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European Regional Development Fund

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Immunological causes for infertility, miscarriages, complications and IVF failures

Know-how exchange on state-of-the-art methods in microbiological and genetic screening and diagnosis in infertility, maternal and fetal health

28 – 29.10.2022, OncoGen Centre

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Partnership for a better future

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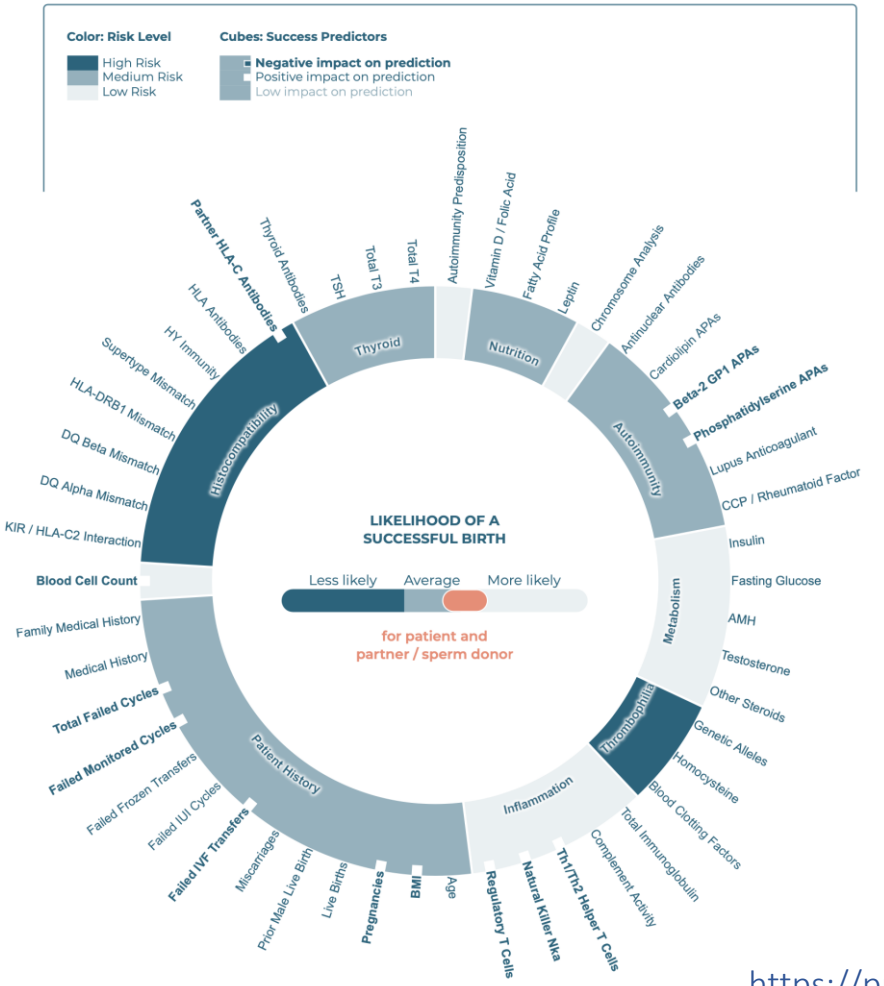
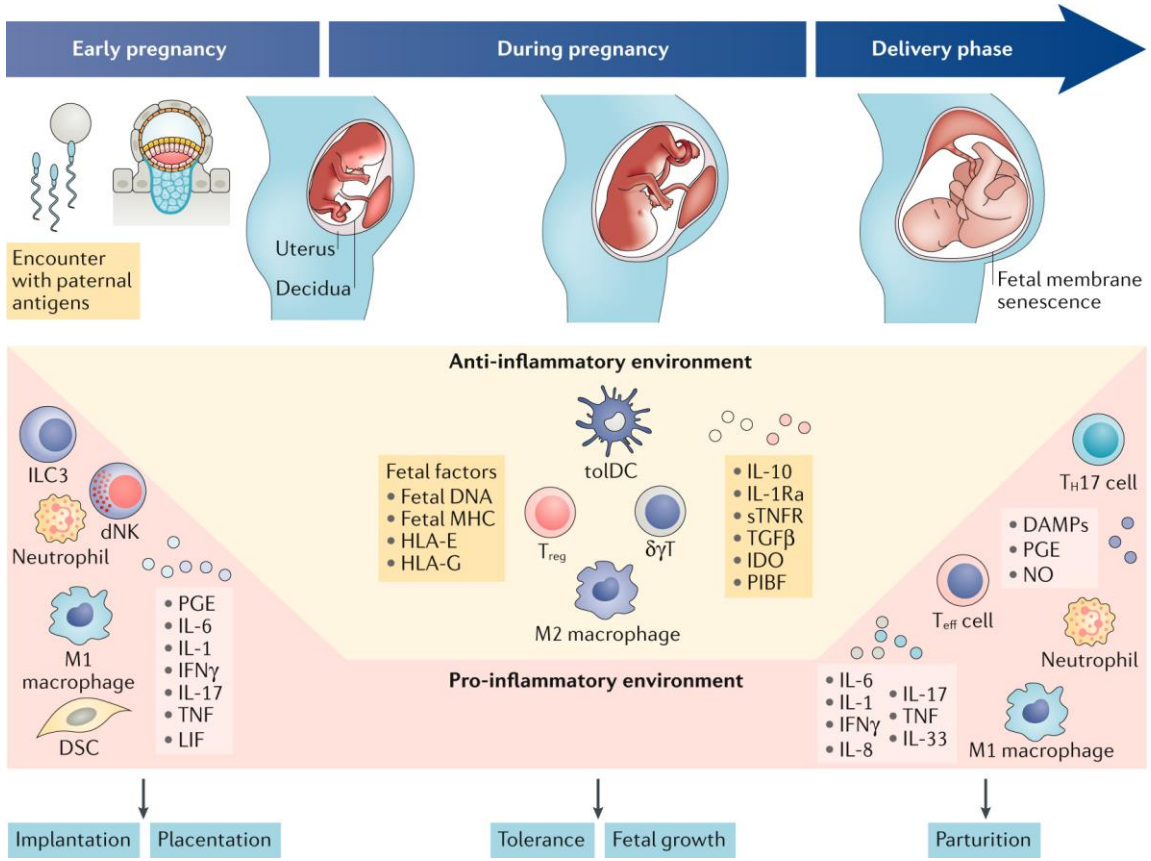
Immunological assessment





