



Immunological Diagnostics for Infertility: Cellular, Molecular, and Genetic Comprehensive Review

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Authors

Sara Rasoul Panah¹, Ali Kolahdoozha¹,
Hamed Mohammadi^{1*}

1- Non-Communicable Diseases, Research
Center, Alborz University of Medical
Sciences, Karaj, Iran

*Corresponding Authors:

Hamed Mohammadi, PhD
Non-Communicable Diseases
Research Center, Alborz University
of Medical Sciences, Karaj, Iran
Email: mohamadi.h86@gmail.com

ABSTRACT

Pregnancy represents a unique immunological state where pregnant women develop tolerance mechanisms to avoid fetal rejection. Various mechanisms modulate the maternal immune system to prevent this rejection. Despite these mechanisms, infertility affects approximately 8-12% of reproductive-age couples, particularly those experiencing recurrent implantation failure and recurrent pregnancy loss. Assisted reproductive techniques have significantly advanced in recent decades, yet success rates remain relatively low. Endometrial immune profiling is crucial in understanding infertility and constitutes a distinct microenvironment during pregnancy. Consequently, research has focused on analyzing specific biomarkers, cytokines, and identifying immune system disorders within this context. This approach aims to provide insights for developing personalized treatments. This review examines cellular immune markers, molecular/genetic markers in endometrial studies, and autoantibodies involved in infertility.

Keywords: Immunological Diagnostics, Infertility, Genetic

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1. Introduction

Miscarriage, the most common pregnancy complication, often occurs unexpectedly and can have devastating psychological and physical effects (1). It is reported that about 10-20% of clinically confirmed pregnancies end in miscarriage (3-5). Potential causes of spontaneous pregnancy loss include metabolic/endocrinological abnormalities, genetic factors, anatomical issues, immune disorders, thrombophilia, male factors, and psychological factors (2, 6). While some couples' miscarriages can be managed, about 50% of cases have no clearly defined clinical etiology (7). Given that the fetus is genetically distinct from the mother, specific immunological events must occur to enable the mother to carry the fetus to term. Disruptions in these immunological mechanisms can lead to recurrent miscarriages. Reduced maternal immune tolerance toward the fetus may contribute to recurrent pregnancy losses (4). Immunology offers potential solutions to common reproductive medicine problems, including implantation issues, infertility, miscarriage, and complications later in pregnancy (8). Various immunological factors, such as autoantibodies and changes in uterine immune cell levels, are implicated in immune-related infertility. This review explores available immunological tests in reproductive disorders and miscarriage to provide optimal diagnostic strategies for patients.

2. Cellular Immune Markers:

2.1. NK Cells

Natural killer (NK) cells, a fundamental component of the innate immune system, play a pivotal role in maintaining maternal-fetal tolerance (9). These cells are instrumental in warding off infections during pregnancy (10). Within the unique uterine environment, NK cells are crucial in fostering a conducive setting for pregnancy. They produce various factors that are essential for the regulation of placental invasion and the development of maternal vasculature. Uterine NK cells are characterized by their CD56⁺superbright, CD16⁻ phenotype (11, 12), distinguishing them from peripheral blood NK (pbNK) cells, which predominantly consist of two subsets: CD56⁺dim (95%) and CD56⁺bright (5%) (14). The resemblance of decidual NK (dNK) cells to the CD56⁺bright subset of pbNK cells suggests a shared lineage, likely originating from CD56⁺bright pbNK cells that migrate to the uterus and undergo differentiation within the uterine microenvironment (15). During implantation and placentation, uterine NK (uNK) cells constitute approximately 70% of the

leukocyte population and interact with trophoblast ligands via specific receptors (16).

Aberrant activity of uNK cells can disrupt vascular patterns, lead to ischemic conditions, and elevate oxidative stress, all of which are particularly detrimental during early trophoblast invasion (11, 17). uNK cells are pivotal for the establishment of normal early placentation and facilitate vascular remodeling at the conclusion of the implantation process. Insufficient trophoblast invasion and altered vascular remodeling are primary pathological features in conditions such as preeclampsia and are thought to contribute to recurrent pregnancy loss (RPL) (18). Furthermore, uNK cells support trophoblast invasion and promote vascular remodeling by inducing extravillous trophoblast (EVT) cells (19) and T regulatory (Treg) cells (FoxP3⁺+Treg), enhancing fetomaternal tolerance.

The interaction between maternal killer-cell immunoglobulin-like receptors (KIRs) expressed on uNK cells and fetal human leukocyte antigen-C (HLA-C) on EVT cells regulates placentation (20). The KIR/HLA interface is complex and highly polymorphic, influencing susceptibility to various diseases, including infectious diseases, autoimmune conditions, malignancies, and transplant rejection (21-24). KIR genes modulate the immune response at the fetomaternal interface, with KIR A lacking stimulatory receptors, while KIR B encompasses both stimulatory and inhibitory receptors. The KIR AA genotype is predominantly inhibitory, whereas KIR AB and BB genotypes express a mix of activating and inhibitory receptors. Studies indicate that both activating and inhibitory KIR-HLA combinations are implicated in pregnancy loss (25, 26).

Each pregnancy involves a unique interaction between inherited maternal KIR genes and potentially varied paternal HLA-C groups, even from the same father, creating a dynamic balance between trophoblast and uNK cells. A retrospective analysis of 291 women undergoing 1,304 cycles of in vitro fertilization (IVF) revealed a correlation between the inhibitory KIR-AA haplotype, miscarriage, and implantation failure post-double embryo transfer (27).

Additionally, elevated uNK cell density in endometrial biopsies from patients with recurrent miscarriage (RM) compared to controls has been reported in several studies (28-30). Hence, HLA-C and KIR genotyping could be beneficial for selecting third-party gametes or gestational carriers to mitigate pregnancy complications, including preeclampsia (PE). Clinically, the implications of uNK cell dynamics in the reproductive process should be considered for patients at risk of PE, and the frequency

of prenatal examinations for these individuals might need to be increased (31).

2.2. TH1/TH2 Dynamics

Despite their critical roles in pregnancy, the levels of non-Th1/2 cytokines, such as those produced by regulatory T cells (Treg) and Th17 cells, are less frequently measured. Th17 cells defend against pathogens and are crucial during pregnancy; stimulation of IL-17 production by Th17 cells enhances progesterone secretion and tissue invasiveness (43). Th17 cells also activate decidual natural killer (dNK) cells and impair the vascular reactivity of uterine arteries, potentially leading to embryo resorption (44). Elevated levels of IL-17+ T cells have been detected in women with RPL (45), and Th17 cells show increased expressions of IL-6, IL-17, and IL-23 in cases of unexplained infertility, correlating negatively with fertility outcomes (46). Treg cells (CD4+CD25+Foxp3+), on the other hand, mediate immunosuppression influenced by Th1 and Th17 cells and regulate maternal-fetal immune tolerance. These cells are often diminished in RPL patients (32). Differences in Treg/Th17 immune profiles have been noted between women with RIF and those who are normally fertile. Treatment with Prednisone has been observed to shift the Treg/Th17 balance towards Treg dominance, promoting favorable pregnancy outcomes (48). While promising, clinical data on Treg and TH17 roles in fertility are limited, necessitating further research."

B cells are critical in pregnancy, serving vital roles in humoral immunity and antibody production which support normal pregnancy development. However, B cells can also contribute to adverse obstetric outcomes such as pregnancy loss, preeclampsia, intrauterine growth restriction, stillbirth, and preterm birth, predominantly through autoantibody production (49-51). Despite the known involvement of B cell dysfunction in benign female reproductive pathologies such as endometriosis, research has primarily addressed peripheral B cells rather than those in the endometrial or tissue-specific contexts (52-54).

Evidence indicates that endometrial B cells play a role in the normal development of the endometrium and are also present in endometrial samples from women with reproductive disorders. Conditions like infertility and endometriosis are linked with a broad spectrum of autoimmune diseases, generally resulting from an expanded population of autoreactive B cells (55-58). The presence of endometrial plasma cells is frequently utilized as a diagnostic marker for chronic endometritis (CE), an inflammatory disorder of the endometrium (59-62).

Contrary to the common perception that B cells are scarce or nonexistent in the endometrium, studies demonstrate consistent expression of endometrial B cells in the normal cyclic endometrium. These cells are

also found in endometrial tissue from women suffering from endometriosis, infertility, repeated implantation failure (RIF), recurrent pregnancy loss (RPL), endometritis, and other conditions such as abnormal uterine bleeding, endometrial polyps, and uterine fibroids (63, 64).

secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) from the uterine epithelium, leading to potential abortion and toxicity (39, 40). Neopterin serves as an indicator of pro-inflammatory immune response; elevated levels in fluids such as cerebrospinal fluid, urine, and serum can activate Th1 cells, promoting immunogenic stimulation during pregnancy and contributing to RPL through the associated production of reactive oxygen species (41, 42). Although the ELISA technique is seldom utilized clinically for monitoring Neopterin levels, routine assessment during pregnancy could enhance prognostic outcomes (34) (Figure 1).

