

A guide to reproductive immunology

Part of the Treatment Guide series of brochures

Attention:

The information provided in this brochure should not be used as a substitute for information or advice provided by a doctor. Your doctor can help you choose the best option for you.

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Introduction

Infertility in both sexes can be caused by immune system disorders. There are several dozen immunopathologies that can result in sterility or infertility.

Reproductive immunology deals with the nature, function and development of germ cells such as sperm and eggs. It deals with immune system interrelationships, especially the development of sperm and oocytes, the embryo and its connection to the mother, and both early and late pregnancy in terms of various immunological pregnancy disorders, including miscarriage, preeclampsia or other complications in

the mother associated with an underlying disease, or linked to in vitro fertilisation itself.

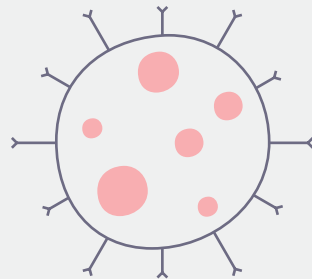
It is estimated that about 10% of couples with fertility disorders have an imbalance in their immune responses.

In IVF, we focus on treating immune system imbalances in both partners, which can affect your reproductive capacity as well as the treatment process. An immunology test will reveal whether a problem with your immune system is causing your infertility. If so, our immunologist will help you treat this imbalance to maximise your chances of conception.

Who are immunology tests recommended for?

An immunology test is indicated:

- after other etiological causes of sterility are ruled out or resolved
- after two unsuccessful IVF cycles or two unsuccessful embryo transfers
- if one of the partners has been diagnosed with an immune disorder (e.g. systemic autoimmune disease, malignancy, thyroid disease, etc.)



The immunologist uses the patient's medical history and current health to plan tests necessary to reveal or rule out an immunological cause of infertility. After analysing the results of the recommended tests, he creates a treatment plan and schedules check-ups to determine the effect of the treatment.



Laboratory tests for women

Sterility

Sterile women are unable to conceive naturally, so we look for the cause of their sterility at the beginning of the process, i.e. when the sperm and egg meet; we also perform tests for basic immunological diseases and cellular immunity tests, monitoring the following:

- ✓ Basic parameters, i.e. serum levels of immunoglobulins, white blood cell count and type, complete blood count, CRP – basic inflammatory markers.
- ✓ Reproductive antibodies, i.e. Antisperm antibodies, ovarian antibodies and zona pellucida (oocyte coat) – the test monitors the response against reproductive antigens.
- ✓ AMH (Anti-Müllerian hormone) – the amount of this hormone correlates with the ovarian reserve.
- ✓ Autoantibodies – antibodies made against substances formed by a person's own body. Anti-nuclear antibodies (ANA) are a general marker of autoimmune

diseases, TPO antibodies are thyroid antibodies, tTG antibodies are found in celiac disease, and antiphospholipid antibodies are directed against phosphorus-fat components of your cell membranes.

- ✓ NK cell activity – we culture the patient's NK lymphocytes along with sperm stimulation, a trophoblastic cell line and a non-specific activator and monitor the expression of the CD69 activation marker on the surface of NK and NKT cells. High expression of activation markers after sperm or trophoblast stimulation is indicative of increased cytotoxic reactivity directed against partner/fetal antigens and may affect the success of conception or increase the risk of miscarriage. NK and NKT cell numbers in the blood must also not be too high.
- ✓ Cytokine production – we culture the patient's T-lymphocytes along with sperm stimulation, a trophoblastic cell line and a non-specific activator and monitor the amount of cytokines inside the cells. The production of embryoprotective IL-4 and IL-10 is desirable, while a high amount of embryotoxic IFN-gamma and TNF-alpha is a sign of pro-inflammatory cytokines that pose a risk in pregnancy.

Infertility

Infertility is a condition characterised by repeated miscarriages. Women with infertility become pregnant but are unable to carry to term and deliver a viable foetus, and an immunological problem can occur at any stage of pregnancy.

- ✓ Basic parameters, i.e. serum levels of immunoglobulins, white blood cell count and type, complete blood count, CRP – basic inflammatory markers.
- ✓ Autoantibodies – antibodies made against substances formed by a person's own body. Anti-nuclear antibodies (ANA) are a general marker of autoimmune diseases, while some of them (ENA antibodies) also pose a risk to foetal development. TPO antibodies are thyroid antibodies, tTG antibodies are found in celiac disease, and antiphospholipid antibodies are directed against phosphorus-fat components of your cell membranes.
- ✓ Complement components – a complement is a complex system of blood plasma proteins that the immune system uses to identify a harmful substance, and it decides how to dispose of this harmful substance. If some complement components do not function sufficiently or correctly, the immune system may have a reduced chance of proper maternal immune tolerance. This can result in early and late miscarriages, preeclampsia and some other pregnancy complications. The complement we monitor is mannose-binding lectin (MBL).

