BRAVERMAN
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Answers for Infertility & Recurrent Pregnancy Loss

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# Patient Consult Report

## Clinical History

- Currently 36 years old
- History of firstborn son now about 5 years old followed by secondary recurrent pregnancy loss with 9 losses (all between 6 and 11 weeks) over a 3-year period
- Family history of thyroid disease (mother)
- Family history of AODM (grandmother)

## KIR Analysis

- HLA-C
  - You
    - 01, 15
    - C1/C2
  - Partner
    - 04, 06
    - C2/C2
- Maternal KIR haplotype
  - Only A haplotype genes present so KIR AA haplotype
- Assessment
  - KIR AA haplotype puts you in the highest risk category.
    - OR 2.09 when fetus contains more C2 than mother
      - 50% chance in any given fetus
    - OR 1.43 when fetus contains same C2 as mother
      - 50% chance in any given fetus
    - OR 0.97 when fetus contains less C2 than mother
      - 0% chance in any given fetus

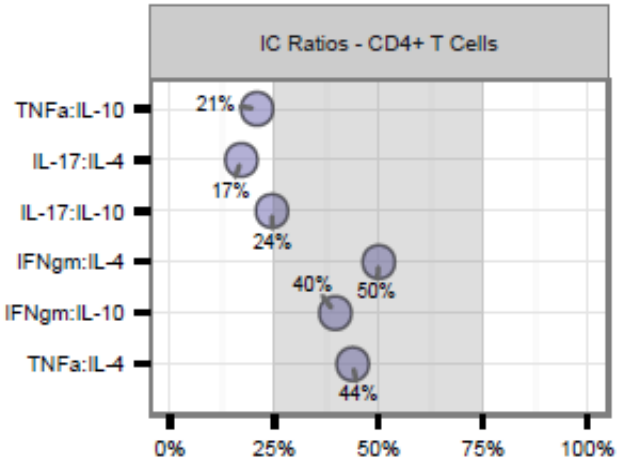
## HLA Analysis

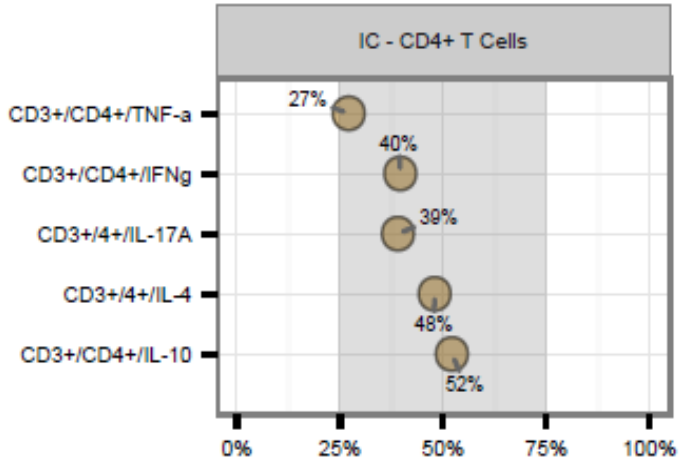
NOTE: The analysis below indicates HLA alleles and haplotypes that have been shown to predispose to certain autoimmune conditions. The presence of one or more predisposing HLA alleles/haplotypes is not diagnostic of the existence of an autoimmune condition. However, when combined with other analysis 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.

