Local and systemic factors and implantation: what is the evidence?

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Significant progress has been made in the understanding of embryonic competence and endometrial receptivity since the inception of assisted reproductive technology. The endometrium is a highly dynamic tissue that plays a crucial role in the establishment and maintenance of normal pregnancy. In response to steroid sex hormones, the endometrium undergoes marked changes during the menstrual cycle that are critical for acceptance of the nascent embryo. There is also a wide body of literature on systemic factors that impact assisted reproductive technology outcomes. Patient prognosis is impacted by an array of factors that tip the scales in her favor or against success. Recognizing the local and systemic factors will allow clinicians to better understand and optimize the maternal environment at the time of implantation. This review will address the current literature on endometrial and systemic factors related to impaired implantation and highlight recent advances in this area of reproductive medicine. (Fertil Steril® 2016;105:873–84. ©2016 by American Society for Reproductive Medicine.)

Key Words: Endometrium, implantation, immune factors, IVF, thyroid, vitamin D

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This review will address the current literature on endometrial and systemic factors related to impaired implantation and highlight recent advances in this area of reproductive medicine. The review will be divided into two major parts: the first section will address endometrial factors; the second part, systemic factors.

IMPAIRED EXPRESSION OF ENDOMETRIAL FACTORS CORRELATES WITH REDUCED IMPLANTATION

Introduction

The human endometrium is a hormone-responsive mucosa that lines the uterine cavity and undergoes cyclic proliferation and differentiation to support embryo implantation (1). During the proliferative phase the endometrium grows in response to estrogen (E), arising from the remaining basalis layer that remains after menstruation. A dynamic transition from proliferation to a secretory morphology occurs after ovulation (2), orchestrated directly and indirectly by the sex steroids E and P (1), and is further mediated by a complex array of secondary autocrine and paracrine factors, including cytokines and chemokines and their receptors and second messengers (3, 4).

Endometrial development after ovulation normally culminates with a defined period of endometrial receptivity. The secretory phase is divided into three recognized stages. The early secretory phase, from postovulatory days 1 to 5, is characterized histologically by initiation of secretory products and characterized by the presence of subnuclear vacuoles that traverse the cells by postovulatory day 6 (5). The mid-secretory phase, representing the window of implantation and time of maximal endometrial receptivity, occurs from postovulatory day 6 to 10. During this period stromal cells are undergoing pseudo-decidualization reactions, and epithelial cells develop specialized structures known as pinopodes (6) and cell adhesion molecules (7–9). The third phase in nonconception cycles represents the late luteal phase (postovulatory days 11–14), during which preparation for menstruation occurs. In the absence of the nidatory hCG signal from the embryo, endometrial breakdown occurs, associated with apoptosis and an orchestrated inflammatory response that leads to an orderly and

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brief episode of menstrual shedding in anticipation of the next cycle (10). When pregnancy occurs, decidualization of the endometrial stroma transforms into a specialized epithelialized mesenchymal structure, essential for pregnancy (11, 12).

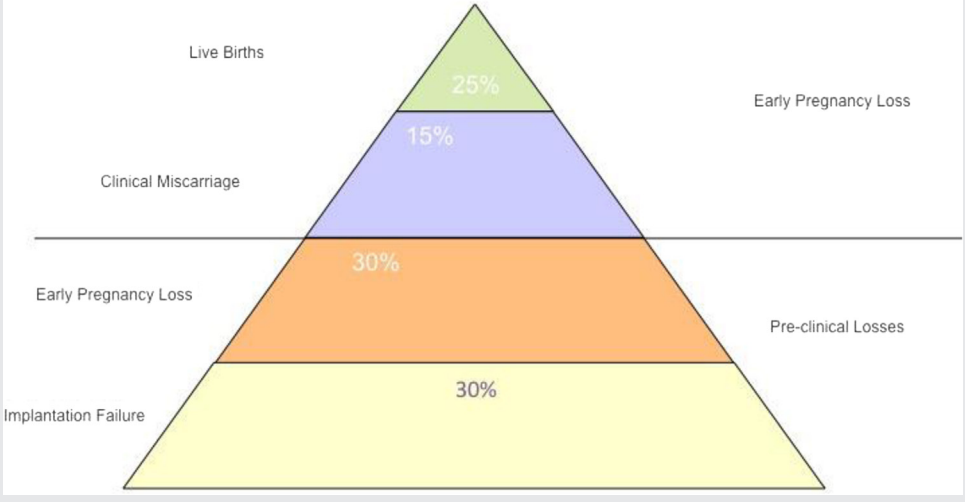
The mid-secretory phase coincides with the entry into the uterine cavity of the preimplantation blastocyst, with the differentiation of trophectoderm by postovulatory day 5. A defined period of endometrial receptivity during the mid-secretory phase also corresponds well to prime responsiveness of the corpus luteum (CL) to hCG (13, 14). In fact, evidence from the 1999 Wilcox study shows that late-implanting embryos are at higher risk for miscarriage than those that implant during the window of implantation (WOI), between postovulatory days 6 and 10 (15). One interpretation for these interesting findings is that a sustained rescue of the corpus luteum occurs best at the time of normal implantation. This hypothesis is supported by early studies that examined CL rescue in response to early or late administration of hCG (13). The CL has a more robust response to hCG administered on postovulatory days 8–10 compared with postovulatory days 11–14, and P may fall more quickly in early losses or implantation failure than after pregnancy is established (16). Our intention in this review, however, will be to focus not on the CL but rather on uterine factors that contribute to a delay in implantation that then contributes to both pregnancy loss and implantation failure.

