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

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Patient and Partner Consult Report

Clinical History

- History of primary infertility for a period of about 2 years
  - Including 6 failed IUI cycles and 3 failed IVF cycles
    - Low success rate of embryos surviving to day 5 blastocycts
    - Developed flu-like symptoms with one embryo transfer
- History of moderate pain with menstruation
- History of internal pain with relations
- History of premenstrual spotting
- History of small posterior subserosal fibroids removed by hysteroscopy on 2014
- Family history of hypothyroidism (Patient's mother)
- Family history of adult onset diabetes mellitus (AODM)
- Family history of multiple sclerosis (Patient's aunt)

KIR Analysis

- HLA-C
  - You
    - 04, 07
    - C2/C1
  - Partner
    - 06, 07
    - C2/C1
- 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
      - 25% 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
      - 25% chance in any given fetus

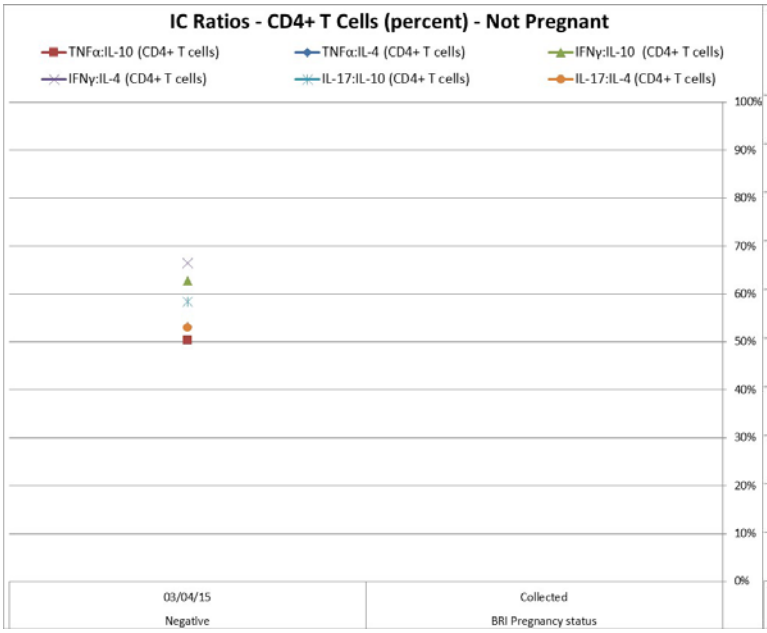
HLA Analysis

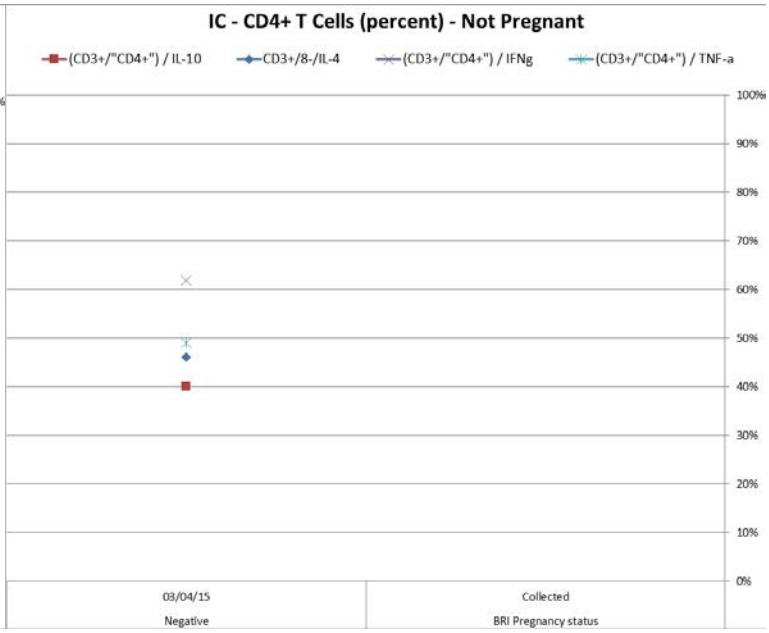
NOTE: The analysis below indicates HLA alleles and haplotypes that have been shown to predispose to certain autoimmune conditions. The presence of one or more predisposing HLA alleles/haplotypes is not diagnostic of the existence of an autoimmune condition. However, when combined with other analysis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