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Parental histocompatibility

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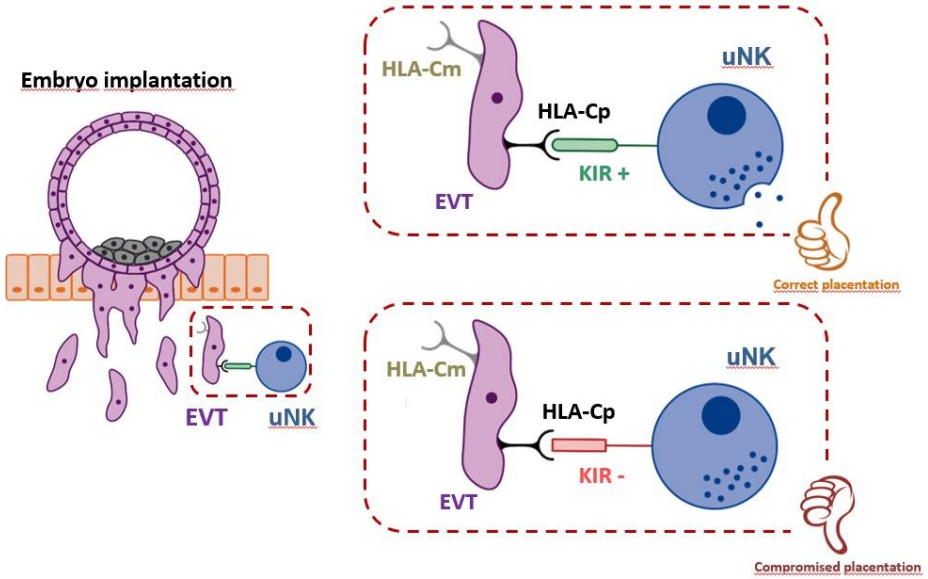
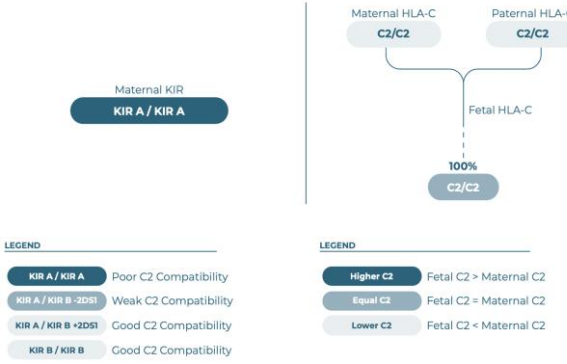
Fetal HLA-C and Maternal KIR interactions





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- Uterine natural killer cells (uNK) secrete a unique repertoire of cytokines and growth factors that regulate blood vessel growth and development (also known as spiral artery remodeling) leading to a healthy placentation that supports embryo growth.
- Killer immunoglobulin-like receptors (KIRs) present on the surface of uNK interact with HLA-C on the trophoblast to promote (activating KIR) or suppress (inhibiting KIR) this vascular remodeling.
- HLA-C genotype of both the father and the mother has a significant impact on the risk for defective placentation which can manifest as recurrent miscarriage, intrauterine growth restriction and/or preeclampsia.