The efficiency of human reproduction is relatively low compared with other mammalian species. As summarized by Macklon et al. (17) (Fig. 1), there are many more implantation failures and early clinical and preclinical losses than successful pregnancies. Although this is obvious to the clinician who treats infertility, our understanding of the basis for defects in endometrial receptivity has remained fragmented. A failed pregnancy can be the result of many diverse factors, including chromosomal defects in the nascent embryo, mechanical causes in the reproductive tract, or inflammatory changes associated with disease. Assigning cause and effect in terms of the embryo or endometrial defects has been problematic. In this era of preimplantation genetic screening, answers may be forthcoming. In a report on a large series of euploid blastocysts, the proportion of euploid embryos failing to implant was approximately 40% (18). For those who study endometrial receptivity defects, those data may be a "smoking gun" regarding the importance of the endometrium.

Do Endometrial Receptivity Defects Exist?

Historically, Georgianna Seegar Jones might have been the first investigator to show that defects in the endometrial histology could be associated with infertility (19). Using the then newly identified morphologic changes in the secretory phase endometrium (5), she noted for the first time that women with infertility could have a lag in predicted endometrial histologic development, a term she coined as "luteal phase deficiency." It is worth noting that the existence and impact of luteal phase deficiency has come under question (20, 21). Nevertheless, the concept of a shifting WOI has been shown to have continued importance. In a landmark study by Wilcox et al. in 1999 (15), it was noted that women who implant beyond the normal window had an increasing chance for pregnancy loss. Biochemical defects have also been described that support a concept of a delayed WOI and retarded histology, including the use of placenta protein-14 (also knows as glycodelin), integrins, MUC-1, pinopods, leukemia inhibitory factor, and many others (22–26). In addition, cycles without histologic lag have been described that display defects in key biomarkers of endometrial receptivity as well (27, 28).

FIGURE 1



The hidden impact of implantation failure. Adapted from Macklon et al. (17).

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Regulation of Endometrial Receptivity

The endometrium undergoes well-defined and regulated gene expression in preparation for implantation (1). The timing of endometrial receptivity coincides with the down-regulation of epithelial E receptor-α (ESR1) in normal mid-secretory endometrium (29), as seen in other mammals studied at the time of implantation (30). Progesterone and its receptor (PR) are essential for successful embryo implantation, but there is a shift in PR out of the epithelium to the stromal compartment that also occurs during the WOI (29). Persistence of ESR1 and PR in the glandular epithelium is associated with infertility and suspected implantation defects (31, 32). Aberrant overexpression of ESR1 and PR at the time of implantation is a sign of P resistance, because P normally down-regulates both endometrial ESR1 and its own receptor (PR) (33). Progesterone resistance is associated with luteal phase deficiency, pregnancy loss, or infertility due to endometriosis.

Progesterone also limits E action through the induction of 17β-hydroxysteroid dehydrogenase-type 2 (HSD17βII) in the endometrium, which converts E_2 to the less-active estrone (34). Through these complex mechanisms of induction and inhibition of gene expression, there is a shift during the WOI from direct actions of P (endocrine factors) to indirect actions (via paracrine and autocrine factors) (3, 35, 36). Failure to make this transition is likely a cause of implantation failure.

Disorders Associated with Implantation Failure

Individual uterine factors associated with some implantation failures in the setting of infertility, recurrent loss, and IVF have previously been reported. These include mechanical, inflammatory, and systemic factors (26, 37, 38). Mechanical factors encompass both congenital uterine anomalies and acquired intracavitary conditions. Congenital uterine anomalies, including uterine septae, have been linked to early miscarriages. One study comparing IVF outcomes between women with untreated septate uteri vs. women who had undergone hysteroscopic metroplasty found that untreated women had worse IVF outcomes (39). Acquired intracavitary conditions, such as submucosal fibroids, endometrial polyps, and intrauterine adhesions depending on size and location, have also been linked to poor obstetric outcomes and may also contribute to recurrent implantation failure (40). Available evidence suggests that surgical correction of these intrauterine pathologies may improve pregnancy outcomes (41, 42).