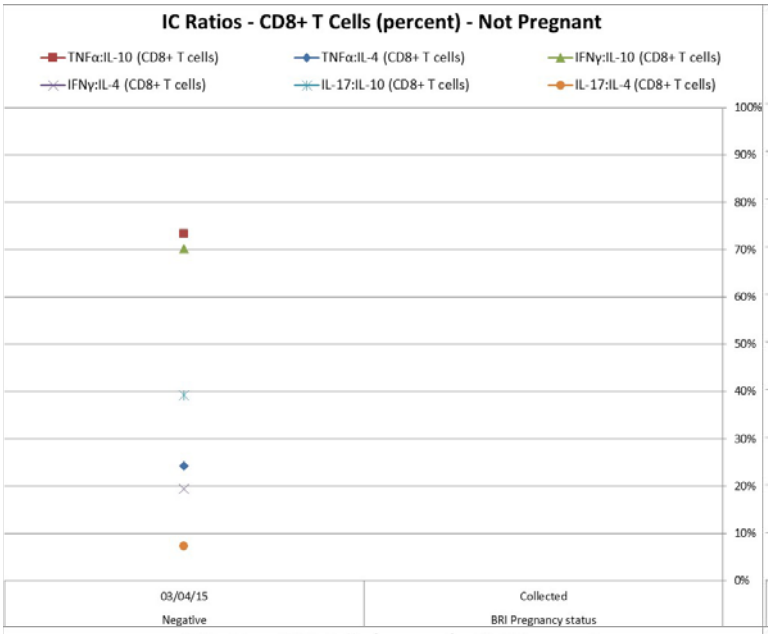
- No significant lack of class II allele mismatching
  - 4 total class II mismatches (out of 6)
  - 1 HLA-DRB1 mismatch (out of 2)
  - 1 HLA-DR supertype mismatch (out of 2)

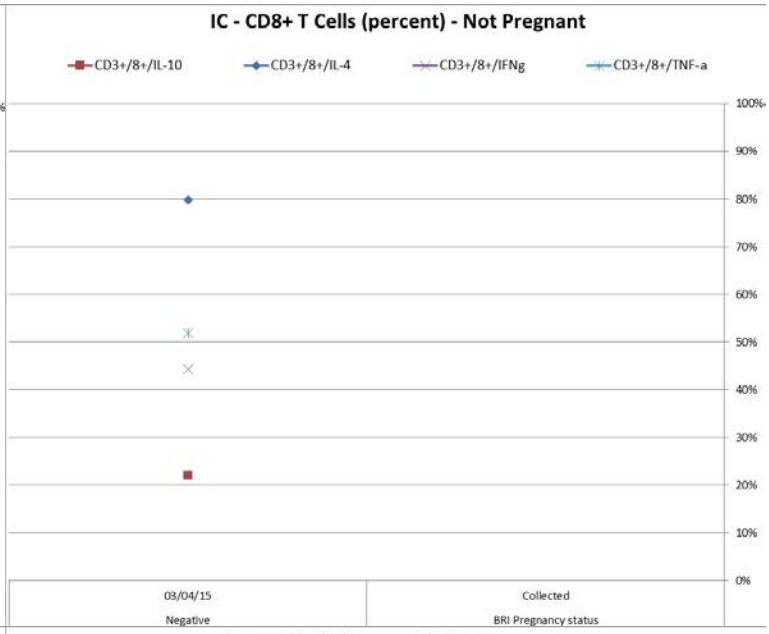
- No significant homozygosity of class II alleles
    - You are homozygous at the DQA1 locus
- You harbor the complete 8.1 extended haplotype which includes DRB1*03:01 in combination with DQB1*02:01
    - DQB1*02:01 in combination with DRB1*03:01 (HLA DR3-DQ2) is a haplotype attributed to a significant 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, sarcoidosis, autoimmune hepatitis, and inclusion body myositis.
- 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
- You harbor DRB1*11 which is part of the DR5 serotype that is associated with several autoimmune conditions, including Hashimoto's thyroiditis, primary antiphospholipid syndrome, and TTP.
- You harbor DRB1*11:04 which have been associated with multiple sclerosis and systemic sclerosis
- You harbor the DRB1*11:04/DQA1*05:01/DQB1*03:01 haplotype which has been associated with systemic sclerosis
- HLA-G 14bp ins
    - You are homozygous for the HLA-G 14bp ins allele
- HLA class II HY restricting alleles (class II HYrHLA)
    - None detected