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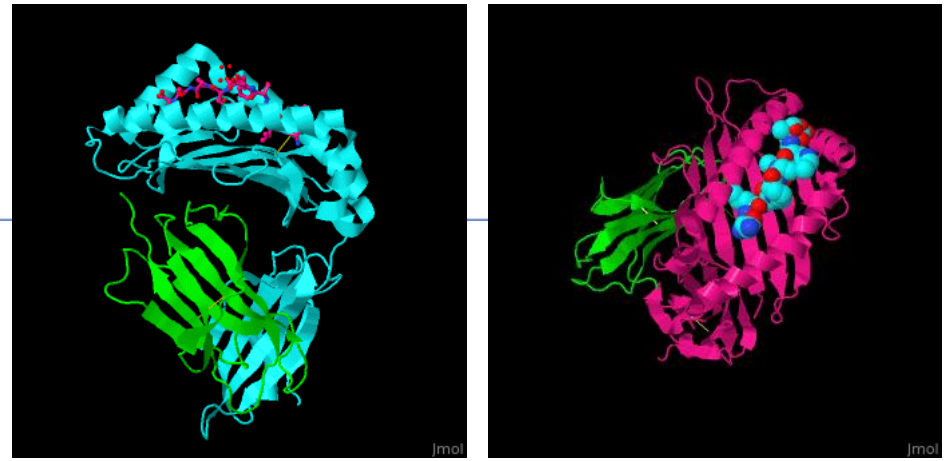
HLA Mismatches



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- Human Leukocyte Antigen (HLA) genes (MHC class I – HLA-A, B, C; MHC class II – HLA-DR, DQ, DP), are a cluster of genes present on the chromosome 6 and are involved in antigen presentation to T cells to initiate an immune response;
- In most cases, this immune reaction leads to the destruction of cells displaying "non-self" peptides. HLA molecules play a key role in organ transplantation and are associated with many diseases including auto-immune disorders;
- While a mismatch in Human Leukocyte Antigen (HLA) between a donor and a recipient in organ transplantation may lead to a graft rejection, most often due to the formation of antibodies, a certain level of difference between the mother's and father's HLA alleles (inherited by the embryo and defined as a mismatch) is necessary to actively generate immune tolerance of the embryo;
- Thus, couples with significant matched alleles for HLA genes may be more prone to infertility, repeated implantation failure, and recurrent pregnancy loss.

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HY Immunity

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- During a first pregnancy with a male fetus, the maternal immune system could be activated by allogenic fetal cells possessing male-specific minor histocompatibility inherited antigens (HYrHLA allele) that are encoded by genes localized on the Y chromosome;
- In some women, this can lead to an acute immune reaction leading to the production of HY antibodies by B cells.
- These antibodies can last for several years in the maternal serum. This may lead to secondary recurrent miscarriage (with male or female embryos) and to giving birth to boys with a low birth weight

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HY Immunity

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- Although present in 30% of women, anti HY antibodies have been linked to secondary recurrent miscarriage in subsequent pregnancy and other pregnancy complications such as stillbirth, placental abruption or fetal growth retardation, all these events being the results of an inflammatory environment;
- A large cohort study including women with unexplained secondary RPL showed that these patients were more prone to miscarriage during their subsequent pregnancy when having a firstborn boy compared to a firstborn girl (46 % versus 24 % respectively);
- In these patients with a first-born son, the presence of H-Y restricting HLA class II alleles (HLA-DRB1*15, HLA-DQB1*05:01/02 and HLA–DRB3*03:01) has been correlated with lower chance of live birth and a low male/female ratio among the subsequent births (increased loss rate of male embryos);
- Patients with no copy of HY restricting alleles have a subsequent live birth rate of 73%, which dropped to 58% with 1 allele copy and 49% with two allele copies.

