. 2013; **5**(1):8.
146. Farhi J, Ben-Haroush A, Andrawus N, Pinkas H, Sapir O, Fisch B, Ashkenazi J. High serum oestradiol concentrations in IVF cycles increase the risk of pregnancy complications related to abnormal placentation. *Reprod Biomed Online*. 2010;**21**(3):331–337.
147. Kalra SK, Ratcliffe SJ, Coutifaris C, Molinaro T, Barnhart KT. Ovarian stimulation and low birth weight in newborns conceived through in vitro fertilization. *Obstet Gynecol*. 2011;**118**(4): 863–871.
148. Senapati S, Wang F, Ord T, Coutifaris C, Feng R, Mainigi M. Superovulation alters the expression of endometrial genes critical to tissue remodeling and placentation. *J Assist Reprod Genet*. 2018;**35**(10):1799–1808.
149. Weinerman R, Ord T, Bartolomei MS, Coutifaris C, Mainigi M. The superovulated environment, independent of embryo vitrification, results in low birthweight in a mouse model. *Biol Reprod*. 2017;**97**(1):133–142.
150. Xu N, Barlow GM, Cui J, Wang ET, Lee B, Akhlaghpour M, Kroener L, Williams J III, Rotter JI, Chen YI, Goodarzi MO, Pisarska MD. Comparison of genome-wide and gene-specific DNA methylation profiling in first-trimester chorionic villi from pregnancies conceived with infertility treatments. *Reprod Sci*. 2017;**24**(7):996–1004.
151. Lee B, Buttle R, Castellano K, Gonzalez TL, Sun T, Sundheimer LW, Wang ET, Williams J, Pisarska MD. Maternal 17-β-estradiol and progesterone remain elevated into the late first trimester in pregnancies conceived with in-vitro fertilization (IVF) despite discontinuation of supplemental therapy. *Fertil Steril*. 2017; **108**(3):e376.
152. Sun T, Lee B, Kinchen J, Wang ET, Gonzalez TL, Chan JL, Rotter JI, Chen YI, Taylor K, Goodarzi MO, Rich SS, Farber CR, Williams J III, Pisarska MD. Differences in first trimester maternal metabolomic profiles in pregnancies conceived from fertility treatments [published online ahead of print 15 November 2018]. *J Clin Endocrinol Metab*. doi: 10.1210/jc.2018-01118.
153. Grimes RW, Pepe GJ, Albrecht ED. Regulation of human placental trophoblast low-density lipoprotein uptake in vitro by estrogen. *J Clin Endocrinol Metab*. 1996;**81**(7):2675–2679.
154. Carr BR, Simpson ER. Cholesterol synthesis by human fetal hepatocytes: effects of hormones. *J Clin Endocrinol Metab*. 1984; **58**(6):1111–1116.
155. Babischkin JS, Grimes RW, Pepe GJ, Albrecht ED. Estrogen stimulation of P450 cholesterol side-chain cleavage activity in cultures of human placental syncytiotrophoblasts. *Biol Reprod*. 1997;**56**(1):272–278.
156. Albrecht ED, Bonagura TW, Burleigh DW, Enders AC, Aberdeen GW, Pepe GJ. Suppression of extravillous trophoblast invasion of uterine spiral arteries by estrogen during early baboon pregnancy. *Placenta*. 2006;**27**(4-5):483–490.
157. Lee B, Kroener LL, Xu N, Wang ET, Banks A, Williams J III, Goodarzi MO, Chen YI, Tang J, Wang Y, Gangalapudi V, Pisarska MD. Function and hormonal regulation of GATA3 in human first trimester placentation. *Biol Reprod*. 2016;**95**(5): 113.
158. Mainigi MA, Sapienza C, Butts S, Coutifaris C. A molecular perspective on procedures and outcomes with assisted reproductive technologies. *Cold Spring Harb Perspect Med*. 2016; **6**(4):a023416.
159. Beltrame JS, Sordelli MS, Cañumil VA, Alonso CAI, Perez Martinez S, Ribeiro ML. Steroid hormones induce in vitro human first trimester trophoblast tubulogenesis by the lysophosphatidic acid pathway. *Mol Cell Endocrinol*. 2018;**478**:126–132.