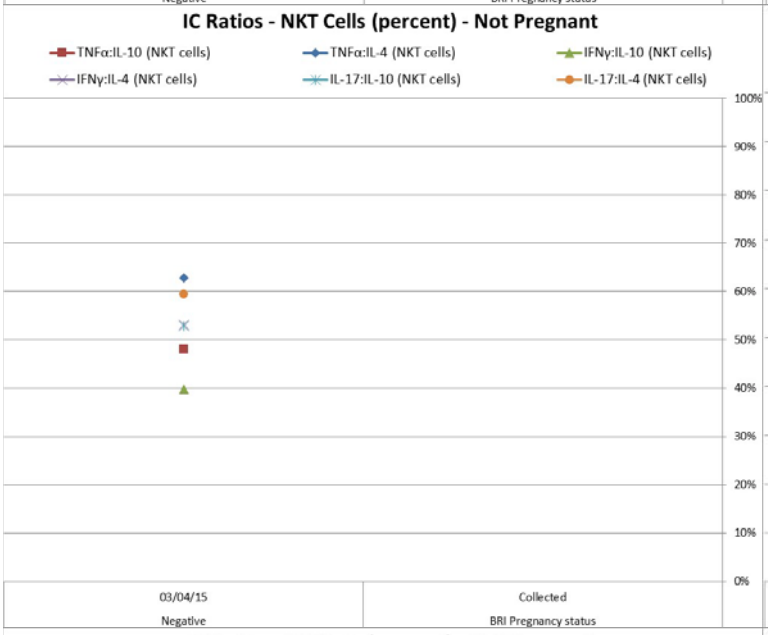
<u>Intracellular Cytokine Analysis (IM-Xpress)</u>