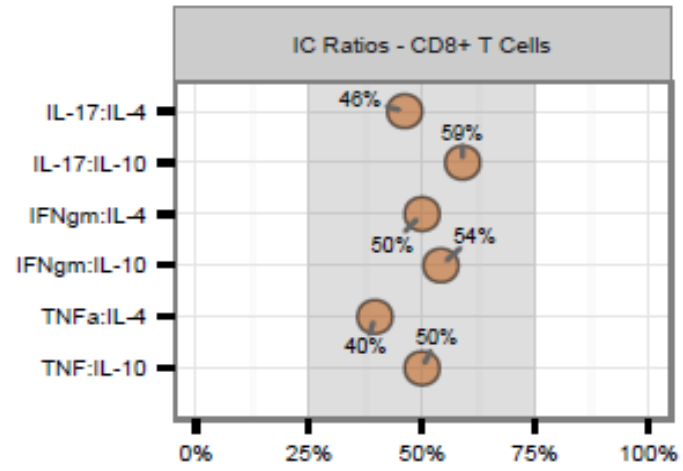
- No significant lack of class II allele mismatching
  - 5 total class II mismatches (out of 6)
  - 2 HLA-DRB1 mismatches (out of 2)
  - 1 HLA-DR supertype mismatch (out of 2)
- No significant homozygosity of class II alleles
  - You are homozygous at the DQA1 and DQB1 loci

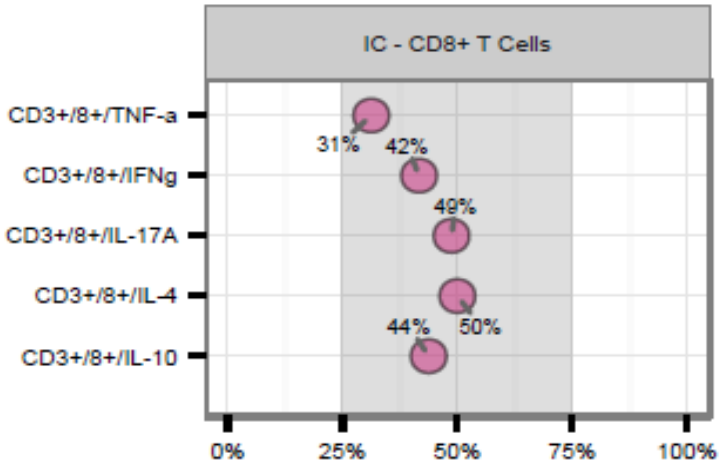
- You harbor DRB1*10:01 in combination with DQA1*01 and DQB1*05 which is associated with rheumatoid arthritis (Pascual, 2001)
- You harbor DRB1*16 and DQB1*05
    - DQB1*05 in combination with DRB1*16 has been found to be associated with a subgroup of myasthenia gravis patients positive for autoantibodies against muscle specific kinase (MuSK)
- HLA-G 14bp ins
    - You have 1 copy of the HLA-G 14bp ins allele
- HLA class II HY restricting alleles (class II HYrHLA)
    - DQB1*05:01 detected
    - DQB1*05:02 detected

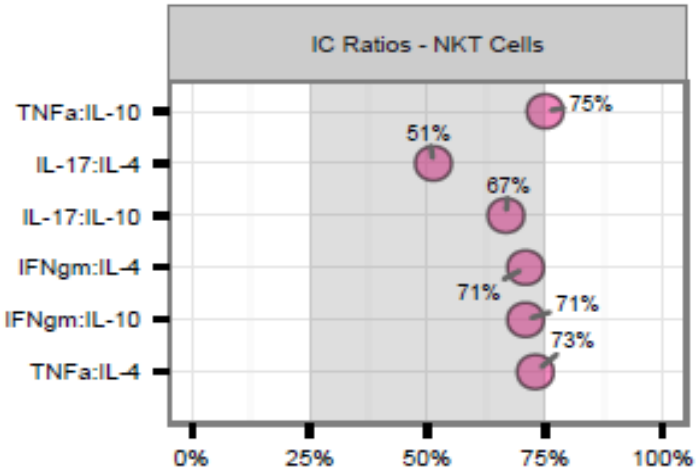
Intracellular Cytokine Analysis (IM-Xpress)

