BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

IVF MANUAL

INTRODUCTION

Dear Madam,

You have been referred to this document because you have not managed to get pregnant, for now, and have requested medical assistance. According to the Ministry of Health's regulations, patients undergoing medically assisted reproduction need to receive in written form all information regarding the procedure following which they need to sign a consent form for the procedure. We will here describe to you in detail various procedures and ask you to carefully read the information pertaining to your procedure.

Participation in assisted reproduction procedures are physically, emotionally and financially one of the most demanding and most intense periods you will experience in your life. If you need psychological help or support during the procedure, it is well-known that consultations with psychologists and/or joining forums and support groups help couples undergoing procedures. Do not hesitate to seek help. It is important that you begin the procedures ready for what awaits you, and also that, in agreement with your reproductive gynecologist, you decide upon the method which suits you best at the given time.

The methods are first most often simple ones, such as ovarian stimulation with medication and timed sexual intercourse or insemination, and IVF/ICSI will most often be recommended only after all other options have been explored. However, there are also exceptions to this rule where we will sooner opt for IVF/ICSI, such as advancing age in patients, many years of infertility regardless of age, more serious causes of infertility, large distances from the assisted reproduction center, and similar.

The methods of Medically Assisted Reproduction **(MAR)** which we perform in BetaPlus Center are:

- artificial insemination by husband **(AIH)**
- in vitro reproduction **(IVF)** and intracytoplasmic sperm injection **(ICSI)**
- cryopreservation and thawing of reproductive cells and embryos

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

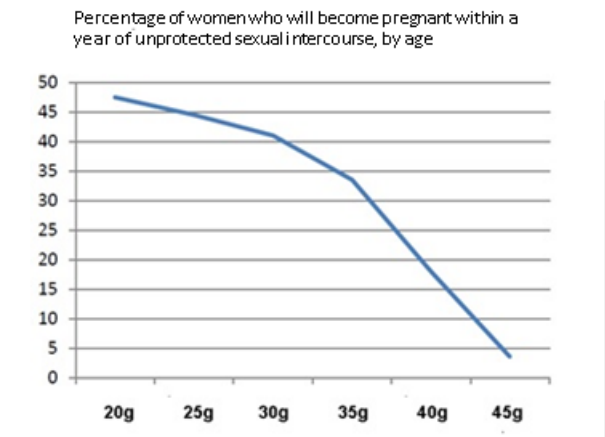
Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

You have been referred for assisted reproduction because you have one or more of the following causes of infertility:

- Tubal factor (obstructed fallopian tubes). Since the reproduction of oocytes with sperm occurs in the fallopian tube, if the fallopian tubes are physically impassable reproduction has nowhere to take place, and therefore, in vitro reproduction (in the laboratory) is therefore performed. Operations and procedures which try to re-establish patency of the fallopian tubes have been abandoned because the fallopian tubes are very fine and delicate structures which, once damaged, are beyond repair.
- PCOS (anovulation). Women who do not ovulate, be it because of polycystic ovary syndrome or because of other endocrinological reasons, have a lower chance of pregnancy because of this and are candidates for one of the methods of assisted reproduction.
- Endometriosis. It is believed that women with endometriosis have poor quality oocytes and/or damaged motility of fallopian tubes or obstructed fallopian tubes, and/or damage to ovarian reserves caused by surgery for endometriosis.
- The woman's age. After the age of 36 a decrease in the woman's fertility occurs because the majority of oocytes which a woman has have already been spent. The remaining ones are of a lesser quality, making pregnancy harder to achieve and miscarriages more frequent.



Adapted from NICE guidelines 2012

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

- Diminished ovarian reserve in young women. This is a newly identified cause of infertility characterized by diminished Anti-Mullarian Hormone values and a low antral follicle count in a woman's ovaries which causes, like advanced age in women, a lower number of remaining oocytes. Although those oocytes can be of a very high quality, the woman is what is called a "low responder", that is, she responds to therapy with medications with a lower number of oocytes than expected for her age.
- Male factor. The sperm count can be less than is standard, there can be low sperm motility or the sperm can be morphologically changed, which leads to infertility.
- Unexplained infertility. Many couples will not find out why they are unable to get pregnant and will rather be diagnosed with unexplained or idiopathic infertility, most likely something medicine has not yet identified as a separate problem.

The experts performing the MAR:

- Human reproductive medicine sub-specialists who are required to be experienced in gynecological endocrinology, reproductive medicine and medical diagnostics.
- Embryologists (biologists) who are biological engineers with extensive experience in working with reproductive cells and embryos.
- Nurses who work in institutions dealing with reproductive medicine who are required to be specially educated in that field, because they are the ones who have the most contact with patients during the assisted reproduction procedure.