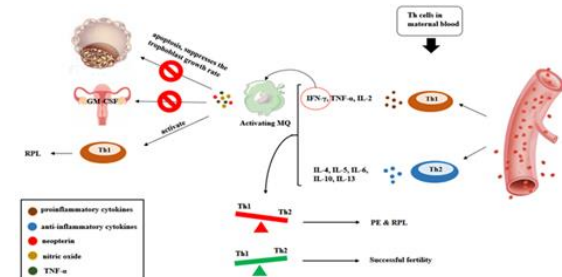


Figure-1. The role of Th1 and Th2 cell responses in fertility and infertility

2.3. Treg/TH17 Interactions

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Table 1. Type of cellular immune markers in infertility

Cellular immune markers	Definition	Mechanism of action
NK cell	one of the innate immune cells that participate in maternal-fetal tolerance while protecting pregnancy from infection	<ul style="list-style-type: none">Regulate placental invasion and maternal vascular development.Account for the majority of leukocytes in the process of implantation and placentation.Establishment of normal early placentation through vascular remodeling.Regulate trophoblast invasion and enhance vascular remodeling induced by EVT cells and Tregs.Regulated Placentation by interactions between maternal KIRs expressed by uNK cells and fetal HLA-C molecules expressed by EVT cells.
TH1/TH2	An essential component of the adaptive immune system in the peripheral blood can be defined by their cytokine production profile. Th1 cells produce IFN- γ , TNF- α , IL-2. / Th2 cells produce IL-4, IL-5, IL-6, IL-10, IL-13	<ul style="list-style-type: none">Pregnancy is associated with a Th2 response, while a Th1 response lead to embryo rejection.IFN-γ production leads to the activation of macrophages and production of signaling mediators induces the apoptosis, suppresses the trophoblast growth rate and inhibit the secretion of GM-CSF and Thus, leading to pregnancy termination and toxicity.
Treg/TH17	Tregs and Th17 cells are two CD4 ⁺ T Cell subsets with antagonist effects. Th17 cells promote inflammation,	<ul style="list-style-type: none">Their cytokine products play a role in successful implantation.IL-17 produced by TH17 increased capacities of progesterone secretion and tissue invasion and leading to

	whereas Tregs are crucial in maintaining immune homeostasis.	embryo resorption by induce activation of dNK cells and impair vascular reactivity of uterine arteries. <ul style="list-style-type: none">Treg cells suppress Th1-and Th17-mediated immunity and lead to maternal immune tolerance to the fetus.
B Cell	B cells make antibodies in response to antigens.	Evidence suggests that B cells is important in the normal endometrium and endometrium obtained from women with reproductive pathologies.

3. Autoantibodies:
3.1. Anti-Phospholipid Antibody (APA)

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the production of antiphospholipid antibodies (aPLs), which are associated with thrombosis and adverse pregnancy outcomes (65). The primary aPLs identified in APS are anticardiolipin antibodies (aCLs), lupus anticoagulant (LA), and anti- β 2-glycoprotein I antibodies (a β 2GPI). These antibodies can disrupt reproductive processes by affecting oocyte development, embryo morphology, uterine receptivity, and decidualization, thereby potentially leading to subfertility (66-68).

Diagnostic criteria for APS are divided into clinical and laboratory categories. Clinically, APS is indicated by vascular thrombosis or specific pregnancy complications, such as fetal death post-10 weeks, preterm delivery before 34 weeks' gestation, or three or more consecutive miscarriages prior to 10 weeks of gestation. Laboratory criteria for APS include the detection of lupus anticoagulant (LA) in plasma, measured twice, 12 weeks apart; anticardiolipin antibody levels in plasma exceeding 40 GPL or MPL, or above the 99th percentile, measured twice, 12 weeks apart; or anti- β 2 glycoprotein-I antibody levels in plasma above the 99th percentile, also measured twice, 12 weeks apart. A diagnosis of APS requires meeting at least one clinical and one laboratory criterion (68).

Table 2. Laboratory clinical criteria in APS syndrome

1- Lupus anticoagulant (LA) measured in the plasma twice and 12 weeks apart
2- Anticardiolipin antibody in plasma >40GPL or MPL or > 99th percentile, measured twice and 12 weeks apart
3- Anti- β 2 glycoprotein-I antibody in plasma >99th percentile measured twice and 12 weeks apart

Antiphospholipid antibodies (aPL) interfere with phospholipids and phospholipid-binding proteins, such as beta-2 glycoprotein 1, protein C, and protein S, impairing the function of these homeostasis regulators and precipitating vascular issues and pregnancy complications (69). Moreover, aPLs activate endothelial cells, escalating the production of arachidonic acid metabolites, adhesion molecules, and cytokines, thereby enhancing the risk of thromboembolism (70). aPL antibodies also impede hormone production by trophoblasts, including hCG, and restrict the invasive capability of extracellular

villous trophoblasts into the maternal decidua (71). Activation of the complement cascade through the classical pathway by aPLs initiates neutrophil recruitment and the subsequent release of proinflammatory cytokines (72).

Miscarriage is a frequent consequence of aPL presence (73-75). During pregnancy, tolerance to fetal alloantigens by the maternal immune system, facilitated by Treg cells, is essential for fetal survival. A reduction in Treg cells may lead to failed embryo implantation and increased production of proinflammatory cytokines (76). Compared to healthy women, those with aPL exhibit fewer Treg cells and more activated T- and pathogenic B-cells (78, 79). Additionally, lower levels of NK and NK T-cells in aPL-positive women contribute to inadequate trophoblast invasion and spiral artery remodeling, emphasizing the altered immune status in these patients (79).

Significantly higher prevalence of autoantibodies against smooth muscle, phospholipids, and nuclear antigens have been observed in women with infertility compared to those with normal pregnancies (80). A notable rise in the prevalence of various autoantibodies, including antinuclear, lupus anticoagulant, anticardiolipin, and anti-double stranded DNA antibodies, is also evident in patients with unexplained infertility versus those with ovulatory infertility (20.5% versus 3.3%) (81). Furthermore, all tested aPLs (IgG, IgM, and IgA anticardiolipin, antiphosphatidyl ethanolamine, antiphosphatidyl inositol, antiphosphatidic acid, antiphosphatidyl glycerol, antiphosphatidyl choline, and antiphosphatidyl serine) are more frequently observed in women with implantation failure (82). Despite this, routine aPL testing in infertility patients lacks sufficient supporting data; further research into APS's pathophysiology is necessary to develop new therapeutic strategies targeting the immune system's inflammatory signaling pathways.

3.2. Anti-Thyroid Antibody (ATA)

Thyroid autoimmunity (TAI) represents the most prevalent autoimmune disorder among childbearing women, affecting between 5% and 20% of this demographic (83, 84). TAI is characterized by the presence of circulating antithyroid autoantibodies such as thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAAb), and thyrotropin receptor antibodies (TRAb), which may or may not impair thyroid function (83, 84).

"Previous studies have established that thyroid autoantibodies are prevalent among women of reproductive age, demonstrating particularly high rates in women with a history of subfertility (with prevalence estimates ranging between 10-31%) (85-87) and recurrent miscarriage (with prevalence estimates between 17-33%) (88, 89).

Thyroid hormone synthesis is critical for the progression and maintenance of pregnancy, with thyroid hormone transporters and receptors present in various reproductive tissues including the ovary, early embryo, endometrium, uterus, and placenta (90). Dysregulation of thyroid hormones impairs the stimulatory effects of gonadotropins on granulosa cells, reducing steroid hormone production and leading to menstrual irregularities and ovulatory dysfunctions (90, 91). Moreover, thyroid dysfunctions detrimentally affect folliculogenesis, fertilization rates, embryo quality, and trophoblast invasion, thereby decreasing the likelihood of a successful pregnancy. Consequently, maintaining euthyroidism is essential during pregnancy (90).

Women with thyroid autoimmunity (TAI) often exhibit insufficient production of thyroid hormones due to antibody interference, potentially culminating in pregnancy loss if unmanaged (92, 93). Thyroid peroxidase antibodies (TPO-Ab) are associated with increased risks of miscarriage, placental abruption, and hypertension induced by pregnancy (94). Furthermore, thyrotropin receptor antibodies (TRAbs) can cross the placental barrier, adversely affecting thyroid function in both the mother and fetus (94).

Additionally, thyroid-stimulating hormone (TSH) enhances the activation of natural killer (NK) cells, promoting their proliferation and cytotoxic activity (95, 96). Thyroid autoantibodies also disrupt the Research indicates that the proportion of peripheral NKT-like cells escalates in women with autoimmune thyroiditis (AIT), contributing to miscarriage and implantation failure (99, 100). Notably, serum levels of interleukin-2 (IL-2) and interleukin-17 (IL-17) are elevated in early pregnancy among patients with AIT compared to controls (101). Th1 cells, through IL-2 and interferon-gamma (INF- γ) production, are crucial in mediating implantation failure and abortion. IL-17, a pro-inflammatory cytokine produced by Th17 cells, plays a significant role in the pathogenesis of abortion (32).

Collective findings from various studies indicate:

- Increased rates of miscarriage and poorer delivery outcomes are observed in the TPOAb-positive group compared to the TPOAb-negative group (102).
- The co-presence of TPOAb and elevated TSH levels in early pregnancy correlates with a heightened risk of gestational diabetes (103).
- TPOAb positivity is associated with placental abruption (104).
- A correlation exists between TPOAb positivity and maternal anemia (105).
- Associations between TPOAb and preterm delivery have shown more consistent findings (106).
- The presence of thyroid autoantibodies significantly elevates the risk of miscarriage across various populations compared to women without these autoantibodies (107).

Consequently, screening for thyroid autoimmunity is recommended as part of the diagnostic workup for women experiencing infertility or early miscarriage to facilitate timely evaluation, diagnosis, and potentially, initial treatment to enhance pregnancy outcomes.

3.3 Anti-Nuclear Antibody (ANA)

Antinuclear antibodies (ANA) target cytoplasmic and nuclear antigens present in all nucleated cells and comprise a broad group that recognizes various cellular components such as double-stranded DNA (ds- DNA), RNA molecules, mitochondrial antigens, and various proteins within the cytoplasm and nucleus, as well as their complexes (108-110). Elevated ANA titers serve as biomarkers for several autoimmune diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis. There is also evidence linking high ANA levels to immunologically induced infertility (111). Significant differences in ANA serum positivity, titer, and pattern have been observed between women with and without recurrent pregnancy loss (RPL), with ANA levels being threefold higher in the RPL group compared to controls (112).

"The involvement of antinuclear antibodies (ANA) in recurrent pregnancy loss (RPL) is a subject of ongoing debate, with numerous studies striving to elucidate their influence on this reproductive issue. Research indicates that ANAs negatively impact pregnancy and implantation rates while also potentially degrading oocyte quality and embryo development (113). Moreover, ANAs can initiate the activation of plasmacytoid dendritic cells via Toll-like receptor-9, which enhances the production of inflammatory cytokines such as interferon α . This cascade stimulates the humoral immune response and leads to further ANA production (114, 115). Additionally, evidence demonstrates that ANA-positive groups exhibit significantly lower rates of Miosis II oocytes, normal fertilization, and pregnancy and implantation rates, coupled with increased rates of abnormal fertilization and early miscarriage (116). The presence of anti-dsDNA antibodies is linked to immunological inflammation in the placenta, adversely affecting pregnancy outcomes (117). High levels of ANAs are associated with detrimental effects on oocyte and embryo development, correlating with repeated implantation failure (RIF) and recurrent miscarriage (118). Given the established roles of ANAs in various infertility-related disorders, measuring ANA titers is advised. Continued research and trials are imperative to explore potential roles and immunotherapeutic strategies in affected individuals.