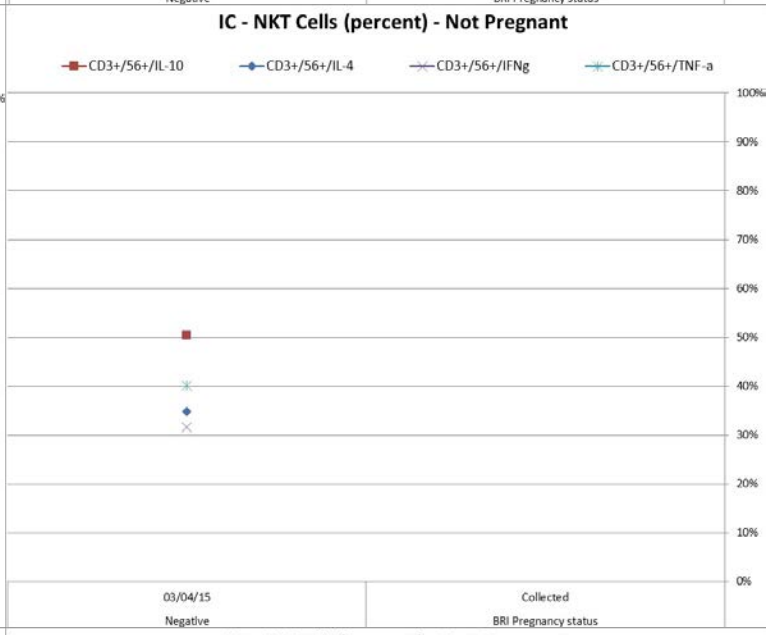


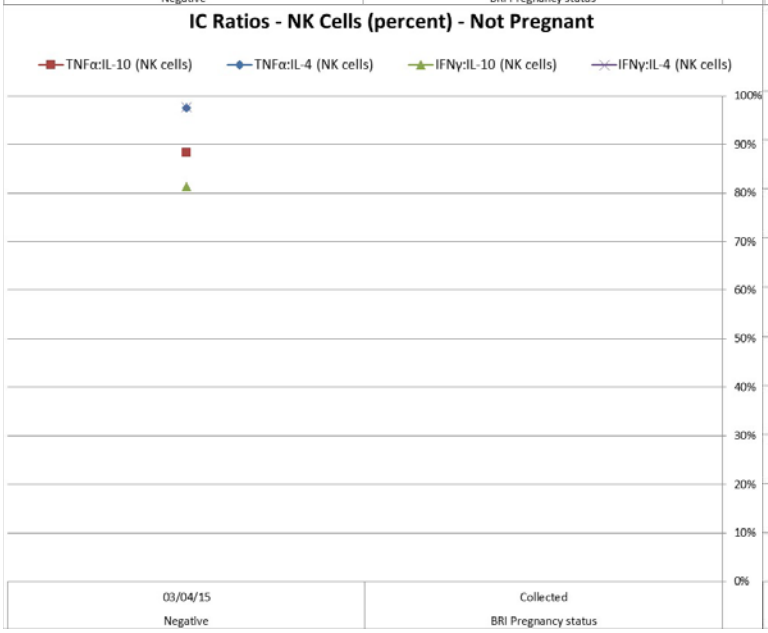


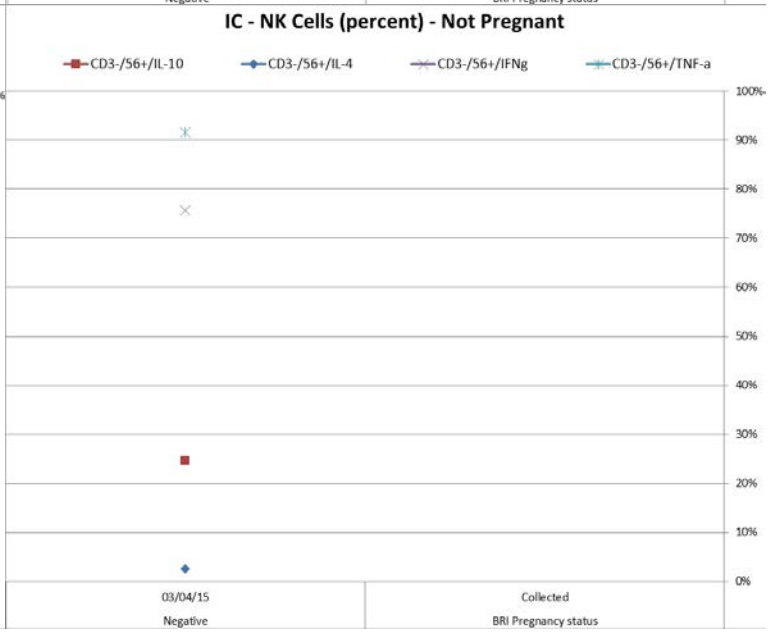

IC Ratios - CD8+ T Cells (percent) - Not Pregnant
TNFα:IL-10 (CD8+ T cells)
TNFα:IL-4 (CD8+ T cells)
IFNγ:IL-10 (CD8+ T cells)
IFNγ:IL-4 (CD8+ T cells)
IL-17:IL-10 (CD8+ T cells)
IL-17:IL-4 (CD8+ T cells)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
03/04/15
Negative
Collected
BRI Pregnancy status


IC - CD8+ T Cells (percent) - Not Pregnant
CD3+/8+/IL-10
CD3+/8+/IL-4
CD3+/8+/IFNg
CD3+/8+/TNF-a
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
03/04/15
Negative
Collected
BRI Pregnancy status


IC Ratios - NKT Cells (percent) - Not Pregnant
TNFα:IL-10 (NKT cells)
TNFα:IL-4 (NKT cells)
IFNγ:IL-10 (NKT cells)
IFNγ:IL-4 (NKT cells)
IL-17:IL-10 (NKT cells)
IL-17:IL-4 (NKT cells)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
03/04/15
Negative
Collected
BRI Pregnancy status

