BRAVERMAN
IVF & Reproductive Immunology, P.C.
Answers for Infertility & Recurrent Pregnancy Loss

800 Woodbury Road, Suite G
Woodbury, NY 11797
T: 516.584.8710
F: 516.584.8711
www.reproductiveimmunology.com
www.preventmiscarriage.com

Patient Consult Report

Clinical History

- Currently 34 years old
- History of 3 chemical pregnancies all resulting from IVF cycles
- History of Sjogren's syndrome
- History of antiphospholipid syndrome (APS)
- History of hypothyroidism – takes synthroid (50 mcg)
- History of 20 eggs retrieved with 1 IVF cycle
- History of moderate pain with menstruation
- Family history of psoriasis (sister) and celiac disease (grandmother)
    - Mother also has autoimmune inner ear disease
- Family history of miscarriages
- Family history of thyroid disease (grandmother), blood clots/strokes, child with ASD, and breast cancer
- Partner with history of varicocele treated surgically
- Partner uses chewing tobacco

KIR Analysis

- HLA-C
    - You
        - 07, 07
        - C1/C1
    - Partner
        - 07, 07
        - C1/C1
- Maternal KIR haplotype
    - A and B haplotype genes present so KIR AB haplotype
    - KIR2DS1 present
- Assessment
    - KIR AB haplotype with KIR2DS1 puts you in the protective effect category

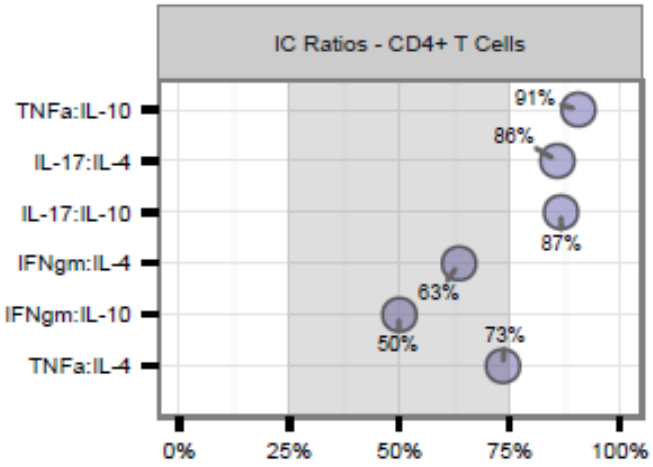
HLA Analysis

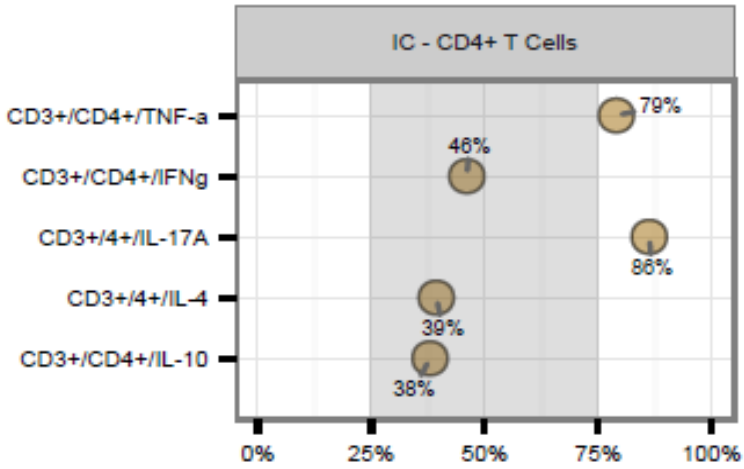
NOTE: The analysis below indicates HLA alleles and haplotypes that have been shown to predispose to certain autoimmune conditions. The presence of one or more predisposing HLA alleles/haplotypes is not diagnostic of the existence of an autoimmune condition. However, when combined with other analysis in this report, the presence of specific alleles/haplotypes can provide valuable insight into the state of the patient's immune system that could be contributing to failure to initiate or maintain pregnancy. HLA alleles and haplotypes that contribute to autoimmunity may also directly lead to an inability to appropriately establish maternal immune tolerance to an embryo or fetus.