The success of MAR depends on a number of factors. Generally, success rates are around 10-25% for insemination and IVF/ICSI during a natural cycle, 25-30% per cycle for IVF/ICSI in cycles where we retrieve fewer oocytes, and as high as 40-60% per procedure for IVF/ICSI in a fully stimulated cycle where we retrieve a larger number of oocytes and also have embryos for cryopreservation. We publish the success rates of MAR in our center for the previous year at the start of each new year on our web page.

Further, with IVF/ICSI procedures it is necessary to differentiate the success rate per embryo transfer (which can be as high as 70% in younger women), which is far higher than the success rate per cycle. The reason for this is that a certain number of women will not have an embryo transfer due to negative aspiration or unfertilized oocytes, and in some women we intentionally will not perform an embryo transfer in order to increase the woman's chances of getting pregnant in the next cycle.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

The success of the procedure depends, among other things, on the following (the web pages were you can read more about each item are listed in parentheses):

- the patient's age (http://www.neplodnost.hr/uzroci-neplodnostiv2/203-neplodnost-dob-ene.html)
- the cause of infertility (http://www.neplodnost.hr/uzroci-neplodnostiv2.html)
- ovarian reserves (http://www.neplodnost.hr/uzroci-neplodnostiv2/203-neplodnost-dob-ene.html)
- quality and experience of the staff (http://betaplus.hr/djelatnici-i-suradnici.html)
- quality of the endocrinology laboratory (http://www.betaplus.hr/uploads/pdf/Postupci_u_IVF_%20laboratoriju.pdf)
- how state-of-the art the apparatus and equipment in the endocrinology laboratory are (http://betaplus.hr/o-poliklinici/oprema-u-ivf-laboratoriju.html)
- quality of the media and other supplies (http://www.betaplus.hr/uploads/pdf/Postupci_u_IVF_%20laboratoriju.pdf)

AIH and IVF/ICSI can be applied during an entirely natural cycle, a cycle with mild ovarian stimulation, and a cycle with traditional (conventional) ovarian stimulation. The number of oocytes we will retrieve with this method of stimulation depends exclusively on the patient's ovarian reserves, and if the reserves are good, we will retrieve 2-5 oocytes with mild stimulation and usually far more than 6 oocytes with traditional stimulation.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

PROVIDING SEMEN SAMPLES

Refrain from sexual activity two to five days prior to insemination or the follicular aspiration. You will give a semen sample before insemination and after the follicular aspiration (in order to be sure we retrieved an egg). The staff will give you a sterile container in which you should provide your sample. Wash your hands and genitals well and open the container carefully. Only touch the outside of the lid and when putting it down, put the outer side face-down. After producing the sample carefully close the container and hand it over to the biologists.

In exceptional circumstances, you can also bring your sample in from home (in that case request a container from us). Follow the above instructions then, as well, and keep the container in a warm place during transport, preferably close to the body.

No more than one hour can pass between the time the sample is produced and the time it is processed in the laboratory. When submitting the sample it is necessary to sign a form confirming the semen is yours.

In some cases the partner cannot provide a semen sample (in cases of illness, inability, etc.) during the insemination or aspiration procedure, and we can freeze his sample ahead of time for use during the procedure.

A body temperature of over 38°C one to two months before providing a semen sample can harm the quality of the sperm. Hot baths and saunas can have a negative effect and should be avoided 2-3 months prior to the procedure. Refrain from sexual relations 2-5 days but absolutely no more than 7 days before providing a sample.

GENERAL INSTRUCTIONS

If you are receiving injections, you need to receive them around 5 p.m. or later. The reason for this is that this way an injection won't be necessary on the last day of stimulation therapy, not including the HCG injection. You can receive injections at the center from Monday through Friday and from Friday through Sunday at your nearest emergency room. We recommend, however, that you learn to give yourself the injections on your own, which your nurse will teach you to do. What is called the trigger shot, that is, the final injection in the procedure, is usually administered later at night (after our office hours). Naturally, if you are not from Zagreb, or if it does not suit you to come for the injection, any nurse or doctor, if they are able to, can administer it to you.

You do not need to bring anything with you to the procedures (insemination, aspiration, transfer). We will provide you with a disposable night grown and slippers as well as medication which will alleviate any potential pain.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

ULTRASOUND CHECK-UPS

An exceptionally important part of the assisted reproduction procedure is ultrasound check-ups. Extensive knowledge and experience in this field are required in order to determine the exact number and size of the follicles, to correlate those findings with the thickness of the uterus lining, to assess the risk of potential complications, and to decide on the best moment for completing the procedure. We at BetaPlus Center are organized so that we have a reproduction gynecologist on duty every day both in the morning and afternoon shift so that you don't have to miss work for your folliculometry scans. If you live far away, it is also possible to arrange for your gynecologist to examine you.