3.4. Antisperm antibody (ASA)

Antisperm antibodies (ASA) were identified in infertile males as early as 1954 by Rumke and Wilson (119). ASAs are immunoglobulins that target sperm antigens and are present in reproductive tract

secretions and blood in both genders. Typically, mature sperm are shielded from immune recognition by the blood-testis barrier, which maintains tight intercellular junctions. However, damage to the testis, epididymis, or vas deferens, exposing sperm to the immune system, can prompt an autoimmune response against sperm. Conditions such as testicular carcinoma (120), testicular torsion (121), epididymal and bilateral orchitis (122), varicocele (123), seminal infections, sexually transmitted diseases (125), prostate inflammation (126), and seminal vesicle inflammation can elevate ASA levels. Similarly, structural disruptions in the male reproductive tract, vasectomy, or erectile dysfunction (128) are associated with higher ASA levels. Chronic bacterial infections, such as chronic prostatitis, increase the likelihood of ASA development threefold compared to controls (129, 130). A recent study also linked human papillomavirus (HPV) infection in men with an increased risk of ASA development (131). The reason for variability in ASA production among females, with some developing ASAs while others do not, remains elusive. Sperm cells introduced into the lower female reproductive tract are recognized as allogeneic antigens, triggering an inflammatory or allergic response leading to ASA production (133, 134). Despite some cases of idiopathic ASA presence (135), ASAs impair sperm capacitation, the acrosome reaction, sperm traversal through female reproductive tract secretions, gamete fusion, and early embryo development (136, 137). While ASAs do not affect sperm volume, viability, progressive motility, or morphology, they significantly reduce sperm liquefaction and motility (120)."

Sperm agglutination serves as a crucial parameter in the Anti-Sperm Antibody (ASA) assay, as evidenced by reference 138. Although there is a weak correlation between sperm agglutination and the presence of ASA, factors other than sperm antibodies can also induce agglutination (139-141). The World Health Organization's 2010 laboratory manual for human semen analysis categorizes sperm agglutinates as indicative of ASA presence (139). Furthermore, the presence of ASA correlates significantly with reductions in sperm count, vitality, and motility (141, 142), and studies suggest that asthenozoospermia may warrant ASA testing (143).

ASA in semen predominantly comprises two classes of immunoglobulins: IgA and IgG (139). Clinically, IgA is more significant, though over 95% of individuals with IgA also possess IgG (reference 139). The detection of ASA on spermatozoa can be accomplished through two direct assays: the Mixed Antiglobulin Reaction (MAR) test, using fresh semen, and the Immunobead (IB) test, employing washed spermatozoa (139). These tests involve incubating the sample with latex beads coated with anti-human antibodies (139). If ASA are present, these antibodies

bind to the sperm surface antibodies, and under microscopic observation, motile spermatozoa coated with beads are identified, with the percentages of coated motile sperm counted (139). Insufficient counts of motile spermatozoa (fewer than 100) necessitate the use of indirect assays (139).

Direct assays yield information confirming the presence and type of immunoglobulins (IgG or IgA) and their specific localization on the sperm's head, midsection, tail, or entire length (139). Conversely, indirect assays assess sperm-specific immunoglobulins in sperm-free fluids such as heat-inactivated serum, seminal plasma, and dissolved cervical mucus, which are incubated with ASA-free donor sperm previously washed from the original seminal fluid, considering the interaction time between sperm and potential antibodies (139).

Indirect testing is advisable in cases of oligozoospermia or stenozoospermia, either alone or in combination, and in scenarios of obstructive azoospermia or when a sample is unavailable for testing, allowing for semen to be frozen and stored until analysis (139). Despite thorough research into immunological infertility, substantial ambiguity remains regarding the application of ASA testing and treatment strategies for men with ASA, underscoring the need for further investigation.

4. Molecular and Genetic Markers for Endometrial Analysis

4.1. Endometrial Immune Profile Test (EIP):

The Endometrial Immune Profile (EIP) test, which utilizes reverse transcription-quantitative polymerase chain reaction (RT-qPCR), quantitatively assesses gene expressions related to immune modulation in the endometrium. This includes the evaluation of interleukin-15 (IL-15), interleukin-18 (IL-18), tumor necrosis factor-like weak inducer of apoptosis (TWEAK), fibroblast growth factor-inducible molecule 14 (Fn14), and CD56 (144).

It is well-documented that a balanced immune cell profile, particularly the equilibrium between TH1 and TH2 cells, along with the activity and cytotoxicity levels of uNK cells, are critical for fostering fetomaternal tolerance. Any imbalance can precipitate reproductive issues, such as compromised implantation processes.

The interaction between TWEAK and its receptor, Fn14, mitigates local cytotoxicity and regulates uNK cell functions, influencing various physiological and pathological outcomes like embryonic development, angiogenesis, inflammation, and apoptosis (145-148). Furthermore, TWEAK modulates the expression of other cytokines such as IL-18 and IL-15, thereby playing a crucial role in controlling uNK cell cytotoxicity and promoting maternal tolerance towards the fetus.

Research indicates that IL-18 is actively expressed in the endometrium during the implantation window (WOI) and is instrumental in managing trophoblast invasion, migration, and uNK cell activity, as well as promoting angiogenesis and placental vascularization essential for maternal-fetal nutrient and oxygen exchange (149).

Excessive or imbalanced IL-18 expression has been linked to reproductive disorders like preterm birth, preeclampsia, and fetal growth restriction (150). IL-18 also influences the TH1/TH2 balance; it can stimulate TH1 immune responses, triggering cytotoxic T cell activation and pro-inflammatory cytokine production (e.g., TNF and INF) (151). Conversely, IL-18 can exhibit TH2-like activity, enhancing eosinophil responses and IL-5 and IL-13 production, thus supporting TH2 responses in synergy with IL-4 (152). IL-15, another crucial immune system cytokine, supports the survival, proliferation, and maturation of immune cells, including uNK cells (153).

In the EIP test, the IL-18/TWEAK mRNA ratio serves as a biomarker for angiogenesis and the TH1/TH2 balance. High IL-18 expression, which typically benefits the immune response, can become deleterious by promoting local cytotoxicity if not balanced by TWEAK expression (146). Elevated TWEAK levels can counteract excessive IL-18 expression, preventing the transformation of uNK cells into cytotoxic entities (146).

IL-15/Fn14 mRNA serves as a biomarker to assess the activation and maturation of uterine natural killer (uNK) cells by evaluating the presence of uNK-CD56+ cells. The activation and maturation status of NK cells during pregnancy is critical. As uterine NK cells are typically immature, they undergo a process of maturation, where IL-15 plays a pivotal role in their recruitment and development.

In a study examining the endometrial immunity of 104 patients with recurrent pregnancy loss (RPL), 75% exhibited signs of endometrial immune dysregulation. Among these, 31% displayed an underactive uterine immune profile, 50% an overactive profile, and 19% a mixed pattern. Notably, uterine immune profiling was significantly correlated with higher live birth rates (LBR) when dysregulation was identified (154).

Another investigation on the endometrial immunity of 394 patients with recurrent implantation failure (RIF) identified overactivation in 56.6% of cases and low activation in 25%. The LBR among these dysregulated/treated patients at their subsequent embryo transfer was 39.8% (155). These findings underscore the need for further research to verify the efficacy of these assessments.

4.2. Endometrial Decidualization Score (EDS)

Decidualization involves the extensive proliferation, secretion, and regression of the endometrium's inner lining in preparation for pregnancy. This process

transforms human endometrial stromal cells into decidual cells, creating a tissue receptive to embryo implantation (156). Decidualization primarily relies on the action of progesterone on estradiol-primed progesterone receptors in endometrial stromal cells (157).

A key progesterone signaling mediator, Forkhead box O1 (FOXO1), induces senescence in a subset of decidualized stromal cells, crucial for tissue remodeling essential for embryo implantation (158). Additionally, decidualization involves increased expression of homeostatic tissue and cellular factors (159), as well as glucose transport molecules like Glut1 and Glut3 in the human endometrium, peaking during the mid-luteal phase to support embryo implantation and growth (160).

Concomitant with these metabolic enhancements, there is a notable increase in uterine natural killer (NK) cells in the endometrium during this phase (161). These uNK cells, secreting growth-promoting, angiogenic, chemotactic, and immunoregulatory factors, play significant roles in angiogenesis, placental growth, and trophoblast invasion regulation (162, 163). Moreover, interleukin 15 levels in the endometrium, which bolster the proliferation and survival of NK cells, also rise during the luteal phase (164, 165).

Molecular diagnostics utilizing targeted RNA sequencing has been employed to detect endometrial gene expressions crucial for progesterone signaling and decidualization (FOXO1) (166, 167), tissue and cellular homeostasis (SGK1, SCNN1A, and SLC2A1) (168-170), and immunoregulatory and tissue remodeling factors (IL-15 and GZMB) (158). This gene expression profile evaluation is referred to as the decidualization score.

Research indicates that among women with reproductive failures, 76% had EDS scores ≤ 4 , and 19% had scores of 0, whereas 89% and 11% of fertile controls had EDS scores ≥ 5 and 4, respectively (171). However, additional studies are necessary to confirm the utility and effectiveness of this score.

