The human periconceptional maternal–embryonic space in health and disease

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Keywords: Periconceptional period, embryo, preimplantation endometrium, decidua, endometrial microbiome, myometrium, periconceptional care.

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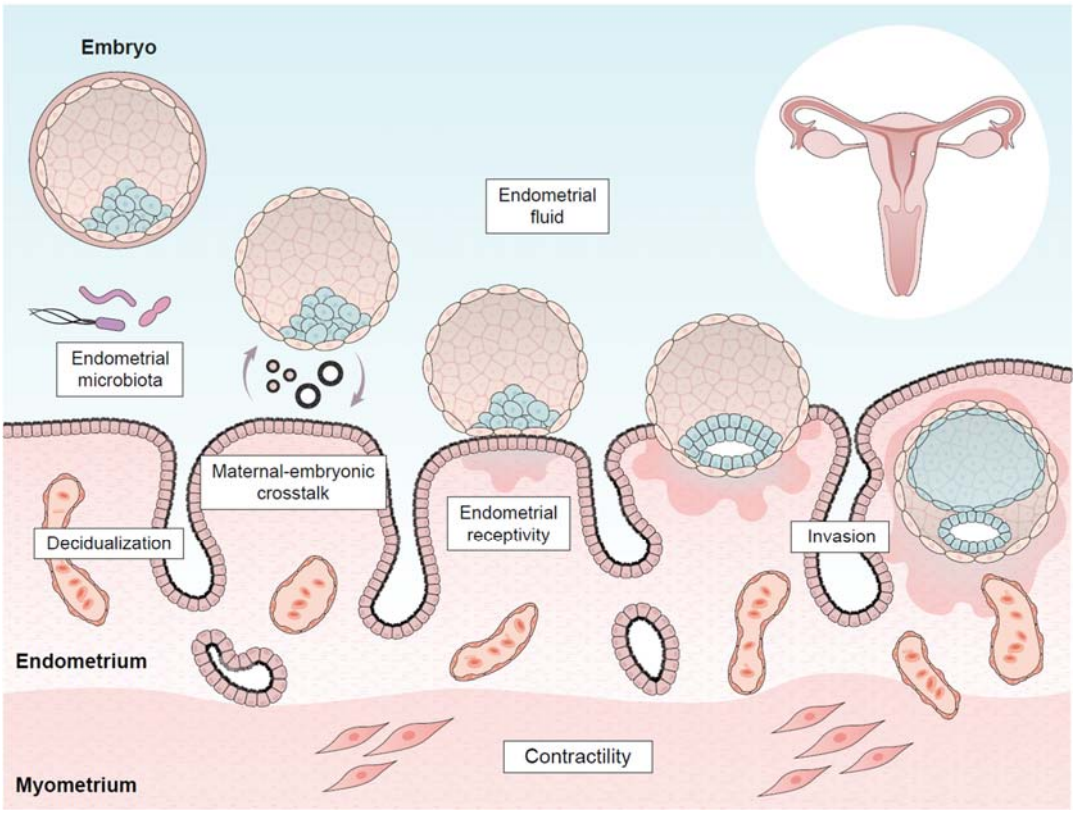
ABSTRACT

Pregnancy is established during the periconceptional period as a continuum beginning with blastocyst attachment to the endometrial epithelial surface followed by embryo invasion and placenta formation. This period sets the foundation for the child and mother's health during pregnancy. Emerging evidence indicates that prevention of downstream pathologies in both the embryo/newborn and pregnant mother may be possible at this stage. In this review, we discuss current advances in the periconceptional space, including the preimplantation human embryo and maternal endometrium. We also discuss the role of the maternal decidua, the periconceptional maternal-embryonic interface, the dialogue between these elements, and the importance of the endometrial microbiome in the implantation process and pregnancy. Finally, we discuss the myometrium in the periconceptional space and review its role in determining pregnancy health.



Graphical abstract

CLINICAL HIGHLIGHTS BOX

The periconceptional period represents a critical stage from a medical and scientific perspective;furthermore is now clinically actionable (to some extent) to prevent downstream pathologies for both the prospective mother and child. The foundations for the general and reproductive health of the prospective child and mother become set during this periconceptional period when the embryo implants into the maternal endometrium. Here, we summarize current knowledge regarding the peri-implantation interface, human embryo, and maternal endometrium and how we may prevent genetic/chromosomal pathologies and implantation failure of endometrial origin.

- Fertile and infertile prospective parents may have the ability to choose diagnosis to avoid genetic/chromosomal alterations for their children
- Embryos can undergo routine chromosomal and genetically analysis prior to implantation to prevent genetic conditions in the child
- The maternal endometrium can be investigated before pregnancy, identifying the personalized window of implantation and the existence of microbial dysbiosis that may lead to initial conception failures and intrauterine infections during pregnancy
- The existence of defective decidualization impairing cytotrophoblast invasion represents a possible cause of late pregnancy complications such as severe preeclampsia, leveraging the development of targeted therapies
- Investment in the optimization of periconceptional health has the potential to decrease significant health burdens and improve lifelong health

I. INTRODUCTION

Humans are the result of genetics (nature) and environment (nurture) in equal measure. The periconceptional space represents the crossroads between reproductive medicine and obstetrics, where nature and nurture come together at the beginning of life. Infertility specialists seek to optimize pregnancy success and first-trimester outcomes, while obstetricians prioritize diagnosing, treating, and

preventing second- and third-trimester obstetrical complications, including preterm labor, preeclampsia (PE), fetal growth disorders, and fetal death. Traditionally, infertility specialists and obstetricians operate in parallel; however, increasing evidence supports the need to understand maternal and fetal health beginning at embryo implantation. The potential to target, prevent, and/or avoid risks before pregnancy occurs by leveraging knowledge of the periconceptional space could shift the focus of reproductive and obstetrical care to a much earlier timeframe, thereby avoiding significant neonatal and maternal morbidity, mortality, and healthcare cost burden.

Common clinical obstetrical practice consists of monitoring fetal growth for congenital or developmental problems that may result in pregnancy termination or, in some cases, additional post-birth interventions may follow. Congenital defects occur in 3%–5% of naturally conceived newborns, contributing to 70% of pediatric hospitalization and 20% of infant mortality. Many of these cases are preventable through embryo selection (1, 2). Most genetic disorders arise due to recessive or X-linked mutations; 84% of asymptomatic patients aiming to conceive are carriers of 549 recessive and X-linked gene mutations involved in severe childhood phenotypes (3). Most important, 5% of couples have coincident pathogenic variants conferring high risk for six different severe diseases to their offspring (3). In the US, 1 in 300 newborns has chromosomal trisomy, predominantly for chromosome 21, which results in Down's syndrome. The risk of Down's syndrome increases with increasing maternal age, and the incidence of this primary genetic cause of intellectual disabilities ranges between 1:40 and 1:2,000 deliveries (4). Embryo selection in the preconceptional period offers the possibility of preventing trisomy in newborns.

Infertility-associated conditions such as endometriosis, recurrent pregnancy loss (RPL), chronic endometritis (CE), or obstetrical conditions such as PE have, at least in part, pathological roots in the endometrium. PE can be life-threatening for the mother and child if not diagnosed and treated promptly. Currently, the only cure for PE is delivery of the placenta and infant, regardless of gestational age, leading to preterm birth (PTB). Conservative estimates attribute more than 76,000 maternal and 500,000 infant deaths to PE worldwide each year (5).

There exists compelling evidence that a deeper scientific understanding of the maternal-embryonic interface is fundamental to understanding human reproduction and preventing some causes of infertility and unwanted pathological consequences for the child and mother during pregnancy. In this review, we discuss i) preimplantation embryo genetics and how it can be leveraged to prevent genetic/chromosomal pathologies; ii) preimplantation endometrial transcriptomics from whole tissue to single-cell resolution and how it can help prevent implantation failure of endometrial origin; iii) the maternal decidua as a distinct autocrine-paracrine tissue modulating pregnancy in health and disease; iv) the endometrial fluid (EF) as the environment where endometrial-embryonic dialogue begins and is maintained through the first trimester of pregnancy; v) the periconceptional maternal-embryonic interface; vi) crosstalk across the maternal-embryonic interface; vii) the endometrial microbiome and its diagnostic possibilities; and viii) the myometrium in health and disease.

Improving our knowledge about the periconceptional space translates to advances in periconceptional care aims to target, prevent, and/or avoid risks before pregnancy occurs. Interventions during this period provide an opportunity not only for the child's medical future but also for their parents. The focus of periconception health is the wellbeing of the developing child, the prevention of late-onset obstetrical pathologies, and the adoption of healthy habits by the parents.

II. THE EMBRYO AND THE CONTINUITY OF THE GENETIC SPACE: CHROMOSOMES, SINGLE GENES, AND POLYGENIC TRAITS

The human zygote is the earliest form of a new, unique diploid individual, comprising haploid genome contributions from male and female gametes. Genomic programming in the mature oocyte guides embryonic development during early cellular divisions in a period when the newly combined embryonic genome is quiescent (6). In humans, embryonic genome activation (also known as the maternal-zygotic transition) occurs around the 4–8-cell stage of preimplantation development (7). In this phase, the embryo becomes transcriptionally active and develops with input from external stimuli in the surrounding tubal/uterine environment.

During the complex processes of gametogenesis and embryogenesis, several crucial biological activities require tight regulation and execution, including meiotic division, acquisition of oocyte cytoplasmic competence to drive early embryo development, correct fertilization, and pronuclei syngamy. Alteration of these molecular processes by genetic or epigenetic defects can result in a broad spectrum of phenotypic consequences, ranging from gross impairment of reproductive competence (leading to developmental arrest, failure to implant, or miscarriage) to minor flaws at the single gene or chromosome level, which may result in genetic disorders. Some genetic defects can be harbored by the parental genome and, following random assortment at meiosis and syngamy, inherited by the embryo/child. Monogenic (i.e., Mendelian) disorders can be dominant (i.e., one defective allele is sufficient to produce a pathological effect) or recessive (i.e., both gene copies must carry the pathological variant to manifest). The pathological effects of dominant defects with variable clinical manifestation may be visible in the parent; however, they may not manifest due to imperfect penetrance. Recessive genetic diseases are usually silent and asymptomatic in heterozygote carriers; however, when the ensuing embryo inherits two hypomorphic alleles from both parental genomes (whether in homozygosity or compound heterozygosity), gene translation results in a heavily compromised protein product that cannot be balanced by a normal allele (as it would for heterozygous carriers). A broad spectrum of genetic conditions falls into this category, such as infertility characterized by preimplantation embryo developmental arrest, fetal structural defects that manifest during pregnancy (fetal anomalies caused by pathogenic sequence variants in developmental genes, such as *COL1A1*, *PTPN11, FGFR3, CHD7,* and *PTPN11*) (8), and severe highly-penetrant childhood-onset recessive genetic diseases (e.g., spinal muscular atrophy and fragile X syndrome) (9). An estimated 3% of newborns are affected by recessive genetic disorders, which are responsible for 70% of pediatric hospitalizations and intensive care admissions in developed countries (1, 2).

De novo genomic alterations also have profound implications for pregnancy and fetal health and include genetic or chromosomal abnormalities not harbored by the parental somatic genome but arising primarily during germ cell division and maturation. This category includes aneuploidy, an incorrect set of chromosomes (partial or entire), which is the leading cause of early embryonic developmental failure,

pregnancy loss, and intellectual disability in humans. De novo events can also involve single genes or imbalances for small genomic regions, with the formation of novel point mutations or small duplications/deletions caused by errors in DNA repair machinery in germ cells. De novo pathogenic variants in critical genes involved in fetal development and clinically significant sub-microscopic chromosome copy number variations (CNVs) are the most frequent causes of fetal structural defects detectable by ultrasound (8, 10), with an incidence of around 1:600 and 1:300 pregnancies, respectively. De novo conditions can also arise during embryonic development and differentiation and can involve both chromosomal and single gene defects, leading to cytogenetic (i.e., chromosomal) and genetic mosaicism, respectively (11) (**Figure 1**).

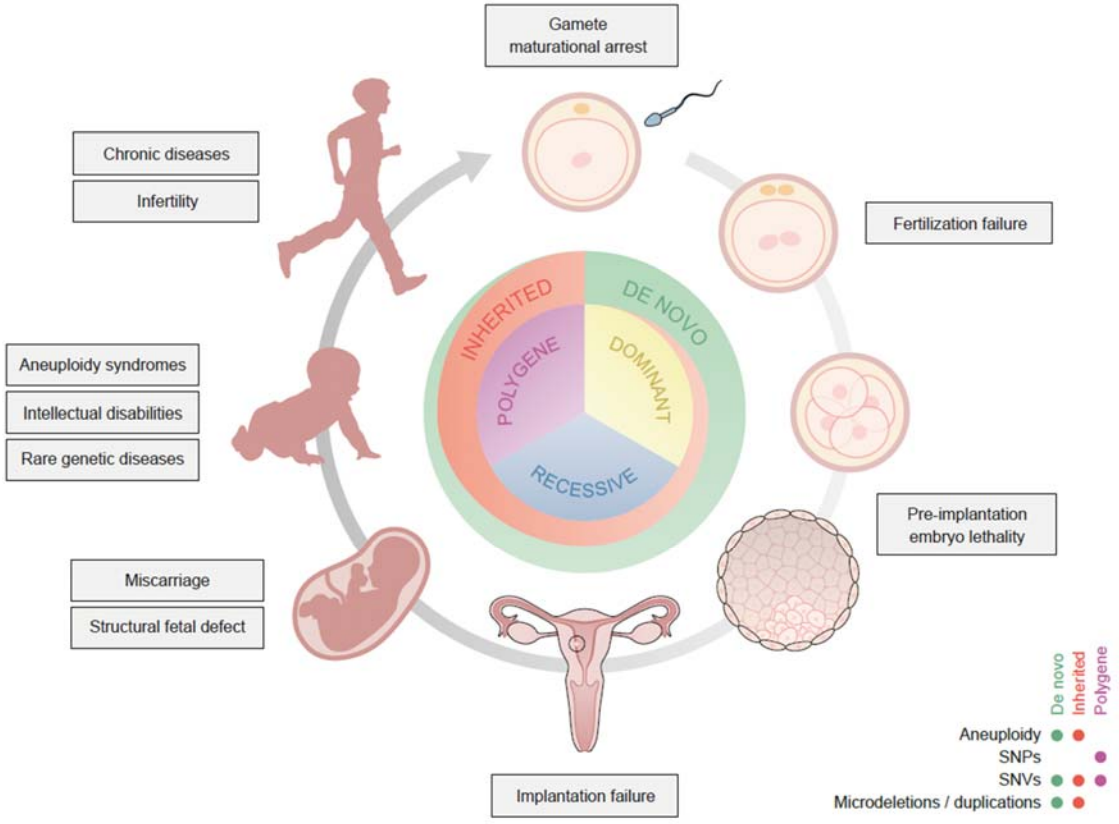


Figure 1. An individual's genome is composed of genetic features inherited from parents (as components of their genome or as de novo variants acquired by germinal cells) or acquired (as de novo variants during the first stages of embryo development). Genetic features that govern and modulate phenotypes primarily include aneuploidy, single nucleotide polymorphisms (SNPs), single nucleotide variants (SNVs), and microdeletions/duplications. Most inheritable genetic conditions are determined by defects in single genes (e.g., *CFTR* for cystic fibrosis) passed on by parents to their offspring. The phenotypic manifestation of genetic defects depends on the inheritance mechanism of their alleles: dominant or recessive. In addition, most genetic traits are

under multifactorial (polygenic) control, where a specific phenotype (e.g., height) is determined by the combined effect of several genes and their interaction with environmental and lifestyle factors. Embryo chromosomal defects causing embryo implantation failure, miscarriage, and aneuploidy syndromes in the newborn (among other conditions) are mostly de novo events due to chromosomal mis-segregation in oocytes prior to conception. Although several genes have been implicated in determining an individual's reproductive fitness, isolated defects in several genes determine early reproductive failure (e.g., gamete maturational arrest, fertilization failure, or preimplantation embryo lethality).

Another type of genetic mechanism causing increased predisposition to multiple chronic diseases (such as cancer, metabolic and cardiovascular conditions) is provided by polygenic interactions of multiple common genomic variants with individually low impact. Indeed, most human traits have a genetic background with numerous differences in common genetic variants, which include height, eye color, cognitive and behavioral characteristics, and individual predisposition to chronic diseases. In contrast with monogenic inheritance, where the phenotype results from the expression of a single gene, the transmission of these traits follows polygenic inheritance, where the trait or disease risk is provided by the cumulative effect of hundreds to millions of genetic variations throughout the genome. According to the World Health Organization, the percentage of individuals severely affected by polygenic diseases (e.g., cardiovascular disease, respiratory disease, cancer, and diabetes) is more than ten times higher than that of monogenic diseases (12).

Remarkable efforts are being made to establish comprehensive biobanks that can expedite research in population health by aggregating large-scale genomics and clinical data representing different ethnicities [e.g., UK BioBank]. These platforms enable the construction of increasingly powerful polygenic risk scores (PRS) from genome-wide association studies (GWAS) that provide an individual estimated genetic predisposition to specific traits or diseases. Summing the relatively small contribution of each genetic variant detected with genome-wide statistical association with a disease provides an additive genetic score. When applied to the general population, PRS identify individuals at increased risk (i.e., top 1^{st} or 5^{th} percentile) or with a low-risk profile (bottom 1^{st} or 5^{th} percentile) to inform more tailored and efficient provision of medical interventions or recommendations for behavioral changes to reduce risks. A recent study demonstrated the ability of PRS to predict polygenic disorders with a risk equivalent to monogenic disorders for several conditions (13). Additional landmark studies found that PRS can be effectively used in combination with other clinical risk factors and standard monogenic

screening workflow, providing an additive and equally powerful adjunct parameter for improving risk prediction and stratification for several chronic diseases in the general population (14). Moreover, using large-scale biobank data from the FinnGen Consortium, Mars and colleagues demonstrated that PRS improved clinical risk prediction determined using routine clinical thresholds for coronary heart disease, type 2 diabetes (T2D), atrial fibrillation, breast cancer, and prostate cancer; importantly, PRS was the best stand-alone predictor for early-onset events (15). In reproductive medicine, recent GWAS studies have highlighted genome-wide significant loci associated with age at natural menopause and its clinical extremes (e.g., premature ovarian insufficiency) (16). The application of PRS has thus emerged as a powerful clinical tool to improve precision medicine in the general and infertile population and empower the discovery of the molecular mechanisms causing common diseases.

Both inherited and acquired genetic and chromosomal defects in the newly formed zygote frame the development and health of the ensuing embryo and child (**Figure 1**). Investigations in preconception, preimplantation, and prenatal genetics offer opportunities for early diagnosis of reproductive risks and the potential to prevent reproductive failures, obstetrical complications, and genetic disorders.

A. Aneuploidies in gametes and embryo development

Oocyte and embryo aneuploidy represent the principal causes of infertility, miscarriage, and cognitive disability in humans. The predominant etiology for aneuploidy is chromosomal segregation errors during female meiosis, for which advanced female reproductive age is the leading risk factor. Indeed, aneuploidy affects 30%–80% of human oocytes (17, 18), and its incidence follows a U-shaped trajectory that characterizes the female reproductive lifespan from menarche to menopause. The presence of meiotically-derived aneuploidies in oocytes is thus the most critical determinant of human embryo reproductive competence. This apparent deficiency derives from the oocyte's unique developmental processes, which entail a maturational suspension in the prophase of meiosis I (dictyate), where chromosomes align on the metaphasic plate for decades before chromosomal segregation completes. This specific configuration facilitates chromosomal recombination through the formation of chiasmata across sister chromatids and homologous chromosomes and helps preserve the gamete until reproduction is possible. Once a month, from puberty to menopause, a cohort of follicles is recruited

for further growth, during which time granulosa cells increase estrogen production, reaching a threshold and triggering a peak in luteinizing hormone (LH) levels (19), which provokes a complex molecular cascade that stimulates ovulation and initiates the final oocyte maturation steps (20). Throughout the years of maturational suspension, defects accumulate in the molecular apparatus connecting chromosomes with the spindle responsible for anaphase resolution, predisposing to suboptimal chromatid detachment and migration to opposite cellular poles (21). Over time, oocyte centromeric chromatin undergoes decompaction due to partial loss of cohesin, a protein complex that consolidates kinetochores of sister chromatids in meiosis I and connects them during meiosis II (21). Progressive deconstruction of this complex destabilizes the integrity of the associated kinetochores, leading to defective connections with spindle microtubules. During anaphase of meiosis I, when homologous chromosomes diverge from the metaphasic plate and migrate to opposite spindles, unstable cohesion between the impaired kinetochores and microtubules, combined with the intense traction exercised by the spindle apparatus, further fragments kinetochores, critically affecting their ability to stabilize and coordinate chromosome migration. During meiosis II, suboptimal cohesion between sister chromatids leads to premature separation, disrupting the anaphase process, and ultimately resulting in aneuploidy. This sequence of events appears to be the main driver for the increased incidence of aneuploidy in the oocytes of women of advanced maternal age (18, 21–23). Commonly, aneuploidies deriving from defective meiotic processes affect every cell of the ensuing embryo, as aberrations are uniformly propagated through all daughter cells at each subsequent cellular division.

The aneuploidy rate in human embryos is highly consistent with that of oocytes, ranging from around 21% at age 30 to >85% for women over 43 (24). Distinct mis-segregation errors cause aneuploidy at different life stages, with whole chromosome non-disjunctions preferentially affecting younger females and sister chromatid cohesion defects characterizing most errors in older individuals (18). Only 2% of sperm are estimated to carry chromosomal aberrations (25); nonetheless, most aneuploidies do not prevent the initial stages of embryo development, demonstrated by the detection of diverse chromosomal defects in preimplantation embryos at both cleavage and blastocyst stages, including both monosomies and trisomies of most chromosomes as well as polyploidy and haploid configurations (26).

Because 50%–60% of first-trimester miscarriages present with chromosomal abnormalities, abnormal karyotypes are responsible for most failed pregnancies, with only some chromosomal defects compatible with later stages of embryonic development (4). For example, other than the 45,X karyotype observed in around 10% of pregnancy losses, monosomies are rarely detected in prenatal testing (<1% of miscarriages with chromosomal abnormalities) (27). This situation may be due to unbalanced epigenetic imprinting or the inability to support the intense transcriptional activities required to compensate for the absent second allele (haploinsufficiency for a whole chromosome) during endometrial invasion. Meanwhile, trisomies are detected in >60% of fetuses with an abnormal karyotype, followed by polyploidies (triploidy and tetraploidy) and structural chromosome anomalies at approximately 21% and 5%, respectively (28). Trisomy 16 represents the most common aneuploidy in late pregnancy losses (i.e., second trimester), followed by trisomy 22 and 21 (28), suggesting that abnormalities involving other chromosomes are severely deleterious for early embryo/fetal development. Most autosomal abnormalities detected in live births involve chromosomes 13 (Patau's syndrome), 18 (Edward's syndrome), and 21 (Down's syndrome), with an incidence in the general population around 1:7,000, 1:3,000, and 1:700 live births, respectively (29).

Both Patau's and Edward's syndromes show a high mortality rate due to complications exacerbated by congenital disabilities. Five-year survival rates for children affected by Patau's and Edward's syndrome are estimated at 9.7% and 12.3%, respectively (30). Most individuals diagnosed with Down's syndrome (~95%) show full trisomy 21, while partial translocation and mosaicism are detected in approximately 3% and 2% of cases, respectively (31). The survival rate of infants with complete trisomy 21 is estimated at 92.9% at one year and 88.6% at ten years (32).

Aneuploidies involving sexual chromosomes show more variable phenotypes, with a longer lifespan and much-improved quality of life compared to aneuploidies involving autosomes. The most common sexual chromosome aneuploidies include 47,XXY (Klinefelter's syndrome), 47,XXX, 47,XYY, and 45,X (Turner's syndrome), with incidences of approximately 1:500–1,000 males, 1:900–1,000, 1:1,000 live births, and 1:2,000–2,500 live female births, respectively (33). Individuals affected by sexual chromosome aneuploidies do not show a highly characteristic phenotype; indeed, although clinical

presentation often includes intellectual, motor, and neurological deficits, most Klinefelter's and XYY cases are undiagnosed (34). Commonly, individuals carrying sexual chromosome aneuploidies show a significant degree of variation in neurological and intellectual performance, suggesting that other genetic determinants related to the parental origin of the extra (or missing) chromosome X, its transcriptional activity, and the pattern of inactivation may affect the overall clinical presentation of the syndrome (34, 35).

In addition to meiotic aneuploidies, chromosome segregation errors can occur during embryo mitotic divisions at pre- and post-implantation stages (28). Depending on the embryo's developmental stage at the time of the mitotic error and the survival capability and biological fitness of the resulting secondary cell lines, a variable portion of the embryo can comprise additional cell lines with karyotypes distinct from the constitution of the zygote of origin. This phenomenon, known as chromosomal mosaicism, affects up to 3%–5% of human embryos generated via in vitro fertilization (IVF) (36–39). In-depth analyses of the genetic composition of mosaic preimplantation embryos demonstrate that abnormal cells in a mosaic embryo are equally distributed in the trophectoderm and inner cell mass (ICM) (36, 40). Most mosaic segmental aneuploidies detected in the trophectoderm are unconfirmed in ICM specimens (41). Chromosomal mosaicism is rarely detected at prenatal stages (true mosaicism ~0.4%) and in newborns (<0.2%) (39), even when derived from embryos diagnosed with mosaicism during preimplantation genetic testing (PGT) (42). Based on these findings, the discrepancy between preimplantation and prenatal/postnatal mosaicism detection frequencies may be due to mechanisms that mitigate the dissemination of secondary clones with abnormal karyotypes (42). These putative reparative processes include containment of the expansion of defective clones through increased apoptotic activity in aberrant cells and/or the targeted isolation of defective cells in extra-embryonic tissues, which prevents the involvement of the ICM and subsequent fetus. Detection of low/moderate chromosomal mosaicism in human preimplantation embryos (e.g., <50%) is not associated with the reduced reproductive capability to implant and progress to term; however, when detected in fetuses, mosaicism for specific chromosomes can result in fetal growth restriction and congenital abnormalities (43).

PGT for aneuploidies (PGT-A) using next-generation sequencing (NGS) represents an effective strategy for identifying embryos with chromosomal abnormalities. Based on non-selection trials data available to date, PGT-A-mediated diagnoses of uniform aneuploidy (i.e., non-mosaic episodes) provide a 99% (300/302; 95%CI:97.6%-99.9%) predictive value for early embryo/fetal lethality and a 100% (95%CI:98.8%-100%) predictive value for abnormal conception (44, 45). When considering only uniform aneuploidy findings, embryo selection minimizes the occurrence of pregnancy loss or chromosomally abnormal birth and improves pregnancy and live birth rate at the first embryo transfer (46, 47).

Mosaic chromosomal findings suggest a much lower impact on the reproductive capabilities of an embryo. A recent non-selection trial reported no differences in positive pregnancy, biochemical loss, miscarriage, and live birth rates between low-grade (<50%) chromosomally mosaic and uniformly euploid embryos (48).

Overall, PGT-A allows the identification of those embryos that carry a chromosomal abnormality and their active deselection from transfer to minimize the chance of failed implantation, miscarriage, and aneuploid pregnancy. Despite several reports describing its advantages, PGT-A technology remains widely debated. Critics are concerned that PGT-A cannot improve the live birth rate per cycle and that embryos can be either damaged by the biopsy procedure or erroneously discarded because of false positive aneuploidy results. In other terms, there exists a risk that viable and healthy embryos might be misclassified as abnormal and discarded from clinical use. This situation becomes even more apparent when PGT-A is employed to diagnose genetic features of uncertain significance (i.e., mosaicism or segmental imbalances) or when test accuracy is compromised in favor of easier application (i.e., non-invasive PGT-A). One recent randomized clinical trial reported a comparable cumulative live-birth rate in conventional IVF and PGT-A when three or more good-quality blastocysts were available (49); however, another similar randomized clinical trial observed no improvements in pregnancy rates, whether embryo transfers or intention to treat were considered (50). Nonetheless, breakdown data from Munne et al. and other studies showed that when PGT-A is employed in selective patient populations of advanced reproductive age, PGT-A may reduce time to pregnancy and miscarriage rates in patients

with RPL (51) and improve clinical outcomes in advanced maternal age (AMA) patients (51), supporting the crucial role of chromosomal abnormalities in the peri- and early post-implantation stages. A recent review of US national surveillance data (SART database) between 2014 and 2018 of day-to-day clinical practice, including over 420,000 cycles (30% of which were PGT-A), described an increase in the live birth rate per cycle in women aged 35 and older but decreased outcomes in those younger than 35, highlighting the critical role of patient selection for the application of PGT-A (47). More trials are required to fully determine the negative and positive contributions of PGT-A to IVF treatments for each subpopulation; furthermore, guidelines for minimum competence required by the embryology and genetics lab to carry out such diagnoses are also needed(52). Nonetheless, due to the complex confounding factors and variations characterizing the IVF and PGT population, it has been impossible, so far, to determine whether PGT-A offers clear benefits or drawbacks (53). Currently, both advantages (e.g., the deselection of aneuploid embryos and the reduction of cryostored embryos) and disadvantages (e.g., increased costs and labor) coexist in this application, with overall cost-effectiveness still undetermined; however, this type of investigation would be highly challenging due to the significant differences in cycle costs and insurance/institutional coverage across the world and the indefinable costs of adverse outcomes, including failed cycles, miscarriages, and chromosomally abnormal pregnancy/birth (54).

B. Inherited and de novo highly penetrant genetic variants

1. Pathogenic DNA variants causing preimplantation embryonic lethality

Pathogenic DNA variants in single genes can cause a broad spectrum of defective phenotypes, including specific infertility endotypes essential for oocyte and early embryo development. Such phenotypes include cases of complete oocyte immaturity, total failure of fertilization, or the inability of embryos to progress through normal preimplantation. GWAS and whole genome/exome sequencing can identify genes involved in defective gametogenesis and embryo developmental arrest and reveal the genetic foundation in unexplained infertility cases that account for up to 30% of the IVF population. Whole genome/exome sequencing and GWAS studies are instrumental in identifying both monogenic and polygenic variants involved in impaired spermatogenesis (e.g., *TEX* gene family), oocyte maturation

(e.g., *TUBB8, PATL2,* and *WEE2*), preimplantation embryo developmental arrest (e.g., *NLRP2, NLRP5,* and *TLE6*), and recurrent formation of hydatidiform moles (e.g., *NLRP7* and*KHDC3L*) (reviewed by (55)). If the causative role of these genes was confirmed in broader populations and by independent studies, preconception screening by NGS for candidate genes involved in reproductive failure in women suffering from isolated and unexplained infertility, whether occurring naturally or following assisted reproductive technology (ART), would be clinically valuable in determining the etiology of infertility.

Preliminary evidence supporting tailored molecular strategies to rescue lethal embryonic phenotypes has recently emerged. In 2018, during an intracytoplasmic sperm injection cycle, Sang and colleagues injected *WEE2* cRNA into the oocytes of a proband that had previously experienced recurrent fertilization failure in multiple IVF attempts and possessed defective variants in both copies of *WEE2*. All seven cRNA-treated oocytes displayed proper fertilization and developed into chromosomally normal blastocysts (56). More recently, a similar strategy was used as part of the infertility treatment of a woman with complete oocyte maturation arrest and homozygosity for *TRP13*. In this trial, all *TRP13* cRNA-injected meiosis I (MI) oocytes extruded the first polar body, and the resulting zygotes exhibited normal progression to the blastocyst stage (57). Although these findings require corroboration in more extensive trials and procedural safety needs to be demonstrated for clinical application, this preliminary result highlights the potential of novel genomic precision medicine to overcome and treat genetically imposed reproductive barriers.

2. Pathogenic DNA variants in fetal structural defects

Ultrasound detects fetal structural anomalies in around 3% of pregnancies, ranging from an isolated inconsequential anomaly to multiple defects fatally aggravating several organs (58). Genetic diagnostics are increasingly important to assess fetal structural anomalies and are associated with either aneuploidy, uniparental disomy, CNVs, and/or intragenic mutations (8). Conventional karyotyping has been used for more than thirty years to investigate prenatal anomalies; however, over the last decade, chromosomal microarray analysis has been increasingly adopted in prenatal genetic diagnostics due to an ability to detect sub-microscopic pathogenic CNVs, which increased anomaly detection frequency by 3%–5% (10, 59, 60). In 2012, Wapner and colleagues evaluated 4,406 abnormal pregnancies using

chromosomal microarray analysis; they identified 6% as having CNVs with clinical significance in fetal structural anomalies, marking a turning point for prenatal testing (10).

Two independent studies provided additional evidence to expand the diagnostic yield toward structural fetal defects and beyond chromosomal microarray analysis. These studies identified 8.5% and 10.3% of fetuses with clinically significant genetic variants through whole exome sequencing (8, 61). Additionally, fetuses with increased nuchal translucency had phenotype-specific variants at an incidence of 3.2% and 6.3%, respectively, and those with multisystem fetal structural anomalies had variants at an incidence of 15.4% and 18.9%, respectively (8, 61). In both studies, dominant de novo mutations were the most significant source of pathogenic variation, representing around 60% of diagnostic variants. For their specific etiology and inheritance pattern, these events are usually not associated with a higher risk of recurrence. Moreover, they cannot be anticipated in the context of preconception carrier screening based on parental DNA analysis from leukocytes. Another considerable fraction of cases (25%) were caused by recessive genes and were thus clinically actionable before a defective pregnancy was established (8, 61).

Once causative genetic variants are identified in the parental genome, and a risk profile is determined for the couple, it is possible to manage risk through PGT for monogenic disorders (PGT-M) by identifying affected embryos and deselecting them from the pool of blastocysts considered for transfer. Although significant improvements have been made in the genetic diagnosis of fetal structural defects, the etiology of a considerable proportion of cases remains unknown, and more extensive genomic translation research studies are required to fill this gap. In light of this evidence, prenatal chromosomal microarray analysis should be complemented by whole exome sequencing to provide the most comprehensive outlook on diagnosing fetal anomalies and improve the clinical management of pregnancies (62).

3. Pathogenic DNA variants and severe recessive genetic disease

Pathogenic variants in the coding portion of the genome can impair embryo development and cause implantation failure/miscarriage or support full-term fetal development but cause genetic disease in fetuses. This last type of genetic abnormality becomes fixed throughout development and cannot be

485 influenced by external factors, such as those mediated by the endometrium/placenta (63). A healthy
486 genome harbors an estimated three pathogenic variants of the approximately 2,000 genes responsible
487 for severe recessive highly penetrant childhood-onset conditions (autosomal recessive), leading to an
488 incidence of about 3-5 of every 1,000 newborns affected by pathogenic variants (2, 64). Unlike
489 autosomal dominant genes, which run in families and have clear clinical manifestations among
490 relatives, autosomal recessive variants often remain concealed until an affected birth occurs. Despite
491 the relatively low frequency of recessive conditions in the general population, there is a risk of an
492 affected child for 2%–4% of couples, even without positive family history or consanguinity (65, 66).
493 Furthermore, certain genetic conditions display more prevalence in geographically isolated populations
494 and societies with frequent consanguineous relationships, increasing the likelihood of homozygosity
495 for a determined pathological variant. Heterozygous carriers of a pathological variant may have a fitness
496 advantage compared to normal homozygous individuals, favoring transmission and propagation of the
497 pathological mutation in the local population (e.g., sickle cell disease in regions affected by malaria).
498 For these reasons, preconception carrier screening has been used for decades to limit the incidence of
499 severe genetic conditions in populations with specific ethnic backgrounds. Systematic carrier screening
500 strategies have demonstrated beneficial results in several regions. For example, the incidence of β-
501 thalassemia in newborns was as high as 1:250 in Cyprus and Sardinia (67); however, the introduction
502 of mandatory premarital disease screening drastically reduced the frequency of affected newborns,
503 bringing the incidence down to 1:4,000 (67).

504 NGS technology has drastically increased the diagnostic sensitivity of expanded carrier screening. As
505 the panel composition of commercially available expanded carrier screening tests differs in terms of
506 disease severity, carrier rate, gene-disease strength of association of the conditions included, and the
507 gene level of coverage (68, 69), the overall efficacy, residual risk, and clinical utility varies significantly
508 across expanded carrier screening panels. Nonetheless, expanded carrier screening can potentially
509 reduce affected pregnancies even when a minimal number of conditions are evaluated. For example, in
510 a study on preconception and early pregnancy care programs in Australia, the combined affected
511 pregnancy rate detected in routine testing of the three most common genetic conditions (cystic fibrosis,

spinal muscular atrophy, and fragile X syndrome) was comparable to the risk for Down's syndrome in the general population (70). These findings suggest that applying systematic expanded carrier screening testing at the preconception stage can effectively minimize the risk of pathological conditions.

Early detection of recessive genetic conditions at preconception offers the opportunity to avoid transmission of the pathologic genetic variant (e.g., IVF/PGT-M). Multiple studies have reported that expanded carrier screening testing displays clinical utility, and most at-risk couples opt for tailored reproductive approaches that minimize genetic risk, such as PGT-M (9, 71, 72). Although expanded carrier screening is an established technology for predicting and preventing genetic reproductive risk, more support is needed before expanded carrier screening can be implemented more universally (73–75). A thorough overview of the advantages, limitations, and ethical aspects of preconception expanded carrier screening was recently published by the European Society of Human Reproduction and Embryology (ESHRE) Ethics Committee (76).

Regardless of the function of the gene targeted for analysis during a PGT-M test, the technology employed is highly accurate and has been employed clinically since the early 1990s (77). PGT-M can be employed in virtually any circumstance where the causative relationship between the gene and the condition is well characterized. The American Society for Reproductive Medicine (ASRM) considers PGT-M ethically justifiable when employed for severe conditions and where no or ineffective interventions are available for the disease (78). Moreover, for less critical conditions, the ASRM deems PGT-M ethically acceptable as a manifestation of reproductive liberty, although clarifying that every patient should be informed of the potential long-term impact of the biopsy procedure. Especially in the case of PGT-M, patients are recommended to undergo counseling sessions with a clinical geneticist to receive detailed information on their conditions and allow for informed decision-making. Notably, adverse outcomes (i.e., affected pregnancy) are exceptional and often related to human errors (e.g., mislabeling of samples or the transfer of incorrect embryos), while misdiagnoses are rare (<0.1%) (79). Overall, when the reproductive genetic risk of a couple is known prior to conception (whether through family anamnesis or expanded carrier screening testing), IVF/PGT-M provides a robust strategy for

avoiding the transmission of the pathogenic trait to the ensuing pregnancy through the identification of the affected embryos and de-selection from the transfer.

C. Polygenic inheritance in the embryo

Until recently, technical limitations associated with the lack of evidence between genetic contribution and polygenic disease (and logistic/informative issues surrounding embryo selection based on polygenic inheritance) had hindered the application of PGT in polygenic diseases (PGT-P). The combination of highly informative population-level biobanks and new technical capabilities in obtaining reliable embryonic genotypes from small numbers of biopsied cells has made PGT-P theoretically applicable in clinical practice. From a technological perspective, it is possible to comprehensively profile human preimplantation embryos for multiple polygenic conditions, together with aneuploidies and monogenic diseases, from a single trophectoderm biopsy (80). The first clinical application of PGT-P was reported in 2019 by Treff and colleagues (81), resulting in the first baby born using this technology; however, certain sensitive aspects beyond technical capabilities currently prevent the broader application of this technology and must be taken into consideration (82, 83). Among the most important considerations is the absolute clinical gain achievable by PRS application in IVF, where only a few euploid/viable embryos are available for selection. Indeed, low intra-familial genetic variability combined with a small number of embryos to choose from does less to reduce disease risk when applied to IVF patients compared to the broader general population. Whatever the predictive power of various polygenic scores may be for clinical decision-making, it will be lower in the within-family embryonic PRS context. Another limitation is that most powerful PRS developed today were defined and verified in European individuals, and an extension to other ethnicities has yet to be performed for current PRS. With particularly relevance to the context of PRS application in human embryos, there exists a risk of pleiotropy - where a genetic variant influences distinct phenotypes. Pleiotropic effects may have juxtaposed results, where low-risk scores for certain conditions coexist with high-risk scores for other undesirable conditions (84). For instance, when discussing the limitations of PRS applications in IVF, Turley and colleagues presented the example of an embryo selected for intellectual abilities, which, in turn, would have a 16% increased risk of bipolar disorder (82). In reverse,

565 however, pleiotropy may also produce converging positive effects, where selection against a
566 pathological trait may include a reduction in risks of pathologies associated with the primary phenotype
567 (e.g., hypercholesterolemia and myocardial infarction) (85). In most cases involving polygenic effects,
568 relationships between genetic variants and phenotypes remain unknown, warranting caution in future
569 preclinical research applying PRS to embryo selection. Other unintended consequences of PRS
570 application for embryo selection are currently debated, including the possible alteration of population
571 demographics, which could aggravate societal inequalities through discrimination and devaluation of
572 particular traits (82).

573 Although numerous technologies have potential utility in IVF, evidence regarding their clinical validity
574 and utility remains unestablished. With an increasing understanding of the genetic basis of disease, a
575 unified (monogenic and polygenic) space of genomic information will likely be leveraged for
576 embryonic reproductive competence and future newborn health.

577

578 **III. THE SOIL. THE PREIMPLANTATION ENDOMETRIUM: FROM WHOLE TISSUE TO**
579 **SINGLE-CELL RESOLUTION**

580 Menstruation is a resetting process that occurs across the reproductive lifespan - from menarche to
581 menopause - to prepare the uterine cavity for synchrony of the blastocyst's arrival during the
582 endometrial receptivity window of implantation (WOI). The WOI allows blastocyst adherence and
583 triggers the completion of decidualization of the stromal compartments that control trophoblast invasion
584 and subsequent placentation. Endometrial receptivity and decidualization are postovulatory processes
585 of endometrial remodeling to prepare for pregnancy. Endometrial receptivity includes all the
586 morphological and molecular modifications known as plasma membrane transformation (see below).
587 During decidualization, uterine glands undergo secretory transformation, and endometrial tissue
588 exhibits vascular remodeling and infiltration by specialized immune cells (see Section IV).

589 Human embryo implantation rates are 50%–65% for euploid embryos (86), which is low compared to
590 other mammals (e.g., 95% for rodents and 96% for rabbit). This difference largely derives from the

591 endometrial control of human implantation and pregnancy compared to the embryonic control of
592 implantation in other species. The rodent embryo can direct implantation by delaying its metabolism
593 via embryonic diapause (87). Performing ovariectomy or hypophysectomy prevents estrogen secretion
594 during preimplantation in rodents, delaying implantation and triggering blastocyst dormancy (88).
595 Nevertheless, exogenous stimulation with injected estrogen rescues this process (89).

596 The human endometrium is formed by luminal and glandular epithelium, stromal fibroblasts, vascular
597 smooth muscle and endothelium, stem/progenitor cells, and immune cells. These cell types organize
598 themselves into two distinct functional layers, the basal layer with regenerative potential and the
599 functional layer, which undergoes proliferation, secretory changes, and tissue degeneration every month
600 (in the absence of pregnancy) from menarche to menopause mediated by ovarian steroids (90). The
601 human menstrual cycle has been historically classified into two major phases: proliferative and
602 secretory, with two subphases (early and late) in the proliferative and three subphases (early, mid, and
603 late) in the secretory phase. Briefly, in the endometrial proliferative phase, increasing estrogen (E2)
604 causes the proliferation of stromal cells and elongation of spiral arteries, while in epithelial cells is
605 associated with proliferation, differentiation, and cellular apoptosis (91). The endometrial proliferative
606 phase is characterized by an increased abundance of genes consistent with mitotic activity and tissue
607 remodeling (92, 93). The early-secretory phase begins with a rise in progesterone (P4) levels, induced
608 by ovulation and the formation of a corpus luteum. During this phase, cell metabolism and transport
609 increase, and there is negative cell proliferation regulation (93). The mid-secretory phase involves more
610 significant metabolic secretory and non-proliferative activity, with immense importance placed on the
611 innate immune response and the response to stress and wounding (93). E2 and P4 levels decrease during
612 the late-secretory phase, triggering extracellular matrix (ECM) degradation, inflammatory response,
613 and apoptosis leading to menstruation (95, 96).

614 **A. Endometrial receptivity**

615 Endometrial receptivity involves the coordinated actions of ovarian steroids (E2 and P4) produced by
616 the ovarian follicle acting through the estrogen (ER) and progesterone receptors (PR) (94) located in
617 the nuclei of endometrial cells. E2 is produced in growing ovarian follicles and drives the extensive

proliferation of epithelial, stromal, and vascular endothelium. At mid-cycle, a luteinizing hormone (LH) surge induced after E2 peak triggers ovulation, and P4 is produced by granulosa-luteinized cells in the corpus luteum. Glands become secretory when stromal cells start to differentiate (pre-decidualization), a process accompanied by endometrial edema (95).

The transition of the endometrium to its unique receptivity status that allows adhesion of the blastocyst involves remodeling of the uterine epithelium structure and function (**Figure 2**). Epithelial cells lose cell polarity due to the downregulation of the adhesion molecule E-cadherin on the cell surface, the inhibition of mucin 1 (MUC1), and the formation of apical pinopodes (96–98). The absence of implantation leads to luteolysis, withdrawal of P4 and, ultimately, menstruation. In contrast, the presence of an implanted blastocyst that secretes human chorionic gonadotropin (hCG) maintains the corpus luteum and P4 production, impacting the formation of the decidua and preventing additional implantation events after pregnancy has started.

1. Estradiol and progesterone receptors

The effects of E2 and P4 are mediated by their interaction with specific receptors. E2 (ERα and ERβ) and P4 receptors (PR-A and PR-B) are present in the cell cytoplasm. When present, hormones bind and activate their receptors, resulting in receptor dimerization and nuclear translocation. Activated hormone receptors can bind to DNA to regulate the expression of target genes (99, 100). The regulation of PR-A and PR-B changes across the menstrual cycle; both receptors are expressed at similar levels in glandular epithelium before subnuclear vacuolization, with decreasing PR-A during the mid-secretory phase. In the stroma, there is a predominant expression of PR-A throughout the cycle (101).

E2 directs early and late proliferative phases in the endometrium while modifying cell cycle gene transcription, DNA synthesis, epithelial mitosis, stromal edema, hyperemia, and immune cell infiltration. E2 drives proliferation via paracrine signaling with fibroblast growth factor (FGF) ligands. ER-induced stromal cell expression of FGF activates epithelial FGF receptors (FGFRs) that drive proliferation through downstream ERK1/2 or phosphoinositide 3-kinase (PI3K) pathways (102, 103). ERα is critical to female fertility and sensitivity to E2's mitogenic effects (104); however, loss of epithelial ERα does not affect the influence of E2 on epithelial cell proliferation, indicating that ERα's

stromal function is critical for cell proliferation. In contrast, loss of ERβ negatively impacts fertility but does not affect uterine functionality (105).

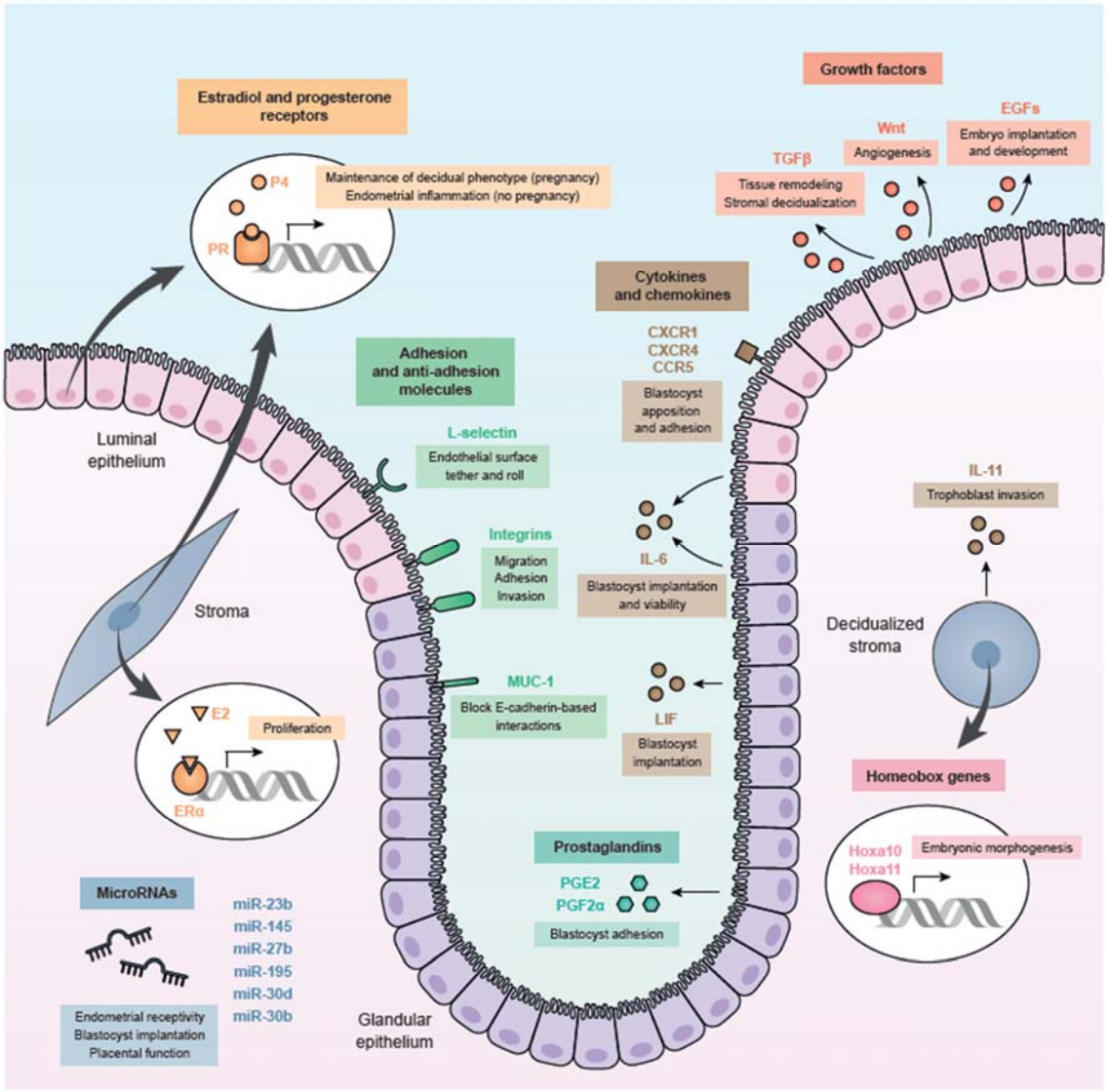


Figure 2. The endometrial plasma membrane transformations and the accompanying molecular processes required for the receptive state. Schematic representation of the processes and molecules implicated in acquiring the window of implantation (WOI).

During the menstrual cycle, steroid hormone signaling changes endometrial morphology. During the postovulatory phase, the most dramatic effects of P4 affecting uterine tissue specific-cells occur when PR-A and PR-B are detected in epithelial and stromal endometrial cells. The importance of PR is

demonstrated by antagonists like mifepristone (RU486), which cause a delay in endometrial maturation even at low doses and induce abortion when administrated during pregnancy (106, 107). P4 also maintains the decidual phenotype of stromal cells during the late luteal phase (108). If pregnancy is not established, decreased in P4 levels induce the expression of inflammatory cytokines, matrix metalloproteinases (MMPs), and chemokines that generate endometrial inflammation to signal the start of menstruation (109). In pregnancy, P4 remains high, playing an important role in maintaining complete decidualization for the first week (110). PR signaling may be involved in the development of certain endometrial diseases such as endometriosis, endometrial cancer, RPL, and the growth of uterine leiomyomas (reviewed by (111)).

Epithelial PR expression in mouse models increases during the days before implantation. Specifically, PR expression decreases in the uterine epithelium at the time of embryo attachment but increases in the endometrial stroma surrounding the implantation site. One of the leading functions driven by endometrial PR is the induction of Indian hedgehog (IHH) in the epithelium during the pre-receptive phase (112). As a result, IHH exerts its effects on its receptors (Patched and Smoothened [PTCH/SMO]) located on the surface of stromal cells. This paracrine signaling pathway drives the proliferation and differentiation of stromal cells in a PR-dependent manner. Thus, the induction of IHH in the epithelium is critical to embryo implantation and stromal cell decidualization (113). Interestingly, murine models lacking PR are infertile, exhibiting an ovulation defect, and hyperplastic uteri non-receptive to embryos (114). Artificially stimulating the hormone-primed uteri of these mice does not trigger a decidual response; furthermore, mice with the epithelial-specific loss of PR exhibit signs of infertility associated with persistent luminal epithelium proliferation (115).

2. Adhesion and anti-adhesion molecules

Specific changes occur in the luminal epithelium during plasma membrane transformation to achieve receptivity. Integrins are transmembrane glycoproteins formed by α and β subunits, each possessing an extracellular domain, a transmembrane section, and an intracellular domain acting as a receptor that interacts by non-covalent bonds. These subunits are activated by intracellular and extracellular signals and participate in various cellular processes such as migration, adhesion, and invasion (116–118). Some

integrins exhibit constitutive expression on the luminal epithelium, while others are regulated in the stroma during the menstrual cycle (116, 119). Integrins act as receptors for ECM ligands such as laminin, fibronectin, and collagen; additionally, they can transduce signals from soluble ligands (e.g., osteopontin) (120). Human α2β1, α3β1, α6β1, α9β1, αvβ1, αvβ3, αvβ5, and αvβ6 are localized on the luminal epithelium. Except for αvβ5 and αvβ6, these proteins also localize to the glandular epithelium, a layer that additionally expresses α1β1 and α4β1 (121, 122). The levels of specific integrins are most increased during the mid-luteal phase of the cycle and are used as receptivity markers for that reason. For example, α4β1 expression correlates with implantation success, as reduced expression in endometrial stromal cells (ESCs) associates with recurrent miscarriage while blocking intrauterine α4β1 with an antibody leads to delayed implantation or implantation failure (123).

The selectin adhesion molecules also participate in endometrial receptivity; these calcium-dependent molecules contain a highly glycosylated extracellular domain with a transmembrane domain and a cytoplasmic tail binding heterotypically to sialylated and fucosylated glycoproteins (116). E-, L-, and P-selectins are responsible for the endothelial surface tether and roll mechanism (124). Carbohydrate ligands (e.g., MECA-79) bind to the L-selectin present on the luminal epithelium, and this event occurs simultaneously with L-selectin expression on the embryo trophectoderm after hatching. Mid-luteal biopsies lacking MECA-79 mRNA expression predict low pregnancy probability (125).

Interestingly, the expression of anti-adhesion molecules in the endometrium is regulated by signals secreted by the blastocyst during the first stages of conception. A key player in the maternal-conceptus dialogue is the membrane glycoprotein MUC1, which is expressed by the apical surface of secretory epithelial cells. The MUC1 protein comprises an amino-terminal region containing a hydrophobic signal sequence and a transmembrane domain with a cytoplasmatic tail in the carboxyl-terminal section and likely acts to sterically block E-cadherin-based cell-to-cell interactions (126). In humans, MUC1 concentration increases during the WOI and decreases in the late secretory phase; however, despite the existence of a general steroid-mediated rise in MUC1 during the WOI, in vitro experiments show that paracrine effects from the blastocyst may cause local clearance of MUC1 at the attachment site (127). Recent studies demonstrate that MUC1 expression in the glandular and luminal epithelium in women

who experience recurrent implantation failure (RIF) is lower than in women with recurrent miscarriage, suggesting that MUC1 could serve for blastocyst selection by the endometrium contributing to reproductive failure in RIF patients; however, the exact mechanism explaining how MUC1 participates in embryo implantation remains unclear (128).

3. Cytokines and chemokines

Cytokines are a family of diffusible proteins that function as signaling agents in an intercellular soluble manner and play a vital role in regulating immune-related events (129). Cytokines are released by the maternal tract and produce paracrine signals that influence embryo development and physiology (130–132) and bind to high-affinity receptors located on specific target cells to activate a broad range of JAK/STAT family tyrosine kinases (133–135). Ovarian steroid hormones regulate the production of endometrial cytokines, and paracrine factors can also be secreted by adjacent endometrial cells or emanate from microorganisms or the trophectoderm. Dysregulation of cytokine production is related to implantation failure and miscarriage, with the most crucial cytokines in the preimplantation endometrium belonging to IL-6 family - specifically IL-6, IL-11, and leukemia inhibitory factor (LIF) (136). Signal transduction in the IL6 cytokine family is dominated by STAT3 activation, with IL-27, whose signals are activated via STAT1, being the only exception (137).

IL-6 employs the common signaling receptor glycoprotein 130 kDa (gp130); the cytokines that use gp130 for signal transduction might also possess specific receptors on target cells. IL-6 regulates several aspects of the acute-phase reaction, immune response, and hematopoiesis, as demonstrated in mouse models (137, 138). IL-6 levels remain low under physiological conditions and increase during processes such as inflammation. IL-6 signaling is mediated by a receptor, soluble (s)IL-6R, during pro-inflammatory processes and the IL-6R receptor during anti-inflammatory processes (137). In the endometrium, IL-6 mRNA and protein are residually expressed during the proliferative phase and increase in the luminal and glandular epithelium during the mid-secretory phase of the menstrual cycle (139). In vitro, IL-6 production is stimulated in endometrial epithelial primary cells by IL-1b in a dose/time-dependent manner acting through a classic ligand-receptor interaction (139). This finding, coupled with the increased concentrations of E2 and P4 in the secretory endometrium, suggests that

737 these hormones ultimately regulate the IL-6 production required for implantation (139, 140). In human
738 stromal explanted cells, IL-1β (together with IL-1α, tumor necrosis factor [TNF], and interferon-γ [IFN-
739 γ]) promotes IL-6 production while E2 inhibits production (139). Anomalous expression of IL-6 is
740 associated with recurrent miscarriage; specifically, endometrial IL-6 mRNA levels (stimulated by IL-
741 1β) remain significantly lower during the mid-secretory phase in recurrent miscarriage patients
742 compared with controls (141), suggesting that IL-6 plays a critical paracrine or autocrine role in embryo
743 implantation (140). Furthermore, elevated levels of IL-6 are linked to reduced implantation rates in
744 patients with endometriosis via oocyte and embryo quality impairment (142). Analysis of the secretome
745 of implanted blastocysts grown in a sequential culture system versus those co-cultured with endometrial
746 epithelial cells has shown that embryo viability is related to IL-6 consumption (143). The same study
747 found that IL-6 is the most abundantly secreted protein in endometrial epithelial cells in the co-culture
748 to be metabolized by blastocysts. Compared to the sequential culture system from blastocysts that did
749 not implant, media from the culture of blastocysts that later successfully implanted is nearly devoid of
750 IL-6 (143), reinforcing the hypothesis that IL-6 consumption and metabolism play an essential role in
751 blastocyst development and perhaps during the implantation process. For this reason, remaining IL-6
752 levels present in single blastocyst-conditioned media after co-culture with endometrial epithelial cells
753 were proposed as an indirect biomarker of embryo implantation to complement morphology; however,
754 this approach is not currently in clinical use due to the high level of inconsistency observed (144).