Inflammatory factors associated with implantation failure include endometriosis, adenomyosis, hydrosalpinges, and endometritis (1). A meta-analysis in 2002 of IVF success rates in patients with endometriosis found not only that pregnancy rates were decreased compared with control patients, but fertilization rates, implantation rates, and number of oocytes retrieved were significantly reduced (43). Hydrosalpinx, or a blocked fallopian tube, is also associated with implantation failure (44), with improvement noted after salpingectomy (45–48). Endometritis, an inflammation of the endometrium, is also associated with infertility and obstetric complications (49). Endometritis is associated with aberrant inflammatory cytokine expression and has been associated with endometriosis (50). Polycystic ovary syndrome, a common cause of infertility and the most common endocrinopathy affecting reproductive-aged women, is associated with reduced endometrial receptivity (51, 52). Progesterone resistance, which is associated with inflammatory changes in the endometrium (36), has been observed in both endometriosis and polycystic ovary syndrome by DNA microarray analysis (53, 54), and both conditions exhibit increased E receptor dominance during the secretory phase (32, 55). Collectively, all of these conditions share an inflammatory component, increasingly considered to be a root cause of impaired implantation (38, 56).

Endometrial Factors as Biomarkers of Receptivity

Pinopodes are protrusions of the endometrial epithelium first identified in mice in 1958 (57) and later identified in human endometrium by electron microscopy (58). Since that time, pinopodes have been identified as markers of endometrial receptivity (59), owing to their putative expression coinciding with the WOI (6). Blastocyst attachment has been shown to occur at the site of endometrial pinopode expression in vitro (60), and pinopodes are the site of expression of uterine receptivity, including $\alpha v\beta 3$ integrin and osteopontin (9, 24). Although the detection of pinopodes has been used for assessment of uterine receptivity, clinical usefulness is limited by technical factors due to the need for electron microscopy, the brief time of expression, and the subjective nature of scoring them (61). In addition, three prospective studies have failed to confirm a precise association between the temporal timing of pinopode expression and the WOI (23, 62, 63), raising substantive doubts related to this endometrial feature as a biomarker of receptivity in humans.

Numerous molecular mediators of early feto-maternal interface have been identified in the literature. These include adhesion molecules, cytokines, growth factors, lipids, and other factors (37, 38). One of the better-described endometrial biomarkers associated with the WOI is the $\alpha v\beta 3$ integrin (7, 24, 64). Integrins are a class of cell adhesion molecules (CAMs) that interact with extracellular matrix ligands, other CAMs, and matrix metalloproteinases. Studies have documented how integrin expression is aberrant in many of the same inflammatory conditions associated with implantation failure, including endometriosis, hydrosalpinges, polycystic ovary syndrome (27, 46, 65, 66), and endometritis (unpublished results). Reduced $\alpha v\beta 3$ integrin expression has been associated with unexplained IVF failure (67, 68), whereas positive integrin expression has been found to predict future IVF success (69). Additional CAMs have been investigated to play a role in endometrial receptivity, including CD 44 (70), trophinin (71), and cadherin-11 (72).

Glycodelin, formerly referred to as placental protein 14, is a major secretory protein from the glandular endometrium expressed during and after the window of implantation (1). It is an immune modulator with a putative role in prevention of maternal immune rejection of the fetal allograft (73). Glycodelin has been investigated as a marker of endometrial receptivity, with conflicting results (25,74,75).

Mucin 1 (MUC-1) is another glycoprotein localized to the luminal surface epithelium of the receptive endometrium. In primates and mice, MUC-1 seems to function as a barrier to implantation during the nonreceptive phase and must be removed at the time of implantation (76, 77). In humans, MUC-1 localizes on the luminal surface but is excluded from cells with pinopodes, suggesting that the antiadhesive molecule may allow the blastocyst to preferentially attach to these specialized structures on the apical surface (78).

Several cytokines and growth factors have been identified whose expression in the endometrium is temporal with implantation and have been suggested as biomarkers for uterine receptivity. These include leukemia inhibitory factor (LIF), heparin binding–epidermal growth factor-like factor, insulin-like growth factor II (1). Leukemia inhibitory factor seems to play a role in events between the endometrium and the blastocyst and is expressed in the endometrium at the time of implantation. In mouse models, female homozygote mice with an LIF null mutation demonstrate complete lack of implantation (79, 80). Normal-appearing blastocysts were found within the uteri of these mice lacking LIF, but successfully implanted when placed into LIF-positive controls. Interestingly, administration of exogenous LIF resulted in a partial reversal of the defect, demonstrating that the implantation abnormalities resulted from a defect of the endometrial protein and not the blastocyst. Examination of LIF in human samples suggests that LIF maintains importance in the human endometrium as well (28, 81). Heparin binding–epidermal growth factor-like factor and insulin-like growth factor II are expressed during the window of implantation and seem to play an important role in successful implantation (82, 83). Other potential markers include calcitonin (84, 85), HOXA-10 transcription factor (86, 87), and L-selectin and L-selectin ligand (8).