NATURAL CYCLE PROCEDURES

If you or your physician have selected natural-cycle IVF (or as it's called in professional literature, modified natural-cycle IVF, because one medication is administered, the "trigger shot"), on the first day of your menstruation you need to call our staff and schedule your first ultrasound between the 6^{th} and 10^{th} day of your cycle. The day of your first U/S exam depends on the length of your cycle, your age, and the expected ovulation time. It will monitor the growth of the follicle, and when the follicle reaches a size of 14-19 mm you will get an injection of HCG (the trigger shot, most often Ovitrelle).

Sometimes the physician will request blood work be done in order to check estradiol levels or an LH-test before the trigger shot in order for us to be sure your ovulation process has not already begun. 36-38 hours after the injection, ovulation will occur, and we will plan the insemination or follicular aspiration an hour or two before that window closes. If IVF/ICSI is planned we will administer an ultrasound exam as soon as you arrive in order to confirm the follicle did not rupture.

MEDICATIONS TO STIMULATE OVULATION AND MAR

We will list some of the medications which you might be prescribed in the MAR procedure as well as their side effects and how they work.

- Pills which block estrogen receptors:

Clomiphene citrate (Clomid, Klomifen) 50 mg pills

Femara pills 2.5 mg

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

These pills are taken starting between the second and fifth day of the cycle, usually on the 5th day.

They stimulate the production of one or two to three follicles, in rare instances more than that. They are most often used in the induction of ovulation in women who do not ovulate, before insemination, or in mild stimulation protocols.

These medications may cause nausea, sudden fevers, headaches, mood swings and bloating or may cause vision disorders, all of which are entirely harmless symptoms which disappear entirely after you stop taking the medication.

After these medications are taken, particularly in women with poor ovarian reserves, cysts which go away on their own in a few months are common. In extremely rare cases the hyper-stimulation of the ovaries is possible with these medications, never in women with poor ovarian reserves. More common than hyper-stimulation is an excessive reaction of the ovary to the medication, in which case we will have to abandon the insemination if it was planned and offer you the option of IVF or abandon the procedure. The limit above which we do not perform insemination is 3-4 follicles.

In insemination after therapy with these pills, if 2-3 follicles are developed, a multiple pregnancy is possible.

- Ampoules which stimulate the production of more follicles (gonadotropins):

Gonal-F ampoules (pure FSH), ampoules and "pen"

Puregon ampoules (pure FSH), ampoules and "pen"

Luveris ampoules (pure LH), ampoules

Menopur ampoules (FSH and LH), ampoules

Menogon ampoules (FSH and LH), ampoules

Elonva ampoules (corifollitropin alfa), ampoules

These ampoules are also taken starting between the second and fifth day of the cycle, or in continuation to therapy with the above listed pills. Depending on a number of factors as assessed by your physician, you may take one, two, three or four ampoules a day, except in the case of Elonve, where one shot replaces 7 days of therapy.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

- Medications which are used in short and long GnRH agonist protocols:

Suprefact spray (Buserelin - agonist Gn-Rh in a nasal spray)

Buserelin ampoules (agonist Gn-Rh for subcutaneous injection)

Decapeptyl ampoules (agonist Gn-Rh for subcutaneous or IM application)

In the long protocol these medications are taken starting the 21st day of the previous cycle or as the physician decides, but certainly before the period during which the procedure will be performed, and in the short procedure the medications are taken starting on the first day of the cycle. Suprefact spray is used in a dose of 3x2 sprays into the nostrils (every 8 hours, as closely as possible) and the ampoules once a day. There are also long-acting preparations which act for 21 days. They are used up until the final day of stimulation, that is, until the injection with the medication which stimulates ovulation. These medications balance out the growth of follicles and prevent their premature rupture.

- Gn-Rh antagonists

Cetrotide ampoules (cetrorelix, Gn-Rh antagonist)

Orgalutran ampoules (ganerelix, Gn-Rh antagonist)

Their application begins either the fourth or fifth day of stimulation, depending on the size of the follicle (usually when the follicle size is over 14mm). They are administered once daily, and there are also long-acting preparations which act for 3 days. They are given until the final HCG injection. As with agonists, these medications balance out the growth of follicles and prevent their premature rupture. Their advantage is in the shorter duration of stimulation and in the lower consumption of gonadotropin ampoules.