755 IL-11 plays a crucial role in tissue remodeling, hematopoiesis, and inflammation. IL-11 gene expression
756 is induced by IL-1α; meanwhile, phorbol myristate acetate (PMA) induces IL-11 mRNA stabilization
757 through tyrosine kinase pathways, as demonstrated in the PU-34 stromal cell line derived from primate
758 bone marrow (145). In the endometrium, IL-11 and its receptor IL-11Rα localize to decidualized
759 stromal cells during the mid-secretory phase. The IL-11Rα receptor is constitutively expressed during
760 normal pregnancy in mice, with the protein maximally expressed during decidualization in response to
761 the presence of the blastocyst (146, 147). In humans, IL-11 is present in stromal cells during the
762 preimplantation stage before becoming reduced in the luteal phase; protein expression remains high
763 during the first trimester of pregnancy but decreases during the second trimester (146, 147). IL-11 is

764 also present in epithelial cells in basal conditions, as proposed using an in vitro model of cultured human
765 cells in which basal protein production was higher in epithelial cells than stromal cells; however, IL-11
766 production in stromal cells is higher when stromal cells are decidualized in vitro (148). The functional
767 relevance of IL-11 in infertility has been demonstrated by mice lacking IL-11, which display diminished
768 fertility and small litter sizes. Furthermore, female mice carrying the null mutation in the IL-11R alpha
769 chain are infertile due to a defective decidual response of the stroma to an implanting blastocyst (147,
770 149). IL-11 is crucial for the decidualization process in response to the presence of the blastocyst in
771 mice; however, decidualization occurs spontaneously during the acquisition of receptivity and does not
772 depend on embryo presence in humans. Of note, IL-11 and IL-11R expression during the secretory
773 phase of the menstrual cycle suggest that they also play important roles in human decidualization. In
774 addition, women with recurrent miscarriages display IL-11 deficiency in the endometrial epithelial
775 compartment during the peri-implantation period, suggesting that a higher IL-11 abundance may
776 prevent miscarriage (147, 150, 151).

777 LIF represents a crucial regulator of cell processes, including proliferation, differentiation, and survival
778 (152). Under maternal control, this pro-inflammatory factor displays increased expression during the
779 WOI. Like other cytokines, LIF binds to its receptor, LIFR, and co-receptor, gp130, to trigger signaling
780 through several pathways (e.g., JAK/STAT, MAPK, and PI3K) (153). LIF is critical to murine
781 reproduction; blastocyst implantation does not occur in LIF knockout female mice (154); however, the
782 possible role of LIF in implantation failure and unexplained infertility in humans remains unclear.
783 During the WOI, LIFR and gp130 secretion is reduced in infertile patients (155). Although the
784 administration of recombinant human (rh)LIF has been hypothesized to help improve pregnancy rates,
785 a clinical trial in women with implantation failure investigating the efficacy of administration of (rh)LIF
786 during the luteal phase failed to show improvements in clinical pregnancy rates after treatment (156).
787 While this result underlines discrepancies in the effect of LIF between mice and humans, the
788 implantation failure in patients in this study could be caused by factors unrelated to LIF, thereby
789 explaining why adding rhLIF does not improve pregnancy rates in these patients.

Cytokines such as IL-4, IL-5, and IL-10 participate in the maintenance of pregnancy, while high levels of IL-2, IFN-γ, and TNFα have been detected in women with unexplained recurrent miscarriages (157). IL-15 is mainly expressed at the maternal-embryonic interface during the peri-implantation period and during pregnancy, with its homeostasis essential for establishing and maintaining healthy pregnancy in both mouse and human (158). IL-15 is involved in hormonal control of the human endometrium, as demonstrated in cultured endometrial epithelial cells showing that P4 induces IL-15 mRNA expression. IL-15 effects are mediated by an IL-2/15Rβ membrane receptor and a specific receptor (IL-15RA) (159).

The matrix metalloproteinases (MMPs) are a group of zinc-dependent proteins that play essential roles in the decomposition of ECM, vascularization processes, and tissue resorption and remodeling. MMPs can cleave different ECM components and non-matrix proteins using a system of proteolytic enzymes (160, 161). These effects confer MMPs an important role in endometrial tissue remodeling across the menstrual cycle and the degradation of ECM proteins during trophoblast invasion into the decidualized stroma (160, 161). MMP-2 and MMP-9 are implicated in placentation and uterine expansion in normal pregnancies but can be altered in obstetrical complications like preeclampsia due to decreased vasodilation that, as a consequence, prompts increased vasoconstriction, which can generate premature labor (162). Studies have also highlighted the significant role of MMP-3 in endometriosis; meanwhile, MMP-2 levels increase in stage III/IV endometriosis (163). A specific maternal polymorphism within the MMP3 gene has been found in patients with RPL, highlighting an association between this polymorphism and recurrent miscarriage (164).

Chemokines are fundamental molecules that regulate leukocyte recruitment from the blood and display directional movement within tissues. Chemokines can be dispersed via the lymphatic system, producing their effects through G protein-coupled receptors (GPCRs) (165). Chemokines are classified through their structural motifs: CXC, CC, CX3C, and C groups, or the α, β, γ, and δ subfamilies (166). A study that co-cultured endometrial epithelial cells with human embryos to explore paracrine effects detected the expression of the chemokine receptors CXCR1, CXCR4, and CCR5 in the endometrial epithelial

monolayer and the presence of a human blastocyst, expression of these receptors substantially increased and became polarized to one pole of these cells (167).

Despite the large number of published studies showing associations of the abundance of certain molecules (cytokines, chemokines and growth factors) with the different phases of the menstrual cycle or certain pathologies, the molecular and cellular mechanisms that produce these changes remain largely unknown. Most of the molecular studies published to date have been carried out in animal models or using in vitro cultures of human cells, suggesting but not convincingly demonstrating the in vivo functional role of these molecules in humans.

4. Growth factors

Growth factors are a group of polypeptides and peptides that interact with cell membrane receptors to activate intracellular signaling pathways (168, 169). The transforming growth factor beta (TGF-β) superfamily encompasses 42 known dimeric proteins (170, 171) divided into two subfamilies: the TGFβ/activating/nodal subfamily and the bone morphogenetic protein (BMP)/Mullerian inhibiting substance/growth and differentiation factor subfamily (172). TGFβ family signaling is associated with reproductive processes and tissue remodeling. TGFβ proteins specifically participate in preparing the endometrium for implantation by promoting the activation of stromal decidualization (173, 174); however, decreased TGFβ expression occurs in patients with RIF (175) suggesting a potential role in endometrial receptivity or embryo implantation.

Wnt proteins function in epithelial-mesenchymal interactions and cell specification, act as growth factors in the uterus, and are required for the growth of ESCs and organoids (176–179). Wnt signaling acts through an unrelated receptor tyrosine kinase or by a family of receptors that induces β-catenin stabilization (180). Wnt signaling also regulates the vascular endothelial growth factor (VEGF) family of angiogenesis effectors. VEGF-A interacts with the two VEGF receptors (VEGF-R1 and VEGF-R2, also known as FLT-1 and KDR), with these interactions playing important roles in angiogenesis and impacting several human pathologies (181). A specific VEGF isoform (VEGF165) binds to neuropilin proteins (NPN1 and NPN2), which are transmembrane proteins unrelated to VEGF-Rs. An interaction between VEGF165 and NPN2 promotes VEGF165 binding to VEGF-R (182). Significantly, pathway

redundancy and crosstalk increase the complexity of biological responses, making it difficult to identify mechanisms underlying paracrine mediation of hormone action (183). In women with unexplained infertility, levels of VEGF165 in the EF are significantly decreased during the WOI. In mice, rhVEGF administration substantially improves preimplantation embryo development, in vitro embryo outgrowth, implantation rates, and fetal growth in vivo. These findings further support the existence of a precise paracrine and autocrine dialogue between the blastocyst and endometrial epithelium during the WOI and highlight the importance of the changing microenvironment as the embryo develops (184).

The EGF family includes EGF itself, heparin-binding EGF (HB-EGF), TGFα, epiregulin, neuregulins, β-cellulin, and amphiregulin (185). These interact with the ErbB receptor family, which consists of four tyrosine kinase receptors: ERBB1 (EGF-R), ERBB2, ERBB3, and ERBB4 (186). EGF and ErbB proteins exhibit spatiotemporal expression patterns in the uterus during the peri-implantation period, suggesting that these growth factors have compartmentalized functions during implantation (187–191). During the late secretory phase, the expression of HB-EGF peaks, suggesting that it plays a critical role in embryo development (192). HB-EGF has a dual role as a growth factor contributing to blastocyst development and as an attachment factor preparing the endometrial luminal epithelium for blastocyst adhesion during implantation. Human blastocysts that express cell surface ERBB4 can adhere to cells that express the transmembrane form of HB-EGF (192), specifically day-4 blastocysts expressing ERBB4 on the cell surface (193). ERB4 is primarily located in connective tissues and sub-myometrial stroma and is expressed at basal levels throughout the stroma (194). Interestingly, the luminal epithelium at the implantation site expresses epiregulin ligands and β-cellulin that could interact with ERBB1 and ERBB4.

5. Homeobox genes

Homeobox (HOX) genes are transcriptional regulators related to differentiation and control of embryonic morphogenesis (195). Specifically, these genes encode a 60-amino acid homeodomain responsible for sequence-specific DNA binding of larger homeodomain proteins. HOX proteins are transcriptional transregulators that can activate or repress the expression of specific target genes (196). Early studies in animal models describe the upregulation of Hoxa10 and Hoxa11 during endometrial

receptivity in a steroid hormone-responsive manner by inducing decidualization in stromal cells (197). *Hoxa10*$^{-/-}$ and *Hoxa11*$^{-/-}$ knockout mice have low receptivity due to defective decidualization (DD), with a stronger phenotype in *Hoxa11*$^{-/-}$ knockout. *Hoxa10*$^{-/-}$ mice exhibit downregulation of the cyclin-dependent kinase (CKD)4/6, cyclin D3, and p21 cell cycle regulatory axis (197, 198). Likewise, *Hoxa11*-/- mouse uteri lack cytokine LIF expression, reinforcing its crucial role in uterine receptivity (199). During a regular human menstrual cycle, the expression of *HOXA10* and *HOXA11* is maximal during the mid-secretory phase and remains elevated afterward; however, these genes are downregulated during the secretory phase of the menstrual cycle in infertility-related disorders like polycystic ovary syndrome, leiomyoma, hydrosalpinx, and endometriosis (200–204).

Other Hox genes may also play a significant role during the implantation process. For example, mutation of H6 homeobox 3 (*Hmx3*) generates implantation failure in mice (205). Hox genes *Msx1* and *Msx2* are tissue morphogenesis regulators important for uterine stromal-epithelial interactions (206). *Msx1* and *Msx2* expression on the luminal and glandular occurs during day 3 and 4 of pregnancy, with downregulation occurring at the end of day 4 as the blastocyst attaches. Knockout of *Msx1* or *Msx2* results in subfertility, while double knockout abolishes fertility, suggesting a compensatory mechanism between these genes (207, 208). Double knockouts exhibit a luminal epithelium that remains polarized and columnar with tightly adhered cells rather than a less polar, cuboidal state that permits blastocyst attachment. Moreover, this enhanced polarity in the uteri of double knockouts correlates with increased formation of complexes of E-cadherin/β-catenin (mediated by Wnt5) at adherens junctions in receptive endometrium (207).

6. Prostaglandins

Prostaglandins (PGs) are secreted into the EF and play specific roles in leukocyte recruitment, vascular permeability, embryo transport, stromal decidualization, trophoblast invasion, and ECM remodeling and prepare a pro-inflammatory environment during embryo implantation (209, 210). PGs are bioactive eicosanoid lipid compounds derived from arachidonic fatty acid (AA) (211) through the activities of PG synthases, which enables the formation of a set of PGs: PGD2, PGE2, PGF2α, and PGI2 (212). PGE2 and PGF2α are crucial for endometrial receptivity (213). The specific action of PG is mediated

by binding to GPCRs, which include four PGE2 subtypes (EP1, EP2, EP3, and EP4) and one PGF2α subtype (FP) (214). Indeed, PGE2 and PGF2α concentrations measured 24 hours before embryo transfer helped predict pregnancy outcomes (213). Moreover, using mouse embryos as a model, blocking PG receptors on the trophectoderm membrane reduces embryo adhesion rates, suggesting that PGs are essential for acquiring a proper adhesion phenotype (213).

The specific role of PGs in embryo implantation is supported by experiments in animal models demonstrating that the inhibition of PG production using female mice lacking phospholipase A2 (PLA2) and cyclooxygenase (COX) enzymes prompts a significant defect in the implantation process (215). Among COX-derived prostaglandins, COX1 and COX2 exhibit spatiotemporal expression during pregnancy (216). In mice, COX1 (PTGS1) expression in the epithelium occurs early on day 4, suggesting a role in luminal closure for blastocyst apposition. COX2 (PTGS2) induction occurs in the luminal epithelium and stroma underlying embryonic attachment sites, indicating roles in attachment and local endometrial vascular permeability. Moreover, *Ptgs2*$^{-/-}$ mice show implantation failure, but PTGS1 compensation partially rescues implantation (216). COX2 activates the uterine peroxisome proliferator-activated receptor-δ (PPAR-δ) and retinoid X receptor (RXR), suggesting a specific role in implantation (217). While maternal PPAR-δ is critical in implantation and decidualization, placentation requires embryonic PPAR-δ (218). PTGS2 localizes to the antimesometrial decidua upon attachment but is displaced to the opposite (mesometrial) side by day 6 of pregnancy (216). In *Ptgs2*$^{-/-}$ females, ovulation, fertilization, decidualization, and placentation are defective; these phenotypes implicate COX2-derived prostaglandin signaling across female reproduction. In addition, epithelial sodium channels must be activated to induce COX2 at the implantation stage (219).

7. MicroRNAs

MicroRNAs (miRNAs) are small sequences (19 to 22 nucleotides in length) of non-coding RNA that function as endogenous epigenetic regulators of gene expression (220, 221). miRNAs are transcribed by RNA polymerase II as large primary miRNAs or pre-miRNAs and subsequently processed by the ribonucleases Drosha and Dicer to give rise to their mature form. Mature miRNAs are then incorporated into the RISC (RNA-induced silencing) complex, where they bind to the mRNA 3' untranslated regions

complementary region, inducing their degradation or inhibiting their translation, resulting in gene silencing (222, 223). The association between mRNA and miRNA is established through short recognition sequences of 6-7 nucleotides, meaning that one single miRNA can target hundreds of different target genes, and at the same time, one gene can be regulated by several miRNAs. Mature miRNAs regulate hundreds of mRNA targets through complete or incomplete complementation with mRNA 3' untranslated regions (224–227). While the general function of miRNAs is to promote translation-inhibition events or RNA degradation, there is evidence that miRNAs play a role in inducing protein expression or mRNA (228). miRNA function can be altered by endogenous sponges or interactions with RNA-binding proteins, such as competing RNAs (228). Extracellular miRNAs can be transported by high- and low-density lipoprotein (229, 230) and can be bound to other proteins such as argonaute2 (AGO2) (231, 232) and nucleophosmin1 (NPM1) (233). Extracellular vesicles (EVs), such as apoptotic bodies (234), microvesicles (235), or exosomes (236) mediate miRNA transport by protecting the molecules from degradation and stabilizing them (237).

Multiple miRNAs have been linked to endometrial receptivity, implantation, placental function, and parturition (238). Some differentially expressed miRNAs (e.g., miR-23b, miR-145, miR-27b, and miR-195) were identified by endometrial biopsies in women with RIF compared to women with proven fertility, suggesting that these miRNAs participate in biological functions related to implantation, such as Wnt signaling (reviewed above), cell adhesion molecules (reviewed above), adherens junctions, p53 signaling, and cancer pathways (239). Additionally, the upregulation of miR-145 has been linked to the downregulation of ERα, suggesting that targeting miR-145 could represent a treatable target for RIF patients.

miRNAs have been evaluated as biomarkers for human endometrial receptivity, and deep sequencing approaches have provided the first miRNA profiles comparing pre-receptive (LH+2) and receptive (LH+7) states in natural and stimulated cycles. In regular cycles, LH+7 exhibits eight upregulated miRNAs and twelve downregulated miRNAs compared to LH+2, and bioinformatics analysis indicates that the target mRNAs are expressed during the WOI (240). At the time of implantation, miR-30b and miR-30d become upregulated, while miR-494 and miR-923 become downregulated in the receptive

endometrium (241). The secreted miRNA profile of the human secretory endometrium was determined using NGS in samples of EF, with 81 predicted interactions of differentially expressed miRNAs in hormone replacement therapy (HRT) cycles. Nevertheless, no differences between natural and HRT cycles were found, emphasizing the functional clinical similarities of the approaches (242). miRNAs also participate in decidualization; in vitro decidualization studies on primary ESCs have described the upregulation of 26 and the downregulation of 17 miRNAs associated with this process (243). Specifically, secreted miR-30d may represent a biomarker of endometrial receptivity and is downregulated in decidualized stromal cells compared to non-decidualized cells (244). A recent study noted that endometrial miRNAs transit from the maternal endometrium to preimplantation embryo trophectoderm cells via a novel cell-to-cell communication mechanism (245). Furthermore, B6C3-derived mouse embryos contain both EV-associated and free miR-30d, which results in the overexpression of adhesion-related genes, including *Itgα7* and *Cdh5* (245).

B. The human endometrium at single-cell resolution

Single-cell sequencing genomics, highlighted as "Method of the Year" in 2013 (246), has become a routine component when investigating cellular heterogeneity in human tissues, and the number and scope of studies and technologies continue to expand. A prominent example is the Human Cell Atlas (247) an initiative to map the numerous cell types and states in all organs and tissues of the human body.

The origin of these studies is rooted in the heterogeneity of mammalian tissues. Based on a bulk tissue sample, the information gathered from expression profiling averages the expression profiles of all constituent cells. Technological advances that enable the study of single cells through automated super high-throughput isolation and analysis have circumvented this problem (248, 249). Furthermore, these methods have advanced from analyzing only small numbers of individual cells, particularly for cell types that are not easily obtained in large numbers (e.g., oocytes or cells from the early embryo) (249), to routine sequencing of hundreds of thousands of cells in one experiment using microfluidics and droplet-based techniques together with combinatorial indexing strategies (250–254).

Circulating steroid hormones within the endometrium's multiple cell types are finely coordinated with their cognate receptors; therefore, biological functions are tightly regulated in time and space, with

clinical abnormalities occurring when the equilibrium is disturbed. The endometrium comprises different cellular components: epithelial cells, stromal cells, vascular wall cells, and immune cells, with each cell type exhibiting a unique gene expression profile that evolves with the progression of the menstrual cycle. The classical histological characteristics of the endometrium at specific sample collection times impact tissue gene expression profiles (255, 256). The molecular signature of endometrial receptivity continues to be refined as RNA-sequencing technologies advance from the tissue (257) to the single-cell level (151). These approaches identify significant changes in epithelial and stromal components as the endometrium transitions from pre-receptive to receptive phases.

Proportions of cell types vary along with the menstrual cycle; however, the precise impact of these variations is not measurable in whole-tissue gene expression profiling (258). Modern single-cell transcriptomics informs endometrial function, adding to gene expression analysis the new dimension of cell populations implicated in each time point or condition of study (151). Many studies have used single-cell RNA-sequencing (scRNA-seq) to define cellular compositions in human reproductive tissues (151, 259, 260). **Figure 3** depicts a schematic representation of the cell types described in these studies.

1. Endometrial cell populations across the menstrual cycle

A hallmark study recently provided molecular and cellular cartographies denoting the endometrial changes occurring across the menstrual cycle using microfluidics (C1 Fluidigm) and nanodroplets (10x Genomics) (154). This study analyzed a total of 73,180 single cells from endometrial tissue samples of 27 healthy donors, identifying six major endometrial cell types: epithelial cells, an epithelial ciliated cell type, stromal fibroblasts, endothelial cells, macrophages, and lymphocytes. Highly differentially-expressed genes can act as markers that clearly distinguish cell types. An interesting cell type with motile cilia functions emerged from an analysis of genes expressed in the endometrial epithelium. Using RNA and protein co-staining for ciliated endometrial epithelial cells, the authors visualized the spatial distribution of cells in situ. Their analysis confirmed four genes as markers (*C11orf88*, *C20orf85*, *FAM183A*, and *CDHR3*) and confirmed the consistent co-expression with the canonical regulator of

cilia functions *FOXJ1*. Ciliated cells were localized in an embedded position within both luminal and glandular epithelium (151); however, the function of these ciliated cells remains undetermined.

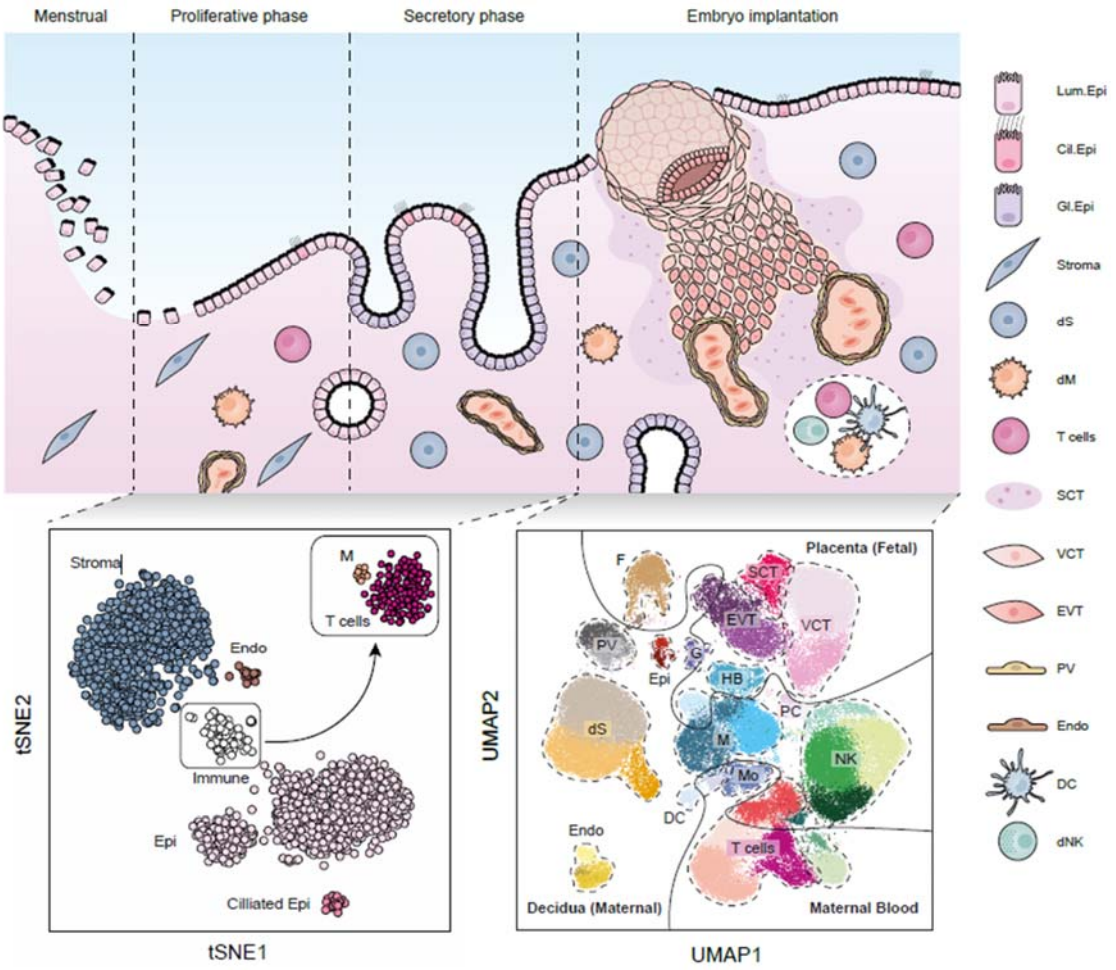


Figure 3. Endometrial cell types and alterations mapped by single-cell RNA-seq during the menstrual cycle and embryo implantation. Left panel; C1 Fluidigim single-cell atlas modified from (151) Right panel; 10X single-cell atlas modified from (261). Cell types present in the upper panel - Lum.Epi: luminal epithelium; Cil.Epi: ciliated epithelium; Gl.Epi: glandular epithelium; dS: decidualized stroma; dM: decidual macrophage; SCT: syncytiotrophoblast; VCT: villous cytotrophoblast; EVT: extravillous trophoblast; PV: perivascular cells; Endo: endothelium; DC: dendritic cells; dNK: decidual natural killer cells. Cell types present in the lower panel - Epi: epithelium; F: fibroblasts; HB: Hofbauer cells; PC: plasma cells; Mo: monocytes; M: macrophages; NK: natural killer cells.

Beyond the descriptive view of cell types constituting the endometrium, there exists emerging evidence of gene expression changes across temporal phases of the menstrual cycle in the stroma and epithelium (151). A major transcriptomic shift occurs in the epithelium when the tissue enters the WOI. These

changes are not exclusive to the epithelium and also occur in the stromal compartment (with the expression of *DKK1* or *CRYAB* (151)). Single-cell data have corroborated findings in bulk tissue during receptivity and revealed different menstrual cycle timing among cell subsets. Decidualization initiates in the stromal compartment before the WOI opens and is marked by *FOXO1* and *IL-15* (151). In the decidualizing endometrium, lymphocytes overexpress markers of uterine natural killer (uNK) cells during pregnancy (*CD69*, *ITGA1*, *CD56*), genes encoding IL-2 receptors, and diverse activating and inhibitory NK receptors. Immunofluorescence assays of ligand-receptor pairs provide support for communication between CD3+/CD56+ lymphocytes and stromal fibroblasts in decidualizing endometrium (262). These findings dissected tissue events along the menstrual timeline from the perspective of each main cellular component, measuring the gap between the decidualizing stroma and the receptive epithelium for the first time.

An integrative approach to combining data from the first menstrual cycle atlas (151) with newly generated single-cell and spatial transcriptomics data (260) revealed additional cell states; this included a population of fibroblasts (C7) restricted to the basal layer and yielded new insights on distinctive signatures in luminal and glandular parts during epithelial gland reconstitution in the proliferative phase. In this work (260) the authors deployed a strategy of spatial mapping with 10x Genomics Visium and quantitative multiplexed Single-molecule fluorescence in situ hybridization (smFISH) that allowed them to determine three-dimensional (3D) cellular arrangements in the endometrium. They allocated epithelial cells into the three main endometrial layers: lumenal, functional, and basal. Focusing on epithelial differentiation lineages, the authors resolved three cellular compartments within the SOX9 population, which have been previously explored as stem/progenitor cell types (263, 264): (1) SOX9+LGR5+ non-cycling cells, enriched in the luminal surface and expressing KRT17 and WNT7A; (2) SOX9+LGR5− cells located in the basal glands, expressing IHH; and (3) proliferative SOX9+ cells, including both LGR5+ and LGR5− cycling cells in the regenerating superficial layer of the glands.

Paying attention to central transcription factor signatures (WNT and NOTCH), the authors (260) described distinct expression distribution across the endometrial regions. NOTCH activation was associated with glandular subsets, and WNT was activated in the ciliated epithelium. An in vitro

1047 assessment with endometrial organoids under hormonal stimuli and inhibitors of WNT or NOTCH
1048 signaling demonstrated the opposing roles of these two regulators, suggesting that in vivo, this is
1049 regulated by the boundaries set by the localization of distinct cellular populations in the lumenal versus
1050 glandular microenvironments.

1051 Altogether, these spatiotemporal single-cell transcriptomic atlases (151, 260) represent the most
1052 detailed picture to date of the healthy endometrium in humans, which is highly exploitable as a reference
1053 source for other studies in disease conditions or altered female fertility.

1054

1055 IV. THE MATERNAL DECIDUA AS A DISTINCT AUTOCRINE-PARACRINE
1056 TISSUE MODULATING PREGNANCY IN HEALTH AND DISEASE

1057 Decidualization is the secretory transformation of the endometrium, which supports the ability to
1058 control embryonic invasion, growth, and development of the placenta. Human decidualization is an
1059 ovarian steroid-regulated differentiation process that begins around the terminal portions of the spiral
1060 arteries underlying the luminal epithelium; if blastocyst implantation occurs, the decidual lining is
1061 formed and maintained throughout pregnancy. These modifications are accompanied by the secretory
1062 transformation of the uterine glands (265) and immune cell infiltration made up mainly of uNK cells
1063 (266).

1064 In contrast to most mammals, human decidualization does not depend on the presence of a conceptus
1065 (267). The biological advantage of this phenomenon remains unclear; however, species with an invasive
1066 type of hemochorial placentation and long-term gestation share cyclic decidualization (268). The
1067 functional relevance of decidualization in humans is incompletely understood but appears as the optimal
1068 maternal solution to ensure appropriate accommodation of the invasive trophoblast.

1069 A. Master regulators of decidualization

1070 Successful pregnancy depends on a healthy maternal periconceptional period with a proper
1071 decidualization process, which plays a critical role in controlling the active embedding of the conceptus

(269). Hormonal, biochemical, and immunological factors regulate endometrial adaptation for the optimal conceptus in the secretory phase of the menstrual cycle.

1. Hormonal regulation

Progesterone is the major driver of decidualization primarily mediated by nuclear PRs encoded by PGR (270). The expression of PR-A and PR-B isoforms is transcriptionally regulated by coregulators and post-translational modifications that target the gene promoter. PR expression increases in ESCs and remains high during the decidualization process, with PR-A predominating (101). The involvement of specific ligand-bound PRs is a paramount mediator of decidualization changes at the transcriptomic and proteomic levels (271, 272).

Adding to the role of progesterone, metabolic and biochemical factors represent critical regulators of decidualization. In this context, hCG induces local secretion of hormones such as relaxin and corticotropin-releasing hormone, which enhance the production of cAMP levels in an autocrine/paracrine manner, promoting decidualization and supporting implantation (273–275). To add additional complexity, PR- and cAMP-dependent pathways are instrumental in maintaining the decidual phenotype, sharing downstream targets (114, 276).

2. Autocrine/paracrine inductors

The main autocrine/paracrine factors secreted by endometrium, namely interleukins IL-1β, IL-11, and LIF (277–280), bone morphogenetic protein 2 (BMP2) (281), left-right determination factor 2 (LEFTY) (282), and TGFβ (283), play important roles in supporting decidualization by promoting cAMP and regulating ECM remodeling (173), angiogenesis, and regulating embryo invasion (185). Additionally, metabolic pathways are critical regulators of implantation and posterior invasion events. Large amounts of cytokines are synthesized in the placental–decidual interface post-implantation, with colony-stimulating factor (CSF) 1, TGFβ, TNFα, granulocyte-macrophage (GM)-CSF, LIF, IFNγ, IL-6, and IL-10 having functionally relevance in trophoblast differentiation and invasion (284). Soluble factors involved in implantation have been identified by in vitro studies using single human hatched blastocysts co-cultured with decidualizing ESCs. Over three days of co-culture, 75% of embryos were arrested,

while the remaining continued normal development. Multiple immunoassays determined the level of fourteen factors secreted by ESCs, showing that normal blastocysts had no significant effect on decidual secretions, but arrested embryos inhibited IL-1β, IL-6, IL-10, IL-17, IL-18, eotaxin, and HB-EGF (285). Furthermore, control of ESC decidualization is spatially facilitated by lipid mediators such as lysophosphatidic acid (286) by modulating the production of HB-EGF, EGF receptor (287), COX2 (216), and PGE2 (288).

B. Cellular and molecular transformation of decidualization

Decidualization is mediated by an orchestrated cellular and molecular transformation (**Figure 4**). Endometrial differentiation is mediated by the phenotypic transformation of human ESCs and secretory activity of the uterine glands, which ultimately results in ECM remodeling, immune cell recruitment, angiogenesis, and resistance to oxidative stress (289–291).

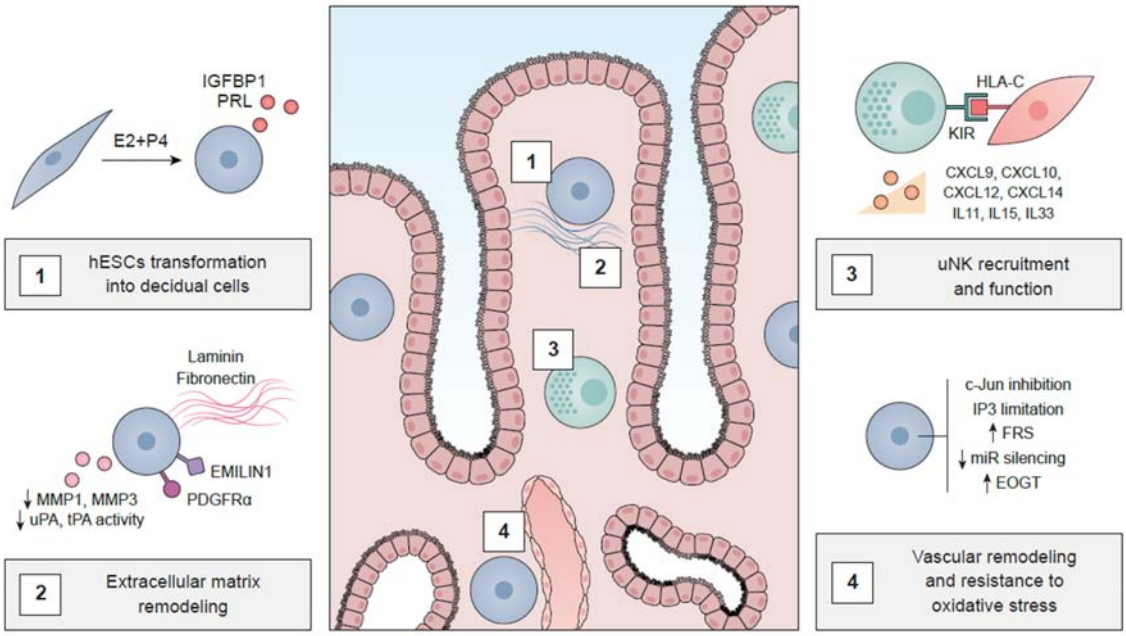


Figure 4. Cellular and molecular transformation during decidualization. Phenotypic transformation of human endometrial stromal cells (hESCs) into enlarged secretory decidual cells (1); extracellular matrix (ECM) changes in composition and function (2); leukocyte infiltration by uterine natural killer (uNK) cells (3); and promotion of angiogenesis and oxidative stress resistance (4).

1. Human endometrial stromal cells transformation into decidual cells

ESC differentiation into a decidualized phenotype includes intensive morphological and secretory changes. Morphological changes include elongated fibroblast-like cells transforming into an enlarged polygonal or round cell population with ultrastructural modifications mediated by complex cytoskeletal rearrangements (297). Decidualized ESCs (DESCs) secrete molecules such as PRL and insulin-like growth factor binding protein 1 (IGFBP1), which signals extravillous trophoblast (EVT) invasion and represent canonical decidualization markers (298). In addition to these markers, global proteomic analysis of in vitro decidualized versus non-decidualized hESCs revealed 60 differentially expressed proteins modulated during this process, including cathepsin B, transglutaminase 2, peroxiredoxin 4, and ACTB (Actin Beta). Secretomic analysis of conditioned media using a human protein cytokine array identified thirteen secreted proteins, which included well-known (e.g., IGFBP-1) and novel (e.g., MPIF-1 and PECAM-1) markers (299).

Endometrial glands undergo an intensive transformation, secreting growth factors and other molecules such as LIF, a vital molecule for promoting embryo implantation (277). Epithelial-stromal crosstalk is essential to orchestrate decidualization and, consequently, to maintain an optimal uterine microenvironment during pregnancy (292–294). The epithelium releases paracrine effectors such as BMP2 acting downstream of the Indian Hedgehog (IHH) pathway, which creates a gradient of the decidualization signal to the stromal compartment (295). Hormonal signaling activates receptors in the epithelium, and the signal is transduced to the stromal compartment. The stromal PR signaling network acts through paracrine mechanisms targeting genes involved in decidualization, such as *MSX2*, *CNR1*, and *MMP9*. Knockout mouse models support the crucial roles of these molecules as primary decidualization regulators. In this sense, Sox17 ablation in the uterine epithelium impairs IHH signaling, leading to embryo implantation failure (302). Interestingly, mice displaying uterine MSX2 deletion prompted defective implantation with loss of Bmp2 expression (207). In this line, the conditional ablation of Bmp2 in mice demonstrated that uterine stroma could not undergo decidual reactions in the mouse (296).

2. Extracellular matrix remodeling

Decidualization is characterized by significant changes in ECM composition and function, which provide anchorage for cells and a favorable environment for trophoblast expansion (297). The distribution of laminin close to DESCs and its absent in the myometrium controls embryo invasion (298, 299). Fibronectin also remains localized around decidual cells containing an arginine-glycine-aspartic acid motif connected with IGFBP1, a decidualization hallmark, and may play an anchoring role (300). Progesterone-mediated signaling mediates the decreased levels of enzymes such as MMP1 and MMP3 and the diminished activity of urokinase-type and tissue-type plasminogen activators (PAs), which amplify ECM synthesis by decidualizing cells (301). EMILIN1, a connective tissue glycoprotein produced by DESCs, is associated with elastic fibers and induces migration of EVT cells in a process called haptotactic directional migration (302). Imaging studies show that decidualizing cells are programmed to migrate toward implantation-competent blastocysts (303). Trophoblast signals, specifically PDGF-AA, produce chemotactic and invasive migration of decidualized cells coincident with increased expression of its receptor PDGF-Rα (304). Together, these studies indicate that decidualization coordinates trophoblast invasion by encapsulating the implanting blastocyst through active engagement.

3. Immune cell recruitment

In the late secretory phase of the menstrual cycle, the functional endometrial layer becomes increasingly compact with leukocyte infiltration by uNK cells. The number of uNK cells increases around the spiral arterioles, near endometrial glands, and adjacent to EVTs in early pregnancy. Immune cell recruitment and distribution of uNK cells determine reproductive success, and their imbalance is associated with reproductive failure, specifically RPL (discussed below) (305). uNKs express killer-cell immunoglobulin-like receptors (KIRs); on placental cells, KIRs bind to human leukocyte antigen (HLA)-C molecules, indicating a role in maternal tolerance of fetal trophoblasts toward the allogeneic conceptus (306). Complex and dynamic gradients of chemokines, such as chemokine (8C-X-C) motif ligand 9, CXCL10, CXCL14, and trophoblast-derived CXCL12, and cytokines produced by DESCs, such as interleukin-11 (IL-11), IL-15, and IL-33, control the spatiotemporal distribution of uNK cells in the peri-implantation endometrium (307). In human first-trimester implantation, the CX3CR1 and

CCR1 chemokine receptors locate in the endovascular EVT. CCR1 is expressed by the placenta and trophoblast cell lines, while CX3CR1, CCR1, CCR2, and CCR5 are also expressed by endometrial cells (308). Macrophage levels also increase in the decidua near invading human EVTs, likely conferring tolerance to invading semiallogenic trophoblast cells (309). Taken together, decidualization is a major contributor to the immunological regulation of pregnancy.

4. Vascular remodeling and resistance to oxidative stress

A key feature of decidualization is the vascular remodeling that fundamentally increases permeability at the site of blastocyst attachment and is associated with oxygen tension fluctuations and reactive oxygen species generation (185). DESCs are programmed to resist the oxidative stress environment, protecting the integrity of the maternal-embryonic interface. A wide range of defense systems are implicated in this adaptation, including inhibition of stress pathways such as JNK, limitation of inositol trisphosphate signaling, and stimulation of free radical scavenger secretion (310–312). Additionally, diminished miRNA silencing in DESCs may be a defense mechanism to protect from trophoblast-derived miRNAs (313) (see Section VII.B). Another essential posttranslational modification linking glucose sensing to cellular stress resistance includes those pathways involved in O-GlcNAcylation. Notably, an EGF domain-specific O-linked GlcNAc transferase is markedly upregulated in DESCs and modifies and secretes membrane proteins (314). Finally, uterine-specific deletion of Trp53 in a mouse model compromises antioxidant responses by decreasing mitochondrial oxidation and ATP production, altering lipid signaling, and causing premature senescence (315, 316). This phenotype could contribute to PTB and/or stillbirth based on evidence that inhibiting mTORC1 activity using rapamycin attenuates decidual senescence and rescues the PTB phenotype (317).

C. The decidua as a maternal tissue essential to the maternal-embryonic unit

Endometrial remodeling reaches maximal specialization with the formation of the decidua, the maternal tissue most closely connected to the fetoplacental unit. "*Decidua*" derives from the Latin verb decider, meaning to die, fall off, or detach, reflecting the temporal function of this tissue during pregnancy. The relationship with the fetoplacental unit establishes three regions of decidua with distinct anatomical and functional roles: the decidual basalis, capsularis, and parietalis. Here, we discuss the area beneath the

site of implantation known as the decidua basalis, which supports the formation of the discoid placenta, representing the maternal-fetal interface.

The decidua is a complex and heterogeneous tissue composed of DESCs, representing 10%–30% of cells in the first trimester and up to 60%–70% of cells in the full-term decidua. DESCs control trophoblast invasion and placenta formation (318). The uterine glands and small blood vessels, including spiral arterioles, provide nutrition to the fetoplacental unit, first by providing histotrophic nutrition through the first trimester of pregnancy (see Section VI.B) and then through the connection of the maternal arterial circulation to the placenta. The fraction of immune cells, particularly uNK cells and macrophages, plays a critical role in regulating trophoblast invasion and spiral artery transformation via the secretion of specific cytokines and chemokines that promote EVT action (319, 320). Recently, a cell atlas of the early maternal-fetal interface defined three major subsets of uNKs cells; interaction analysis also predicted that their function mediated the extent of trophoblast invasion and coordinated immunomodulatory pathways involving myeloid cells, T cells, and stromal cells (261, 320). Overall, the complexity and dynamism of decidual tissue reflect its pivotal role in pregnancy.

The decidua is crucial in orchestrating trophoblast invasion, which occurs in two steps. The first step occurs between days seven and ten post-conception, starting with the trophectoderm that actively invades the uterine tissue; then, the conceptus is wholly embedded in the stromal compartment. Before 8 weeks of gestation, the second step begins with the transformation of EVTs into an invasive phenotype. These cells change their adhesion molecule phenotype and invade from the placental villi to the full thickness of decidua, as far as the inner third of the myometrium (321). EVTs invade and remodel maternal spiral arteries by destroying the muscle layer that replaces the endothelial lining, forming a chimeric feto-maternal endothelium (322). This process is usually completed by eighteen weeks of gestation and is critical for establishing uteroplacental circulation. Invasion is partly promoted by oxygen tension (323) and active recruitment of uNKs and macrophages (324, 325). The demands of the fetoplacental unit increase during pregnancy and maternal spiral arteries must dilate enormously to allow 10% of maternal cardiac output (~800 mL) to pass through the placenta per minute (326).

D. Defective decidualization in the origin of infertility and pregnancy complications

The maternal endometrium is traditionally considered an inert reservoir without an active role in the implantation process and subsequent pregnancy success. Much attention has focused on the embryo because most early pregnancy losses result from chromosomally abnormal embryos (see Section II). Evidence suggests that, in addition to the seed (the embryo), improper endometrial preparation may contribute to reproductive and obstetrical disorders (327).

The inability of maternal tissue to undergo a molecular program of hormone-driven differentiation signals, known as DD, leads to implantation/placentation aberrations and adverse pregnancy outcomes. DD has been described at the cellular and molecular level as (i) a defect in cellular shape changes and the expected cytoskeletal reorganization, (ii) a failure to display an increase in stage-specific antigens of classical markers (PRL and IGFBP1), and (iii) an alteration in the global transcriptional profile of decidualized cells, including downregulation of genes such as *IGFBP1*, *CNR1*, and *IL-1B* that are functionally annotated with pathways associated with differentiation failures such as cytokine-cytokine interactions, E2 and TFG-β signaling, and inflammation.

Although infertility and pregnancy disorders are typically classified into distinct categories, these conditions occur along a continuum. The decidua represents the common factor, driving placental establishment and supporting the concept that pregnancy health is determined even before the arrival of the embryo into the uterine cavity. Therefore, pregnancy complications are not two-stage disorders with the placental defect leading to an abnormal maternal response to clinical manifestations; instead, they could be three-stage disorders triggered by deficient endometrial decidualization that predates embryo arrival and leads to aberrant implantation/placentation and finally, clinical disease. Accumulated evidence demonstrates that DD is present in pathological conditions such as endometriosis and RPL and late-onset pregnancy complications such as PE, intrauterine growth restriction (IUGR), and placenta accrete (328, 329) that will be discussed below (also see **Figure 5**).

1. Endometriosis

Endometriosis is a chronic inflammatory disease of unknown origin characterized by endometrium-like glands and stroma outside the uterus, mainly involving the pelvic organs. Endometriosis affects 6%–10% of reproductive-aged women and is a leading cause of severe pelvic pain and subfertility.

Retrograde menstruation was classically thought to be the source of ectopic endometrial tissue within the peritoneal cavity (330); however, other factors must be involved, as nearly 90% of menstruating women have retrograde reflux of menstrual blood, yet only a fraction develop endometriosis. Other hypotheses include disturbances in embryological development (metaplasia of the coelom) (331) possibly combined with pluripotent stem cell reprogramming (332–334), vascular or lymphatic spread (335), neonatal bleeding (336), and genetic factors (337). Accumulated evidence also suggests DD of ESCs in the eutopic endometrium of patients with endometriosis (338–340). Endometrial transcriptomic differences between women with and without endometriosis are evident and even accentuated in primary ESC cultures under in vitro progesterone treatment, with 172 genes affected (341). These findings gave rise to the term progesterone resistance, which is DD resistance to P4. Reduced expression of the PR-B isoform and P4 target genes (e.g., 17β-hydroxysteroid dehydrogenase-2 and glycodelin) in endometriotic tissue has been reported (342–344). Endometriotic tissue exhibits downregulation of other genes important for endometrial stromal decidualization, such as *HOXA-10* and *HOXA-11* (345). Based on these findings, endometriosis could be partially associated with P4 resistance or DD that precedes deregulation in stopping epithelial proliferation and impaired stromal decidualization in endometriotic implants (346).

2. Recurrent pregnancy loss

RPL, defined as three or more consecutive pregnancy failures, affects 1%–2% of all couples attempting to conceive. RPL is widely attributed to either embryo chromosomal abnormalities or uterine defects; however, no underlying causes are found compared to uncomplicated pregnancies in most patients. Aberrant decidualization governed by disruption of hormonal signaling leading to dysregulation of PRL and prokineticin has been suggested in RPL (347). Like endometriosis, ESCs obtained from RPL patients also display an aberrant decidual response when decidualized in vitro; however, in contrast to the highly debilitated response detected in endometriosis, this effect results from an imbalanced pro-inflammatory response (348).

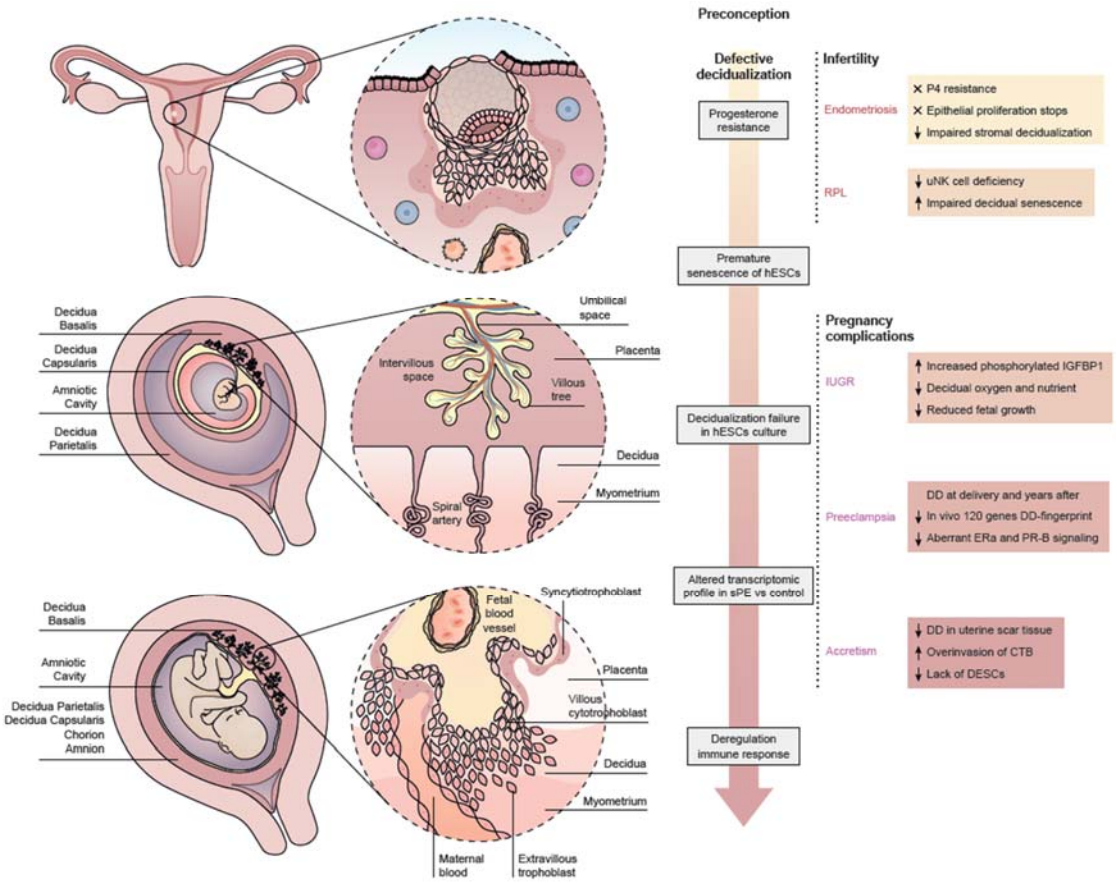


Figure 5. Defective decidualization in infertility and pregnancy complications. Defective decidualization (DD) mediated by progesterone resistance, premature human endometrial stromal cell (hESC) senescence, transcriptomic alterations, and disbalance of immune response is present with infertility such as endometriosis, recurrent pregnancy loss (RPL), and late-onset pregnancy complications such as preeclampsia (PE), intrauterine growth restriction (IUGR), and placenta accreta.

Aberrant decidualization is related to a pro-senescence decidual response during the peri-implantation window (349). Single-cell reconstruction of in vitro decidualization suggests that DESCs require the recruitment of immune uNK cells to eliminate senescent ESCs. In this context, the endometrium from RPL patients is characterized by uNK cell deficiency correlated with excessive decidual senescence. Indeed, recent single-cell profiling efforts of human decidual in patients with RPL revealed a remarkable decrease in the number of a particular subset of uterine-specific decidual NK cells (dNK1) and a significant increase in the number of a cytokine-producing pro-inflammatory subset of dNK cells (dNK3) (350). Furthermore, lower levels of SCARA5 (Scavenger Receptor Class A Member 5) but higher DIO2 (Iodothyronine Deiodinase 2) percentiles in RPL samples indicate the predominant

senescence of DESCs. DD may play a role in the lack of adaptation to significant oxidative stress due to active maternal perfusion of the placenta, leading to miscarriage (351, 352).

3. Preeclampsia

PE is a major obstetric complication that affects 5%–7% of all pregnancies, with short- and long-term, life-threatening consequences to the mother and child (5, 353). Individuals with PE exhibit hypertension and other maternal vascular damage, such as proteinuria and edema. Pathogenesis may involve shallow cytotrophoblast (CTB) invasion through the uterine decidua into spiral arteries, although the cause of deficient arterial invasion remains unknown.

The current model posits that suboptimal endovascular invasion is triggered by the failure of the maternal decidua to regulate trophoblast invasion. In support of this hypothesis, DD occurs in vitro in ESCs isolated from women with previous severe PE (sPE) pregnancy linked to impaired CTB invasion. DD is detected at the time of delivery and persists five years after the affected pregnancy (354). This maternal deficiency is influenced, at least in part, by an annexin A2 (*ANXA2*) defect associated with aberrant decidualization that could underlie shallow trophoblast invasion and contribute to the aberrant establishment of the maternal-fetal interface (355). In support of this theory, a microarray analysis of decidual tissue obtained from a chorionic villous biopsy from an 11.5-week pregnant patient who later developed PE corroborated a molecular signature of suboptimal endometrial decidualization and altered uNK cell function before PE development (356, 357).

Recent data corroborate the existence of a transcriptomic signature of the preconceptional endometrium compatible with dysregulated decidualization in patients who develop sPE (358). The molecular DD fingerprint includes 120 genes associated with the development of sPE dist, reinforcing the concept of the periconceptional continuum of decidual defect that may be leveraged to predict sPE risk. Interestingly, 43 genes (45.7%) overlap with the transcriptome and cistrome associated with PR signaling (359), and 45 genes (47.9%) in this signature are involved in ERα (ER1) function (360). Endometrial dysregulated genes are highly connected with PR-B and ERα signaling in a dynamic network of DD in sPE. Additional findings revealed that ERα and PR-B gene expression and protein

abundance were remarkably disrupted in sPE, supporting that sPE that may result from an aberrant endometrial response to E2 and P4 via ERα and PR-B signaling (358).

4. Placenta accreta spectrum

Placenta accreta spectrum (PAS) is a complex obstetric complication that includes a range of pathologic placental adherence, including placenta accreta, increta, and percreta (361). PAS is characterized by abnormal trophoblast invasion into the myometrium of the uterine wall. The hallmark of PAS is overly deep invasion, increasing the risk of preterm delivery, hemorrhage, and death (362). The primary defect of CTB biology involves impaired penetration into the uterus, leading to excessive invasion (363); however, the reduction of decidual tissue overall in the first trimester of PAS cases counters previous suggestions that the decidual layer is normal at the initiation of gestation and atrophies as pregnancy progresses (364). The current hypothesis regarding its etiology is that defects in the endometrial-myometrial interface lead to DD in uterine scar tissue areas, which can result in abnormally deep penetration of placental anchoring villi and trophoblast invasion into the uterus (365). In support of this hypothesis, a recent study detected over-invasion of CTB and faulty decidualization at the maternal-fetal interface in PAS tissue sections (366). Specifically, samples with PAS lack the decidual signals that may normally constrain invasion, and there is a lack of DESCs corroborated by vimentin-positive staining adjacent to the CTBs that form the smooth chorion layer of the fetal membranes, allowing EVTs to invade deeper, resulting in PAS. Thus, the most acceptable explanation is the lack of proper decidualization at the placental attachment site.

5. Intrauterine growth restriction

IUGR, defined by fetal growth below its in-utero growth potential, represents a major cause of fetal and neonate morbidity and mortality. In IUGR, ESCs fail to decidualize into large secretory decidualized cells and instead remain small and cuboidal and express low levels of PR-B, IGFBP1, and CD10. Skewed decidual immunology is found in IUGR second-trimester tissues, illustrated by increased CD8 T cells, mature CD83 dendritic cells, and lymphatic vessels packed with decidual leukocytes (367). This suggests that DD is associated with a deficiency in P4 responses and a dysregulated immune response, impacting invading endovascular EVTs to remodel spiral arteries and leading to placenta

insufficiency in IUGR pregnancies. In addition, increased levels of phosphorylated IGFBP1 are observed in decidual tissue at the time of delivery and in the maternal plasma in the first trimester in women delivering IUGR infants at term compared to normal pregnancies (368). These observations suggest that IGFBP1 phosphorylation is linked to decreased decidual oxygen and nutrient availability and reduced fetal growth, resulting in decreased trophoblast invasion and poor placental function.

V. THE ENDOMETRIAL FLUID: THE UTERINE MILK

The endometrial glands generate fluid in the uterine cavity at approximately fifteen openings per mm^2 of the uterine luminal surface. EF (endometrial fluid) is a primary source of nutrients for the pre-and post-implantation conceptus during the first trimester (377–379) and the fluid environment where endometrial-embryo molecular dialogue occurs (245, 369, 370).

During preimplantation, histotrophic nutrition constitutes the conceptus's initial nutrition in all species before the placenta is established (371). In sheep, endometrial gland morphogenesis occurs postnatally and can be inhibited by continuous exposure to progestin, creating a uterine gland knockout model (UGKO) with the full development of extra-uterine reproductive tract and a normal hypothalamic–pituitary–ovarian axis but no gland formation and glandular secretion. In the UGKO model, no pregnancies are achieved after transferring blastocysts from normal superovulated ewes, supporting that defects in conceptus elongation and survival in UGKO ewes are due to the absence of endometrial glands and their secretions (372, 373). In mice, progesterone treatment during the neonatal phase inhibits the differentiation of uterine glands producing adults with aglandular uteri. Direct evidence that endometrial glands and their secretions are essential for implantation comes from the absence of endometrial glands in the sheep and mouse (372–376).