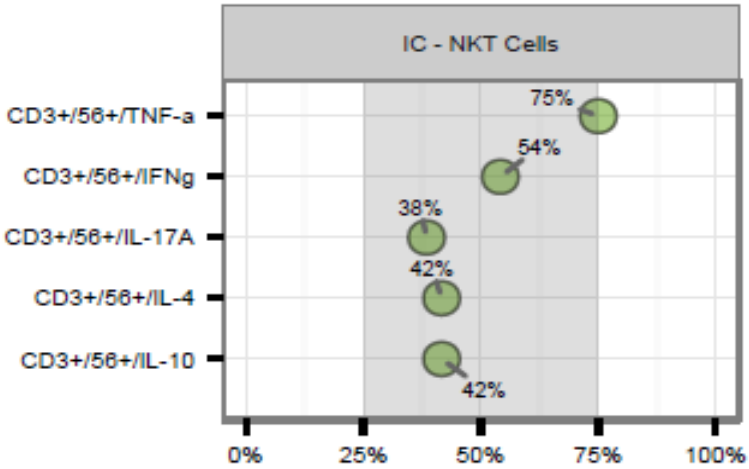


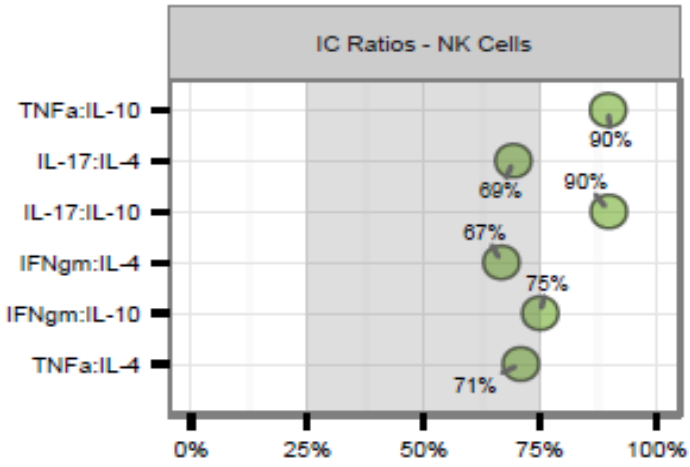



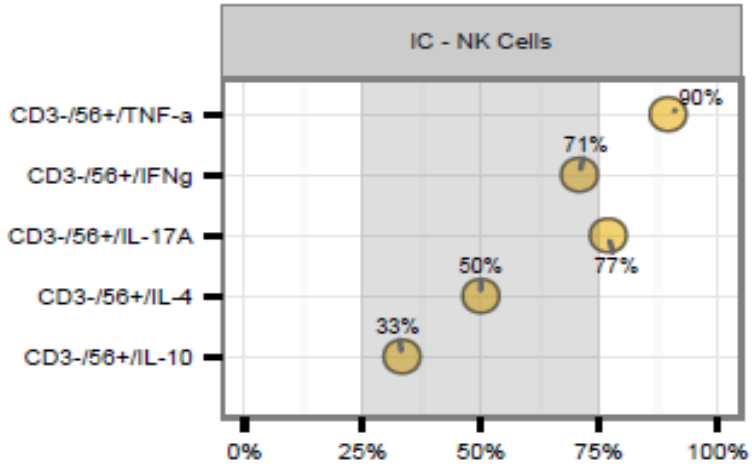


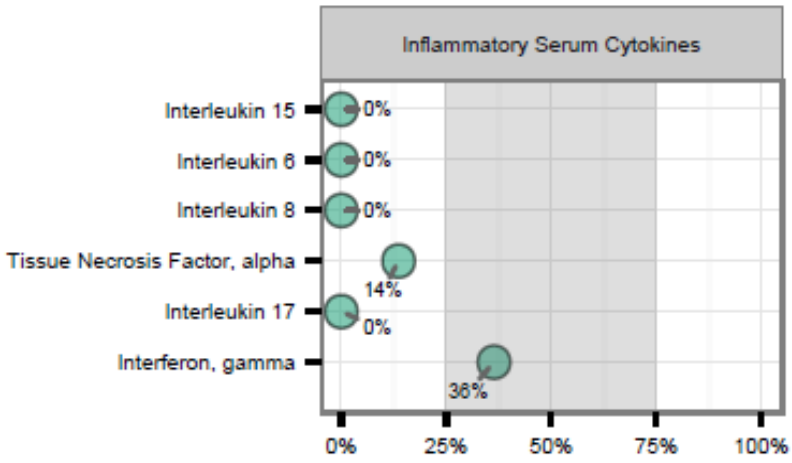



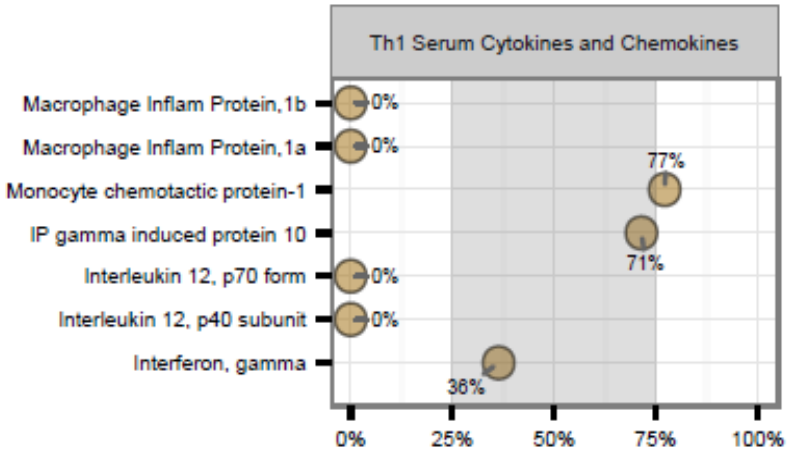



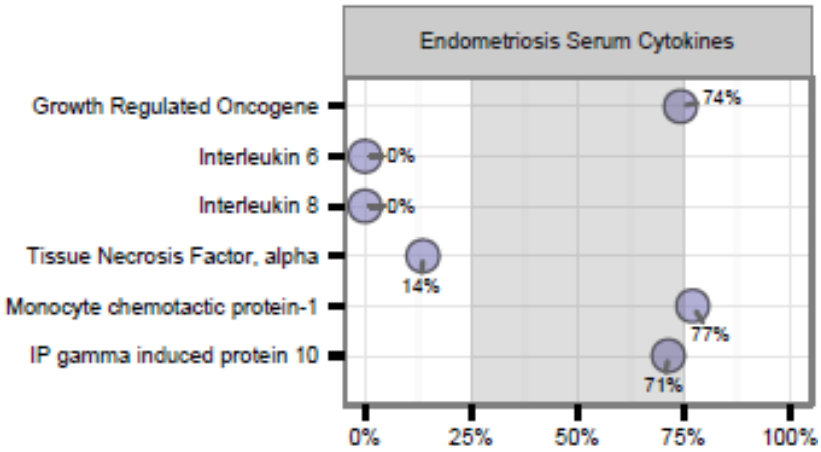




- Your intracellular cytokine (IC) ratios indicate a neutral Th1/Th2 balance with a CD4+ T cell IFNγ:IL-4 ratio in the 50$^{th}$ percentile. Together with the neutral Th1/Th2 balance, all of your CD4+ T cell, CD8+ T cell, and NKT cell IC ratios are within normal ranges or low. All of your NK cell IC ratios however are either mildly elevated or borderline elevated. This is largely a result of elevated levels of TNFα positive and IL-17 positive NK cells and a borderline elevated level of IFNγ positive NK cells.