- Medications which are used to stimulate ovulation:

Ovitrelle ampoules (coriogonadotropin)

Choragon ampoules (coriogonadotropin)

Brevactide ampoules (coriogonadotropin)

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

The final injection in the ovarian stimulation process, popularly called the trigger shot, or HCG shot. Ovulation occurs 36-38 hours after this injection.

- Medications which are used with the frozen embryo transfer

Estrofem pills of 1-2 mg

Utrogestan 100 mg pills for vaginal preparation

Duphaston 10 mg pills

Crinone gel

Medications which are used to stimulate ovulation (Ovitrelle and similar) in the natural cycle

Side effects of medications:

When estradiol levels become mildly to moderately elevated, you may notice bloating, water retention with an increase in body weight, spotting, nausea or diarrhea, discomfort in the pelvis caused by enlarged ovaries, tender breasts, fatigue, headaches or mood swings. These side effects are harmless and are no cause for worry. During ovarian stimulation, however, ovarian hyperstimulation syndrome (OHSS) can occur and is the most serious side effect of the assisted reproduction procedure.

It can ONLY develop in women with high AMH and a large number of antral follicles.

Symptoms of potential OHSS are rapid weight gain, rapidly increased waist size, shortness of breath, abdominal and lower abdominal pain, nausea and vomiting. Further development of the syndrome may lead to fluid in the belly and lungs, and the risk for thrombosis and embolism is increased, which requires admittance to the hospital. Today we have numerous methods with which we can reduce the chances of OHSS or its severity (skipping treatment, aspiration of one ovary a day earlier, not using HCG, abandoning ET and freezing all embryos), so even if you are at risk of developing OHSS we do not expect a serious form of this syndrome to develop. The best way to alleviate symptoms of mild OHSS is strict bed rest along with drinking many liquids and pain medicine, as needed, and we therefore ask you to adhere to these steps if you notice any of these symptoms.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

STIMULATED CYCLE PROCEDURES

If stimulated cycle treatment is planned for you, be it with mild or traditional ovarian stimulation, carefully follow the instructions you received during your consultations. In what is called the long stimulation protocol, therapy begins on the 21st day of the previous cycle (or a few days later, depending on the length of your cycle) and it is possible your menstruation is late because of the therapy. In that protocol, a spray or injection (Suprefact, Decapeptyl) is used all the way up until the final injection of Ovitrelle. It is important to remember that once you begin receiving therapy, you must continue to do so every day until your physician terminates it.

In other stimulation protocols stimulation begins between the first and third day of your cycle, or a whole cycle before assisted reproduction. Sometimes you will begin oral contraception a month or two before stimulation in order to better regulate your cycle.

If you were not told otherwise, you should call our staff on the first day of your menstruation and schedule your first ultrasound for between the 5th and 8th days of stimulation, depending upon the procedure and what you and your physician have agreed on. At that ultrasound a folliculometry will be performed, estradiol levels might be measured, and therapy will be adjusted if needed. You will also then receive instructions on when you need to come in for your next visit. Depending on your reaction to medications, 1-4 folliculometries will be required before aspiration. The growth of the follicle will then be monitored and when they reach a size of around 17mm you will get an HCG injection. 36-38 hours after that injection ovulation will occur, and we will plan the insemination or follicular aspiration around that time. There is no need for you to change your normal lifestyle during stimulation. Sexual intercourse is permitted all the way up to 4-5 days before ovulation, unless your physician warns you otherwise.

POTENTIAL REASONS FOR A FAILED PROCEDURE OR FOR ABANDONING THE PROCEDURE

During the procedure of medically assisted reproduction, a number of unpredictable situations which require the procedure be abandoned are possible:

- stimulation does not at all lead to the development of a follicle. This is possible in women with exhausted ovarian reserves (low AMH an AFC)

- stimulation creates too many oocytes leading to an increased risk of ovarian hyperstimulation syndrome. Too many oocytes usually means there are more poor quality ones and the chances of pregnancy are no greater than in moderate stimulation. This is possible in women whose ovaries appear polycystic at the ultrasound.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

- in a spontaneous cycle, premature ovulation is possible and in that case the aspiration is not performed. Occasionally, if your fallopian tubes are passable and the spermiogram results are satisfactory we will in this case offer to perform insemination

- an egg is not retrieved during the aspiration. Very common with women with a poor ovarian reserve

- the oocytes are of a poor quality and do not become fertilized. Such oocytes are called degenerative oocytes and they are far more common in patients over the age of forty

- the oocytes appear to be of a good quality but reproduction still does not occur. This sometimes happens with traditional IVF, particularly in couples with unknown causes of infertility or endometriosis

- the embryos divide unevenly and stop developing (triploidy)

In all the above cases, the procedure is abandoned, and the couple is offered an explanation as well as instructions for the next potential procedure.