In humans, the syncytiotrophoblast mantle enlarges to encircle and erode into the necks of the glands during post-implantation. This step connects the glandular lumen with the placenta's developing intervillous space as early as day seventeen post-fertilization (377), a connection that persists through the first trimester (378). The importance of EF during the first trimester is evident in that full

establishment of maternal arterial circulation to the placenta does not occur until ten to twelve weeks of pregnancy (379, 380). At this point, ultrasound can detect significant flow within the intervillous space (381). Until then, EF from uterine gland secretions fills the intervillous space (Carnegie collection. http://virtualhumanembryo.lsuhsc.edu). Walter Needham (1631–1691) named this substance "uterine milk," arguing that it was distinct from lymph and important for fetal nutrition (382). In the late 19th century, von Hoffman observed that "fetal villi in the placenta do not float naked in the maternal blood but are surrounded by cells whose function is to secrete a special fluid serving for the nutrition of the fetus and called "uterine milk" " (383).

Under physiological conditions, human endometrial glands secrete a wide range of varied factors (e.g., ions, carbohydrates, glucose, polyols, lipids, miRNAs, and various proteins, including cytokines, enzymes, hormones, growth factors, proteases, and their inhibitors, and transporters); however, menstrual cycle stage affects EF composition, especially during the transition from the proliferative to the secretory phase (384, 385).

Electrolytes secreted into the EF include sodium (~120 mmol/L; the most abundant cation), potassium (~25 mmol/L), and calcium (~1.5 mmol/L) (386). Carbohydrates like glucose (~5 mmol/L) and fructose (~0.2 mmol/L) may also be present (386). Proteins have been identified in EF using uterine lavages and include the protease inhibitor A2M, alpha-1-antitrypsin (A1AT), ANT3, alpha-1-antichymotrypsin (AACT), angiotensinogen (ANGT), inter-alpha inhibitor H4 (ITIH4), apolipoproteins A1 and A4 (APOA1, APOA4), serine protease 3 (PRSS3), vitamin D-binding protein (DBP), transthyretin (TTHY), and activin receptor type-2B (AVT2B) (387). ANT3 plays a role in blood coagulation during angiogenesis, and its mutation contributes to thrombophilia (388). A2M binds to metalloproteinases, limiting trophoblast invasion (389, 390) and specifically the dialog between A2M and IL-11, which plays a role in embryo implantation (391). AACT and A1AT impact blastocyst implantation by possibly regulating the immunological endometrial response (392). APOA1 and APOA4 are reduced in EF from women with endometriosis (393) while reductions in ANGT and DBP levels have been observed in patients with unexplained infertility (394, 395). Additionally, cytokines (see Section III.A.3) such as LIF, glycodelin (PP14), macrophage colony-stimulating factor (M-CSF), EGF, VEGF, IGFBP1, and

ILs, and steroid and non-steroid hormones (E2, P4, prolactin (PRL), hCG, and precursors) have been identified in EF (396–398).

Lipids secreted to the EF include triglycerides, eicosanoids, endocannabinoids, and sphingolipids (399–402). The most abundant are the PGs, which play roles in leukocyte recruitment, vascular permeability, embryo transport, stromal decidualization, trophoblast invasion, and ECM remodeling and prepare a pro-inflammatory environment during embryo implantation (210, 403). Recent work has demonstrated that PGE2 and PGF2α secretion increases significantly during the WOI in natural menstrual cycles and ART patients. In an in vitro model of embryo adhesion, inhibiting PGE2 and PGF2α or PG receptors (EP2 and FP) blocks embryo adhesion, with this effect reversed by restoring these molecules or adding agonists. Additionally, detecting EF levels of PGE2 and PGF2α 24 hours before embryo transfer predicted pregnancy outcomes in a pilot study (213).

miRNAs are secreted to the EF by vesicles or proteins and transported from endometrial cells to the EF (213, 242, 369, 404). A recent report detailed a miRNA "roadmap" of the secretory phase of the human endometrium using NGS in samples of EF and described 81 predicted interactions of differentially expressed miRNAs in HRT cycles. There were no observed differences between natural and HRT cycles (242). miRNAs are also crucial for decidualization; indeed, an in vitro model demonstrated 26 upregulated and 17 downregulated miRNAs (405, 406). Specifically, secreted miR-30d was tightly correlated with reproduction; the secretion of miRNA from the maternal endometrium and internalized on the preimplantation embryo trophectoderm was associated with the adhesion processes (404) (see Section III.A).

Secretomic analysis of EF provides reliable real-time read-outs of individual molecules throughout the menstrual cycle. Furthermore, the collection of EF is a minimally invasive method that does not affect pregnancy rates even when performed at the time of embryo transfer (407), thereby offering a timely assessment of endometrial biology for diagnostic and/or therapeutic purposes (213, 242, 402).

VI. PERICONCEPTIONAL MATERNAL-EMBRYONIC INTERFACE: FROM WHOLE TISSUE TO SINGLE-CELL RESOLUTION

Following fertilization in the distal section of the Fallopian tube, the embryo is propelled toward the proximal section and the uterine cavity through the combined transporting action of cilia and tubal peristalsis (408). The time required for the oocyte to transfer from the tubal fimbriae to the uterine cavity is approximately three to four days. During this journey, the embryo undergoes cellular compaction, followed by cavitation and formation of the blastocoele (i.e., blastulation). Concurrently, the demethylation of the embryo genome occurs under the control of maternally inherited proteins and mRNA, resulting in transcriptional waves of embryo-encoded molecules and transcription factors (409). The surrounding environment also changes as the embryo develops and progresses through the Fallopian tube. A gradual reduction in oxygen tension in the reproductive tract characterizes species such as primates, humans, mice, and cows (410). The female reproductive tract maintains physiological O_2 levels below atmospheric gas composition (20.9% O_2) - ranging from 8.7% in the distal tube of Rhesus monkeys to a progressively lower concentrationat the site of ideal embryo implantation within the uterine cavity (i.e., 1.5% in Rhesus monkeys, ~2% in humans) (411, 412). Environmental oxygen tension affects features of embryo developmental, ultimately affecting the metabolism and reproductive capabilities (413). For example, when investigating in vitro generated mouse embryos, a 5% O_2 concentration comparable to a physiological normoxic state in vivo associated with the reduced production of reactive oxygen species (414), a higher number of normal mitochondria (415), and lower blastocyst senescence (416) when compared to the O_2 concentration found under normal atmospheric composition (20.9%). Reactive oxygen species are known to cause DNA damage, induce peroxidation of lipids, and trigger cellular apoptosis, eventually resulting in detrimental effects on embryo development, as seen in mice, cows, and humans (417–419). Moreover, from a metabolic standpoint, pre-compaction mouse embryos display lower amino acid and pyruvate consumption when cultured in a low oxygen tension environment (5% O_2), while glucose consumption increased in the post-compaction stages (420). In the murine model, ammonium, a known toxic agent for embryo development that accumulates during in vitro culture, was promptly metabolically removed under 5%

O_2 conditions while persisting in culture at 20% O_2, suggesting the involvement of oxygen levels in modulating metabolic response by the embryo (421). From a genomic standpoint, when compared to in vivo generated embryos, gene and protein expression of the mouse blastocyst appeared more similar to in vitro generated embryos cultured at 5% O_2 than those cultured at 20% O_2 (422, 423). Similarly, global DNA methylation levels of bovine blastocysts were lower in blastocysts cultured at 5% O_2 compared to environments with higher oxygen concentration (424), suggesting the additional impact of oxygen on the embryo's epigenetic landscape. Although no differences in fertilization rates were detected when human oocytes were cultured in environments with low (5%) and atmospheric (20.9%) O_2, blastocyst formation and quality, implantation, pregnancy, and live birth rate per transferred embryo increased in the 5% O_2 group (425–429). These data suggest that higher oxygen tension does not significantly affect fertilization and early developmental stages (i.e., those naturally occurring in the Fallopian tubes where the oxygen tension is relatively higher); however, higher oxygen concentration (i.e., 20%) may have a more considerable impact on the following stages when promoting cell lineage differentiation (i.e., trophectoderm) and actively pursuing specific metabolic requirements and associated subcellular modification (e.g., mitochondrial maturation). More specifically, the effects of environmental oxygen levels on molecular processes active in embryos and human embryonic stem cells have been recently reviewed in detail by Houghton (413). To mimic the environmental characteristics of natural conception, IVF-generated embryos are also incubated at low O_2 concentration (5%) throughout development until transfer to the uterine cavity (usually five days after fertilization). For these reasons, 5% O_2 concentration during embryo culture can be considered normoxic, while previous culture systems involving atmospheric O_2 levels (20.9%) can be referred to as hyperoxic.

Pregnancy is established during the periconception period as a continuum beginning with blastocysts attachment to the endometrial epithelial surface, followed by embryo invasion and formation of the placenta. The placenta forms the interface between the mother and fetus, and its development is established by the end of the first trimester. Each stage in the process is tightly regulated to ensure pregnancy success and optimal maternal and fetal health; this is of considerable importance—failure of these processes can lead to infertility, placental insufficiency, and many associated pregnancy disorders.

A. Blastocyst adhesion to the endometrium

Initial adhesion or apposition of activated hatched blastocysts to the endometrial luminal epithelium is unstable and followed by firm adhesion, which initiates blastocyst implantation and invasion into an adequately prepared or receptive endometrium (290) (**Figure 6A**). A lack of firm adhesion leads to implantation failure. The human embryo at the blastocyst stage enters the uterine cavity for up to 72 hours before implanting; during this time, blastocyst-endometrial interactions are mediated via soluble and cell surface factors (328). Blastocyst attachment to the endometrial luminal epithelium requires extensive remodeling of the luminal epithelium plasma membrane. The glycocalyx that covers the apical surface of the endometrial epithelial plasma membrane consists of more than forty glycoproteins that require redistribution to facilitate blastocyst adhesion. Many additional locally-produced factors from blastocysts and endometrial cells in humans have been investigated for their roles in blastocyst adhesion and have been extensively reviewed elsewhere (430). Recent studies have identified endometrial epithelial cell proteins that are dysregulated in women with unexplained infertility block epithelial cell adhesive capacity (431, 432). Environmental toxicants such as bisphenol A analogs have also recently been shown to block endometrial epithelial cell capacity (433). Epithelial cells display apical/basal polarity; endometrial luminal epithelial cell polarity is an important regulator of the cell's adhesive capacity (434, 435).

The uterine fluid is a rich source of soluble factors and microvesicles released by blastocysts and endometrial cells that carry cargos that include proteins and non-coding (nc)RNA, which are transported extracellularly bound to RNA-binding proteins (436). The cell source of locally produced factors at the time of blastocyst adhesion includes the blastocyst trophectoderm, endometrial luminal and glandular epithelium, and likely other cell types in the endometrium such as sub-epithelial stromal cells, as recent studies suggest that endometrial epithelial cells may transfer cytokines between cells via transcytosis (437). Blastocysts release miRNAs, and their patterns correlate with their implantation and pregnancy outcomes after IVF (436). MicroRNA-661, released by embryos that did not implant, was uptaken by primary human endometrial epithelial cells and blocked trophoblast spheroid adhesion to the cells in vitro (436, 438). The uterine fluid contains bacterial factors, and recent microbiomal characterization

efforts suggest a role in regulating blastocyst attachment and implantation into the endometrium (439, 440) (see Section VIII for a review); however, functional studies have yet to investigate the role of the microbiome in blastocyst attachment and implantation.

Most mechanistic findings in humans come from in vitro studies using primary endometrial cells and endometrial/trophoblast cell lines that identified the role of specific factors in blastocyst-endometrial interactions. Endometrial organoids maintained in long-term culture represent cutting-edge models to further understand these endometrial-embryo interactions (179). Similarly, recent studies have reported 2D and 3D culture systems of human embryos cultured to the equivalent of day fourteen post-fertilization (441), a time by which embryos would have implanted and wholly contained within the endometrium. This approach has allowed direct investigation of embryo development and trophoblast differentiation in the absence of the maternal counter side, while embryo competence for implantation could not be investigated in this system.

B. Embryo invasion and placentation

After firm attachment, embryos invade between epithelial cells and move into the underlying stroma via an unknown process (**Figure 6B**). The trophoblast derived from the blastocyst trophectoderm then rapidly proliferates and differentiates along villous and extravillous pathways, initiating placentation (442). EVT cells invade the endometrial stroma interstitially and endovascularly and plug the maternal arteries (443) resulting in a low oxygen environment in the placental intervillous space at less than ten weeks gestation. The EVT plugging the spiral arteries are then removed or lost by twelve weeks of gestation, increasing oxygen tension to the placenta (381). By this time, the EVT remodels arteries in the decidua and inner third of the myometrium, ensuring adequate blood supply to the placenta essential for fetal-maternal exchange (444). The decidua contains a rich immune milieu, including uNK cells, macrophages, T-cells, and DCs (445), and is required to limit EVT invasion and provide a tolerogenic environment; this is evident in studies showing that over- or under-invasion of EVT cells causes disorders including placenta accreta, IUGR, and PE (327, 446).

Cells within the decidua, including decidual and immune cells, regulate EVT invasion (309, 447), although the interplay between these and other cell types, including endometrial epithelial and vascular cells, is only beginning to be realized thanks to the advent of scRNA-seq. Single-cell transcriptomics of human first-trimester placenta and decidua recently defined the unique cellular milieu during placental formation. Recent characterization of 3D organoid cultures of the human first-trimester placenta may help study cellular interactions at the implantation site (448). Organoid systems are in their infancy and require refining to determine whether the main cell types at the implantation site can be recapitulated in 3D. Such systems will further enable the determination of the main pathways that lead to placental damage and drive the development of pregnancy disorders.

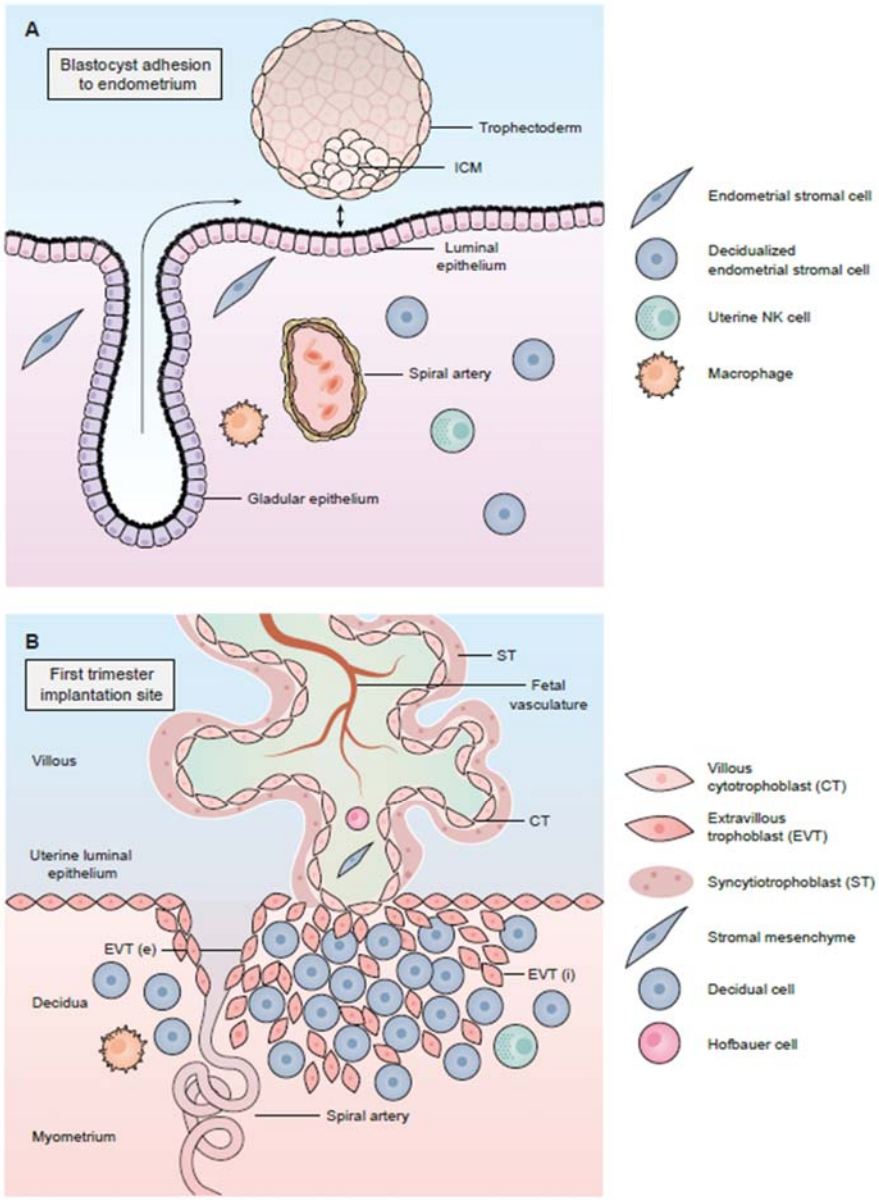


Figure 6. Blastocyst implantation and placenta development. (**A**) Blastocyst adhesion to the endometrial lining. The endometrium becomes receptive to an implanting blastocyst for a brief period during the mid-secretory phase of the menstrual cycle. Blastocysts anchor to a suitable region in the endometrial luminal epithelium (LE), where there is reciprocal communication between the trophectoderm and the endometrial LE. The glandular epithelium (GE) secretes factors in the endometrial lumen that can act on the trophectoderm and the endometrial LE. Blastocysts firmly adhere to the endometrial LE, initiating implantation into the endometrium. During the window of implantation (WOI), the endometrial stroma undergoes remodeling whereby ESCs differentiate into decidualized stromal cells, which coincides with the recruitment of uterine natural killer (uNK) cells. Other immune cells, including macrophages, are present in the stroma. (**B**) The blastocyst trophectoderm differentiates into different placental trophoblast subtypes, forming the placental villous. The placental villous consists of an outer multinucleated syncytiotrophoblast with an underlying cytotrophoblast (CTB) surrounding a mesenchymal stromal core that contains specialized immune cells known as Hofbauer cells. The villous tip attaches to the decidua, enabling the CTB to proliferate and form a column structure anchoring the villous to the decidua. The CTB differentiates to extravillous trophoblast (EVT), migrates, and invades endovascularly or interstitially into the decidua to plug the uterine spiral arteries, reducing oxygen supply to the placenta during a critical time in fetal development where the primary source of nutrition is from the endometrial glands. Around ten weeks gestation,

EVT remodeling of the spiral arteries is completed, enabling adequate blood supply to the placenta for gas and nutrient exchange.

1. The maternal-embryonic interphase at the single-cell level

The maternal-fetal interface has been investigated by scRNA-seq with distinct levels of coverage (449–453). Vento-Tormo et al. (261) identified 33 cell clusters unique to this maternal-fetal interface at early pregnancy, inferring an extensive interactive network based on an integrated database of single-cell gene expression and protein-protein interactions (CellPhoneDB) that favors immunological tolerance and nurtures fetal growth. These data, which cover the entire transcriptome of ~70,000 single cells, provide information about fetal and maternal cell types. The placental interphase includes villous CTB cells (cover placental structures called villi), the syncytiotrophoblast (a multinucleated cell type), the syncytium (covers the villus surface of the entire placenta), and EVT cells (line maternal blood vessels and are mixed with maternal cells in the decidua). The maternal decidua includes T cell subtypes (mainly T reg cells), three dNK cell subsets, two perivascular cell types, and three decidual stromal cell types (structurally support the decidua).

NK cells are the most abundant maternal immune cells in the decidua in the first trimester of pregnancy (319). In most tissues, NK cells kill infected cells and tumor cells; however, NK cells secrete soluble proteins that promote the remodeling of maternal blood vessels during pregnancy. dNK cells also regulate the invasion of EVT cells into the decidua. Each dNK subset has immunological activity regulated by its interactions with maternal and fetal cells in the decidua, promoting fetal growth and restraining the immune attack of fetal cells. Decidual stromal cells express IL-15 and can interact with NK cells via a heterodimeric inhibitory receptor IL-15RB/G on NK cells to promote the suppression of their inflammatory reactions. IL-15-uNK cell interactions are essential for NK cell survival and proliferation (261, 319).

Additional studies have focused on cell subsets in decidual and placental fractions of implantation sites and throughout human pregnancy (350, 449, 454, 455). Pique-Regi and collaborators (449) analyzed scRNA-seq profiles of the placental villous tree, basal plate, and chorioamniotic membranes of women

with term and preterm labor and found significant differences in cell type composition among groups and identified two new cell types: (i) lymphatic endothelial decidual stromal cells in chorioamniotic membranes, and (ii) non-proliferative interstitial CTBs in the placental villi. NK-cell-specific signatures increased in normal-term labor; meanwhile, macrophages, monocytes, and activated T-cells signatures increased in preterm labor. This outcome was broadened by Guo and colleagues (350), who specifically profiled immune cells of human decidua in patients with RPL and healthy controls. They discovered that the differential distribution of immune cell subsets was primarily assigned to dNK cells that support embryo growth, which might be associated with the defective development of this NK subset in RPL patients. Macrophage populations shifted from a predominantly immunomodulatory M2-state in controls to a predominantly T-cell activating M1-state in RPL. Microenvironmental cell-to-cell interactions between immune cells with stromal decidua or EVT were also determined from the data.

In general, cellular components and events during the periconceptional processes are only beginning to be elucidated. Furthermore, single-cell transcriptomics alone cannot resolve tissue architecture and the spatial arrangement of cells; spatial transcriptomics will help to understand cells and their regulating interactions in their topological context.

VII. MATERNAL-EMBRYONIC CROSSTALK DURING THE PRECONCEPTIONAL PERIOD

In the 1990s, Barker identified a link between fetal and infant growth with cardiovascular diseases, modifying the adult disease model by including environmental programming during fetal and infant life (456). Studies on the Pima Native American community in Arizona, a population with a high prevalence of T2D and obesity, demonstrated that the offspring of mothers with T2D during pregnancy have a substantially higher risk of developing both T2D and obesity than offspring born to the same mother after non-diabetic pregnancies (457, 458). Subsequently, a larger cohort of Caucasian women showed the same results (459), suggesting that the Barker hypothesis on environmental programming during the fetal period as a non-demonstrated hypothesis could be related to possible modifications at the

transcriptomic or epigenetic level by the maternal intrauterine environment of the preimplantation embryo. This hypothesis proposes additional potential mechanisms, independent of the genetic background, in which the mother can imprint some traits in her offspring and supports the exchange of information between the mother and the embryo during the pre- and peri-implantation process.

Intercellular communication is essential to allow multicellular and unicellular organisms to interact with their environment and between hosts and other cells (460). In the past, intracellular communication was classically described as involving cell-to-cell contact and the release/uptake of chemical factors, including growth factors, neurotransmitters, and hormones (461). Recently, EVs were proposed as a third mechanism of cellular communication in both physiological and pathological conditions (462, 463). EVs are membrane-enclosed particles released by almost all cell types that directly interact with cell surface receptors or transmit their contents to target cells via endocytosis, phagocytosis, or membrane fusion, with target cell specificity driven by cell and EV receptors (370, 463–465).

EVs are classified into three categories based on biogenesis, composition, and physical characteristics (e.g., size, density): apoptotic bodies, microvesicles, and exosomes (464, 466–468). EVs mediate their effects through their molecular cargo, specifically through coding and ncRNAs (including miRNAs), metabolites, proteins, and lipids delivered to recipient cells (370, 469–471). EVs derived from reproductive tissues have been identified in amniotic fluid (472, 473), breast milk (474), cervical mucus (475), EF (404, 476), oviductal fluid (477, 478), follicular fluid (479, 480), and seminal fluid (481, 482). There is growing interest in the EVs secreted by the male and female reproductive tracts, as these could represent a novel communication mechanism between the mother and developing embryo, with potential implications for establishing a successful pregnancy (reviewed by (370)).

A. Maternal to embryo communication

The first manuscript describing EV production by primary endometrial epithelial cells in culture identified EVs containing a specific subset of miRNAs distinct from parent cells (476), suggesting the regulated packaging of miRNAs into EVs. Moreover, bioinformatic studies revealed that some target genes encoding miRNAs enclosed in EVs participated in embryo implantation (476). Using sheep as a model, another laboratory isolated a comparable population of EVs from uterine luminal fluid,

demonstrating that EVs are enriched in proteins from the endometrial epithelia and conceptus trophectoderm, with a specific cargo pattern present in pregnancy (483). Other cargo molecules, such as RNAs, were mainly molecules of less than 200 bp, which is in contrast to larger RNAs in parental cells. Among such RNAs, the *gag* and *env* RNAs from endogenous Jaagsiekte sheep retrovirus (enJSRV) were identified within EVs from uterine fluid and demonstrated to be transmissible to surrounding cells. EVs are the proposed transport mechanism for *env* genes from the endometrium to the conceptus, where these genes play crucial roles in trophectoderm differentiation (483). EVs produced by the endometrial epithelium in vivo (using a sheep model) are present in EF and uptaken by the embryo and epithelium, suggesting bidirectional maternal-embryo crosstalk through EVs (484).

Exosomes are present in uterine fluid containing mRNA coding sequences for ILs, IFN regulatory factors, and enJSRV-*env* genes, with a decrease in mRNA levels for enJSRV-*env* genes as pregnancy progresses from day ten/twelve (preimplantation) to sixteen (attachment). Using co-incubation of uterine fluid exosomes with trophectoderm cell lines (oTr1) in vitro, enJSRV-*env* genes promote the proliferation of trophectoderm cells and the secretion of IFN-tau in an exosome dose-dependent manner. These exosomes are uptaken by cells, likely via toll-like receptor signaling. IFN-tau signals pregnancy recognition in sheep, which announces the presence of embryos in the uterus around day ten-to-twelve of pregnancy for attachment on day sixteen (485).

One study demonstrated maternal–embryonic communication in humans through EVs. This work first confirmed the presence of EVs in human EF and demonstrated that exosomes containing hsa-miR-30d are actively transferred from endometrial epithelial cells to EF and uptaken by the embryo, where miRNA is internalized in trophectodermal cells. The study additionally indicated that murine embryos treated with a synthetic analog of human miR-30d display transcriptomic modifications, with increased expression of genes coding for molecules involved in embryo adhesion (e.g., *Itgb3*, *Itga7*, and *Cdh5*), highlighting the importance of miR-30d maternal-embryo transference via exosomes (404) (**Figure 7**).

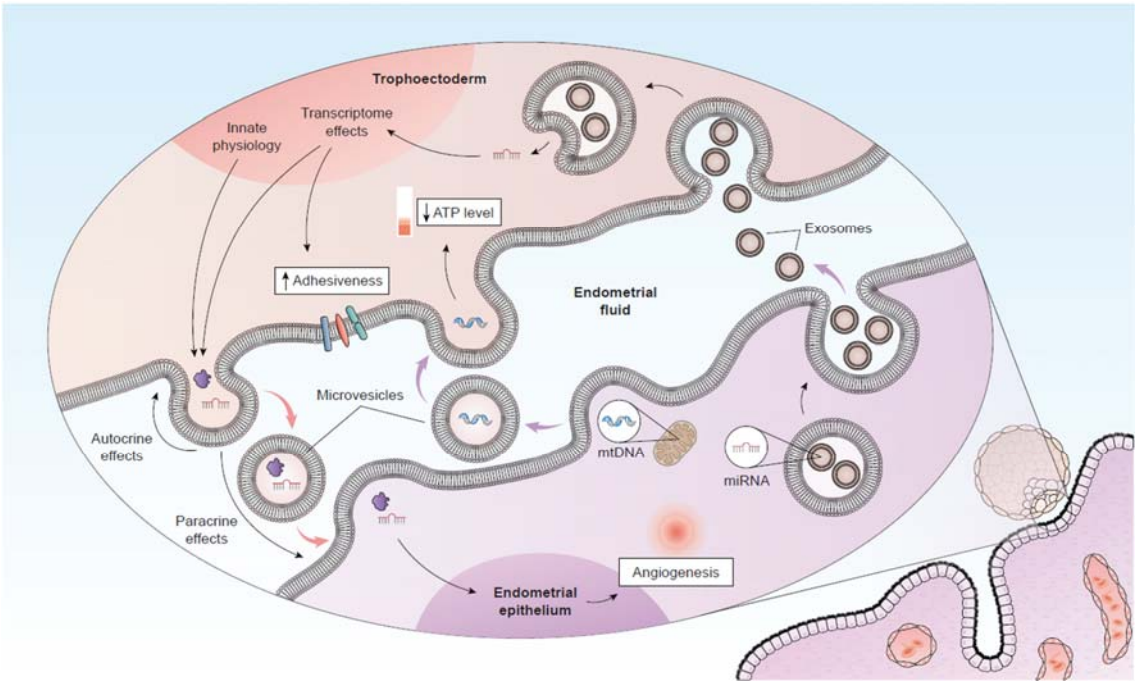


Figure 7. Maternal-embryo crosstalk during preimplantation. Schematic representation of the bidirectional communication occurring at the maternal-embryonic interface through the secretion of miRNAs, proteins, and mitochondrial DNA enclosed in exosomes and/or microvesicles.

Other studies demonstrated that the EV proteome is regulated by steroid hormones and thus exhibits differences depending on menstrual cycle progression and stage. The exosome proteome produced under hormonal conditions (E2 and E2+P4) of the receptive phase may be implicated in embryo implantation, and exosomes are internalized by human trophoblastic cells, enhancing their ability to adhere, partially through the focal adhesion kinase cascade (486).

A novel crosstalk mechanism regarding DNA transmission by EVs has recently been reported. Single- and double-stranded DNA are present in EVs, with the latter being the most abundant form (487). EV-DNA horizontal transfer has been proposed as a new mechanism for transferring genetic material across species, potentially impacting genome evolution (488). Exosomes are also transporters of mitochondrial DNA (mtDNA) cargo as a possible mechanism to transmit altered mtDNA (489) but also to restore mtDNA (489). Interestingly, exposure of the first-trimester placenta to antiphospholipid antibodies increases mtDNA levels in secreted microvesicles and exosomes and subsequently enhances the activation of endothelial cell response (490). Vertical transmission of maternal mtDNA to the embryo

has been suggested as a mechanism to modulate embryo bioenergetics during the periconceptional period (491). Isolation of microvesicles in the EF shows 11.12±0.53-fold enrichment in the thirteen mtDNA-encoded genes for electron transport chain complexes and 7.1–26.7-fold enrichment in transcription factor binding sites. These microvesicles are internalized in the cytoplasm and nuclei of trophectoderm cells (491) (**Figure 7**).

B. Embryo to maternal communication

The preimplantation embryo can release molecular effectors into the extracellular environment, likely affecting the downstream endometrial cell gene expression landscape (352, 492). Embryo-derived miRNAs have been proposed as principal mediators of this dialogue. They are: (i) exported from donor cells under different physiological conditions, (ii) extremely resistant to biological and physical insults (e.g., RNase A digestion, sequential freezing and thawing, extreme pH, and DNase treatment (493)), and (iii) potent regulators of gene expression when internalized by recipient cells (494). During IVF cycles, spent culture media enriched with embryo-derived molecules can be easily collected, allowing the characterization of paracrine/autocrine processes occurring at early embryo developmental stages by direct profiling of extracellular miRNAs released exclusively by embryonic cells into the extracellular environment.

This approach can help identify biological processes regulating embryonic-maternal crosstalk at the implantation interphase and non-invasive biomarkers of embryo competence (436, 495–498). Capalbo and colleagues characterized miRNAs secreted by human preimplantation embryos in blastocyst spent culture media collected at different time-points of preimplantation development to investigate whether secreted miRNA markers predict clinical outcomes in IVF cycles. Culture media samples were collected from the same embryos at cleavage, morula, and expanded blastocyst stages. miRNA analysis of cleavage and morula-stage media revealed a profile comparable to that observed in controls, suggesting little or no release of embryonic miRNAs at early preimplantation stages. However, miRNAs were consistently detected at a much larger scale at the blastocyst stage, indicating that this type of signaling mechanism begins soon after blastulation in a period concomitant with endometrial invasion. In a subsequent study, a blinded analysis of 221 spent culture media samples from blastocysts undergoing

euploid single embryo transfer investigated whether miRNA profiling from blastocyst spent culture media provides a novel approach for embryo assessment (499). No miRNAs in blastocyst spent culture media readily predicted the embryonic implantation potential, warranting further investigation with the use of novel technologies for comprehensive small RNA analysis from extremely low-input samples (i.e., blastocyst spent culture media specimens).

The mechanisms mediating miRNA secretion from preimplantation embryos and, more precisely, in which form they are released (i.e., EVs or bound to stabilizing proteins) are unknown. Giacomini and colleagues characterized the embryonic secretome in IVF cycles, demonstrating that secreted embryo-specific exosomes are significantly increased from cleavage to blastocyst stages (500). Furthermore, Pallinger and colleagues and Abu-Halima and colleagues then quantified embryonic EVs (i.e., possibly exosomes) in blastocyst spent culture media samples, highlighting a direct association between EVs and reduced embryo implantation potential (498, 501). Although further functional studies are required to determine the mechanisms mediating miRNA secretion from preimplantation embryos, these data suggest preferential vesicle-mediated secretion.

Although endometrial cells may uptake vesicles containing miRNAs (370, 404), there is no direct evidence that endometrial cells internalize blastocyst-secreted miRNA-containing complexes. Additionally, secreted miRNAs may transmit information to neighboring trophectoderm cells rather than the uterus. Further investigations of this potential communication between embryos and implantation sites are needed to better understand the biologic processes underlying implantation.

EVs are also produced by the embryo and participate both in crosstalk with the endometrium (469, 484) and autocrine regulation (502). Microvesicles produced by ICM cells enhance the migration of trophoblast cells in vitro, either as isolated cells or as whole embryos. This effect is carried out via the laminin and fibronectin cargo of ICM microvesicles, which attach to integrins in the trophoblast cell surface and stimulate JNK and FAK kinase cascades leading to increased trophoblast migration. Injection of these microvesicles directly in the uterine cavity in mice at E3.5 days increases blastocyst implantation efficiency (502). Additionally, EVs isolated from a trophectoderm cell line stimulate the proliferation of endothelial cells in vitro, making them potential regulators of endometrial angiogenesis.

Moreover, the vesicles contain a miRNA and protein cargo profile, including species with annotated functions in the angiogenesis process (503). In line with these results, a previous study on the roles of exosomes derived from two EVT cell lines (HTR-8/SVneo and Jeg3) demonstrated that these vesicles promote vascular smooth muscle cell migration, a process involved in human uterine spiral artery remodeling in successful pregnancies (504).

VIII. THE ENDOMETRIAL MICROBIOME: MICROBIAL INFLUENCE ON INFERTILITY AND PREGNANCY COMPLICATIONS

The human microbiome, represented by the community of microorganisms inhabiting the body, including bacteria, viruses, yeasts, and archaea, influences all aspects of human life. Microorganisms exert their influence by expressing microbial genes (estimated to be several million), which, apart from coding for bacterial proteins and functions, also modify host physiology by interacting with the human genome of ~23,000 genes (505). The microbiome trains the immune system to activate defenses against pathogens, produces vitamins and metabolites, and regulates food and drug metabolism (506). The vast diversity of microorganisms colonizing humans and their potential combinations make the human microbiome a significant contributor to human genetic diversity (507).

Disease states originate from an imbalance of the microbial population (known as dysbiosis), which entails altered host-microbial interactions. These imbalances lead to chronic inflammation, immune disorders, metabolic syndrome, cancers, and mental disorders such as autism and depression (508, 509). Early-life gut microbiota composition contributes to infant development and modulates life-long immune modulation and metabolism, among other effects (510–513).

The timing of bacterial colonization of infants is under debate. Some studies have revealed similarities between the microbiota present in the placenta, amniotic fluid, and meconium of preterm babies and term neonates delivered by c-section, suggesting that microbial transfer may initiate at the maternal-fetal interface leading to the “in utero colonization hypothesis” (514, 515). Recent research describing the presence of bacterial DNA and potential microorganisms in the fetal gut using different scientific

strategies supports these findings (516, 517). Others have argued this hypothesis and defended the idea of a sterile womb in healthy pregnancies, proposing that microbiota is first established during/after birth (518). This hypothesis has gained support thanks to the successful generation of germ-free individuals in different mammal species, including humans. There also exists an association between cesarean section and intrapartum antibiotics administration with the colonization of the neonatal gut by opportunistic pathogens (519) which favors the colonization of infants after birth. Consistent with this hypothesis, multiple adult diseases, including obesity, inflammatory bowel disease, allergies, and asthma, are associated with cesarean deliveries, the absence of breastfeeding, and/or antibiotic administration during the first year of life (515), suggesting that the microbiome can be manipulated throughout this period.

The elevated levels of inconsistency between results obtained regarding the composition of the placental microbiota from a range of laboratories have fueled this controversy (520). The fact that not all samples analyzed identified a placental microbiota may indicate the transitory nature of the bacterial presence in the maternal-fetal interface. Furthermore, these data suggest that the presence of bacteria at the maternal-fetal interface, in some women and circumstances, does not indicate the presence of an indigenous microbiome permanently colonizing this niche. In this sense, the current evidences for the existence of a stable, resident, and metabolic active microbiome remain insufficient, as the identification of residual amounts of bacterial DNA in fetal samples could derive from dead bacterial cells, contaminants present in laboratory reagents, false positive results due to insufficient/inadequate controls, or failure to differentiate the noise/signal ratio during the analysis of low biomass samples (521).

Of note, even if the fetus remains uncolonized by a resident microbiome before pregnancy, it would be exposed to a cascade of metabolites and signals produced by the maternal microbiota during pregnancy even if this microbiota does not become vertically transmitted from the mother to the fetus. Therefore, even given the lack of initial colonization of the fetal microbiome in utero, the maternal microbiome may shape the fetal immune system and predispose offspring to conditions related to microbial symbiotic or parasitic communities (522). Thus, functional studies that aim to analyze the impact of

products derived from the maternal microbiome on the fetus will help to understand host-response mechanisms and improve the health of offspring.

A. Reproductive tract microbiota

The vaginal microbiota in reproductive-age women accounts for approximately 9% of the total human microbiota. Compared to the gut microbiota, the vaginal microbiota is characterized by low diversity and richness (523).

Growth and differentiation of vaginal epithelial cells are regulated by E2 levels, which have also been associated with the bacterial composition of the vaginal microbiome throughout reproductive development. In children, the vaginal microbiota is mainly composed of aerobic and anaerobic bacteria, while *Lactobacillus* species are practically non-existent until E2 levels rise in the pre-menarche period (524, 525), which leads to increased glycogen deposits and acidification of the vaginal niche, favoring *Lactobacillus* growth and inhibiting dysbiotic or pathogenic growth in the vagina (526). During puberty, the vaginal microbiota is stabilized; meanwhile, *Lactobacillus* species predominate at the menarche to create one of the *Lactobacillus*-dominated community state types (CST - I, II, III, or V, driven by *Lactobacillus crispatus*, *Lactobacillus gasserii*, *Lactobacillus iners,* or *Lactobacillus jensenii*, respectively) observed in reproductive-aged women. Deviations from *Lactobacillus*-dominated status, represented by CST IV, are associated with reproductive tract dysbiosis such as bacterial vaginosis (527, 528). The vaginal microbiota is dynamic throughout the reproductive age and can shift from CST in response to hormonal status, hygiene, sexual behavior, and other factors (529, 530). After menopause, E2 decreases, leading to a shift from the *Lactobacillus*-dominated communities to a CST-IV enriched microbiota with a high abundance of anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella* spp., *Escherichia coli,* and other dysbiotic bacteria (531, 532), with a substantial abundance of *Lactobacillus* spp. in menopausal women receiving HRT (533).

Pregnancy is also characterized by increased E2, which enhances *Lactobacilli* growth in the vagina of women with healthy pregnancies until term to the detriment of other bacteria (534–536). The vaginal presence of dysbiotic species such as *Gardnerella vaginalis*, *Prevotella* spp., *Sneathia* spp., and *Ureaplasma* spp. is increased in white women delivering preterm or presenting with obstetric

complications (534–541); however, this correlation is not significant in pregnant Black women (539, 542, 543), supporting the hypothesis that racial traits may predispose to specific commensal microbiota and immune adaptation in non-pregnant and pregnant women (544). Associations between abnormal vaginal microbiota and PTB have been observed in cases of early membrane rupture and immediate delivery. Low *Lactobacillus* and high bacterial diversity have been found early in pregnancy in vaginal samples of pregnant women undergoing a first-trimester miscarriage and in those supporting pregnancy after a threatened miscarriage with subsequent PTB and preterm premature rupture of membranes (PPROM) (539, 545, 546). Additionally, pathogens related to early-onset neonatal sepsis, such as *E. coli*, *Fusobacterium nucleatum,* and group B *Streptococcus,* are found in vaginal samples of pregnant women as early as twenty-eight weeks of pregnancy (547). These results suggest that alterations of the vaginal microbiota early in pregnancy can be a risk factor for pregnancy complications and early-onset infections and can predict adverse obstetric and neonatal outcomes.

Although most studies related to reproductive tract microbiota analyze vaginal samples, there is an increasing interest in the characterization of upper genital tract microbiota, as microbial profiles in the ovaries, fallopian tubes, and uterus may directly impact oocyte formation, fertilization, and embryo implantation, respectively. Although traditionally considered sterile, new evidence reveals that microorganisms colonize the upper genital tract. Characterization of the bacterial communities in the vagina, cervix, endometrium, tubes, ovaries, and peritoneal cavity suggests that the reproductive tract presents a microbiomal continuum that progressively decreases in bacterial load and increases in taxonomical diversity from the lower to upper organs (548).

B. The existence of the endometrial microbiota

The existence of microbiota inhabiting the uterine cavity has been debated for decades, but molecular techniques have revealed that the endometrium presents an indigenous microbiota in reproductive-age women. Recent research using functional meta-transcriptomics reveals that around 15% of the total RNA detected in endometrial samples belongs to microorganisms, with a distribution of 85% bacteria, 10% yeasts, 5% viruses, and 0.3% archaea (549). Detection of mRNA sequences of more than 5,000 different microorganisms in the endometrium demonstrates that the uterine cavity harbors a living and

active microbiota; however, its composition is nearly indistinguishable from background DNA controls in nearly 40% of women (550, 551).

The endometrial microbiota is a low-biomass community compared to bacterial load in the vagina, which harbors up to 10,000-fold more bacteria (10^7–10^8 colony-forming units of *Lactobacillus* per gram of vaginal fluid) in asymptomatic premenopausal women (548, 552, 553), or the gastrointestinal tract microbiota, with approximately 10^{11} bacterial cells per gram of fecal material (554). Three mechanisms have been described for colonization of the upper genital tract: (i) ascension of vaginal communities through the cervical canal, (ii) translocation of bacteria from the gut, and (iii) migration of oral microbiota to other organs through the bloodstream (509, 555). The most studied mechanism in healthy women is ascension from the vagina. In this scenario, conformational changes of endocervical mucins may create permeability of the cervical plug, leading to endometrial colonization with bacteria from the lower reproductive tract during specific periods of the menstrual cycle or under certain circumstances (556). This hypothesis is supported by the isolation of *Lactobacillus* spp., *Gardnerella vaginalis*, *Enterobacter* spp., and *Mycoplasma hominis* upon the culture of uterine samples collected by hysterectomy (557, 558) and the similarities between the bacterial composition observed by molecular methods in paired endometrial and vaginal samples collected from the same woman (553, 559, 560). Of note, a comparative study analyzing sequencing of the bacterial 16S rRNA gene in asymptomatic reproductive-aged women revealed that although the endometrial microbiota seems similar to that of the vagina, it is not identical in approximately 20% of women, supporting the existence of an endometrial microbiota (560).

The composition of the endometrial microbiota in healthy individuals is challenging to study due to the invasive nature of uterine sampling and the variability between studies in terms of sample type, phase of the cycle, and sequencing techniques make the core microbiota challenging to assess. While some studies indicate *Lactobacillus* as the major taxa in the endometrium and genera from the *Bifidobacteriaceae* and *Streptococcaceae* families (551, 560–563), others have found *Acinetobacter*, *Pseudomonas*, *Sphingobium,* or *Vagococcus* to dominate the endometrial niche (548, 550, 564, 565). The latter taxa have been characterized as potential contaminants from laboratory materials or reagents

in taxonomical studies using NGS in low-biomass samples; therefore, defining the core endometrial microbiome remains particularly challenging (566–573). Guidelines for good practice in endometrial microbiota analysis have recently been published (570, 574, 575); therefore, following these recommendations for study design, experimental work, and data analysis should improve the standardization of endometrial microbiota studies and support a consensus concerning the composition of the endometrial microbiota.

C. Microbial-host interactions in the periconceptional space

Molecular studies analyzing the endometrial microbiome using shotgun metagenomics reveal that bacteria in the endometrium participate in functions related to genomic stability, metabolism, energy production, motility, cell signaling, and immunity (548, 576, 577). Consistent with these findings, several mechanisms have been proposed to modulate the interplay between bacteria and the host in the reproductive tract through regulation of (i) endometrial cell survival, (ii) epithelial integrity, (iii) secretion of ligands and metabolites, (iv) immune activation, and (v) protection against pathogens (555, 578, 579) (**Figure 8**). Three of these mechanisms have major relevance in the context of reproduction.

1. Protection against pathogens

Endometrial epithelial and stromal cells express pattern recognition receptors (PRRs) as a first-line defense mechanism of the innate immune system that detects pathogen-associated molecular patterns (PAMPs) from invading bacteria, viruses, or yeasts.

Once endometrial PRRs sense the presence of pathogens in the reproductive tract, an inflammatory cascade activates an adaptative immune response to secrete antimicrobial peptides (AMPs) with antiviral, antifungal, and bactericidal properties into the EF. Interestingly, endometrial expression of PRRs and AMPs is hormonally regulated during the menstrual cycle (580, 581) and increases from the proliferative to the secretory phase, providing a safe niche for the embryo to implant. Colonizing the reproductive tract with commensal bacteria constitutes a defense mechanism against pathogens, as resident bacteria presenting adaptation advantages will occupy the niche and produce unfavorable

conditions for other bacterial species to grow, thereby preventing the invasion of pathogenic counterparts (578).

2. Integrity of the endometrial epithelium

Embryo adhesion and subsequent trophoblast invasion require loosening intercellular junctions and desmosomes during the WOI (582, 583). This remodeling is regulated by the secretion of cytokines, among other molecules (584). Commensal bacteria in the reproductive tract may contribute to the secretion of remodeling factors beneficial during the periconceptional period, promoting embryo implantation. In turn, pathogenic bacteria may alter this mechanism, leading to dysregulated epithelial barrier permeability that can impact embryo implantation but can provide a permissive environment for the movement of bacterial pathogens or viruses between epithelial cells, ultimately affecting embryo viability and pregnancy (578). Another potential mechanism leading to the loss of endometrial epithelial integrity is ECM degradation by bacterially-synthesized MMPs (555).

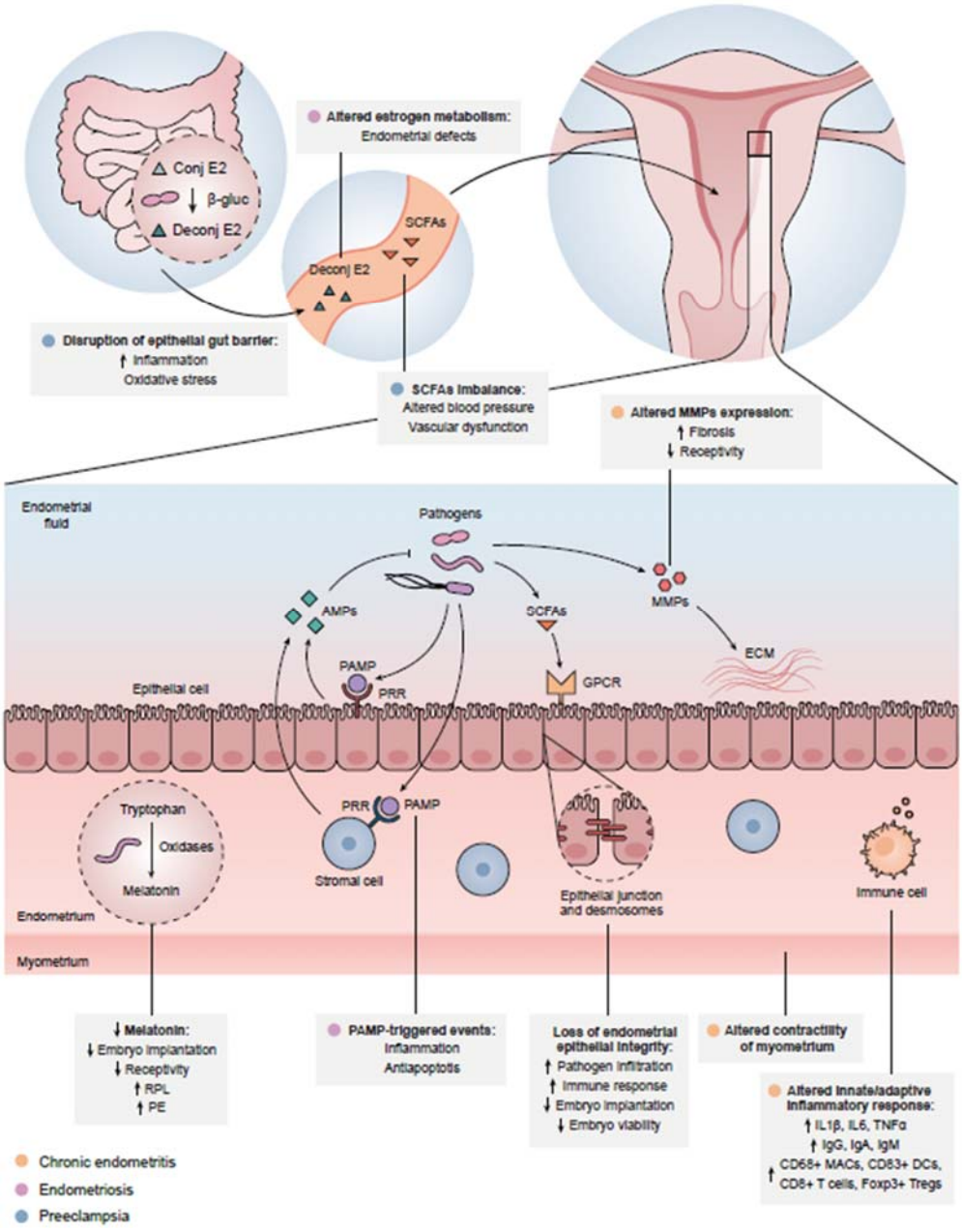


Figure 8. Microbial-host interactions involved in infertility and pregnancy complications. Several mechanisms between pathogenic bacteria or their by-products and human cells may be responsible for the loss of endometrial homeostasis, leading to impaired embryo implantation and gynecological or obstetrical conditions. Locally, altered endometrial immune balance, matrix metalloproteinase (MMP) expression, and uterine contractility may lead to chronic endometritis (CE). This inflammatory scenario and the antiapoptotic events caused by pathogen-associated molecular patterns (PAMPs) and pathogen recognition receptors (PRRs) in host cells have been observed in patients with endometriosis. In preeclampsia (PE), the intrauterine microbiome and an altered gut microbiome can contribute to the onset of disease by disrupting the epithelial barrier, releasing pathogen-associated reactive oxidative species and short-chain fatty acids to the bloodstream that may facilitate vascular dysfunction and subsequent increase in blood pressure. Dysbiosis in the gut or the endometrium may impair human reproduction and overall maternal health through the altered metabolism of systemic compounds such as estrogen, melatonin, and serotonin.

3. Secretion of promiscuous metabolites

Bacteria in the reproductive tract synthesize and secrete various molecules, including short-chain fatty acids, reactive oxygen species, vitamins, and oligosaccharides. Due to similarities in size, chemistry, and structure to human molecules, bacterial substances may function as ligands to effectively bind to human receptors, thereby activating host cellular processes (585). This mechanism occurs for GPCRs, the largest family of human membrane receptors responsible (among other functions) for hormonal and neurotransmitter signaling in the hypothalamus-pituitary-gonadal axis; additionally, GPCRs represent the pharmacological targets of nearly 33% of approved drugs (586, 587). In some instances, metabolic products from dysbiotic bacteria mimic human ligand binding to GPCRs, thereby altering intracellular cascades and interfering with drug metabolism. These metabolic products can also impact reproductive function if they target the signaling receptors of gonadotropin-releasing hormone, LH, and follicle-stimulating hormone (588, 589).

D. Impact of endometrial microbiota on human reproduction

Studies analyzing the vaginal microbiota of infertile women have indicated that a *Lactobacillus*-dominated microbiota is associated with better ART outcomes. In contrast, increased bacterial pathogens associated with bacterial vaginosis (*Atopobium vaginae*, *G. vaginalis*, *Leptotrichia* spp., *Prevotella* spp., *Sneathia* spp., and *Ureaplasma* spp.) are associated with failed ART cycles (590–599); however, multiple studies have failed to reveal any significant association between vaginal microbiota and pregnancy outcome (600, 601). The beneficial effects of vaginal *Lactobacillus* in ART outcomes may spread to the endometrium, where a higher abundance of *Lactobacilli* is found in fertile subjects compared to infertile patients receiving IVF (561).

Before molecular characterization of the endometrial microbiota using NGS, several groups used conventional microbial cultures to associate the isolation of *Lactobacillus* spp. from the distal tip of the embryo transfer catheter with better implantation and clinical pregnancy rates in IVF (602–604). In contrast, isolation of common endometrial pathogens, such as *Enterobacteriaceae* (including *E. coli*), *Staphylococcus* spp., *Streptococcus* spp., and other gram-negative bacteria, have been associated with a significant decrease in implantation and clinical pregnancy rates and an increase in miscarriage rates

(602, 603, 605–608). Recently, 16S rRNA gene sequencing has been used to investigate the relationship between endometrial microbiota composition and ART outcomes. The *Lactobacillus*-dominated endometrial microbiota influences reproduction, with poorer reproductive outcomes observed in IVF patients receiving embryo transfer with a non-*Lactobacillus*-dominated microbiota (560, 561). A pilot study conducted in thirty-five IVF patients with RIF receiving personalized embryo transfer provided evidence that women who had a live birth presented higher levels of *Lactobacillus* compared to women who miscarried or did not become pregnant, for whom reproductive tract dysbiotic taxa (e.g., *Gardnerella*, *Streptococcus*, *Veillonella*) were increased (560). However, other authors failed to find statistically significant associations between endometrial microbiota composition and reproductive outcomes (562, 609, 610).

The impact of the endometrial microbiota on ART outcomes was confirmed in the largest prospective observational study to date, which includes 342 infertile patients undergoing IVF in eight countries in America, Europe, and Asia in 2017–2020. The uterine microbiome in matched EF and endometrial biopsies were investigated during the WOI in these patients. *Lactobacilli* were more abundant than the rest of the taxa in patients with successful ART, while the dysbiotic microbiota was significantly associated with no pregnancy or clinical miscarriage. Predictive probability analysis demonstrated that patients with a higher frequency of *Lactobacillus* compared to other taxa were more likely to have a live birth. The bacterial genera significantly associated with poor reproductive outcomes were *Atopobium*, *Bifidobacterium*, *Chryseobacterium*, *Gardnerella*, *Haemophilus*, *Klebsiella*, *Neisseria*, and *Staphylococcus* (551) (**Table 1**). These results link taxon profiles with clinical outcomes, implicating the endometrial microbiome in infertility and implantation failure. The regulatory mechanisms of each bacterial taxa on embryo implantation are unknown, and one can only hypothesize that bacterial pathogens in the upper reproductive tract may trigger an inflammatory response in the endometrium that impairs embryo implantation. Two case reports have analyzed the mechanisms through which endometrial microbiota may affect human reproduction. The first clinical case describes taxonomic metagenomics of six EF samples collected longitudinally during an eighteen-month follow-up from an infertile patient presenting repeated reproductive failure (one ectopic pregnancy and two clinical

miscarriages) associated with *G. vaginalis* infection. At the functional level, *G. vaginalis* was likely responsible for 80% of the expressed genes in the sample collected before personalized embryo transfer, resulting in a clinical miscarriage. Among the detected genes associated with *G. vaginalis*, epithelial adhesion and biofilm formation, antimicrobial resistance, cytotoxicity, and other virulence factors were present, supporting the pathogenic potential of endometrial *G. vaginalis* in pregnancy outcomes (576).

Table 1. Endometrial microbiota profiles associated with poor reproductive outcomes in endometrial samples from women with failed IVF cycles compared to women with live birth

	ART outcome	Comparison to Live Birth (LB)	Bacterial taxa	p-value
Endometrial fluid	No pregnancy	Above 95% CI of LB	*Atopobium*	0.021
			Bifidobacterium	0.0001
			Gardnerella	0.00001
			Klebsiella	0.049
			Streptococcus	0.018
		Below 95% CI of LB	*Chryseobacterium*	0.008
			Lactobacillus	0.000003
			Micrococcus	0.028
	Biochemical pregnancy	Above 95% CI of LB	*Enterococcus*	0.011
		Below 95% CI of LB	n.s.	n.s.
	Clinical miscarriage	Above 95% CI of LB	*Haemophilus*	0.039
			Klebsiella	0.019
			Staphylococcus	0.031
		Below 95% CI of LB	*Lactobacillus*	0.002
Endometrial biopsy	No pregnancy	Above 95% CI of LB	*Bifidobacterium*	0.004
			Gardnerella	0.011
			Klebsiella	0.016
			Neisseria	0.023
		Below 95% CI of LB	*Cupriavidus*	0.038
			Enterococcus	0.015
			Finegoldia	0.029
			Lactobacillus	0.008
			Tepidimonas	0.004

Data from (551). ART: Assisted reproductive technology; CI: confidence interval; LB: Live birth.

The second case report described the endometrial microbiome of an early (four-week) pregnancy compared to just before personalized embryo transfer, resulting in miscarriage in the same patient.