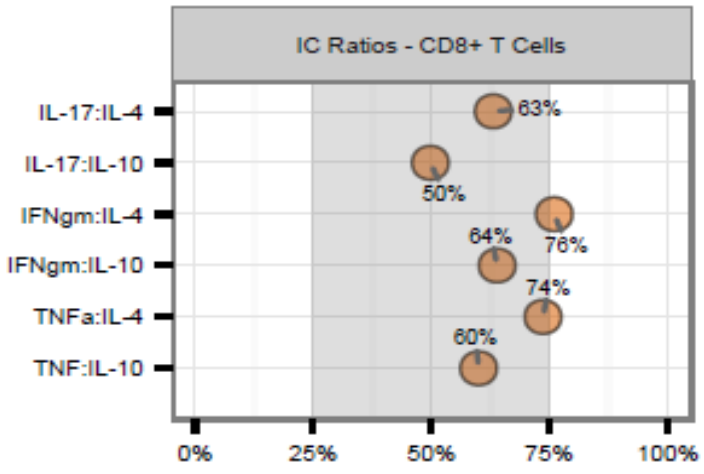
- Partial lack of class II allele mismatching
    - 1 total class II mismatch (out of 6)
    - 1 HLA-DRB1 mismatch (out of 2)
    - 1 HLA-DR supertype mismatch (out of 2)

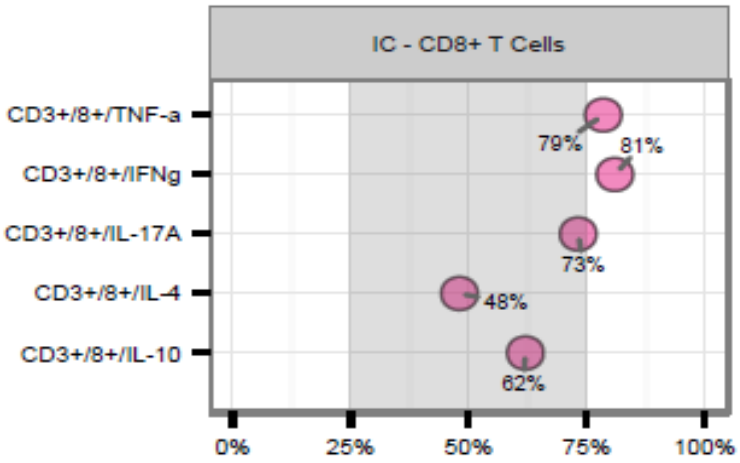
- Significant homozygosity of class II alleles
  - Partner is homozygous for all class II alleles
- You harbor the complete 8.1 extended haplotype which includes DRB1*03:01 in combination with DQB1*02:01
  - DQB1*02:01 in combination with DRB1*03:01 (HLA DR3-DQ2) is a haplotype attributed to a significant proportion of the predisposition to autoimmunity in humans. Autoimmune diseases that this haplotype predispose to include, but are not limited to, myasthenia gravis, systemic lupus erythematosus, Sjogren's, sarcoidosis, autoimmune hepatitis, and inclusion body myositis.
- You harbor an extended HLA haplotype consisting of DQA1*01:02, DQB1*06:04 and DRB1*13:02 that is associated with development of early-onset myasthenia gravis and systemic sclerosis
- You have 1 copy of B*18 which has been associated with complement C2 deficiency which can lead to autoimmunity
- You harbor DQ2 (DQA1*05 with DQB1*02) which is predisposing for celiac disease
  - Celiac disease cannot be excluded and further testing may be warranted
- HLA-G 14bp ins
  - You are homozygous for the HLA-G 14bp ins allele
- HLA class II HY restricting alleles (class II HYrHLA)
  - None

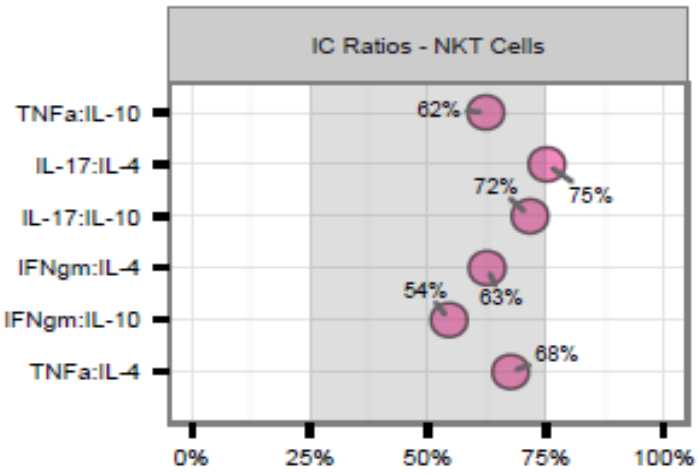
Intracellular Cytokine Analysis (IM-Xpress)