Aromatase (p450arom) is overexpressed in inflammatory conditions involving the endometrium, including endometriosis (88). Aromatase overexpression shows promise as an important predictor of implantation failure in assisted reproductive technology (ART) cycles (68, 89). Overexpression of this enzyme, coupled with decreased expression of the E-metabolizing enzyme (17-hydroxysteroid dehydrogenase II) (88, 90, 91), increases bioavailable E in the endometrium, potentially accounting for aberrantly high ESR1 and proliferation (32, 34, 92, 93). Estrogen is a potent inhibitor of endometrial $\alpha v\beta 3$ integrin (94), a prime CAM involved in embryo attachment and invasion (7,95–98). Alterations in eutopic endometrial metabolism of E in endometriosis is regulated by complex changes in autocrine and paracrine signaling associated with inflammation (36, 92, 99–102), driven in part by prostaglandin E2 (103, 104), produced in response to E-regulated cycylo-oyxgenase 2 (105) and hypoxia-induced factor-1 (106). Hypoxia-induced factor-1α is stabilized by activation of signal transducer and activator of transcription-3 (STAT3) that we recently showed are both overexpressed in women with endometriosis and infertility (107). Cycylo-oyxgenase 2 and STAT3 expression have been linked to inflammatory cytokines such as IL-17 and IL-6 (107–109), which are also elevated in women with endometriosis (107, 110). Inhibitors of aromatase can reverse the negative effects of endometriosis in general (111) and improve outcomes in IVF for patients with suspected defects in endometrial receptivity (68).

Whereas STAT5 is central to P-mediated signaling (112), STAT3 seems central to P resistance (113). One of the proteins induced by activated STAT3 is B-cell lymphoma protein 6 (BCL6), while it is also inhibited by STAT5 (114). B-cell lymphoma protein 6 seems to be a reliable single biomarker for the detection of endometriosis (115). B-cell lymphoma protein 6 targets GLI1 (116), a signaling factor involved in the Indian Hedgehog pathway, making it a prime candidate driving the P resistance observed in endometriosis. This relationship between inflammatory changes, E dominance, and P resistance represents a unifying theory of the link between inflammation, E dominance, and P resistance (Fig. 2).

Progesterone resistance is a hallmark of implantation failure and associated with measurable changes in endometrial gene expression (113, 117). With the advent of transcriptome microarrays, the signatures of gene expression throughout the menstrual cycle have been well-documented in normal women (118, 119) and in those with gynecologic disorders (53, 54, 120, 121). New panels of selected biomarkers are now becoming available, with the potential to screen for a receptive and nonreceptive endometrium. A commercialized test based on transcriptomics (Endometrial Receptivity Assay or ERA) has been offered from the IVI group in Valencia, Spain (122–125). Interestingly, although the ERA test is accurate at assigning histologic stage (126), this array of biomarkers in aggregate does not differ significantly in women with endometriosis (127), a known cause of endometrial receptivity defects and P resistance (54).