Taxonomical and functional analyses revealed that *L. iners* was the only bacterium present in the uterine cavity of the pregnant woman, in contrast to a highly diverse microbiota found in the sample associated with miscarriage. Functional differences were also observed in the microbiome between the two samples, suggesting that *Lactobacillus* has a protective role in endometrial and reproductive health. In contrast, dysbiosis of the reproductive tract may impact the physiology of endometrial cells leading to altered embryo implantation or pregnancy (577). Although these results come from clinical case reports and conclusions should be made cautiously, they provide the first evidence supporting a role for endometrial microbiota in maternal-embryonic interactions during the periconceptional period.

E. Endometrial dysbiosis and the maternal-embryonic interface implicated in gynecological and obstetrical conditions

With increasing evidence of the role of microorganisms in human health, studies are underway to establish the specific role of the microbiota in the reproductive tract. Bacterial composition and the host-microbial functional interactions supporting homeostasis are important to understanding and determining how dysbiosis influences obstetrical and gynecological conditions (**Figure 8**).

1. Chronic endometritis

Chronic endometritis (CE) results when common bacterial pathogens cause persistent inflammation of the endometrial mucosa. CE is rarely suspected and clinically diagnosed, although symptoms can include pelvic pain, abnormal uterine bleeding, dyspareunia, and leukorrhea (611). CE diminishes the success of both spontaneous and ART conceptions and contributes to obstetric and neonatal complications such as chronic deciduitis and PTB (612–618).

The prevalence of CE in infertile patients is estimated at 2.8%–39% (619, 622–628) but can be as high as 60% or 66% in women diagnosed with unexplained RPL or RIF, respectively (619, 620). Because the pathogenic agents most frequently responsible for CE are *Enterococcus faecalis*, *Enterobacteriaceae*, *Streptococcus* and *Staphylococcus* spp., *G. vaginalis*, and *Mycoplasma* spp., and genital pathogens associated with sexually transmitted infections (e.g., *Ureaplasma urealyticum*,

Chlamydia trachomatis, and *Neisseria gonorrhoeae* (621, 622), CE is often treated with broad-spectrum antibiotics, which promotes antibiotic resistance and high rates of CE relapse.

The infectious etiology of CE is demonstrated by its reduction after antibiotic treatment in up to 80% of cases (623, 624); however, some patients with CE and a history of RIF only display a 28% response rate to the first course of antibiotics, with up to 25% patients potentially resistant to treatment after the third regimen of antibiotics (620). At the clinical level, pregnancy and ongoing pregnancy rates increase after antimicrobial therapy for CE in IVF patients with RIF, RPL, or unexplained infertility (619, 620, 625–627).

Several mechanisms may contribute to the onset and maintenance of CE and subsequent infertility. Infection with CE pathogens may produce an innate and adaptative inflammatory response by increasing levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), antibodies (IgA, IgG, and IgM), and endometrial infiltration of immune cells (CD68+ macrophages, CD83+ dendritic cells, CD8+ T lymphocytes, and Foxp3+ regulatory cells) (628–631). Additionally, women with CE show altered expression of MMPs, which may induce endometrial fibrosis and impair endometrial receptivity (631). Another mechanism linking CE with implantation failure is the altered frequency and direction of myometrial contractility during the mid-luteal phase of the menstrual cycle of CE patients, which may contribute to uterine bleeding, infertility, and endometriosis (632).

Recently, microbial infection as an exclusive cause of CE has been questioned, and new theories (including impaired endometrial hormonal status and autoimmune stimuli) have been proposed to contribute to the etiology of CE (633). Targeted therapeutic approaches based on a better understanding of bacterial ligand-human receptor interactions will be of immense value to the clinical management of these patients.

2. Endometriosis

Abnormalities in the reproductive tract microbiome may also play a role in the development of endometriosis and the progression and maintenance of ectopic endometrial lesions. This hypothesis has been coined the “bacterial contamination hypothesis” (634). Indeed, *E. coli* contamination of the

menstrual blood of women with endometriosis has been reported (635). Similarly, comparisons of bacterial 16S metagenome assays in the cystic fluid of endometriomas and non-endometrioma cysts demonstrate a significantly higher proportion of *Streptococcus* and *Staphylococcus* spp. in endometriomas (636). In addition, endometrial smears from women with endometriosis show an aberrant microbiota dominated by pathogens such as *Gardnerella*, *Enterococcus*, *Streptococcus*, *Staphylococcus*, and, to a lesser extent, *Actinomyces*, *Corynebacterium*, *Fusobacterium*, *Prevotella,* and *Propionibacterium,* which is in contrast to the *Lactobacillus*-dominated microbiota found in control women (637). Analysis of the reproductive tract microbiota via NGS demonstrates that microbiota-based models can distinguish subjects with and without endometriosis (548).

In support of the endometrial microbiota´s role in endometriosis, CE has been associated with endometriosis in a comparative immunohistochemical study examining endometrial samples obtained from a hysterectomy (638). Furthermore, microbial cultures of eutopic endometrium from endometriosis patients possess significantly higher amounts of *E. coli*, *Gardnerella*, α-*Streptococcus*, and *Enterococcus* spp. than tissues from women without endometriosis (639). A retrospective study in more than 140,000 subjects also demonstrated that patients with pelvic inflammatory disease presented an increased risk for endometriosis, suggesting that dysbiosis of the reproductive tract and transport of these bacteria from the lower to upper genital tract may cause endometriosis (640). Uterine dysbiosis involves an initial bacterial endometrial colonization by common pathogens that, together with the iron in blood from retrograde menstruation, may induce oxidative stress and initiate a cascade of PAMPs and damage-associated molecular patterns (DAMPs) that trigger persistent inflammation and activate antiapoptotic events (641). Interestingly, patients with endometriosis have significantly increased risk for spontaneous miscarriage, PTB, and other obstetrical complications such as gestational diabetes mellitus, hypertensive disorders of pregnancy, and placenta previa (652, 653). Thus, changes in the reproductive microbiome associated with endometriosis may provide helpful information to identify women at risk for developing infertility or chronic pelvic pain and which women may respond well to treatment.

In the last decade, it has become evident that the gut microbiome is a significant regulator of inflammatory responses inside and outside the gut (642). The gut microbiome contributes to the development and progression of inflammatory bowel disease, arthritis, psoriasis, and cancer (643). Endometriosis is a similar complex, polygenic condition involving pathologic inflammation, and similar pre-disposing genetic alterations associated with an immune response may be present. Microbiota could drive endometriosis by promoting inflammation, hormonal dysregulation, and oxidative stress, altering the cellular proliferation/apoptosis ratio and metabolism, and increasing angiogenesis/vascularization. Animal models have demonstrated an interconnection between endometriosis and the intestinal microbiome (644). Mouse models with transplanted endometriotic lesions have been shown to induce dysbiosis in the gut microbiota in terms of decreased α-diversity, imbalanced *Firmicutes*/*Bacteroidetes* ratio, and altered abundance of specific taxa. Although no consensus exists on the microbial profile associated with endometriosis (644–646) all the studies found abnormal levels of microbial taxa, with their by-products potentially inducing altered estrogen metabolism in endometriotic mice (644). Antibiotics also reduce the size and proliferation of endometriotic lesions and the inflammatory response in a mouse endometriosis model, while oral gavage of gut microbiota from endometriotic mice into metronidazole-treated mice restored the disease (646). Importantly, we must consider the limitations of these studies as (i) mice do not spontaneously develop endometriosis, and mouse models with surgical transplantation of endometrial tissue in the peritoneal cavity may not recapitulate the pathophysiology of endometriosis, (ii) some antibiotics (e.g., metronidazole) possess anti-inflammatory properties that could reduce endometriosis symptoms in treated mice, and (iii) sample size and experimental differences (e.g., animal age or hormonal treatments) represent essential factors to consider when extracting conclusions from studies. Meanwhile, the results of a bacterial culture-based study in rhesus monkeys that spontaneously developed endometriosis demonstrated an altered profile of the intestinal microflora in animals with the disease (647), together with the observation that women with endometriosis have a 50% increased risk of inflammatory bowel disease, support that gut dysbiosis is associated with endometriosis (648). A systematic review analyzing the link between endometriosis and inflammatory bowel disease concluded the existence of a positive association between these two diseases based on the results of

epidemiological studies; however, differences between studies have made a meta-analysis of the published literature challenging to undertake(649). It also remains unclear whether gut dysbiosis contributes to the etiology and progression of endometriosis as a consequence of the epithelial barrier loss, which could deregulate the immune function and estrogen metabolism, whether endometriosis produces dysbiosis through the secretion of pro-inflammatory molecules that affect the intestinal niche and alter the physiological microbiome in the gut; or if a bidirectional relationship exists. Furthermore, the pathophysiological mechanisms behind this association remain unknown.

3. Preeclampsia

The role of the microbiota in obstetric outcomes has been suggested by some studies standing up for the existence of a healthy placental microbiota in healthy pregnancies (514, 650–652) while others have reported increased bacterial species in the placenta and amniotic fluid of patients with PE using microbial culture and targeted PCR for the bacterial 16S rRNA gene (653–656). Bacteria associated with gastrointestinal (*Bacillus*, *Escherichia*, *Listeria*, and *Salmonella*), respiratory (*Anoxybacillus* and *K. pneumoniae*), and periodontal infections (*Dialister*, *Porphyromonas*, *Prevotella*, and *Variovorax*) were associated with PE (655). These findings suggested an intrauterine microbiome in the development of PE, with bacterial infection at the implantation site likely to harm local immune cells, EVT migration, and spiral artery remodeling (578). Under intrauterine infection, pathogens could trigger the untimely activation of steroid hormone receptors, leading to insufficient P4 action and deficient differentiation of endometrial stroma cells that secrete lower levels of IGFBP1 and PRL and contribute to defective trophoblast invasion (657). As a secondary event, the cellular immune response accompanying uterine infections is associated with acute atherosis of spiral arteries in the decidua basalis in patients with PE (658).

Notably, some studies describing a placental microbiome report the presence of bacteria that do not colonize humans (e.g., *Thioalkalivibrio*, *Arthrobacter*, and *Planctomycetes*) or are commonly present in negative controls (kitome) within the microbial communities in the placenta or the amniotic fluid, casting doubts on the robustness and plausibility of results. As the bacterial load in the placenta is minimal, if any, placental microbiome composition may have been influenced by potential

contamination or traces of bacterial DNA present in laboratory reagents (570, 652). These inconsistencies have questioned the existence of a stable and functional intrauterine microbiome in healthy pregnancies and have led to more rigorous studies, including experimental controls (both positive and negative) and an exhaustive biological interpretation of the data obtained. A recent study following the recommendations for analysis of low-biomass samples and controlling for false positive results has challenged the existence of a bonafide placental microbiota (656). The results of this prospective study analyzing more than 500 placental samples have shown that there are several sources of contamination (contact with bacteria during labor and delivery, contamination during sample collection and manipulation, microbiome associated with reagents used for extraction, amplification, and sequencing) that may impact the analysis of placental samples leading to artifactual microbiome profiles (656). This study concludes that the placenta does not have a stable and unique microbiome, although it can harbor pathogenic bacteria under certain conditions. For example, *Streptococcus agalactiae* has been found in 5% of placental samples before the onset of labor, although a significant association with PE has not been demonstrated. These results suggest that leading causes for PE other than the presence or composition of the placental microbiota may exist in most cases; however, intrauterine infections during pregnancy may contribute to a higher risk for the development of PE or other pregnancy-related complications.

The gut-placental axis has been suggested to play a role in the onset of PE, while gut dysbiosis could explain specific pre-eclamptic symptoms, as the gut microbiota profile of women with PE is similar to that of hypertensive non-pregnant women (659, 660). A consensus has not been reached regarding the composition of the gut microbiota associated with PE compared to normal pregnancies (673–676); however, it has been proposed that reducing bacteria producing short-chain fatty acids could be responsible for altered blood pressure in PE via irregular trophoblast function, endothelial dysfunction, and an insufficient supply of oxygen and nutrients to the placenta (661). At the molecular level, an imbalance in bacterial species producing short-chain fatty acids may impact blood pressure through dysregulation of plasminogen activator inhibitor-1, while butyrate and other short-chain fatty acids in the bloodstream can bind to GPCRs in target tissues to modulate blood pressure (661). For example,

Fusobacterium may disrupt the gut epithelial barrier and translocate to the placenta during PE, where the immune response is activated by the secretion of NF-κB and inflammatory cytokines that produce oxidative stress, vascular dysfunction, and ultimately induce rejection of the fetus and hypertension (660). Therefore, deciphering the impact of the gut microbiome on sPE onset could provide a new approach to targeting the pathogenesis of this significant pregnancy complication.

4. Chorioamnionitis, chronic deciduitis, and preterm birth

Chorioamnionitis is the inflammation of fetal membranes and is usually caused by bacterial infection. The association of chorioamnionitis with bacterial vaginosis suggests that the intrauterine infection responsible for chorioamnionitis may originate in the vagina (678); however, preconceptional CE also could represent the origin of microbial invasion of the amniotic cavity and has been described in gestational chronic deciduitis (662).

Colonization with *Corynebacterium* spp., *E. coli*, *Fusobacterium nucleatum*, *Peptostreptococcus* spp., *Prevotella* spp., group B *Streptococcus*, *Ureaplasma parvum,* and genital mycoplasmas are hallmarks of inflammation of the amnion and chorion and are linked to complications such as premature rupture of membranes (PROM), PTB, and neonatal sepsis (663–667).

As the endometrium is responsible for developing a healthy placenta that can carry a pregnancy to term, the microbiota and/or microbial compounds present in the intrauterine cavity at the time of conception may be important for embryo implantation and placentation. An association between chorioamnionitis and PE has been suggested; however, the increased bacterial load, together with pathogenic bacteria characteristic of microbial invasion of the amniotic cavity, has only been found in 1%–9% of patients with PE, so a causative association between the pathologies remains unlikely (654).

PTB is a major cause of infant morbidity and mortality and has also been associated with dysbiotic intrauterine microbiota. There is an increased abundance of bacterial pathogens such as *Bacteroides*, *Streptococcus* spp., *Ureaplasma* spp., *E. coli*, *F. nucleatum*, *G. vaginalis*, *M. hominis*, and *Sneathia sanguinegens* to the detriment of *L. crispatus* abundance in the choriodecidual space, amnion, chorion, amniotic fluid, placenta, and umbilical cord blood (667, 668). Colonization with dysbiotic bacteria and

resultant secretion of pro-inflammatory cytokines and PGs may induce uterine contractility and cervical shortening, leading to PTB.

F. The reproductive–brain axis

Studies in animal models confirm the presence of bidirectional trafficking of molecules between the enteric and nervous systems modulated by the secretion of microbial metabolites, which involves a role of the gut microbiota in neuropsychiatric disorders through the so-called microbiota-gut-brain axis (669). The impact of the gut microbiota on the brain, metabolic, and reproductive functions is mediated by the "estrobolome," the collection of genes responsible for E2 metabolism. Gut bacteria secrete the enzyme β-glucuronidase, which deconjugates and activates E2s to induce cellular responses upon binding to ERα and ERβ in E2-regulated organs, activating signaling pathways, and/or leading to epigenetic modifications (670). In the context of gut dysbiosis, defective deconjugation of E2 precursors decreases levels of circulating active E2s that can reach target organs, leading to pathologies such as obesity, metabolic syndrome, cardiovascular disease, endometriosis, polycystic ovary syndrome, endometrial hyperplasia, breast cancer, and infertility (670). This remote regulation of distant organs or functions by the microbiome is not exclusive to E2-regulated organs.

Mounting evidence suggests that gut microbiota regulate brain function. Microbial molecular mimicry produced upon gut dysbiosis may lead to serious neural conditions such as Alzheimer's or Parkinson's disease, autism spectrum disorder, or amyotrophic lateral sclerosis (671). For example, accumulation of misfolded α-synuclein that forms Lewy bodies in certain Parkinson's disease cases can be triggered by a structurally similar amyloid protein called curli produced in the gut by the bacterium *E. coli* that travels to the brain through the vagus nerve (672, 673). Interestingly, brain regulation exerted by the microbiota can influence a fetus if exposed to maternal infection while in utero, which was demonstrated in a Swedish cohort of patients born after exposure to maternal microbial infection (e.g., sepsis, pneumonia, chorioamnionitis, urinary tract infection) during fetal life, showing an increased risk of autism and depression during childhood and adulthood (674). Maternal immune activation (increased levels of cytokines and T cells) in the presence of pathogens could be responsible for neurodevelopmental disorders in the offspring if this occurs during fetal brain growth; however, these

long-term sequelae could have been caused by maternal pyrexia due to the altered maternal immune activation induced by fever independent of the presence of pathogens or their direct impact on fetal brain development (675).

Like other estrobolome-targeted organs, the endometrium is regulated by cycling hormones such as including E2 and P4. The gut microbiota may influence endometrial function by modulating the action of hormones that subsequently modulate the secretion of AMPs and immune response in the endometrium across the menstrual cycle, influencing the growth of specific commensal or pathogenic bacteria in a cyclical fashion (549).

Another link between microbiota and reproductive-brain function involves tryptophan oxidation to produce serotonin and metabolize melatonin. Several bacteria colonizing human tissues, including *Lactobacillus* and *Ruminococcus*, degrade tryptophan (676). Interestingly, the first step in tryptophan conversion to serotonin and melatonin is catalyzed by oxidases produced in the endometrium in a menstrual cycle-dependent manner in animal models (677). Both neurotransmitters play important roles in reproductive function. Maternal serotonin levels also have been linked to neural development in murine embryos (678) while melatonin contributes to oocyte quality, endometrial receptivity, embryo implantation, and pregnancy, with abnormal melatonin production in the endometrium and placenta associated with RPL and PE, respectively (679).

Clinical studies must now confirm the roles of certain bacteria in the reproductive-brain axis. The action of microbiota in regulating distant organs may help prevent neural disorders or as a therapeutic option to target inaccessible organs like the brain.

IX. THE MYOMETRIUM AND THE PERICONCEPTIONAL SPACE IN HEALTH AND DISEASE

The human myometrium comprises the middle layer of the uterine wall, located between the endometrium and the serosa or perimetrium. This muscular layer is subdivided into three separate layers: the inner stratum subvasculare, middle stratum vasculare, and outer stratum supravasculare. The

outer stratum supravasculare is the thickest and contains numerous venous plexuses and lymphatic vessels (680). While the inner third of the myometrium (junctional or sub-endometrial layer) may derive from the Müllerian duct and shows peristaltic activity, the external layer may derive from non-Müllerian tissue and is the primary contractile tissue during childbirth (681, 682).

Regarding cellular composition, the human myometrium mainly comprises smooth muscle cells (uterine myocytes) intermingling and spiraling throughout an ECM of connective and vascular tissue and lymphatic vessels and contributing to the normal tonicity of the uterus (683, 684). Additionally, interstitial cells of Cajal (ICC), which are closely related to smooth muscle cells, may play a crucial role as pacemaker cells by producing rhythmic oscillations in the resting membrane potential of smooth muscle cells (685).

The ability of the myometrium to grow and stretch supports the substantial changes that occur during pregnancy and childbirth; however, the primary function of this muscular tissue is to induce uterine contractions throughout a woman's reproductive timespan. Additional functions include those related to the physiological processes that occur during the periconceptional space, such as sperm transport and embryo implantation (682); nevertheless, the regulation of myometrial contractility in the non-pregnant state remains unclear.

A. Physiology of myometrial function

Myometrial contractility depends on the ability of smooth muscle cells to maintain, through specific ion channels, the difference in ion concentrations between the intracellular and extracellular spaces, undergoing phasic cycles of depolarization and repolarization (686–688).

In the early 20th century, Julius Bernstein, a pioneering neurobiologist and biophysicist, revealed through the "membrane theory of electrical potentials" that living muscle cells have a bioelectric membrane (689). Specifically, uterine smooth muscle possesses phasic action potentials consisting of a contractile pattern and maintenance to a resting tone, with intermittent contractions that display varied frequency, amplitude, and duration (682). Since then, additional knowledge has been gained regarding regulating muscle cells by hormonal and mechanical factors.

1. Hormonal regulation

Hormones and paracrine mediators may induce myometrial cells to enter quiescence or promote muscle contraction through regulation of gene expression, direct modulation of ion channel action, or activation of membrane receptors, activating a cascade of further cellular messengers (690–693).

Interestingly, uterine peristalsis increases during the menstrual cycle's early and mid-follicular phases. This process is probably controlled by estradiol secreted by the growing dominant follicle (694, 695) and is accompanied by an increase of oxytocin and prostaglandins, which could play a possible role in sperm transport (696, 697). Conversely, a decrease in uterine peristalsis has been observed in the luteal phase, accompanied by increased progesterone produced by the corpus luteum (685, 698). Consequently, the uterus enters a quiescent state that may allow embryo implantation in the case of fertilization (699) .

2. Mechanical factors

Distinguishing gross uterine activity of the outer two-thirds of the myometrium from uterine peristalsis of the inner one-third of the myometrium remains a challenging task (685) when discussing mechanical stretch. Indeed, the physical forces generated by distention or contraction of the myometrium, among other tissues, are mechanically transduced into intracellular signals, gene transcription, ECM production and degradation, and cell adhesion (700), ultimately producing inflammatory cytokines and ECM remodeling (701).

Data derived from intrauterine pressure and ultrasound research has suggested uterine peristalsis changes throughout the menstrual cycle (694, 702). Contractions from the fundus to the cervix have been detected in the early follicular phase, probably assisting in sperm transport, while a switch in the direction of contractility occurs in the late follicular phase to provide nutrients and position the embryo before implantation (685). Therefore, considering that uterine contractions also occur in the periconceptional space, this pressure-gradient force due to mechanical stimulation in the fundal segment of the uterus during reproductive-assisted treatments can influence the distribution or even expel the transferred load in the uterine cavity during embryo transfer (685, 703, 704).

B. Myometrial alterations in the origin of infertility

During implantation, the embryo adheres to and is encapsulated by the endometrium, forming a unique structure supported by the myometrium until the pregnancy ends. Consequently, myometrial physiological and/or morphological abnormalities, such as dysfunctional uterine contractility, anatomical distortion of the uterine cavity, and subsequent poor placentation, could affect the initiation and maintenance of pregnancy to term (705).

1. Uterine peristalsis impairment as the origin of unexplained infertility

Uterine contractility or peristalsis is influenced by ovarian hormones undergoing periodic changes with the menstrual cycle phases, increasing during the follicular phase due to E2 stimulation and changing to relative quiescence in response to P4 after ovulation. Indeed, contractions in the luteal phase are low amplitude, bidirectional, and believed to assist with the site and process of implantation (706, 707). Moreover, some authors have suggested that the contractile activity of the junctional zone may alter the shape of the uterus from an elongated configuration in the follicular phase to a piriform configuration in the luteal phase, making the uterine cavity more spherical (708). Nevertheless, if conception does not occur, strong antegrade contractions start simultaneously with progesterone withdrawal, facilitating the emptying of menstrual blood and tissue (707).

Notably, the peristaltic activity of the uterus represents a critical process in human reproduction, mainly during fertilization (709) and embryo implantation (710); furthermore, excessive peristalsis of myometrial tissue might cause unexplained infertility and embryo implantation failure (685, 707, 711, 712). In ART, a comparison between stimulated versus natural cycles revealed two-day delayed contractile activity following ovulation in the stimulated condition (712, 713). Higher estradiol concentrations due to ovarian stimulation treatment may be involved in this process, which is inversely related to the chances of pregnancy success (707). Additionally, women with pathologies such as endometriosis (714, 715), uterine fibroids (716), and/or adenomyosis (717, 718) exhibit altered uterine peristalsis, which may also contribute to infertility. Specifically, peristaltic detection rate and frequency are significantly less for patients with endometriosis and/or adenomyosis than healthy patients, which may contribute to an altered sperm transport during the periovulatory phase (714, 715, 717, 718).

Moreover, submucosal fibroids may also be associated with loss of peristalsis and focal myometrial movements related to pregnancy loss (716).

Based on the significance of uterine contractility under physiological and pathological conditions, there exists a need to better understand the role of therapeutic compounds such as anticholinergic agents (719–721) and prostaglandin synthetase inhibitors (722) in regulating uterine contractility and thus contributing to clinical outcomes in ART (685). Interestingly, using a combined oxytocin and vasopressin receptor antagonist (atosiban) may increase the amplitude of uterine contractions and thus decrease uterine peristalsis in women with RIF (723–725); however, studies evaluating the effects of these drugs show conflicting results (726). Thus, novel selective peptide oxytocin antagonists such as barusiban and/or nolasiban are the subject of current clinical trials.

2. Uterine fibroids

Uterine fibroids, also known as leiomyomas (uLM), are benign tumors arising from the myometrium composed of disorganized smooth-muscle cells surrounded by ECM. These benign myometrial tumors may exhibit myometrial cell morphology and phenotype changes due to menstrual cycle-related injury and repair (684, 727). Nevertheless, it remains unknown whether new smooth muscle cells arise from differentiated or tissue-specific stem cells (728).

In 2011, a detailed classification of uLM was established by the International Federation of Gynaecology and Obstetrics, which used a nine-point system to describe the location of uLM in relation to the endometrium and the serosal surface (729). According to this classification, it has been reported how uLM may affect fertility outcomes in women, including those undergoing assisted reproduction strategies (730). Specifically, subserous fibroids do not affect pregnancy or implantation rates(731, 732), but submucosal and large intramural uLM, which protrude into the endometrial cavity, associate with poor clinical outcomes due to their potential for growth under hormonal influence (733–735) (**Figure 9**). Indeed, they can physically block the tubal ostia or the cervix and cause changes such as cavity distortion, which affects sperm transport and embryo implantation (736).

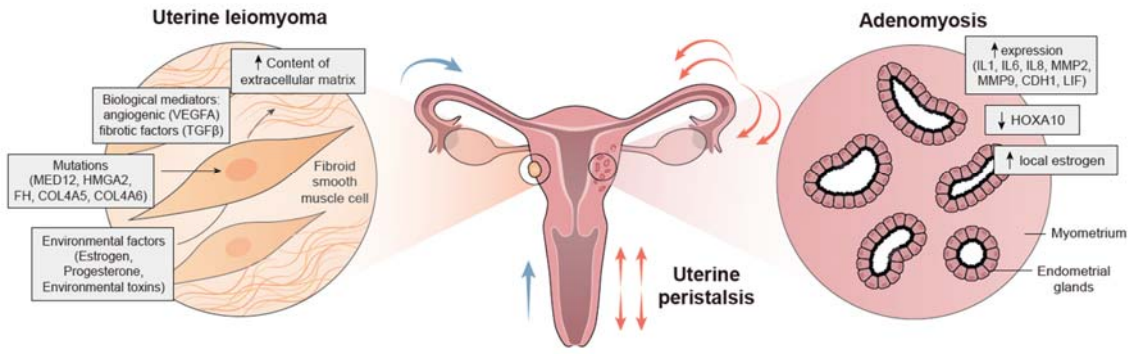


Figure 9. Schematic of main myometrial alterations as the origin of infertility conditions. Excessive peristalsis of myometrial tissue may cause infertility and embryo implantation failure. Myometrial tumors such as leiomyoma (LM) significantly impact fertility outcomes. Environmental factors, chromosomal abnormalities, and specific mutations may be involved in disease etiology and progression. Finally, the growth of ectopic endometrial glands into the myometrium (adenomyosis) could be triggered by factors such as local hyperestrogenism, elevated prolactin (PRL), and autoimmune factors.

The number of uLMs has also been examined in studies comparing the presence of one versus multiple tumors, although the underlying mechanisms of infertility remain unclear (731, 737). Independent of the number, size, or location of uLMs, several studies have examined the influence of uLM on embryo implantation in terms of endometrial receptivity (734, 738, 739), showing that uLMs adversely affect the decidualization response in the overlying endometrium (740, 741) and are responsible for excessive uterine bleeding or defective implantation (742–744). Indeed, a strong correlation between the expression of genes controlling the WOI, such as *TGFβ*, *osteopontin*, *GPX3*, and *glycodelin*, and uLM presence has been demonstrated (745, 746). *HOXA10* and *HOXA11* genes and LIFs, which are also thought to be crucial in the implantation process, display decreased expression in women with fibroids (747, 748). Other mechanisms involving uterine contractility dysfunction (749) and impaired vascularization (750, 751) also have been proposed to explain how uLM affects reproductive function.

Finally, available research has demonstrated increased pregnancy rates after surgery to remove large tumors or those abutting the endometrial cavity (733) ; however, surgical treatments can delay fertility treatment by at least six months while waiting for wound healing. Moreover, women are advised to deliver by cesarean section to prevent rupture of the uterine wall during contractions if the surgery involves opening the uterine cavity. Consequently, a balance must be reached between removing tumors

and the consequences of surgery. Reaching this balance requires a better understanding of the mechanisms through which myometrial tumors influence fertility outcomes.

3. Adenomyosis

Adenomyosis is an estrogen-dependent uterine disorder characterized by the growth of ectopic endometrial glands into hyperplastic myometrial fibers of the myometrium induced by enhanced cell survival, EMT, and cell migration, which leads to thickening of the uterus. It is also presented as an adenomyoma together with a uLM. This condition triggers dysmenorrhea, heavy menstrual bleeding, pelvic pain, and infertility (735, 752–754).

Although the etiologic mechanisms of adenomyosis remain unknown, the bulk of evidence supports an association with uterine trauma that may have broken the junctional zone between the endometrium and myometrium, followed by de novo metaplasia from adult stem cells and embryonic Mullerian remnants (754). Consequently, physiological damage caused by excessive uterine hyperperistalsis induced by an increase of local estrogen during early reproductive life most likely results in the same causative cascade (755, 756), which is likely the mechanism underlying the observation that adenomyosis often gets more severe after each pregnancy and childbirth (757). Hormonal factors, such as local hyperestrogenism (758), elevated PRL, and dysregulated autoimmune factors (759) have been suggested as possible risk factors for adenomyosis.

Adenomyosis affects both the myometrium and endometrial stroma and has a complex origin, including multifactorial genetic and biochemical factors (760, 761). Several studies show altered cytokines (IL-1, IL-6, and IL-8) and growth factors such as MMP2 and MMP9 in ectopic lesions of human endometrium (762) Further, the expression of other integrin adhesion molecules, such as E-cadherin is significantly increased in this condition (763), while *LIF* (764) and *HOXA10* expression was significantly reduced in adenomyosis (203) (**Figure 9**).

Overall, infertile women with adenomyosis are less likely to become pregnant and more likely to experience a miscarriage, perhaps due to an altered uterine environment, abnormal uterine contractility, increased inflammation, and abnormal eutopic endometrial function (765, 766). Conversely, women

who successfully achieve pregnancy experience preterm labor and PROM at high rates (767, 768). Treatments to improve this estrogen-dependent disease are based on only a few studies, mainly case reports, showing the regression of lesions and reduced hypertrophy by combining agonists of gonadotropin-releasing hormone (769) and surgery (770), also improving tissue remodeling and blood supply (771, 772). These procedures may lead to an elevated risk of uterine rupture during pregnancy since the removal of adenomyotic tissue is often accompanied by the excision of healthy myometrium (773, 774). Accordingly, further knowledge integrating molecular, genetic, clinical, and epidemiologic approaches will be essential to better understand this prevalent uterine disorder's pathogenesis and preventive strategies.

X. CONCLUSIONS AND FUTURE PERSPECTIVES

Humans are the result of genetics (nature) and environment (nurture) in equal measure. Timely assessments of a couple's reproductive genetic risk through expanded carrier screening represent an accepted and effective method to prevent genetic disorders in offspring.

The human embryo now undergoes consistent chromosomal assessment, with 50% of IVF cycles performed in the USA using PGT-A (47); however, many "healthy" embryos fail to produce healthy offspring, and most genetic defects underlying embryo developmental arrest, failed implantation, and miscarriage of euploid embryos remain undetermined. Identifying genes involved in embryonic lethality and some forms of idiopathic infertility has been hindered by their low incidence in the overall population, resulting in a slow discovery process and the development of targeted testing genetic panels. This difficulty is likely to be partially overcome by deploying large-scale genomics studies comprising infertile individuals with rare embryonic phenotypes (e.g., early embryonic lethality or recurrent miscarriage). Additionally, instead of using PGT to diagnose and deselect affected embryos, it may be possible to correct the molecular defect carried by the embryo through ex vivo genome editing during IVF cycles. Any future applications of PGT will require the implementation of additional regulatory frameworks to prioritize service access and ensure equity.

From the maternal side, single-cell analysis followed by systems biology approaches will be essential to integrate cellular, molecular, and spatial multi-omics data in the periconceptional space. The maternal endometrium and myometrium can be prospectively investigated before pregnancy to diagnose preexisting conditions before conception, facilitating targeted therapies to prevent infertility and pregnancy complications, such as sPE. While we possess a vast knowledge of endometrial physiology and its hormonal regulation, we remain far from understanding how the endometrium regenerates every month, which progenitor cells orchestrate endometrial architecture, and what stimuli participate in endometrial regeneration and dynamics.

The main challenge is the production of non-invasive diagnostic strategies for endometrial pathologies through liquid biopsy. Circulating plasma-derived human and bacterial DNA/RNA transcripts represent a promising translational source. The myometrium affects the initiation and maintenance of pregnancy. Our lack of knowledge of myometrial physiology requires effective model systems to better understand uterine contractility in the presence and absence of myometrial disorders. Myometrium-on-a-chip systems could establish hormonal and mechanical contributions to myometrial function and the influence of uterine contractility on fertilization and embryo implantation. On-chip approaches will also enhance drug discovery, with strategies to stimulate or inhibit uterine contractions likely to improve pregnancy rates after assisted reproductive technologies.

Regarding the environment, crosstalk during the preconceptional period remains far from being understood. In this regard, reliable 3D models of embryonic implantation could help describe the environmental challenges that might perturb embryo development and human implantation. Continued evaluations of the endometrial microbiome and its metabolic by-products may also identify interventions that could prevent intrauterine infections before and during pregnancy. Understanding bi-directional functional interactions between microbiome and host genetics will be meaningful in the dissection of the biological mechanisms of reproductive disorders and help discover new therapeutic targets that may not require antibiotics.

Periconceptional care that promotes a healthy maternal and fetal pregnancy is hugely cost-effective; therefore, investment in research into human periconceptional maternal-embryonic space and its timely translation to clinical care will likely result in significant improvements in lifelong health.

FIGURES LEGENDS

Figure 1. An individual's genome is composed of genetic features inherited from parents (as components of their genome or as de novo variants acquired by germinal cells) or acquired (as de novo variants during the first stages of embryo development). Genetic features that govern and modulate phenotypes primarily include aneuploidy, single nucleotide polymorphisms (SNPs), single nucleotide variants (SNVs), and microdeletions/duplications. Most inheritable genetic conditions are determined by defects in single genes (e.g., *CFTR* for cystic fibrosis) passed on by parents to their offspring. The phenotypic manifestation of genetic defects depends on the inheritance mechanism of their alleles: dominant or recessive. In addition, most genetic traits are under multifactorial (polygenic) control, where a specific phenotype (e.g., height) is determined by the combined effect of several genes and their interaction with environmental and lifestyle factors. Embryo chromosomal defects causing embryo implantation failure, miscarriage, and aneuploidy syndromes in the newborn (among other conditions) are mostly de novo events due to chromosomal mis-segregation in oocytes prior to conception. Although several genes have been implicated in determining an individual's reproductive fitness, isolated defects in several genes determine early reproductive failure (e.g., gamete maturational arrest, fertilization failure, or preimplantation embryo lethality).

Figure 2. The endometrial plasma membrane transformations and the accompanying molecular processes required for the receptive state. Schematic representation of the processes and molecules implicated in acquiring the window of implantation (WOI).

Figure 3. Endometrial cell types and alterations mapped by single-cell RNA-seq during the menstrual cycle and embryo implantation. Left panel; C1 Fluidigim single-cell atlas modified from (151) Right panel; 10X single-cell atlas modified from (261). Cell types present in the upper panel -

Lum.Epi: luminal epithelium; Cil.Epi: ciliated epithelium; Gl.Epi: glandular epithelium; dS: decidualized stroma; dM: decidual macrophage; SCT: syncytiotrophoblast; VCT: villous cytotrophoblast; EVT: extravillous trophoblast; PV: perivascular cells; Endo: endothelium; DC: dendritic cells; dNK: decidual natural killer cells. Cell types present in the lower panel - Epi: epithelium; F: fibroblasts; HB: Hofbauer cells; PC: plasma cells; Mo: monocytes; M: macrophages; NK: natural killer cells.

Figure 4. Cellular and molecular transformation during decidualization. Phenotypic transformation of human endometrial stromal cells (hESCs) into enlarged secretory decidual cells (1); extracellular matrix (ECM) changes in composition and function (2); leukocyte infiltration by uterine natural killer (uNK) cells (3); and promotion of angiogenesis and oxidative stress resistance (4).

Figure 5. Defective decidualization in infertility and pregnancy complications. Defective decidualization (DD) mediated by progesterone resistance, premature human endometrial stromal cell (hESC) senescence, transcriptomic alterations, and disbalance of immune response is present with infertility such as endometriosis, recurrent pregnancy loss (RPL), and late-onset pregnancy complications such as preeclampsia (PE), intrauterine growth restriction (IUGR), and placenta accreta.

Figure 6. Blastocyst implantation and placenta development. (**A**) Blastocyst adhesion to the endometrial lining. The endometrium becomes receptive to an implanting blastocyst for a brief period during the mid-secretory phase of the menstrual cycle. Blastocysts anchor to a suitable region in the endometrial luminal epithelium (LE), where there is reciprocal communication between the trophectoderm and the endometrial LE. The glandular epithelium (GE) secretes factors in the endometrial lumen that can act on the trophectoderm and the endometrial LE. Blastocysts firmly adhere to the endometrial LE, initiating implantation into the endometrium. During the window of implantation (WOI), the endometrial stroma undergoes remodeling whereby ESCs differentiate into decidualized stromal cells, which coincides with the recruitment of uterine natural killer (uNK) cells. Other immune cells, including macrophages, are present in the stroma. (**B**) The blastocyst trophectoderm differentiates into different placental trophoblast subtypes, forming the placental villous. The placental villous consists of an outer multinucleated syncytiotrophoblast with an underlying cytotrophoblast (CTB)

surrounding a mesenchymal stromal core that contains specialized immune cells known as Hofbauer cells. The villous tip attaches to the decidua, enabling the CTB to proliferate and form a column structure anchoring the villous to the decidua. The CTB differentiates to extravillous trophoblast (EVT), migrates, and invades endovascularly or interstitially into the decidua to plug the uterine spiral arteries, reducing oxygen supply to the placenta during a critical time in fetal development where the primary source of nutrition is from the endometrial glands. Around ten weeks gestation, EVT remodeling of the spiral arteries is completed, enabling adequate blood supply to the placenta for gas and nutrient exchange.

Figure 7. Maternal-embryo crosstalk during preimplantation. Schematic representation of the bidirectional communication occurring at the maternal-embryonic interface through the secretion of miRNAs, proteins, and mitochondrial DNA enclosed in exosomes and/or microvesicles.

Figure 8. Microbial-host interactions involved in infertility and pregnancy complications. Several mechanisms between pathogenic bacteria or their by-products and human cells may be responsible for the loss of endometrial homeostasis, leading to impaired embryo implantation and gynecological or obstetrical conditions. Locally, altered endometrial immune balance, matrix metalloproteinase (MMP) expression, and uterine contractility may lead to chronic endometritis (CE). This inflammatory scenario and the antiapoptotic events caused by pathogen-associated molecular patterns (PAMPs) and pathogen recognition receptors (PRRs) in host cells have been observed in patients with endometriosis. In preeclampsia (PE), the intrauterine microbiome and an altered gut microbiome can contribute to the onset of disease by disrupting the epithelial barrier, releasing pathogen-associated reactive oxidative species and short-chain fatty acids to the bloodstream that may facilitate vascular dysfunction and subsequent increase in blood pressure. Dysbiosis in the gut or the endometrium may impair human reproduction and overall maternal health through the altered metabolism of systemic compounds such as estrogen, melatonin, and serotonin.

Figure 9. Schematic of main myometrial alterations as the origin of infertility conditions. Excessive peristalsis of myometrial tissue may cause infertility and embryo implantation failure. Myometrial tumors such as leiomyoma (LM) significantly impact fertility outcomes. Environmental

factors, chromosomal abnormalities, and specific mutations may be involved in disease etiology and progression. Finally, the growth of ectopic endometrial glands into the myometrium (adenomyosis) could be triggered by factors such as local hyperestrogenism, elevated prolactin (PRL), and autoimmune factors.

Table 1. Endometrial microbiota profiles associated with poor reproductive outcomes in endometrial samples from women with failed IVF cycles compared to women with live birth

	ART outcome	Comparison to LB	Bacterial taxa	p-value
Endometrial fluid	No pregnancy	Above 95% CI of LB	*Atopobium*	0.021
			Bifidobacterium	0.0001
			Gardnerella	0.00001
			Klebsiella	0.049
			Streptococcus	0.018
		Below 95% CI of LB	*Chryseobacterium*	0.008
			Lactobacillus	0.000003
			Micrococcus	0.028
	Biochemical pregnancy	Above 95% CI of LB	*Enterococcus*	0.011
		Below 95% CI of LB	n.s.	n.s.
	Clinical miscarriage	Above 95% CI of LB	*Haemophilus*	0.039
			Klebsiella	0.019
			Staphylococcus	0.031
		Below 95% CI of LB	*Lactobacillus*	0.002
Endometrial biopsy	No pregnancy	Above 95% CI of LB	*Bifidobacterium*	0.004
			Gardnerella	0.011
			Klebsiella	0.016
			Neisseria	0.023
		Below 95% CI of LB	*Cupriavidus*	0.038
			Enterococcus	0.015
			Finegoldia	0.029
			Lactobacillus	0.008
			Tepidimonas	0.004

Data from (551). ART: Assisted reproductive technology; CI: confidence interval; LB: Live birth.

ACKNOWLEDGEMENTS

We thank Fresh Eyes Editing, LLC, and Stuart P. Atkinson for manuscript editing and proofreading and Antonio Garcia from Bio-graphics for figure preparation.

GRANTS

IM is supported by the FIS project (PI21/00235) from the Instituto de Salud Carlos III (ISCIII), Spain. AM is supported by the Miguel Servet Program CP19/00162 and FIS Project Grant PI20/00942 from ISCIII. BR is supported by the HUTER project (European Union's Horizon 2020 Framework Programme for Research and Innovation under grant agreement 874867). ED is supported by the

National Health Medical Research Council of Australia Project Grant #1120689 and Senior Research Fellowship #1117033. FV is supported by the Miguel Servet Program Type II (MSII18/00020), the FIS Project Grant PI21/00528 from ISCIII, and the European Union's Horizon 2020 Framework Programme for Research and Innovation under grant agreement number 874867. CS was supported by the Spanish Government MINECO/FEDER (grant RTI2018-094946-B-I00), the Valencian Innovation Council (ref. PROMETEO/2018/161), and the European Union's Horizon 2020 Framework Programme for Research and Innovation under grant agreement number 874867.

DISCLOSURES

AC is a full-time employee of Juno Genetics. MP is partially employed by Igenomix Italy. The rest of the authors declare no conflict of interest.

REFERENCES

1. McCandless SE, Brunger JW, Cassidy SB. The Burden of Genetic Disease on Inpatient Care in a Children's Hospital. *Am J Hum Genetics* 74: 121–127, 2004. doi: 10.1086/381053.

2. Baird PA, Anderson TW, Newcombe HB, Lowry RB. Genetic disorders in children and young adults: a population study. *Am J Hum Genet* 42: 677–93, 1988.

3. Martin J, Asan, Yi Y, Alberola T, Rodríguez-Iglesias B, Jiménez-Almazán J, Li Q, Du H, Alama P, Ruiz A, Bosch E, Garrido N, Simon C. Comprehensive carrier genetic test using next-generation deoxyribonucleic acid sequencing in infertile couples wishing to conceive through assisted reproductive technology. *Fertil Steril* 104: 1286–1293, 2015. doi: 10.1016/j.fertnstert.2015.07.1166.

4. Burgoyne PS, Holland K, Stephens R. Incidence of numerical chromosome anomalies in human pregnancy estimation from induced and spontaneous abortion data. *Hum Reprod* 6: 555–565, 1991. doi: 10.1093/oxfordjournals.humrep.a137379.

5. Ghulmiyyah L, Sibai B. Maternal Mortality From Preeclampsia/Eclampsia. *Semin Perinatol* 36: 56–59, 2012. doi: 10.1053/j.semperi.2011.09.011.

6. Li L, Guo F, Gao Y, Ren Y, Yuan P, Yan L, Li R, Lian Y, Li J, Hu B, Gao J, Wen L, Tang F, Qiao J. Single-cell multi-omics sequencing of human early embryos. *Nat Cell Biol* 20: 847–858, 2018. doi: 10.1038/s41556-018-0123-2.

7. Schulz KN, Harrison MM. Mechanisms regulating zygotic genome activation. *Nat Rev Genet* 20: 221–234, 2019. doi: 10.1038/s41576-018-0087-x.

8. Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, Prigmore E, Keelagher R, Best SK, Carey GK, Mellis R, Robart S, Berry IR, Chandler KE, Cilliers D, Cresswell L, Edwards SL, Gardiner C, Henderson A, Holden ST, Homfray T, Lester T, Lewis RA, Newbury-Ecob R, Prescott K, Quarrell OW, Ramsden SC, Roberts E, Tapon D, Tooley MJ, Vasudevan PC, Weber AP, Wellesley DG, Westwood P, White H, Parker M, Williams D, Jenkins L, Scott RH, Kilby MD, Chitty LS, Hurles ME, Maher ER, Consortium PA of G and E, Bateman M, Berry IR, Best SK, Campbell C, Campbell J, Carey G, Chandler KE, Chitty LS, Cilliers D, Cohen K, Collingwood E, Constantinou P, Cresswell L, Delmege C, Eberhardt RY, Edwards SL, Ellis R, Evans J, Everett T, Pinto CF, Forrester N, Fowler E, Gardiner C, Hamilton S, Healey K, Henderson A, Holden ST, Homfray T, Hudson R, Hurles ME, Jenkins L, Keelagher R, Kilby MD, Lester T, Lewis R, Lord J, Maher ER, Marton T, McMullan DJ, Mehta S, Mellis R, Newbury-Ecob R, Park S-M, Parker M, Prescott K, Prigmore E, Quarrell OW, Quinlan-Jones E, Ramsden SC, Rinck G, Robart S, Roberts E, Rowland J, Scott RH, Steer J, Tapon D, Taylor EJ, Tooley MJ, Vasudevan PC, Weber AP, Wellesley DG, Westwood P, White H, Williams D, Wilson E. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet Lond Engl* 393: 747–757, 2019. doi: 10.1016/s0140-6736(18)31940-8.

9. Capalbo A, Fabiani M, Caroselli S, Poli M, Girardi L, Patassini C, Favero F, Cimadomo D, Vaiarelli A, Simon C, Rienzi LF, Ubaldi FM. Clinical validity and utility of preconception expanded carrier screening for the management of reproductive genetic risk in IVF and general population. *Hum Reprod* 36: 2050–2061, 2021. doi: 10.1093/humrep/deab087.

10. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L. Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. *New Engl J Medicine* 367: 2175–2184, 2012. doi: 10.1056/nejmoa1203382.

11. Acuna-Hidalgo R, Bo T, Kwint MP, van de Vorst M, Pinelli M, Veltman JA, Hoischen A, Vissers LELM, Gilissen C. Post-zygotic Point Mutations Are an Underrecognized Source of De Novo Genomic Variation. *Am J Hum Genetics* 97: 67–74, 2015. doi: 10.1016/j.ajhg.2015.05.008.

12. Organisation WH. WHO reveals leading causes of death and disability worldwide: 2000-2019 [Online]. https://www.who.int/news/item/09-12-2020-who-reveals-leading-causes-of-death-and-disability-worldwide-2000-2019.

13. Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, Natarajan P, Lander ES, Lubitz SA, Ellinor PT, Kathiresan S. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet* 50: 1219–1224, 2018. doi: 10.1038/s41588-018-0183-z.

14. Hassanin E, May P, Aldisi R, Spier I, Forstner AJ, Nöthen MM, Aretz S, Krawitz P, Bobbili DR, Maj C. Breast and prostate cancer risk: The interplay of polygenic risk, rare pathogenic germline variants, and family history. *Genet Med* 24: 576–585, 2022. doi: 10.1016/j.gim.2021.11.009.

15. Mars N, Koskela JT, Ripatti P, Kiiskinen TTJ, Havulinna AS, Lindbohm JV, Ahola-Olli A, Kurki M, Karjalainen J, Palta P, Palotie A, Daly M, Jacob H, Matakidou A, Runz H, John S, Plenge R, McCarthy M, Hunkapiller J, Ehm M, Waterworth D, Fox C, Malarstig A, Klinger K, Call K, Mäkelä T, Kaprio J, Virolainen P, Pulkki K, Kilpi T, Perola M, Partanen J, Pitkäranta A, Kaarteenaho R, Vainio S, Savinainen K, Kosma V-M, Kujala U, Tuovila O, Hendolin M, Pakkanen R, Waring J, Riley-Gillis B, Matakidou A, Runz H, Liu J, Biswas S, Hunkapiller J, Waterworth D, Ehm M, Diogo D, Fox C, Malarstig A, Marshall C, Hu X, Call K, Klinger K, Gossel M, Ripatti S, Schleutker J, Perola M, Arvas M, Carpen O, Hinttala R, Kettunen J, Laaksonen R, Mannermaa A, Paloneva J, Kujala U, Tuovila O, Hendolin M, Pakkanen R, Soininen H, Julkunen V, Remes A, Kälviäinen R, Hiltunen M, Peltola J, Tienari P, Rinne J, Ziemann A, Waring J, Esmaeeli S, Smaoui N, Lehtonen A, Eaton S, Runz H, Lahdenperä S, Biswas S, Michon J, Kerchner G, Hunkapiller J, Bowers N, Teng E, Eicher J, Mehta V, Gormley P, Linden K, Whelan C, Xu F, Pulford D, Färkkilä M, Pikkarainen S, Jussila A, Blomster T, Kiviniemi M, Voutilainen M, Georgantas B, Heap G, Waring J, Smaoui N, Rahimov F, Lehtonen A, Usiskin K, Maranville J, Lu T, Bowers N, Oh D, Michon J, Mehta V, Kalpala K, Miller M, Hu X, McCarthy L, Eklund K, Palomäki A, Isomäki P, Pirilä L, Kaipiainen-Seppänen O, Huhtakangas J, Georgantas B, Waring J, Rahimov F, Lertratanakul A, Smaoui N, Lehtonen A, Close D, Hochfeld M, Bowers N, Michon J, Diogo D, Mehta V, Kalpala K, Bing N, Hu X, Gordillo JE, Mars N, Laitinen T, Pelkonen M, Kauppi P, Kankaanranta H, Harju T, Smaoui N, Close D, Greenberg S, Chen H, Bowers N, Michon J, Mehta V, Betts J, Ghosh S, Salomaa V, Niiranen T, Juonala M, Metsärinne K, Kähönen M, Junttila J, Laakso M, Pihlajamäki J, Sinisalo J, Taskinen M-R, Tuomi T, Laukkanen J, Challis B, Peterson A, Hunkapiller J, Bowers N, Michon J, Diogo D, Chu A, Mehta V, Parkkinen J, Miller M, Muslin A, Waterworth D, Joensuu H, Meretoja T, Carpen O, Aaltonen L, Auranen A, Karihtala P, Kauppila S, Auvinen P, Elenius K, Popovic R, Waring J, Riley-Gillis B,

Lehtonen A, Matakidou A, Schutzman J, Hunkapiller J, Bowers N, Michon J, Mehta V, Loboda A, Chhibber A, Lehtonen H, McDonough S, Crohns M, Kulkarni D, Kaarniranta K, Turunen J, Ollila T, Seitsonen S, Uusitalo H, Aaltonen V, Uusitalo-Järvinen H, Luodonpää M, Hautala N, Runz H, Strauss E, Bowers N, Chen H, Michon J, Podgornaia A, Mehta V, Diogo D, Hoffman J, Tasanen K, Huilaja L, Hannula-Jouppi K, Salmi T, Peltonen S, Koulu L, Harvima I, Kalpala K, Wu Y, Choy D, Michon J, Smaoui N, Rahimov F, Lehtonen A, Waterworth D, Jalanko A, Kajanne R, Lyhs U, Kaunisto M, Davis JW, Riley-Gillis B, Quarless D, Petrovski S, Liu J, Chen C-Y, Bronson P, Yang R, Maranville J, Biswas S, Chang D, Hunkapiller J, Bhangale T, Bowers N, Diogo D, Holzinger E, Gormley P, Wang X, Chen X, Hedman Å, Auro K, Wang C, Xu E, Auge F, Chatelain C, Kurki M, Ripatti S, Daly M, Karjalainen J, Havulinna A, Jalanko A, Palin K, Palta P, Parolo PDB, Zhou W, Lemmelä S, Rivas M, Harju J, Palotie A, Lehisto A, Ganna A, Llorens V, Karlsson A, Kristiansson K, Arvas M, Hyvärinen K, Ritari J, Wahlfors T, Koskinen M, Carpen O, Kettunen J, Pylkäs K, Kalaoja M, Karjalainen M, Mantere T, Kangasniemi E, Heikkinen S, Mannermaa A, Laakkonen E, Kononen J, Loukola A, Laiho P, Sistonen T, Kaiharju E, Laukkanen M, Järvensivu E, Lähteenmäki S, Männikkö L, Wong R, Kristiansson K, Mattsson H, Lemmelä S, Hiekkalinna T, Jiménez MG, Donner K, Palta P, Pärn K, Nunez-Fontarnau J, Harju J, Kilpeläinen E, Sipilä TP, Brein G, Dada A, Awaisa G, Shcherban A, Sipilä T, Laivuori H, Havulinna A, Lemmelä S, Kiiskinen T, Laitinen T, Siirtola H, Tabuenca JG, Kallio L, Soini S, Partanen J, Pitkänen K, Vainio S, Savinainen K, Kosma V-M, Kuopio T, Neale BM, Daly M, Salomaa V, Palotie A, Widén E, Ripatti S. Polygenic and clinical risk scores and their impact on age at onset and prediction of cardiometabolic diseases and common cancers. *Nat Med* 26: 549–557, 2020. doi: 10.1038/s41591-020-0800-0.

16. Ruth KS, Day FR, Hussain J, Martínez-Marchal A, Aiken CE, Azad A, Thompson DJ, Knoblochova L, Abe H, Tarry-Adkins JL, Gonzalez JM, Fontanillas P, Claringbould A, Bakker OB, Sulem P, Walters RG, Terao C, Turon S, Horikoshi M, Lin K, Onland-Moret NC, Sankar A, Hertz EPT, Timshel PN, Shukla V, Borup R, Olsen KW, Aguilera P, Ferrer-Roda M, Huang Y, Stankovic S, Timmers PRHJ, Ahearn TU, Alizadeh BZ, Naderi E, Andrulis IL, Arnold AM, Aronson KJ, Augustinsson A, Bandinelli S, Barbieri CM, Beaumont RN, Becher H, Beckmann MW, Benonisdottir S, Bergmann S, Bochud M, Boerwinkle E, Bojesen SE, Bolla MK, Boomsma DI, Bowker N, Brody JA, Broer L, Buring JE, Campbell A, Campbell H, Castelao JE, Catamo E, Chanock SJ, Chenevix-Trench G, Ciullo M, Corre T, Couch FJ, Cox A, Crisponi L, Cross SS, Cucca F, Czene K, Smith GD, Geus EJCN de, Mutsert R de, Vivo ID, Demerath EW, Dennis J, Dunning AM, Dwek M, Eriksson M, Esko T, Fasching PA, Faul JD, Ferrucci L, Franceschini N, Frayling TM, Gago-Dominguez M, Mezzavilla M, García-Closas M, Gieger C, Giles GG, Grallert H, Gudbjartsson DF, Gudnason V, Guénel P, Haiman CA, Håkansson N, Hall P, Hayward C, He C, He W, Heiss G, Høffding MK, Hopper JL, Hottenga JJ, Hu F, Hunter D, Ikram MA, Jackson RD, Joaquim MDR, John EM, Joshi PK, Karasik D, Kardia SLR, Kartsonaki C, Karlsson R, Kitahara CM, Kolcic I, Kooperberg C, Kraft P, Kurian AW, Kutalik Z, Bianca ML, LaChance G, Langenberg C, Launer LJ, Laven JSE, Lawlor DA, Marchand LL, Li J, Lindblom A, Lindstrom S, Lindstrom T, Linet M, Liu Y, Liu S, Luan J, Mägi R, Magnusson PKE, Mangino M, Mannermaa A, Marco B, Marten J, Martin NG, Mbarek H, McKnight B, Medland SE, Meisinger C, Meitinger T, Menni C, Metspalu A, Milani L, Milne RL, Montgomery GW, Mook-Kanamori DO, Mulas A, Mulligan AM, Murray A, Nalls MA, Newman A, Noordam R, Nutile T, Nyholt DR, Olshan AF, Olsson H, Painter JN, Patel AV, Pedersen NL, Perjakova N, Peters A, Peters U, Pharoah PDP, Polasek O, Porcu E, Psaty BM, Rahman I, Rennert G, Rennert HS, Ridker PM, Ring SM, Robino A, Rose LM, Rosendaal FR, Rossouw J, Rudan I, Rueedi R, Ruggiero D, Sala CF, Saloustros E, Sandler DP, Sanna S, Sawyer EJ, Sarnowski C, Schlessinger D, Schmidt MK, Schoemaker MJ, Schraut KE, Scott C,

Shekari S, Shrikhande A, Smith AV, Smith BH, Smith JA, Sorice R, Southey MC, Spector TD, Spinelli JJ, Stampfer M, Stöckl D, Meurs JBJ van, Strauch K, Styrkarsdottir U, Swerdlow AJ, Tanaka T, Teras LR, Teumer A, Þorsteinsdottir U, Timpson NJ, Toniolo D, Traglia M, Troester MA, Truong T, Tyrrell J, Uitterlinden AG, Ulivi S, Vachon CM, Vitart V, Völker U, Vollenweider P, Völzke H, Wang Q, Wareham NJ, Weinberg CR, Weir DR, Wilcox AN, Dijk KW van, Willemsen G, Wilson JF, Wolffenbuttel BHR, Wolk A, Wood AR, Zhao W, Zygmunt M, Consortium BIOS (BIOS), Consortium eQTLGen, Project BJ, Group CKBC, Investigators kConFab, Study LC, consortium I, Team 23andMe Research, Chen Z, Li L, Franke L, Burgess S, Deelen P, Pers TH, Grøndahl ML, Andersen CY, Pujol A, Lopez-Contreras AJ, Daniel JA, Stefansson K, Chang-Claude J, Schouw YT van der, Lunetta KL, Chasman DI, Easton DF, Visser JA, Ozanne SE, Namekawa SH, Solc P, Murabito JM, Ong KK, Hoffmann ER, Murray A, Roig I, Perry JRB. Genetic insights into biological mechanisms governing human ovarian ageing. *Nature* 596: 393–397, 2021. doi: 10.1038/s41586-021-03779-7.

17. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: mechanisms and new insights into an age-old problem. *Nat Rev Genet* 13: 493–504, 2012. doi: 10.1038/nrg3245.

18. Gruhn JR, Zielinska AP, Shukla V, Blanshard R, Capalbo A, Cimadomo D, Nikiforov D, Chan AC-H, Newnham LJ, Vogel I, Scarica C, Krapchev M, Taylor D, Kristensen SG, Cheng J, Ernst E, Bjørn A-MB, Colmorn LB, Blayney M, Elder K, Liss J, Hartshorne G, Grøndahl ML, Rienzi L, Ubaldi F, McCoy R, Lukaszuk K, Andersen CY, Schuh M, Hoffmann ER. Chromosome errors in human eggs shape natural fertility over reproductive life span. *Science* 365: 1466–1469, 2019. doi: 10.1126/science.aav7321.

19. Holesh J, Bass A, Lord M. Physiology, Ovulation. StatPearls, StatPearls Publishing, 2021. .

20. Arroyo A, Kim B, Yeh J. Luteinizing Hormone Action in Human Oocyte Maturation and Quality: Signaling Pathways, Regulation, and Clinical Impact. *Reprod Sci* 27: 1223–1252, 2020. doi: 10.1007/s43032-019-00137-x.

21. Zielinska AP, Bellou E, Sharma N, Frombach A-S, Seres KB, Gruhn JR, Blayney M, Eckel H, Moltrecht R, Elder K, Hoffmann ER, Schuh M. Meiotic Kinetochores Fragment into Multiple Lobes upon Cohesin Loss in Aging Eggs. *Curr Biol* 29: 3749-3765.e7, 2019. doi: 10.1016/j.cub.2019.09.006.

22. Capalbo A, Hoffmann ER, Cimadomo D, Ubaldi FM, Rienzi L. Human female meiosis revised: new insights into the mechanisms of chromosome segregation and aneuploidies from advanced genomics and time-lapse imaging. *Hum Reprod Update* 23: 706–722, 2017. doi: 10.1093/humupd/dmx026.

23. Zielinska AP, Holubcova Z, Blayney M, Elder K, Schuh M. Sister kinetochore splitting and precocious disintegration of bivalents could explain the maternal age effect. *Elife* 4: e11389, 2015. doi: 10.7554/elife.11389.

24. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, Scott RT. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril* 101: 656-663.e1, 2014. doi: 10.1016/j.fertnstert.2013.11.004.

2716 25. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat*
2717 *Rev Genet* 2: 280–291, 2001. doi: 10.1038/35066065.

2718 26. Shahbazi MN, Wang T, Tao X, Weatherbee BAT, Sun L, Zhan Y, Keller L, Smith GD,
2719 Pellicer A, Scott RT, Seli E, Zernicka-Goetz M. Developmental potential of aneuploid human
2720 embryos cultured beyond implantation. *Nat Commun* 11: 3987, 2020. doi: 10.1038/s41467-
2721 020-17764-7.

2722 27. Soler A, Morales C, Mademont-Soler I, Margarit E, Borrell A, Borobio V, Muñoz M,
2723 Sánchez A. Overview of Chromosome Abnormalities in First Trimester Miscarriages: A Series
2724 of 1,011 Consecutive Chorionic Villi Sample Karyotypes. *Cytogenet Genome Res* 152: 81–89,
2725 2017. doi: 10.1159/000477707.

2726 28. Lebedev I. Mosaic Aneuploidy in Early Fetal Losses. *Cytogenet Genome Res* 133: 169–
2727 183, 2011. doi: 10.1159/000324120.

2728 29. Mai CT, Isenburg JL, Canfield MA, Meyer RE, Correa A, Alverson CJ, Lupo PJ, Riehle-
2729 Colarusso T, Cho SJ, Aggarwal D, Kirby RS, Network NBDP. National population-based
2730 estimates for major birth defects, 2010–2014. *Birth Defects Res* 111: 1420–1435, 2019. doi:
2731 10.1002/bdr2.1589.

2732 30. Meyer RE, Liu G, Gilboa SM, Ethen MK, Aylsworth AS, Powell CM, Flood TJ, Mai CT,
2733 Wang Y, Canfield MA, Network NBDP. Survival of children with trisomy 13 and trisomy 18:
2734 A multi-state population-based study. *Am J Med Genet A* 170: 825–837, 2015. doi:
2735 10.1002/ajmg.a.37495.

2736 31. Shin M, Siffel C, Correa A. Survival of children with mosaic Down syndrome. *Am J Med*
2737 *Genet A* 152A: 800–801, 2010. doi: 10.1002/ajmg.a.33295.

2738 32. Rasmussen SA, Wong L-Y, Correa A, Gambrell D, Friedman JM. Survival in infants with
2739 Down syndrome, Metropolitan Atlanta, 1979-1998. *J Pediatrics* 148: 806-812.e1, 2006. doi:
2740 10.1016/j.jpeds.2006.01.010.

2741 33. NORD (National Organization for Rare Disorders). https://rarediseases.org/for-patients-
2742 and-families/information-resources/rare-disease-information/. .

2743 34. Hong DS, Reiss AL. Cognitive and neurological aspects of sex chromosome aneuploidies.
2744 *Lancet Neurology* 13: 306–318, 2014. doi: 10.1016/s1474-4422(13)70302-8.

2745 35. Gall JL, Nizon M, Pichon O, Andrieux J, Audebert-Bellanger S, Baron S, Beneteau C,
2746 Bilan F, Boute O, Busa T, Cormier-Daire V, Ferec C, Fradin M, Gilbert-Dussardier B, Jaillard
2747 S, Jønch A, Martin-Coignard D, Mercier S, Moutton S, Rooryck C, Schaefer E, Vincent M,
2748 Sanlaville D, Caignec CL, Jacquemont S, David A, Isidor B. Sex chromosome aneuploidies
2749 and copy-number variants: a further explanation for neurodevelopmental prognosis variability?
2750 *Eur J Hum Genet* 25: 930–934, 2017. doi: 10.1038/ejhg.2017.93.

2751 36. Starostik MR, Sosina OA, McCoy RC. Single-cell analysis of human embryos reveals
2752 diverse patterns of aneuploidy and mosaicism. *Genome Res* 30: 814–825, 2020. doi:
2753 10.1101/gr.262774.120.

2754 37. Treff NR, Marin D. The “mosaic” embryo: misconceptions and misinterpretations in
2755 preimplantation genetic testing for aneuploidy. *Fertil Steril* 116: 1205–1211, 2021. doi:
2756 10.1016/j.fertnstert.2021.06.027.

2757 38. Capalbo A, Ubaldi FM, Rienzi L, Scott R, Treff N. Detecting mosaicism in trophectoderm
2758 biopsies: current challenges and future possibilities. *Hum Reproduction Oxf Engl* 32: 492–498,
2759 2017. doi: 10.1093/humrep/dew250.

2760 39. Popovic M, Dhaenens L, Boel A, Menten B, Heindryckx B. Chromosomal mosaicism in
2761 human blastocysts: the ultimate diagnostic dilemma. *Hum Reprod Update* 26: 313–334, 2020.
2762 doi: 10.1093/humupd/dmz050.

2763 40. Victor AR, Tyndall JC, Brake AJ, Lepkowsky LT, Murphy AE, Griffin DK, McCoy RC,
2764 Barnes FL, Zouves CG, Viotti M. One hundred mosaic embryos transferred prospectively in a
2765 single clinic: exploring when and why they result in healthy pregnancies. *Fertil Steril* 111:
2766 280–293, 2019. doi: 10.1016/j.fertnstert.2018.10.019.

2767 41. Girardi L, Serdarogullari M, Patassini C, Poli M, Fabiani M, Caroselli S, Coban O, Findikli
2768 N, Boynukalin FK, Bahceci M, Chopra R, Canipari R, Cimadomo D, Rienzi L, Ubaldi F,
2769 Hoffmann E, Rubio C, Simon C, Capalbo A. Incidence, Origin, and Predictive Model for the
2770 Detection and Clinical Management of Segmental Aneuploidies in Human Embryos. *Am J*
2771 *Hum Genetics* 106: 525–534, 2020. doi: 10.1016/j.ajhg.2020.03.005.

2772 42. Coorens THH, Oliver TRW, Sanghvi R, Sovio U, Cook E, Vento-Tormo R, Haniffa M,
2773 Young MD, Rahbari R, Sebire N, Campbell PJ, Charnock-Jones DS, Smith GCS, Behjati S.
2774 Inherent mosaicism and extensive mutation of human placentas. *Nature* 592: 80–85, 2021. doi:
2775 10.1038/s41586-021-03345-1.

2776 43. Lestou VS, Kalousek DK. Confined placental mosaicism and intrauterine fetal growth.
2777 *Archives Dis Child - Fetal Neonatal Ed* 79: F223, 1998. doi: 10.1136/fn.79.3.f223.

2778 44. Tiegs AW, Tao X, Zhan Y, Whitehead C, Kim J, Hanson B, Osman E, Kim TJ, Patounakis
2779 G, Gutmann J, Castelbaum A, Seli E, Jalas C, Scott RT. A multicenter, prospective, blinded,
2780 nonselection study evaluating the predictive value of an aneuploid diagnosis using a targeted
2781 next-generation sequencing-based preimplantation genetic testing for aneuploidy assay and
2782 impact of biopsy. *Fertil Steril* 115: 627–637, 2020. doi: 10.1016/j.fertnstert.2020.07.052.

2783 45. Marin D, Xu J, Treff NR. Preimplantation genetic testing for aneuploidy: A review of
2784 published blastocyst reanalysis concordance data. *Prenatal Diag* 41: 545–553, 2021. doi:
2785 10.1002/pd.5828.

2786 46. Rubio C, Bellver J, Rodrigo L, Castillón G, Guillén A, Vidal C, Giles J, Ferrando M,
2787 Cabanillas S, Remohí J, Pellicer A, Simón C. In vitro fertilization with preimplantation genetic
2788 diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. *Fertil*
2789 *Steril* 107: 1122–1129, 2017. doi: 10.1016/j.fertnstert.2017.03.011.

2790 47. Hipp HS, Crawford S, Boulet S, Toner J, Sparks AAE, Kawwass JF. Trends and Outcomes
2791 for Preimplantation Genetic Testing in the United States, 2014-2018. *Jama* 327: 1288, 2022.
2792 doi: 10.1001/jama.2022.1892.

48. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, Cimadomo D, Benini F, Farcomeni A, Cuzzi J, Rubio C, Albani E, Sacchi L, Vaiarelli A, Figliuzzi M, Findikli N, Coban O, Boynukalin FK, Vogel I, Hoffmann E, Livi C, Levi-Setti PE, Ubaldi FM, Simón C. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. *Am J Hum Genet* 108: 2238–2247, 2021. doi: 10.1016/j.ajhg.2021.11.002.

49. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, Sun X, Ling X, Li H, Hao C, Tan J, Yang J, Zhu Y, Liu F, Chen D, Wei D, Lu J, Ni T, Zhou W, Wu K, Gao Y, Shi Y, Lu Y, Zhang T, Wu W, Ma X, Ma H, Fu J, Zhang J, Meng Q, Zhang H, Legro RS, Chen Z-J. Live Birth with or without Preimplantation Genetic Testing for Aneuploidy. *New Engl J Med* 385: 2047–2058, 2021. doi: 10.1056/nejmoa2103613.

50. Munne S, Kaplan B, Frattarelli JL, Gysler M, Child TJ, Nakhuda G, Shamma FN, Silverberg K, Kalista T, Oliver K, Katz-Jaffe M, Wells D, Gordon T, Willman S. Global multicenter randomized controlled trial comparing single embryo transfer with embryo selected by preimplantation genetic screening using next-generation sequencing versus morphologic assessment. *Fertil Steril* 108: e19, 2017. doi: 10.1016/j.fertnstert.2017.07.079.

51. Sacchi L, Albani E, Cesana A, Smeraldi A, Parini V, Fabiani M, Poli M, Capalbo A, Levi-Setti PE. Preimplantation Genetic Testing for Aneuploidy Improves Clinical, Gestational, and Neonatal Outcomes in Advanced Maternal Age Patients Without Compromising Cumulative Live-Birth Rate. *J Assist Reprod Gen* 36: 2493–2504, 2019. doi: 10.1007/s10815-019-01609-4.

52. Committee EPCS, Carvalho F, Coonen E, Goossens V, Kokkali G, Rubio C, Meijer-Hoogeveen M, Moutou C, Vermeulen N, Rycke MD. ESHRE PGT Consortium good practice recommendations for the organisation of PGT. *Hum Reproduction Open* 2020: hoaa021, 2020. doi: 10.1093/hropen/hoaa021.

53. Technology PC of the AS for RM and the S for AR, Penzias A, Bendikson K, Butts S, Coutifaris C, Falcone T, Fossum G, Gitlin S, Gracia C, Hansen K, Barbera AL, Mersereau J, Odem R, Paulson R, Pfeifer S, Pisarska M, Rebar R, Reindollar R, Rosen M, Sandlow J, Vernon M, Widra E. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. *Fertil Steril* 109: 429–436, 2018. doi: 10.1016/j.fertnstert.2018.01.002.

54. Somigliana E, Busnelli A, Paffoni A, Vigano P, Riccaboni A, Rubio C, Capalbo A. Cost-effectiveness of preimplantation genetic testing for aneuploidies. *Fertil Steril* 111: 1169–1176, 2019. doi: 10.1016/j.fertnstert.2019.01.025.

55. Capalbo A, Poli M, Riera-Escamilla A, Shukla V, Høffding MK, Krausz C, Hoffmann ER, Simon C. Preconception genome medicine: current state and future perspectives to improve infertility diagnosis and reproductive and health outcomes based on individual genomic data. *Hum Reprod Update* 27: 254–279, 2020. doi: 10.1093/humupd/dmaa044.

56. Sang Q, Li B, Kuang Y, Wang X, Zhang Z, Chen B, Wu L, Lyu Q, Fu Y, Yan Z, Mao X, Xu Y, Mu J, Li Q, Jin L, He L, Wang L. Homozygous Mutations in WEE2 Cause Fertilization Failure and Female Infertility. *Am J Hum Genetics* 102: 649–657, 2018. doi: 10.1016/j.ajhg.2018.02.015.

57. Zhang Z, Li B, Fu J, Li R, Diao F, Li C, Chen B, Du J, Zhou Z, Mu J, Yan Z, Wu L, Liu S, Wang W, Zhao L, Dong J, He L, Liang X, Kuang Y, Sun X, Sang Q, Wang L. Bi-allelic Missense Pathogenic Variants in TRIP13 Cause Female Infertility Characterized by Oocyte Maturation Arrest. *Am J Hum Genetics* 107: 15–23, 2020. doi: 10.1016/j.ajhg.2020.05.001.

58. Persson M, Cnattingius S, Villamor E, Söderling J, Pasternak B, Stephansson O, Neovius M. Risk of major congenital malformations in relation to maternal overweight and obesity severity: cohort study of 1.2 million singletons. *Bmj* 357: j2563, 2017. doi: 10.1136/bmj.j2563.

59. Robson SC, Chitty LS, Morris S, Verhoef T, Ambler G, Wellesley DG, Graham R, Leader C, Fisher J, Crolla JA. Evaluation of Array Comparative genomic Hybridisation in prenatal diagnosis of fetal anomalies:a multicentre cohort study with cost analysis and assessment of patient, health professional and commissioner preferences for array comparative genomic hybridisation. *Efficacy Mech Evaluation* 4: 1–104, 2017. doi: 10.3310/eme04010.

60. Hillman SC, Pretlove S, Coomarasamy A, Mcmullan DJ, Davison EV, Maher ER, Kilby MD. Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systematic review and meta-analysis. *Ultrasound Obst Gyn* 37: 6–14, 2011. doi: 10.1002/uog.7754.

61. Petrovski S, Aggarwal V, Giordano JL, Stosic M, Wou K, Bier L, Spiegel E, Brennan K, Stong N, Jobanputra V, Ren Z, Zhu X, Mebane C, Nahum O, Wang Q, Kamalakaran S, Malone C, Anyane-Yeboa K, Miller R, Levy B, Goldstein DB, Wapner RJ. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. *Lancet* 393: 758–767, 2019. doi: 10.1016/s0140-6736(18)32042-7.

62. Talkowski ME, Rehm HL. Introduction of genomics into prenatal diagnostics. *Lancet* 393: 719–721, 2019. doi: 10.1016/s0140-6736(19)30193-x.

63. Fay RA, Ellwood DA. Categories of intrauterine growth retardation. *Fetal Maternal Medicine Rev* 5: 203–212, 1993. doi: 10.1017/s0965539500000899.

64. Samuels ME. Saturation of the Human Phenome. *Curr Genomics* 11: 482–499, 2010. doi: 10.2174/138920210793175886.

65. Capalbo A, Valero RA, Jimenez-Almazan J, Pardo PM, Fabiani M, Jiménez D, Simon C, Rodriguez JM. Optimizing clinical exome design and parallel gene-testing for recessive genetic conditions in preconception carrier screening: Translational research genomic data from 14,125 exomes. *Plos Genet* 15: e1008409, 2019. doi: 10.1371/journal.pgen.1008409.

66. Wakap SN, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, Murphy D, Cam YL, Rath A. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. *Eur J Hum Genet* 28: 165–173, 2020. doi: 10.1038/s41431-019-0508-0.

67. Cao A. Carrier screening and genetic counselling in β-thalassemia. *Int J Hematol* 76: 105–113, 2002. doi: 10.1007/bf03165098.

68. Fabiani M, Cogo F, Poli M, Capalbo A. Technical factors to consider when developing an Expanded Carrier Screening platform. *Curr Opin Obstet Gyn* 33: 178–183, 2021. doi: 10.1097/gco.0000000000000706.

69. Chokoshvili D, Vears D, Borry P. Expanded carrier screening for monogenic disorders: where are we now? *Prenatal Diag* 38: 59–66, 2018. doi: 10.1002/pd.5109.

70. Archibald AD, Smith MJ, Burgess T, Scarff KL, Elliott J, Hunt CE, Barns-Jenkins C, Holt C, Sandoval K, Kumar VS, Ward L, Allen EC, Collis SV, Cowie S, Francis D, Delatycki MB, Yiu EM, Massie RJ, Pertile MD, Sart D du, Bruno D, Amor DJ. Reproductive genetic carrier screening for cystic fibrosis, fragile X syndrome, and spinal muscular atrophy in Australia: outcomes of 12,000 tests. *Genet Med* 20: 513–523, 2018. doi: 10.1038/gim.2017.134.

71. Franasiak JM, Olcha M, Bergh PA, Hong KH, Werner MD, Forman EJ, Zimmerman RS, Scott RT. Expanded carrier screening in an infertile population: how often is clinical decision making affected? *Genet Med* 18: 1097–1101, 2016. doi: 10.1038/gim.2016.8.

72. Ghiossi CE, Goldberg JD, Haque IS, Lazarin GA, Wong KK. Clinical Utility of Expanded Carrier Screening: Reproductive Behaviors of At-Risk Couples. *J Genet Couns* 27: 616–625, 2018. doi: 10.1007/s10897-017-0160-1.

73. Azimi M, Schmaus K, Greger V, Neitzel D, Rochelle R, Dinh T. Carrier screening by next-generation sequencing: health benefits and cost effectiveness. *Mol Genetics Genom Medicine* 4: 292–302, 2016. doi: 10.1002/mgg3.204.

74. Beauchamp KA, Taber KAJ, Muzzey D. Clinical impact and cost-effectiveness of a 176-condition expanded carrier screen. *Genet Med* 21: 1948–1957, 2019. doi: 10.1038/s41436-019-0455-8.

75. Zhang L, Bao Y, Riaz M, Tiller J, Liew D, Zhuang X, Amor DJ, Huq A, Petelin L, Nelson M, James PA, Winship I, McNeil JJ, Lacaze P. Population genomic screening of all young adults in a health-care system: a cost-effectiveness analysis. *Genet Med* 21: 1958–1968, 2019. doi: 10.1038/s41436-019-0457-6.

76. Wert G de, Hout S van der, Goddijn M, Vassena R, Frith L, Vermeulen N, Eichenlaub-Ritter U, Committee EE, Blanchet V, D'Angelo A, Wert G de, Eguizabal C, Eichenlaub-Ritter U, Frith L, Geril A, Goddijn M, Heindryckx B, Mertes H, Ombelet W, Rautakallio-Hokkanen S, Strowitzki T, Tapanainen J, Tarlatzis BC, Eede BV den, Westin C. The ethics of preconception expanded carrier screening in patients seeking assisted reproduction. *Hum Reproduction Open* 2021: hoaa063, 2021. doi: 10.1093/hropen/hoaa063.

77. Handyside AH, Lesko JG, Tarín JJ, Winston RML, Hughes MR. Birth of a Normal Girl after in Vitro Fertilization and Preimplantation Diagnostic Testing for Cystic Fibrosis. *New Engl J Medicine* 327: 905–909, 1992. doi: 10.1056/nejm199209243271301.

78. Medicine EC of the AS for R, Daar J, Benward J, Collins L, Davis J, Davis O, Francis L, Gates E, Ginsburg E, Gitlin S, Klipstein S, McCullough L, Paulson R, Reindollar R, Ryan G, Sauer M, Tipton S, Westphal L, Zweifel J. Use of preimplantation genetic testing for monogenic defects (PGT-M) for adult-onset conditions: an Ethics Committee opinion. *Fertil Steril* 109: 989–992, 2018. doi: 10.1016/j.fertnstert.2018.04.003.

79. Rycke MD, Goossens V, Kokkali G, Meijer-Hoogeveen M, Coonen E, Moutou C. ESHRE PGD Consortium data collection XIV-XV: cycles from January 2011 to December 2012 with

pregnancy follow-up to October 2013. *Hum Reproduction Oxf Engl* 32: 1974–1994, 2017. doi: 10.1093/humrep/dex265.

80. Treff NR, Zimmerman R, Bechor E, Hsu J, Rana B, Jensen J, Li J, Samoilenko A, Mowrey W, Alstine JV, Leondires M, Miller K, Paganetti E, Lello L, Avery S, Hsu S, Tellier LCAM. Validation of concurrent preimplantation genetic testing for polygenic and monogenic disorders, structural rearrangements, and whole and segmental chromosome aneuploidy with a single universal platform. *Eur J Med Genet* 62: 103647, 2019. doi: 10.1016/j.ejmg.2019.04.004.

81. Treff NR, Eccles J, Lello L, Bechor E, Hsu J, Plunkett K, Zimmerman R, Rana B, Samoilenko A, Hsu S, Tellier LCAM. Utility and First Clinical Application of Screening Embryos for Polygenic Disease Risk Reduction. *Front Endocrinol* 10: 845, 2019. doi: 10.3389/fendo.2019.00845.

82. Turley P, Meyer MN, Wang N, Cesarini D, Hammonds E, Martin AR, Neale BM, Rehm HL, Wilkins-Haug L, Benjamin DJ, Hyman S, Laibson D, Visscher PM. Problems with Using Polygenic Scores to Select Embryos. *New Engl J Med* 385: 78–86, 2021. doi: 10.1056/nejmsr2105065.

83. Karavani E, Zuk O, Zeevi D, Barzilai N, Stefanis NC, Hatzimanolis A, Smyrnis N, Avramopoulos D, Kruglyak L, Atzmon G, Lam M, Lencz T, Carmi S. Screening Human Embryos for Polygenic Traits Has Limited Utility. *Cell* 179: 1424-1435.e8, 2019. doi: 10.1016/j.cell.2019.10.033.

84. Zheutlin AB, Dennis J, Linnér RK, Moscati A, Restrepo N, Straub P, Ruderfer D, Castro VM, Chen C-Y, Ge T, Huckins LM, Charney A, Kirchner HL, Stahl EA, Chabris CF, Davis LK, Smoller JW. Penetrance and Pleiotropy of Polygenic Risk Scores for Schizophrenia in 106,160 Patients Across Four Health Care Systems. *Am J Psychiat* 176: 846–855, 2019. doi: 10.1176/appi.ajp.2019.18091085.

85. Treff N, Marin D, Lello L, Hsu S, Tellier LC. Preimplantation Genetic Testing for Polygenic Disease Risk. *Reproduction* 1: A13–A17, 2020. doi: 10.1530/rep-20-0071.

86. Gingold JA, Lee JA, Rodriguez-Purata J, Whitehouse MC, Sandler B, Grunfeld L, Mukherjee T, Copperman AB. Endometrial pattern, but not endometrial thickness, affects implantation rates in euploid embryo transfers. *Fertil Steril* 104: 620-628.e5, 2015. doi: 10.1016/j.fertnstert.2015.05.036.

87. Cha J, Dey SK. Cadence of procreation: Orchestrating embryo–uterine interactions. *Semin Cell Dev Biol* 34: 56–64, 2014. doi: 10.1016/j.semcdb.2014.05.005.

88. Yoshinaga K, Adams C. Delayed implantation in the spayed, progesterone treated adult mouse. *Reproduction* 12: 593–595, 1966. doi: 10.1530/jrf.0.0120593.

89. Yoshinaga K. Uterine Receptivity for Blastocyst Implantation. *Ann Ny Acad Sci* 541: 424–431, 1988. doi: 10.1111/j.1749-6632.1988.tb22279.x.

90. Gómez E, Ruíz-Alonso M, Miravet J, Simón C. Human Endometrial Transcriptomics: Implications for Embryonic Implantation. *Csh Perspect Med* 5: a022996, 2015. doi: 10.1101/cshperspect.a022996.

91. Medina-Estrada I, Alva-Murillo N, López-Meza JE, Ochoa-Zarzosa A. Immunomodulatory Effects of 17β-Estradiol on Epithelial Cells during Bacterial Infections. *J Immunol Res* 2018: 6098961, 2018. doi: 10.1155/2018/6098961.

92. Ruiz-Alonso M, Blesa D, Simón C. The genomics of the human endometrium. *BBA - Molecular Basis of Disease* 1822: 1931–1942, 2012. doi: 10.1016/j.bbadis.2012.05.004.

93. Critchley HOD, Maybin JA, Armstrong GM, Williams ARW. Physiology of the Endometrium and Regulation of Menstruation. *Physiol Rev* 100: 1149–1179, 2020. doi: 10.1152/physrev.00031.2019.

94. Adams NR, DeMayo FJ. Regulation of Implantation and Establishment of Pregnancy in Mammals, Tribute to 45 Year Anniversary of Roger V. Short's "Maternal Recognition of Pregnancy." *Adv Anat Embryology Cell Biology* 216: 27–50, 2015. doi: 10.1007/978-3-319-15856-3_3.

95. Hess A, Nayak N, Giudice L. Oviduct and Endometrium Cyclic Changes in the Primate Oviduct and Endometrium. In: , edited by Neill's RK and N of. K and. Physiology of Reproduction, 2006, p. 337–381.

96. Thie M, Harrach-Ruprecht B, Sauer H, Fuchs P, Albers A, Denker HW. Cell adhesion to the apical pole of epithelium: a function of cell polarity. *European journal of cell biology* 66: 180–191, 1995.

97. Nikas G, Psychoyos A. Uterine Pinopodes in Peri-implantation Human Endometrium Clinical Relevance. *Ann Ny Acad Sci* 816: 129–142, 1997. doi: 10.1111/j.1749-6632.1997.tb52136.x.

98. Surveyor GA, Gendler SJ, Pemberton L, Das SK, Chakraborty I, Julian J, Pimental RA, Wegner CC, Dey SK, Carson DD. Expression and steroid hormonal control of Muc-1 in the mouse uterus. *Endocrinology* 136: 3639–3647, 1995. doi: 10.1210/endo.136.8.7628404.

99. Hewitt SC, Li L, Grimm SA, Chen Y, Liu L, Li Y, Bushel PR, Fargo D, Korach KS. Research Resource: Whole-Genome Estrogen Receptor α Binding in Mouse Uterine Tissue Revealed by ChIP-Seq. *Mol Endocrinol* 26: 887–898, 2012. doi: 10.1210/me.2011-1311.

100. Rubel CA, Lanz RB, Kommagani R, Franco HL, Lydon JP, DeMayo FJ. Research Resource: Genome-Wide Profiling of Progesterone Receptor Binding in the Mouse Uterus. *Mol Endocrinol* 26: 1428–1442, 2012. doi: 10.1210/me.2011-1355.

101. Mote PA, Balleine RL, McGowan EM, Clarke CL. Colocalization of Progesterone Receptors A and B by Dual Immunofluorescent Histochemistry in Human Endometrium during the Menstrual Cycle1. *J Clin Endocrinol Metabolism* 84: 2963–2971, 1999. doi: 10.1210/jcem.84.8.5928.

2986 102. Fujimoto J, Hori M, Ichigo S, Tamaya T. Ovarian Steroids Regulate the Expression of
2987 Basic Fibroblast Growth Factor and its mRNA in Fibroblasts Derived from Uterine
2988 Endometrium. *Ann Clin Biochem* 34: 91–96, 1996. doi: 10.1177/000456329703400114.

2989 103. Tsai S-J, Wu M-H, Chen H-M, Chuang P-C, Wing L-YC. Fibroblast Growth Factor-9 Is
2990 an Endometrial Stromal Growth Factor. *Endocrinology* 143: 2715–2721, 2002. doi:
2991 10.1210/endo.143.7.8900.

2992 104. Hewitt S, Korach K. Oestrogen receptor knockout mice: roles for oestrogen receptors
2993 alpha and beta in reproductive tissues. *Reproduction* 125: 143–149, 2003. doi:
2994 10.1530/rep.0.1250143.

2995 105. Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS,
2996 Gustafsson J-Å, Smithies O. Generation and reproductive phenotypes of mice lacking estrogen
2997 receptor β. *Proc National Acad Sci* 95: 15677–15682, 1998. doi: 10.1073/pnas.95.26.15677.

2998 106. Lakha F, Ho PC, Spuy ZMV der, Dada K, Elton R, Glasier AF, Critchley HOD, Williams
2999 ARW, Baird DT. A novel estrogen-free oral contraceptive pill for women: multicentre, double-
3000 blind, randomized controlled trial of mifepristone and progestogen-only pill (levonorgestrel).
3001 *Hum Reprod* 22: 2428–2436, 2007. doi: 10.1093/humrep/dem177.

3002 107. Shaw KA, Topp NJ, Shaw JG, Blumenthal PD. Mifepristone–Misoprostol Dosing Interval
3003 and Effect on Induction Abortion Times. *Obstetrics Gynecol* 121: 1335–1347, 2013. doi:
3004 10.1097/aog.0b013e3182932f37.

3005 108. Das A, Mantena SR, Kannan A, Evans DB, Bagchi MK, Bagchi IC. De novo synthesis of
3006 estrogen in pregnant uterus is critical for stromal decidualization and angiogenesis. *Proc*
3007 *National Acad Sci* 106: 12542–12547, 2009. doi: 10.1073/pnas.0901647106.

3008 109. Jabbour HN, Kelly RW, Fraser HM, Critchley HOD. Endocrine regulation of
3009 menstruation. *Endocrine reviews* 27: 17–46, 2006. doi: 10.1210/er.2004-0021.

3010 110. Tan J, Paria BC, Dey SK, Das SK. Differential Uterine Expression of Estrogen and
3011 Progesterone Receptors Correlates with Uterine Preparation for Implantation and
3012 Decidualization in the Mouse. *Endocrinology* 140: 5310–5321, 1999. doi:
3013 10.1210/endo.140.11.7148.

3014 111. Patel B, Elguero S, Thakore S, Dahoud W, Bedaiwy M, Mesiano S. Role of nuclear
3015 progesterone receptor isoforms in uterine pathophysiology. *Hum Reprod Update* 21: 155–173,
3016 2015. doi: 10.1093/humupd/dmu056.

3017 112. Takamoto N, Zhao B, Tsai SY, DeMayo FJ. Identification of Indian Hedgehog as a
3018 Progesterone-Responsive Gene in the Murine Uterus. *Mol Endocrinol* 16: 2338–2348, 2002.
3019 doi: 10.1210/me.2001-0154.

3020 113. Takamoto N, Kurihara I, Lee K, DeMayo FJ, Tsai M-J, Tsai SY. Haploinsufficiency of
3021 Chicken Ovalbumin Upstream Promoter Transcription Factor II in Female Reproduction. *Mol*
3022 *Endocrinol* 19: 2299–2308, 2005. doi: 10.1210/me.2005-0019.

3023 114. Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA, Shyamala G,
3024 Conneely OM, O'Malley BW. Mice lacking progesterone receptor exhibit pleiotropic
3025 reproductive abnormalities. *Gene Dev* 9: 2266–2278, 1995. doi: 10.1101/gad.9.18.2266.

3026 115. Wetendorf M, DeMayo FJ. Progesterone receptor signaling in the initiation of pregnancy
3027 and preservation of a healthy uterus. *Int J Dev Biol* 58: 95–106, 2014. doi:
3028 10.1387/ijdb.140069mw.

3029 116. Singh H, Aplin JD. Adhesion molecules in endometrial epithelium: tissue integrity and
3030 embryo implantation. *J Anat* 215: 3–13, 2009. doi: 10.1111/j.1469-7580.2008.01034.x.

3031 117. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, Buck CA. Integrin
3032 adhesion molecules in the human endometrium. Correlation with the normal and abnormal
3033 menstrual cycle. *J Clin Invest* 90: 188–195, 1992. doi: 10.1172/jci115835.

3034 118. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo
3035 implantation. *Hum Reprod Update* 12: 731–746, 2006. doi: 10.1093/humupd/dml004.

3036 119. Bowen JA, Hunt JS. The Role of Integrins in Reproduction. *P Soc Exp Biol Med* 223:
3037 331–343, 2000. doi: 10.1046/j.1525-1373.2000.22348.x.

3038 120. Aplin JD. Embryo implantation: the molecular mechanism remains elusive. *Reproductive*
3039 *biomedicine online* 13: 833–839, 2006. doi: doi:10.1016/s1472-6483(10)61032-2.

3040 121. Minas V, Loutradis D, Makrigiannakis A. Factors controlling blastocyst implantation.
3041 *Reprod Biomed Online* 10: 205–216, 2005. doi: 10.1016/s1472-6483(10)60942-x.

3042 122. Hoozemans DA, Schats R, Lambalk CB, Homburg R, Hompes PG. Human embryo
3043 implantation: current knowledge and clinical implications in assisted reproductive technology.
3044 *Reprod Biomed Online* 9: 692–715, 2004. doi: 10.1016/s1472-6483(10)61781-6.

3045 123. Basak S, Dhar R, Das C. Steroids Modulate the Expression of α4 Integrin in Mouse
3046 Blastocysts and Uterus During Implantation. *Biol Reprod* 66: 1784–1789, 2002. doi:
3047 10.1095/biolreprod66.6.1784.

3048 124. Torry DS, Leavenworth J, Chang M, Maheshwari V, Groesch K, Ball ER, Torry RJ.
3049 Angiogenesis in implantation. *J Assist Reprod Gen* 24: 303–315, 2007. doi: 10.1007/s10815-
3050 007-9152-7.

3051 125. Foulk RA, Genbacev O, Prakobphol A. Expression of L-selectin ligand MECA-79 as a
3052 predictive marker of human uterine receptivity. *Fertil Steril* 88: S102, 2007. doi:
3053 10.1016/j.fertnstert.2007.07.332.

3054 126. Komatsu M, Carraway CAC, Fregien NL, Carraway KL. Reversible Disruption of Cell-
3055 Matrix and Cell-Cell Interactions by Overexpression of Sialomucin Complex*. *J Biol Chem*
3056 272: 33245–33254, 1997. doi: 10.1074/jbc.272.52.33245.

3057 127. Meseguer M, Pellicer A, Simón C. MUC1 and endometrial receptivity. *Mol Hum Reprod*
3058 4 (12): 1089–1098, 1998. doi: 10.1093/molehr/4.12.1089.

3059 128. Wu F, Chen X, Liu Y, Liang B, Xu H, Li TC, Wang CC. Decreased MUC1 in
3060 endometrium is an independent receptivity marker in recurrent implantation failure during
3061 implantation window. *Reproductive biology and endocrinology : RB&E* 16: 60–7, 2018. doi:
3062 10.1186/s12958-018-0379-1.

3063 129. Robertson S, Aplin J, A F. Cytokine and chemokine regulation of endometrial
3064 immunobiology. *The endometrium Second ed* : 547–569, 2008.

3065 130. Kaye P. Preimplantation growth factor physiology. *Rev Reprod* 2: 121–127, 1997. doi:
3066 10.1530/ror.0.0020121.

3067 131. Hardy K, Spanos S. Growth factor expression and function in the human and mouse
3068 preimplantation embryo. *J Endocrinol* 172: 221–236, 2002. doi: 10.1677/joe.0.1720221.

3069 132. Richter KS. The importance of growth factors for preimplantation embryo development
3070 and in-vitro culture. *Curr Opin Obstetrics Gynecol* 20: 292–304, 2008. doi:
3071 10.1097/gco.0b013e3282fe743b.

3072 133. Ihle JN. Cytokine receptor signalling. *Nature* 377: 591–594, 1995. doi:
3073 10.1038/377591a0.

3074 134. Gadina M, Hilton D, Johnston JA, Morinobu A, Lighvani A, Zhou Y-J, Visconti R,
3075 O'Shea JJ. Signaling by Type I and II cytokine receptors: ten years after. *Curr Opin Immunol*
3076 13: 363–373, 2001. doi: 10.1016/s0952-7915(00)00228-4.

3077 135. Ihle JN. The Stat family in cytokine signaling. *Curr Opin Cell Biol* 13: 211–217, 2001.
3078 doi: 10.1016/s0955-0674(00)00199-x.

3079 136. Cork BA, Tuckerman EM, Li TC, Laird SM. Expression of interleukin (IL)-11 receptor
3080 by the human endometrium in vivo and effects of IL-11, IL-6 and LIF on the production of
3081 MMP and cytokines by human endometrial cells in vitro. *Mol Hum Reprod* 8: 841–848, 2002.
3082 doi: 10.1093/molehr/8.9.841.

3083 137. Rose-John S. Interleukin-6 Family Cytokines. *Csh Perspect Biol* 10: a028415, 2017. doi:
3084 10.1101/cshperspect.a028415.

3085 138. Robertson SA, Christiaens I, Dorian CL, Zaragoza DB, Care AS, Banks AM, Olson DM.
3086 Interleukin-6 Is an Essential Determinant of On-Time Parturition in the Mouse. *Endocrinology*
3087 151: 3996–4006, 2010. doi: 10.1210/en.2010-0063.

3088 139. Tabibzadeh S, Babaknia A. The signals and molecular pathways involved in implantation,
3089 a symbiotic interaction between blastocyst and endometrium involving adhesion and tissue
3090 invasion. *Mol Hum Reprod* 1: 179–202, 1995. doi: 10.1093/molehr/1.4.179.

3091 140. Vandermolen DT, Gu Y. Human endometrial interleukin-6 (IL-6): in vivo messenger
3092 ribonucleic acid expression, in vitro protein production, and stimulation thereof by IL-1 beta.
3093 *Fertil Steril* 66: 741–7, 1996.

141. Wolff M von, Thaler CJ, Strowitzki T, Broome J, Stolz W, Tabibzadeh S. Regulated expression of cytokines in human endometrium throughout the menstrual cycle: dysregulation in habitual abortion. 6: 627–634, 2000.

142. Pellicer A, Ardiles G, Neuspiller F, Remohí J, Simón C, Bonilla-Musoles F. Evaluation of the ovarian reserve in young low responders with normal basal levels of follicle-stimulating hormone using three-dimensional ultrasonography. *Fertil Steril* 70: 671–675, 1998. doi: 10.1016/s0015-0282(98)00268-4.

143. Dominguez F, Gadea B, Mercader A, Esteban FJ, Pellicer A, Simón C. Embryologic outcome and secretome profile of implanted blastocysts obtained after coculture in human endometrial epithelial cells versus the sequential system. *Fertility and sterility* 93: 774-782.e1, 2010. doi: 10.1016/j.fertnstert.2008.10.019.

144. Zhong H, Sun Q, Chen P, Xiong F, Li G, Wan C, Yao Z, Zeng Y. Detection of IL-6, IL-10, and TNF-α level in human single-blastocyst conditioned medium using ultrasensitive Single Molecule Array platform and its relationship with embryo quality and implantation: a pilot study. *J Assist Reprod Gen* 37: 1695–1702, 2020. doi: 10.1007/s10815-020-01805-7.

145. Du X, Williams DA. Interleukin-11: review of molecular, cell biology, and clinical use. *Blood* 89: 3897–908, 1997.

146. Rango U von, Alfer J, Kertschanska S, Kemp B, Müller-Newen G, Heinrich PC, Beier HM, Classen-Linke I. Interleukin-11 expression: its significance in eutopic and ectopic human implantation. *Mhr Basic Sci Reproductive Medicine* 10: 783–792, 2004. doi: 10.1093/molehr/gah107.

147. Dimitriadis E, Salamonsen LA, Robb L. Expression of interleukin-11 during the human menstrual cycle: coincidence with stromal cell decidualization and relationship to leukaemia inhibitory factor and prolactin. 6: 907–914, 2000.

148. Cork BA, Li TC, Warren MA, Laird SM. Interleukin-11 (IL-11) in human endometrium: expression throughout the menstrual cycle and the effects of cytokines on endometrial IL-11 production in vitro. *J Reprod Immunol* 50: 3–17, 2001. doi: 10.1016/s0165-0378(00)00089-9.

149. Makrigiannakis A, Minas V, Kalantaridou SN, Nikas G, Chrousos GP. Hormonal and cytokine regulation of early implantation. *Trends Endocrinol Metabolism* 17: 178–185, 2006. doi: 10.1016/j.tem.2006.05.001.

150. Linjawi S, Li TC, Tuckerman EM, Blakemore AIF, Laird SM. Expression of interleukin-11 receptor α and interleukin-11 protein in the endometrium of normal fertile women and women with recurrent miscarriage. *J Reprod Immunol* 64: 145–155, 2004. doi: 10.1016/j.jri.2004.08.007.

151. Wang W, Vilella F, Alama P, Moreno I, Mignardi M, Isakova A, Pan W, Simon C, Quake SR. Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle. *Nat Med* 26: 1644–1653, 2020. doi: 10.1038/s41591-020-1040-z.

152. Stewart CL. Leukaemia inhibitory factor and the regulation of pre-implantation development of the mammalian embryo. *Mol Reprod Dev* 39: 233–238, 1994. doi: 10.1002/mrd.1080390217.

153. Cha J, Vilella F, Dey SK, Simón C. Molecular Interplay in Successful Implantation. *Science (New York, NY)* Ten critical Topics in Reproductive Medicine: 44–48, 2013.

154. Song H, Lim H, Das SK, Paria BC, Dey SK. Dysregulation of EGF Family of Growth Factors and COX-2 in the Uterus during the Preattachment and Attachment Reactions of the Blastocyst with the Luminal Epithelium Correlates with Implantation Failure in LIF- Deficient Mice. *Mol Endocrinol* 14: 1147–1161, 2000. doi: 10.1210/mend.14.8.0498.

155. Aghajanova L. Leukemia Inhibitory Factor and Human Embryo Implantation. *Ann Ny Acad Sci* 1034: 176–183, 2004. doi: 10.1196/annals.1335.020.

156. Brinsden PR, Alam V, Moustier B de, Engrand P. Recombinant human leukemia inhibitory factor does not improve implantation and pregnancy outcomes after assisted reproductive techniques in women with recurrent unexplained implantation failure. *Fertil Steril* 91: 1445–1447, 2009. doi: 10.1016/j.fertnstert.2008.06.047.

157. Piccinni M-P, Raghupathy R, Saito S, Szekeres-Bartho J. Cytokines, Hormones and Cellular Regulatory Mechanisms Favoring Successful Reproduction. *Front Immunol* 12: 717808, 2021. doi: 10.3389/fimmu.2021.717808.

158. Gordon SM. Interleukin-15 in Outcomes of Pregnancy. *Int J Mol Sci* 22: 11094, 2021. doi: 10.3390/ijms222011094.

159. Okada H, Tsuzuki T, Shindoh H, Nishigaki A, Yasuda K, Kanzaki H. Regulation of decidualization and angiogenesis in the human endometrium: Mini review. *J Obstet Gynaecol Re* 40: 1180–1187, 2014. doi: 10.1111/jog.12392.

160. Zitka O, Kukacka J, Krizkov S, Huska D, Adam V, Masarik M, Prusa R, Kizek R. Matrix Metalloproteinases. *Curr Med Chem* 17: 3751–3768, 2010. doi: 10.2174/092986710793213724.

161. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat Rev Mol Cell Bio* 8: 221–233, 2007. doi: 10.1038/nrm2125.

162. Chen J, Khalil RA. Matrix Metalloproteinases in Normal Pregnancy and Preeclampsia. *Prog Mol Biol Transl* 148: 87–165, 2017. doi: 10.1016/bs.pmbts.2017.04.001.

163. Ke J, Ye J, Li M, Zhu Z. The Role of Matrix Metalloproteinases in Endometriosis: A Potential Target. *Biomol* 11: 1739, 2021. doi: 10.3390/biom11111739.

164. Wysocka U, Sakowicz A, Jakubowski L, Pinkier I, Rybak-Krzyszkowska M, Alaszewski W, Dudarewicz L, Gach A. Association between idiopathic recurrent pregnancy loss and genetic polymorphisms in cytokine and matrix metalloproteinase genes. Ginekol Pol. 2021;10.5603/GP.a2021.0089. .

165. Du M-R, Wang S-C, Li D-J. The integrative roles of chemokines at the maternal–fetal interface in early pregnancy. *Cell Mol Immunol* 11: 438–448, 2014. doi: 10.1038/cmi.2014.68.

166. Carvalho-Gaspar M, Billing JS, Spriewald BM, Wood KJ. Chemokine gene expression during allograft rejection: Comparison of two quantitative PCR techniques. *J Immunol Methods* 301: 41–52, 2005. doi: 10.1016/j.jim.2005.03.003.

167. Dominguez F, Galan A, Martin JJL, Remohi J, Pellicer A, Simón C. Hormonal and embryonic regulation of chemokine receptors CXCR1, CXCR4, CCR5 and CCR2B in the human endometrium and the human blastocyst. *Mhr Basic Sci Reproductive Medicine* 9: 189–198, 2003. doi: 10.1093/molehr/gag024.

168. Giudice LC, Saleh W. Growth factors in reproduction. *Trends Endocrinol Metabolism* 6: 60–69, 1995. doi: 10.1016/1043-2760(94)00205-i.

169. Sporn MB, Roberts AB, Wakefield LM, Assoian RK. Transforming Growth Factor-β: Biological Function and Chemical Structure. *Science* 233: 532–534, 1986. doi: 10.1126/science.3487831.

170. Piek E, Heldin C, Dijke P. Specificity, diversity, and regulation in TGF-β superfamily signaling. *Faseb J* 13: 2105–2124, 1999. doi: 10.1096/fasebj.13.15.2105.

171. Kingsley DM. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. *Gene Dev* 8: 133–146, 1994. doi: 10.1101/gad.8.2.133.

172. Chimote N, Chimote M, Mehta B, Nath N. Cytokines and Growth Factors in Implantation. *J Reproductive Biotechnology Fertility* 1: 219–243, 2010. doi: 10.1177/205891581000100209.

173. Jones RL, Findlay JK, Farnworth PG, Robertson DM, Wallace E, Salamonsen LA. Activin A and Inhibin A Differentially Regulate Human Uterine Matrix Metalloproteinases: Potential Interactions during Decidualization and Trophoblast Invasion. *Endocrinology* 147: 724–732, 2006. doi: 10.1210/en.2005-1183.

174. Ni N, Li Q. TGFβ superfamily signaling and uterine decidualization. *Reproductive Biology Endocrinol Rb E* 15: 84, 2017. doi: 10.1186/s12958-017-0303-0.

175. Guo F, Si C, Zhou M, Wang J, Zhang D, Leung PCK, Xu B, Zhang A. Decreased PECAM1-mediated TGF-β1 expression in the mid-secretory endometrium in women with recurrent implantation failure. *Hum Reprod* 33: 832–843, 2018. doi: 10.1093/humrep/dey022.

176. Bui T, Zhang L, Rees M, Bicknell R, Harris A. Expression and hormone regulation of Wnt2, 3, 4, 5a, 7a, 7b and 10b in normal human endometrium and endometrial carcinoma. *Brit J Cancer* 75: 1131–1136, 1997. doi: 10.1038/bjc.1997.195.

177. Tulac S, Nayak NR, Kao LC, Waes M van, Huang J, Lobo S, Germeyer A, Lessey BA, Taylor RN, Suchanek E, Giudice LC. Identification, Characterization, and Regulation of the Canonical Wnt Signaling Pathway in Human Endometrium. *J Clin Endocrinol Metabolism* 88: 3860–3866, 2003. doi: 10.1210/jc.2003-030494.

178. Dunlap KA, Filant J, Hayashi K, Rucker EB, Song G, Deng JM, Behringer RR, DeMayo FJ, Lydon J, Jeong J-W, Spencer TE. Postnatal Deletion of Wnt7a Inhibits Uterine Gland Morphogenesis and Compromises Adult Fertility in Mice. *Biol Reprod* 85: 386–396, 2011. doi: 10.1095/biolreprod.111.091769.

179. Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, Hollinshead M, Marsh SGE, Brosens JJ, Critchley HO, Simons BD, Hemberger M, Koo B-K, Moffett A, Burton GJ. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat Cell Biol* 19: 568–577, 2017. doi: 10.1038/ncb3516.

180. Izhar B-S. Sharing of unrelated receptors and ligands by cognate partners: possible implications for ovarian and endometrial physiology. *Reprod Biomed Online* 11: 259–269, 2005. doi: 10.1016/s1472-6483(10)60967-4.

181. Mamer SB, Wittenkeller A, Imoukhuede PI. VEGF-A splice variants bind VEGFRs with differential affinities. *Sci Rep-uk* 10: 14413, 2020. doi: 10.1038/s41598-020-71484-y.

182. Pavelock K, Braas KM, Ouafik L, Osol G, May V. Differential Expression and Regulation of the Vascular Endothelial Growth Factor Receptors Neuropilin-1 and Neuropilin-2 in Rat Uterus. *Endocrinology* 142: 613–622, 2001. doi: 10.1210/endo.142.2.7947.

183. Bigsby RM, Bethin KE. Paracrine mediators of endometrial growth and differentiation, in Aplin, J., Fazleabas, A., Glasser, S. & Giudice, G. (eds.) The endometrium. Boca Ratón, FL: CRC Press, 2008. pp. 319-330. .

184. Binder NK, Evans J, Gardner DK, Salamonsen LA, Hannan NJ. Endometrial signals improve embryo outcome: functional role of vascular endothelial growth factor isoforms on embryo development and implantation in mice. *Hum Reprod* 29: 2278–2286, 2014. doi: 10.1093/humrep/deu211.

185. Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H. Molecular Cues to Implantation. *Endocrine reviews* 25: 341–373, 2004. doi: 10.1210/er.2003-0020.

186. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. *Embo J* 19: 3159–3167, 2000. doi: 10.1093/emboj/19.13.3159.

187. Bass KE, Morrish D, Roth I, Bhardwaj D, Taylor R, Zhou Y, Fisher SJ. Human cytotrophoblast invasion is up-regulated by epidermal growth factor: Evidence that paracrine factors modify this process. *Dev Biol* 164: 550–561, 1994. doi: 10.1006/dbio.1994.1223.

188. Dakour J, Li H, Chen H, Morrish DW. EGF Promotes Development of a Differentiated Trophoblast Phenotype Having c-myc and junB Proto-oncogene Activation. *Placenta* 20: 119–126, 1999. doi: 10.1053/plac.1998.0336.

189. Li RH, Zhuang LZ. The effects of growth factors on human normal placental cytotrophoblast cell proliferation. *Hum Reprod* 12: 830–834, 1997. doi: 10.1093/humrep/12.4.830.

190. Maruo T, Matsuo H, Otani T, Mochizuki M. Role of epidermal growth factor (EGF) and its receptor in the development of the human placenta. *Reproduction Fertility Dev* 7: 1465–1470, 1995. doi: 10.1071/rd9951465.

191. Staun-Ram E, Goldman S, Gabarin D, Shalev E. Expression and importance of matrix metalloproteinase 2 and 9 (MMP-2 and -9) in human trophoblast invasion. *Reproductive Biology Endocrinol Rb E* 2: 59–59, 2004. doi: 10.1186/1477-7827-2-59.

192. Chobotova K, Spyropoulou I, Carver J, Manek S, Heath JK, Gullick WJ, Barlow DH, Sargent IL, Mardon HJ. Heparin-binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. *Mech Develop* 119: 137–144, 2002. doi: 10.1016/s0925-4773(02)00342-8.

193. Paria BC, Elenius K, Klagsbrun M, Dey SK. Heparin-binding EGF-like growth factor interacts with mouse blastocysts independently of ErbB1: a possible role for heparan sulfate proteoglycans and ErbB4 in blastocyst implantation. *Development (Cambridge, England)* 126: 1997–2005, 1999. doi: 10.1242/dev.126.9.1997.

194. Lim H et al. erbB Genes in the Mouse Uterus: Cell-Specific Signaling by Epidermal Growth Factor (EGF) Family of Growth Factors during Implantation. *Dev Biol* 1: 97–110, 1998. doi: 10.1006/dbio.1998.9072.

195. Krumlauf R. Hox genes in vertebrate development. *Cell* 78: 191–201, 1994. doi: 10.1016/0092-8674(94)90290-9.

196. Mark M, Rijli FM, Chambon P. Homeobox Genes in Embryogenesis and Pathogenesis. *Pediatr Res* 42: 421–429, 1997. doi: 10.1203/00006450-199710000-00001.

197. Lim H, Ma L, Ma W, Maas RL, Dey SK. Hoxa-10 Regulates Uterine Stromal Cell Responsiveness to Progesterone during Implantation and Decidualization in the Mouse. *Mol Endocrinol* 13: 1005–1017, 1999. doi: 10.1210/mend.13.6.0284.

198. Tan J, Raja S, Davis MK, Tawfik O, Dey SK, Das SK. Evidence for coordinated interaction of cyclin D3 with p21 and cdk6 in directing the development of uterine stromal cell decidualization and polyploidy during implantation. *Mech Develop* 111: 99–113, 2002. doi: 10.1016/s0925-4773(01)00614-1.

199. Gendron RL, Paradis H, Hsieh-Li HM, Lee DW, Potter SS, Markoff E. Abnormal uterine stromal and glandular function associated with maternal reproductive defects in Hoxa-11 null mice. *Biology of reproduction* 56: 1097–1105, 1997. doi: 10.1095/biolreprod56.5.1097.

200. Kwon H, Taylor H. The Role of HOX Genes in Human Implantation. *Ann Ny Acad Sci* 1034: 1–18, 2004. doi: 10.1196/annals.1335.001.

201. Matsuzaki S, Canis M, Darcha C, Pouly J-L, Mage G. HOXA-10 expression in the mid-secretory endometrium of infertile patients with either endometriosis, uterine fibromas or unexplained infertility. *Hum Reprod* 24: 3180–3187, 2009. doi: 10.1093/humrep/dep306.

202. Taylor HS. The role of HOX genes in human implantation. *Hum Reprod Update* 6: 75–79, 2014. doi: 10.1093/humupd/6.1.75.

3278 203. Fischer C, Kayisli U, Taylor H. HOXA10 expression is decreased in endometrium of
3279 women with adenomyosis. *Fertil Steril* 94: S175, 2010. doi: 10.1016/j.fertnstert.2010.07.693.

3280 204. Özcan C, Özdamar Ö, Gökbayrak ME, Doğer E, Çakıroğlu Y, Çine N. HOXA-10 gene
3281 expression in ectopic and eutopic endometrium tissues: Does it differ between fertile and
3282 infertile women with endometriosis? *Eur J Obstet Gyn R B* 233: 43–48, 2019. doi:
3283 10.1016/j.ejogrb.2018.11.027.

3284 205. Wang W, Water TVD, Lufkin T. Inner ear and maternal reproductive defects in mice
3285 lacking the Hmx3 homeobox gene. *Dev Camb Engl* 125: 621–34, 1998.

3286 206. ALAPPAT S, ZHANG ZY, CHEN YP. Msx homeobox gene family and craniofacial
3287 development. *Cell Res* 13: 429–442, 2003. doi: 10.1038/sj.cr.7290185.

3288 207. Daikoku T, Cha J, Sun X, Tranguch S, Xie H, Fujita T, Hirota Y, Lydon J, DeMayo F,
3289 Maxson R, Dey SK. Conditional Deletion of MSX Homeobox Genes in the Uterus Inhibits
3290 Blastocyst Implantation by Altering Uterine Receptivity. *Dev Cell* 21: 1014–1025, 2011. doi:
3291 10.1016/j.devcel.2011.09.010.

3292 208. Nallasamy S, Li Q, Bagchi MK, Bagchi IC. Msx Homeobox Genes Critically Regulate
3293 Embryo Implantation by Controlling Paracrine Signaling between Uterine Stroma and
3294 Epithelium. *Plos Genet* 8: e1002500, 2012. doi: 10.1371/journal.pgen.1002500.