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HLA Antibodies

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- Pregnancy is marked by profound changes of the maternal immune system that allow the semi-allogeneic fetus to implant and grow within the uterus;
- Among immune cells, B lymphocytes (B cells) are key players in the maternal immune adaptation towards fetal implantation;
- Some B cell subsets are capable of producing antibodies that target components of the embryo/fetus encoded by the paternal genetics – most notably paternally derived HLA molecules;
- Anti-HLA antibodies are present in one third of healthy successful pregnancies;
- The presence of partner-specific anti-HLA antibodies (particularly those that fix complement) can be harmful to pregnancy maintenance and can induce miscarriage, or later complications such as preeclampsia, intrauterine growth restriction, or stillbirth.

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Systemic inflammation

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Total Immunoglobulin

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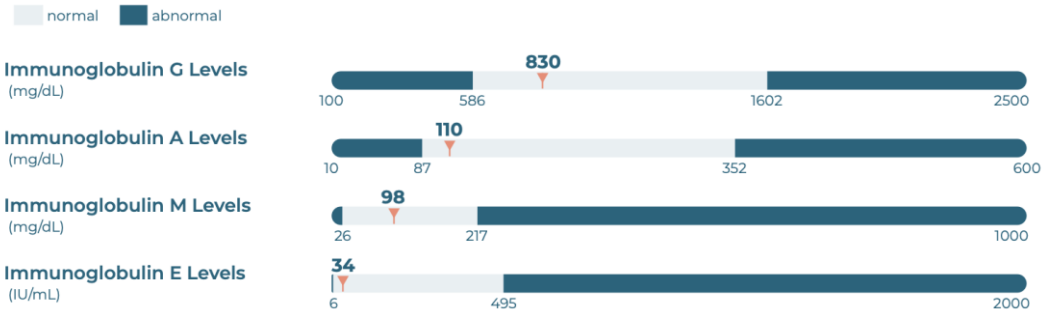

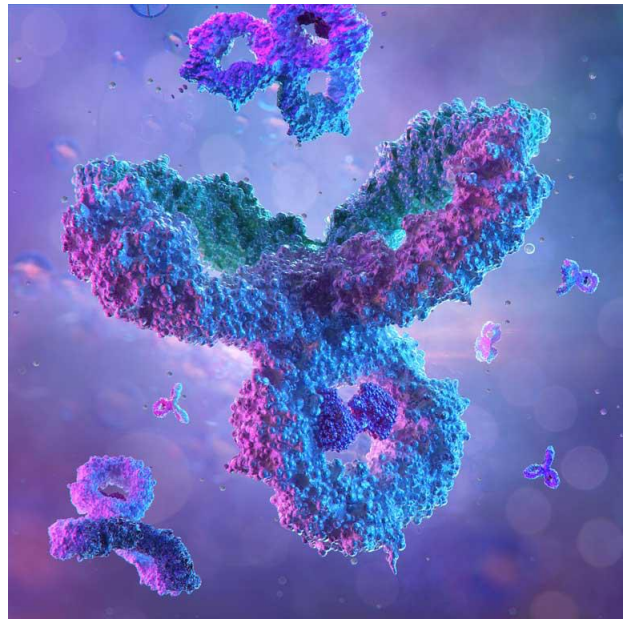

- Immunoglobulins are key mediators of the immune system that identify and neutralize pathogens and other targets;
- Antibody levels can help indicate allergies and other inflammatory reactions.

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Complement activity

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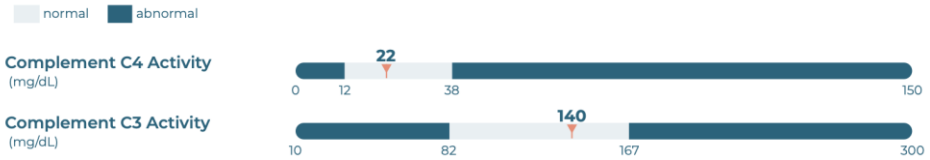