It is also important to know:

- that the number of follicles during stimulation can decrease because not all follicles have good growth potential

- that a certain number of follicles do not necessarily contain an egg, so we retrieve fewer oocytes than there were follicles

- that not all oocytes are fertilized (usually around 70% are fertilized)

- that not all fertilized oocytes with lead to embryos (embryos develop with 70-80% of fertilized oocytes)

- therefore the average procedure in women with a normal ovarian reserve looks like this: if we retrieve, for example, 10 oocytes, we will cultivate 9 because one won't be of a good quality, 7 oocytes will be fertilized, two embryos will not continue to develop. Of the five remaining embryos, we can transfer one or two the second or third day. We will leave the rest in a growth medium until the 5th day, when we will cryopreserve them if they managed to develop into blastocysts.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

FOLLICULAR ASPIRATION

Before the aspiration you will most often need to place into your vagina a vaginal tablet we will provide you with, unless you have an allergy or we are planning an alternative method of disinfection before the aspiration.

On the day the procedure is planned for, you will arrive at the center at the agreed time. Do not eat anything after dinner the previous night, or if your procedure has been planned for the afternoon, do not eat or drink anything 6 hours prior to the procedure.

We will take you to a room where you will lie down after the aspiration, where you will put on a night gown. It is important to empty your bladder entirely immediately before the aspiration. The aspiration (aspiration) is performed transvaginally and under ultrasound guidance. You will lie down on the table. If you have more than one follicle, a nurse will give you an injection to make the procedure as painless as possible. We will introduce the ultrasound probe.

A special aspiration needle is passed through the wall of the uterus and into the ovary. The needle is connected with plastic tubes to containers for collecting the content which has been sucked out.

The aspiration follows, where the oocyte complex is pulled from the follicle wall when the fluid is aspirated through the needle. Although the needle is long, which sometimes frightens patients, it is inserted no deeper than 5mm for the superficial follicles. The needle is not too painful and patients who opt to undergo the procedure without analgesia generally tolerate it well.

The biologist immediately checks the delivered material with a microscope so you will know before you leave how many cells we retrieved and what their quality is like.

After the procedure you will stay and lie down for another hour or two. Before going home you will receive instructions on the therapy you need to take and a discharge letter which states how many cells we retrieved.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

COMPLICATIONS AND RISKS WITH THE MEDICAL PROCEDURE

Although complications after aspiration are exceptionally rare, they occur nonetheless, and we therefore need to familiarize you with them. They are injuries to the internal organs (ex.: intestines, blood vessels, bladder). These injuries are more common in women who have undergone surgery or had infections or endometriosis in the abdomen (adhesions) and in cases of repeated aspiration of the follicle. There are also potential complications and risks with introducing the cannula through which we administer the intravenous injections, allergic reactions and similar.

- mild bleeding occurs with almost every aspiration; it stops within a few minutes and does not require further therapy
- heavier bleeding might require surgical attention (stitches)
- bleeding from the bladder after aspiration is possible in patients with a distorted anatomy. This bleeding can last a few days and sometimes requires hospital treatment but usually does not require any other procedures or have long-term effects
- in patients with a distorted anatomy damage to the intestines is possible and can lead to bleeding, intestinal infections and sometimes inflammation of the peritoneum, which requires surgery and potentially the resection of part of the intestine
- infections are a very rare complication of aspiration. They are delayed and occur in women who generally have problems with infections; in extreme cases they may require surgery to remove the accumulation of pus in the abdomen and in extremely rare instances they may require the removal of the uterus and ovaries
- bruising and haematomas, damage to the skin and nerves at the site of the introduction of the cannula
- inflammation of the superficial veins which, if left untreated, can lead to a generalized infection and hospitalization
- nausea and vomiting following the administration of opioid narcotics used with aspirations
- allergic reactions to drugs which can be light (itching, irritation of the skin) or severe (anaphylactic shock with serious consequences)
- pneumonia after the leaking of stomach contents into the lungs in the case of eating and drinking before the anesthesia

The only known long-term side-effect of hormonal stimulation in healthy women is an increased risk of borderline ovarian tumors.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

INTRAUTERINE INSEMINATION (AIH - Artificial Insemination by Husband)

On the day of the planned procedure you will arrive at the center at the scheduled time. Your partner will provide a semen sample. You will wait for our biologists to process the semen, after which we will take you to our procedure room. You will lie down on the table, the gynecologist will place the instrument in your vagina and show the cervix. Your doctor will then introduce a thin catheter into your uterus or fallopian tube and squeeze the processed semen in through it. The procedure is painless; you might just feel mild cramping similar to menstrual cramps during the procedure.