3295 209. Waclawik A. Novel insights into the mechanisms of pregnancy establishment: regulation
3296 of prostaglandin synthesis and signaling in the pig. *Reproduction* 142: 389–399, 2011. doi:
3297 10.1530/rep-11-0033.

3298 210. Salleh N. Diverse roles of prostaglandins in blastocyst implantation.
3299 *TheScientificWorldJournal* 2014: 968141, 2014. doi: 10.1155/2014/968141.

3300 211. Irvine RF. How is the level of free arachidonic acid controlled in mammalian cells?
3301 *Biochem J* 204: 3–16, 1982. doi: 10.1042/bj2040003.

3302 212. Hara S, Kamei D, Sasaki Y, Tanemoto A, Nakatani Y, Murakami M. Prostaglandin E
3303 synthases: Understanding their pathophysiological roles through mouse genetic models.
3304 *Biochimie* 92: 651–659, 2010. doi: 10.1016/j.biochi.2010.02.007.

3305 213. Vilella F, Ramirez L, Berlanga O, Martinez S, Alama P, Meseguer M, Pellicer A, Simón
3306 C. PGE2 and PGF2 Concentrations in Human Endometrial Fluid as Biomarkers for Embryonic
3307 Implantation. *Journal of Clinical Endocrinology & Metabolism* 98: 4123–4132, 2013. doi:
3308 10.1210/jc.2013-2205.

3309 214. Gu G, Gao Q, Yuan X, Huang L, Ge L. Immunolocalization of Adipocytes and
3310 Prostaglandin E2 and Its Four Receptor Proteins EP1, EP2, EP3, and EP4 in the Caprine Cervix
3311 During Spontaneous Term Labor1. *Biol Reprod* 86: Article 159, 1-10, 2012. doi:
3312 10.1095/biolreprod.111.096040.

3313 215. Song H, Lim H, Paria BC, Matsumoto H, Swift LL, Morrow J, Bonventre JV, Dey SK.
3314 Cytosolic phospholipase A2alpha is crucial [correction of A2alpha deficiency is crucial] for

"on-time" embryo implantation that directs subsequent development. *Development (Cambridge, England)* 129: 2879–2889, 2002. doi: 10.1242/dev.129.12.2879.

216. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91: 197–208, 1997. doi: 10.1016/s0092-8674(00)80402-x.

217. Lim H, Gupta RA, Ma W, Paria BC, Moller DE, Morrow JD, DuBois RN, Trzaskos JM, Dey SK. Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ. *Gene Dev* 13: 1561–1574, 1999. doi: 10.1101/gad.13.12.1561.

218. Wang H, Xie H, Sun X, Tranguch S, Zhang H, Jia X, Wang D, Das SK, Desvergne B, Wahli W, DuBois RN, Dey SK. Stage-specific Integration of Maternal and Embryonic Peroxisome Proliferator-activated Receptor δ Signaling Is Critical to Pregnancy Success*. *J Biol Chem* 282: 37770–37782, 2007. doi: 10.1074/jbc.m706577200.

219. Ruan YC, Guo JH, Liu X, Zhang R, Tsang LL, Dong JD, Chen H, Yu MK, Jiang X, Zhang XH, Fok KL, Chung YW, Huang H, Zhou WL, Chan HC. Activation of the epithelial Na+ channel triggers prostaglandin E2 release and production required for embryo implantation. *Nat Med* 18: 1112–1117, 2012. doi: 10.1038/nm.2771.

220. Bartel DP. MicroRNAs. *Cell* 116: 281–297, 2004. doi: 10.1016/s0092-8674(04)00045-5.

221. Ambros V, Chen X. The regulation of genes and genomes by small RNAs. *Development* 134: 1635–1641, 2007. doi: 10.1242/dev.002006.

222. Jackson RJ, Standart N. How Do MicroRNAs Regulate Gene Expression? *Sci Stke* 2007, 2007. doi: 10.1126/stke.3672007re1.

223. Nilsen TW. Mechanisms of microRNA-mediated gene regulation in animal cells. *Trends in genetics : TIG* 23: 243–249, 2007. doi: 10.1016/j.tig.2007.02.011.

224. Maccani MA, Marsit CJ. REVIEW ARTICLE: Epigenetics in the Placenta. *Am J Reprod Immunol* 62: 78–89, 2009. doi: 10.1111/j.1600-0897.2009.00716.x.

225. Prieto DMM, Markert UR. MicroRNAs in pregnancy. *J Reprod Immunol* 88: 106–111, 2011. doi: 10.1016/j.jri.2011.01.004.

226. Wilkins-Haug L. Epigenetics and assisted reproduction. *Curr Opin Obstetrics Gynecol* 21: 201–206, 2009. doi: 10.1097/gco.0b013e32832d7b95.

227. Ambros V. microRNAs Tiny Regulators with Great Potential. *Cell* 107: 823–826, 2001. doi: 10.1016/s0092-8674(01)00616-x.

228. Mehta A, Baltimore D. MicroRNAs as regulatory elements in immune system logic. *Nat Rev Immunol* 16: 279–294, 2016. doi: 10.1038/nri.2016.40.

229. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are Transported in Plasma and Delivered to Recipient Cells by High-Density Lipoproteins. *Nat Cell Biol* 13: 423–433, 2011. doi: 10.1038/ncb2210.

3351 230. Michell DL, Vickers KC. Lipoprotein carriers of microRNAs. *Biochimica et biophysica*
3352 *acta* 1861: 2069–2074, 2016. doi: 10.1016/j.bbalip.2016.01.011.

3353 231. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular
3354 circulating microRNA. *Nucleic Acids Res* 39: 7223–7233, 2011. doi: 10.1093/nar/gkr254.

3355 232. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS,
3356 Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2
3357 complexes carry a population of circulating microRNAs independent of vesicles in human
3358 plasma. *Proc National Acad Sci* 108: 5003–5008, 2011. doi: 10.1073/pnas.1019055108.

3359 233. Wang K, Zhang S, Weber J, Baxter D, Galas DJ. Export of microRNAs and microRNA-
3360 protective protein by mammalian cells. *Nucleic Acids Res* 38: 7248–7259, 2010. doi:
3361 10.1093/nar/gkq601.

3362 234. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, Hristov M,
3363 Köppel T, Jahantigh MN, Lutgens E, Wang S, Olson EN, Schober A, Weber C. Delivery of
3364 microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Science*
3365 *signaling* 2: ra81, 2009. doi: 10.1126/scisignal.2000610.

3366 235. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee M-LT,
3367 Schmittgen TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microRNA Expression
3368 in Human Peripheral Blood Microvesicles. 3: e3694, 2008. doi:
3369 10.1371/journal.pone.0003694.t004.

3370 236. Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. *J*
3371 *Cell Biology* 200: 373–383, 2013. doi: 10.1083/jcb.201211138.

3372 237. Pieters BCH, Arntz OJ, Bennink MB, Broeren MGA, Caam APM van, Koenders MI, Lent
3373 PLEM van, Berg WB van den, Vries M de, Kraan PM van der, Loo FAJ van de. Commercial
3374 Cow Milk Contains Physically Stable Extracellular Vesicles Expressing Immunoregulatory
3375 TGF-β. *Plos One* 10: e0121123, 2015. doi: 10.1371/journal.pone.0121123.

3376 238. Bidarimath M, Khalaj K, Wessels JM, Tayade C. MicroRNAs, immune cells and
3377 pregnancy. *Cellular and Molecular Immunology* 11: 538–547, 2014. doi:
3378 10.1038/cmi.2014.45.

3379 239. Revel A, Achache H, Stevens J, Smith Y, Reich R. MicroRNAs are associated with human
3380 embryo implantation defects. *Human reproduction (Oxford, England)* 26: 2830–2840, 2011.
3381 doi: 10.1093/humrep/der255.

3382 240. Sha A-G, Liu J-L, Jiang X-M, Ren J-Z, Ma C-H, Lei W, Su R-W, Yang Z-M. Genome-
3383 wide identification of micro-ribonucleic acids associated with human endometrial receptivity
3384 in natural and stimulated cycles by deep sequencing. *Fertility and sterility* 96: 150-155.e5,
3385 2011. doi: 10.1016/j.fertnstert.2011.04.072.

3386 241. Altmäe S, Martinez-Conejero JA, Esteban FJ, Ruiz-Alonso M, Stavreus-Evers A,
3387 Horcajadas JA, Salumets A. MicroRNAs miR-30b, miR-30d, and miR-494 Regulate Human
3388 Endometrial Receptivity. *Reprod Sci* 20: 308–317, 2013. doi: 10.1177/1933719112453507.

242. Grasso A, Navarro R, Balaguer N, Moreno I, Alamá P, Jimenez J, Simón C, Vilella F. Endometrial Liquid Biopsy Provides a miRNA Roadmap of the Secretory Phase of the Human Endometrium. *J Clin Endocrinol Metabolism* 105, 2020. doi: 10.1210/clinem/dgz146.

243. Estella C, Herrer I, Moreno-Moya JM, Quiñonero A, Martínez S, Pellicer A, Simón C. miRNA Signature and Dicer Requirement during Human Endometrial Stromal Decidualization In Vitro. *Plos One* 7: e41080, 2012. doi: 10.1371/journal.pone.0041080.

244. Qian K, Hu L, Chen H, Li H, Liu N, Li Y, Ai J, Zhu G, Tang Z, Zhang H. Hsa-miR-222 Is Involved in Differentiation of Endometrial Stromal Cells in Vitro. *Endocrinology* 150: 4734–4743, 2009. doi: 10.1210/en.2008-1629.

245. Vilella F, Moreno-Moya JM, Balaguer N, Grasso A, Herrero M, Martínez S, Marcilla A, Simón C. Hsa-miR-30d, secreted by the human endometrium, is taken up by the pre-implantation embryo and might modify its transcriptome. *Development* 142: 3210–3221, 2015. doi: 10.1242/dev.124289.

246. Method of the Year 2013. *Nat Methods* 11: 1–1, 2014. doi: 10.1038/nmeth.2801.

247. Regev A, Teichmann SA, Lander ES, Amit I, Benoist C, Birney E, Bodenmiller B, Campbell P, Carninci P, Clatworthy M, Clevers H, Deplancke B, Dunham I, Eberwine J, Eils R, Enard W, Farmer A, Fugger L, Göttgens B, Hacohen N, Haniffa M, Hemberg M, Kim S, Klenerman P, Kriegstein A, Lein E, Linnarsson S, Lundberg E, Lundeberg J, Majumder P, Marioni JC, Merad M, Mhlanga M, Nawijn M, Netea M, Nolan G, Pe'er D, Phillipakis A, Ponting CP, Quake S, Reik W, Rozenblatt-Rosen O, Sanes J, Satija R, Schumacher TN, Shalek A, Shapiro E, Sharma P, Shin JW, Stegle O, Stratton M, Stubbington MJT, Theis FJ, Uhlen M, Oudenaarden A van, Wagner A, Watt F, Weissman J, Wold B, Xavier R, Yosef N, Participants HCAM. The Human Cell Atlas. *Elife* 6: e27041, 2017. doi: 10.7554/elife.27041.

248. Tang F, Barbacioru C, Wang Y, Nordman E, Lee C, Xu N, Wang X, Bodeau J, Tuch BB, Siddiqui A, Lao K, Surani MA. mRNA-Seq whole-transcriptome analysis of a single cell. *Nat Methods* 6: 377–382, 2009. doi: 10.1038/nmeth.1315.

249. Tang F, Barbacioru C, Bao S, Lee C, Nordman E, Wang X, Lao K, Surani MA. Tracing the Derivation of Embryonic Stem Cells from the Inner Cell Mass by Single-Cell RNA-Seq Analysis. *Cell Stem Cell* 6: 468–478, 2010. doi: 10.1016/j.stem.2010.03.015.

250. Zilionis R, Nainys J, Veres A, Savova V, Zemmour D, Klein AM, Mazutis L. Single-cell barcoding and sequencing using droplet microfluidics. *Nat Protoc* 12: 44–73, 2017. doi: 10.1038/nprot.2016.154.

251. Vitak SA, Torkenczy KA, Rosenkrantz JL, Fields AJ, Christiansen L, Wong MH, Carbone L, Steemers FJ, Adey A. Sequencing thousands of single-cell genomes with combinatorial indexing. *Nat Methods* 14: 302–308, 2017. doi: 10.1038/nmeth.4154.

252. Svensson V, Vento-Tormo R, Teichmann SA. Exponential scaling of single-cell RNA-seq in the past decade. *Nat Protoc* 13: 599–604, 2018. doi: 10.1038/nprot.2017.149.

253. Luo T, Fan L, Zhu R, Sun D. Microfluidic Single-Cell Manipulation and Analysis: Methods and Applications. *Micromachines-basel* 10: 104, 2019. doi: 10.3390/mi10020104.

254. Gao D, Jin F, Zhou M, Jiang Y. Recent advances in single cell manipulation and biochemical analysis on microfluidics. *Analyst* 144: 766–781, 2018. doi: 10.1039/c8an01186a.

255. 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-uk* 7: 10077, 2017. doi: 10.1038/s41598-017-10098-3.

256. Talbi S, Hamilton AE, Vo KC, Tulac S, Overgaard MT, Dosiou C, Shay NL, 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* 147: 1097–1121, 2006. doi: 10.1210/en.2005-1076.

257. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, Esteban FJ, Alamá P, Pellicer A, Simón C. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertility and sterility* 95: 50–60, 60 e1–15, 2011. doi: 10.1016/j.fertnstert.2010.04.063.

258. Suhorutshenko M, Kukushkina V, Velthut-Meikas A, Altmäe S, Peters M, Mägi R, Krjutškov K, Koel M, Codoñer FM, Martinez-Blanch JF, Vilella F, Simón C, Salumets A, Laisk T. Endometrial receptivity revisited: endometrial transcriptome adjusted for tissue cellular heterogeneity. *Hum Reprod* 33: 2074–2086, 2018. doi: 10.1093/humrep/dey301.

259. Krjutškov K, Katayama S, Saare M, Vera-Rodriguez M, Lubenets D, Samuel K, Laisk-Podar T, Teder H, Einarsdottir E, Salumets A, Kere J. Single-cell transcriptome analysis of endometrial tissue. *Hum Reproduction Oxf Engl* 31: 844–853, 2016. doi: 10.1093/humrep/dew008.

260. Garcia-Alonso L, Handfield L-F, Roberts K, Nikolakopoulou K, Fernando RC, Gardner L, Woodhams B, Arutyunyan A, Polanski K, Hoo R, Sancho-Serra C, Li T, Kwakwa K, Tuck E, Lorenzi V, Massalha H, Prete M, Kleshchevnikov V, Tarkowska A, Porter T, Mazzeo CI, Dongen S van, Dabrowska M, Vaskivskyi V, Mahbubani KT, Park J, Jimenez-Linan M, Campos L, Kiselev VYu, Lindskog C, Ayuk P, Prigmore E, Stratton MR, Saeb-Parsy K, Moffett A, Moore L, Bayraktar OA, Teichmann SA, Turco MY, Vento-Tormo R. Mapping the temporal and spatial dynamics of the human endometrium in vivo and in vitro. *Nat Genet* 53: 1698–1711, 2021. doi: 10.1038/s41588-021-00972-2.

261. Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, Park J-E, Stephenson E, Polański K, Goncalves A, Gardner L, Holmqvist S, Henriksson J, Zou A, Sharkey AM, Millar B, Innes B, Wood L, Wilbrey-Clark A, Payne RP, Ivarsson MA, Lisgo S, Filby A, Rowitch DH, Bulmer JN, Wright GJ, Stubbington MJT, Haniffa M, Moffett A, Teichmann SA. Single-cell reconstruction of the early maternal–fetal interface in humans. *Nature* 563: 347–353, 2018. doi: 10.1038/s41586-018-0698-6.

262. Wang W, Vilella F, Alama P, Moreno I, Mignardi M, Isakova A, Pan W, Simon C, Quake SR. Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle. *Nat Med* 26: 1644–1653, 2020. doi: 10.1038/s41591-020-1040-z.

263. Hapangama DK, Drury J, Silva LD, Al-Lamee H, Earp A, Valentijn AJ, Edirisinghe DP, Murray PA, Fazleabas AT, Gargett CE. Abnormally located SSEA1+/SOX9+ endometrial epithelial cells with a basalis-like phenotype in the eutopic functionalis layer may play a role in the pathogenesis of endometriosis. *Hum Reproduction Oxf Engl* 34: 56–68, 2019. doi: 10.1093/humrep/dey336.

264. Tempest N, Baker AM, Wright NA, Hapangama DK. Does human endometrial LGR5 gene expression suggest the existence of another hormonally regulated epithelial stem cell niche? *Hum Reproduction Oxf Engl* 33: 1052–1062, 2018. doi: 10.1093/humrep/dey083.

265. Kelleher AM, Milano-Foster J, Behura SK, Spencer TE. Uterine glands coordinate on-time embryo implantation and impact endometrial decidualization for pregnancy success. *Nat Commun* 9: 2435, 2018. doi: 10.1038/s41467-018-04848-8.

266. Croy BA, Heuvel MJVD, Borzychowski AM, Tayade C. Uterine natural killer cells: a specialized differentiation regulated by ovarian hormones. *Immunol Rev* 214: 161–185, 2006. doi: 10.1111/j.1600-065x.2006.00447.x.

267. Brosens JJ, Parker MG, McIndoe A, Pijnenborg R, Brosens IA. A role for menstruation in preconditioning the uterus for successful pregnancy. *Am J Obstet Gynecol* 200: 615.e1-615.e6, 2009. doi: 10.1016/j.ajog.2008.11.037.

268. Emera D, Romero R, Wagner G. The evolution of menstruation: A new model for genetic assimilation. *Bioessays* 34: 26–35, 2012. doi: 10.1002/bies.201100099.

269. Gellersen B, Reimann K, Samalecos A, Aupers S, Bamberger A-M. Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. *Hum Reprod* 25: 862–873, 2010. doi: 10.1093/humrep/dep468.

270. Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *Embo J* 9: 1603–1614, 1990. doi: 10.1002/j.1460-2075.1990.tb08280.x.

271. Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem* 63: 451–486, 1994. doi: 10.1146/annurev.bi.63.070194.002315.

272. Maruyama T, Yoshimura Y. Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium. *Endocrine journal* 55: 795–810, 2008. doi: 10.1507/endocrj.k08e-067.

273. Gravanis A, Stournaras C, Margioris A. Paracrinology of Endometrial Neuropeptides: Corticotropin-Releasing Hormone and Opioids. *Semin Reprod Endocr* 17: 29–38, 1999. doi: 10.1055/s-2007-1016209.

274. Palejwala S, Tseng L, Wojtczuk A, Weiss G, Goldsmith LT. Relaxin Gene and Protein Expression and Its Regulation of Procollagenase and Vascular Endothelial Growth Factor in Human Endometrial Cells1. *Biol Reprod* 66: 1743–1748, 2002. doi: 10.1095/biolreprod66.6.1743.

275. Einspanier A, Lieder K, Husen B, Ebert K, Lier S, Einspanier R, Unemori E, Kemper M. Relaxin Supports Implantation and Early Pregnancy in the Marmoset Monkey. *Ann Ny Acad Sci* 1160: 140–146, 2009. doi: 10.1111/j.1749-6632.2009.03947.x.

276. Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci U S A* 90: 11162–11166, 1993.

277. Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL. Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. *Proc National Acad Sci* 93: 3115–3120, 1996. doi: 10.1073/pnas.93.7.3115.

278. Salamonsen LA, Dimitriadis E, Robb L. Cytokines in implantation. *Seminars in reproductive medicine* 18: 299–310, 2000. doi: 10.1055/s-2000-12567.

279. Christian M, Marangos P, Mak I, McVey J, Barker F, White J, Brosens JJ. Interferon-γ Modulates Prolactin and Tissue Factor Expression in Differentiating Human Endometrial Stromal Cells 1. *Endocrinology* 142: 3142–3151, 2001. doi: 10.1210/endo.142.7.8231.

280. Tamura M, Sebastian S, Yang S, Gurates B, Fang Z, Bulun SE. Interleukin-1β Elevates Cyclooxygenase-2 Protein Level and Enzyme Activity via Increasing Its mRNA Stability in Human Endometrial Stromal Cells: An Effect Mediated by Extracellularly Regulated Kinases 1 and 2. *J Clin Endocrinol Metabolism* 87: 3263–3273, 2002. doi: 10.1210/jcem.87.7.8594.

281. Li Q, Kannan A, Wang W, DeMayo FJ, Taylor RN, Bagchi MK, Bagchi IC. Bone Morphogenetic Protein 2 Functions via a Conserved Signaling Pathway Involving Wnt4 to Regulate Uterine Decidualization in the Mouse and the Human*. *J Biol Chem* 282: 31725–31732, 2007. doi: 10.1074/jbc.m704723200.

282. Tang M, Naidu D, Hearing P, Handwerger S, Tabibzadeh S. LEFTY, a Member of the Transforming Growth Factor-β Superfamily, Inhibits Uterine Stromal Cell Differentiation: A Novel Autocrine Role. *Endocrinology* 151: 1320–1330, 2010. doi: 10.1210/en.2009-1081.

283. Kim MR, Park DW, Lee JH, Choi DS, Hwang KJ, Ryu HS, Min CK. Progesterone-dependent release of transforming growth factor-beta1 from epithelial cells enhances the endometrial decidualization by turning on the Smad signalling in stromal cells. *Mhr Basic Sci Reproductive Medicine* 11: 801–808, 2005. doi: 10.1093/molehr/gah240.

284. Robertson SA, Seamark RF, Guilbert LJ, Wegmann TG. The Role of Cytokines in Gestation. *Crit Rev Immunol* 14: 239–292, 1994. doi: 10.1615/critrevimmunol.v14.i3-4.30.

285. Teklenburg G, Salker M, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, Roelen BAJ, Quenby S, Kuijk EW, Kavelaars A, Heijnen CJ, Regan L, Brosens JJ, Macklon NS. Natural Selection of Human Embryos: Decidualizing Endometrial Stromal Cells Serve as Sensors of Embryo Quality upon Implantation. 5: e10258, 2010. doi: 10.1371/journal.pone.0010258.g004.

286. Aikawa S, Kano K, Inoue A, Wang J, Saigusa D, Nagamatsu T, Hirota Y, Fujii T, Tsuchiya S, Taketomi Y, Sugimoto Y, Murakami M, Arita M, Kurano M, Ikeda H, Yatomi Y,

Chun J, Aoki J. Autotaxin–lysophosphatidic acid–LPA3 signaling at the embryo-epithelial boundary controls decidualization pathways. *Embo J* 36: 2146–2160, 2017. doi: 10.15252/embj.201696290.

287. Chobotova K, Karpovich N, Carver J, Manek S, Gullick WJ, Barlow DH, Mardon HJ. Heparin-Binding Epidermal Growth Factor and Its Receptors Mediate Decidualization and Potentiate Survival of Human Endometrial Stromal Cells. *J Clin Endocrinol Metabolism* 90: 913–919, 2005. doi: 10.1210/jc.2004-0476.

288. Milne SA, Perchick GB, Boddy SC, Jabbour HN. Expression, localization, and signaling of PGE(2) and EP2/EP4 receptors in human nonpregnant endometrium across the menstrual cycle. *Journal of Clinical Endocrinology & Metabolism* 86: 4453–4459, 2001. doi: 10.1210/jcem.86.9.7856.

289. Gellersen1 B, Brosens2 I, Brosens3 J. Decidualization of the Human Endometrium: Mechanisms, Functions, and Clinical Perspectives. *Semin Reprod Med* 25: 445–453, 2007. doi: 10.1055/s-2007-991042.

290. Evans J, Salamonsen LA, Winship A, Menkhorst E, Nie G, Gargett CE, Dimitriadis E. Fertile ground: human endometrial programming and lessons in health and disease. *Nature reviews Endocrinology* 11: 654–667, 2016. doi: 10.1038/nrendo.2016.116.

291. Schatz F, Guzeloglu-Kayisli O, Arlier S, Kayisli UA, Lockwood CJ. The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding. *Hum Reprod Update* 22: 497–515, 2016. doi: 10.1093/humupd/dmw004.

292. Wetendorf M, DeMayo FJ. The progesterone receptor regulates implantation, decidualization, and glandular development via a complex paracrine signaling network. *Mol Cell Endocrinol* 357: 108–118, 2012. doi: 10.1016/j.mce.2011.10.028.

293. Wang X, Wu S-P, DeMayo FJ. Hormone dependent uterine epithelial-stromal communication for pregnancy support. *Placenta* 60: S20–S26, 2017. doi: 10.1016/j.placenta.2017.07.003.

294. Wang X, Li X, Wang T, Wu S-P, Jeong J-W, Kim TH, Young SL, Lessey BA, Lanz RB, Lydon JP, DeMayo FJ. SOX17 regulates uterine epithelial–stromal cross-talk acting via a distal enhancer upstream of Ihh. *Nat Commun* 9: 4421, 2018. doi: 10.1038/s41467-018-06652-w.

295. Bhurke AS, Bagchi IC, Bagchi MK. Progesterone-Regulated Endometrial Factors Controlling Implantation. *Am J Reprod Immunol* 75: 237–245, 2016. doi: 10.1111/aji.12473.

296. Lee KY, Jeong J-W, Wang J, Ma L, Martin JF, Tsai SY, Lydon JP, DeMayo FJ. Bmp2 is critical for the murine uterine decidual response. *Mol Cell Biol* 27: 5468–78, 2007. doi: 10.1128/mcb.00342-07.

297. McKay DB, Vazquez MA, Redline RW, Lu CY. Macrophage functions are regulated by murine decidual and tumor extracellular matrices. *J Clin Invest* 89: 134–142, 1992. doi: 10.1172/jci115553.

298. Irwin JC, Kirk D, King RJB, Quigley MM, Gwatkin RBL. Hormonal regulation of human endometrial stromal cells in culture: an in vitro model for decidualization. *Fertil Steril* 52: 761–768, 1989. doi: 10.1016/s0015-0282(16)61028-2.

299. Loke YW, Gardner L, Burland K, King A. Laminin in human trophoblast—decidua interaction. *Hum Reprod* 4: 457–463, 1989. doi: 10.1093/oxfordjournals.humrep.a136926.

300. Aplin JD, Charlton AK, Ayad S. An immunohistochemical study of human endometrial extracellular matrix during the menstrual cycle and first trimester of pregnancy. *Cell Tissue Res* 253: 231–240, 1988. doi: 10.1007/bf00221758.

301. Schatz F, Aigner S, Papp C, Toth-Pal E, Hausknecht V, Lockwood CJ. Plasminogen activator activity during decidualization of human endometrial stromal cells is regulated by plasminogen activator inhibitor 1. *J Clin Endocrinol Metabolism* 80: 2504–2510, 1995. doi: 10.1210/jcem.80.8.7629251.

302. Spessotto P, Bulla R, Danussi C, Radillo O, Cervi M, Monami G, Bossi F, Tedesco F, Doliana R, Colombatti A. EMILIN1 represents a major stromal element determining human trophoblast invasion of the uterine wall. *J Cell Sci* 119: 4574–4584, 2006. doi: 10.1242/jcs.03232.

303. Haouzi D, Dechaud H, Assou S, Monzo C, Vos J de, Hamamah S. Transcriptome analysis reveals dialogues between human trophectoderm and endometrial cells during the implantation period. *Hum Reprod* 26: 1440–1449, 2011. doi: 10.1093/humrep/der075.

304. Schwenke M, Knöfler M, Velicky P, Weimar CHE, Kruse M, Samalecos A, Wolf A, Macklon NS, Bamberger A-M, Gellersen B. Control of Human Endometrial Stromal Cell Motility by PDGF-BB, HB-EGF and Trophoblast-Secreted Factors. *Plos One* 8: e54336, 2013. doi: 10.1371/journal.pone.0054336.

305. Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J, Bulmer J. Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod* 24: 45–54, 2009. doi: 10.1093/humrep/den348.

306. Chazara O, Xiong S, Moffett A. Maternal KIR and fetal HLA-C: a fine balance. *J Leukocyte Biol* 90: 703–716, 2011. doi: 10.1189/jlb.0511227.

307. Heuvel MJ van den, Chantakru S, Xie X, Evans SS, Tekpetey F, Mote PA, Clarke CL, Croy BA. Trafficking of Circulating Pro-NK Cells to the Decidualizing Uterus: Regulatory Mechanisms in the Mouse and Human. *Immunol Invest* 34: 273–293, 2009. doi: 10.1081/imm-200064488.

308. Hannan NJ, Jones RL, White CA, Salamonsen LA. The Chemokines, CX3CL1, CCL14, and CCL4, Promote Human Trophoblast Migration at the Feto-Maternal Interface. *Biol Reprod* 74: 896–904, 2006. doi: 10.1095/biolreprod.105.045518.

309. Co EC, Gormley M, Kapidzic M, Rosen DB, Scott MA, Stolp HAR, McMaster M, Lanier LL, Brcena A, Fisher SJ. Maternal Decidual Macrophages Inhibit NK Cell Killing of Invasive Cytotrophoblasts During Human Pregnancy1. *Biol Reprod* 88: Article 155, 1-9, 2013. doi: 10.1095/biolreprod.112.099465.

310. Kajihara T, Jones M, Fusi L, Takano M, Feroze-Zaidi F, Pirianov G, Mehmet H, Ishihara O, Higham JM, Lam EW-F, Brosens JJ. Differential Expression of FOXO1 and FOXO3a Confers Resistance to Oxidative Cell Death upon Endometrial Decidualization. *Mol Endocrinol* 20: 2444–2455, 2006. doi: 10.1210/me.2006-0118.

311. Leitao B, Jones MC, Fusi L, Higham J, Lee Y, Takano M, Goto T, Christian M, Lam EW-F, Brosens JJ. Silencing of the JNK pathway maintains progesterone receptor activity in decidualizing human endometrial stromal cells exposed to oxidative stress signals. *Faseb J* 24: 1541–1551, 2010. doi: 10.1096/fj.09-149153.

312. Muter J, Brighton PJ, Lucas ES, Lacey L, Shmygol A, Quenby S, Blanks AM, Brosens JJ. Progesterone-Dependent Induction of Phospholipase C-Related Catalytically Inactive Protein 1 (PRIP-1) in Decidualizing Human Endometrial Stromal Cells. *Endocrinology* 157: 2883–2893, 2016. doi: 10.1210/en.2015-1914.

313. Shah KM, Webber J, Carzaniga R, Taylor DM, Fusi L, Clayton A, Brosens JJ, Hartshorne G, Christian M. Induction of microRNA resistance and secretion in differentiating human endometrial stromal cells. *J Mol Cell Biol* 5: 67–70, 2013. doi: 10.1093/jmcb/mjs058.

314. Muter J, Alam MT, Vrljicak P, Barros FSV, Ruane PT, Ewington LJ, Aplin JD, Westwood M, Brosens JJ. The Glycosyltransferase EOGT Regulates Adropin Expression in Decidualizing Human Endometrium. *Endocrinology* 159: 994–1004, 2017. doi: 10.1210/en.2017-03064.

315. Burnum KE, Hirota Y, Baker ES, Yoshie M, Ibrahim YM, Monroe ME, Anderson GA, Smith RD, Daikoku T, Dey SK. Uterine Deletion of Trp53 Compromises Antioxidant Responses in the Mouse Decidua. *Endocrinology* 153: 4568–4579, 2012. doi: 10.1210/en.2012-1335.

316. Lanekoff I, Cha J, Kyle JE, Dey SK, Laskin J, Burnum-Johnson KE. Trp53 deficient mice predisposed to preterm birth display region-specific lipid alterations at the embryo implantation site. *Sci Rep-uk* 6: 33023, 2016. doi: 10.1038/srep33023.

317. Hirota Y, Cha J, Yoshie M, Daikoku T, Dey SK. Heightened uterine mammalian target of rapamycin complex 1 (mTORC1) signaling provokes preterm birth in mice. *Proc National Acad Sci* 108: 18073–18078, 2011. doi: 10.1073/pnas.1108180108.

318. Dunn CL, Kelly RW, Critchley HO. Decidualization of the human endometrial stromal cell: an enigmatic transformation. *Reprod Biomed Online* 7: 151–161, 2003. doi: 10.1016/s1472-6483(10)61745-2.

319. Murata H, Tanaka S, Okada H. Immune Tolerance of the Human Decidua. *J Clin Medicine* 10: 351, 2021. doi: 10.3390/jcm10020351.

320. Choudhury RH, Dunk CE, Lye SJ, Aplin JD, Harris LK, Jones RL. Extravillous Trophoblast and Endothelial Cell Crosstalk Mediates Leukocyte Infiltration to the Early Remodeling Decidual Spiral Arteriole Wall. *J Immunol* 198: 4115–4128, 2017. doi: 10.4049/jimmunol.1601175.

3660 321. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human
3661 cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular
3662 invasion in this syndrome? *J Clin Invest* 99: 2152–2164, 1997. doi: 10.1172/jci119388.

3663 322. Roberts VHJ, Morgan TK, Bednarek P, Morita M, Burton GJ, Lo JO, Frias AE. Early first
3664 trimester uteroplacental flow and the progressive disintegration of spiral artery plugs: new
3665 insights from contrast-enhanced ultrasound and tissue histopathology. *Hum Reprod* 32: 2382–
3666 2393, 2017. doi: 10.1093/humrep/dex301.

3667 323. Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, Fisher SJ.
3668 Trophoblast differentiation during embryo implantation and formation of the maternal-fetal
3669 interface. *J Clin Invest* 114: 744–754, 2004. doi: 10.1172/jci22991.

3670 324. Gaynor LM, Colucci F. Uterine Natural Killer Cells: Functional Distinctions and
3671 Influence on Pregnancy in Humans and Mice. *Front Immunol* 8: 467, 2017. doi:
3672 10.3389/fimmu.2017.00467.

3673 325. Harris LK, Benagiano M, D'Elios MM, Brosens I, Benagiano G. Placental bed research:
3674 II. Functional and immunological investigations of the placental bed. *Am J Obstet Gynecol*
3675 221: 457–469, 2019. doi: 10.1016/j.ajog.2019.07.010.

3676 326. Redman CWG, Tannetta DS, Dragovic RA, Gardiner C, Southcombe JH, Collett GP,
3677 Sargent IL. Review: Does size matter? Placental debris and the pathophysiology of pre-
3678 eclampsia. *Placenta* 33: S48–S54, 2012. doi: 10.1016/j.placenta.2011.12.006.

3679 327. Ng S-W, Norwitz GA, Pavlicev M, Tilburgs T, Simón C, Norwitz ER. Endometrial
3680 Decidualization: The Primary Driver of Pregnancy Health. *Int J Mol Sci* 21: 4092, 2020. doi:
3681 10.3390/ijms21114092.

3682 328. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the Survival of Early Pregnancy. *New*
3683 *Engl J Medicine* 345: 1400–1408, 2001. doi: 10.1056/nejmra000763.

3684 329. Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy.
3685 *Nature medicine* 18: 1754–1767, 2012. doi: 10.1038/nm.3012.

3686 330. Sampson J. Peritoneal Endometriosis, Due to the Menstrual Dissemination of Endometrial
3687 Tissue into the Peritoneal Cavity. *Am J Obstet Gynecol* 14 (4): 422–469, 1927. doi:
3688 10.1016/s0002-9378(15)30003-x.

3689 331. Ferguson BR, Bennington JL, Haber SL. Histochemistry of mucosubstances and histology
3690 of mixed müllerian pelvic lymph node glandular inclusions. Evidence for histogenesis by
3691 müllerian metaplasia of coelomic epithelium. *Obstet Gynecol* 33: 617–25, 1969.

3692 332. Figueira PGM, Abrão MS, Krikun G, Taylor HS, Taylor H. Stem cells in endometrium
3693 and their role in the pathogenesis of endometriosis. *Ann Ny Acad Sci* 1221: 10–17, 2011. doi:
3694 10.1111/j.1749-6632.2011.05969.x.

3695 333. Du H, Taylor HS. Contribution of Bone Marrow-Derived Stem Cells to Endometrium and
3696 Endometriosis. *Stem Cells* 25: 2082–2086, 2007. doi: 10.1634/stemcells.2006-0828.

334. Gargett CE, Masuda H. Adult stem cells in the endometrium. *Mhr Basic Sci Reproductive Medicine* 16: 818–834, 2010. doi: 10.1093/molehr/gaq061.

335. Mechsner S, Weichbrodt M, Riedlinger WFJ, Bartley J, Kaufmann AM, Schneider A, Köhler C. Estrogen and progestogen receptor positive endometriotic lesions and disseminated cells in pelvic sentinel lymph nodes of patients with deep infiltrating rectovaginal endometriosis: a pilot study. *Hum Reprod* 23: 2202–2209, 2008. doi: 10.1093/humrep/den259.

336. Gargett CE, Schwab KE, Brosens JJ, Puttemans P, Benagiano G, Brosens I. Potential role of endometrial stem/progenitor cells in the pathogenesis of early-onset endometriosis. *Mhr Basic Sci Reproductive Medicine* 20: 591–598, 2014. doi: 10.1093/molehr/gau025.

337. Borghese B, Zondervan KT, Abrao MS, Chapron C, Vaiman D. Recent insights on the genetics and epigenetics of endometriosis. *Clin Genet* 91: 254–264, 2017. doi: 10.1111/cge.12897.

338. Su R-W, Strug MR, Joshi NR, Jeong J-W, Miele L, Lessey BA, Young SL, Fazleabas AT. Decreased Notch Pathway Signaling in the Endometrium of Women With Endometriosis Impairs Decidualization. *J Clin Endocrinol Metabolism* 100: E433–E442, 2015. doi: 10.1210/jc.2014-3720.

339. Maggiore ULR, 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* 22: 70–103, 2016. doi: 10.1093/humupd/dmv045.

340. Klemmt PAB, Carver JG, Kennedy SH, Koninckx PR, Mardon HJ. Stromal cells from endometriotic lesions and endometrium from women with endometriosis have reduced decidualization capacity. *Fertil Steril* 85: 564–572, 2006. doi: 10.1016/j.fertnstert.2005.08.046.

341. Aghajanova L, Tatsumi K, Horcajadas JA, Zamah AM, Esteban FJ, Herndon CN, Conti M, Giudice LC. Unique Transcriptome, Pathways, and Networks in the Human Endometrial Fibroblast Response to Progesterone in Endometriosis. *Biol Reprod* 84: 801–815, 2011. doi: 10.1095/biolreprod.110.086181.

342. Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S, Johns A, Meng L, Putman M, Carr B, Bulun SE. Deficient 17β-Hydroxysteroid Dehydrogenase Type 2 Expression in Endometriosis: Failure to Metabolize 17β-Estradiol 1. *J Clin Endocrinol Metabolism* 83: 4474–4480, 1998. doi: 10.1210/jcem.83.12.5301.

343. Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone Receptor Isoform A But Not B Is Expressed in Endometriosis1. *J Clin Endocrinol Metabolism* 85: 2897–2902, 2000. doi: 10.1210/jcem.85.8.6739.

344. Taylor RN, Lundeen SG, Giudice LC. Emerging role of genomics in endometriosis research. *Fertil Steril* 78: 694–698, 2002. doi: 10.1016/s0015-0282(02)03325-3.

345. Taylor HS, Bagot C, Kardana A, Olive D, Arici A. HOX gene expression is altered in the endometrium of women with endometriosis. *Hum Reprod* 14: 1328–1331, 1999. doi: 10.1093/humrep/14.5.1328.

346. Marquardt RM, Kim TH, Shin J-H, Jeong J-W. Progesterone and Estrogen Signaling in the Endometrium: What Goes Wrong in Endometriosis? *Int J Mol Sci* 20: 3822, 2019. doi: 10.3390/ijms20153822.

347. Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, Roelen BAJ, Quenby S, Kuijk EW, Kavelaars A, Heijnen CJ, Regan L, Macklon NS, Brosens JJ. Natural Selection of Human Embryos: Impaired Decidualization of Endometrium Disables Embryo-Maternal Interactions and Causes Recurrent Pregnancy Loss. *Plos One* 5: e10287, 2010. doi: 10.1371/journal.pone.0010287.

348. Salker MS, Nautiyal J, Steel JH, Webster Z, Šućurović S, Nicou M, Singh Y, Lucas ES, Murakami K, Chan Y-W, James S, Abdallah Y, Christian M, Croy BA, Mulac-Jericevic B, Quenby S, Brosens JJ. Disordered IL-33/ST2 Activation in Decidualizing Stromal Cells Prolongs Uterine Receptivity in Women with Recurrent Pregnancy Loss. *Plos One* 7: e52252, 2012. doi: 10.1371/journal.pone.0052252.

349. Lucas ES, Vrljicak P, Muter J, Diniz-da-Costa MM, Brighton PJ, Kong C-S, Lipecki J, Fishwick KJ, Odendaal J, Ewington LJ, Quenby S, Ott S, Brosens JJ. Recurrent pregnancy loss is associated with a pro-senescent decidual response during the peri-implantation window. *Commun Biology* 3: 37, 2020. doi: 10.1038/s42003-020-0763-1.

350. Guo C, Cai P, Jin L, Sha Q, Yu Q, Zhang W, Jiang C, Liu Q, Zong D, Li K, Fang J, Lu F, Wang Y, Li D, Lin J, Li L, Zeng Z, Tong X, Wei H, Qu K. Single-cell profiling of the human decidual immune microenvironment in patients with recurrent pregnancy loss. *Cell Discov* 7: 1, 2021. doi: 10.1038/s41421-020-00236-z.

351. Salker MS, Christian M, Steel JH, Nautiyal J, Lavery S, Trew G, Webster Z, Al-Sabbagh M, Puchchakayala G, Föller M, Landles C, Sharkey AM, Quenby S, Aplin JD, Regan L, Lang F, Brosens JJ. Deregulation of the serum- and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. *Nat Med* 17: 1509–1513, 2011. doi: 10.1038/nm.2498.

352. Weimar CHE, Kavelaars A, Brosens JJ, Gellersen B, Vreeden-Elbertse JMT de, Heijnen CJ, Macklon NS. Endometrial Stromal Cells of Women with Recurrent Miscarriage Fail to Discriminate between High- and Low-Quality Human Embryos. *Plos One* 7: e41424, 2012. doi: 10.1371/journal.pone.0041424.

353. Roberts J, Cooper D. Pathogenesis and genetics of pre-eclampsia. *Lancet* 357: 53–56, 2001. doi: 10.1016/s0140-6736(00)03577-7.

354. Garrido-Gómez T, Dominguez F, Quiñonero A, Díaz-Gimeno P, Kapidzic M, Gormley M, Ona K, Padilla-Iserte P, McMaster M, Genbacev O, Perales A, Fisher SJ, Simón C. Defective decidualization during and after severe preeclampsia reveals a possible maternal contribution to the etiology. *Proceedings of the National Academy of Sciences* 1: 201706546, 2017. doi: 10.1242/dev.066589.

3774 355. Garrido-Gomez T, Quiñonero A, Dominguez F, Rubert L, Perales A, Hajjar KA, Simon
3775 C. Preeclampsia: a defect in decidualization is associated with deficiency of Annexin A2. *Am*
3776 *J Obstet Gynecol* 222: 376.e1-376.e17, 2020. doi: 10.1016/j.ajog.2019.11.1250.

3777 356. Conrad KP, Rabaglino MB, Uiterweer EDP. Emerging role for dysregulated
3778 decidualization in the genesis of preeclampsia. *Placenta* 60: 119–129, 2017. doi:
3779 10.1016/j.placenta.2017.06.005.

3780 357. Rabaglino MB, Conrad KP. Evidence for shared molecular pathways of dysregulated
3781 decidualization in preeclampsia and endometrial disorders revealed by microarray data
3782 integration. *Faseb J* 33: 11682–11695, 2019. doi: 10.1096/fj.201900662r.

3783 358. Garrido-Gomez T, Castillo-Marco N, Clemente-Ciscar M, Cordero T, Muñoz-Blat I,
3784 Amadoz A, Jimenez-Almazan J, Monfort-Ortiz R, Climent R, Perales-Marin A, Simon C.
3785 Disrupted PGR-B and ESR1 signaling underlies defective decidualization linked to severe
3786 preeclampsia. *Elife* 10: e70753, 2021. doi: 10.7554/elife.70753.

3787 359. Mazur EC, Vasquez YM, Li X, Kommagani R, Jiang L, Chen R, Lanz RB, Kovanci E,
3788 Gibbons WE, DeMayo FJ. Progesterone Receptor Transcriptome and Cistrome in Decidualized
3789 Human Endometrial Stromal Cells. *Endocrinology* 156: 2239–2253, 2015. doi:
3790 10.1210/en.2014-1566.

3791 360. Hewitt SC, Kissling GE, Fieselman KE, Jayes FL, Gerrish KE, Korach KS. Biological
3792 and biochemical consequences of global deletion of exon 3 from the ERα gene. *Faseb J* 24:
3793 4660–4667, 2010. doi: 10.1096/fj.10-163428.

3794 361. Bartels HC, Postle JD, Downey P, Brennan DJ. Placenta Accreta Spectrum: A Review of
3795 Pathology, Molecular Biology, and Biomarkers. *Dis Markers* 2018: 1–11, 2018. doi:
3796 10.1155/2018/1507674.

3797 362. Jauniaux E, Collins S, Burton GJ. Placenta accreta spectrum: pathophysiology
3798 and evidence-based anatomy for prenatal ultrasound imaging. *Am J Obstet Gynecol* 218: 75–
3799 87, 2018. doi: 10.1016/j.ajog.2017.05.067.

3800 363. Goh WA, Zalud I. Placenta accreta: diagnosis, management and the molecular biology of
3801 the morbidly adherent placenta. *J Maternal-fetal Neonatal Medicine* 29: 1–6, 2015. doi:
3802 10.3109/14767058.2015.1064103.

3803 364. Tantbirojn P, Crum CP, Parast MM. Pathophysiology of Placenta Creta: The Role of
3804 Decidua and Extravillous Trophoblast. *Placenta* 29: 639–645, 2008. doi:
3805 10.1016/j.placenta.2008.04.008.

3806 365. Jauniaux E, Jurkovic D. Placenta accreta: Pathogenesis of a 20th century iatrogenic uterine
3807 disease. *Placenta* 33: 244–251, 2012. doi: 10.1016/j.placenta.2011.11.010.

3808 366. McNally L, Zhou Y, Robinson JF, Zhao G, Chen L, Chen H, Kim MY, Kapidzic M,
3809 Gormley M, Hannibal R, Fisher SJ. Up-regulated cytotrophoblast DOCK4 contributes to over-
3810 invasion in placenta accreta spectrum. *Proc National Acad Sci* 117: 15852–15861, 2020. doi:
3811 10.1073/pnas.1920776117.

3812 367. Dunk C, Kwan M, Hazan A, Walker S, Wright JK, Harris LK, Jones RL, Keating S,
3813 Kingdom JCP, Whittle W, Maxwell C, Lye SJ. Failure of Decidualization and Maternal
3814 Immune Tolerance Underlies Uterovascular Resistance in Intra Uterine Growth Restriction.
3815 *Front Endocrinol* 10: 160, 2019. doi: 10.3389/fendo.2019.00160.

3816 368. Gupta MB, Shehab MA, Nygard K, Biggar K, Singal SS, Santoro N, Powell TL, Jansson
3817 T. IUGR Is Associated With Marked Hyperphosphorylation of Decidual and Maternal Plasma
3818 IGFBP-1. *J Clin Endocrinol Metabolism* 104: 408–422, 2018. doi: 10.1210/jc.2018-00820.

3819 369. Moreno-Moya JM, Vilella F, Simón C. MicroRNA: key gene expression regulators.
3820 *Fertility and sterility* 101 (6): 1516–1523, 2013. doi: 10.1016/j.fertnstert.2013.10.042.

3821 370. Simón C, Greening DW, Bolumar D, Balaguer N, Salamonsen LA, Vilella F. Extracellular
3822 Vesicles in Human Reproduction in Health and Disease. *Endocrine reviews* 39: 292–332, 2018.
3823 doi: 10.1210/er.2017-00229.

3824 371. Wooding P, Burton G. Comparative Placentation, Structures, Functions and Evolution.
3825 Springer, Berlin, Heidelberg, 2008. https://doi.org/10.1007/978-3-540-78797-6. .

3826 372. Gray CA, Bartol FF, Taylor KM, Wiley AA, Ramsey WS, Ott TL, Bazer FW, Spencer
3827 TE. Ovine Uterine Gland Knock-Out Model: Effects of Gland Ablation on the Estrous Cycle1.
3828 *Biol Reprod* 62: 448–456, 2000. doi: 10.1095/biolreprod62.2.448.

3829 373. Gray C, Burghardt R, Johnson G, Bazer F, Spencer T. Evidence that absence of
3830 endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival
3831 and elongation. *Reproduction* 124: 289–300, 2002. doi: 10.1530/rep.0.1240289.

3832 374. Cooke PS, Ekman GC, Kaur J, Davila J, Bagchi IC, Clark SG, Dziuk PJ, Hayashi K,
3833 Bartol FF. Brief Exposure to Progesterone During a Critical Neonatal Window Prevents
3834 Uterine Gland Formation in Mice. *Biol Reprod* 86: 63, 2012. doi:
3835 10.1095/biolreprod.111.097188.

3836 375. Filant J, Spencer TE. Endometrial Glands Are Essential for Blastocyst Implantation and
3837 Decidualization in the Mouse Uterus. *Biology of reproduction* 88: 93–93, 2013. doi:
3838 10.1095/biolreprod.113.107631.

3839 376. Gray CA, Taylor KM, Ramsey WS, Hill JR, Bazer FW, Bartol FF, Spencer TE.
3840 Endometrial Glands Are Required for Preimplantation Conceptus Elongation and Survival1.
3841 *Biol Reprod* 64: 1608–1613, 2001. doi: 10.1095/biolreprod64.6.1608.

3842 377. Hamilton WJ, Gladstone RJ. A presomite human embryo (Shaw): the implantation. *J Anat*
3843 76: 187–203, 1942.

3844 378. Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniaux E. Uterine glands provide
3845 histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *J Clin*
3846 *Endocrinol Metabolism* 87: 2954–2959, 2002. doi: 10.1210/jcem.87.6.8563.

3847 379. Burton GJ, Scioscia M, Rademacher TW. Endometrial secretions: creating a stimulatory
3848 microenvironment within the human early placenta and implications for the aetiopathogenesis
3849 of preeclampsia. *J Reprod Immunol* 89: 118–125, 2011. doi: 10.1016/j.jri.2011.02.005.

380. Jauniaux E, Gulbis B, Burton GJ. Physiological implications of the materno–fetal oxygen gradient in human early pregnancy. *Reprod Biomed Online* 7: 250–253, 2003. doi: 10.1016/s1472-6483(10)61760-9.

381. Jauniaux E, Watson AL, Hempstock J, Bao Y-P, Skepper JN, Burton GJ. Onset of Maternal Arterial Blood Flow and Placental Oxidative Stress. *Am J Pathology* 157: 2111–2122, 2000. doi: 10.1016/s0002-9440(10)64849-3.

382. Cooke PS, Spencer TE, Bartol FF, Hayashi K. Uterine glands: development, function and experimental model systems. *Mhr Basic Sci Reproductive Medicine* 19: 547–558, 2013. doi: 10.1093/molehr/gat031.

383. Hoffmann G von. Uterine Milk. *Am J Medical Sci* 87: 254–255, 1884. doi: 10.1097/00000441-188401000-00040.

384. Gaast MH van der, Macklon NS, Beier-Hellwig K, Krusche CA, Fauser BCJM, Beier HM, Classen-Linke I. The feasibility of a less invasive method to assess endometrial maturation--comparison of simultaneously obtained uterine secretion and tissue biopsy. *BJOG : an international journal of obstetrics and gynaecology* 116: 304–312, 2009. doi: 10.1111/j.1471-0528.2008.02039.x.

385. Simón C, Piquette GN, Frances A, Polan ML. Localization of interleukin-1 type I receptor and interleukin-1 beta in human endometrium throughout the menstrual cycle. *J Clin Endocrinol Metabolism* 77: 549–555, 1993. doi: 10.1210/jcem.77.2.8345061.

386. Casslén B, Nilsson B. Human uterine fluid, examined in undiluted samples for osmolarity and the concentrations of inorganic ions, albumin, glucose, and urea. *Am J Obstet Gynecol* 150: 877–881, 1984. doi: 10.1016/0002-9378(84)90466-6.

387. Hannan NJ, Stephens AN, Rainczuk A, Hincks C, Rombauts LJF, Salamonsen LA. 2D-DiGE analysis of the human endometrial secretome reveals differences between receptive and nonreceptive states in fertile and infertile women. *Journal of proteome research* 9: 6256–6264, 2010. doi: 10.1021/pr1004828.

388. Yin ET, Wessler S, Stoll PJ. Identity of plasma-activated factor X inhibitor with antithrombin 3 and heparin cofactor. *J Biological Chem* 246: 3712–9, 1971.

389. Cross J, Werb Z, Fisher S. Implantation and the placenta: key pieces of the development puzzle. *Science* 266: 1508–1518, 1994. doi: 10.1126/science.7985020.

390. Esadeg S, He H, Pijnenborg R, Leuven FV, Croy BA. Alpha-2 Macroglobulin Controls Trophoblast Positioning in Mouse Implantation Sites. *Placenta* 24: 912–921, 2003. doi: 10.1016/s0143-4004(03)00148-6.

391. Dimitriadis E, Stoikos C, Stafford-Bell M, Clark I, Paiva P, Kovacs G, Salamonsen LA. Interleukin-11, IL-11 receptorα and leukemia inhibitory factor are dysregulated in endometrium of infertile women with endometriosis during the implantation window. *J Reprod Immunol* 69: 53–64, 2006. doi: 10.1016/j.jri.2005.07.004.

392. Marshall RJ, Braye SG. Immunohistochemical Demonstration of α-1-Antitrypsin and α-1− Antichymotrypsin in Normal Human Endometrium. *Int J Gynecol Pathol* 6: 49–54, 1987. doi: 10.1097/00004347-198703000-00006.

393. Fowler PA, Tattum J, Bhattacharya S, Klonisch T, Hombach-Klonisch S, Gazvani R, Lea RG, Miller I, Simpson WG, Cash P. An investigation of the effects of endometriosis on the proteome of human eutopic endometrium: A heterogeneous tissue with a complex disease. *Proteomics* 7: 130–142, 2007. doi: 10.1002/pmic.200600469.

394. Corbo RM, Ulizzi L, Piombo L, Scacchi R. Association of ACE I/D polymorphism and recurrent miscarriages in an Italian population with a pre-modern reproductive pattern. *Ann Hum Biol* 38: 102–105, 2010. doi: 10.3109/03014460.2010.481265.

395. White P, Cooke N. The Multifunctional Properties and Characteristics of Vitamin D-binding Protein. *Trends Endocrinol Metabolism* 11: 320–327, 2000. doi: 10.1016/s1043-2760(00)00317-9.

396. Kane M, Morgan P, Coonan C. Peptide growth factors and preimplantation development. *Hum Reprod Update* 3: 137–157, 1997. doi: 10.1093/humupd/3.2.137.

397. Garrido-Gómez T, Domínguez F, Simón C. Proteomics of embryonic implantation. *Handb Exp Pharmacol* : 67–78, 2010. doi: 10.1007/978-3-642-02062-9_5.

398. Hempstock J, Cindrova-Davies T, Jauniaux E, Burton GJ. Endometrial glands as a source of nutrients, growth factors and cytokines during the first trimester of human pregnancy: A morphological and immunohistochemical study. *Reproductive Biology Endocrinol Rb E* 2: 58, 2004. doi: 10.1186/1477-7827-2-58.

399. Wang H, Dey SK. Lipid signaling in embryo implantation. *Prostaglandins & other lipid mediators* 77: 84–102, 2005. doi: 10.1016/j.prostaglandins.2004.09.013.

400. Ye X, Hama K, Contos JJ, Anliker B, Inoue A, Skinner MK, Suzuki H, Amano T, Kennedy G, Arai H, Aoki J, Chun J. LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. *Nature* 435: 104–108, 2005. doi: 10.1038/nature03505.

401. Paria BC, Ma W, Tan J, Raja S, Das SK, Dey SK, Hogan BL. Cellular and molecular responses of the uterus to embryo implantation can be elicited by locally applied growth factors. *Proc Natl Acad Sci U S A* 98: 1047–1052, 2001. doi: 10.1073/pnas.98.3.1047.

402. Berlanga O, Bradshaw HB, Vilella-Mitjana F, Garrido-Gómez T, Simón C. How endometrial secretomics can help in predicting implantation. *Placenta* 32 Suppl 3: S271-5, 2011. doi: 10.1016/j.placenta.2011.06.002.

403. Waclawik A, Jabbour HN, Blitek A, Ziecik AJ. Estradiol-17 , Prostaglandin E2 (PGE2), and the PGE2 Receptor Are Involved in PGE2 Positive Feedback Loop in the Porcine Endometrium. *Endocrinology* 150: 3823–3832, 2009. doi: 10.1210/en.2008-1499.

404. Vilella F, Moreno-Moya JM, Balaguer N, Grasso A, Herrero M, Martinez S, Marcilla A, Simón C. Hsa-miR-30d, secreted by the human endometrium, is taken up by the pre-

implantation embryo and might modify its transcriptome. *Development (Cambridge, England)* 142: 3210–3221, 2015. doi: 10.1242/dev.124289.

405. Estella C, Herrer I, Atkinson SP, Quiñonero A, Martínez S, Pellicer A, Simón C. Inhibition of Histone Deacetylase Activity in Human Endometrial Stromal Cells Promotes Extracellular Matrix Remodelling and Limits Embryo Invasion. 7: e30508, 2012. doi: 10.1371/journal.pone.0030508.

406. Estella C, Herrer I, Moreno-Moya JM, Quiñonero A, Martínez S, Pellicer A, Simón C. miRNA signature and Dicer requirement during human endometrial stromal decidualization in vitro. 7: e41080, 2012. doi: 10.1371/journal.pone.0041080.

407. Gaast MH van der, Beier-Hellwig K, Fauser BCJM, Beier HM, Macklon NS. Endometrial secretion aspiration prior to embryo transfer does not reduce implantation rates. *Reproductive biomedicine online* 7: 105–109, 2003. doi: 10.1016/s1472-6483(10)61737-3.