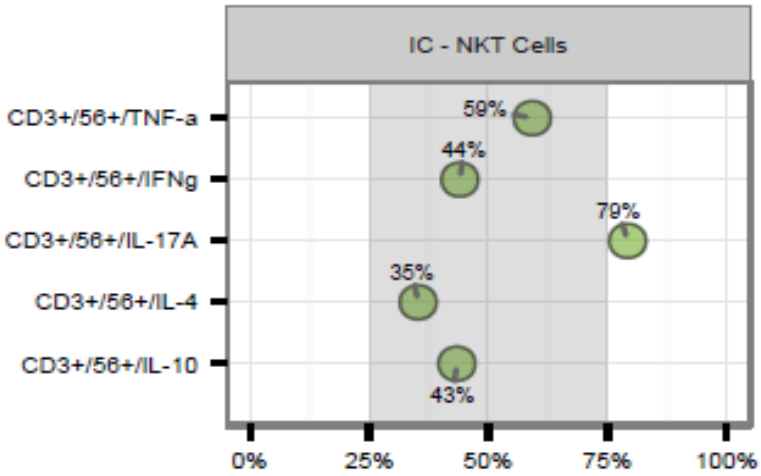


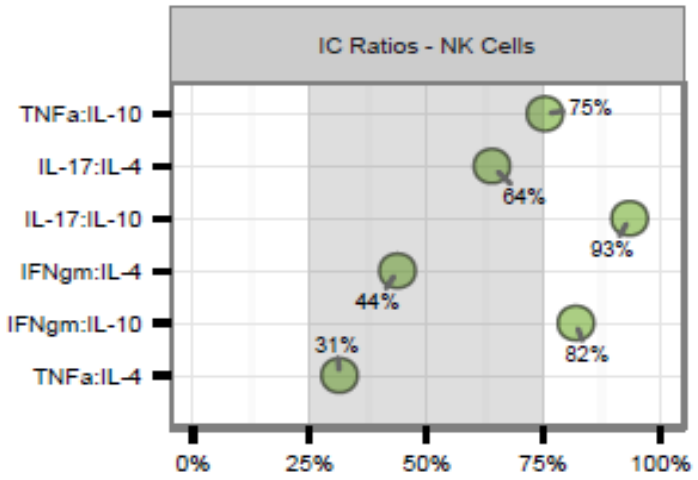


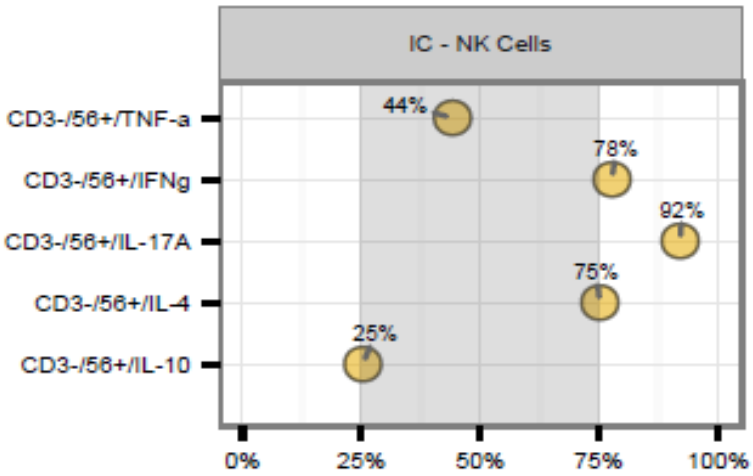






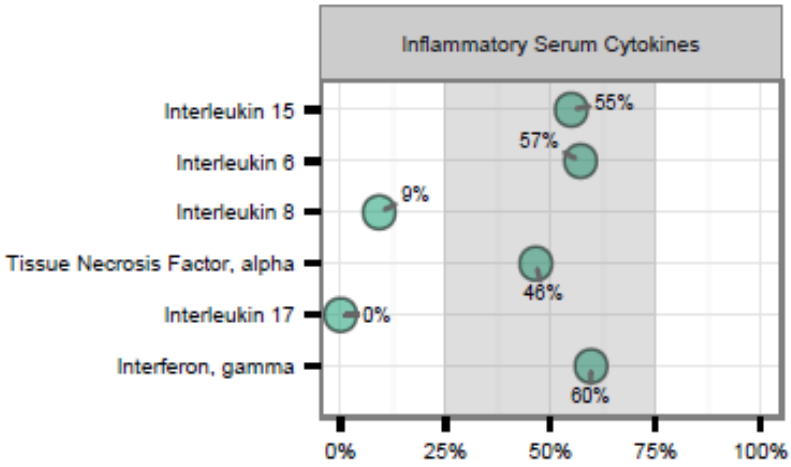


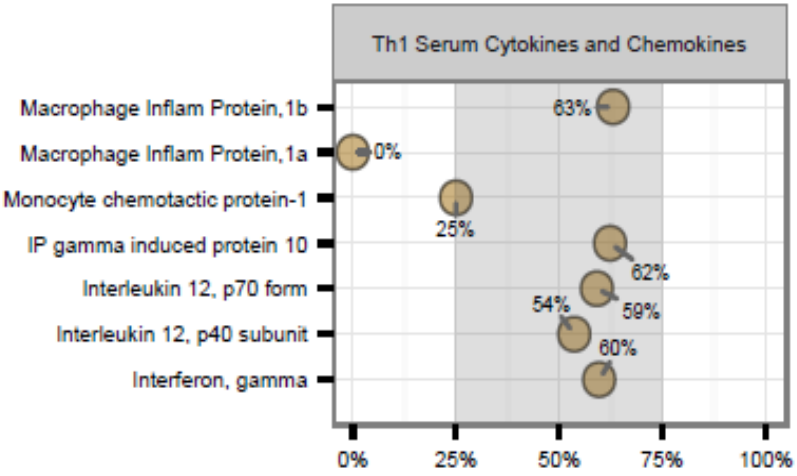


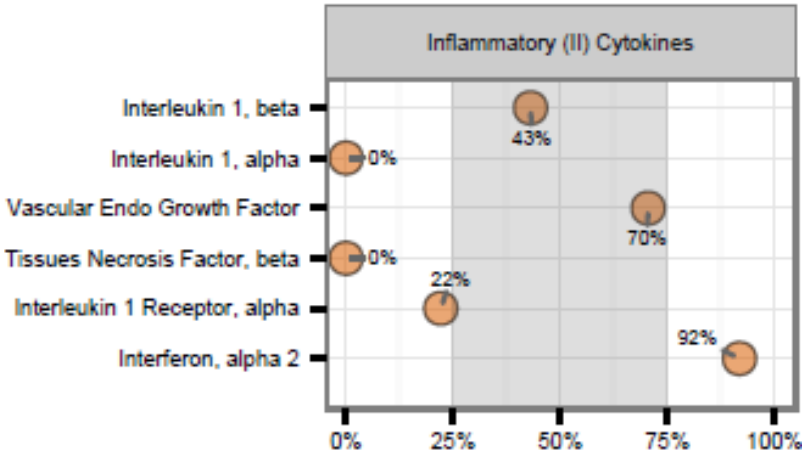


- Your intracellular cytokine (IC) ratios indicate a borderline Th1 bias with a CD4+ T cell IFNγ:IL-4 ratio in the 64th percentile. Together with the borderline Th1 bias, all of your CD4+ T cell TNFα and IL-17 IC ratios are elevated, as well as your CD8+ T cell TNFα:IL-4 and IFNγ:IL-4 IC ratios, your NKT cell IL-17 IC ratios, and all NK cell IL-10 IC ratios (TNFα:IL-10, IFNγ:IL-10, IL-17:IL-10). These elevated IC ratios are partially a function of elevated levels of TNFα positive CD4+ and CD8+ T cells, IFNγ positive CD8+ T cells and NK cells, and IL-17 positive CD4+ T cells, NKT cells, and NK cells.