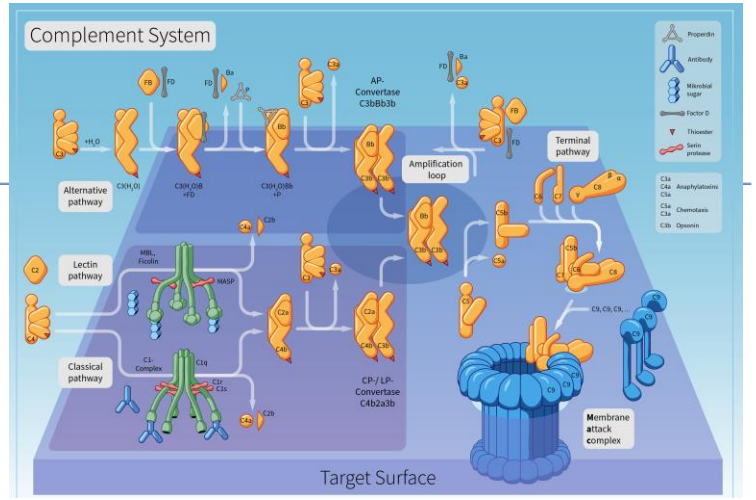

- These pathways converge and their activation results in the generation of C3a, C4a, and C5a anaphylatoxins (potent inflammatory molecules) and the membrane attack complex MAC (C5b, 6,7,8, and 9);
- The complement and coagulation pathways are closely interacting with C5a inducing procoagulant activity and reducing fibrinolysis;
- C3a and C5a activate endothelial cells and platelets, inducing increased levels of adhesion factors and procoagulant activity;
- Some proteins of the complement activation (factor B and H) were found to be useful biomarkers to predict preterm birth as early as 15 weeks of pregnancy;
- Complement activation has dramatic effects on placenta, causing inflammation and placental injury, leading to pre-eclampsia and fetal loss.

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NK Cytotoxic Activity As

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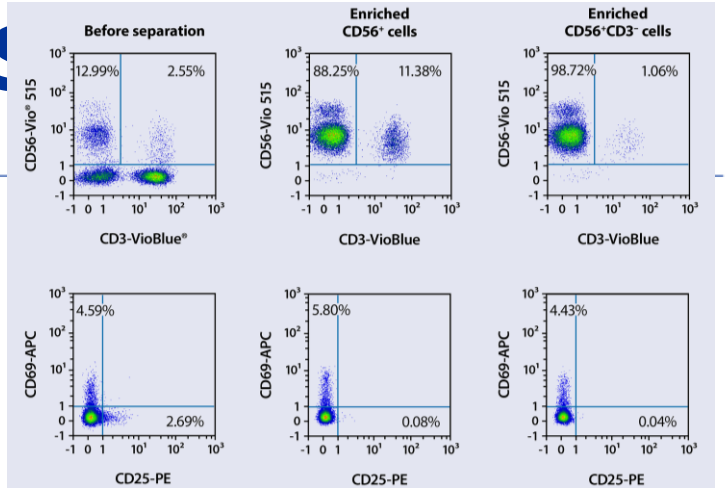

- NK cell activity has been associated with the pathogenesis of recurrent pregnancy loss, even when the embryo was chromosomally normal;
- A clinical study has shown that women with high NK cell activity pre-conception have a higher risk for pregnancy loss (3.5- fold higher risk) than women with normal levels;
- More recent studies showed a strong correlation between high NK cell activity and recurrent spontaneous abortion and suggested that NK cell activity can be used as a predictive biomarker;
- IVIG can induce a down-regulation of NK cell activity among women with recurrent pregnancy losses which may help improve a patient's pregnancy outcome;
- The significance of pre-conceptional NK activity as a predictive value remains debated as a study showed no correlation between NK activity and risk for a subsequent loss.

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Regulatory T cells (Tregs)

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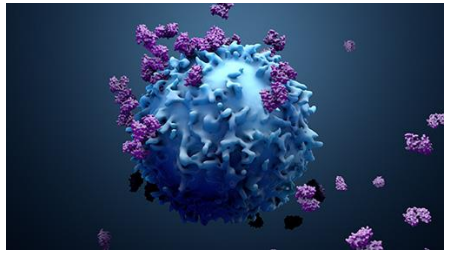

- Tregs cells play a critical role in regulating tolerance to the semi-allogenic fetus presenting paternal alloantigens; these cells may prevent fetal rejection by the maternal immune system;
- Tregs may promote fetal survival by inhibiting effector T cells and secreting anti-inflammatory factors such as IL-10 and TGF-β;
- These cells are enriched in the fetal-maternal interface during early pregnancy; they were shown to migrate from the peripheral blood to the decidua in pregnant subjects; thus, they might be recruited and expanded by the recognition to fetal antigens.