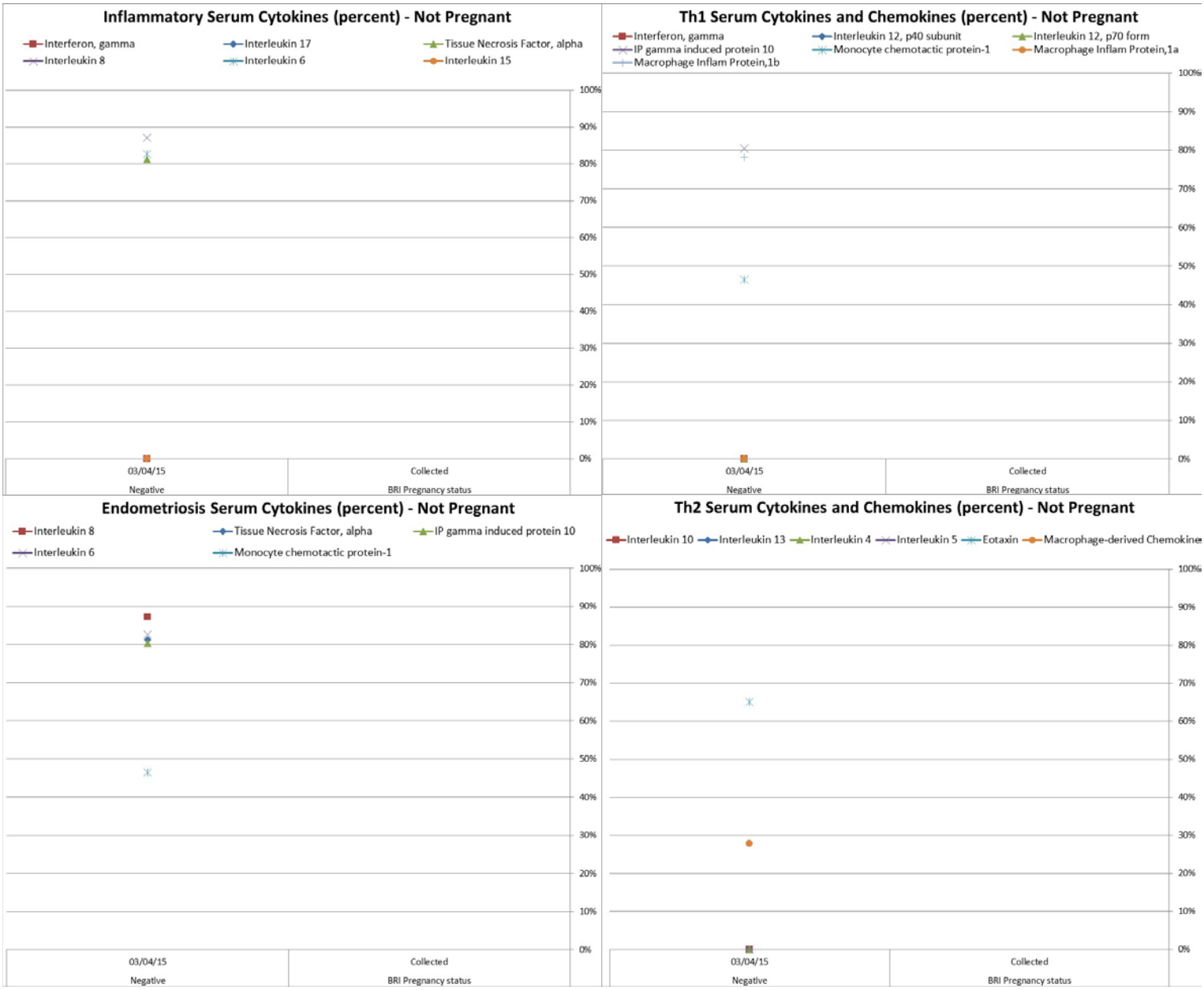

IC - NKT Cells (percent) - Not Pregnant
CD3+/56+/IL-10
CD3+/56+/IL-4
CD3+/56+/IFNg
CD3+/56+/TNF-a
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
03/04/15
Negative
Collected
BRI Pregnancy status


IC Ratios - NK Cells (percent) - Not Pregnant
TNFα:IL-10 (NK cells)
TNFα:IL-4 (NK cells)
IFNγ:IL-10 (NK cells)
IFNγ:IL-4 (NK cells)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
03/04/15
Negative
Collected
BRI Pregnancy status


IC - NK Cells (percent) - Not Pregnant
CD3-/56+/IL-10
CD3-/56+/IL-4
CD3-/56+/IFNg
CD3-/56+/TNF-a
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
03/04/15
Negative
Collected
BRI Pregnancy status