160. Cunningham FG, Leveno KJ. Childbearing among older women—the message is cautiously optimistic. *N Engl J Med*. 1995;**333**(15):1002–1004.
161. Kenny LC, Lavender T, McNamee R, O'Neill SM, Mills T, Khashan AS. Advanced maternal age and adverse pregnancy outcome: evidence from a large contemporary cohort. *PLoS One*. 2013;**8**(2):e56583.
162. Kaaja RJ, Pöyhönen-Alho MK. Insulin resistance and sympathetic overactivity in women. *J Hypertens*. 2006;**24**(1):131–141.

163. Kaaja RJ, Greer IA. Manifestations of chronic disease during pregnancy. *JAMA*. 2005;**294**(21):2751–2757.
164. Stekkinger E, Zandstra M, Peeters LL, Spaanderman ME. Early-onset preeclampsia and the prevalence of postpartum metabolic syndrome. *Obstet Gynecol*. 2009;**114**(5):1076–1084.
165. Zandstra M, Stekkinger E, van der Vlugt MJ, van Dijk AP, Lotgering FK, Spaanderman ME. Cardiac diastolic dysfunction and metabolic syndrome in young women after placental syndrome. *Obstet Gynecol*. 2010;**115**(1):101–108.
166. van Rijn BB, Nijdam ME, Bruinse HW, Roest M, Uiterwaal CS, Grobbee DE, Bots ML, Franx A. Cardiovascular disease risk factors in women with a history of early-onset preeclampsia. *Obstet Gynecol*. 2013;**121**(5):1040–1048.
167. Tobias DK, Chavarro JE, Williams MA, Buck Louis GM, Hu FB, Rich-Edwards J, Missmer SA, Zhang C. History of infertility and risk of gestational diabetes mellitus: a prospective analysis of 40,773 pregnancies. *Am J Epidemiol*. 2013;**178**(8):1219–1225.
168. Barry JA, Azizia MM, Hardiman PJ. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod Update*. 2014; **20**(5):748–758.
169. Gottschau M, Kjaer SK, Jensen A, Munk C, Mellemkjaer L. Risk of cancer among women with polycystic ovary syndrome: a Danish cohort study. *Gynecol Oncol*. 2015;**136**(1):99–103.
170. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS. Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2006;**91**(4):1357–1363.
171. Wang ET, Calderon-Margalit R, Cedars MI, Daviglus ML, Merkin SS, Schreiner PJ, Sternfeld B, Wellons M, Schwartz SM, Lewis CE, Williams OD, Siscovick DS, Bibbins-Domingo K. Polycystic ovary syndrome and risk for long-term diabetes and dyslipidemia. *Obstet Gynecol*. 2011;**117**(1):6–13.
172. Wang ET, Cirillo PM, Vittinghoff E, Bibbins-Domingo K, Cohn BA, Cedars MI. Menstrual irregularity and cardiovascular mortality. *J Clin Endocrinol Metab*. 2011;**96**(1):E114–E118.
173. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN, Group PCTS; PCOS/Troglitazone Study Group. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2006;**91**(1): 48–53.
174. Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. *Fertil Steril*. 2002;**77**(5): 861–870.
175. Melo AS, Rosa-e-Silva JC, Rosa-e-Silva AC, Poli-Neto OB, Ferriani RA, Vieira CS. Unfavorable lipid profile in women with endometriosis. *Fertil Steril*. 2010;**93**(7):2433–2436.
176. Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Missmer SA. Endometriosis and risk of coronary heart disease. *Circ Cardiovasc Qual Outcomes*. 2016;**9**(3):257–264.
177. Nielsen NM, Jørgensen KT, Pedersen BV, Rostgaard K, Frisch M. The co-occurrence of endometriosis with multiple sclerosis, systemic lupus erythematosus and Sjögren syndrome. *Hum Reprod*. 2011;**26**(6):1555–1559.
178. Brinton LA, Gridley G, Persson I, Baron J, Bergqvist A. Cancer risk after a hospital discharge diagnosis of endometriosis. *Am J Obstet Gynecol*. 1997;**176**(3):572–579.