You will remain lying down some 15 minutes, after which time you can leave. Refrain from heavy activity the remainder of the day, but you do not need to lie down. You might feel cramping in your abdomen after insemination.

IVF and ICSI

On the day of the planned procedure you will come to the center at the scheduled time. Follicular aspiration will be performed as described above. If we retrieve oocytes through the aspiration, your husband will provide his semen. The biologist will examine the semen and decide, depending on the quality of the semen that day, if the oocyte will be fertilized using IVF or ICSI. ICSI is recommended in cases of severe male infertility, previous failed reproductions or in cases where the woman is over 42 or has a diminished ovarian reserve.

With most couples, the choice of methods will have been specified earlier based on a review of medical documentation, but in a certain number of cases, depending on the number and quality of oocytes as well as the quality of the sperm, it is possible that a method other than the one which was planned will offer you greater chances for success, and we will therefore recommend it.

The IVF procedure is as follows. The retrieved oocytes are inseminated (by adding the processed sperm into the medium with the oocyte) and incubated in strictly controlled laboratory conditions. The following day each individual oocyte is examined and the success of reproduction is assessed, after which we inform you of the results of the reproduction.

The ICSI procedure is as follows. The oocytes are cleaned of the granulosa cells surrounding them immediately after the aspiration. One cell is placed in a lab dish and held with a special pipette. Another pipette is used to select a single sperm and to inject it into the oocyte. 16-20 hours later the oocyte is examined to see if adequate reproduction and division was achieved.

The ICSI procedure results in a somewhat higher rate of deformations in the child, while the rate in IVF procedures is equal to that of spontaneous pregnancies.

BetaPlus
POLIKLINIKA ZA GINEKOLOGIJU, PORODNIŠTVO I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

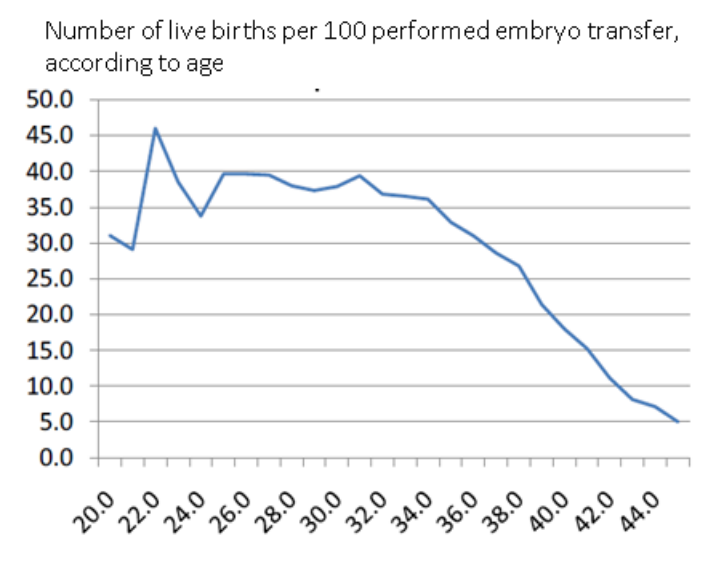
EMBRYO TRANSFER

Embryo transfer is performed at earliest twenty-four hours after the aspiration, although that is rare in practice. The earliest we transfer an embryo is after 48 hours. In exceptional situations this period is extended to between 72 hours and five days.

If only one or two embryos are developed, they will be transferred the second or third day, because we have nothing to gain by extending cultivation to five days, we are just unnecessarily manipulating the embryos. According to the Croatian Medically Assisted Reproduction Act, we are allowed to transfer two embryos, in exceptional circumstances three, if the patient is older or has many failed procedures behind her or in very serious cases of male infertility. With a larger number of embryos the risk of multiple pregnancy increases and with it the risk of miscarriages, premature births and complications during pregnancy.

On the day of the planned procedure you will arrive at the center at the scheduled time. We will take you into a room where you will change into a night gown. You will lie down on the table, the gynecologist will insert an instrument into your vagina and show the cervix. Your doctor will then introduce a thin catheter and squeeze the processed semen in through it. The procedure is painless; you might just feel mild cramping similar to menstrual cramps during the procedure.