4.3. Human Herpesvirus 6A Test (HHV6A)

Human Herpesvirus 6A (HHV-6A) is categorized within the beta-herpesviruses and is recognized as part of the Roseolovirus genus (172-175). It exhibits a broad cellular tropism, infecting numerous cell types across various tissues, including: 1) diverse immune cells—such as CD4⁺ T cells, CD8⁺ T cells, and NK cells; 2) various nervous system cells—such as astrocytes, microglial cells, oligodendrocytes, and neuronal cells; 3) and cells from other tissues including liver cells, human fibroblasts, epithelial cells, and endothelial cells (174-176). Moreover, HHV-6A is capable of infecting different cells within the female reproductive tract, being detected in the vaginal canal, uterus, and cervix (177, 178, 179). The

viral infection of immune cells leads to an increased production of pro-inflammatory cytokines including IL-1 β , TNF α , IFN- α , IFN- γ , and IL-6, while concurrently reducing the levels of the anti-inflammatory cytokine IL-10 (180-186). Furthermore, infection by HHV-6A enhances the toxicity of NK cells in non-pregnant women, particularly when endometrial epithelial cells are involved, resulting in elevated pro-inflammatory cytokine levels that may inhibit implantation (9, 187). This suggests that endometrial NK cell contamination plays a role in the pathogenesis of primary infertility. Conversely, during pregnancy, NK cells exhibit reduced susceptibility to foreign antigens due to interactions with HLA-G and HLA-E on cytotrophoblasts, which inhibit attacks against paternal antigens (188). Theoretically, HHV-6A infection could disrupt this protective interaction, leading to impaired implantation and contributing to primary unexplained infertility and preeclampsia (PE). Research indicates that Human herpesvirus 6A deoxyribonucleic acid was found in 43% of endometrial samples from women with primary unexplained subfertility, in contrast to 0% in fertile controls (177). Additionally, cases of PE show a higher prevalence of inherited chromosomally integrated HHV-6A (iciHHV-6A) and possibly acquired infections, suggesting susceptibility to PE (189, 190). The evidence thus far is compelling and merits further investigation.

4.4. B Cell CLL/Lymphoma 6 Test (BCL6)

B-cell lymphoma 6 (BCL6), a crucial proto-oncogene, plays a predominant role in regulating humoral immunity and lymphoma survival (191, 192) (Figure 2). This transcriptional repressor is involved in cellular differentiation, cell cycle control, and apoptosis inhibition (193). Elevated BCL6 expression correlates with unexplained infertility, endometriosis-associated infertility, and common pregnancy diseases such as preeclampsia (PE) (194-198). Notably, BCL6 is frequently altered in pre-eclamptic placentas as shown through systematic meta-analysis and expression network analysis (199, 200). Its overexpression stimulates ARNT2 (aryl hydrocarbon receptor nuclear translocator 2) production, which partners with hypoxia-inducible factor 1 α (HIF-1 α) to influence trophoblast invasion and contribute to PE pathogenesis (198, 201-203). A study revealed that 2977 genes, enriched with metabolism-related pathways and transporter functions, were differentially expressed in severe early-onset PE (EO-PE), while 375 genes associated with immune pathways were more prevalent in severe late-onset PE (LO-PE), with BCL6 being upregulated in both conditions (196). Aberrant BCL6 expression exhibits high sensitivity and specificity for diagnosing all stages of endometriosis, indicating its potential as a biomarker (204). The prevalence of elevated

endometrial BCL6 expression in women with unexplained infertility (UI) is reported at 75.3% and 80% (194, 195). Although some progress has been made, further research is necessary to fully elucidate the molecular mechanisms through which BCL6 exerts its diverse functions in the placenta and endometrium.

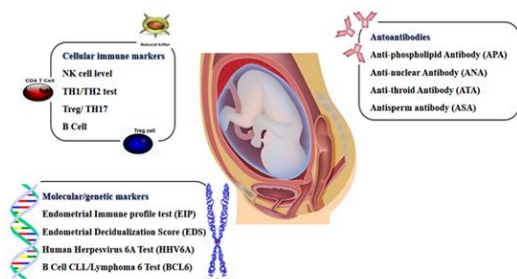


Figure-2. Different methods of identifying immune system disorders in infertility

Conclusion

The establishment of pregnancy and its maintenance, involve complex states, tightly regulated by intricate relationships among the different cell subsets of the immune system. Endometrial immune status has been a neglected factor in reproductive medicine and management. However, the uterine immune profiling represents a clinical innovation which can significantly increase the appropriate assisted reproductive technology (ART) through personalization. Currently, infertility is a growing problem, affecting 8-12% of couples of reproductive age worldwide. Therefore, it is clear that there is a great need in this field for progress in the development of diagnostic tests that provide the possibility of assessing the risk of these infertility, such as RPL and RIF, etc.

Ethical Issue

There was no ethical issue in this review.

Conflict of Interests

There was no conflict of interest in this study.

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Author's ORCID

Reference:

1. Woolner AM, Raja EA, Bhattacharya S, Danielian P, Bhattacharya S. Inherited susceptibility to miscarriage: a nested case-control study of 31,565 women from an intergenerational cohort. *American Journal of Obstetrics and Gynecology*. 2020;222(2):168. e1-. e8.
2. RPL EGG0, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, et al. ESHRE guideline: recurrent pregnancy loss. *Human reproduction open*. 2018;2018(2):hoy004.
3. Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. *Reviews in obstetrics and gynecology*. 2009;2(2):76.
4. Mohamad BN, Alsakkal G. The effect of consanguinity on reproductive outcomes in Maternity Teaching Hospital in Erbil city. *AMJ (Advanced Medical Journal) is the scientific journal of Kurdistan Higher Council of Medical Specialties*. 2023;8(1):54-61.
5. Tasadduq R, Ajmal L, Batool F, Zafar T, Babar A, Riasat A, Shakoori A-R. Interplay of immune components and their association with recurrent pregnancy loss. *Human Immunology*. 2021;82(3):162-9.
6. Medicine PCotASfR. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertility and sterility*. 2012;98(5):1103-11.
7. Daya S, Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. *Fertility and sterility*. 1996;66(1):24-9.
8. Norman RJ. Immunology in reproductive medicine: is current testing and therapy justified by science? *Fertility and Sterility*. 2022;117(6):1105-6.
9. Bortolotti D, Gentili V, Caselli E, Siculo M, Soffritti I, D'Accolti M, et al. DNA sensors' signaling in NK cells during HHV-6A, HHV-6B and HHV-7 infection. *Frontiers in Microbiology*. 2020;11:226.
10. Kwak-Kim J, Gilman-Sachs A. Clinical implication of natural killer cells and reproduction. *American journal of reproductive immunology*. 2008;59(5):388-400.
11. Donoghue J, Paiva P, Teh W, Cann L, Nowell C, Rees H, et al. Endometrial uNK cell counts do not predict successful implantation in an IVF population. *Human Reproduction*. 2019;34(12):2456-66.
12. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *The Journal of experimental medicine*. 2003;198(8):1201-12.
13. Moffett A, Shreeve N. First do no harm: uterine natural killer (NK) cells in assisted reproduction. *Human reproduction*. 2015;30(7):1519-25.
14. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends in immunology*. 2001;22(11):633-40.
15. King A, Jokhi P, Burrows TD, Gardner L, Sharkey A, Lore Y. Functions of human decidual NK cells. *American Journal of Reproductive Immunology*. 1996;35(3):258-60.

16. Sargent I, Borzychowski A, Redman C. NK cells and pre-eclampsia. *Journal of reproductive immunology*. 2007;76(1-2):40-4.
17. Kwak-Kim J, Bao S, Lee SK, Kim JW, Gilman-Sachs A. Immunological modes of pregnancy loss: inflammation, immune effectors, and stress. *American journal of reproductive immunology*. 2014;72(2):129-40.
18. Arck PC, Hecher K. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. *Nature medicine*. 2013;19(5):548-56.
19. Moffett A, Colucci F. Co-evolution of NK receptors and HLA ligands in humans is driven by reproduction. *Immunological reviews*. 2015;267(1):283-97.
20. Hiby S, Regan L, Lo W, Farrell L, Carrington M, Moffett A. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. *Human reproduction*. 2008;23(4):972-6.
21. McLaren PJ, Carrington M. The impact of host genetic variation on infection with HIV-1. *Nature immunology*. 2015;16(6):577-83.
22. Ahn R, Moslehi H, Martin M, Abad-Santos M, Bowcock A, Carrington M, Liao W. Inhibitory KIR3DL1 alleles are associated with psoriasis. *British Journal of Dermatology*. 2016;174(2):449-51.
23. Mancusi A, Ruggeri L, Urbani E, Pierini A, Massei MS, Carotti A, et al. Haploidentical hematopoietic transplantation from KIR ligand-mismatched donors with activating KIRs reduces nonrelapse mortality. *Blood, The Journal of the American Society of Hematology*. 2015;125(20):3173-82.
24. Hollenbach JA, Pando MJ, Caillier SJ, Gourraud P-A, Oksenberg JR. The killer immunoglobulin-like receptor KIR3DL1 in combination with HLA-Bw4 is protective against multiple sclerosis in African Americans. *Genes & Immunity*. 2016;17(3):199-202.
25. Yang X, Yang E, Wang W-J, He Q, Jubiz G, Katukurundage D, et al. Decreased HLA-C1 alleles in couples of KIR2DL2 positive women with recurrent pregnancy loss. *Journal of Reproductive Immunology*. 2020;142:103186.
26. Dambaeva SV, Lee DH, Sung N, Chen CY, Bao S, Gilman-Sachs A, et al. Recurrent pregnancy loss in women with killer cell immunoglobulin-like receptor KIR2DS1 is associated with an increased HLA-C2 allelic frequency. *American journal of reproductive immunology*. 2016;75(2):94-103.
27. Alecsandru D, Garrido N, Vicario J, Barrio A, Aparicio P, Requena A, García-Velasco J. Maternal KIR haplotype influences live birth rate after double embryo transfer in IVF cycles in patients with recurrent miscarriages and implantation failure. *Human reproduction*. 2014;29(12):2637-43.
28. Quenby S, Bates M, Doig T, Brewster J, Lewis-Jones D, Johnson P, Vince G. Pre-implantation endometrial leukocytes in women with recurrent miscarriage. *Human reproduction*. 1999;14(9):2386-91.
29. Tuckerman E, Laird S, Prakash A, Li T. Prognostic value of the measurement of uterine natural killer cells in the endometrium of women with recurrent miscarriage. *Human reproduction*. 2007;22(8):2208-13.
30. Clifford K, Flanagan A, Regan L. Endometrial CD56+ natural killer cells in women with recurrent miscarriage: a histomorphometric study. *Human reproduction*. 1999;14(11):2727-30.
31. Yang X, Yang Y, Yuan Y, Liu L, Meng T. The roles of uterine natural killer (NK) cells and KIR/HLA-C combination in the development of preeclampsia: a systematic review. *BioMed research international*. 2020;2020.
32. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *American journal of reproductive immunology*. 2010;63(6):601-10.
33. Bashiri A, Halper KI, Orvieto R. Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions. *Reproductive Biology and Endocrinology*. 2018;16:1-18.
34. Ünüvar S, Tanrıverdi Z, editors. Neopterin And Recurrent Spontaneous Abortion (Rsa): The Effect Of Cellular Immune System Activation On Subsequent Pregnancy. *CBU International Conference Proceedings*; 2017.
35. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Pregnancy and obstetrics. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Human Reproduction*. 2000;15(3).
36. Lee SK, Na BJ, Kim JY, Hur SE, Lee M, Gilman-Sachs A, Kwak-Kim J. Determination of clinical cellular immune markers in women with recurrent pregnancy loss. *American journal of reproductive immunology*. 2013;70(5):398-411.
37. Giannubilo SR, Landi B, Pozzi V, Sartini D, Cecati M, Stortoni P, et al. The involvement of inflammatory cytokines in the pathogenesis of recurrent miscarriage. *Cytokine*. 2012;58(1):50-6.
38. Liang P-Y, Diao L-H, Huang C-Y, Lian R-C, Chen X, Li G-G, et al. The pro-inflammatory and anti-inflammatory cytokine profile in peripheral blood of women with recurrent implantation failure. *Reproductive biomedicine online*. 2015;31(6):823-6.
39. Todt JC, Yang Y, Lei J, Lauria MR, Sorokin Y, Cotton DB, Yelian FD. Effects of tumor necrosis factor-alpha on human trophoblast cell adhesion and motility. *American Journal of Reproductive Immunology*. 1996;36(2):65-71.