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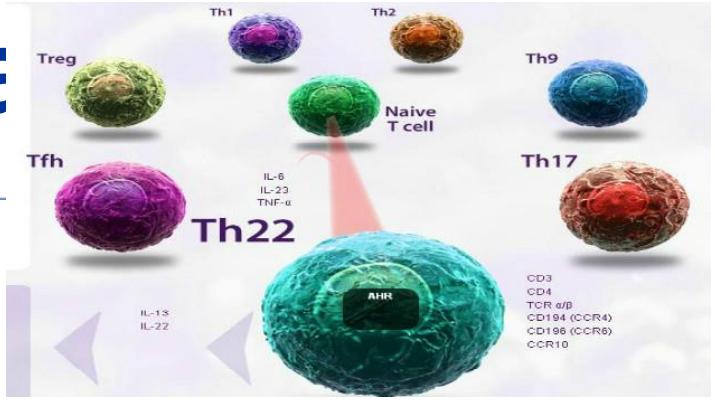
Th1/Th2 Helper T Cell Ra



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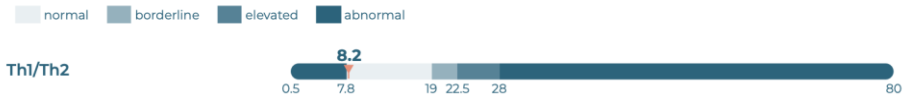

- CD4+ T cells, CD8+ T cells, NKT cells, and NK cells comprise four of the major effector cell types of the immune system; these cells can respond to the presence of foreign antigens, including paternally derived antigens present on conceptuses, and elicit either immunogenic or tolerogenic responses;
- These lineages include Th1, Th2, Th17, and Treg cells (regulatory T cells) which are distinguished by unique cytokine expression profiles. Cells expressing these cytokines (IFN-γ for Th1, IL-4 for Th2, IL-17 for Th17, and IL-10 for Treg) can be identified by flow cytometry and ratios of these cells can be determined to characterize the CD4+ T cell lineage profile for an individual;
- Analogous lineages also exist for CD8+ T cells, NKT cells, and NK cells which can be similarly characterized. Levels of TNFa positive cells can also be used as a general marker of cellular activation;
- Relative balances of these lineages within each of these cell types can be used to help characterize the nature of any underlying immune conditions.

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Th1/Th2 Helper T Cell Rat

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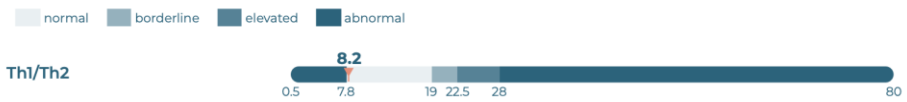

- Some autoimmune conditions such as rheumatoid arthritis are Th1-dominant, whereas other autoimmune conditions such as systemic lupus erythematosus are Th2-dominant;
- Other conditions including endometriosis, PCOS, and atopy are also characterized by differential intracellular cytokine profiles. These profiles can be present many years prior to the full clinical manifestation and diagnosis of an underlying autoimmune/inflammatory immune condition and therefore, in combination with other genetic and cellular data, can be used to characterize preclinical/asymptomatic conditions that may affect the immune response to foreign antigens;
- Changes in intracellular cytokine profiles can also be used to track the maternal immune response to pregnancy, assess the efficacy of immune treatment, and determine if any failures of pregnancy are immunological in nature.

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Th1/Th2 Helper T Cell Rat

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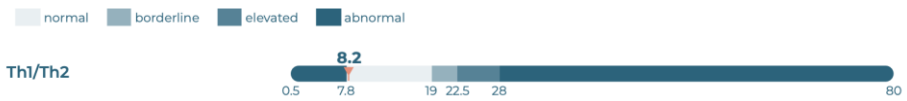

- Altered intracellular cytokine profiles have been found in the peripheral blood of women with a history of recurrent implantation failure or recurrent miscarriage;
- These include an elevated ratio of Th1 to Th2 cells, elevated levels of TNFa positive cells, decreased levels of IL-10 positive cells, and increased levels of Th17 cells;
- The normal (non-pathological) response to pregnancy is characterized by a tolerogenic response to paternal antigens present on the conceptus. This tolerogenic response is characterized by specific changes to the intracellular cytokine profile of various immune cell types;
- These tolerogenic responses include a shift to Th2 dominance (a decrease in the Th1/Th2 ratio) and an increase in levels of IL-10 positive cells.

