

Fertility diagnostics

- Determination of intracellular cytokines
- NK-cell assay
- Partner lymphocyte vaccination

General:

Since the rate of successful pregnancies decreases to 50% after 3 miscarriages and since every unsuccessful pregnancy (incl. IVF treatment) represents a significant problem for the affected couple, specific diagnostics, searching for possible (treatable) causes are recommended. Autoimmune, genetic, hemostasiological, alloimmunological as well as cellular immune factors should be considered.

Autoimmune risk factors: the ANA IFT will provide most information as a first screening test (including ENA), phospholipid antibodies are the most frequent cause of repeated abortion in up to 60% - 70 % of cases.

See **ANA-profile, Autoantibodies panel, ANA**

The pathological mechanism is not clarified in detail. While phospholipid antibodies lead to increased aggregation of thrombocytes (influence on the arachidonic acid metabolism of the thrombocytes), ANA are described more often in association with vasculitis. Disturbances of the implantation phase or premature pregnancy might occur. As a further cause of infertility, thyroid gland autoantibodies are discussed, however the pathological mechanism is unclear. Autoantibodies may lead to generalized activation of humoral and cellular immune reactions with negative effects on pregnancy. For the treatment of autoimmune disorders prednisolone in a dosage of 10- 15 mg/day and acetylsalicylic acid 100 mg/day and/or low molecular heparin are used. Depending on the concentration level of the autoantibodies, the daily dose of prednisolone can be increased to 25 mg/day for a short time. Also a treatment with polyvalent immune globulins is possible.

Alloimmune risk factors: Diagnosing alloimmunological factors in infertility represents an exclusion diagnosis after excluding other well defined causes. Working hypothesis for corresponding diagnostics and therapy is the following strategy: The human fetus induces a protective immune answer on the basis of his own antigens which are different from those of the mother. It is decisive how different the antigens of mother and child are. If there is an increased compatibility, a delayed or failing identification of the unknown antigens occurs and subsequent pregnancy protective reactions are not effective. In embryo transfer (IVF) another aspect is considered due to the 'in

vitro' situation of sperms and oocytes within the first 48 to 72 hours: naturally (in vivo) an immunological conditioning of the maternal immune system might occur, which is absent during the first 48 to 72 hours. The induction of a protective maternal immune answer by partner lymphocyte vaccination is a therapeutic approach. Lymphocytes are extracted from the partner from 40 ml of stabilized blood and injected subcutaneously.

Cellular immune factors: Cellular immune response is mostly modulated by TH1-cells; Interferon- γ (IFN- γ) is the most important effector and at the same time marker cytokine of the TH1-helper cells. IFN-gamma mediates a pro-inflammatory immune response. The processes involved in this immune response effect the efficient elimination of intracellular pathogens (i.e. viruses, intracellular bacteria), but can also cause tissue damage if an overreaction or inadequate reaction (type IV immune reaction) takes place. The most important parameter for TH1-immunity is TNF-alpha, which is the one therapeutically targeted during immune modulation.

The population of TH2-cells mainly regulates the humoral immune response. The effector cytokines of TH2-cells are Interleukin-4 (IL-4) and Interleukin5. These promote the differentiation of B-cells and stimulate their antibody production. An increased IL-4 production indicates a prevailing TH2-immune response and often occurs in atopic patients and those with autoimmune diseases. A TH2-shift may also take place in the advanced stages of various chronic infections. TH2-immunity has protective effects during pregnancy.

Function: Both TH-populations regulate each other via their respective cytokines. If one response is up-regulated (called switch or shift), the other is automatically down-regulated. And to prevent an ongoing immune response from turning in to a continual inflammation, there are cytokines in charge of down-regulating the immune response. IFN- γ and IL-4/IL-10 act reciprocally in inhibiting the production of the other TH-type, meaning IL-4 prevents a TH1-response and IFN- γ prevents a TH2-response.