40. Yui J, Garcia-Lloret M, Wegmann Tea, Guilbert L. Cytotoxicity of tumour necrosis factor-alpha and gamma-interferon against primary human placental trophoblasts. *Placenta*. 1994;15(8):819-35.
41. Sencan H, Keskin N, Khatib G. The role of neopterin and anti-Mullerian hormone in unexplained recurrent pregnancy loss—A case-control study. *Journal of Obstetrics and Gynaecology*. 2019;39(7):996-9.
42. Wang Z, Dong M, Chu H, He J. Increased serum levels of neopterin and soluble interleukin-2 receptor in intrahepatic cholestasis of pregnancy. *Acta obstetrica et gynecologica Scandinavica*. 2004;83(11):1067-70.
43. Pongcharoen S, Supalap K. Interleukin-17 increased progesterone secretion by JEG-3 human choriocarcinoma cells. *American journal of reproductive immunology*. 2009;61(4):261-4.
44. Travis OK, White D, Pierce WA, Ge Y, Stubbs CY, Spradley FT, et al. Chronic infusion of interleukin-17 promotes hypertension, activation of cytolytic natural killer cells, and vascular dysfunction in pregnant rats. *Physiological Reports*. 2019;7(7):e14038.
45. Lee S, Kim J, Hur S, Kim C, Na B, Lee M, et al. An imbalance in interleukin-17-producing T and Foxp3+ regulatory T cells in women with idiopathic recurrent pregnancy loss. *Human reproduction*. 2011;26(11):2964-71.
46. Saifi B, Rezaee SA, Tajik N, Ahmadpour ME, Ashrafi M, Vakili R, et al. Th17 cells and related cytokines in unexplained recurrent spontaneous miscarriage at the implantation window. *Reproductive biomedicine online*. 2014;29(4):481-9.
47. Ozkan ZS, Deveci D, Kumbak B, Simsek M, Ilhan F, Sekercioglu S, Sapmaz E. What is the impact of Th1/Th2 ratio, SOCS3, IL17, and IL35 levels in unexplained infertility? *Journal of reproductive immunology*. 2014;103:53-8.
48. Huang Q, Wu H, Li M, Yang Y, Fu X. Prednisone improves pregnancy outcome in repeated implantation failure by enhance regulatory T cells bias. *Journal of Reproductive Immunology*. 2021;143:103245.
49. Jensen F, Wallukat G, Herse F, Budner O, El-Mousleh T, Costa S-D, et al. CD19+ CD5+ cells as indicators of preeclampsia. *Hypertension*. 2012;59(4):861-8.
50. Muzzio D, Zenclussen AC, Jensen F. The role of B cells in pregnancy: the good and the bad. *American Journal of Reproductive Immunology*. 2013;69(4):408-12.
51. Saccone G, Berghella V, Maruotti GM, Ghi T, Rizzo G, Simonazzi G, et al. Antiphospholipid antibody profile based obstetric outcomes of primary antiphospholipid syndrome: the PREGNANTS study. *American journal of obstetrics and gynecology*. 2017;216(5):525. e1-. e12.
52. Gagné D, Rivard M, Pagé M, Shazand K, Hugo P, Gosselin D. Blood leukocyte subsets are modulated in patients with endometriosis. *Fertility and sterility*. 2003;80(1):43-53.
53. Riccio LG, Baracat EC, Chapron C, Batteux F, Abrão MS. The role of the B lymphocytes in endometriosis: a systematic review. *Journal of reproductive immunology*. 2017;123:29-34.
54. Danaii S, Ghorbani F, Ahmadi M, Abbaszadeh H, Koushaeian L, Soltani-Zangbar MS, et al. IL-10-producing B cells play important role in the pathogenesis of recurrent pregnancy loss. *International Immunopharmacology*. 2020;87:106806.
55. Shigesu N, Kvaskoff M, Kirtley S, Feng Q, Fang H, Knight JC, et al. The association between endometriosis and autoimmune diseases: a systematic review and meta-analysis. *Human reproduction update*. 2019;25(4):486-503.
56. Khizroeva J, Nalli C, Bitsadze V, Lojacono A, Zatti S, Andreoli L, et al. Infertility in women with systemic autoimmune diseases. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2019;33(6):101369.
57. Rawlings DJ, Metzler G, Wray-Dutra M, Jackson SW. Altered B cell signalling in autoimmunity. *Nature reviews Immunology*. 2017;17(7):421-36.
58. Hershberg U, Luning Prak ET. The analysis of clonal expansions in normal and autoimmune B cell repertoires. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2015;370(1676):20140239.
59. Song D, Li T-C, Zhang Y, Feng X, Xia E, Huang X, Xiao Y. Correlation between hysteroscopy findings and chronic endometritis. *Fertility and sterility*. 2019;111(4):772-9.
60. Cicinelli E, Bettocchi S, de Ziegler D, Loizzi V, Cormio G, Marinaccio M, et al. Chronic endometritis, a common disease hidden behind endometrial polyps in premenopausal women: first evidence from a case-control study. *Journal of minimally invasive gynecology*. 2019;26(7):1346-50.
61. Liu Y, Chen X, Huang J, Wang C-C, Yu M-Y, Laird S, Li T-C. Comparison of the prevalence of chronic endometritis as determined by means of different diagnostic methods in women with and without reproductive failure. *Fertility and sterility*. 2018;109(5):832-9.
62. 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. *American journal of reproductive immunology*. 2014;72(4):386-91.
63. Weisel NM, Weisel FJ, Farber DL, Borghesi LA, Shen Y, Ma W, et al. Comprehensive analyses of B-cell compartments across the human body reveal novel subsets and a gut-resident memory phenotype. *Blood, The Journal*

of the American Society of Hematology. 2020;136(24):2774-85.

64. Farstad I, Carlsen H, Morton H, Brandtzaeg P. Immunoglobulin A cell distribution in the human small intestine: phenotypic and functional characteristics. *Immunology*. 2000;101(3):354-63.

65. Rodrigues VdO, Soligo AdG, Pannain GD. Síndrome Anticorpo Antifosfolípide e Infertilidade. *Revista Brasileira de Ginecologia e Obstetrícia*. 2019;41:621-7.

66. Stoeber ZM, Mozes E, Tartakovsky B. Anti-cardiolipin antibodies induce pregnancy failure by impairing embryonic implantation. *Proceedings of the National Academy of Sciences*. 1993;90(14):6464-7.

67. Di Simone N, Meroni P, Del Papa N, Raschi E, Caliendo D, De Carolis S, et al. Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered β 2-glycoprotein I. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*. 2000;43(1):140-50.

68. Di Simone N, Meroni P, D'Asta M, Di Nicuolo F, D'Alessio MC, Caruso A. Pathogenic role of anti- β 2-glycoprotein I antibodies on human placenta: functional effects related to implantation and roles of heparin. *Human Reproduction Update*. 2007;13(2):189-96.

69. Chighizola CB, Raimondo MG, Meroni PL. Does APS impact women's fertility? *Current rheumatology reports*. 2017;19:1-9.

70. Pattison NS, Chamley LW, Birdsall M, Zanderigo AM, Liddell HS, McDougall J. Does aspirin have a role in improving pregnancy outcome for women with the antiphospholipid syndrome? A randomized controlled trial. *American journal of obstetrics and gynecology*. 2000;183(4):1008-12.

71. Tong M, Viall C, Chamley L. Antiphospholipid antibodies and the placenta: a systematic review of their in vitro effects and modulation by treatment. *Human reproduction update*. 2015;21(1):97-118.

72. Abrahams VM, Chamley LW, Salmon JE. Antiphospholipid syndrome and pregnancy: pathogenesis to translation. *Arthritis & rheumatology (Hoboken, NJ)*. 2017;69(9):1710.

73. Chauleur C, GALANAUD JP, Alonso S, Cochery-Nouvellon E, BALDUCCHI JP, Marès P, et al. Observational study of pregnant women with a previous spontaneous abortion before the 10th gestation week with and without antiphospholipid antibodies. *Journal of Thrombosis and Haemostasis*. 2010;8(4):699-706.

74. Ibrahim I, Mamman A, Adaji S, Hassan A, Babadoko A. Prevalence of lupus anticoagulant in women with spontaneous abortion in Zaria. *Nigerian journal of clinical practice*. 2017;20(9):1145-9.

75. Abdullahi ZG, Abdul MA, Aminu SM, Musa BO, Amadu L, Jibril E-BM. Antiphospholipid antibodies among pregnant women with recurrent fetal wastage in a tertiary hospital in Northern Nigeria. *Annals of African Medicine*. 2016;15(3):133.

76. La Rocca C, Carbone F, Longobardi S, Matarese G. The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. *Immunology letters*. 2014;162(1):41-8.