Serum Cytokines Analysis

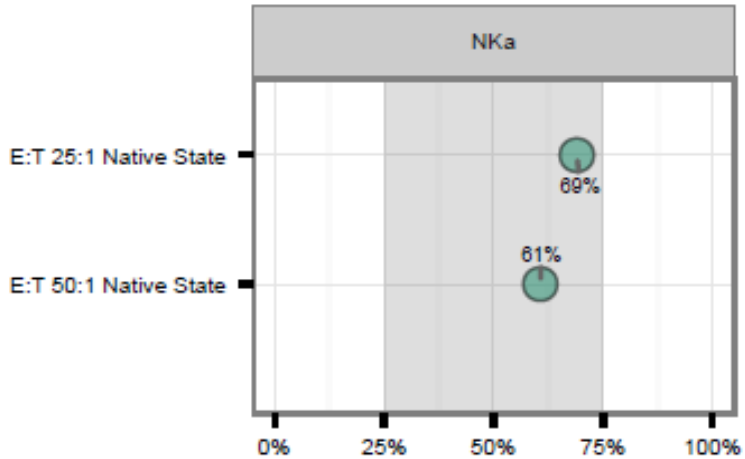






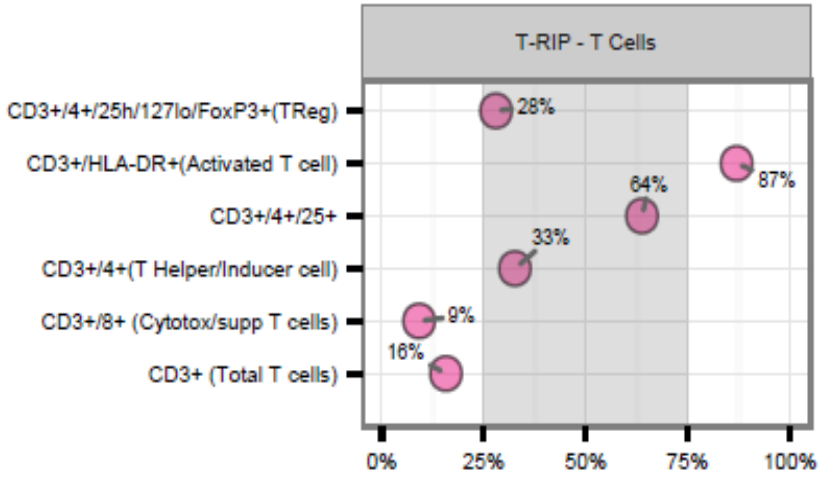
- You have elevated serum levels of IFNα2 and GM-CSF.