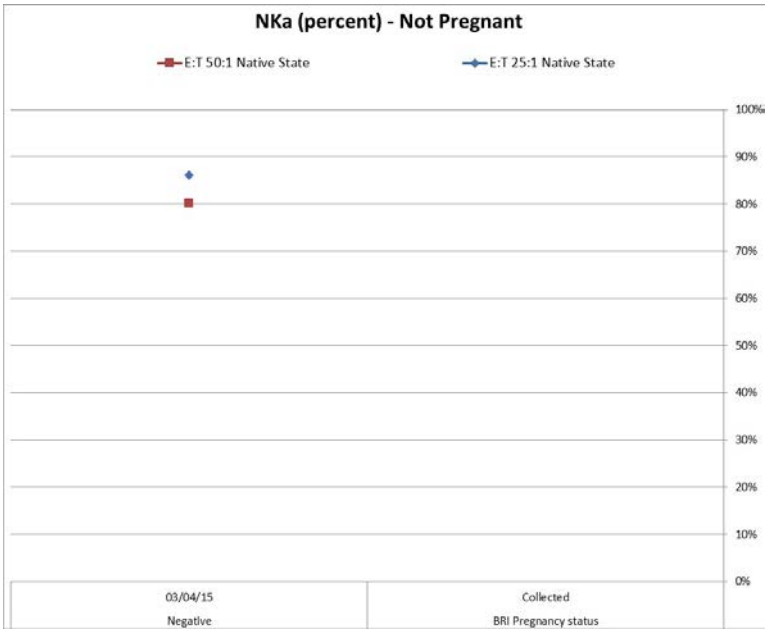
- Your intracellular cytokine (IC) ratios indicate a neutral Th1/Th2 balance with a CD4+ T cell IFNγ:IL-4 ratio in the 66th percentile. Together with your neutral Th1/Th2 balance, all IC ratios in your CD4+ T cells, CD8+ T cells, and NKT cells are within normal ranges except for your CD8+ T cell TNFα:IL-4 and IFNγ:IL-4 ratios which are slightly low. However, you have elevated levels of TNFα positive and IFNγ positive NK cells, and as a result, all of your NK cell IC ratios are elevated with your NK cell TNFα:IL-4 and IFNγ:IL-4 ratios both in the 98th percentile.

Serum Cytokines Analysis



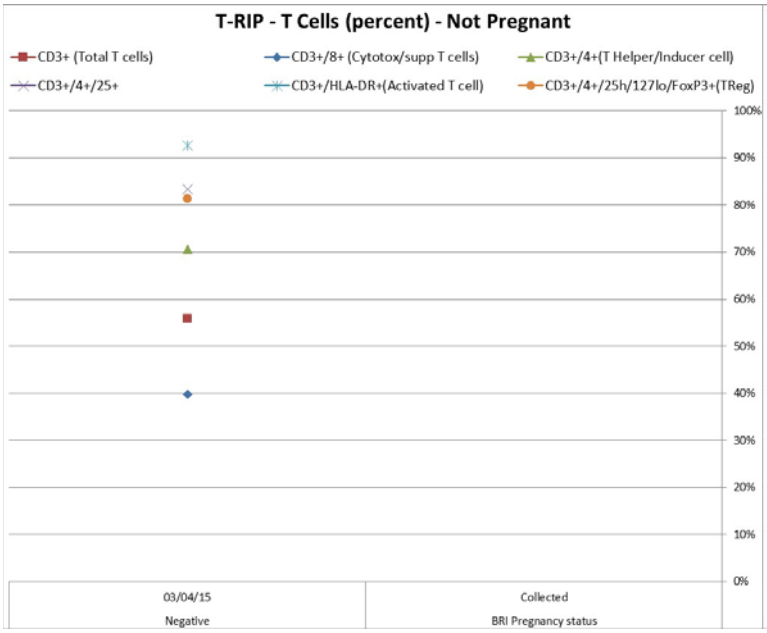
- Your serum levels of TNFα, IL-6, IL-8, IP-10, and MIP-1β are elevated all elevated. Your serum level of eotaxin is also borderline elevated. Serum levels of all other tested cytokines and chemokines are within normal ranges or low.