In immunologically healthy individuals the TH1 and TH2 populations are in equilibrium and over-reactions by one of the cell types are averted. There are many causes of a switch, or an imbalance. A physiological shift to prevailing TH2 populations takes place after age 40. This is influenced by certain factors, such as tobacco, soot, UV-radiation, psychological stress, oxidative stress, medication, poisoning, vaccines, protein deficiency etc. A healthy pregnancy is accompanied by a TH2-switch (TH2-immunity).

Recurring miscarriages or unsuccessful IVFs may be caused by a cytokine imbalance, which when treated may lead to a successful pregnancy.

Literature for review: Toth B, et al., Recurrent miscarriage: current concepts in diagnosis and treatment. J Reprod Immunol. 2010 May; 85(1): 25-32.

An example treatment is giving TNF-alpha blockers to reverse a TH1- dominated imbalance. Additional information can be gained by determining the activation status of NK-cells, allowing better therapeutic advice.

Indication:

Required diagnostics in infertility: after at least 3 miscarriages, > 35-years old woman after 2 miscarriages, after 3-4 technically and hormonally unsuccessful IVFs .

The following tests are available:

- **Determination of intracellular cytokines**

General:

The levels of the following cytokines are determined: TNF-alpha, Interferon-gamma, Interleukin 4, Interleukin 10.

Indication: immunologically-related pregnancy problems, such as recurrent miscarriages or unsuccessful IVF. Prior to treatment with TNF-alpha-blockers or assisted reproductive techniques. Therapy monitoring can only be done if initial levels are known.

Material: 10 ml heparin-blood. **Please call FML before submitting the blood!!**

TAT: 7 - 10 days*

Method: Flow cytometry

- **NK-cell assay**

General:

The lytic activity of natural killer (NK) cells on a myeloid tumor cell (K562) is determined. This activity reflects the strength of an unspecific immune response by NK-cells.

Indication: Stimulatory test to determine basal activity and ability of cells to be stimulated.

Material: 15-20ml fresh CPDA-blood (few hours old only), do not chill. NK-cells are very sensitive, so valid results are dependent on proper pre-analytics. **Please call FML before submitting the blood!!**

TAT: 7-10 days*

Method: Flow cytometry

- **Partner lymphocyte vaccination**

General:

A partner lymphocyte vaccination can be carried out at MVZ Heidelberg, Wasserturmstrasse 71, D-69214 Eppelheim/Germany, Tel.: +49-6221-793130, www.immu-baby.de, email: kinderwunsch@synlab.de by Dr. med. S. Reichel-Fentz.

Fertility diagnostics

Examinations in general	
<i>Anatomical</i>	hysteroscopy, laparoscopy
<i>Genetic</i>	karyotyping of both parents, miscarriage material (as of 3 rd miscarriage)
<i>Endocrine</i>	prolactin, diabetes?, testosterone, DHEAS, LH, FSH, progesterone, estradiol, TSH
<i>Autoimmune</i>	antinuclear antibodies, phospholipid antibodies, β 2-glycoprotein antibodies, thyroid gland auto-antibodies (TPO, TG, TSHR), sperm antibodies, cellular immune status, TNF alpha.
<i>Alloimmune</i>	HLA class I+II typing of both partners, cross match, immune differentiation, TNF alpha, further interleukins.
<i>Coagulation</i>	PTT, Lupus anticoagulant, Factor V and Factor II mutation, protein C, protein S, antithrombin 3, homocysteine, thrombocyte count, factor XII act., MTHFR gene mutation, PA 1-gene.
<i>Infectious</i>	toxoplasmosis, chlamydia infection, chlamydial heat shock proteins (HSP); ureaplasma, mycoplasma.
<i>Environmental</i>	lead, cadmium, mercury, pesticide, ethylene glycol etc.

For complete list of laboratory test offered at Freiburg Medical Laboratory, please visit
<http://www.fml-dubai.com/parameter-listings/>