179. Melin A, Sparén P, Persson I, Bergqvist A. Endometriosis and the risk of cancer with special emphasis on ovarian cancer. *Hum Reprod*. 2006;**21**(5):1237–1242.
180. Verit FF, Yildiz Zeyrek F, Zebitay AG, Akyol H. Cardiovascular risk may be increased in women with unexplained infertility. *Clin Exp Reprod Med*. 2017;**44**(1):28–32.
181. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ*. 2007;**335**(7627):974.
182. Heida KY, Franx A, van Rijn BB, Eijkemans MJ, Boer JM, Verschuren MW, Oudijk MA, Bots ML, van der Schouw YT. Earlier age of onset of chronic hypertension and type 2 diabetes mellitus after a hypertensive disorder of pregnancy or gestational diabetes mellitus. *Hypertension*. 2015;**66**(6):1116–1122.
183. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort. *Hypertension*. 2010; **56**(1):166–171.
184. Kessous R, Shoham-Vardi I, Pariente G, Sherf M, Sheiner E. An association between gestational diabetes mellitus and long-term maternal cardiovascular morbidity. *Heart*. 2013;**99**(15): 1118–1121.
185. Smith GD, Whitley E, Gissler M, Hemminki E. Birth dimensions of offspring, premature birth, and the mortality of mothers. *Lancet*. 2000;**356**(9247):2066–2067.
186. Heitritter SM, Solomon CG, Mitchell GF, Skali-Ounis N, Seely EW. Subclinical inflammation and vascular dysfunction in women with previous gestational diabetes mellitus. *J Clin Endocrinol Metab*. 2005;**90**(7):3983–3988.
187. Retnakaran R, Qi Y, Connelly PW, Sermer M, Zinman B, Hanley AJ. Glucose intolerance in pregnancy and postpartum risk of metabolic syndrome in young women. *J Clin Endocrinol Metab*. 2010;**95**(2):670–677.
188. Retnakaran R, Qi Y, Connelly PW, Sermer M, Hanley AJ, Zinman B. The graded relationship between glucose tolerance status in pregnancy and postpartum levels of low-density-lipoprotein cholesterol and apolipoprotein B in young women: implications for future cardiovascular risk. *J Clin Endocrinol Metab*. 2010;**95**(9):4345–4353.
189. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet*. 2009;**373**(9677):1773–1779.
190. Shah BR, Retnakaran R, Booth GL. Increased risk of cardiovascular disease in young women following gestational diabetes mellitus. *Diabetes Care*. 2008;**31**(8):1668–1669.
191. Carr DB, Utzschneider KM, Hull RL, Tong J, Wallace TM, Kodama K, Shofer JB, Heckbert SR, Boyko EJ, Fujimoto WY, Kahn SE. Gestational diabetes mellitus increases the risk of cardiovascular disease in women with a family history of type 2 diabetes. *Diabetes Care*. 2006;**29**(9):2078–2083.
192. Fadl H, Magnuson A, Östlund I, Montgomery S, Hanson U, Schwarcz E. Gestational diabetes mellitus and later cardiovascular disease: a Swedish population based case-control study. *BJOG*. 2014;**121**(12):1530–1536.
193. Ling HY, Ou HS, Feng SD, Zhang XY, Tuo QH, Chen LX, Zhu BY, Gao ZP, Tang CK, Yin WD, Zhang L, Liao DF. Changes in microRNA (miR) profile and effects of miR-320 in insulin-resistant 3T3-L1 adipocytes. *Clin Exp Pharmacol Physiol*. 2009;**36**(9):e32–e39.
194. Panir K, Schjenken JE, Robertson SA, Hull ML. Non-coding RNAs in endometriosis: a narrative review. *Hum Reprod Update*. 2018;**24**(4):497–515.
195. Cosar E, Mamillapalli R, Moridi I, Duleba A, Taylor HS. Serum microRNA biomarkers regulated by simvastatin in a primate model of endometriosis [published online ahead of print 1 January 2018]. *Reprod Sci*. doi: 10.1177/19337191876597.1
196. Nematian SE, Mamillapalli R, Kadakia TS, Majidi Zolbin M, Moustafa S, Taylor HS. Systemic inflammation induced by microRNAs: endometriosis-derived alterations in circulating microRNA 125b-5p and Let-7b-5p regulate macrophage cytokine production. *J Clin Endocrinol Metab*. 2018;**103**(1):64–74.