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Sometimes reproductive immunology tests come back normal, but we still suspect an immunological cause of the fertility. This applies to both sterility and infertility. In this case, we can perform tests on white blood cells present directly in the endometrium – this is called uNK immunophenotyping. At a certain stage in the menstrual cycle, a painless procedure is performed in which a small piece of mucous membrane is taken from the uterine cavity (approx. 1 mm), and then the white blood cells, especially uterine NK cells, are tested.

There may be very few of these useful blood cells in the uterus (insufficient in situ activation), or there may be plenty of them, but they may exhibit the wrong 'killer' activity (cytotoxic immunophenotype); in addition to the predominance of 'killer' NK lymphocytes, there may also be a lack of protective/embryoprotective Nk lymphocytes. The doctor can use these results to tailor therapeutic immunomodulation.

For a comprehensive view of the issue, it is essential that the male partner also undergo tests, focusing on a semen analysis.



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Clinical Immunology and Allergy

Laboratory tests for men

The first choice for immunological testing in men is a semen analysis according to WHO standards from 2010. For accurate results, it is important to follow the basic rules of semen collection. Sexual abstinence is recommended for a minimum of three to four days before sampling. The sample is collected in a sterile container. The ejaculate cannot be taken from a condom, which usually contains spermicidal substances. The sample must be transported to the laboratory within 60 minutes at a temperature close to body temperature.

A combination of classic light microscopy (motility, morphology) and flow cytometry is used to determine the above parameters of the semen; the sperm count and leukocytes are examined along with sperm vitality. This method also verifies the integrity of the sperm acrosome and the presence of intra-acrosomal protein. The acrosome contains the enzymes hyaluronidase and acrosin, which break down the outer membrane of the egg called the zona pellucida, allowing the haploid sperm nucleus to enter the egg.

The main parameters we evaluate include

- ✓ ejaculate volume and pH
- ✓ sperm concentration, motility, morphology and vitality
- ✓ leukocyte count

Sperm vitality

sperm vitality is measured with propidium iodide, which penetrates the disrupted membrane of dead cells and binds to DNA. We calculate vitality as a percentage of live sperm in the total count.



Sperm and leukocyte count

Sperm and leukocyte counts are based on the addition of an internal standard (fluorescent beads of a known number) to the ejaculate sample.

Oligozoospermia is characterized by a sperm count of less than 15 million/ml. Leukocytes in the sample are detected with the anti-CD45 PE-Cy™5 antibody. With an increased number of leukocytes ($> 1 \times 10^6/\text{ml}$), we recommend a microbiological analysis of the ejaculate to rule out an infectious cause.

Acrosome integrity measurement

The acrosome integrity is measured with a test for intra-acrosomal protein (IAP), which is present in the acrosome. If the sperm acrosome is not disrupted, this protein cannot be detected. In sperm with a disrupted membrane, this protein is accessible to and detected by the anti-IAP antibody. Normal values: $< 30\%$ abnormal sperm.



Presence of intra-acrosomal protein

After disruption or permeabilisation of the sperm membrane, the intra-acrosomal protein (IAP) becomes accessible to the anti-IAP antibody and is detected. If the sperm do not contain the intraacrosomal protein, it is not detected after permeabilisation of the sperm with an anti-IAP antibody. Normal values: $> 65\%$ sperm. If the blood-testis barrier is breached (injury, inflammation,

surgery, tumour...), immunocompetent cells reach the surface of the sperm and anti-spermatozoal antibodies can be formed. We measure antisperm antibodies in the IgA and IgG class with a direct MAR test, and we evaluate them under a microscope. Normal finding: $< 50\%$ motile sperm with sperm-bound antibody.

Testing in couples with fertility problems

Immunological testing of both partners should be an integral part of care for couples with fertility problems. About 10% of all sterility is believed to have an immunological cause. Disorders can occur both in humoral (antibody-mediated) and cell-mediated immunity. Immunopathological diseases are often covert with no obvious issues, but they trigger an inflammatory reaction in the body, disrupting the balance of immune mechanisms. Pregnancy requires

immunological tolerance of the foetus by the mother's immune system, as inflammation can harm it.

The aim of immunological testing is to determine the cause of the imbalance and then treat and restore it appropriately. Without sufficient long-term, targeted immunological treatment, IVF treatment fails repeatedly, partners become stressed and their health continues to deteriorate.

Target group for treatment

- ✓ Couples (especially over 35 years of age) who have not conceived after one year of regular unprotected intercourse.
- ✓ Couples that have failed to conceive after one, two or more IVF cycles (age factor is important).
- ✓ Patients that have experienced two or more miscarriages (the age factor must be taken into account, especially in women).
- ✓ Patients that have experienced a late miscarriage, with a history of preeclampsia, HELLP syndrome or other pregnancy complications with a possible immune disorder.
- ✓ Couples with an autoimmune disease, oncological disease or other serious underlying diagnosis.
- ✓ Before cryopreservation of sperm or eggs due to a serious oncological diagnosis and before medical procedures (surgery, chemotherapy).

Immunotherapy - immunointervention

The term immunointervention refers to artificial and targeted modulation of the body's immune responses.

Immunointerventional therapy includes immunosuppression (suppresses the immune response), immunosubstitution (supplements missing components of immunity), immunonormalisation and immunomodulation (only affects certain processes in immune reactions).