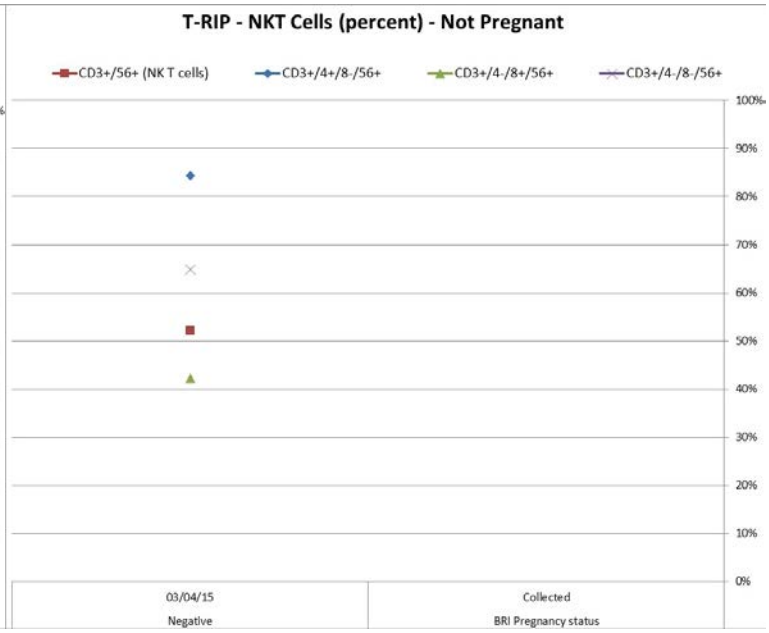
NK Cell Activity Analysis

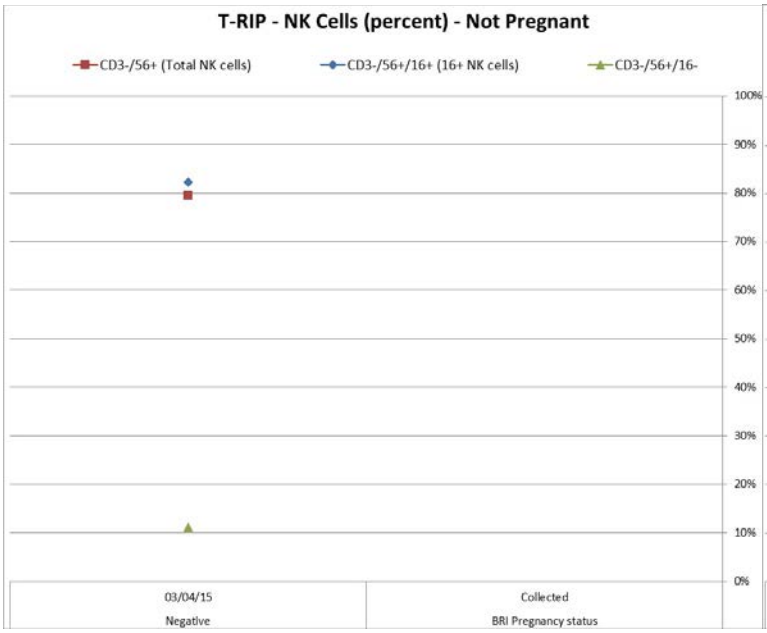


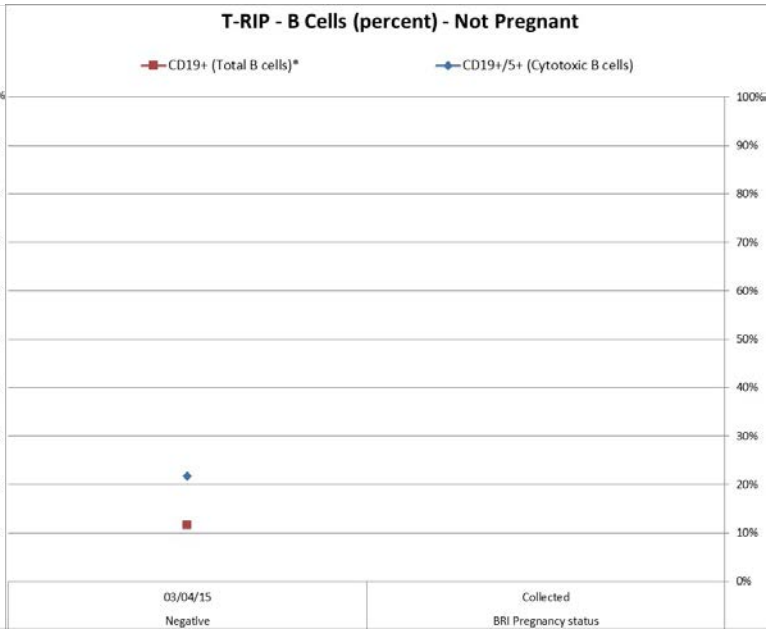
- Your NK cell cytotoxic activity (NKa) is elevated, consistent with your elevated NK cell IC ratios.