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Th1/Th2 Helper T Cell Rat

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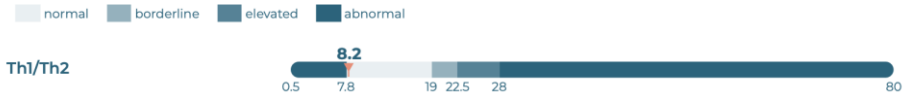

- Pathological immune responses to a conceptus involve a failure to properly develop immunological tolerance to paternally derived antigens. Rather, an immunogenic response is elicited which can lead to cellular and/or humoral (antibody-mediated) responses;
- Such pathological immune responses to a conceptus can result in various clinical manifestations including implantation failure, spontaneous abortion, preeclampsia, intrauterine growth restriction (IUGR), and stillbirth;
- Defective development of immunological tolerance during pregnancy is characterized in the peripheral blood in part by a failure in a shift to Th2 dominance, a failure in the increase in IL-10 positive cells and increases in levels of IL-17 positive cells.

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Autoimmunity

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Antinuclear Antibodies (ANAs)

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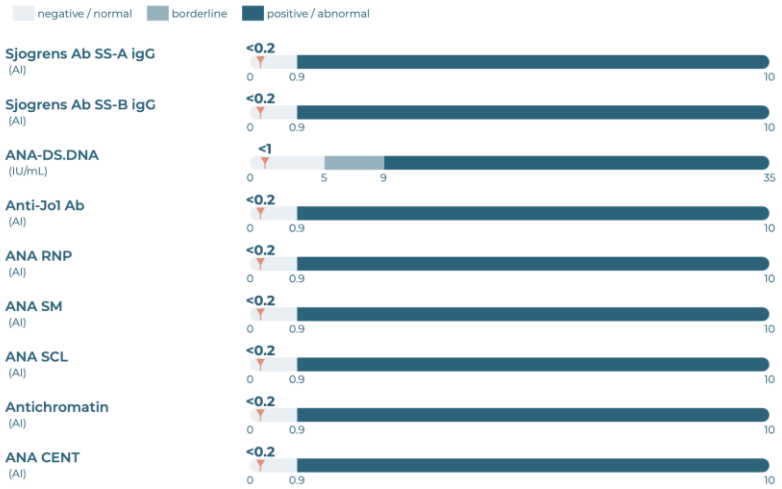

- ANA may play a role in early pregnancy as well as in recurrent pregnancy losses (RPL) by negatively impacting embryo development;
- ANA have been detected more frequently in women with RPL than in control women. They are also associated with poor outcomes of IVF/ICSI cycles;
- ANA positivity may predict a subsequent miscarriage in women with RPL; ANA+ RPL women had a higher number of miscarriages and lower number of successful subsequent pregnancies than ANA− women; miscarriages can occur in women ANA+ before pregnancy and who remained ANA+ in the first trimester;
- Pregnancy in RA patients is associated with higher risk for complications such as IUGR (intra uterine growth retardation) and premature rupture of membranes with rates that are 1.5–2 times higher than in the general obstetric population.

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Antiphospholipid Antibodies (APAs)

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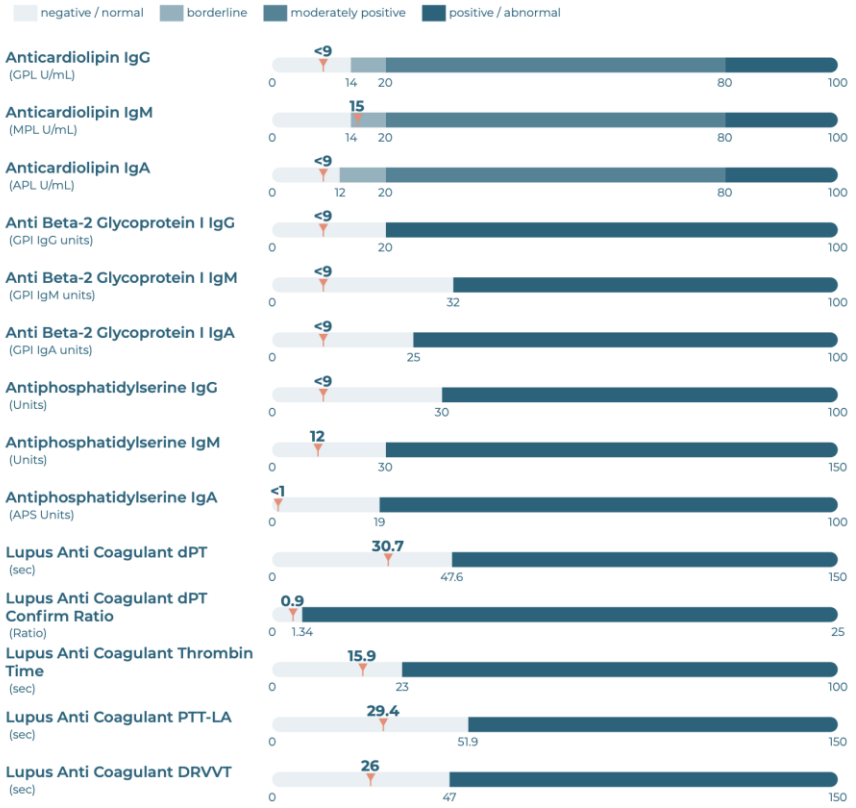