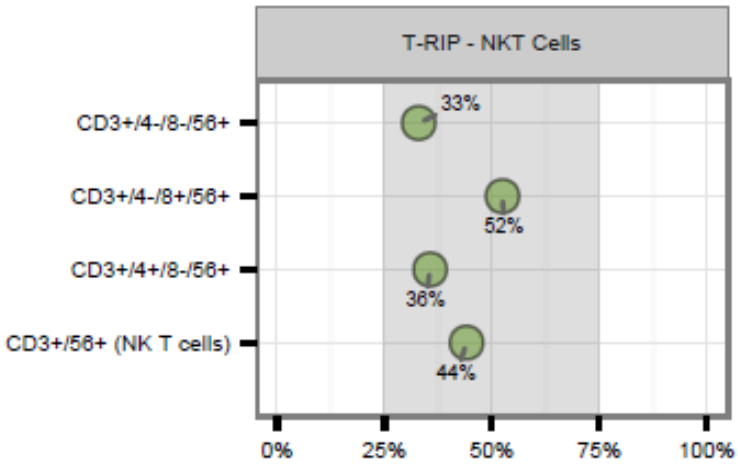
NK Cell Activity Analysis

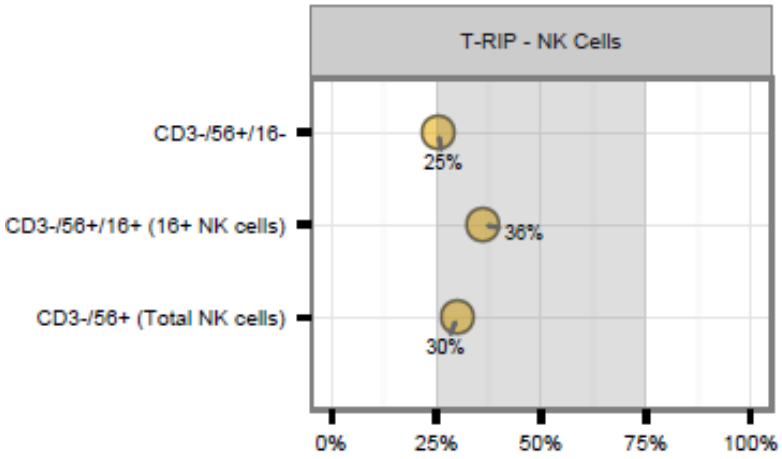


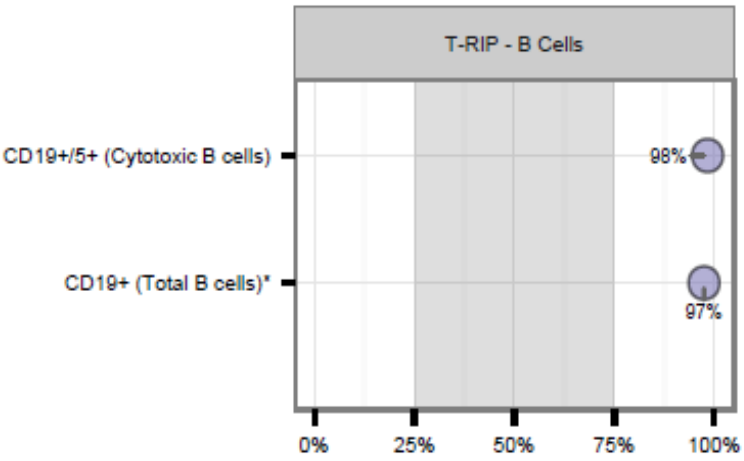
- Your NK cell cytotoxic activity (NKa) is within normal ranges.

Reproductive Immunophenotype and CBC Analysis









- T-RIP
  - You have highly elevated levels of total and CD5+ B cells, as well as an elevated level of HLA-DR+ T cells.
- CBC
  - WBCs = 4.0

Anti-HLA Antibodies Analysis

- Class I antibodies
  - 4K-10K MFI:
    - B*44:02
  - 1.5K-4K MFI:
    - B45
  - Partner-specific
    - None
- Class II antibodies
  - None

Other

- Strong positive for:
  - ANA-LA(SS-B) (82.69)
  - ANA-RO(SS-A) (175.8)
- Borderline for ANA-Histone (0.7)
- Positive for RF (96.0)
- Indeterminate levels of:
  - APhL IgG (17)
  - Anticardiolipin IgM (17)
  - Antiphosphatidylethanolamine IgG (18)
  - Antiphosphatidylserine IgM (19)
  - Antiphosphatidylglycerol IgM (22)
- Elevated total IgG (2830)
- Elevated total IgA (454)
- Low C4 complement activity (16)
- Heterozygous for the MTHFR Al298C polymorphism
- Normal total IgM (170)
- Normal total IgE (44)
- Normal C3 complement activity (87)
- Negative for anti-CCP antibodies (<16)
- Negative for anti-TPO/THAB
  - Anti-TPO = <1.0
  - THAB = <1.0
- Normal TSH (0.97)
- Negative for TSH receptor antibody (7.80)
- Total 25 Hydroxy Vit D sufficiency (41.6)
  - 25 Hydroxy Vit D2 = <5.0
  - 25 Hydroxy Vit D3 = 41.6
- Normal homocysteine (5.3)
- Negative (normal) for the MTHFR C677T polymorphism

Summary

You and your partner have a partial lack of HLA class II allele mismatching with only 1 total mismatched class II allele. A lack of HLA class II allele mismatching can lead to a deficiency in the ability of the maternal immune system to generate tolerance for paternal antigens present on the embryo. Your class II allele mismatch is at the DRB1 locus, however and it includes a DRB supertype mismatch indicating that the DRB1 allele mismatch is likely to be sufficiently antigenically distinct to be immunogenic. This partial lack of class II allele mismatching is due in part to your partner being homozygous for all class II loci (DQA1/DQB1/DRB1). Homozygosity of HLA class II alleles leads to a limitation in the repertoire of paternal class II antigens that can be presented to the maternal immune system which in turn leads to a deficiency in the ability of the maternal immune system to generate tolerance for paternal antigens found on the embryo.