Serum Cytokines Analysis

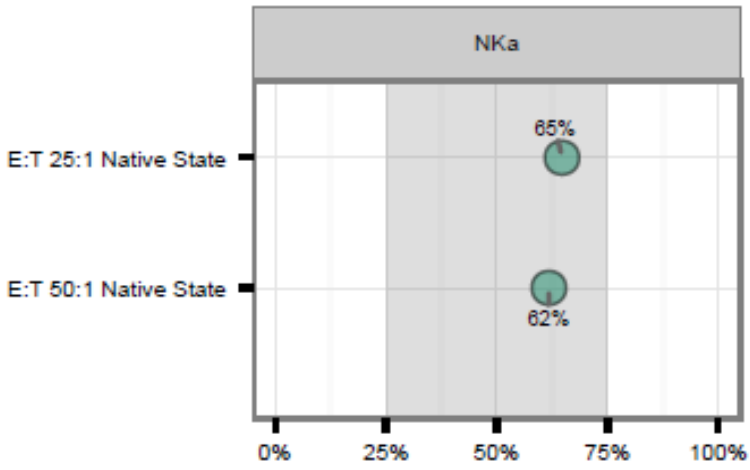


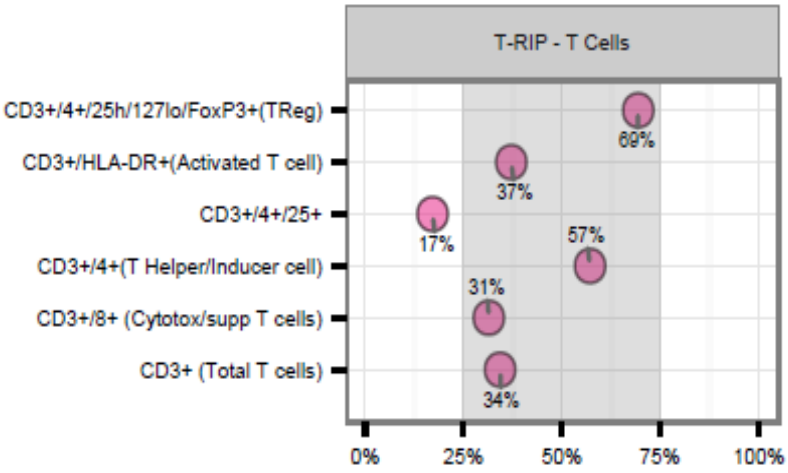

- You have borderline/mildly elevated serum levels of GRO, MCP-1, and IP-10. Serum levels of all other tested cytokines and chemokines are within normal ranges or low.