Immunointervention for immunological causes of infertility has been developing rapidly in recent years. Our medical armamentarium currently includes

corticosteroids, antimalarials, thiopurines, phosphodiesterase inhibitors, antiaggregants, intralipids, polyspecific immunoglobulins and more.

We follow the recommendations of the European Society for Reproductive Immunology (ESRI) and the International Society for Immunology of Reproduction (ISIR) in our preparation of treatment procedures. In the Czech Republic, we are also a member of the Czech Society of Allergy and Clinical Immunology under the J.E. Purkyně Czech Medical Association.



You can find lots of information adapted to various stages in a couple's journey on our website www.europeivf.com/en.

Are you ready to take the first step?

If you're ready, you can book your first appointment at our clinic. Our coordinators will arrange an in-person meeting, video meeting or phone call. You can contact us during normal business hours, or we can arrange a time that suits you best.

Book a consultation with one of our fertility specialists

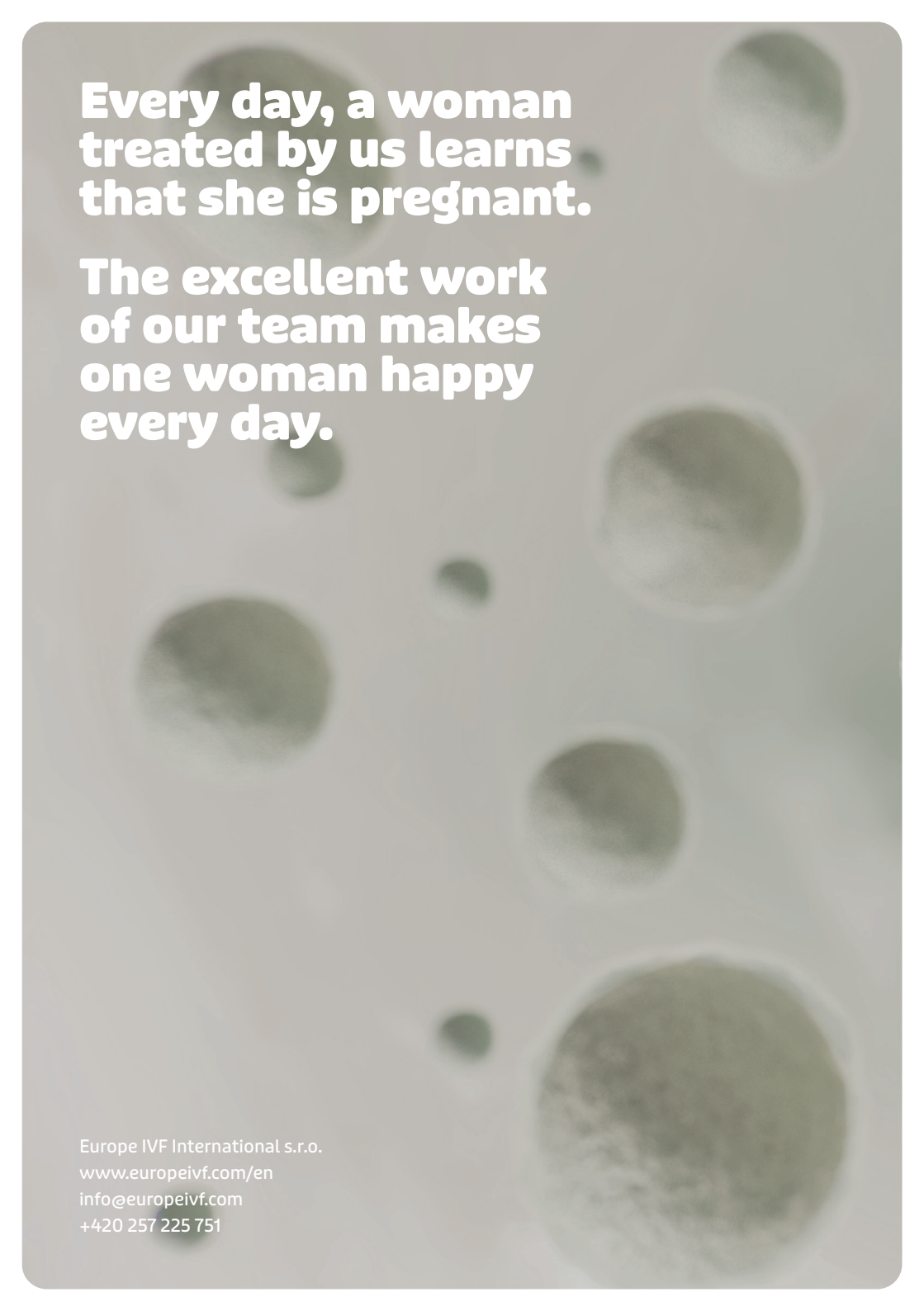


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**If you have any questions,
ask us.**



You dream. We care.



**Every day, a woman
treated by us learns
that she is pregnant.**

**The excellent work
of our team makes
one woman happy
every day.**

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