You have a family history of autoimmune diseases and a personal history of Sjogren's syndrome, APS, and hypothyroidism, and you harbor several HLA alleles/haplotypes known to predispose to the development of various autoimmune conditions. These include the complete 8.1 extended haplotype which predisposes to the development of several autoimmune conditions including not limited to, myasthenia gravis, systemic lupus erythematosus, Sjogren's, sarcoidosis, autoimmune hepatitis, and inclusion body myositis. The 8.1 haplotype also included the DQ2 serotype (DQA1*05/DQB1*02) which predisposes to the development of celiac disease. You also harbor a copy of B*18 which is associated with complement C2 deficiency that can lead to

autoimmunity, and DQA1*01:02/DQB1*06:04/DRB1*13:02 which predisposes to the development of early-onset myasthenia gravis and systemic sclerosis. As stated above, these and other HLA predispositions are not diagnostic for the presence of one or more of these autoimmune conditions. However, HLA alleles and haplotypes are known to predispose to autoimmunity by inducing failure in mechanisms that promote tolerance for self-antigens. A failure in these same mechanisms can also cause a deficiency in the ability of the immune system to generate tolerance for non-self-antigens, such as when the maternal immune system is exposed to antigens present on an embryo of paternal origin.

You are also homozygous for the HLA-G 14bp ins allele. Homozygosity for this allele is found at increased frequency in women experiencing infertility, repeated implantation failure, and recurrent miscarriage, and leads to decreased expression of the tolerogenic HLA-G protein which results in a reduced ability of the maternal immune system to generate tolerance for paternal antigens.

You have elevated levels of TNFα positive CD4+ and CD8+ T cells, IFNγ positive CD8+ T cells and NK cells, and IL-17 positive CD4+ T cells, NKT cells, and NK cells. Largely as a result of these elevated levels of individual intracellular cytokine (IC) positive cells, your immune system has a borderline Th1 bias together with elevation of all CD4+ T cell TNFα and IL-17 IC ratios, your CD8+ T cell TNFα:IL-4 and IFNγ:IL-4 IC ratios, your NKT cell IL-17 IC ratios, and all NK cell IL-10 IC ratios. You also have highly elevated levels of total and CD5+ B cells, and an elevated level of HLA-DR+ T cells. Your serum levels of IFNα2 and GM-CSF are also elevated, indicative of an elevated level of systemic inflammation.

Consistent with your history of Sjogren's syndrome, you are strongly positive for ANA-LA(SS-B) and ANA-RO(SS-A), as well as borderline positive for ANA-Histone. Consistent with your history of APS, you also have indeterminate levels of APhL IgG, anticardiolipin IgM, antiphosphatidylethanolamine IgG, antiphosphatidylserine IgM, and antiphosphatidylglycerol IgM antiphospholipid antibodies (APAs).

You are also positive for rheumatoid factor (RF). Although elevated levels of RF can be associated with the presence of rheumatoid arthritis, RF can also be elevated in patients with other connective tissue autoimmune diseases including systemic lupus erythematosus (SLE), sarcoidosis, and Sjogren's syndrome, as well as with infectious diseases, including viral hepatitis, syphilis, tuberculosis, and infectious mononucleosis, as well as in liver disease, including autoimmune hepatitis.

Consistent with your elevated levels of ANAs, APAs, and RF, you also have elevated levels of total IgG and IgA. You also have low C4 complement activity. This is partially due to a null allele of C4 associated with the extended 8.1 haplotype, but likely also reflects elevated consumption of this factor due to peripheral complement cascade activation.

You are negative for partner-specific class I and class II anti-HLA antibodies.

You have a history of 3 chemical pregnancies and moderate pain with menstruation which is consistent with the possible presence of endometriosis. Your elevated levels of TNFα positive, IFNγ positive, and IL-17 positive NK cells, as well as elevated NK cell IL-10 IC ratios, are consistent with this possibility. Your history of hypothyroidism is also consistent with this possibility, as autoimmune thyroiditis is strongly associated with the presence of endometriosis in our patient population. Borderline positive levels of ANA-Histone and indeterminate/positive levels of APAs are also frequently found in our patients with endometriosis.

You have a history of 20 eggs retrieved with 1 IVF cycle and fasting bloodwork to investigate the possibility of PCOS is recommended.

You are heterozygous for the MTHFR Al298C polymorphism and use of a methylated form of folate during pregnancy could be considered.