77. Care AS, Bourque SL, Morton JS, Hjartarson EP, Robertson SA, Davidge ST. Reduction in regulatory T cells in early pregnancy causes uterine artery dysfunction in mice. *Hypertension*. 2018;72(1):177-87.

78. Kwak-Kim J, Chung-Bang H, Ng S, Ntrivalas E, Mangubat C, Beaman K, et al. Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. *Human reproduction*. 2003;18(4):767-73.

79. Krivonos MI, Kh. Khizroeva J, Zainulina MS, Ereemeeva DR, Selkov SA, Chugunova A, et al. The role of lymphocytic cells in infertility and reproductive failures in women with antiphospholipid antibodies. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2022;35(5):871-7.

80. Taylor PV, Campbell JM, Scott JS. Presence of autoantibodies in women with unexplained infertility. *American journal of obstetrics and gynecology*. 1989;161(2):377-9.

81. Kim CH, Cho YK, Mok JE. The efficacy of immunotherapy in patients who underwent superovulation with intrauterine insemination. *Fertility and sterility*. 1996;65(1):133-8.

82. Coulam CB, Kaider BD, Kaider AS, Janowicz P, Roussev RG. Antiphospholipid antibodies associated with implantation failure after IVF/ET. *Journal of Assisted Reproduction and Genetics*. 1997;14:603-8.

83. Plowden TC, Schisterman EF, Sjaarda LA, Zarek SM, Perkins NJ, Silver R, et al. Subclinical hypothyroidism and thyroid autoimmunity are not associated with fecundity, pregnancy loss, or live birth. *The Journal of Clinical Endocrinology & Metabolism*. 2016;101(6):2358-65.

84. Cueva S, Burks C, McQueen D, Barkoff MS, Stephenson MD. Maternal antithyroid antibodies and euploid miscarriage in women with recurrent early pregnancy loss. *Fertility and Sterility*. 2018;110(3):452-8.

85. Glinier D, Delange F. The potential repercussions of maternal, fetal, and neonatal hypothyroxinemia on the progeny. *Thyroid*. 2000;10(10):871-87.

86. Sakar M, Unal A, Atay A, Zebitay A, Verit F, Demir S, et al. Is there an effect of thyroid autoimmunity on the outcomes of assisted reproduction? *Journal of Obstetrics and Gynaecology*. 2016;36(2):213-7.

87. Muller A, Verhoeff A, Mantel M, Berghout A. Thyroid autoimmunity and abortion: a prospective study in women undergoing in vitro fertilization. *Fertility and sterility*. 1999;71(1):30-4.
88. Bussen S, Steck T. Thyroid autoantibodies in euthyroid non-pregnant women with recurrent spontaneous abortions. *Human reproduction*. 1995;10(11):2938-40.
89. Kutteh WH, Yetman DL, Carr AC, Beck LA, Scott Jr RT. Increased prevalence of antithyroid antibodies identified in women with recurrent pregnancy loss but not in women undergoing assisted reproduction. *Fertility and sterility*. 1999;71(5):843-8.
90. Vissenberg R, Manders V, Mastenbroek S, Fliers E, Afink G, Ris-Stalpers C, et al. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. *Human reproduction update*. 2015;21(3):378-87.
91. Poppe K, Velkeniers B, Glinoe D. Thyroid disease and female reproduction. *Clinical endocrinology*. 2007;66(3):309-21.
92. Colicchia M, Campagnolo L, Baldini E, Ulisse S, Valensise H, Moretti C. Molecular basis of thyrotropin and thyroid hormone action during implantation and early development. *Human reproduction update*. 2014;20(6):884-904.
93. Bliddal S, Boas M, Hilsted L, Friis-Hansen L, Tabor A, Feldt-Rasmussen U. Thyroid function and autoimmunity in Danish pregnant women after an iodine fortification program and associations with obstetric outcomes. *European Journal of Endocrinology*. 2015;173(6):709-18.
94. Tierney K, Delpachitra P, Grossmann M, Onwude J, Sikaris K, Wallace EM, et al. Thyroid function and autoantibody status among women who spontaneously deliver under 35 weeks of gestation. *Clinical endocrinology*. 2009;71(6):892-5.
95. Provinciali M, Di Stefano G, Fabris N. Improvement in the proliferative capacity and natural killer cell activity of murine spleen lymphocytes by thyrotropin. *International journal of immunopharmacology*. 1992;14(5):865-70.
96. Hidaka Y, Amino N, Iwatani Y, Kaneda T, Nasu M, Mitsuda N, et al. Increase in peripheral natural killer cell activity in patients with autoimmune thyroid disease. *Autoimmunity*. 1992;11(4):239-46.
97. Kim NY, Cho HJ, Kim HY, Yang KM, Ahn HK, Thornton S, et al. Thyroid autoimmunity and its association with cellular and humoral immunity in women with reproductive failures. *American Journal of Reproductive Immunology*. 2011;65(1):78-87.
98. Pratt D, Novotny M, Kaberlein G, Dudkiewicz A, Gleicher N. Antithyroid antibodies and the association with non-organ-specific antibodies in recurrent pregnancy loss. *American Journal of Obstetrics and Gynecology*. 1993;168(3):837-41.
99. Svensson J, Oderup C, Åkesson C, Uvebrant K, Hallengren B, Ericsson U, et al. Maternal autoimmune thyroid disease and the fetal immune system. *Experimental and clinical endocrinology & diabetes*. 2011;445-50.
100. Miko E, Meggyes M, Doba K, Farkas N, Bogar B, Barakonyi A, et al. Characteristics of peripheral blood NK and NKT-like cells in euthyroid and subclinical hypothyroid women with thyroid autoimmunity experiencing reproductive failure. *Journal of reproductive immunology*. 2017;124:62-70.
101. Iyidir OT, Degertekin CK, Sonmez C, Yucel AA, Erdem M, Akturk M, Ayvaz G. The effect of thyroid autoimmunity on T-cell responses in early pregnancy. *Journal of reproductive immunology*. 2015;110:61-6.
102. Negro R, Mangieri T, Coppola L, Presicce G, Casavola EC, Gismondi R, et al. Levothyroxine treatment in thyroid peroxidase antibody-positive women undergoing assisted reproduction technologies: a prospective study. *Human reproduction*. 2005;20(6):1529-33.
103. Karakosta P, Alegakis D, Georgiou V, Roumeliotaki T, Fthenou E, Vassilaki M, et al. Thyroid dysfunction and autoantibodies in early pregnancy are associated with increased risk of gestational diabetes and adverse birth outcomes. *The Journal of Clinical Endocrinology & Metabolism*. 2012;97(12):4464-72.
104. Abbassi-Ghanavati M, Casey BM, Spong CY, McIntire DD, Halvorson LM, Cunningham FG. Pregnancy outcomes in women with thyroid peroxidase antibodies. *Obstetrics & Gynecology*. 2010;116(2 Part 1):381-6.
105. Meena M, Chopra S, Jain V, Aggarwal N. The effect of anti-thyroid peroxidase antibodies on pregnancy outcomes in euthyroid women. *Journal of clinical and diagnostic research: JCDR*. 2016;10(9):QC04.
106. He X, Wang P, Wang Z, He X, Xu D, Wang B. Endocrinology in pregnancy: thyroid antibodies and risk of preterm delivery: a meta-analysis of prospective cohort studies. *European journal of endocrinology*. 2012;167(4):455-64.
107. Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J, Coomarasamy A. Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. *Bmj*. 2011;342.
108. Xu L, Chang V, Murphy A, Rock JA, Damewood M, Schlaff W, Zacur HA. Antinuclear antibodies in sera of patients with recurrent pregnancy wastage. *American journal of obstetrics and gynecology*. 1990;163(5):1493-7.
109. Deocharan B, Qing X, Beger E, Putterman C. Antigenic triggers and molecular targets for anti-double-stranded DNA antibodies. *Lupus*. 2002;11(12):865-71.