197. Pateisky P, Pils D, Szabo L, Kuessel L, Husslein H, Schmitz A, Wenzl R, Yotova I. hsa-miRNA-154-5p expression in plasma of endometriosis patients is a potential diagnostic marker for the disease. *Reprod Biomed Online*. 2018;**37**(4):449–466.
198. Lin SC, Wang CC, Wu MH, Yang SH, Li YH, Tsai SJ. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic stromal cells. *J Clin Endocrinol Metab*. 2012;**97**(8):E1515–E1523.

199. Suryawanshi S, Vlad AM, Lin HM, Mantia-Smaldone G, Laskey R, Lee M, Lin Y, Donnellan N, Klein-Patel M, Lee T, Mansuria S, Elishaev E, Budiu R, Edwards RP, Huang X. Plasma microRNAs as novel biomarkers for endometriosis and endometriosis-associated ovarian cancer. *Clin Cancer Res*. 2013;19(5):1213–1224.
200. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update*. 2012;**18**(5):485–503.
201. Männistö T, Mendola P, Vääräsmäki M, Järvelin MR, Hartikainen AL, Pouta A, Suvanto E. Elevated blood pressure in pregnancy and subsequent chronic disease risk. *Circulation*. 2013;**127**(6):681–690.
202. Libby G, Murphy DJ, McEwan NF, Greene SA, Forsyth JS, Chien PW, Morris AD; DARTS/MEMO Collaboration. Pre-eclampsia and the later development of type 2 diabetes in mothers and their children: an intergenerational study from the Walker cohort. *Diabetologia*. 2007;**50**(3):523–530.
203. Brown MC, Best KE, Pearce MS, Waugh J, Robson SC, Bell R. Cardiovascular disease risk in women with pre-eclampsia: systematic review and meta-analysis. *Eur J Epidemiol*. 2013;**28**(1):1–19.
204. Kvehaugen AS, Dechend R, Ramstad HB, Troisi R, Fugelseth D, Staff AC. Endothelial function and circulating biomarkers are disturbed in women and children after preeclampsia. *Hypertension*. 2011;**58**(1):63–69.
205. Davis EF, Lazdam M, Lewandowski AJ, Worton SA, Kelly B, Kenworthy Y, Adwani S, Wilkinson AR, McCormick K, Sargent I, Redman C, Leeson P. Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review. *Pediatrics*. 2012;**129**(6):e1552–e1561.
206. Thoulass JC, Robertson L, Denadai L, Black C, Crilly M, Iversen L, Scott NW, Hannaford PC. Hypertensive disorders of pregnancy and adult offspring cardiometabolic outcomes: a systematic review of the literature and meta-analysis. *J Epidemiol Community Health*. 2016;**70**(4):414–422.
207. Miettola S, Hartikainen AL, Vääräsmäki M, Bloigu A, Ruokonen A, Järvelin MR, Pouta A. Offspring's blood pressure and metabolic phenotype after exposure to gestational hypertension in utero. *Eur J Epidemiol*. 2013;**28**(1):87–98.
208. Kajantie E, Eriksson JG, Osmond C, Thornburg K, Barker DJ. Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study. *Stroke*. 2009;**40**(4):1176–1180.
209. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet*. 1986;**1**(8489):1077–1081.
210. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. *Lancet*. 1998;**351**(9097):173–177.
211. Turan N, Ghalwash MF, Katari S, Coutifaris C, Obradovic Z, Sapienza C. DNA methylation differences at growth related genes correlate with birth weight: a molecular signature linked to developmental origins of adult disease? *BMC Med Genomics*. 2012;**5**(1):10.
212. Rimoldi SF, Sartori C, Rexhaj E, Bailey DM, de Marchi SF, McEneny J, Arx R, Cerny D, Duplain H, Germond M, Allemann Y, Scherrer U. Antioxidants improve vascular function in children conceived by assisted reproductive technologies: a randomized double-blind placebo-controlled trial. *Eur J Prev Cardiol*. 2015;**22**(11):1399–1407.