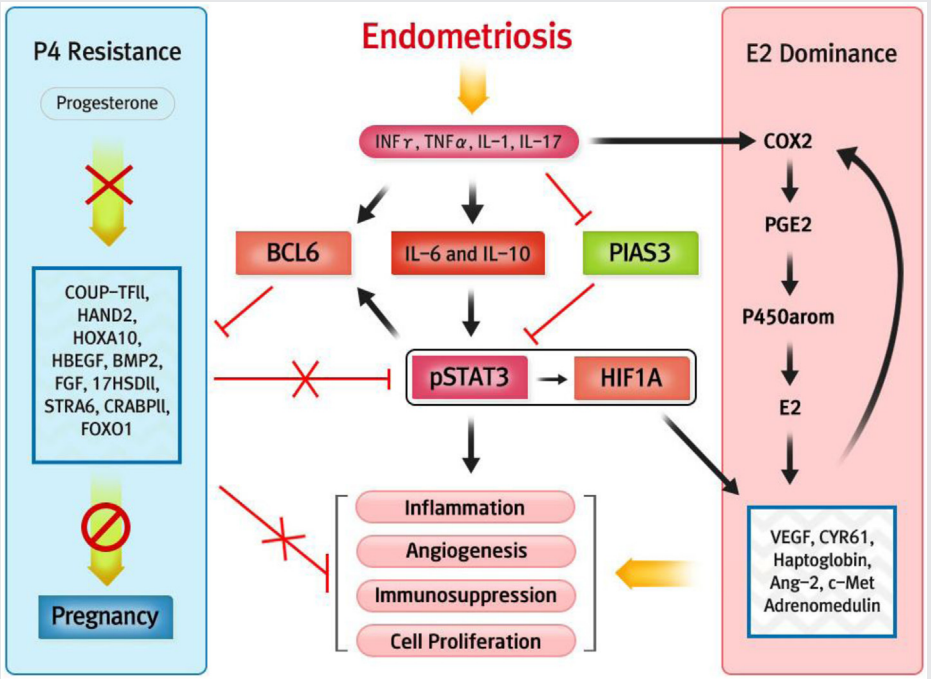
Individual tests for endometrial receptivity, including the E-tegrity test based on integrin expression (Innovative Reproductive Solutions), are also available. As discussed above, the presence or absence of the $\alpha v\beta 3$ integrin indicates potential defects in endometrial receptivity. This specific integrin is highly specific to the initiation of the window of implantation on postovulatory day 5 to 6, and is always absent in histologically delayed endometrium before the opening of the WOI (19). This test could be complemented by additional uterine factors that do not depend as heavily on histology, including LIF (25).

Another commercialized endometrial function test, from Yale University, is based on alterations in cyclin E and on p27 expression (128, 129). These biomarkers are associated with cell proliferation, as seen in eutopic endometrium of women with endometriosis (93), and therefore reacting to E dominance. In aggregate, the evidence showing benefit or utility for any of these tests remains relatively weak, and validation of these and future tests as predictors of IVF outcomes or implantation failure need to be rigorously studied in prospective, randomized trials to fully evaluate their performance and reliability.

Future Directions

Implantation concerns arise frequently in couples with infertility, especially in the setting of ART cycles. Implantation rates have been relatively stagnant over the past 10 years, suggesting that progress in solving implantation problems may have slowed. In vitro fertilization failure in more than half

FIGURE 2



Like menstruation, endometriosis is associated with inflammation. Inflammatory cytokines including interferon-gamma (Infg), tumor necrosis factor alpha (TNFa), interleukin-1 (IL-1) and interleukin-17 (IL-17), have down-stream effects on the endometrium. IL-17 has been shown to stimulate COX-2 expression and prostaglandin (PGE2) production that stimulates aromatase expression. Endometrial IL-6 is associated with these changes that activates signal transducer and activator of transcription-3 (STAT3). Phosphorylated STAT3 (pSTAT3), as seen in endometriosis and infertility (104), stabilizes hypoxia-induced factor-1 alpha (HIF-1a) which together with STAT3 stimulates many of the estrogen-dependent changes associated with endometriosis, including estrogen receptor beta (ESR2). STAT3 also induces BCL6 that is a candidate signaling protein that is associated with endometriosis and progesterone resistance (112). The imbalance between estrogen and progesterone actions likely plays a critical role in the implantation defects found associated with various inflammatory states including endometriosis, adenomyosis and hydrosalpinges. Other abbreviations: Chicken ovalbumin protein transcription factor II (COUP-TFII), Heart- and neural crest derivatives-expressed protein-2 (HAND2), homeobox A10 (HOXA10), heparin- binding epidermal growth factor-like growth factor (HBEGF), bone morphogenic protein 2 (BMP2), fibroblast growth factor (FGF), 17 hydroxysteroid dehydrogenase-2 (17HSDII), Stimulated by retinoic acid 6 (STRA6), cellular retinoic acid binding-protein 2 (CRABPII), Foxhead box protein O1 (FOXO1), interleukin 6 (IL-6), interleukin 10 (IL-10), protein inhibitor of activated STAT3 (PIAS3), cyclooxygenase 2 (COX2), prostaglandin E2 (PGE2), P450 Aromatase (P450AROM), estradiol (E2), vascular endothelial growth factor (VEGF), cysteine-rich angiogenic inducer 61 (CYR61), Angiopoietin (Ang-2), hepatocyte growth factor receptor (c-MET).

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of all cases in women across all age groups seems to be concentrated in specific subgroups of patients, including those with unexplained causes. Future directions are now focused on identifying new biomarkers that alone or together reliably predict implantation success or failure. In an era in which the underlying causes of infertility are increasingly not being identified or surgically addressed, availability of such biomarkers could be a key to identifying and better treating these women. Until such a time when reliable endometrial receptivity tests are available and adequately tested, that subset of women with implantation failure will continue to go largely unrecognized. In the future, our goal should be to make repetitive implantation failure an exceedingly rare occurrence.