You will remain lying for some time, after which time you can leave. Refrain from heavy activity the remainder of the day, but you do not need to lie down. If you live more than a 3-hour drive away, we recommend you do not travel home the same day. You might feel cramping in your abdomen after the embryo transfer.



BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

The image shows the percentage of live births of children which are born for each 100 performed embryo transfers according to the woman's age (the data is from HFEA, adapted from the NICE Guidelines, 2012). We can see from the image that in 100 embryo transfers in women over 30 we can expect 37 children to be born, and in women over 45, 5 children.

FREEZING UNUSED EMBRYOS AND OOCYTES

Under the provisions of the Croatian Medically Assisted Reproduction Act, up to 12 oocytes can be fertilized in one cycle, and potential unused embryos are cryopreserved for later use. Couples can choose to also fertilized one or two oocytes and cryopreserve the unused oocytes. Also, if we have an embryo for embryo transfer, and for some medical reason it is not advisable to perform the embryo transfer (the most common reason being the threat of serious ovarian hyperstimulation syndrome), that surplus of embryos can be saved for later embryo transfer by a controlled freezing and thawing procedure.

The embryos and oocytes will be frozen at -196 degrees through a vitrification procedure used in the majority of IVF centers. The physical procedure of freezing and thawing itself is strictly controlled in order to avoid damage to or loss of material. The frozen embryos and oocytes will be preserved in special laboratory dishes placed in liquid nitrogen. The origin, division phase it is in and exact time and date of cryopreservation is separately taken down for each individual embryo or oocyte.

The reproductive cells and embryos will remain cryopreserved until the couple requests their use. When thawing oocytes, ovulation in the natural cycle will be monitored, and the oocytes will be thawed on the day ovulation occurs. The partner will provide his semen and an intracitoplasmatic sperm injection will be performed. 2-5 days later an ET will be performed.

With cryopreserved embryos, when the gynecologist prepares the lining of the uterus to receive the embryo and performs an ultrasound examination to assess whether the conditions required for an embryo transfer have been reached, the controlled thawing of the required number of embryos is performed.

It is important to be aware that sometimes the oocytes and embryos are not of an adequate quality for controlled freezing and thawing. Bearing that in mind, we cannot offer all patients the option of cryopreservation. Further, some reproductive cells may not survive the process of cryopreservation and thawing.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

CRYO EMBRIO TRANSFER (FET - FROZEN EMBYOTRANSFER)

FET, or cryo embryo transfer, can be performed during the natural cycle or with the preparation of the lining with medications. In the natural cycle the requirement is that the woman is ovulating, while that is not necessary when preparing the lining with medications.

Natural cycle: The woman is usually told to come to her first ultrasound around the 10th day of her cycle (if she has regular 28-day cycles), when a check-up is performed to see if the dominant follicle in that cycle is developing and the day the patient needs to begin using LH strips to identify ovulation is set. The LH strips are used in the morning or evening hours, one strip every 24 hours, and they are a very reliable way of identifying the day of ovulation. Please hold onto the strips, and after you are sure the strip is positive, do another one in 24 hours in order to be sure it is no longer positive. Afterwards, line all the strips up on a white piece of paper, and beside each strip, write down the date it was done. Take a photo of all of the strips together on your cell phone and send us the photo. We will get back to you with instructions on when the embryo transfer will be.

Preparing the lining with medications: 2 mg Estrofem tablets are used in various doses starting with the first day of your cycle as determined by your doctor. Sometimes, especially if the woman has a short cycle or if we want to be entirely certain that ovulation will not occur, GnRh agonist injections are used over the course of 7 days. The first ultrasound is set for between the 6th and 10th day of the cycle, depending on the dose of medications, when the dose of Estrofem is either increased and/or gestagen (usually Utrogesetan 3x200mg) is introduced. After introducing gestagen, the embryo transfer is planned for a few days later, depending on the age of the embryo.

It is important to remember that it is necessary to take the pills every day until at least the 8th week of pregnancy. The embryo transfer itself is performed the same way, regardless of whether the embryo is fresh or was frozen.

AFTER THE EMBRYO TRANSFER

You are required to adhere to the therapy you were prescribed in the discharge letter, because in vitro pregnancy is unlikely without it.

Limit intensive activities the first couple of days. This does not mean you have to spend them lying down, but rather, do not strain yourself physically. Instead, read or go for walks, etc. Do not take baths, shower instead. Do not use tampons. Refrain from sexual intercourse until your pregnancy test and until at least 4-5 days after ET in a stimulated cycle. You can go back to your everyday activities a few days after the embryo transfer. There is no evidence that bedrest increases the chances of success of IVF.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

Approximately fifteen days after aspiration or insemination, by measuring beta-HCG levels, we determine the outcome of the procedure. You can get your Beta HCG levels tested with us or in any hospital or private laboratory.