110. Derksen R, Bast E, Strooisma T, Jacobs J. A comparison between the Farr radioimmunoassay and a new automated fluorescence immunoassay for the detection of antibodies against double stranded DNA in serum. *Annals of the rheumatic diseases*. 2002;61(12):1099-102.
111. Deroux A, Dumestre-Perard C, Dunand-Faure C, Bouillet L, Hoffmann P. Female infertility and serum auto-antibodies: a systematic review. *Clinical reviews in allergy & immunology*. 2017;53:78-86.
112. Ticconi C, Rotondi F, Veglia M, Pietropolli A, Bernardini S, Ria F, et al. Antinuclear autoantibodies in women with recurrent pregnancy loss. *American Journal of Reproductive Immunology*. 2010;64(6):384-92.
113. Reimand K, Talja I, Metsküla K, Kadastik Ü, Matt K, Uibo R. Autoantibody studies of female patients with reproductive failure. *Journal of Reproductive Immunology*. 2001;51(2):167-76.
114. Zeng M, Wen P, Duan J. Association of antinuclear antibody with clinical outcome of patients undergoing in vitro fertilization/intracytoplasmic sperm injection treatment: A meta-analysis. *American Journal of Reproductive Immunology*. 2019;82(3):e13158.
115. Papadimitraki ED, Choulaki C, Koutala E, Bertsias G, Tsatsanis C, Gergianaki I, et al. Expansion of toll-like receptor 9-expressing B cells in active systemic lupus erythematosus: Implications for the induction and maintenance of the autoimmune process. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*. 2006;54(11):3601-11.
116. Zhu Q, Wu L, Xu B, Hu M-H, Tong X-H, Ji J-J, Liu Y-S. A retrospective study on IVF/ICSI outcome in patients with anti-nuclear antibodies: the effects of prednisone plus low-dose aspirin adjuvant treatment. *Reproductive Biology and Endocrinology*. 2013;11(1):1-9.
117. Tanacan A, Beksac MS, Orgul G, Duru S, Sener B, Karaagaoglu E. Impact of extractable nuclear antigen, anti-double stranded DNA, antiphospholipid antibody, and anticardiolipin antibody positivity on obstetrical complications and pregnancy outcomes. *Human antibodies*. 2019;27(2):135-41.
118. Ying Y, Zhong Y-p, Zhou C-q, Xu Y-w, Wang Q, Li J, et al. Antinuclear antibodies predicts a poor IVF-ET outcome: impaired egg and embryo development and reduced pregnancy rate. *Immunological investigations*. 2012;41(5):458-68.
119. Wilson L. Sperm agglutinins in human semen and blood. *Proceedings of the Society for Experimental Biology and Medicine*. 1954;85(4):652-5.
120. AS V, Dhama K, Chakraborty S, Abdul Samad H, K. Latheef S, Sharun K, et al. Role of antisperm antibodies in infertility, pregnancy, and potential for contraceptive and antifertility vaccine designs: Research progress and pioneering vision. *Vaccines*. 2019;7(3):116.
121. Sinisi AA, FINIZIO BD, Pasquali D, Scurini C, D'apuzzo A, Bellastella A. Prevalence of antisperm antibodies by SpermMARtest in subjects undergoing a routine sperm analysis for infertility. *International journal of andrology*. 1993;16(5):311-4.
122. Marconi M, Pilatz A, Wagenlehner F, Diemer T, Weidner W. Are antisperm antibodies really associated with proven chronic inflammatory and infectious diseases of the male reproductive tract? *European urology*. 2009;56(4):708-15.
123. Mazumdar S, Levine AS. Antisperm antibodies: etiology, pathogenesis, diagnosis, and treatment. *Fertility and sterility*. 1998;70(5):799-810.
124. Eggert-Kruse W, Rohr G, Probst S, Rusu R, Hund M, Demirakca T, et al. Antisperm antibodies and microorganisms in genital secretions—a clinically significant relationship? *Andrologia*. 1998;30(S1):61-71.
125. Eggert-Kruse W, Buhlinger-Göpfarth N, Rohr G, Probst S, Aufenanger J, Näher H, Runnebaum B. Immunology: Antibodies to Chlamydia trachomatis in semen and relationship with parameters of male fertility. *Human reproduction*. 1996;11(7):1408-17.
126. Kortebani G, Gonzales G, Barrera C, Mazzolli A. Leucocyte populations in semen and male accessory gland function: relationship with antisperm antibodies and seminal quality. *Andrologia*. 1992;24(4):197-204.
127. Komori K, Tsujimura A, Miura H, Shin M, Takada T, Honda M, et al. Serial follow-up study of serum testosterone and antisperm antibodies in patients with non-obstructive azoospermia after conventional or microdissection testicular sperm extraction. *international journal of andrology*. 2004;27(1):32-6.
128. Kendirci M, Hellstrom WJ. Antisperm antibodies and varicocele. *Southern medical journal*. 2006;99(1):13-5.
129. Jiang Y, Cui D, Du Y, Lu J, Yang L, Li J, et al. Association of anti-sperm antibodies with chronic prostatitis: a systematic review and meta-analysis. *Journal of Reproductive Immunology*. 2016;118:85-91.
130. Condorelli R, Russo GI, Calogero A, Morgia G, La Vignera S. Chronic prostatitis and its detrimental impact on sperm parameters: a systematic review and meta-analysis. *Journal of Endocrinological Investigation*. 2017;40:1209-18.
131. Piroozmand A, Nasab SDM, Erami M, Hashemi SMA, Khodabakhsh E, Ahmadi N, Vahedpoor Z. Distribution of human papillomavirus and antisperm antibody in semen and its association with semen parameters among infertile men. *Journal of Reproduction & Infertility*. 2020;21(3):183.
132. Zini A, Fahmy N, Belzile E, Ciampi A, Al-Hathal N, Kotb A. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis. *Human reproduction*. 2011;26(6):1288-95.

133. Zini A, Lefebvre J, Kornitzer G, Bissonnette F, Kadoch IJ, Dean N, Phillips S. Anti-sperm antibody levels are not related to fertilization or pregnancy rates after IVF or IVF/ICSI. *Journal of reproductive immunology*. 2011;88(1):80-4.
134. Clarke GN. Etiology of sperm immunity in women. *Fertility and sterility*. 2009;91(2):639-43.
135. Djaladat H, Mehraei A, Rezazade M, Djaladat Y, Pourmand G. Varicocele and antisperm antibody: fact or fiction? *Southern medical journal*. 2006;99(1):44-7.
136. Marín-Briggiler CI, Vazquez-Levin MH, Gonzalez-Echeverría F, Blaquier JA, Miranda PV, Tezón JG. Effect of antisperm antibodies present in human follicular fluid upon the acrosome reaction and sperm–zona pellucida interaction. *American Journal of Reproductive Immunology*. 2003;50(3):209-19.
137. Taneichi A, Shibahara H, Takahashi K, Sasaki S, Kikuchi K, Sato I, Yoshizawa M. Effects of sera from infertile women with sperm immobilizing antibodies on fertilization and embryo development in vitro in mice. *American Journal of Reproductive Immunology*. 2003;50(2):146-51.
138. Francavilla F, Santucci R, Barbonetti A, Francavilla S. Naturally-occurring antisperm antibodies in men: interference with fertility and clinical implications. An update. *Frontiers in Bioscience-Landmark*. 2007;12(8):2890-911.
139. Menkveld R. Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. *Asian journal of andrology*. 2010;12(1):47.
140. Heidenreich A, Bonfig R, Wilbert DM, Strohmaier WL, Engelmann UH. Risk factors for antisperm antibodies in infertile men. *American Journal of Reproductive Immunology*. 1994;31(2-3):69-76.
141. Dimitrov D, Urbanek V, Zvěřina J, Madar J, Nouza K, Kinský R. Correlation of asthenozoospermia with increased antisperm cell-mediated immunity in men from infertile couples. *Journal of reproductive immunology*. 1994;27(1):3-12.
142. Verón GL, Molina RI, Tissera AD, Estofan GM, Marín-Briggiler CI, Vazquez-Levin MH. Incidence of sperm surface autoantibodies and relationship with routine semen parameters and sperm kinematics. *American Journal of Reproductive Immunology*. 2016;76(1):59-69.
143. Cui D, Han G, Shang Y, Liu C, Xia L, Li L, Yi S. Antisperm antibodies in infertile men and their effect on semen parameters: a systematic review and meta-analysis. *Clinica Chimica Acta*. 2015;444:29-36.
144. Lédée N, Petitbarat M, Prat-Ellenberg L, Dray G, Cassuto G, Chevrier L, et al. The uterine immune profile: A method for individualizing the management of women who have failed to implant an embryo after IVF/ICSI. *Journal of Reproductive Immunology*. 2020;142:103207.
145. Qi X, Lei M, Qin L, Xie M, Zhao D, Wang J. Endogenous TWEAK is critical for regulating the function of mouse uterine natural killer cells in an immunological model of pregnancy loss. *Immunology*. 2016;148(1):70-82.
146. Petitbarat M, Rahmati M, Sérazin V, Dubanchet S, Morvan C, Wainer R, et al. TWEAK appears as a modulator of endometrial IL-18 related cytotoxic activity of uterine natural killers. *PLoS One*. 2011;6(1):e14497.
147. Petitbarat M, Serazin V, Dubanchet S, Wayner R, de Mazancourt P, Chaouat G, Lédée N. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK)/fibroblast growth factor inducible-14 might regulate the effects of interleukin 18 and 15 in the human endometrium. *Fertility and sterility*. 2010;94(3):1141-3.
148. Winkles JA. The TWEAK–Fn14 cytokine–receptor axis: discovery, biology and therapeutic targeting. *Nature reviews Drug discovery*. 2008;7(5):411-25.
149. Croy B, Gambel P, Rossant J, Wegmann T. Characterization of murine decidual natural killer (NK) cells and their relevance to the success of pregnancy. *Cellular immunology*. 1985;93(2):315-26.
150. Chaouat G, Ledee-bataille N, Zourbas S, Dubanchet S, Sandra O, Martal J, et al. Implantation: can immunological parameters of implantation failure be of interest for pre-eclampsia? *Journal of reproductive immunology*. 2003;59(2):205-17.
151. Lédée-Bataille N, Bonnet-Chea K, Hosny G, Dubanchet S, Frydman R, Chaouat G. Role of the endometrial tripod interleukin-18,-15, and-12 in inadequate uterine receptivity in patients with a history of repeated in vitro fertilization–embryo transfer failure. *Fertility and sterility*. 2005;83(3):598-605.
152. Zhao Y, Niu C, Cui J. Gamma-delta ($\gamma\delta$) T cells: friend or foe in cancer development? *Journal of translational medicine*. 2018;16(1):1-13.
153. Manaster I, Mizrahi S, Goldman-Wohl D, Sela HY, Stern-Ginossar N, Lankry D, et al. Endometrial NK cells are special immature cells that await pregnancy. *The Journal of Immunology*. 2008;181(3):1869-76.
154. Cheloufi M, Kazhalawi A, Pinton A, Rahmati M, Chevrier L, Prat-Ellenberg L, et al. The endometrial immune profiling may positively affect the management of recurrent pregnancy loss. *Frontiers in Immunology*. 2021;12:656701.
155. Lédée N, Petitbarat M, Chevrier L, Vitoux D, Vezmar K, Rahmati M, et al. The uterine immune profile may help women with repeated unexplained embryo implantation failure after in vitro fertilization. *American Journal of Reproductive Immunology*. 2016;75(3):388-401.