THE EFFECT OF SYSTEMIC FACTORS ON IMPLANTATION AFTER IVF

Introduction

Implantation rates after IVF have increased over the past 30 years as a result of advances in the basic understanding of reproductive science and the implementation of new technologies and practices. However, the primary focus of research aimed at improving IVF outcomes has focused on two areas: assessment of embryonic competence, and optimization of endometrial receptivity. Research in embryonic competence has led to advanced diagnostics that have enhanced embryo selection and substantially improved implantation rates (130). Investigative efforts focused on the endometrium have uncovered the concept of embryo–endometrial synchrony and led to the characterization of the transcriptomic signature of the receptive endometrium (131).

However, despite our progress, a substantial portion of patients fail to become pregnant after IVF. Many of these patients fail even after the transfer of a euploid embryo into a seemingly receptive endometrium. A portion of these failures undoubtedly reflects the limitation of current diagnostic tools to select the most competent embryo. However, many of these failures are due to systemic factors that affect the maternal environment and negatively impact an embryo's ability to implant. Although research into these systemic factors has

received less focus than the preimplantation embryo and the perinidatory endometrium, many have been clearly demonstrated to affect IVF success. Although a factor in isolation may not preclude a successful pregnancy, the combination of deleterious effects decreases the chance that an individual embryo transfer results in a pregnancy. Thus, it is essential these factors are optimized to give each patient the best chance at success.

Thyroid Dysfunction

Thyroid hormones influence the feto-maternal interface through interactions with thyroid hormone receptors and TSH receptors present in the endometrium and trophoblast during implantation. This interaction is mediated by a variety of downstream effects, including altered transcription and translation of essential cellular proteins during implantation (132). Thyroid dysfunction has been mostly studied in the context of ART, in terms of pregnancy success as well as miscarriage. However, the threshold TSH values that confer implantation success and those that predispose patients to adverse outcomes may be different. Thus, when attempting to isolate the effect of thyroid function on implantation, the threshold values used in the study must be noted.

The upper limit of the reference range for TSH levels was established by the National Health and Nutritional Examination Survey to be 4.5–5 mIU/L (133). Thus, the classic definition of subclinical hypothyroidism (SCH) is a TSH level >4.5 mIU/L, with normal free thyroxine levels. Using this definition, Kim et al. (134) performed a randomized, controlled trial of 64 patients to assess the effect of levothyroxine on IVF patients with SCH. In this study, patients were randomized to either levothyroxine (50 μg) or no treatment. The implantation rate was significantly higher in the treatment arm than in the control group (26.9% vs. 14.9%, P=.044). A similar study used a TSH cutoff of 4.2 mIU/L to diagnose SCH and randomized 70 patients to levothyroxine (50–100 μg daily) or placebo. In this study, the clinical pregnancy rate was significantly higher in the treatment group (35% vs. 10%, P=.02) (135). Thus, there are high-quality data demonstrating that untreated subclinical hypothyroidism negatively impacts the implantation rate after ART.

In practice, most ART programs use 2.5 mIU/L as a threshold for initiating levothyroxine treatment. Green et al. (136), by evaluating TSH levels under 2.5 with 1,599 euploid transfers, determined that no level under that cutoff is more favorable. This strategy follows recommendations of the Endocrine Society to maintain TSH levels below 2.5 mIU/L during the first trimester of pregnancy. However, no studies have evaluated whether treatment of TSH levels between 2.5 mIU/L and the upper limit of normal impacts implantation rates or miscarriage after IVF, although in one study levels between 2.5 and 5 mIU/L in the first 11 weeks of pregnancy were associated with a significant increase in pregnancy loss (6.1% vs. 3.6%, P=.006) (137). However, this investigation did not control for the chromosomal status of the embryo. As a result, it is possible that lower hCG levels associated with aneuploid gestations may have contributed to the failure of TSH to fall below 2.5 mIU/L in this group. Thus, the ideal TSH level within the normal range for optimizing implantation success is unclear, but levels above 2.5 mIU/L during early pregnancy seem to increase miscarriage.

Additionally, multiple studies have assessed the effect of thyroid autoimmunity (either anti-thyroperoxidase or anti-thyroglobulin antibodies) on IVF success. A meta-analysis of seven studies including 330 thyroid antibody–positive patients and 1,430 controls demonstrated no difference in clinical pregnancy rate after IVF (odds ratio 0.67, 95% confidence interval 0.36–1.4, P=.67) (132). One prospective, randomized, controlled trial evaluated empiric treatment with levothyroxine in euthyroid patients with evidence of thyroid autoimmunity. In that study there was no difference in clinical pregnancy rates between the treated and untreated patients (56% vs. 49%, P=.71); however, transfer order was not reported, limiting the conclusion (138). Available evidence does not support the notion that thyroid autoimmunity significantly impacts implantation, although a systematic review and a randomized study of levothyroxine therapy suggested that thyroid autoimmunity increased miscarriage and premature delivery (132, 139), which could be prevented by replacement therapy (139). If a decision is made to not treat women with TSH levels between 2.5 and 5.0 mIU/L, it may be prudent to measure thyroid peroxidase antibodies in those women and to treat if positive.