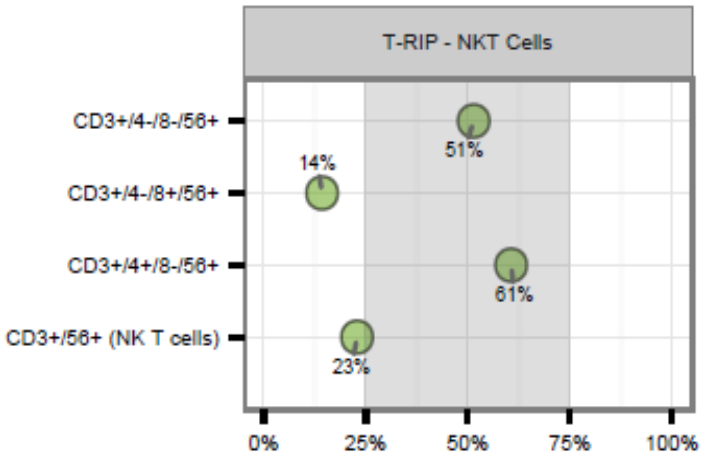
NK Cell Activity Analysis

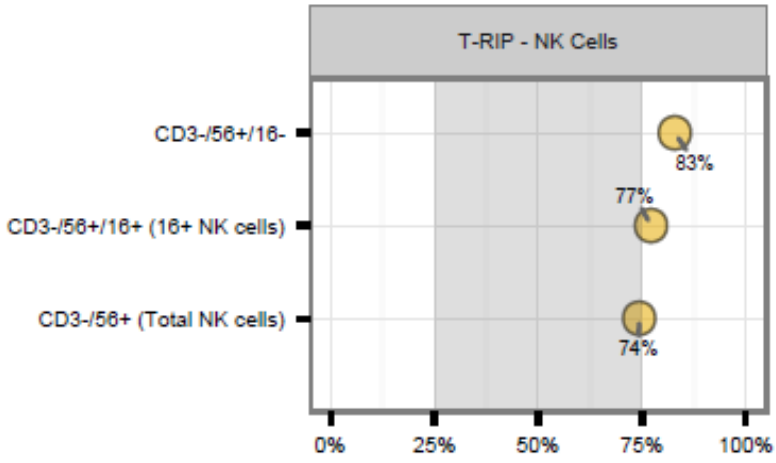


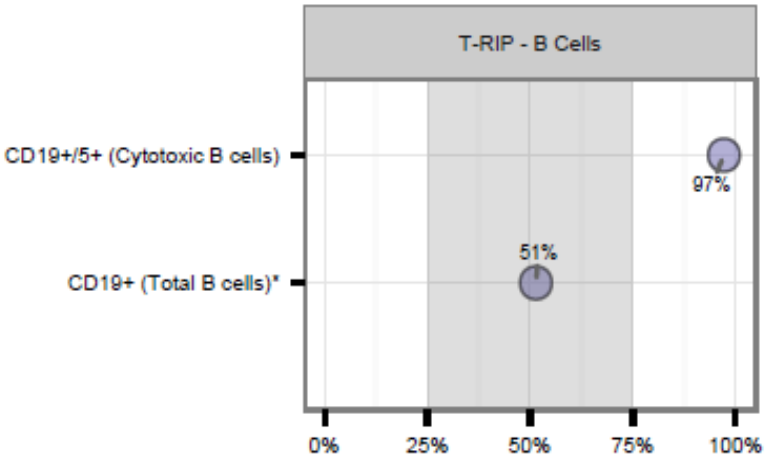

- Your NK cell cytotoxic activity (NKa) is within normal ranges.

Reproductive Immunophenotype and CBC Analysis




- T-RIP
  - You have mildly elevated levels of total and CD16+ NK cells. Your CD5+ B cells are also elevated.
- CBC
  - WBCs = 7.0

Anti-HLA Antibodies Analysis

- Class I antibodies
  - >10K MFI:
    - B76, B82, B45
  - 4K-10K MFI:
    - B44, Cw14, Cw4, Cw6, Cw18, Cw16, Cw5, Cw12, Cw1, A1
  - 1.5K-4K MFI:
    - B46, Cw9, Cw10, B73, B8, Cw4, Cw8, A69, A2, Cw15, B64, B61, B54
  - Partner-specific
    - B44 (10,000), Cw4 (7,000), and Cw6 (8,000)
  - Complement-fixing
    - >10K MFI:
      - B73, Cw8, B46, Cw10, Cw9, Cw1, Cw12, Cw14, Cw4, Cw6, Cw5, Cw18
    - 1.5K-4K MFI:
      - Cw4
  - Complement-fixing and partner-specific
    - Cw4 (24,000), Cw6 (25,000)
- No class II antibodies detected

Other

- Indeterminate levels of anticardiolipin IgM (10)
- Low C3 complement activity (82)
- Heterozygous for the MTHFR C677T polymorphism
- Negative for RF (9)
- Negative for anti-CCP antibodies (<16)
- Negative for anti-TPO/THAB
  - Anti-TPO = 1.0
  - THAB = <1.0
- Normal TSH (1.29)
- Negative for TSH receptor antibody (<6.00)
- Negative for ANAs
- Normal total IgG (1210)
- Normal total IgM (112)
- Normal total IgA (137)
- Normal total IgE (42)
- Total 25 Hydroxy Vit D sufficiency (33.9)
  - 25 Hydroxy Vit D2 = <5.0
  - 25 Hydroxy Vit D3 = 33.9
- Normal C4 complement activity (20)
- Normal homocysteine (6.6)
- Negative (normal) for the MTHFR A1298C polymorphism