- APS is considered primary if it occurs in a patient with no underlying disease and secondary if it is related to an underlying pathology such as systemic lupus erythematous (SLE);
- Among the different antiphospholipid antibodies, anti-beta 2 glycoprotein I (anti-β2-GP1) antibodies is the best to support the diagnosis of APS;
- Many studies supports the fact that anti-β2-GP1 are more specific for APS than anti-cardiolipin antibodies;
- All three isotypes of anti-β2-GP1 (IgG, IgM, and IgA) have been associated with thrombosis;
- The presence of one or both β2-GP1 IgG and IgM antibodies is an independent risk factor for thrombosis and pregnancy complications.

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CCP Antibodies and Rheumatoid Factor

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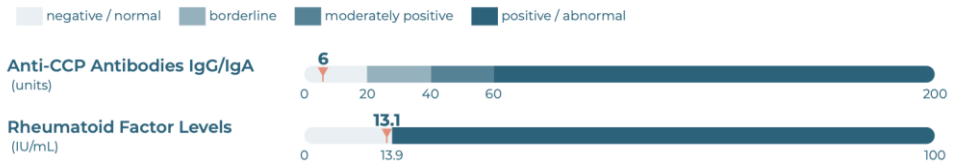

- Anti-citrullinated protein antibodies (anti-CCP) and rheumatoid factor (RF) are two tests used to detect rheumatoid arthritis (RA);
- Elevated RF levels are found in collagen vascular diseases such as SLE, RA, scleroderma, Sjogren's Syndrome or thyroid disease;
- Hypothyroidism is the most common thyroid alteration observed during pregnancy (Hashimoto's thyroiditis); untreated hypothyroidism can lead to fertility issues: miscarriage, preterm delivery, gestational hypertension or reduced cognitive function in the offspring;
- Anti-TPO antibodies may directly attack the fetoplacental units leading to embryo losses or obstetrical complications.

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Results of immunological assays

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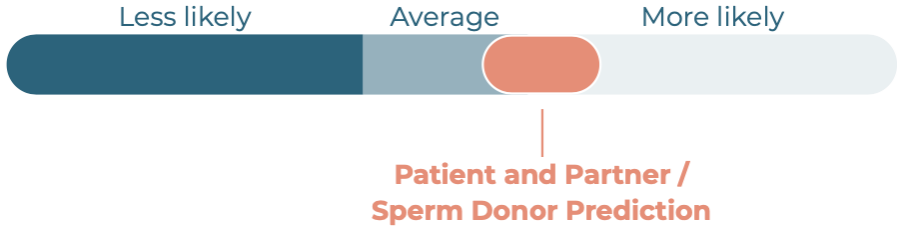
	LOW RISK/ NORMAL	MEDIUM RISK/ BORDERLINE	HIGH RISK/ ABNORMAL
1. Parental Histocompatibility			
Fetal HLA-C and Maternal KIR Interaction			■
HLA Mismatches		■	
HY Immunity	■		
HLA Antibodies	■		
2. Parental Chromosome Analysis			
Maternal Chromosomes	■		
Paternal Chromosomes	■		
3. Thrombophilia			
Prothrombin Factor II Alleles	■		
Leiden Factor V Alleles	■		
Plasminogen Activator Inhibitor Type I Alleles			■
Homocysteine	■		
Blood Clotting Measurements	■		
4. Blood Count			
Cell Blood Count (CBC)	■		
5. Inflammation			
Total Immunoglobulin	■		
Complement Activity	■		
Th1/Th2 Helper T Cell Ratio	■		
Natural Killer Cell Cytotoxic Activity (NKa)	■		
Regulatory T Cells	■		
6. Autoimmunity			
Antinuclear Antibodies (ANAs)	■		
Antiphospholipid Antibodies (APAs)		■	
CCP Antibodies and Rheumatoid Factor	■		

LIKELIHOOD OF A SUCCESSFUL BIRTH



Thank you for your attention!

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