Vitamin D Deficiency

The current vitamin D (25OHD) deficiency epidemic in the developed world has led to increased interest in the role of 25OHD in ART. This interest is based on evidence that calcitriol, the active form of 25OHD, is secreted by the endometrium and regulates expression of target genes that are essential for implantation. In an attempt to control for the effect of 25OHD on the oocyte and resultant embryo, multiple studies have examined the association between 25OHD levels in donor oocyte recipients on egg and embryo quality, with conflicting results. Rudick et al. (140) performed a retrospective cohort study of 99 recipients and found that clinical pregnancy rates were lower among 25OHD deficient patients than 25OHD replete patients (37% vs. 78%, P=.004). A subsequent study by Fabris et al. (141) retrospectively examined 267 oocyte donation cycles and found no difference in implantation rate among 25OHD replete, deficient, or insufficient patients (61% vs. 63.4% vs. 65.2%, P=.894).

The largest analysis was performed by Franasiak et al. (142) and controlled for the chromosomal status of embryos by analyzing euploid transfers. In this study the average serum 25OHD level was no different between women with and without ongoing pregnancies. A multivariate logistic regression demonstrated no association between 25OHD levels and pregnancy rates. Thus, although more attention to the 25OHD deficiency epidemic in reproductive-age women is warranted given its impact on a general health, it does not seem to be a significant determinant of success after IVF.

Prolactin

Circulating levels of PRL are elevated during ovarian stimulation cycles in some women. Limited investigations regarding PRL levels and IVF outcomes have reported associations of higher PRL levels with an improved ovarian response and pregnancy (143, 144), whereas others have failed to demonstrate this association (145–148). Doldi et al. (149) treated a group of women with dopamine agonists and observed a higher ovarian response and improved oocyte morphology and fertilization in an untreated control group, suggesting a beneficial effect of PRL on the ovary. Jinno et al. (150) applied a "bromocriptine rebound" to elevate PRL levels and observed an increase in follicles, fertilized oocytes, embryo quality, clinical pregnancy, and live birth. These investigations are important because of evidence of decidual production of PRL and the suggestion that it may mediate events associated with implantation. However, none of the above-noted studies have sought to isolate the effect of PRL levels on implantation rates. Future studies may benefit from measuring PRL levels in the endometrial secretome, because the local effects of PRL production may be more relevant in determining implantation success than circulating levels of the hormone.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) has been linked to both endometriosis and subfertility. It is unclear whether this effect manifests itself as diminished ovarian reserve or an increased risk for implantation failure. To better characterize this association, Oza et al. (151) published a retrospective cohort study comparing IVF outcomes in 120 patients with IBD with 470 age-matched controls. Although implantation rate was not calculated, the mean number of embryos transferred (two) was the same for each group. There was no difference in clinical pregnancy rate in the first cycle for each patient (40.9% in non-IBD patients vs. 46.7% in IBD patients, $P=.18$). Furthermore, the cumulative live birth rate after up to six IVF cycles was equivalent between the groups (63% vs. 53%, $P=.13$). Thus, although further research is needed, there are no current data to suggest that IBD negatively impacts implantation.

Obesity

The incidence of obesity in the United States has increased substantially since the inception of ART 35 years ago. Today, more than 35% of reproductive-age women are obese (body mass index [BMI] ≥ 30 kg/m^2). Obese women are more likely to be infertile and have poor obstetric outcomes. Thus, obesity is a common and modifiable risk factor for poor pregnancy rates and maternal and neonatal morbidity after IVF.

The association between obesity and IVF outcomes has been widely studied. The most effective study design to isolate obesity's effect on implantation rates after IVF examines donor oocyte recipients. Two large retrospective reviews have used this design. The largest examined the 2008–2010 Society for Assisted Reproductive Technology Registry and included 22,317 donor oocyte cycles (152). Recipients with BMIs between 30 and 34.9 kg/m^2 had a lower implantation rate than normal-range (BMI 18.5–24.9 kg/m^2) patients (42.6% vs. 49.3%, $P<.001$). However, this study did not provide information on the BMI of the oocyte donors, limiting the ability to isolate obesity's impact on implantation. In contrast, Bellver et al. (153) examined the effect of increasing recipient BMI on IVF outcomes, using only oocyte donors with BMI <25 kg/m^2. In this study the implantation rate for recipients with BMI ≥ 30 kg/m^2 was significantly lower than for those with BMI <30 kg/m^2 (30.9% vs. 40%, $P<.001$). Thus, although prospective studies are needed to better characterize this phenomenon, the available evidence suggests that obesity negatively impacts the ability of good-prognosis embryos to implant.