Days following aspiration or insemination	Average Beta HCG value
14	48
15	59
16	95
17	132
18	292
19	303

We would like to emphasize that these values are just guidelines, because Beta HCG values vary greatly, and if your results are far lower or far higher than this, that is not necessarily reason for concern. A positive test (which varies from one lab to the next, but usually more than 0 or more than 5) is proof of what is called biochemical pregnancy. In a certain number of cases biochemical pregnancy continues to develop without clinical pregnancy occurring. This is more frequent in women over 40. Clinical pregnancy is proven with an ultrasound finding of the gestational sac, some 25 days after follicular aspiration.

When clinical pregnancy is determined, the risk of miscarriage is the same as in a spontaneous pregnancy, around 10-15%. After an ultrasound shows the beating of the embryo's heart, the risk of a miscarriage is 3-5%. The risk of an ectopic pregnancy in IVF is around 2%. The risk of a heterotopic pregnancy (where one embryo develops in the uterus and another in the fallopian tube) is around 1%.

It is important to perform a Beta HCG test regardless of any potential bleeding, because bleeding can occur in early pregnancy as well. The earliest an HCG test can be performed is 12-14 days after follicular aspiration, and if it is negative, there is no pregnancy.

For statistical purposes, please inform us of the results by phone, fax, or email as found in our letterhead.

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

MULTIPLE PREGNANCIES IN IVF PROCEDURES

For a number of years now multiple pregnancies have been considered unsuccessful medically assisted reproduction procedures because they are high-risk pregnancies. There is an increased rate of miscarriages, premature births, developmental difficulties in one or more of the children as well as perinatal mortality and morbidity in multiple pregnancies. In BetaPlus Center we strive to keep down the number of multiple pregnancies in our institution and we will recommend the transfer of only one embryo if we assess that you are a multiple pregnancy candidate. The final decision of whether we will transfer one or two embryos is yours. It is important to know that single pregnancies resulting from medically assisted reproduction procedures have the same risks and dangers as spontaneous pregnancies.

DEFINITIONS

SEMEN ANALYSIS determining the number, motility and morphology of the semen sample

AIH (IUI) Artificial Insemination by Husband, Intra Uterine Insemination. Introducing sperm into the uterus

ANALGESIA collective name for procedures which reduce pain

ASPIRATION the procedure where a thin needle, guided by ultrasound, empties the content of a follicle from the ovary and collects oocytes

BETA HCG a hormone which is produced in early pregnancy

BLASTOCYST an embryo on the fifth day after reproduction, containing more than 100 cells

ESTRADIOL (E2) most powerful estrogen. The basic reproductive hormone in women which stimulates the growth of all tissues and organs

FOLLICLE basic functional unit of the ovary. Contains the oocyte, numerous hormone-producing cells, and follicular fluid. With ovulation the mature follicle ruptures and releases an oocyte.

FOLLICULOGENESIS maturation of the ovarian follicle

FOLLICULOMETRY ultrasound monitoring of the growth of a follicle

GN-RH Gonadotropin-releasing hormone

BetaPlus
POLIKLINIKA ZA
GINEKOLOGIJU, PORODNIŠTVO
I REPRODUKCIJSKU MEDICINU

Av. V. Holjevca 23
HR - 10 000 Zagreb

Tel: +385 (0)1 5580 250
Fax: +385 (0)1 5580 251
E-mail: poliklinika@betaplus.hr

www.betaplus.hr
www.neplodnost.hr

GONADOTROPIN FSH and LH (ex. Gonal F, Menopur) injections which are administered in the process of stimulating the growth of follicles

HCG Human chorionic gonadotropin. A hormone produced by pregnancy. Also found in Ovirella injections, which stimulate ovulation

ITI Intratubal insemination. Introducing the partner's semen into the fallopian tube

ICSI Intracytoplasmic sperm injection, injecting sperm directly into the egg

IVF in vitro reproduction

CATHETER very thin plastic tube with which we introduce processed semen into the uterus in insemination and embryos in embryo transfers

CRYOSTORAGE freezing, storing, and thawing embryos

LH Luteinizing hormone (pituitary hormone). Necessary for ovulation in women

LH test – a test which determines LH increases in urine samples using strips. A positive LH test means LH growth has already begun and that it's late for an HCG injection

OVULATION discarding of mature oocytes from the follicle, most often the 13th-15th day of the menstrual cycle