408. Maia HS, Coutinho EM. Peristalsis and Antiperistalsis of the Human Fallopian Tube During the Menstrual Cycle1. *Biol Reprod* 2: 305–314, 1970. doi: 10.1095/biolreprod2.2.305.

409. Guo H, Zhu P, Yan L, Li R, Hu B, Lian Y, Yan J, Ren X, Lin S, Li J, Jin X, Shi X, Liu P, Wang X, Wang W, Wei Y, Li X, Guo F, Wu X, Fan X, Yong J, Wen L, Xie SX, Tang F, Qiao J. The DNA methylation landscape of human early embryos. *Nature* 511: 606–610, 2014. doi: 10.1038/nature13544.

410. Keeley TP, Mann GE. Defining Physiological Normoxia for Improved Translation of Cell Physiology to Animal Models and Humans. *Physiol Rev* 99: 161–234, 2019. doi: 10.1152/physrev.00041.2017.

411. Fischer B, Bavister BD. Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. *Reproduction* 99: 673–679, 1993. doi: 10.1530/jrf.0.0990673.

412. Ottosen LD, Hindkjær J, Husth M, Petersen DE, Kirk J, Ingerslev HJ. Observations on intrauterine oxygen tension measured by fibre-optic microsensors. *Reprod Biomed Online* 13: 380–385, 2006. doi: 10.1016/s1472-6483(10)61443-5.

413. Houghton FD. HYPOXIA AND REPRODUCTIVE HEALTH: Hypoxic regulation of preimplantation embryos: lessons from human embryonic stem cells. *Reproduction* 161: F41–F51, 2021. doi: 10.1530/rep-20-0322.

414. Goto Y, Noda Y, Mori T, Nakano M. Increased generation of reactive oxygen species in embryos cultured in vitro. *Free Radical Bio Med* 15: 69–75, 1993. doi: 10.1016/0891-5849(93)90126-f.

415. Belli M, Zhang L, Liu X, Donjacour A, Ruggeri E, Palmerini MG, Nottola SA, Macchiarelli G, Rinaudo P. Oxygen concentration alters mitochondrial structure and function in in vitro fertilized preimplantation mouse embryos. *Hum Reproduction Oxf Engl* 34: 601–611, 2019. doi: 10.1093/humrep/dez011.

416. Meuter A, Rogmann L-M, Winterhoff BJ, Tchkonia T, Kirkland JL, Morbeck DE. Markers of cellular senescence are elevated in murine blastocysts cultured in vitro: molecular

consequences of culture in atmospheric oxygen. *J Assist Reprod Gen* 31: 1259–67, 2014. doi: 10.1007/s10815-014-0299-8.

417. Ma Y-Y, Chen H-W, Tzeng C-R. Low oxygen tension increases mitochondrial membrane potential and enhances expression of antioxidant genes and implantation protein of mouse blastocyst cultured in vitro. *J Ovarian Res* 10: 47, 2017. doi: 10.1186/s13048-017-0344-1.

418. Takahashi M, Keicho K, Takahashi H, Ogawa H, Schulte RM, Okano A. Effect of oxidative stress on development and DNA damage in in-vitro cultured bovine embryos by comet assay. *Theriogenology* 54: 137–145, 2000. doi: 10.1016/s0093-691x(00)00332-0.

419. Bedaiwy MA, Falcone T, Mohamed MS, Aleem AAN, Sharma RK, Worley SE, Thornton J, Agarwal A. Differential growth of human embryos in vitro: Role of reactive oxygen species. *Fertil Steril* 82: 593–600, 2004. doi: 10.1016/j.fertnstert.2004.02.121.

420. Wale PL, Gardner DK. Oxygen Regulates Amino Acid Turnover and Carbohydrate Uptake During the Preimplantation Period of Mouse Embryo Development1. *Biol Reprod* 87: 24, 1–8, 2012. doi: 10.1095/biolreprod.112.100552.

421. Wale PL, Gardner DK. Oxygen Affects the Ability of Mouse Blastocysts to Regulate Ammonium1. *Biol Reprod* 89: Article 75, 1-10, 2013. doi: 10.1095/biolreprod.113.109256.

422. Rinaudo P, Giritharan G, Talbi S, Dobson A, Schultz R. Effects of oxygen tension on gene expression in preimplantation mouse embryos. *Fertil Steril* 86: 1265.e1-1265.e36, 2006. doi: 10.1016/j.fertnstert.2006.05.017.

423. Katz-Jaffe MG, Linck DW, Schoolcraft WB, Gardner DK. A proteomic analysis of mammalian preimplantation embryonic development. *Reproduction* 130: 899–905, 2005. doi: 10.1530/rep.1.00854.

424. Li W, Goossens K, Poucke MV, Forier K, Braeckmans K, Soom AV, Peelman LJ. High oxygen tension increases global methylation in bovine 4-cell embryos and blastocysts but does not affect general retrotransposon expression. *Reproduction Fertility Dev* 28: 948, 2016. doi: 10.1071/rd14133.

425. Dumoulin JCM, Vanvuchelen RCM, Land JA, Pieters MHEC, Geraedts JPM, Evers JLH. Effect of oxygen concentration on in vitro fertilization and embryo culture in the human and the mouse. *Fertil Steril* 63: 115–119, 1995. doi: 10.1016/s0015-0282(16)57305-1.

426. Dumoulin JCM, Meijers CJJ, Bras M, Coonen E, Geraedts JPM, Evers JLH. Effect of oxygen concentration on human in-vitro fertilization and embryo culture. *Hum Reprod* 14: 465–469, 1999. doi: 10.1093/humrep/14.2.465.

427. Kovačič B, Vlaisavljević V. Influence of atmospheric versus reduced oxygen concentration on development of human blastocysts in vitro: a prospective study on sibling oocytes. *Reprod Biomed Online* 17: 229–236, 2008. doi: 10.1016/s1472-6483(10)60199-x.

428. Ciray HN, Aksoy T, Yaramanci K, Karayaka I, Bahceci M. In vitro culture under physiologic oxygen concentration improves blastocyst yield and quality: a prospective

3999 randomized survey on sibling oocytes. *Fertil Steril* 91: 1459–61, 2008. doi:
4000 10.1016/j.fertnstert.2008.07.1707.

4001 429. Kasterstein E, Strassburger D, Komarovsky D, Bern O, Komsky A, Raziel A, Friedler S,
4002 Ron-El R. The effect of two distinct levels of oxygen concentration on embryo development in
4003 a sibling oocyte study. *J Assist Reprod Gen* 30: 1073–9, 2013. doi: 10.1007/s10815-013-0032-
4004 z.

4005 430. Salamonsen LA, Evans J, Nguyen HPT, Edgell TA. The Microenvironment of Human
4006 Implantation: Determinant of Reproductive Success. *Am J Reprod Immunol* 75: 218–225,
4007 2016. doi: 10.1111/aji.12450.

4008 431. Zafir S, Zhou W, Menkhorst E, Santos L, Dimitriadis E. MAML1: a coregulator that alters
4009 endometrial epithelial cell adhesive capacity. *Fertility Res Pract* 7: 8, 2021. doi:
4010 10.1186/s40738-021-00100-y.

4011 432. Santos LL, Ling CK, Dimitriadis E. Tripeptidyl peptidase I promotes human endometrial
4012 epithelial cell adhesive capacity implying a role in receptivity. *Reproductive Biology*
4013 *Endocrinol Rb E* 18: 124, 2020. doi: 10.1186/s12958-020-00682-0.

4014 433. Fan H, Fernando SR, Jiang L, Wang Z, Kodithuwakku SP, Wong CKC, Ng EHY, Yeung
4015 WSB, Lee K-F. Bisphenol A Analogues Suppress Spheroid Attachment on Human
4016 Endometrial Epithelial Cells through Modulation of Steroid Hormone Receptors Signaling
4017 Pathway. *Cells* 10: 2882, 2021. doi: 10.3390/cells10112882.

4018 434. Whitby S, Zhou W, Dimitriadis E. Alterations in Epithelial Cell Polarity During
4019 Endometrial Receptivity: A Systematic Review. *Front Endocrinol* 11: 596324, 2020. doi:
4020 10.3389/fendo.2020.596324.

4021 435. Peng J, Li X, Zhang Y, Hu J, Shang Y, Yin Y, Xiao Z. Par3/integrin β1 regulates embryo
4022 adhesion via changing endometrial luminal epithelium polarity†. *Biol Reprod* 104: 1228–1238,
4023 2021. doi: 10.1093/biolre/ioab033.

4024 436. Cuman C, Sinderen MV, Gantier MP, Rainczuk K, Sorby K, Rombauts L, Osianlis T,
4025 Dimitriadis E. Human Blastocyst Secreted microRNA Regulate Endometrial Epithelial Cell
4026 Adhesion. *Ebiomedicine* 2: 1528–1535, 2015. doi: 10.1016/j.ebiom.2015.09.003.

4027 437. Monhasery N, Moll J, Cuman C, Franke M, Lamertz L, Nitz R, Görg B, Häussinger D,
4028 Lokau J, Floss DM, Piekorz R, Dimitriadis E, Garbers C, Scheller J. Transcytosis of IL-11 and
4029 Apical Redirection of gp130 Is Mediated by IL-11α Receptor. *Cell Reports* 16: 1067–1081,
4030 2016. doi: 10.1016/j.celrep.2016.06.062.

4031 438. Winship A, Ton A, Sinderen MV, Menkhorst E, Rainczuk K, Griffiths M, Cuman C,
4032 Dimitriadis E. Mouse double minute homologue 2 (MDM2) downregulation by miR-661
4033 impairs human endometrial epithelial cell adhesive capacity. *Reproduction Fertility Dev* 30:
4034 477, 2018. doi: 10.1071/rd17095.

4035 439. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J,
4036 Alonso R, Alamá P, Remohí J, Pellicer A, Ramon D, Simon C. Evidence That the Endometrial

4037 Microbiota Has an Effect on Implantation Success or Failure. *Obstet Gynecol Surv* 72: 341–
4038 342, 2017. doi: 10.1097/01.ogx.0000520608.85323.04.

4039 440. Moreno I, Franasiak JM. Endometrial microbiota—new player in town. *Fertil Steril* 108:
4040 32–39, 2017. doi: 10.1016/j.fertnstert.2017.05.034.

4041 441. Deglincerti A, Croft GF, Pietila LN, Zernicka-Goetz M, Siggia ED, Brivanlou AH. Self-
4042 organization of the in vitro attached human embryo. *Nature* 533: 251–254, 2016. doi:
4043 10.1038/nature17948.

4044 442. James JL, Carter AM, Chamley LW. Human placentation from nidation to 5 weeks of
4045 gestation. Part I: What do we know about formative placental development following
4046 implantation? *Placenta* 33: 327–334, 2012. doi: 10.1016/j.placenta.2012.01.020.

4047 443. Burton GJ, Jauniaux E, Watson AL. Maternal arterial connections to the placental
4048 intervillous space during the first trimester of human pregnancy: The Boyd Collection revisited.
4049 *Am J Obstet Gynecol* 181: 718–724, 1999. doi: 10.1016/s0002-9378(99)70518-1.

4050 444. Pijnenborg R, Robertson WB, Brosens I, Dixon G. Review article: Trophoblast invasion
4051 and the establishment of haemochorial placentation in man and laboratory animals. *Placenta*
4052 2: 71–91, 1981. doi: 10.1016/s0143-4004(81)80042-2.

4053 445. Erlebacher A. Immunology of the Maternal-Fetal Interface. *Annu Rev Immunol* 31: 387–
4054 411, 2013. doi: 10.1146/annurev-immunol-032712-100003.

4055 446. Gellersen B, Brosens JJ. Cyclic Decidualization of the Human Endometrium in
4056 Reproductive Health and Failure. *Endocrine reviews* 35: 851–905, 2014. doi: 10.1210/er.2014-
4057 1045.

4058 447. Menkhorst EM, Sinderen MV, Correia J, Dimitriadis E. Trophoblast function is altered
4059 by decidual factors in gestational-dependant manner. *Placenta* 80: 8–11, 2019. doi:
4060 10.1016/j.placenta.2019.03.013.

4061 448. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, McWhinnie A,
4062 Esposito L, Fernando R, Skelton H, Reimann F, Gribble FM, Sharkey A, Marsh SGE,
4063 O'Rahilly S, Hemberger M, Burton GJ, Moffett A. Trophoblast organoids as a model for
4064 maternal–fetal interactions during human placentation. *Nature* 564: 263–267, 2018. doi:
4065 10.1038/s41586-018-0753-3.

4066 449. Pique-Regi R, Romero R, Tarca AL, Sendler ED, Xu Y, Garcia-Flores V, Leng Y, Luca
4067 F, Hassan SS, Gomez-Lopez N. Single cell transcriptional signatures of the human placenta in
4068 term and preterm parturition. *Elife* 8: e52004, 2019. doi: 10.7554/elife.52004.

4069 450. Liu Y, Fan X, Wang R, Lu X, Dang Y-L, Wang H, Lin H-Y, Zhu C, Ge H, Cross JC,
4070 Wang H. Single-cell RNA-seq reveals the diversity of trophoblast subtypes and patterns of
4071 differentiation in the human placenta. *Cell Res* 28: 819–832, 2018. doi: 10.1038/s41422-018-
4072 0066-y.

4073 451. Tsang JCH, Vong JSL, Ji L, Poon LCY, Jiang P, Lui KO, Ni Y-B, To KF, Cheng YKY,
4074 Chiu RWK, Lo YMD. Integrative single-cell and cell-free plasma RNA transcriptomics

4075 elucidates placental cellular dynamics. *Proc National Acad Sci* 114: E7786–E7795, 2017. doi:
4076 10.1073/pnas.1710470114.

4077 452. Nelson AC, Mould AW, Bikoff EK, Robertson EJ. Single-cell RNA-seq reveals cell type-
4078 specific transcriptional signatures at the maternal–foetal interface during pregnancy. *Nat*
4079 *Commun* 7: 11414, 2016. doi: 10.1038/ncomms11414.

4080 453. Pavličev M, Wagner GP, Chavan AR, Owens K, Maziarz J, Dunn-Fletcher C, Kallapur
4081 SG, Muglia L, Jones H. Single-cell transcriptomics of the human placenta: inferring the cell
4082 communication network of the maternal-fetal interface. *Genome research* 27: 349–361, 2017.
4083 doi: 10.1101/gr.207597.116.

4084 454. Huhn O, Ivarsson MA, Gardner L, Hollinshead M, Stinchcombe JC, Chen P, Shreeve N,
4085 Chazara O, Farrell LE, Theorell J, Ghadially H, Parham P, Griffiths G, Horowitz A, Moffett
4086 A, Sharkey AM, Colucci F. Distinctive phenotypes and functions of innate lymphoid cells in
4087 human decidua during early pregnancy. *Nat Commun* 11: 381, 2020. doi: 10.1038/s41467-019-
4088 14123-z.

4089 455. Suryawanshi H, Morozov P, Straus A, Sahasrabudhe N, Max KEA, Garzia A, Kustagi M,
4090 Tuschl T, Williams Z. A single-cell survey of the human first-trimester placenta and decidua.
4091 *Sci Adv* 4: eaau4788, 2018. doi: 10.1126/sciadv.aau4788.

4092 456. Barker DJ. The fetal and infant origins of adult disease. *BMJ : British Medical Journal*
4093 301: 1111, 1990. doi: 10.1136/bmj.301.6761.1111.

4094 457. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, Roumain J,
4095 Bennett PH, Knowler WC. Intrauterine exposure to diabetes conveys risks for type 2 diabetes
4096 and obesity: a study of discordant sibships. *Diabetes* 49: 2208–2211, 2000.

4097 458. Dabelea D, Knowler WC, Pettitt DJ. Effect of diabetes in pregnancy on offspring: Follow-
4098 up research in the pima indians. *J Maternal-fetal Neonatal Medicine* 9: 83–88, 2000. doi:
4099 10.3109/14767050009020519.

4100 459. Lawlor DA, Lichtenstein P, Langstrom N. Association of Maternal Diabetes Mellitus in
4101 Pregnancy With Offspring Adiposity Into Early Adulthood: Sibling Study in a Prospective
4102 Cohort of 280 866 Men From 248 293 Families. *Circulation* 123: 258–265, 2011. doi:
4103 10.1161/circulationaha.110.980169.

4104 460. Yáñez-Mó M, Siljander PRM, Andreu Z, Zavec AB, Borràs FE, Buzás EI, Buzas K, Casal
4105 E, Cappello F, Carvalho J, Colás E, Silva AC, Fais S, Falcon-Perez JM, Ghobrial IM, Giebel
4106 B, Gimona M, Graner M, Gursel I, Gursel M, Heegaard NHH, Hendrix A, Kierulf P, Kokubun
4107 K, Kosanovic M, Kralj-Iglic V, Krämer-Albers E-M, Laitinen S, Lässer C, Lener T, Ligeti E,
4108 Linē A, Lipps G, Llorente A, Lötvall J, Manček-Keber M, Marcilla A, Mittelbrunn M,
4109 Nazarenko I, Hoen ENMN-'t, Nyman TA, O'Driscoll L, Olivan M, Oliveira C, Pállinger É,
4110 Portillo HA del, Reventós J, Rigau M, Rohde E, Sammar M, Sánchez-Madrid F, Santarém N,
4111 Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Grein SGV der, Vasconcelos MH,
4112 Wauben MHM, Wever OD. Biological properties of extracellular vesicles and their
4113 physiological functions. *Journal of Extracellular Vesicles* 4: 27066, 2015. doi:
4114 10.3402/jev.v4.27066.

461. Hardie DG. *Biochemical Messengers: Hormones, Neurotransmitters and Growth Factors - D.G. Hardie - Google Books*. 1991.

462. Yuana Y, Oosterkamp TH, Bahatyrova S, Ashcroft B, Rodriguez PG, Bertina RM, Osanto S. Atomic force microscopy: a novel approach to the detection of nanosized blood microparticles. *Journal of thrombosis and haemostasis : JTH* 8: 315–323, 2010. doi: 10.1111/j.1538-7836.2009.03654.x.

463. Andaloussi SE, Mäger I, Breakefield XO, Wood MJA. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov* 12: 347–357, 2013. doi: 10.1038/nrd3978.

464. Colombo M, Raposo G, Théry C. Biogenesis, Secretion, and Intercellular Interactions of Exosomes and Other Extracellular Vesicles. *Annual Review of Cell and Developmental Biology* 30: 255–289, 2014. doi: 10.1146/annurev-cellbio-101512-122326.

465. Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study. *Bioscience* 65: 783–797, 2015. doi: 10.1093/biosci/biv084.

466. Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *Journal of neuro-oncology* 113: 1–11, 2013. doi: 10.1007/s11060-013-1084-8.

467. György B, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B, László V, Pállinger É, Pap E, Kittel Á, Nagy G, Falus A, Buzás EI. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cellular and molecular life sciences : CMLS* 68: 2667–2688, 2011. doi: 10.1007/s00018-011-0689-3.

468. Niel G van, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nature Reviews Molecular Cell Biology* 19: 213–228, 2018. doi: 10.1038/nrm.2017.125.

469. Simon B, Bolumar D, Amadoz A, Jimenez-Almazán J, Valbuena D, Vilella F, Moreno I. Identification and Characterization of Extracellular Vesicles and Its DNA Cargo Secreted During Murine Embryo Development. *Genes-basel* 11: 203, 2020. doi: 10.3390/genes11020203.

470. Almiñana C, Bauersachs S. Extracellular vesicles: Multi-signal messengers in the gametes/embryo-oviduct cross-talk. *Theriogenology* 150: 59–69, 2020. doi: 10.1016/j.theriogenology.2020.01.077.

471. Gatien J, Mermillod P, Tsikis G, Bernardi O, Idrissi SJ, Uzbekov R, Bourhis DL, Salvetti P, Almiñana C, Saint-Dizier M. Metabolomic Profile of Oviductal Extracellular Vesicles across the Estrous Cycle in Cattle. *Int J Mol Sci* 20: 6339, 2019. doi: 10.3390/ijms20246339.

472. Uszyński W, Zekanowska E, Uszyński M, Zyliński A, Kuczyński J. New observations on procoagulant properties of amniotic fluid: microparticles (MPs) and tissue factor-bearing MPs (MPs-TF), comparison with maternal blood plasma. *Thrombosis Research* 132: 757–760, 2013. doi: 10.1016/j.thromres.2013.10.001.

4154 473. Keller S, Ridinger J, Rupp A-K, Janssen JWG, Altevogt P. Body fluid derived exosomes
4155 as a novel template for clinical diagnostics. *Journal of translational medicine* 9: 86, 2011. doi:
4156 10.1186/1479-5876-9-86.

4157 474. Admyre C, Johansson SM, Qazi KR, Filén J-J, Lahesmaa R, Norman M, Neve EPA,
4158 Scheynius A, Gabrielsson S. Exosomes with immune modulatory features are present in human
4159 breast milk. *Journal of immunology (Baltimore, Md : 1950)* 179: 1969–1978, 2007. doi:
4160 10.4049/jimmunol.179.3.1969.

4161 475. Flori F, Secciani F, Capone A, Paccagnini E, Caruso S, Ricci MG, Focarelli R. Menstrual
4162 cycle-related sialidase activity of the female cervical mucus is associated with exosome-like
4163 vesicles. *Fertility and sterility* 88: 1212–1219, 2007. doi: 10.1016/j.fertnstert.2007.01.209.

4164 476. Ng YH, Rome S, Jalabert A, Forterre A, Singh H, Hincks CL, Salamonsen LA.
4165 Endometrial exosomes/microvesicles in the uterine microenvironment: a new paradigm for
4166 embryo-endometrial cross talk at implantation. 8: e58502, 2013. doi:
4167 10.1371/journal.pone.0058502.

4168 477. Al-Dossary AA, Strehler EE, Martin-DeLeon PA. Expression and secretion of plasma
4169 membrane Ca2+-ATPase 4a (PMCA4a) during murine estrus: association with oviductal
4170 exosomes and uptake in sperm. 8: e80181, 2013. doi: 10.1371/journal.pone.0080181.g008.

4171 478. Al-Dossary AA, Bathala P, Caplan JL, Martin-DeLeon PA. Oviductosome-Sperm
4172 Membrane Interaction in Cargo Delivery: DETECTION OF FUSION AND UNDERLYING
4173 MOLECULAR PLAYERS USING THREE-DIMENSIONAL SUPER-RESOLUTION
4174 STRUCTURED ILLUMINATION MICROSCOPY (SR-SIM). *Journal of Biological*
4175 *Chemistry* 290: 17710–17723, 2015. doi: 10.1074/jbc.m114.633156.

4176 479. Franz C, Böing AN, Montag M, Strowitzki T, Markert UR, Mastenbroek S, Nieuwland
4177 R, Toth B. Extracellular vesicles in human follicular fluid do not promote coagulation.
4178 *Reproductive biomedicine online* 33: 652–655, 2016. doi: 10.1016/j.rbmo.2016.08.005.

4179 480. Santonocito M, Vento M, Guglielmino MR, Battaglia R, Wahlgren J, Ragusa M,
4180 Barbagallo D, Borzì P, Rizzari S, Maugeri M, Scollo P, Tatone C, Valadi H, Purrello M, Pietro
4181 CD. Molecular characterization of exosomes and their microRNA cargo in human follicular
4182 fluid: bioinformatic analysis reveals that exosomal microRNAs control pathways involved in
4183 follicular maturation. *Fertility and sterility* 102: 1751–61.e1, 2014. doi:
4184 10.1016/j.fertnstert.2014.08.005.

4185 481. Goss DM, Vasilescu SA, Sacks G, Gardner DK, Warkiani ME. Microfluidics facilitating
4186 the use of small extracellular vesicles in innovative approaches to male infertility. .

4187 482. Höög JL, Lötvall J. Diversity of extracellular vesicles in human ejaculates revealed by
4188 cryo-electron microscopy. *J Extracell Vesicles* 4: 28680, 2015. doi: 10.3402/jev.v4.28680.

4189 483. Burns G, Brooks K, Wildung M, Navakanitworakul R, Christenson LK, Spencer TE.
4190 Extracellular Vesicles in Luminal Fluid of the Ovine Uterus. *Plos One* 9: e90913, 2014. doi:
4191 10.1371/journal.pone.0090913.

4192 484. Burns GW, Brooks KE, Spencer TE. Extracellular Vesicles Originate from the Conceptus
4193 and Uterus During Early Pregnancy in Sheep. *Biology of reproduction* 94: 56, 2016. doi:
4194 10.1095/biolreprod.115.134973.

4195 485. Ruiz-González I, Xu J, Wang X, Burghardt RC, Dunlap KA, Bazer FW. Exosomes,
4196 endogenous retroviruses and toll-like receptors: pregnancy recognition in ewes. *Reproduction*
4197 *(Cambridge, England)* 149: 281–291, 2015. doi: 10.1530/rep-14-0538.

4198 486. Greening DW, Nguyen HPT, Elgass K, Simpson RJ, Salamonsen LA. Human
4199 Endometrial Exosomes Contain Hormone-Specific Cargo Modulating Trophoblast Adhesive
4200 Capacity: Insights into Endometrial-Embryo Interactions. *Biology of reproduction* 94: 38,
4201 2016. doi: 10.1095/biolreprod.115.134890.

4202 487. Thakur BK, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, Zheng Y, Hoshino A,
4203 Brazier H, Xiang J, Williams C, Rodriguez-Barrueco R, Silva JM, Zhang W, Hearn S,
4204 Elemento O, Paknejad N, Manova-Todorova K, Welte K, Bromberg J, Peinado H, Lyden D.
4205 Double-stranded DNA in exosomes: a novel biomarker in cancer detection. *Cell research* 24:
4206 766–769, 2014. doi: 10.1038/cr.2014.44.

4207 488. Kawamura Y, Yamamoto Y, Sato T, Ochiya T. Extracellular vesicles as trans-genomic
4208 agents: Emerging roles in disease and evolution. *Cancer Sci* 108: 824–830, 2017. doi:
4209 10.1111/cas.13222.

4210 489. Guescini M, Guidolin D, Vallorani L, Casadei L, Gioacchini AM, Tibollo P, Battistelli
4211 M, Falcieri E, Battistin L, Agnati LF, Stocchi V. C2C12 myoblasts release micro-vesicles
4212 containing mtDNA and proteins involved in signal transduction. *Exp Cell Res* 316: 1977–1984,
4213 2010. doi: 10.1016/j.yexcr.2010.04.006.

4214 490. Tong M, Tsai BW, Chamley LW. Antiphospholipid antibodies and extracellular vesicles
4215 in pregnancy. *Am J Reprod Immunol* 85: e13312, 2021. doi: 10.1111/aji.13312.

4216 491. Bolumar D, Amadoz A, Moreno I, Marín C, Juan AD, Simon C, Vilella F. Endometrial
4217 mitochondrial dna secreted in extracellular vesicles: a novel maternal mechanism modulating
4218 embryo bioenergetics. *Fertil Steril* 114: e34, 2020. doi: 10.1016/j.fertnstert.2020.08.121.

4219 492. Godakumara K, Ord J, Lättekivi F, Dissanayake K, Viil J, Boggavarapu NR, Faridani OR,
4220 Jääger K, Velthut-Meikas A, Jaakma Ü, Salumets A, Fazeli A. Trophoblast derived
4221 extracellular vesicles specifically alter the transcriptome of endometrial cells and may
4222 constitute a critical component of embryo-maternal communication. *Reprod Biol Endocrin* 19:
4223 115, 2021. doi: 10.1186/s12958-021-00801-5.

4224 493. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The
4225 MicroRNA Spectrum in 12 Body Fluids. *Clinical Chemistry* 56: 1733–1741, 2010. doi:
4226 10.1373/clinchem.2010.147405.

4227 494. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li
4228 X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H,
4229 Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang C-Y. Characterization of
4230 microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases.
4231 *Cell Res* 18: 997–1006, 2008. doi: 10.1038/cr.2008.282.

4232 495. Rosenbluth EM, Shelton DN, Wells LM, Sparks AET, Voorhis BJV. Human embryos
4233 secrete microRNAs into culture media—a potential biomarker for implantation. *Fertil Steril*
4234 101: 1493–1500, 2014. doi: 10.1016/j.fertnstert.2014.01.058.

4235 496. Jr EB, Setti AS, Braga DPAF, Geraldo MV, Figueira R de CS, Jr AI. miR-142-3p as a
4236 biomarker of blastocyst implantation failure - A pilot study. *Jbra Assisted Reproduction* 20:
4237 200–205, 2016. doi: 10.5935/1518-0557.20160039.

4238 497. Capalbo A, Ubaldi FM, Cimadomo D, Noli L, Khalaf Y, Farcomeni A, Ilic D, Rienzi L.
4239 MicroRNAs in spent blastocyst culture medium are derived from trophectoderm cells and can
4240 be explored for human embryo reproductive competence assessment. *Fertility and sterility* 105:
4241 225-35.e1–3, 2016. doi: 10.1016/j.fertnstert.2015.09.014.

4242 498. Abu-Halima M, Häusler S, Backes C, Fehlmann T, Staib C, Nestel S, Nazarenko I, Meese
4243 E, Keller A. Micro-ribonucleic acids and extracellular vesicles repertoire in the spent culture
4244 media is altered in women undergoing In Vitro Fertilization. *Sci Rep-uk* 7: 13525, 2017. doi:
4245 10.1038/s41598-017-13683-8.

4246 499. Cimadomo D, Rienzi L, Giancani A, Alviggi E, Dusi L, Canipari R, Noli L, Ilic D, Khalaf
4247 Y, Ubaldi FM, Capalbo A. Definition and validation of a custom protocol to detect miRNAs
4248 in the spent media after blastocyst culture: searching for biomarkers of implantation. *Hum*
4249 *Reprod* 34: 1746–1761, 2019. doi: 10.1093/humrep/dez119.

4250 500. Giacomini E, Vago R, Sanchez AM, Podini P, Zarovni N, Murdica V, Rizzo R, Bortolotti
4251 D, Candiani M, Viganò P. Secretome of in vitro cultured human embryos contains extracellular
4252 vesicles that are uptaken by the maternal side. *Sci Rep-uk* 7: 5210, 2017. doi: 10.1038/s41598-
4253 017-05549-w.

4254 501. Pállinger É, Bognar Z, Bodis J, Csabai T, Farkas N, Godony K, Varnagy A, Buzas E,
4255 Szekeres-Bartho J. A simple and rapid flow cytometry-based assay to identify a competent
4256 embryo prior to embryo transfer. *Sci Rep* 7: 39927–8, 2017. doi: 10.1038/srep39927.

4257 502. Desrochers LM, Bordeleau F ccedil ois, Reinhart-King CA, Antonyak MA, Cerione RA.
4258 Microvesicles provide a mechanism for intercellular communication by embryonic stem cells
4259 during embryo implantation. *Nature Communications* 7: 1–11, 2016. doi:
4260 10.1038/ncomms11958.

4261 503. Bidarimath M, Khalaj K, Kridli RT, Kan FWK, Koti M, Tayade C. Extracellular vesicle
4262 mediated intercellular communication at the porcine maternal-fetal interface: A new paradigm
4263 for conceptus-endometrial cross-talk. *Sci Rep* 7: 40476, 2017. doi: 10.1038/srep40476.

4264 504. Salomon C, Torres MJ, Kobayashi M, Scholz-Romero K, Sobrevia L, Dobierzewska A,
4265 Illanes SE, Mitchell MD, Rice GE. A Gestational Profile of Placental Exosomes in Maternal
4266 Plasma and Their Effects on Endothelial Cell Migration. *Plos One* 9: e98667, 2014. doi:
4267 10.1371/journal.pone.0098667.

4268 505. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The Human
4269 Microbiome Project. *Nature* 449: 804–810, 2007. doi: 10.1038/nature06244.

4270 506. Grice EA, Segre JA. The Human Microbiome: Our Second Genome. *Annual Review of*
4271 *Genomics and Human Genetics* 13: 151–170, 2012. doi: 10.1146/annurev-genom-090711-
4272 163814.

4273 507. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. The role of
4274 microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* 5: 355–362, 2007.
4275 doi: 10.1038/nrmicro1635.

4276 508. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on
4277 brain and behaviour. *Nat Rev Neurosci* 13: 701–712, 2012. doi: 10.1038/nrn3346.

4278 509. García-Peñarrubia P, Ruiz-Alcaraz AJ, Martínez-Esparza M, Marín P, Machado-Linde F.
4279 Hypothetical roadmap towards endometriosis: prenatal endocrine-disrupting chemical
4280 pollutant exposure, anogenital distance, gut-genital microbiota and subclinical infections. *Hum*
4281 *Reprod Update* 26: 214–246, 2020. doi: 10.1093/humupd/dmz044.

4282 510. Cho I, Yamanishi S, Cox L, Methé BA, Zavadil J, Li K, Gao Z, Mahana D, Raju K, Teitler
4283 I, Li H, Alekseyenko AV, Blaser MJ. Antibiotics in early life alter the murine colonic
4284 microbiome and adiposity. *Nature* 488: 621–626, 2012. doi: 10.1038/nature11400.

4285 511. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, Kim SG, Li H, Gao
4286 Z, Mahana D, Zárate Rodriguez JG, Rogers AB, Robine N, Loke P, Blaser MJ. Altering the
4287 Intestinal Microbiota during a Critical Developmental Window Has Lasting Metabolic
4288 Consequences. *Cell* 158: 705–721, 2014. doi: 10.1016/j.cell.2014.05.052.

4289 512. Azad MB, Bridgman SL, Becker AB, Kozyrskyj AL. Infant antibiotic exposure and the
4290 development of childhood overweight and central adiposity. *International journal of obesity*
4291 *(2005)* 38: 1290–1298, 2014. doi: 10.1038/ijo.2014.119.

4292 513. Arrieta M-C, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S,
4293 Kuzeljevic B, Gold MJ, Britton HM, Lefebvre DL, Subbarao P, Mandhane P, Becker A,
4294 McNagny KM, Sears MR, Kollmann T, Investigators the CS, Mohn WW, Turvey SE, Finlay
4295 BB. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci*
4296 *Transl Med* 7: 307ra152-307ra152, 2015. doi: 10.1126/scitranslmed.aab2271.

4297 514. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may
4298 be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci*
4299 *Rep* 6: 23129, 2016. doi: 10.1038/srep23129.

4300 515. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early
4301 life shapes the immune system. *Science* 352: 539–544, 2016. doi: 10.1126/science.aad9378.

4302 516. Rackaityte E, Halkias J, Fukui EM, Mendoza VF, Hayzelden C, Crawford ED, Fujimura
4303 KE, Burt TD, Lynch SV. Corroborating evidence refutes batch effect as explanation for fetal
4304 bacteria. *Microbiome* 9: 10, 2021. doi: 10.1186/s40168-020-00948-0.

4305 517. Rackaityte E, Halkias J, Fukui EM, Mendoza VF, Hayzelden C, Crawford ED, Fujimura
4306 KE, Burt TD, Lynch SV. Viable bacterial colonization is highly limited in the human intestine
4307 in utero. *Nat Med* 26: 599–607, 2020. doi: 10.1038/s41591-020-0761-3.

518. Perez-Muñoz ME, Arrieta M-C, Ramer-Tait AE, Walter J. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. *Microbiome* 5: 48, 2017. doi: 10.1186/s40168-017-0268-4.

519. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, Simpson N, Kumar N, Stares MD, Rodger A, Brocklehurst P, Field N, Lawley TD. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature* 574: 117–121, 2019. doi: 10.1038/s41586-019-1560-1.

520. Silverstein RB, Mysorekar IU. Group therapy on in utero colonization: seeking common truths and a way forward. *Microbiome* 9: 7, 2021. doi: 10.1186/s40168-020-00968-w.

521. Blaser MJ, Devkota S, McCoy KD, Relman DA, Yassour M, Young VB. Lessons learned from the prenatal microbiome controversy. *Microbiome* 9: 8, 2021. doi: 10.1186/s40168-020-00946-2.

522. Macpherson AJ, Agüero MG de, Ganal-Vonarburg SC. How nutrition and the maternal microbiota shape the neonatal immune system. *Nat Rev Immunol* 17: 508–517, 2017. doi: 10.1038/nri.2017.58.

523. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, Creasy HH, Earl AM, FitzGerald MG, Fulton RS, Giglio MG, Hallsworth-Pepin K, Lobos EA, Madupu R, Magrini V, Martin JC, Mitreva M, Muzny DM, Sodergren EJ, Versalovic J, Wollam AM, Worley KC, Wortman JR, Young SK, Zeng Q, Aagaard KM, Abolude OO, Allen-Vercoe E, Alm EJ, Alvarado L, Andersen GL, Anderson S, Appelbaum E, Arachchi HM, Armitage G, Arze CA, Ayyaz T, Baker CC, Begg L, Belachew T, Bhonagiri V, Bihan M, Blaser MJ, Bloom T, Bonazzi V, Brooks JP, Buck GA, Buhay CJ, Busam DA, Campbell JL, Canon SR, Cantarel BL, Chain PSG, Chen I-MA, Chen L, Chhibba S, Chu K, Ciulla DM, Clemente JC, Clifton SW, Conlan S, Crabtree J, Cutting MA, Davidovics NJ, Davis CC, DeSantis TZ, Deal C, Delehaunty KD, Dewhirst FE, Deych E, Ding Y, Dooling DJ, Dugan SP, Dunne WM, Durkin AS, Edgar RC, Erlich RL, Farmer CN, Farrell RM, Faust K, Feldgarden M, Felix VM, Fisher S, Fodor AA, Forney LJ, Foster L, Francesco VD, Friedman J, Friedrich DC, Fronick CC, Fulton LL, Gao H, Garcia N, Giannoukos G, Giblin C, Giovanni MY, Goldberg JM, Goll J, Gonzalez A, Griggs A, Gujja S, Haake SK, Haas BJ, Hamilton HA, Harris EL, Hepburn TA, Herter B, Hoffmann DE, Holder ME, Howarth C, Huang KH, Huse SM, Izard J, Jansson JK, Jiang H, Jordan C, Joshi V, Katancik JA, Keitel WA, Kelley ST, Kells C, King NB, Knights D, Kong HH, Koren O, Koren S, Kota KC, Kovar CL, Kyrpides NC, Rosa PSL, Lee SL, Lemon KP, Lennon N, Lewis CM, Lewis L, Ley RE, Li K, Liolios K, Liu B, Liu Y, Lo C-C, Lozupone CA, Lunsford RD, Madden T, Mahurkar AA, Mannon PJ, Mardis ER, Markowitz VM, Mavromatis K, McCorrison JM, McDonald D, McEwen J, McGuire AL, McInnes P, Mehta T, Mihindukulasuriya KA, Miller JR, Minx PJ, Newsham I, Nusbaum C, O'Laughlin M, Orvis J, Pagani I, Palaniappan K, Patel SM, Pearson M, Peterson J, Podar M, Pohl C, Pollard KS, Pop M, Priest ME, Proctor LM, Qin X, Raes J, Ravel J, Reid JG, Rho M, Rhodes R, Riehle KP, Rivera MC, Rodriguez-Mueller B, Rogers Y-H, Ross MC, Russ C, Sanka RK, Sankar P, Sathirapongsasuti JF, Schloss JA, Schloss PD, Schmidt TM, Scholz M, Schriml L, Schubert AM, Segata N, Segre JA, Shannon WD, Sharp RR, Sharpton TJ, Shenoy N, Sheth NU, Simone GA, Singh I, Smillie CS, Sobel JD, Sommer DD, Spicer P, Sutton GG, Sykes SM, Tabbaa DG, Thiagarajan M, Tomlinson CM, Torralba M, Treangen TJ, Truty RM, Vishnivetskaya TA, Walker J, Wang L, Wang Z, Ward DV, Warren W, Watson MA, Wellington C, Wetterstrand KA, White JR, Wilczek-Boney K, Wu Y, Wylie KM, Wylie T,

4353 Yandava C, Ye L, Ye Y, Yooseph S, Youmans BP, Zhang L, Zhou Y, Zhu Y, Zoloth L, Zucker
4354 JD, Birren BW, Gibbs RA, Highlander SK, Methé BA, Nelson KE, Petrosino JF, Weinstock
4355 GM, Wilson RK, White O. Structure, Function and Diversity of the Healthy Human
4356 Microbiome. *Nature* 486: 207–214, 2012. doi: 10.1038/nature11234.

4357 524. Ranđelović G, Mladenović V, Ristić L, Otašević S, Branković S, Mladenović-Antić S,
4358 Bogdanović M, Bogdanović D. Microbiological aspects of vulvovaginitis in prepubertal girls.
4359 *Eur J Pediatr* 171: 1203–1208, 2012. doi: 10.1007/s00431-012-1705-9.

4360 525. Huang B, Huang B, Fettweis JM, Fettweis JM, Brooks JP, Brooks JP, Jefferson KK,
4361 Jefferson KK, Buck GA, Buck GA. The changing landscape of the vaginal microbiome. *Clin*
4362 *Lab Med* 34: 747–761, 2014. doi: 10.1016/j.cll.2014.08.006.

4363 526. Cruickshank R. The conversion of the glycogen of the vagina into lactic acid. *J Pathol*
4364 *Bacteriol* : 213–219, 1934.

4365 527. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, Karlebach S, Gorle
4366 R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ. Vaginal
4367 microbiome of reproductive-age women. *Proc National Acad Sci* 108 Suppl 1: 4680–4687,
4368 2011. doi: 10.1073/pnas.1002611107.

4369 528. Hickey RJ, Zhou X, Settles ML, Erb J, Malone K, Hansmann MA, Shew ML, Pol BVD,
4370 Fortenberry JD, Forney LJ. Vaginal microbiota of adolescent girls prior to the onset of
4371 menarche resemble those of reproductive-age women. *Mbio* 6: 885, 2015. doi:
4372 10.1128/mbio.00097-15.

4373 529. Gajer P, Brotman RM, Bai G, Sakamoto J, Schütte UME, Zhong X, Koenig SSK, Fu L,
4374 Ma ZS, Zhou X, Abdo Z, Forney LJ, Ravel J. Temporal dynamics of the human vaginal
4375 microbiota. *Sci Transl Med* 4: 132ra52-132ra52, 2012. doi: 10.1126/scitranslmed.3003605.

4376 530. Kroon SJ, Ravel J, Huston WM. Cervicovaginal microbiota, women's health, and
4377 reproductive outcomes. *Fertil Steril* 110: 327–336, 2018. doi: 10.1016/j.fertnstert.2018.06.036.

4378 531. Brotman RM, Shardell MD, Gajer P, Fadrosh D, Chang K, Silver MI, Viscidi RP, Burke
4379 AE, Ravel J, Gravitt PE. Association between the vaginal microbiota, menopause status, and
4380 signs of vulvovaginal atrophy. *Menopause* 21: 450–458, 2014. doi:
4381 10.1097/gme.0b013e3182a4690b.

4382 532. Muhleisen AL, Herbst-Kralovetz MM. Menopause and the vaginal microbiome.
4383 *Maturitas* 91: 42–50, 2016. doi: 10.1016/j.maturitas.2016.05.015.

4384 533. Pabich WL, Fihn SD, Stamm WE, Scholes D, Boyko EJ, Gupta K. Prevalence and
4385 determinants of vaginal flora alterations in postmenopausal women. *J Infect Dis* 188: 1054–
4386 1058, 2003. doi: 10.1086/378203.

4387 534. Aagaard K, Riehle K, Ma J, Segata N, Mistretta T-A, Coarfa C, Raza S, Rosenbaum S,
4388 Veyver IV den, Milosavljevic A, Gevers D, Huttenhower C, Petrosino J, Versalovic J. A
4389 metagenomic approach to characterization of the vaginal microbiome signature in pregnancy.
4390 7: e36466, 2012. doi: 10.1371/journal.pone.0036466.

4391 535. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, Galuppi M, Lamont
4392 RF, Chaemsaithong P, Miranda J, Chaiworapongsa T, Ravel J. The composition and stability
4393 of the vaginal microbiota of normal pregnant women is different from that of non-pregnant
4394 women. *Microbiome* 2: 4, 2014. doi: 10.1186/2049-2618-2-4.

4395 536. MacIntyre DA, Chandiramani M, Lee YS, Kindinger L, Smith A, Angelopoulos N, Lehne
4396 B, Arulkumaran S, Brown R, Teoh TG, Holmes E, Nicoholson JK, Marchesi JR, Bennett PR.
4397 The vaginal microbiome during pregnancy and the postpartum period in a European
4398 population. *Sci Rep-uk* 5: 8988, 2015. doi: 10.1038/srep08988.

4399 537. Hyman RW, Fukushima M, Jiang H, Fung E, Rand L, Johnson B, Vo KC, Caughey AB,
4400 Hilton JF, Davis RW, Giudice LC. Diversity of the vaginal microbiome correlates with preterm
4401 birth. *Reproductive sciences (Thousand Oaks, Calif)* 21: 32–40, 2014. doi:
4402 10.1177/1933719113488838.

4403 538. Stout MJ, Conlon B, Landeau M, Lee I, Bower C, Zhao Q, Roehl KA, Nelson DM,
4404 Macones GA, Mysorekar IU. Identification of intracellular bacteria in the basal plate of the
4405 human placenta in term and preterm gestations. *Am J Obstet Gynecol* 208: 226.e1-226.e7,
4406 2013. doi: 10.1016/j.ajog.2013.01.018.

4407 539. Fettweis JM, Serrano MG, Brooks JP, Edwards DJ, Girerd PH, Parikh HI, Huang B, Arodz
4408 TJ, Edupuganti L, Glascock AL, Xu J, Jimenez NR, Vivadelli SC, Fong SS, Sheth NU, Jean S,
4409 Lee V, Bokhari YA, Lara AM, Mistry SD, Duckworth RA, Bradley SP, Koparde VN, Orenda
4410 XV, Milton SH, Rozycki SK, Matveyev AV, Wright ML, Huzurbazar SV, Jackson EM,
4411 Smirnova E, Korlach J, Tsai Y-C, Dickinson MR, Brooks JL, Drake JI, Chaffin DO, Sexton
4412 AL, Gravett MG, Rubens CE, Wijesooriya NR, Hendricks-Muñoz KD, Jefferson KK, Strauss
4413 JF, Buck GA. The vaginal microbiome and preterm birth. *Nature medicine* 25: 1012–1021,
4414 2019. doi: 10.1038/s41591-019-0450-2.

4415 540. Hočevar K, Maver A, Šimic MV, Hodžić A, Haslberger A, Seršen TP, Peterlin B. Vaginal
4416 Microbiome Signature Is Associated With Spontaneous Preterm Delivery. *Frontiers Medicine*
4417 6: 201, 2019. doi: 10.3389/fmed.2019.00201.

4418 541. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun
4419 CL, Goltsman DSA, Wong RJ, Shaw G, Stevenson DK, Holmes SP, Relman DA. Temporal
4420 and spatial variation of the human microbiota during pregnancy. *Proc National Acad Sci* 112:
4421 11060–11065, 2015. doi: 10.1073/pnas.1502875112.

4422 542. Freitas AC, Bocking A, Hill JE, Money DM, Money D, Bocking A, Hemmingsen S, Hill
4423 J, Reid G, Dumonceaux T, Gloor G, Links M, O'Doherty K, Tang P, Schalkwyk J van, Yudin
4424 M. Increased richness and diversity of the vaginal microbiota and spontaneous preterm birth.
4425 *Microbiome* 6: 117, 2018. doi: 10.1186/s40168-018-0502-8.

4426 543. Tabatabaei N, Eren A, Barreiro L, Yotova V, Dumaine A, Allard C, Fraser W. Vaginal
4427 microbiome in early pregnancy and subsequent risk of spontaneous preterm birth: a case–
4428 control study. *Bjog Int J Obstetrics Gynaecol* 126: 349–358, 2019. doi: 10.1111/1471-
4429 0528.15299.

4430 544. Callahan BJ, DiGiulio DB, Goltsman DSA, Sun CL, Costello EK, Jeganathan P, Biggio
4431 JR, Wong RJ, Druzin ML, Shaw GM, Stevenson DK, Holmes SP, Relman DA. Replication

and refinement of a vaginal microbial signature of preterm birth in two racially distinct cohorts of US women. *Proc National Acad Sci* 114: 9966–9971, 2017. doi: 10.1073/pnas.1705899114.

545. Al-Memar M, Bobdiwala S, Fourie H, Mannino R, Lee Y, Smith A, Marchesi J, Timmerman D, Bourne T, Bennett P, MacIntyre D. The association between vaginal bacterial composition and miscarriage: a nested case–control study. *Bjog Int J Obstetrics Gynaecol* 127: 264–274, 2020. doi: 10.1111/1471-0528.15972.

546. Al-Memar M, Vaulet T, Fourie H, Nikolic G, Bobdiwala S, Saso S, Farren J, Pipi M, Calster BV, Moor B, Stalder C, Bennett P, Timmerman D, Bourne T. Early-pregnancy events and subsequent antenatal, delivery and neonatal outcomes: prospective cohort study. *Ultrasound Obst Gyn* 54: 530–537, 2019. doi: 10.1002/uog.20262.

547. Bennett PR, Brown RG, MacIntyre DA. Vaginal Microbiome in Preterm Rupture of Membranes. *Obstet Gyn Clin N Am* 47: 503–521, 2020. doi: 10.1016/j.ogc.2020.08.001.

548. Chen C, Song X, Wei W, Zhong H, Dai J, Lan Z, Li F, Yu X, Feng Q, Wang Z, Xie H, Chen X, Zeng C, Wen B, Zeng L, Du H, Tang H, Xu C, Xia Y, Xia H, Yang H, Wang J, Wang J, Madsen L, Brix S, Kristiansen K, Xu X, Li J, Wu R, Jia H. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nature Communications* 8: 875, 2017. doi: 10.1038/s41467-017-00901-0.

549. Sola-Leyva A, Andrés-León E, Molina NM, Terron-Camero LC, Plaza-Díaz J, Sáez-Lara MJ, Gonzalvo MC, Sánchez R, Ruíz S, Martínez L, Altmäe S. Mapping the entire functionally active endometrial microbiota. *Hum Reprod* 36: deaa372, 2021. doi: 10.1093/humrep/deaa372.

550. Winters AD, Romero R, Gervasi MT, Gomez-Lopez N, Tran MR, Garcia-Flores V, Pacora P, Jung E, Hassan SS, Hsu C-D, Theis KR. Does the endometrial cavity have a molecular microbial signature? *Sci Rep-uk* 9: 9905, 2019. doi: 10.1038/s41598-019-46173-0.

551. Moreno I, Garcia-Grau I, Perez-Villaroya D, Gonzalez-Monfort M, Bahçeci M, Barrionuevo MJ, Taguchi S, Puente E, Dimattina M, Lim MW, Meneghini G, Aubuchon M, Leondires M, Izquierdo A, Perez-Olgiati M, Chavez A, Seethram K, Bau D, Gomez C, Valbuena D, Vilella F, Simon C. Endometrial microbiota composition is associated with reproductive outcome in infertile patients. *Microbiome* 10 (1): 1, 2022. doi: 10.1101/2021.02.05.21251207.

552. Sobel JD. Vaginitis and vaginal flora: controversies abound. *Curr Opin Infect Dis* 9: 42–47, 1996. doi: 10.1097/00001432-199602000-00011.

553. Mitchell CM, Haick A, Nkwopara E, Garcia R, Rendi M, Agnew K, Fredricks DN, Eschenbach D. Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. *Am J Obstet Gynecol* 212: 611.e1-611.e9, 2015. doi: 10.1016/j.ajog.2014.11.043.

554. Vandeputte D, Kathagen G, D'hoe K, Vieira-Silva S, Valles-Colomer M, Sabino J, Wang J, Tito RY, Commer LD, Darzi Y, Vermeire S, Falony G, Raes J. Quantitative microbiome profiling links gut community variation to microbial load. *Nature* 551: 507–511, 2017. doi: 10.1038/nature24460.

4471 555. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine Microbiota: Residents, Tourists, or
4472 Invaders? *Frontiers in immunology* 9: 208, 2018. doi: 10.3389/fimmu.2018.00208.

4473 556. Brunelli R, Papi M, Arcovito G, Bompiani A, Castagnola M, Parasassi T, Sampaolese B,
4474 Vincenzoni F, Spirito MD. Globular structure of human ovulatory cervical mucus. *Faseb J* 21:
4475 3872–3876, 2007. doi: 10.1096/fj.07-8189com.

4476 557. Cowling P, McCoy DR, Marshall RJ, Padfield CJH, Reeves DS. Bacterial colonization of
4477 the non-pregnant uterus: A study of pre-menopausal abdominal hysterectomy specimens.
4478 *European J Clin Microbiol Infect Dis* 11: 204–205, 1992. doi: 10.1007/bf01967084.

4479 558. Møller BR, Kristiansen FV, Thorsen P, Frost L, Mogensen SC. Sterility of the uterine
4480 cavity. *Acta Obstet Gyn Scan* 74: 216–219, 1995. doi: 10.3109/00016349509008942.

4481 559. Swidsinski A, Verstraelen H, Loening-Baucke V, Swidsinski S, Mendling W, Halwani Z.
4482 Presence of a Polymicrobial Endometrial Biofilm in Patients with Bacterial Vaginosis. 8:
4483 e53997, 2013. doi: 10.1371/journal.pone.0053997.t003.

4484 560. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J,
4485 Alonso R, Alamá P, Remohí J, Pellicer A, Ramón D, Simón C. Evidence that the endometrial
4486 microbiota has an effect on implantation success or failure. *The American Journal of Obstetrics*
4487 *& Gynecology* 215: 684–703, 2016. doi: 10.1016/j.ajog.2016.09.075.

4488 561. Kyono K, Hashimoto T, Nagai Y, Sakuraba Y. Analysis of endometrial microbiota by
4489 16S ribosomal RNA gene sequencing among infertile patients: a single-center pilot study.
4490 *Reproductive medicine and biology* 17: 297–306, 2018. doi: 10.1002/rmb2.12105.

4491 562. Hashimoto T, Kyono K. Does dysbiotic endometrium affect blastocyst implantation in
4492 IVF patients? *J Assist Reprod Gen* 36: 2471–2479, 2019. doi: 10.1007/s10815-019-01630-7.

4493 563. Koedooder R, Mackens S, Budding A, Fares D, Blockeel C, Laven J, Schoenmakers S.
4494 Identification and evaluation of the microbiome in the female and male reproductive tracts.
4495 *Human reproduction update* 25: 298–325, 2019. doi: 10.1093/humupd/dmy048.

4496 564. Leoni C, Ceci O, Manzari C, Fosso B, Volpicella M, Ferrari A, Fiorella P, Pesole G,
4497 Cicinelli E, Ceci LR. Human Endometrial Microbiota at Term of Normal Pregnancies. *Genes-*
4498 *basel* 10: 971, 2019. doi: 10.3390/genes10120971.

4499 565. Younge N, McCann JR, Ballard J, Plunkett C, Akhtar S, Araújo-Pérez F, Murtha A,
4500 Brandon D, Seed PC. Fetal exposure to the maternal microbiota in humans and mice. *Jci Insight*
4501 4, 2019. doi: 10.1172/jci.insight.127806.

4502 566. Laurence M, Hatzis C, Brash DE. Common Contaminants in Next-Generation Sequencing
4503 That Hinder Discovery of Low-Abundance Microbes. *Plos One* 9: e97876, 2014. doi:
4504 10.1371/journal.pone.0097876.

4505 567. Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J,
4506 Loman NJ, Walker AW. Reagent and laboratory contamination can critically impact sequence-
4507 based microbiome analyses. *Bmc Biol* 12: 87, 2014. doi: 10.1186/s12915-014-0087-z.

568. Glassing A, Dowd SE, Galandiuk S, Davis B, Chiodini RJ. Inherent bacterial DNA contamination of extraction and sequencing reagents may affect interpretation of microbiota in low bacterial biomass samples. *Gut Pathog* 8: 24, 2016. doi: 10.1186/s13099-016-0103-7.

569. Lauder AP, Roche AM, Sherrill-Mix S, Bailey A, Laughlin AL, Bittinger K, Leite R, Elovitz MA, Parry S, Bushman FD. Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. *Microbiome* 4: 29, 2016. doi: 10.1186/s40168-016-0172-3.

570. Eisenhofer R, Minich JJ, Marotz C, Cooper A, Knight R, Weyrich LS. Contamination in Low Microbial Biomass Microbiome Studies: Issues and Recommendations. *Trends Microbiol* 27: 105–117, 2018. doi: 10.1016/j.tim.2018.11.003.

571. Stinson LF, Keelan JA, Payne MS. Identification and removal of contaminating microbial DNA from PCR reagents: impact on low-biomass microbiome analyses. *Lett Appl Microbiol* 68: 2–8, 2019. doi: 10.1111/lam.13091.

572. Weyrich LS, Farrer AG, Eisenhofer R, Arriola LA, Young J, Selway CA, Handsley-Davis M, Adler CJ, Breen J, Cooper A. Laboratory contamination over time during low-biomass sample analysis. *Mol Ecol Resour* 19: 982–996, 2019. doi: 10.1111/1755-0998.13011.

573. Garcia-Grau I, Simon C, Moreno I. Uterine microbiome—low biomass and high expectations†. *Biol Reprod* 101: 1102–1114, 2018. doi: 10.1093/biolre/ioy257.

574. Molina NM, Sola-Leyva A, Haahr T, Aghajanova L, Laudanski P, Castilla JA, Altmäe S. Analysing endometrial microbiome: methodological considerations and recommendations for good practice. *Hum Reprod* 36: 859–879, 2021. doi: 10.1093/humrep/deab009.

575. Amos GCA, Logan A, Anwar S, Fritzsche M, Mate R, Bleazard T, Rijpkema S. Developing standards for the microbiome field. *Microbiome* 8: 98, 2020. doi: 10.1186/s40168-020-00856-3.

576. Garcia-Grau I, Perez-Villaroya D, Bau D, Gonzalez-Monfort M, Vilella F, Moreno I, Simon C. Taxonomical and Functional Assessment of the Endometrial Microbiota in A Context of Recurrent Reproductive Failure: A Case Report. *Pathogens* 8: 205, 2019. doi: 10.3390/pathogens8040205.