Cigarette Smoking

Cigarette smoking represents another modifiable factor that substantially impacts reproductive success. Although the incidence of cigarette smoking has dropped substantially, one in five adults between the ages of 25 and 44 years still smoke cigarettes (154). The negative impact of cigarette smoking on ovarian function among smokers is well established. A number of studies have also addressed the effect of cigarette smoking on implantation after IVF.

Most studies that have examined the effect of cigarette use on ART outcomes are confounded by the positive correlation between cigarette use and maternal age. However, in their meta-analysis, Waylen et al. (155) identified nine studies that controlled for maternal age. This pooled analysis of 1,480 patients demonstrated that the odds of a clinical pregnancy after IVF were significantly lower for smokers than for nonsmokers (odds ratio 0.51, 95% confidence interval 0.32–0.79, $P<.0001$). To isolate cigarette smoking's effect on endometrial receptivity, Soares et al. (156) used the donor oocyte recipient model. In this retrospective study of 785 recipients, the authors compared heavy-smoking recipients (>10 cigarettes per day) with light smokers and nonsmokers, after controlling for the tobacco use of the oocyte donors. The clinical pregnancy rate was significantly lower for heavy-smoking recipients (34.1% vs. 52.2%, $P=.02$).

Interestingly, the negative impact of cigarette smoke extends to nonsmokers who are exposed to secondhand smoke. Benedict et al. (157) found cotinine, a nicotine metabolite, to be present in the follicular fluid of 1,909 nonsmoking women during IVF treatment. These patients had a 52% increased risk of implantation failure when compared with cotinine-negative nonsmokers. Thus, chronic exposure to secondhand smoke also results in decreased implantation efficiency, and patients should be counseled to avoid any exposure.

Autoimmunity

The potential role of autoimmune disorders in limiting ART outcomes has been extensively investigated. The established relationship between second-trimester loss and antiphospholipid antibodies (APLAs) led some investigators to evaluate a potential role for these antibodies in failed implantation and early clinical losses. A meta-analysis of multiple studies demonstrated that the presence of APLAs does not impact

pregnancy rates (158). Thus, clinical screening of APLAs in patients whose clinical diagnosis is infertility is not indicated.

Natural killer (NK) cells are prominent in the perinidatory and postimplantation endometrium. It is almost intuitive that abnormalities in NK cell activity might impact clinical outcomes. Unfortunately, the literature has been confused by efforts to measure NK cells in the peripheral circulation as a way of prognosticating NK cell density or function in the endometrium. There is no physiologic reason to assume that any such relationship exists (159). In fact, NK cell numbers in the peripheral circulation and the endometrium are unrelated (160). Prospective clinical studies have failed to demonstrate meaningful relationships with ART outcomem and clinical screening of NK cells (either peripheral or endometrial) is not indicated (161).

Although simple studies of NK cell concentration have not been clinically useful, studies evaluating variation in their function are more intriguing. Natural killer cells are involved in early remodeling of the maternal stromal and vascular compartments and play important roles in villous formation (162). The NK cells are activated by human leukocyte antigen-C (HLA-C), which is expressed on the surface of the invading trophoblast. Combinations of killer immunoglobin receptor types and the nature of HLA-C expression have been associated with increased risk of clinical pregnancy loss and placental insufficiency in the third trimester (163). Recent data have extended these findings to suggest that adverse combinations of the maternal killer immunoglobin receptor genotype and embryonic HLA-C genotypes prognosticate reduced outcomes in oocyte donation cycles (164). Clinical screening is not indicated at this time, but this remains an area of active investigation.

No marker of autoimmune dysfunction evaluated to date has demonstrated clinical value. However, some investigators remain concerned that impaired immune function adversely impacts clinical outcomes. Trials of empiric treatments using anticoagulants, intralipid, or intravenous immunoglobulin have produced mixed but generally negative results (165–167). Empiric treatment is not indicated at this time.

In conclusion, significant progress has been made in understanding many significant embryonic and endometrial factors that mediate ART success. Although much work remains in optimizing embryo selection, we must not lose sight of the systemic factors that modulate the perinidatory environment. Even the transfer of a euploid embryo into a synchronous endometrium will fail in an inhospitable maternal environment. Thus, as clinicians, we must reduce the negative impact of systemic factors that decrease the odds of a given embryo implanting and progressing to a healthy delivery. As investigators, more research is needed to identify epidemiologic factors that affect IVF success and to better understand the molecular mechanisms that govern these relationships.

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