156. Okada H, Tsuzuki T, Murata H. Decidualization of the human endometrium. *Reproductive medicine and biology*. 2018;17(3):220-7.
157. Brar AK, Frank GR, Kessler CA, Cedars MI, Handwerger S. Progesterone-dependent decidualization of the human endometrium is mediated by cAMP. *Endocrine*. 1997;6:301-7.
158. Brighton PJ, Maruyama Y, Fishwick K, Vrljicak P, Tewary S, Fujihara R, et al. Clearance of senescent decidual cells by uterine natural killer cells in cycling human endometrium. *elife*. 2017;6:e31274.
159. Feroze-Zaidi F, Fusi L, Takano M, Higham J, Salker MS, Goto T, et al. Role and regulation of the serum-and glucocorticoid-regulated kinase 1 in fertile and infertile human endometrium. *Endocrinology*. 2007;148(10):5020-9.
160. Thorens B, Mueckler M. Glucose transporters in the 21st Century. *American Journal of Physiology-Endocrinology and Metabolism*. 2010;298(2):E141-E5.
161. Russell P, Sacks G, Tremellen K, Gee A. The distribution of immune cells and macrophages in the endometrium of women with recurrent reproductive failure. III: Further observations and reference ranges. *Pathology*. 2013;45(4):393-401.
162. Rätsep MT, Felker AM, Kay VR, Tolusso L, Hofmann AP, Croy BA. Uterine natural killer cells: supervisors of vasculature construction in early decidua basalis. *Reproduction*. 2015;149(2):R91-R102.
163. Gaynor LM, Colucci F. Uterine natural killer cells: functional distinctions and influence on pregnancy in humans and mice. *Frontiers in immunology*. 2017;8:467.
164. Kitaya K, Yasuda J, Yagi I, Tada Y, Fushiki S, Honjo H. IL-15 expression at human endometrium and decidua. *Biology of reproduction*. 2000;63(3):683-7.
165. Okada S, Okada H, Sanesumi M, Nakajima T, Yasuda K, Kanzaki H. Expression of interleukin-15 in human endometrium and decidua. *Molecular human reproduction*. 2000;6(1):75-80.
166. Kajihara T, Brosens JJ, Ishihara O. The role of FOXO1 in the decidual transformation of the endometrium and early pregnancy. *Medical molecular morphology*. 2013;46:61-8.
167. Vasquez YM, Wang X, Wetendorf M, Franco HL, Mo Q, Wang T, et al. FOXO1 regulates uterine epithelial integrity and progesterone receptor expression critical for embryo implantation. *PLoS genetics*. 2018;14(11):e1007787.
168. Ruan YC, Guo JH, Liu X, Zhang R, Tsang LL, Da Dong J, et al. Activation of the epithelial Na⁺ channel triggers prostaglandin E2 release and production required for embryo implantation. *Nature medicine*. 2012;18(7):1112-7.
169. Salker MS, Christian M, Steel JH, Nautiyal J, Lavery S, Trew G, et al. Deregulation of the serum-and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. *Nature medicine*. 2011;17(11):1509-13.
170. von Wolff M, Ursel S, Hahn U, Steldinger R, Strowitzki T. Glucose transporter proteins (GLUT) in human endometrium: expression, regulation, and function throughout the menstrual cycle and in early pregnancy. *The Journal of Clinical Endocrinology & Metabolism*. 2003;88(8):3885-92.
171. Dambaeva S, Bilal M, Schneiderman S, Germain A, Fernandez E, Kwak-Kim J, et al. Decidualization score identifies an endometrial dysregulation in samples from women with recurrent pregnancy losses and unexplained infertility. *F&S Reports*. 2021;2(1):95-103.
172. Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and clinical aspects of human herpesvirus 6 infections. *Clinical microbiology reviews*. 2015;28(2):313-35.
173. Braun DK, Dominguez G, Pellett PE. Human herpesvirus 6. *Clinical microbiology reviews*. 1997;10(3):521-67.
174. De Bolle L, Naesens L, De Clercq E. Update on human herpesvirus 6 biology, clinical features, and therapy. *Clinical microbiology reviews*. 2005;18(1):217-45.
175. Komaroff AL, Rizzo R, Ecker JL. Human Herpesviruses 6A and 6B in reproductive diseases. *Frontiers in Immunology*. 2021;12:648945.
176. Komaroff AL, Pellett PE, Jacobson S. Human herpesviruses 6a and 6b in brain diseases: Association versus causation. *Clinical microbiology reviews*. 2020;34(1):10.1128/cmr.00143-20.
177. Marci R, Gentili V, Bortolotti D, Lo Monte G, Caselli E, Bolzani S, et al. Presence of HHV-6A in endometrial epithelial cells from women with primary unexplained infertility. *PloS one*. 2016;11(7):e0158304.
178. Okuno T, Oishi H, Hayashi K, Nonogaki M, Tanaka K, Yamanishi K. Human herpesviruses 6 and 7 in cervixes of pregnant women. *Journal of clinical microbiology*. 1995;33(7):1968-70.
179. Caserta MT, Hall CB, Schnabel K, Lofthus G, McDermott MP. Human herpesvirus (HHV)-6 and HHV-7 infections in pregnant women. *The Journal of infectious diseases*. 2007;196(9):1296-303.
180. Flamand L, Gosselin J, D'addario M, Hiscott J, Ablashi D, Gallo R, Menezes J. Human herpesvirus 6 induces interleukin-1 beta and tumor necrosis factor alpha, but not interleukin-6, in peripheral blood mononuclear cell cultures. *Journal of virology*. 1991;65(9):5105-10.
181. TAKAHASHI K, SEGAL E, MUKAI T, MORIYAMA M, TAKAHASHI M, YAMANISHI K. Interferon and

natural killer cell activity in patients with exanthem subitum. *The Pediatric infectious disease journal*. 1992;11(5):369-73.

182. Arena A, Capozza A, Di Luca D. Role of IFN gamma on TNF alpha, IL-1 beta and IL-6 release during HHV-6 infection. *The New microbiologica*. 1996;19(3):183-91.

183. Mayne M, Cheadle C, Soldan SS, Cermelli C, Yamano Y, Akhyani N, et al. Gene expression profile of herpesvirus-infected T cells obtained using immunomicroarrays: induction of proinflammatory mechanisms. *Journal of virology*. 2001;75(23):11641-50.

184. Tallóczy Z, Virgin I, Herbert, Levine B. PKR-dependent xenophagic degradation of herpes simplex virus type 1. *Autophagy*. 2006;2(1):24-9.

185. Flamand L, Stefanescu I, Menezes J. Human herpesvirus-6 enhances natural killer cell cytotoxicity via IL-15. *The Journal of clinical investigation*. 1996;97(6):1373-81.

186. Meeuwssen S, Persoon-Deen C, Bsibsi M, Bajramovic JJ, Ravid R, De Bolle L, van Noort JM. Modulation of the cytokine network in human adult astrocytes by human herpesvirus-6A. *Journal of neuroimmunology*. 2005;164(1-2):37-47.

187. Rizzo R, Soffritti I, D'Accolti M, Bortolotti D, Di Luca D, Caselli E. HHV-6A/6B infection of NK cells modulates the expression of miRNAs and transcription factors potentially associated to impaired NK activity. *Frontiers in Microbiology*. 2017;8:2143.

188. Rizzo R, Di Luca D. Human herpesvirus 6A and 6B and NK cells. *Acta Microbiologica et Immunologica Hungarica*. 2018;65(2):119-25.

189. Gaccioli F, Lager S, de Goffau MC, Sovio U, Dopierala J, Gong S, et al. Fetal inheritance of chromosomally integrated human herpesvirus 6 predisposes the mother to pre-eclampsia. *Nature microbiology*. 2020;5(7):901-8.

190. Miura H, Kawamura Y, Ohye T, Hattori F, Kozawa K, Ihira M, et al. Inherited chromosomally integrated human herpesvirus 6 is a risk factor for spontaneous abortion. *The Journal of infectious diseases*. 2021;223(10):1717-23.

191. Bunting KL, Melnick AM. New effector functions and regulatory mechanisms of BCL6 in normal and malignant lymphocytes. *Current opinion in immunology*. 2013;25(3):339-46.

192. Cardenas MG, Oswald E, Yu W, Xue F, MacKerell Jr AD, Melnick AM. The expanding role of the BCL6 oncoprotein as a cancer therapeutic target. *Clinical Cancer Research*. 2017;23(4):885-93.

193. Shaffer A, Yu X, He Y, Boldrick J, Chan EP, Staudt LM. BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. *Immunity*. 2000;13(2):199-212.

194. Evans-Hoeker E, Lessey BA, Jeong JW, Savaris RF, Palomino WA, Yuan L, et al. Endometrial BCL6 overexpression in eutopic endometrium of women with endometriosis. *Reproductive Sciences*. 2016;23(9):1234-41.

195. Almquist LD, Likes CE, Stone B, Brown KR, Savaris R, Forstein DA, et al. Endometrial BCL6 testing for the prediction of in vitro fertilization outcomes: a cohort study. *Fertility and sterility*. 2017;108(6):1063-9.

196. Ren Z, Gao Y, Gao Y, Liang G, Chen Q, Jiang S, et al. Distinct placental molecular processes associated with early-onset and late-onset preeclampsia. *Theranostics*. 2021;11(10):5028.

197. Guo F, Zhang B, Yang H, Fu Y, Wang Y, Huang J, et al. Systemic transcriptome comparison between early-And late-onset pre-eclampsia shows distinct pathology and novel biomarkers. *Cell Proliferation*. 2021;54(2):e12968.

198. Waldmann H, Melton PE, Manfredi AA, Than NG, Romero R, Papp Z, et al. integrated systems Biology approach identifies novel Maternal and Placental Pathways of Preeclampsia. *Fetal-Maternal Immune Interactions in Pregnancy*. 2020.

199. Brew O, Sullivan MH, Woodman A. Comparison of normal and pre-eclamptic placental gene expression: a systematic review with meta-analysis. *PloS one*. 2016;11(8):e0161504.

200. Vaiman D, Calicchio R, Miralles F. Landscape of transcriptional deregulations in the preeclamptic placenta. *PloS one*. 2013;8(6):e65498.

201. Maltepe E, Keith B, Arsham AM, Brorson JR, Simon MC. The role of ARNT2 in tumor angiogenesis and the neural response to hypoxia. *Biochemical and biophysical research communications*. 2000;273(1):231-8.

202. Chakraborty D, Cui W, Rosario GX, Scott RL, Dhakal P, Renaud SJ, et al. HIF-KDM3A-MMP12 regulatory circuit ensures trophoblast plasticity and placental adaptations to hypoxia. *Proceedings of the National Academy of Sciences*. 2016;113(46):E7212-E21.

203. Rosario GX, Konno T, Soares MJ. Maternal hypoxia activates endovascular trophoblast cell invasion. *Developmental biology*. 2008;314(2):362-75.

204. Nezhat C, Rambhatla A, Miranda-Silva C, Asaia A, Nguyen K, Eyvazzadeh A, et al. BCL-6 overexpression as a predictor for endometriosis in patients undergoing in vitro fertilization. *JSLS: Journal of the Society of Laparoscopic & Robotic Surgeons*. 2020;24(4).

205. Prapas Y, Goudakou M, Matalliotakis I, Kalogeraki A, Matalliotaki C, Panagiotidis Y, et al. History of endometriosis may adversely affect the outcome in menopausal recipients of sibling oocytes. *Reproductive biomedicine online*. 2012;25(5):543-8.