577. Moreno I, Garcia-Grau I, Bau D, Perez-Villaroya D, Gonzalez-Monfort M, Vilella F, Romero R, Simón C. The first glimpse of the endometrial microbiota in early pregnancy. *Am J Obstet Gynecol* 222: 296–305, 2020. doi: 10.1016/j.ajog.2020.01.031.

578. Benner M, Ferwerda G, Joosten I, Molen RG van der. How uterine microbiota might be responsible for a receptive, fertile endometrium. *Hum Reprod Update* 24: 393–415, 2018. doi: 10.1093/humupd/dmy012.

579. Molina NM, Sola-Leyva A, Saez-Lara MJ, Plaza-Diaz J, Tubić-Pavlović A, Romero B, Clavero A, Mozas-Moreno J, Fontes J, Altmäe S. New Opportunities for Endometrial Health by Modifying Uterine Microbial Composition: Present or Future? *Biomol* 10: 593, 2020. doi: 10.3390/biom10040593.

4546 580. Agostinis C, Mangogna A, Bossi F, Ricci G, Kishore U, Bulla R. Uterine Immunity and
4547 Microbiota: A Shifting Paradigm. *Front Immunol* 10: 2387, 2019. doi:
4548 10.3389/fimmu.2019.02387.

4549 581. Crha K, Ventruba P, Žáková J, Ješeta M, Pilka R, Vodička J, Crha I. The role of uterine
4550 microbiome and epithelial-mesenchymal transition in endometrial function. *Medical J Cell*
4551 *Biology* 7: 146–151, 2019. doi: 10.2478/acb-2019-0020.

4552 582. Murphy CR, Rogers PAW, Hosie MJ, Leeton J, Beaton L. Tight Junctions of Human
4553 Uterine Epithelial Cells Change during the Menstrual Cycle: A Morphometric Study. *Cells*
4554 *Tissues Organs* 144: 36–38, 1992. doi: 10.1159/000147282.

4555 583. Buck VU, Windoffer R, Leube RE, Classen-Linke I. Redistribution of adhering junctions
4556 in human endometrial epithelial cells during the implantation window of the menstrual cycle.
4557 *Histochem Cell Biol* 137: 777–790, 2012. doi: 10.1007/s00418-012-0929-0.

4558 584. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 9:
4559 799–809, 2009. doi: 10.1038/nri2653.

4560 585. Cohen LJ, Esterhazy D, Kim S-H, Lemetre C, Aguilar RR, Gordon EA, Pickard AJ, Cross
4561 JR, Emiliano AB, Han SM, Chu J, Vila-Farres X, Kaplitt J, Rogoz A, Calle PY, Hunter C,
4562 Bitok JK, Brady SF. Commensal bacteria make GPCR ligands that mimic human signalling
4563 molecules. *Nature* 549: 48–53, 2017. doi: 10.1038/nature23874.

4564 586. Satake H, Matsubara S, Aoyama M, Kawada T, Sakai T. GPCR Heterodimerization in the
4565 Reproductive System: Functional Regulation and Implication for Biodiversity. *Frontiers in*
4566 *endocrinology* 4: 100, 2013. doi: 10.3389/fendo.2013.00100.

4567 587. Sriram K, Insel PA. GPCRs as targets for approved drugs: How many targets and how
4568 many drugs? *Mol Pharmacol* 93: mol.117.111062, 2018. doi: 10.1124/mol.117.111062.

4569 588. Cryan JF, Clarke G, Dinan TG, Schellekens H. A Microbial Drugstore for Motility. *Cell*
4570 *Host Microbe* 23: 691–692, 2018. doi: 10.1016/j.chom.2018.05.020.

4571 589. Codagnone MG, Spichak S, O'Mahony SM, O'Leary OF, Clarke G, Stanton C, Dinan
4572 TG, Cryan JF. Programming Bugs: Microbiota and the Developmental Origins of Brain Health
4573 and Disease. *Biol Psychiat* 85: 150–163, 2019. doi: 10.1016/j.biopsych.2018.06.014.

4574 590. Hyman RW, Herndon CN, Jiang H, Palm C, Fukushima M, Bernstein D, Vo KC, Zelenko
4575 Z, Davis RW, Giudice LC. The dynamics of the vaginal microbiome during infertility therapy
4576 with in vitro fertilization-embryo transfer. *Journal of Assisted Reproduction and Genetics* 29:
4577 105–115, 2012. doi: 10.1007/s10815-011-9694-6.

4578 591. Kasprzykowska U, Elias J, Elias M, Mączyńska B, Sobieszczańska BM. Colonization of
4579 the lower urogenital tract with Ureaplasma parvum can cause asymptomatic infection of the
4580 upper reproductive system in women: a preliminary study. *Archives of gynecology and*
4581 *obstetrics* 289: 1129–1134, 2014. doi: 10.1007/s00404-013-3102-7.

592. Haahr T. Abnormal vaginal microbiota may be associated with poor reproductive outcomes: A prospective study in IVF patients. *J Reprod Immunol* 115: 47–48, 2016. doi: 10.1016/j.jri.2016.04.144.

593. Babu G, Singaravelu BG, Srikumar R, Reddy SV, Kokan A. Comparative Study on the Vaginal Flora and Incidence of Asymptomatic Vaginosis among Healthy Women and in Women with Infertility Problems of Reproductive Age. *J Clin Diagnostic Res* 11: DC18–DC22, 2017. doi: 10.7860/jcdr/2017/28296.10417.

594. Campisciano G, Florian F, D'Eustacchio A, Stanković D, Ricci G, Seta FD, Comar M. Subclinical alteration of the cervical-vaginal microbiome in women with idiopathic infertility. *Journal of Cellular Physiology* 232: 1681–1688, 2017. doi: 10.1002/jcp.25806.

595. Wee BA, Thomas M, Sweeney EL, Frentiu FD, Samios M, Ravel J, Gajer P, Myers G, Timms P, Allan JA, Huston WM. A retrospective pilot study to determine whether the reproductive tract microbiota differs between women with a history of infertility and fertile women. *Australian New Zealand J Obstetrics Gynaecol* 58: 341–348, 2018. doi: 10.1111/ajo.12754.

596. Graspeuntner S, Bohlmann MK, Gillmann K, Speer R, Kuenzel S, Mark H, Hoellen F, Lettau R, Griesinger G, König IR, Baines JF, Rupp J. Microbiota-based analysis reveals specific bacterial traits and a novel strategy for the diagnosis of infectious infertility. *Plos One* 13: e0191047, 2018. doi: 10.1371/journal.pone.0191047.

597. Koedooder R, Singer M, Schoenmakers S, Savelkoul PHM, Morré SA, Jonge JD de, Poort L, Cuypers WJSS, Beckers NGM, Broekmans FJM, Cohlen BJ, Hartog JE den, Fleischer K, Lambalk CB, Smeenk JMJS, Budding AE, Laven JSE. The vaginal microbiome as a predictor for outcome of in vitro fertilization with or without intracytoplasmic sperm injection: a prospective study. *Hum Reprod* 34: 1042–1054, 2019. doi: 10.1093/humrep/dez065.

598. Fu M, Zhang X, Liang Y, Lin S, Qian W, Fan S. Alterations in Vaginal Microbiota and Associated Metabolome in Women with Recurrent Implantation Failure. *Mbio* 11: e03242-19, 2020. doi: 10.1128/mbio.03242-19.

599. Kong Y, Liu Z, Shang Q, Gao Y, Li X, Zheng C, Deng X, Chen T. The Disordered Vaginal Microbiota Is a Potential Indicator for a Higher Failure of in vitro Fertilization. *Frontiers Medicine* 7: 217, 2020. doi: 10.3389/fmed.2020.00217.

600. Mangot-Bertrand J, Fenollar F, Bretelle F, Gamerre M, Raoult D, Courbiere B. Molecular diagnosis of bacterial vaginosis: impact on IVF outcome. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 32: 535–541, 2013. doi: 10.1007/s10096-012-1770-z.

601. Vergaro P, Tiscornia G, Barragán M, García D, Rodriguez A, Santaló J, Vassena R. Vaginal microbiota profile at the time of embryo transfer does not affect live birth rate in IVF cycles with donated oocytes. *Reprod Biomed Online* 38: 883–891, 2019. doi: 10.1016/j.rbmo.2018.12.019.

602. Moore DE, Soules MR, Klein NA, Fujimoto VY, Agnew KJ, Eschenbach DA. Bacteria in the transfer catheter tip influence the live-birth rate after in vitro fertilization. *Fertility and sterility* 74: 1118–1124, 2000. doi: 10.1016/s0015-0282(00)01624-1.

603. Salim R, Ben-Shlomo I, Colodner R, Keness Y, Shalev E. Bacterial colonization of the uterine cervix and success rate in assisted reproduction: results of a prospective survey. *Hum Reprod* 17: 337–340, 2002. doi: 10.1093/humrep/17.2.337.

604. Verstraelen H, Senok AC. Vaginal lactobacilli, probiotics, and IVF. *Reprod Biomed Online* 11: 674–675, 2005. doi: 10.1016/s1472-6483(10)61683-5.

605. Egbase PE, al-Sharhan M, al-Othman S, al-Mutawa M, Udo EE, Grudzinskas JG. Incidence of microbial growth from the tip of the embryo transfer catheter after embryo transfer in relation to clinical pregnancy rate following in-vitro fertilization and embryo transfer. *Human reproduction (Oxford, England)* 11: 1687–1689, 1996.

606. Fanchin R, Harmas A, Benaoudia F, Lundkvist U, Olivennes F, Frydman R. Microbial flora of the cervix assessed at the time of embryo transfer adversely affects in vitro fertilization outcome. *Fertil Steril* 70: 866–870, 1998. doi: 10.1016/s0015-0282(98)00277-5.

607. Egbase PE, Udo EE, al-Sharhan M, Grudzinskas JG. Prophylactic antibiotics and endocervical microbial inoculation of the endometrium at embryo transfer. *Lancet* 354: 651–652, 1999.

608. Selman H, Mariani M, Barnocchi N, Mencacci A, Bistoni F, Arena S, Pizzasegale S, Brusco GF, Angelini A. Examination of bacterial contamination at the time of embryo transfer, and its impact on the IVF/pregnancy outcome. *Journal of Assisted Reproduction and Genetics* 24: 395–399, 2007. doi: 10.1007/s10815-007-9146-5.

609. Franasiak JM, Werner MD, Juneau CR, Tao X, Landis J, Zhan Y, Treff NR, Scott RT. Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit. *J Assist Reprod Gen* 33: 129–136, 2016. doi: 10.1007/s10815-015-0614-z.

610. Verstraelen H, Vilchez-Vargas R, Desimpel F, Jauregui R, Vankeirsbilck N, Weyers S, Verhelst R, Sutter PD, Pieper DH, Wiele TVD. Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. *Peerj* 4: e1602, 2016. doi: 10.7717/peerj.1602.

611. Greenwood SM, Moran JJ. Chronic endometritis: morphologic and clinical observations. *Obstet Gynecol* 58: 176–84, 1981.

612. Romero R, Mazor M. Infection and Preterm Labor. *Clin Obstet Gynecol* 31: 553, 1988. doi: 10.1097/00003081-198809000-00006.

613. Taylor S, Frydman R. [Hysteroscopy and sperm infection]. *Contraception, fertilite, sexualite (1992)* 24: 549–551, 1996.

614. Gravett MG, Hitti J, Hess DL, ESCHENBACH DA. Intrauterine infection and preterm delivery: Evidence for activation of the fetal hypothalamic-pituitary-adrenal axis. *American journal of obstetrics and gynecology* 182: 1404–1413, 2000. doi: 10.1067/mob.2000.106180.

615. Espinoza J, Erez O, Romero R. Preconceptional antibiotic treatment to prevent preterm birth in women with a previous preterm delivery. *American journal of obstetrics and gynecology* 194: 630–637, 2006. doi: 10.1016/j.ajog.2005.11.050.

616. Féghali J, Bakar J, Mayenga JM, Ségard L, Hamou J, Driguez P, Belaisch-Allart J. [Systematic hysteroscopy prior to in vitro fertilization]. *Gynécologie, obstétrique & fertilité* 31: 127–131, 2003. doi: 10.1016/s1297-9589(03)00007-9.

617. Knudtson EJ, Shellhaas C, Stephens JA, Senokozlieff M, Ye H, Iams JD. The association of chronic endometritis with preterm birth. *American journal of obstetrics and gynecology* 196: 337.e1-337.e4, 2007. doi: 10.1016/j.ajog.2006.11.004.

618. Kitaya K, Matsubayashi H, Yamaguchi K, Nishiyama R, Takaya Y, Ishikawa T, Yasuo T, Yamada H. Chronic Endometritis: Potential Cause of Infertility and Obstetric and Neonatal Complications. *American journal of reproductive immunology (New York, NY : 1989)* 75: 13–22, 2015. doi: 10.1371/journal.pone.0133738.

619. Cicinelli E, Matteo M, Tinelli R, Pinto V, Marinaccio M, Indraccolo U, Ziegler DD, Resta L. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reproductive sciences (Thousand Oaks, Calif)* 21: 640–647, 2014. doi: 10.1177/1933719113508817.

620. 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. *Human Reproduction* 30: 323–330, 2015. doi: 10.1093/humrep/deu292.

621. Cicinelli E, Ziegler DD, Nicoletti R, Colafiglio G, Saliani N, Resta L, Rizzi D, Vito DD. Chronic endometritis: correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. *Fertility and sterility* 89: 677–684, 2008. doi: 10.1016/j.fertnstert.2007.03.074.

622. Cicinelli E, Ziegler DD, Nicoletti R, Tinelli R, Saliani N, Resta L, Bellavia M, Vito DD. Poor reliability of vaginal and endocervical cultures for evaluating microbiology of endometrial cavity in women with chronic endometritis. *Gynecologic and Obstetric Investigation* 68: 108–115, 2009. doi: 10.1159/000223819.

623. Vitagliano A, Saccardi C, Litta PS, Noventa M. Chronic endometritis: Really so relevant in repeated IVF failure? *Am J Reprod Immunol* 78: e12758, 2017. doi: 10.1111/aji.12758.

624. Cicinelli E, Matteo M, Trojano G, Mitola PC, Tinelli R, Vitagliano A, Crupano FM, Lepera A, Miragliotta G, Resta L. Chronic endometritis in patients with unexplained infertility: Prevalence and effects of antibiotic treatment on spontaneous conception. *Am J Reprod Immunol* 79: e12782, 2018. doi: 10.1111/aji.12782.

625. McQueen DB, Bernardi LA, Stephenson MD. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. *Fertil Steril* 101: 1026–1030, 2014. doi: 10.1016/j.fertnstert.2013.12.031.

626. Vitagliano A, Saccardi C, Noventa M, Sardo ADS, Saccone G, Cicinelli E, Pizzi S, Andrisani A, Litta PS. Effects of chronic endometritis therapy on in vitro fertilization outcome in women with repeated implantation failure: a systematic review and meta-analysis. *Fertil Steril* 110: 103-112.e1, 2018. doi: 10.1016/j.fertnstert.2018.03.017.

627. Buzzaccarini G, Vitagliano A, Andrisani A, Santarsiero CM, Cicinelli R, Nardelli C, Ambrosini G, Cicinelli E. Chronic endometritis and altered embryo implantation: a unified pathophysiological theory from a literature systematic review. *J Assist Reprod Gen* 37: 2897–2911, 2020. doi: 10.1007/s10815-020-01955-8.

628. Tortorella C, Piazzolla G, Matteo M, Pinto V, Tinelli R, Sabbà C, Fanelli M, Cicinelli E. Interleukin-6, interleukin-1β, and tumor necrosis factor α in menstrual effluents as biomarkers of chronic endometritis. *Fertility and sterility* 101: 242–247, 2014. doi: 10.1016/j.fertnstert.2013.09.041.

629. Kitaya K, Tada Y, Hayashi T, Taguchi S, Funabiki M, Nakamura Y. Comprehensive Endometrial Immunoglobulin Subclass Analysis in Infertile Women Suffering from Repeated Implantation Failure with or without Chronic Endometritis. *Am J Reprod Immunol* 72: 386–391, 2014. doi: 10.1111/aji.12277.

630. Li Y, Yu S, Huang C, Lian R, Chen C, Liu S, Li L, Diao L, Markert UR, Zeng Y. Evaluation of peripheral and uterine immune status of chronic endometritis in patients with recurrent reproductive failure. *Fertil Steril* 113: 187-196.e1, 2020. doi: 10.1016/j.fertnstert.2019.09.001.

631. Xu Y, Mei J, Diao L, Li Y, Ding L. Chronic endometritis and reproductive failure: Role of syndecan-1. *Am J Reprod Immunol* 84: e13255, 2020. doi: 10.1111/aji.13255.

632. Pinto V, Matteo M, Tinelli R, Mitola PC, Ziegler DD, Cicinelli E. Altered uterine contractility in women with chronic endometritis. *Fertility and sterility* 103: 1049–1052, 2015. doi: 10.1016/j.fertnstert.2015.01.007.

633. Drizi A, Djokovic D, Laganà AS, Herendael B van. Impaired inflammatory state of the endometrium: a multifaceted approach to endometrial inflammation. Current insights and future directions. *Menopausal Rev* 19: 90–100, 2020. doi: 10.5114/pm.2020.97863.

634. Plous RH, Sunshine R, Goldman H, Schwartz IS. Ureteral endometriosis in post menopausal women. *Urology* 26: 408–411, 1985. doi: 10.1016/0090-4295(85)90196-7.

635. Khan KN, Kitajima M, Hiraki K, Yamaguchi N, Yamaguchi N, Katamine S, Katamine S, Matsuyama T, Matsuyama T, Nakashima M, Fujishita A, Ishimaru T, Ishimaru T, Masuzaki H. Escherichia coli contamination of menstrual blood and effect of bacterial endotoxin on endometriosis. *Fertility and sterility* 94: 2860-3.e1-3, 2010. doi: 10.1016/j.fertnstert.2010.04.053.

636. Khan KN, Fujishita A, Masumoto H, Muto H, Kitajima M, Masuzaki H, Kitawaki J. Molecular detection of intrauterine microbial colonization in women with endometriosis. *European journal of obstetrics, gynecology, and reproductive biology* 199: 69–75, 2016. doi: 10.1016/j.ejogrb.2016.01.040.

637. Khan KN, Fujishita A, Kitajima M, Hiraki K, Nakashima M, Masuzaki H. Intra-uterine microbial colonization and occurrence of endometritis in women with endometriosis. *Hum Reprod* 29: 2446–2456, 2014. doi: 10.1093/humrep/deu222.

638. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, Takahashi K, Suginami H, Murakami T. The association between endometriosis and chronic endometritis. 9: e88354, 2014. doi: 10.1371/journal.pone.0088354.

639. Khan KN, Fujishita A, Kitajima M, Hiraki K, Nakashima M, Masuzaki H. Intra-uterine microbial colonization and occurrence of endometritis in women with endometriosis†. *Human Reproduction* 29: 2446–2456, 2014. doi: 10.1093/humrep/deu222.

640. Tai F-W, Chang CY-Y, Chiang J-H, Lin W-C, Wan L. Association of Pelvic Inflammatory Disease with Risk of Endometriosis: A Nationwide Cohort Study Involving 141,460 Individuals. *Journal of clinical medicine* 7: 379, 2018. doi: 10.3390/jcm7110379.

641. Kobayashi H, Higashiura Y, Shigetomi H, Kajihara H. Pathogenesis of endometriosis: The role of initial infection and subsequent sterile inflammation (Review). *Mol Med Rep* 9: 9–15, 2014. doi: 10.3892/mmr.2013.1755.

642. Karmarkar D, Rock KL. Microbiota signalling through MyD88 is necessary for a systemic neutrophilic inflammatory response. *Immunology* 140: 483–492, 2013. doi: 10.1111/imm.12159.

643. Lazar V, Ditu L-M, Pircalabioru GG, Gheorghe I, Curutiu C, Holban AM, Picu A, Petcu L, Chifiriuc MC. Aspects of Gut Microbiota and Immune System Interactions in Infectious Diseases, Immunopathology, and Cancer. *Front Immunol* 9: 1830, 2018. doi: 10.3389/fimmu.2018.01830.

644. Salliss ME, Farland LV, Mahnert ND, Herbst-Kralovetz MM. The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain. *Hum Reprod Update* 28: 92–131, 2021. doi: 10.1093/humupd/dmab035.

645. Yuan M, Li D, Zhang Z, Sun H, An M, Wang G. Endometriosis induces gut microbiota alterations in mice. *Hum Reproduction Oxf Engl* 33: 607–616, 2018. doi: 10.1093/humrep/dex372.

646. Chadchan SB, Cheng M, Parnell LA, Yin Y, Schriefer A, Mysorekar IU, Kommagani R. Antibiotic therapy with metronidazole reduces endometriosis disease progression in mice: a potential role for gut microbiota. *Hum Reproduction Oxf Engl* 34: 1106–1116, 2019. doi: 10.1093/humrep/dez041.

647. Bailey MT, Coe CL. Endometriosis is associated with an altered profile of intestinal microflora in female rhesus monkeys. *Hum Reprod* 17: 1704–1708, 2002. doi: 10.1093/humrep/17.7.1704.

648. Laschke MW, Menger MD. The gut microbiota: a puppet master in the pathogenesis of endometriosis? *Am J Obstet Gynecol* 215: 68.e1-68.e4, 2016. doi: 10.1016/j.ajog.2016.02.036.

649. Chiaffarino F, Cipriani S, Ricci E, Roncella E, Mauri PA, Parazzini F, Vercellini P. Endometriosis and inflammatory bowel disease: A systematic review of the literature. *European J Obstetrics Gynecol Reproductive Biology* 252: 246–251, 2020. doi: 10.1016/j.ejogrb.2020.06.051.

650. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The Placenta Harbors a Unique Microbiome. *Sci Transl Med* 6: 237ra65-237ra65, 2014. doi: 10.1126/scitranslmed.3008599.

651. Seferovic MD, Pace RM, Carroll M, Belfort B, Major AM, Chu DM, Racusin DA, Castro ECC, Muldrew KL, Versalovic J, Aagaard KM. Visualization of microbes by 16S in situ hybridization in term and preterm placentas without intraamniotic infection. *Am J Obstet Gynecol* 221: 146.e1-146.e23, 2019. doi: 10.1016/j.ajog.2019.04.036.

652. Goffau MC de, Lager S, Salter SJ, Wagner J, Kronbichler A, Charnock-Jones DS, Peacock SJ, Smith GCS, Parkhill J. Recognizing the reagent microbiome. *Nat Microbiol* 3: 851–853, 2018. doi: 10.1038/s41564-018-0202-y.

653. Barak S, Oettinger-Barak O, Machtei EE, Sprecher H, Ohel G. Evidence of periopathogenic microorganisms in placentas of women with preeclampsia. *Journal of periodontology* 78: 670–676, 2007. doi: 10.1902/jop.2007.060362.

654. DiGiulio DB, Gervasi M, Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Seok KS, Gómez R, Mittal P, Gotsch F, Chaiworapongsa T, Oyarzún E, Kim CJ, Relman DA. Microbial invasion of the amniotic cavity in preeclampsia as assessed by cultivation and sequence-based methods. *J Perinat Med* 38: 503–513, 2010. doi: 10.1515/jpm.2010.078.

655. Amarasekara R, Amarasekara R, Jayasekara RW, Jayasekara RW, Senanayake H, Senanayake H, Dissanayake VHW, Dissanayake VHW. Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia. *The journal of obstetrics and gynaecology research* 41: 662–669, 2015. doi: 10.1111/jog.12619.

656. Goffau MC de, Lager S, Sovio U, Gaccioli F, Cook E, Peacock SJ, Parkhill J, Charnock-Jones DS, Smith GCS. Human placenta has no microbiome but can harbour potential pathogens. *Nature* 572: 329–334, 2019. doi: 10.1038/s41586-019-1451-5.

657. Wu D, Kimura F, Zheng L, Ishida M, Niwa Y, Hirata K, Takebayashi A, Takashima A, Takahashi K, Kushima R, Zhang G, Murakami T. Chronic endometritis modifies decidualization in human endometrial stromal cells. *Reprod Biol Endocrin* 15: 16, 2017. doi: 10.1186/s12958-017-0233-x.

658. Bardos J, Fiorentino D, Longman RE, Paidas M. Immunological Role of the Maternal Uterine Microbiome in Pregnancy: Pregnancies Pathologies and Alterated Microbiota. *Front Immunol* 10: 2823, 2020. doi: 10.3389/fimmu.2019.02823.

659. Chen HJ, Gur TL. Intrauterine Microbiota: Missing, or the Missing Link? *Trends Neurosci* 42: 402–413, 2019. doi: 10.1016/j.tins.2019.03.008.

660. Chen X, Li P, Liu M, Zheng H, He Y, Chen M-X, Tang W, Yue X, Huang Y, Zhuang L, Wang Z, Zhong M, Ke G, Hu H, Feng Y, Chen Y, Yu Y, Zhou H, Huang L. Gut dysbiosis

induces the development of pre-eclampsia through bacterial translocation. *Gut* 69: 513, 2020. doi: 10.1136/gutjnl-2019-319101.

661. Ahmadian E, Saadat YR, Khatibi SMH, Nariman-Saleh-Fam Z, Bastami M, Vahed FZ, Ardalan M, Vahed SZ. Pre-Eclampsia: Microbiota possibly playing a role. *Pharmacol Res* 155: 104692, 2020. doi: 10.1016/j.phrs.2020.104692.

662. Kim CJ, Romero R, Chaemsaithong P, Kim J-S. Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. *Am J Obstet Gynecol* 213: S53–S69, 2015. doi: 10.1016/j.ajog.2015.08.041.

663. Hecht JL, Onderdonk A, Delaney M, Allred EN, Kliman HJ, Zambrano E, Pflueger SM, Livasy CA, Bhan I, Leviton A, investigators for the E study. Characterization of Chorioamnionitis in 2nd-Trimester C-Section Placentas and Correlation with Microorganism Recovery from Subamniotic Tissues. *Pediatr Devel Pathol* 11: 15–22, 2008. doi: 10.2350/07-06-0285.1.

664. DiGiulio DB, Gervasi MT, Romero R, Vaisbuch E, Mazaki-Tovi S, Kusanovic JP, Seok KS, Gómez R, Mittal P, Gotsch F, Chaiworapongsa T, Oyarzún E, Kim CJ, Relman DA. Microbial invasion of the amniotic cavity in pregnancies with small-for-gestational-age fetuses. *J Perinat Med* 38: 495–502, 2010. doi: 10.1515/jpm.2010.076.

665. DiGiulio DB, Romero R, Kusanovic JP, Gómez R, Gómez R, Kim CJ, Seok KS, Seok KS, Gotsch F, Gotsch F, Mazaki-Tovi S, Mazaki-Tovi S, Vaisbuch E, Vaisbuch E, Sanders K, Sanders K, Bik EM, Bik EM, Chaiworapongsa T, Oyarzún E, Oyarzún E, Relman DA. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. *American journal of reproductive immunology (New York, NY : 1989)* 64: 38–57, 2010. doi: 10.1111/j.1600-0897.2010.00830.x.

666. Solt I. The human microbiome and the great obstetrical syndromes: a new frontier in maternal-fetal medicine. *Best practice & research Clinical obstetrics & gynaecology* 29: 165–175, 2015. doi: 10.1016/j.bpobgyn.2014.04.024.

667. Prince AL, Ma J, Kannan PS, Alvarez M, Gisslen T, Harris RA, Sweeney EL, Knox CL, Lambers DS, Jobe AH, Chougnet CA, Kallapur SG, Aagaard KM. The placental membrane microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis. *Am J Obstet Gynecol* 214: 627.e1-627.e16, 2016. doi: 10.1016/j.ajog.2016.01.193.

668. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet Lond Engl* 371: 75–84, 2008. doi: 10.1016/s0140-6736(08)60074-4.

669. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Guzzetta KE, Jaggar M, Long-Smith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'Connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S, Teichman EM, Wouw M van de, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, Dinan TG. The Microbiota-Gut-Brain Axis. *Physiol Rev* 99: 1877–2013, 2019. doi: 10.1152/physrev.00018.2018.

4853 670. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen-gut microbiome axis:
4854 Physiological and clinical implications. *Maturitas* 103: 45–53, 2017. doi:
4855 10.1016/j.maturitas.2017.06.025.

4856 671. Willyard C. How gut microbes could drive brain disorders. *Nature* 590: 22–25, 2021. doi:
4857 10.1038/d41586-021-00260-3.

4858 672. Sampson TR, Challis C, Jain N, Moiseyenko A, Ladinsky MS, Shastri GG, Thron T,
4859 Needham BD, Horvath I, Debelius JW, Janssen S, Knight R, Wittung-Stafshede P, Gradinaru
4860 V, Chapman M, Mazmanian SK. A gut bacterial amyloid promotes α-synuclein aggregation
4861 and motor impairment in mice. *Elife* 9: e53111, 2020. doi: 10.7554/elife.53111.

4862 673. Kim S, Kwon S-H, Kam T-I, Panicker N, Karuppagounder SS, Lee S, Lee JH, Kim WR,
4863 Kook M, Foss CA, Shen C, Lee H, Kulkarni S, Pasricha PJ, Lee G, Pomper MG, Dawson VL,
4864 Dawson TM, Ko HS. Transneuronal Propagation of Pathologic α-Synuclein from the Gut to
4865 the Brain Models Parkinson's Disease. *Neuron* 103: 627-641.e7, 2019. doi:
4866 10.1016/j.neuron.2019.05.035.

4867 674. al-Haddad BJS, Jacobsson B, Chabra S, Modzelewska D, Olson EM, Bernier R,
4868 Enquobahrie DA, Hagberg H, Östling S, Rajagopal L, Waldorf KMA, Sengpiel V. Long-term
4869 Risk of Neuropsychiatric Disease After Exposure to Infection In Utero. *Jama Psychiat* 76:
4870 594–602, 2019. doi: 10.1001/jamapsychiatry.2019.0029.

4871 675. Antoun S, Ellul P, Peyre H, Rosenzwajg M, Gressens P, Klatzmann D, Delorme R. Fever
4872 during pregnancy as a risk factor for neurodevelopmental disorders: results from a systematic
4873 review and meta-analysis. *Mol Autism* 12: 60, 2021. doi: 10.1186/s13229-021-00464-4.

4874 676. Williams BB, Van Benschoten AH, Cimermancic P, Donia MS, Zimmermann M,
4875 Taketani M, Ishihara A, Kashyap PC, Fraser JS, Fischbach MA. Discovery and
4876 Characterization of Gut Microbiota Decarboxylases that Can Produce the Neurotransmitter
4877 Tryptamine. *Cell Host Microbe* 16: 495–503, 2014. doi: 10.1016/j.chom.2014.09.001.

4878 677. Doherty LF, Kwon HE, Taylor HS. Regulation of tryptophan 2,3-dioxygenase by
4879 HOXA10 enhances embryo viability through serotonin signaling. *Am J Physiol-endoc M* 300:
4880 E86–E93, 2011. doi: 10.1152/ajpendo.00439.2010.

4881 678. Côté F, Fligny C, Bayard E, Launay J-M, Gershon MD, Mallet J, Vodjdani G. Maternal
4882 serotonin is crucial for murine embryonic development. *Proc National Acad Sci* 104: 329–334,
4883 2007. doi: 10.1073/pnas.0606722104.

4884 679. Chuffa LG de A, Lupi LA, Cucielo MS, Silveira HS, Reiter RJ, Seiva FRF. Melatonin
4885 Promotes Uterine and Placental Health: Potential Molecular Mechanisms. *Int J Mol Sci* 21:
4886 300, 2019. doi: 10.3390/ijms21010300.

4887 680. Ross M, Pawlina W. 2006. Female reproductive system in Histology: A Text and Atlas
4888 (5th ed., pp.772-809) Philadelphia: Lippincott Williams & Wilkins. .

4889 681. Noe M, Kunz G, Herbertz M, Mall G, Leyendecker G. The cyclic pattern of the
4890 immunocytochemical expression of oestrogen and progesterone receptors in human

myometrial and endometrial layers: characterization of the endometrial–subendometrial unit. *Hum Reprod* 14: 190–197, 1999. doi: 10.1093/humrep/14.1.190.

682. Aguilar HN, Mitchell BF. Physiological pathways and molecular mechanisms regulating uterine contractility. *Hum Reprod Update* 16: 725–744, 2010. doi: 10.1093/humupd/dmq016.

683. Mas A, Cervello I, Gil-Sanchis C, Simón C. Current understanding of somatic stem cells in leiomyoma formation. *Fertil Steril* 102: 613–620, 2014. doi: 10.1016/j.fertnstert.2014.04.051.

684. Commandeur AE, Styer AK, Teixeira JM. Epidemiological and genetic clues for molecular mechanisms involved in uterine leiomyoma development and growth. *Hum Reprod Update* 21: 593–615, 2015. doi: 10.1093/humupd/dmv030.

685. Kuijsters NPM, Methorst WG, Kortenhorst MSQ, Rabotti C, Mischi M, Schoot BC. Uterine peristalsis and fertility: current knowledge and future perspectives: a review and meta-analysis. *Reprod Biomed Online* 35: 50–71, 2017. doi: 10.1016/j.rbmo.2017.03.019.

686. Wray S. Uterine contraction and physiological mechanisms of modulation. *Am J Physiol-cell Ph* 264: C1–C18, 1993. doi: 10.1152/ajpcell.1993.264.1.c1.

687. Matthew A, Shmygol A, Wray S. Ca2+ entry, efflux and release in smooth muscle. *Biol Res* 37: 617–24, 2004. doi: 10.4067/s0716-97602004000400017.

688. Hutchings G, Williams O, Cretoiu D, Ciontea SM. Myometrial interstitial cells and the coordination of myometrial contractility. *J Cell Mol Med* 13: 4268–4282, 2009. doi: 10.1111/j.1582-4934.2009.00894.x.

689. Seyfarth E-A. Julius Bernstein (1839–1917): pioneer neurobiologist and biophysicist. *Biol Cybern* 94: 2–8, 2006. doi: 10.1007/s00422-005-0031-y.

690. Gimpl G, Fahrenholz F. The Oxytocin Receptor System: Structure, Function, and Regulation. *Physiol Rev* 81: 629–683, 2001. doi: 10.1152/physrev.2001.81.2.629.

691. Buxton ILO. Regulation of Uterine Function: a Biochemical Conundrum in the Regulation of Smooth Muscle Relaxation. *Mol Pharmacol* 65: 1051–1059, 2004. doi: 10.1124/mol.65.5.1051.

692. Bernal AL. The regulation of uterine relaxation. *Semin Cell Dev Biol* 18: 340–347, 2007. doi: 10.1016/j.semcdb.2007.05.002.

693. Mesiano1 S. Myometrial Progesterone Responsiveness. *Semin Reprod Med* 25: 005–013, 2007. doi: 10.1055/s-2006-956771.

694. Bulletti C, Ziegler D de, Polli V, Diotallevi L, Ferro ED, Flamigni C. Uterine contractility during the menstrual cycle. *Hum Reprod* 15: 81–89, 2000. doi: 10.1093/humrep/15.suppl_1.81.

695. Kunz G, Noe M, Herbertz M, Leyendecker G. Uterine peristalsis during the follicular phase of the menstrual cycle: effects of oestrogen, antioestrogen and oxytocin. *Hum Reprod Update* 4: 647–654, 1998. doi: 10.1093/humupd/4.5.647.

4927 696. Vijayakumar R, Walters WAW. Myometrial prostaglandins during the human menstrual
4928 cycle. *Am J Obstet Gynecol* 141: 313–318, 1981. doi: 10.1016/s0002-9378(16)32639-4.

4929 697. Amico JA, Seif SM, Robinson AG. Oxytocin in Human Plasma: Correlation with
4930 Neurophysin and Stimulation with Estrogen*. *J Clin Endocrinol Metabolism* 52: 988–993,
4931 1981. doi: 10.1210/jcem-52-5-988.

4932 698. Fanchin R, Ayoubi J-M, Olivennes F, Righini C, Ziegler D de, Frydman R. Hormonal
4933 influence on the uterine contractility during ovarian stimulation. *Hum Reprod* 15: 90–100,
4934 2000. doi: 10.1093/humrep/15.suppl_1.90.

4935 699. Bulletti C, Ziegler D de. Uterine contractility and embryo implantation. *Curr Opin*
4936 *Obstetrics Gynecol* 17: 265–276, 2005. doi: 10.1097/01.gco.0000169104.85128.0e.

4937 700. Ruoslahti E. Stretching Is Good for a Cell. *Science* 276: 1345–1345, 1997. doi:
4938 10.1126/science.276.5317.1345.

4939 701. Hua R, Pease JE, Sooranna SR, Viney JM, Nelson SM, Myatt L, Bennett PR, Johnson
4940 MR. Stretch and Inflammatory Cytokines Drive Myometrial Chemokine Expression Via NF-
4941 κB Activation. *Endocrinology* 153: 481–491, 2012. doi: 10.1210/en.2011-1506.

4942 702. Ijland MM, Evers JLH, Dunselman GAJ, Katwijk C van, Lo CR, Hoogland HJ.
4943 Endometrial wavelike movements during the menstrual cycle. *Fertil Steril* 65: 746–749, 1996.
4944 doi: 10.1016/s0015-0282(16)58207-7.

4945 703. Kozikowska M, Grusza M, Mrugacz G, Gagan J, Zbucka-Krętowska M, Grygoruk C. The
4946 Influence Of Intrauterine Pressure On Embryo Retention In A Catheter After Embryo Transfer.
4947 *Sci Rep-uk* 9: 11969, 2019. doi: 10.1038/s41598-019-48077-5.

4948 704. IJland MM, Hoogland HJ, Dunselman GAJ, Lo CR, Evers JLH. Endometrial wave
4949 direction switch and the outcome of in vitro fertilization. *Fertil Steril* 71: 476–481, 1999. doi:
4950 10.1016/s0015-0282(98)00501-9.

4951 705. Cakmak H, Taylor HS. Implantation failure: molecular mechanisms and clinical
4952 treatment. *Hum Reprod Update* 17: 242–53, 2010. doi: 10.1093/humupd/dmq037.

4953 706. Fanchin R. Assessing Uterine Receptivity in 2001: Ultrasonographic Glances at the New
4954 Millennium. *Ann Ny Acad Sci* 943: 185–202, 2001. doi: 10.1111/j.1749-6632.2001.tb03802.x.

4955 707. Hunt S, Abdallah K, Ng E, Rombauts L, Vollenhoven B, Mol B. Impairment of Uterine
4956 Contractility Is Associated with Unexplained Infertility. *Semin Reprod Med* 38: 061–073,
4957 2020. doi: 10.1055/s-0040-1716409.

4958 708. Bruce A. Lessey, Steven L. Young, Chapter 9 - Structure, Function, and Evaluation of the
4959 Female Reproductive Tract, Yen and Jaffe's Reproductive Endocrinology (Eighth Edition),
4960 Elsevier, Pages 206-247.e13, ISBN 9780323479127. 2019.

4961 709. Kunz G, Beil D, Deininger H, Wildt L, Leyendecker G. The dynamics of rapid sperm
4962 transport through the female genital tract: evidence from vaginal sonography of uterine

peristalsis and hysterosalpingoscintigraphy. *Hum Reprod* 11: 627–632, 1996. doi: 10.1093/humrep/11.3.627.

710. Pusey J, Kelly WA, Bradshaw JMC, Porter DG. Myometrial Activity and the Distribution of Blastocysts in the Uterus of the Rat: Interference by Relaxin. *Biol Reprod* 23: 394–397, 1980. doi: 10.1095/biolreprod23.2.394.

711. Kunz G, Leyendecker G. Uterine peristaltic activity during the menstrual cycle: characterization, regulation, function and dysfunction. *Reprod Biomed Online* 4: 5–9, 2002. doi: 10.1016/s1472-6483(12)60108-4.

712. Zhu L, Li Y, Xu A. Influence of controlled ovarian hyperstimulation on uterine peristalsis in infertile women. *Hum Reprod* 27: 2684–2689, 2012. doi: 10.1093/humrep/des257.

713. IJland MM, Evers JLH, Dunselman GAJ, Hoogland HJ. Endometrial wavelike activity, endometrial thickness, and ultrasound texture in controlled ovarian hyperstimulation cycles. *Fertil Steril* 70: 279–283, 1998. doi: 10.1016/s0015-0282(98)00132-0.

714. Leyendecker G, Kunz G, Wildt L, Beil D, Deininger H. Uterine hyperperistalsis and dysperistalsis as dysfunctions of the mechanism of rapid sperm transport in patients with endometriosis and infertility. *Hum Reprod* 11: 1542–1551, 1996. doi: 10.1093/oxfordjournals.humrep.a019435.

715. Kido A, Togashi K, Nishino M, Miyake K, Koyama T, Fujimoto R, Iwasaku K, Fujii S, Hayakawa K. Cine MR imaging of uterine peristalsis in patients with endometriosis. *Eur Radiol* 17: 1813–1819, 2007. doi: 10.1007/s00330-006-0494-9.

716. Nishino M, Togashi K, Nakai A, Hayakawa K, Kanao S, Iwasaku K, Fujii S. Uterine contractions evaluated on cine MR imaging in patients with uterine leiomyomas. *Eur J Radiol* 53: 142–146, 2005. doi: 10.1016/j.ejrad.2004.01.009.

717. Kissler S, Hamscho N, Zangos S, Wiegratz I, Schlichter S, Menzel C, Doebert N, Gruenwald F, Vogl T, Gaetje R, Rody A, Siebzehnruebl E, Kunz G, Leyendecker G, Kaufmann M. Uterotubal transport disorder in adenomyosis and endometriosis—a cause for infertility. *Bjog Int J Obstetrics Gynaecol* 113: 902–908, 2006. doi: 10.1111/j.1471-0528.2006.00970.x.

718. Novellas S, Chassang M, Delotte J, Toullalan O, Chevallier A, Bouaziz J, Chevallier P. MRI Characteristics of the Uterine Junctional Zone: From Normal to the Diagnosis of Adenomyosis. *Am J Roentgenol* 196: 1206–1213, 2011. doi: 10.2214/ajr.10.4877.

719. Nakai A, Togashi K, Kosaka K, Kido A, Kataoka M, Koyama T, Fujii S. Do Anticholinergic Agents Suppress Uterine Peristalsis and Sporadic Myometrial Contractions at Cine MR Imaging? *Radiology* 246: 489–496, 2008. doi: 10.1148/radiol.2461062091.

720. Kido A, Togashi K, Hatayama H, Nakayama T, Yamamoto A, Kataoka M, Tulandi T. Uterine Peristalsis in Women With Repeated IVF Failures: Possible Therapeutic Effect of Hyoscine Bromide. *J Obstetrics Gynaecol Can* 31: 732–735, 2009. doi: 10.1016/s1701-2163(16)34278-5.

5000 721. Daido S, Nakai A, Kido A, Okada T, Kamae T, Fujimoto K, Ito I, Togashi K.
5001 Anticholinergic agents result in weaker and shorter suppression of uterine contractility
5002 compared with intestinal motion: time course observation with cine MRI. *J Magn Reson*
5003 *Imaging* 38: 1196–1202, 2013. doi: 10.1002/jmri.24072.

5004 722. Liang Z, Sooranna SR, Engineer N, Tattersall M, Khanjani S, Bennett PR, Myatt L,
5005 Johnson MR. Prostaglandin F2-alpha receptor regulation in human uterine myocytes. *Mhr*
5006 *Basic Sci Reproductive Medicine* 14: 215–223, 2008. doi: 10.1093/molehr/gan008.

5007 723. Pierzynski P. Oxytocin and vasopressin V1A receptors as new therapeutic targets in
5008 assisted reproduction. *Reprod Biomed Online* 22: 9–16, 2011. doi:
5009 10.1016/j.rbmo.2010.09.015.

5010 724. Lan VTN, Khang VN, Nhu GH, Tuong HM. Atosiban improves implantation and
5011 pregnancy rates in patients with repeated implantation failure. *Reprod Biomed Online* 25: 254–
5012 260, 2012. doi: 10.1016/j.rbmo.2012.05.014.

5013 725. Ng EHY, Li RHW, Chen L, Lan VTN, Tuong HM, Quan S. A randomized double blind
5014 comparison of atosiban in patients undergoing IVF treatment. *Hum Reprod* 29: 2687–2694,
5015 2014. doi: 10.1093/humrep/deu263.

5016 726. Huang Q-Y, Rong M-H, Lan A-H, Lin X-M, Lin X-G, He R-Q, Chen G, Li M-J. The
5017 impact of atosiban on pregnancy outcomes in women undergoing in vitro fertilization-embryo
5018 transfer: A meta-analysis. *Plos One* 12: e0175501, 2017. doi: 10.1371/journal.pone.0175501.

5019 727. Walker CL, Stewart EA. Uterine Fibroids: The Elephant in the Room. *Science* 308: 1589–
5020 1592, 2005. doi: 10.1126/science.1112063.

5021 728. Maruyama T, Ono M, Yoshimura Y. Somatic Stem Cells in the Myometrium and in
5022 Myomas. *Semin Reprod Med* 31: 077–081, 2013. doi: 10.1055/s-0032-1331801.

5023 729. Munro MG, Critchley HOD, Broder MS, Fraser IS, Disorders FWG on M. FIGO
5024 classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid
5025 women of reproductive age. *Int J Gynecol Amp Obstetrics* 113: 3–13, 2011. doi:
5026 10.1016/j.ijgo.2010.11.011.

5027 730. Whynott RM, Vaught KCC, Segars JH. The Effect of Uterine Fibroids on Infertility: A
5028 Systematic Review. *Semin Reprod Med* 35: 523–532, 2017. doi: 10.1055/s-0037-1607295.

5029 731. Somigliana E, Vercellini P, Daguati R, Pasin R, Giorgi OD, Crosignani PG. Fibroids and
5030 female reproduction: a critical analysis of the evidence. *Hum Reprod Update* 13: 465–476,
5031 2007. doi: 10.1093/humupd/dmm013.

5032 732. Oliveira FG, Abdelmassih VG, Diamond MP, Dozortsev D, Melo NR, Abdelmassih R.
5033 Impact of subserosal and intramural uterine fibroids that do not distort the endometrial cavity
5034 on the outcome of in vitro fertilization–intracytoplasmic sperm injection. *Fertil Steril* 81: 582–
5035 587, 2004. doi: 10.1016/j.fertnstert.2003.08.034.

5036 733. Pritts EA, Parker WH, Olive DL. Fibroids and infertility: an updated systematic review
5037 of the evidence. *Fertil Steril* 91: 1215–1223, 2009. doi: 10.1016/j.fertnstert.2008.01.051.

5038 734. Longo DL, Bulun SE. Uterine Fibroids. *New Engl J Medicine* 369: 1344–1355, 2013. doi:
5039 10.1056/nejmra1209993.

5040 735. Galliano D, Bellver J, Díaz-García C, Simón C, Pellicer A. ART and uterine pathology:
5041 how relevant is the maternal side for implantation? *Hum Reprod Update* 21: 13–38, 2015. doi:
5042 10.1093/humupd/dmu047.

5043 736. Horne A, Critchley H. The Effect of Uterine Fibroids on Embryo Implantation. *Semin*
5044 *Reprod Med* 25: 483–489, 2007. doi: 10.1055/s-2007-991046.

5045 737. Klatsky PC, Lane DE, Ryan IP, Fujimoto VY. The effect of fibroids without cavity
5046 involvement on ART outcomes independent of ovarian age. *Hum Reprod* 22: 521–526, 2007.
5047 doi: 10.1093/humrep/del370.

5048 738. Rashid NA, Lalitkumar S, Lalitkumar PG, Gemzell-Danielsson K. Endometrial
5049 Receptivity and Human Embryo Implantation. *Am J Reprod Immunol* 66: 23–30, 2011. doi:
5050 10.1111/j.1600-0897.2011.01048.x.

5051 739. Makker A, Goel MM. Uterine Leiomyomas: Effects on Architectural, Cellular, and
5052 Molecular Determinants of Endometrial Receptivity. *Reprod Sci* 20: 631–638, 2013. doi:
5053 10.1177/1933719112459221.

5054 740. Unlu C, Celik O, Celik N, Otlu B. Expression of Endometrial Receptivity Genes Increase
5055 After Myomectomy of Intramural Leiomyomas not Distorting the Endometrial Cavity. *Reprod*
5056 *Sci* 23: 31–41, 2016. doi: 10.1177/1933719115612929.

5057 741. Sagi-Dain L, Ojha K, Bider D, Levron J, Zinchenko V, Walster S, Sagi S, Dirnfeld M.
5058 Pregnancy outcomes in oocyte recipients with fibroids not impinging uterine cavity. *Arch*
5059 *Gynecol Obstet* 295: 497–502, 2017. doi: 10.1007/s00404-016-4273-9.

5060 742. Donnez J, Dolmans M-M. Uterine fibroid management: from the present to the future.
5061 *Hum Reprod Update* 22: 665–686, 2016. doi: 10.1093/humupd/dmw023.

5062 743. Ikhena DE, Bulun SE. The Endometrial Factor A Reproductive Precision Medicine
5063 Approach. CRC Press, 2017, p. 120-129. .

5064 744. Giuliani E, As-Sanie S, Marsh EE. Epidemiology and management of uterine fibroids. *Int*
5065 *J Gynecol Obstet* 149: 3–9, 2020. doi: 10.1002/ijgo.13102.

5066 745. Horcajadas JA, Goyri E, Higón MA, Martínez-Conejero JA, Gambadauro P, García G,
5067 Meseguer M, Simón C, Pellicer A. Endometrial Receptivity and Implantation Are Not Affected
5068 by the Presence of Uterine Intramural Leiomyomas: A Clinical and Functional Genomics
5069 Analysis. *J Clin Endocrinol Metabolism* 93: 3490–3498, 2008. doi: 10.1210/jc.2008-0565.

5070 746. Doherty LF, Taylor HS. Leiomyoma-derived transforming growth factor-β impairs bone
5071 morphogenetic protein-2-mediated endometrial receptivity. *Fertil Steril* 103: 845–852, 2015.
5072 doi: 10.1016/j.fertnstert.2014.12.099.

747. Du H, Taylor HS. The Role of Hox Genes in Female Reproductive Tract Development, Adult Function, and Fertility. *Csh Perspect Med* 6: a023002, 2015. doi: 10.1101/cshperspect.a023002.

748. Makker A, Goel MM, Nigam D, Bhatia V, Mahdi AA, Das V, Pandey A. Endometrial Expression of Homeobox Genes and Cell Adhesion Molecules in Infertile Women With Intramural Fibroids During Window of Implantation. *Reproductive Sci Thousand Oaks Calif* 24: 435–444, 2016. doi: 10.1177/1933719116657196.

749. Surrey ES, Lietz AK, Schoolcraft WB. Impact of intramural leiomyomata in patients with a normal endometrial cavity on in vitro fertilization–embryo transfer cycle outcome. *Fertil Steril* 75: 405–410, 2001. doi: 10.1016/s0015-0282(00)01714-3.

750. Ng EHY, Chan CCW, Tang OS, Yeung WSB, Ho PC. Endometrial and subendometrial blood flow measured by three-dimensional power Doppler ultrasound in patients with small intramural uterine fibroids during IVF treatment. *Hum Reprod* 20: 501–506, 2005. doi: 10.1093/humrep/deh594.

751. Moon J-W, Kim C-H, Kim J-B, Kim S-H, Chae H-D, Kang B-M. Alterations in uterine hemodynamics caused by uterine fibroids and their impact on in vitro fertilization outcomes. *Clin Exp Reproductive Medicine* 42: 163–8, 2015. doi: 10.5653/cerm.2015.42.4.163.

752. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JYH, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biology* 5: R80, 2004. doi: 10.1186/gb-2004-5-10-r80.

753. Guo S-W, Mao X, Ma Q, Liu X. Dysmenorrhea and its severity are associated with increased uterine contractility and overexpression of oxytocin receptor (OTR) in women with symptomatic adenomyosis. *Fertil Steril* 99: 231–240, 2013. doi: 10.1016/j.fertnstert.2012.08.038.

754. Zhai J, Vannuccini S, Petraglia F, Giudice LC. Adenomyosis: Mechanisms and Pathogenesis. *Semin Reprod Med* 38: 129–143, 2020. doi: 10.1055/s-0040-1716687.

755. Leyendecker G, Wildt L, Mall G. The pathophysiology of endometriosis and adenomyosis: tissue injury and repair. *Arch Gynecol Obstet* 280: 529–538, 2009. doi: 10.1007/s00404-009-1191-0.

756. Brosens I, Derwig I, Brosens J, Fusi L, Benagiano G, Pijnenborg R. The enigmatic uterine junctional zone: the missing link between reproductive disorders and major obstetrical disorders? *Hum Reprod* 25: 569–574, 2010. doi: 10.1093/humrep/dep474.

757. Leyendecker G, Bilgicyildirim A, Inacker M, Stalf T, Huppert P, Mall G, Böttcher B, Wildt L. Adenomyosis and endometriosis. Re-visiting their association and further insights into the mechanisms of auto-traumatisation. An MRI study. *Arch Gynecol Obstet* 291: 917–932, 2015. doi: 10.1007/s00404-014-3437-8.

5111 758. Kitawaki J. Adenomyosis: the pathophysiology of an oestrogen-dependent disease. *Best*
5112 *Pract Res Cl Ob* 20: 493–502, 2006. doi: 10.1016/j.bpobgyn.2006.01.010.

5113 759. Ota H, Igarashi S, Hatazawa J, Tanaka T. Is adenomyosis an immune disease? *Hum*
5114 *Reprod Update* 4: 360–367, 1998. doi: 10.1093/humupd/4.4.360.

5115 760. Bergeron C, Amant F, Ferenczy A. Pathology and physiopathology of adenomyosis. *Best*
5116 *Pract Res Cl Ob* 20: 511–521, 2006. doi: 10.1016/j.bpobgyn.2006.01.016.

5117 761. Nepomnyashchikh LM, Lushnikova EL, Molodykh OP, Pichigina AK.
5118 Immunocytochemical Analysis of Proliferative Activity of Endometrial and Myometrial Cell
5119 Populations in Focal and Stromal Adenomyosis. *B Exp Biol Med*+ 155: 512–517, 2013. doi:
5120 10.1007/s10517-013-2190-5.

5121 762. Bruner KL, Matrisian LM, Rodgers WH, Gorstein F, Osteen KG. Suppression of matrix
5122 metalloproteinases inhibits establishment of ectopic lesions by human endometrium in nude
5123 mice. *J Clin Invest* 99: 2851–2857, 1997. doi: 10.1172/jci119478.

5124 763. Ota H, Tanaka T. Integrin Adhesion Molecules in the Endometrial Glandular Epithelium
5125 in Patients with Endometriosis or Adenomyosis. *J Obstet Gynaecol Re* 23: 485–491, 1997. doi:
5126 10.1111/j.1447-0756.1997.tb00877.x.

5127 764. Xiao Y, Sun X, Yang X, Zhang J, Xue Q, Cai B, Zhou Y. Leukemia inhibitory factor is
5128 dysregulated in the endometrium and uterine flushing fluid of patients with adenomyosis
5129 during implantation window. *Fertil Steril* 94: 85–89, 2010. doi:
5130 10.1016/j.fertnstert.2009.03.012.

5131 765. Vercellini P, Consonni D, Dridi D, Bracco B, Frattaruolo MP, Somigliana E. Uterine
5132 adenomyosis and in vitro fertilization outcome: a systematic review and meta-analysis. *Hum*
5133 *Reprod* 29: 964–977, 2014. doi: 10.1093/humrep/deu041.

5134 766. Campo S, Campo V, Benagiano G. Adenomyosis and infertility. *Reprod Biomed Online*
5135 24: 35–46, 2012. doi: 10.1016/j.rbmo.2011.10.003.

5136 767. Juang C, Chou P, Yen M, Twu N, Horng H, Hsu W. Adenomyosis and risk of preterm
5137 delivery. *Bjog Int J Obstetrics Gynaecol* 114: 165–169, 2007. doi: 10.1111/j.1471-
5138 0528.2006.01186.x.

5139 768. Maheshwari A, Gurunath S, Fatima F, Bhattacharya S. Adenomyosis and subfertility: a
5140 systematic review of prevalence, diagnosis, treatment and fertility outcomes. *Hum Reprod*
5141 *Update* 18: 374–392, 2012. doi: 10.1093/humupd/dms006.

5142 769. Huang FJ, Kung FT, Chang SY, Hsu TY. Effects of short-course buserelin therapy on
5143 adenomyosis. A report of two cases. *J Reproductive Medicine* 44: 741–4, 1999.

5144 770. Grimbizis GF, Mikos T, Tarlatzis B. Uterus-sparing operative treatment for adenomyosis.
5145 *Fertil Steril* 101: 472-487.e8, 2014. doi: 10.1016/j.fertnstert.2013.10.025.

5146 771. Bruijn AM de, Smink M, Lohle PNM, Huirne JAF, Twisk JWR, Wong C, Schoonmade
5147 L, Hehenkamp WJK. Uterine Artery Embolization for the Treatment of Adenomyosis: A

Systematic Review and Meta-Analysis. *J Vasc Interv Radiol* 28: 1629-1642.e1, 2017. doi: 10.1016/j.jvir.2017.07.034.

772. Soave I, Wenger J-M, Pluchino N, Marci R. Treatment options and reproductive outcome for adenomyosis-associated infertility. *Curr Med Res Opin* 34: 1–29, 2017. doi: 10.1080/03007995.2017.1393404.

773. Wang C-J, Yuen L-T, Chang S-D, Lee C-L, Soong Y-K. Use of laparoscopic cytoreductive surgery to treat infertile women with localized adenomyosis. *Fertil Steril* 86: 462.e5-462.e8, 2006. doi: 10.1016/j.fertnstert.2005.12.066.

774. Osada H. Uterine adenomyosis and adenomyoma: the surgical approach. *Fertil Steril* 109: 406–417, 2018. doi: 10.1016/j.fertnstert.2018.01.032.