Summary

You have the KIR AA haplotype which lacks activating KIR genes and predisposes to inefficient activation of uterine NK (uNK) cells by HLA-C on trophoblasts. This in turn leads to defective cytokine production by uNK cells and thereby impaired spiral artery remodeling and shallow embryo implantation. The effect of this haplotype on defective implantation is most pronounced when the fetus contains more HLA-C2 alleles than the mother by virtue of contribution of an HLA-C2 allele from the father. Analysis of your and your partner's HLA-C allotypes indicates that 50% of embryos will contain the same HLA-C2 as you and 50% of embryos will contain more HLA-C2 than you.

You harbor some HLA haplotypes known to predispose to the development of autoimmune conditions. These are DRB1*10:01/DQA1*01/DQB1*05 which predisposes to the development of rheumatoid arthritis, and DRB1*16/DQB1*05 which predisposes to a subtype of myasthenia gravis. As stated above, these and other HLA predispositions are not diagnostic for the presence of one or more 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 also have a history of a firstborn son and you have copies of both the DQB1*05:01 and DQB1*05:02 HLA class II HY restricting HLA (HYrHLA) alleles. Class II HYrHLA alleles allow the maternal immune system to detect and react to male antigens encoded on the Y chromosome of a male fetus and can lead to the generation of anti-HY immunity including the generation of anti-HY antibodies. These alleles are found at increased frequency in women experiencing secondary infertility or recurrent miscarriage following the birth of a son and the presence of 2 or more class II HYrHLA further increases this association.

You have elevated levels of TNFα positive and IL-17 positive NK cells and a borderline elevated level of IFNγ positive NK cells. Largely as a result of these elevated/borderline elevated levels of individual intracellular cytokine (IC) positive cells, all of your NK cell IC ratios are either elevated or borderline elevated. You also have mildly elevated levels of total and CD16+ NK cells, as well as an elevated level of CD5+ B cells. You also have borderline/mildly elevated serum levels of GRO, MCP-1, and IP-10 indicative of a mildly elevated level of systemic inflammation.

You are positive for 3 partner-specific anti-HLA antibodies that are all present between 7K to 10K MFI (B44, Cw4, and Cw6). The Cw4 and Cw6 antibodies were also found to fix complement (C1q). Every embryo that you and your partner generate will have one of these HLA-C alleles and HLA-C (including HLA-C of paternal origin) is expressed on the surface of the very early-stage embryo. The presence of partner-specific HLA-C antibodies has been associated with an increased risk of recurrent miscarriage. The presence of partner-specific anti-HLA antibodies (particularly those that fix complement) also significantly increases the likelihood that you also have anti-HY antibodies which you are predisposed to through your history of a firstborn son and possession of 2 class II HYrHLA alleles (see discussion above).

You have an indeterminate level of anticardiolipin IgM antiphospholipid antibodies (APAs). Your C3 complement activity is also low, suggesting elevated consumption of this factor due to peripheral complement cascade activation. You are negative for all tested ANAs and ATAs.

Your elevated levels of TNFα positive and IL-17 positive NK cells, elevated/borderline elevated NK cell IC ratios, elevated levels of total and CD16+ NK cells, and borderline/mildly elevated serum levels of GRO, MCP-1, and IP-10 are all consistent with the possible presence of endometriosis. Indeterminate levels of APAs are also frequently noted in our patients with endometriosis.

You are heterozygous for the MTHFR C677T polymorphism and use of a methylated form of folate during pregnancy could be considered.