Reproductive Immunophenotype and CBC Analysis









- T-RIP
  - Consistent with your elevated NK cell IC ratios and elevated NKa, you also have elevated levels of total and CD16+ NK cells. Your CD4+ NKT cells, activated CD4+ T cells, and HLA-DR+ T cells are also elevated. Your total and CD5+ B cells are low.
- CBC
  - WBCs = 5.7

Anti-HLA Antibodies Analysis

- No class I antibodies detected
- No class II antibodies detected

Other

- Positive for anti-TPO (376.2)
- Indeterminate levels of:
  - Antiphosphatidylglycerol IgG
  - Antiphosphatidylglycerol IgM
- Negative for RF (9.4)
- Negative for anti-CCP antibodies (3)
- Negative for THAB (0.2)
- Negative for ANAs

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, 25% of embryos will contain more HLA-C2 than you, and 25% of embryos will contain less HLA-C2 than you.

You harbor several HLA alleles and haplotypes associated with a predisposition 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 but not limited to, myasthenia gravis, systemic lupus

erythematosus, sarcoidosis, autoimmune hepatitis, and inclusion body myositis. The 8.1 haplotype also includes the DQ2 serotype (DQA1*05/DQB1*02) which predisposes to the development of celiac disease. You also harbor a copy of DRB1*11 which predisposes to the development of Hashimoto's thyroiditis, primary antiphospholipid syndrome, and TTP. Your specific DRB1*11 allele is DRB1*11:04 which is present as part of the DRB1*11:04/DQA1*05:01/DQB1*03:01 haplotype which predisposes to the development of systemic sclerosis. As stated above, these and other HLA predispositions are not diagnostic for the presence of one or more autoimmune conditions. However, these 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 leads to decreased expression of the tolerogenic HLA-G protein which leads to a reduced ability of the maternal immune system to generate tolerance for paternal antigens, and is found at increased frequency in women experiencing infertility, repeated implantation failure, and recurrent miscarriage.

Your intracellular cytokine (IC) ratios indicate a neutral Th1/Th2 balance with all CD4+ T cell, CD8+ T cell, and NKT cell IC ratios within normal ranges or low. However, all of your NK cell IC ratios are elevated with your NK cell TNFα:IL-4 and IFNγ:IL-4 ratios both in the 98th percentile, partially as a result of elevated levels of TNFα positive and IFNγ positive NK cells. Consistent with your elevated NK cell IC ratios, your NKa and levels of total and CD16+ NK cells are also elevated. Your CD4+ NKT cells, activated CD4+ T cells, and HLA-DR+ T cells are also elevated. This activation of your immune system at the cellular level has also resulted in elevated serum levels of several cytokines and chemokines, TNFα, IL-6, IL-8, IP-10, and MIP-1β (and borderline elevated eotaxin), indicative of elevated levels of systemic inflammation.

You are positive for anti-TPO antibodies, indicative of autoimmune thyroiditis. Combined with the presence of a DRB1*11 allele, this is very likely to be Hashimoto's thyroiditis. You also have indeterminate levels of antiphosphatidylglycerol IgG and antiphosphatidylglycerol IgM antiphospholipid antibodies (APAs). You are negative for all tested ANAs, as well as for anti-HLA antibodies.

You have a history of primary infertility including multiple failed IUI and IVF cycles, moderate pain with menstruation, internal pain with relations, and premenstrual spotting which are all consistent with the possible presence of endometriosis. Consistent with this possibility, you harbor a copy of DRB1*11 which is the most frequent HLA allele found in our patients with endometriosis, as well as a copy of DQB1*03:01 which has been independently associated with a predisposition to the development of endometriosis. Your elevated NK cell parameters (NK cell IC ratios, NKa, and levels of total and CD16+ NK cells), and elevated serum levels of TNFα, IL-6, IL-8, and IP-10 are also highly consistent with the possible presence of endometriosis. Endometriosis is also highly associated with the presence of elevated levels of anti-TPO antibodies and Hashimoto's thyroiditis in our patient population, and indeterminate levels of APAs are also frequently found in our endometriosis patients.