eugonia

Assisted Reproduction Unit

In Vitro Fertilization

Everything you need to know

eugonia

Assisted Reproduction Unit

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Opening times:
Daily from 9am to 6pm,
Saturday from 9am to 3:30pm.

Κύριε Λαγκά
Άξια υπερβήκατε την
εμπιστοσύνη μας.
Καλή συνέχεια στο έργο σας

Για το πολυαγαπημένο
δωρο σας δωσατε
ενα χαμόγελο από τα
βάθη της καρδιάς μας

Together we can make your dream come true!

Ευχόμαστε να είστε πάντα καλά και
με το λειτούργημα που επιτελείτε να
δίνετε ευτυχία & ζωή σε τόσα μωράκια

Το ευχαριστώ είναι πολύ
λίγο για να εκφράσει
την ευγνωμοσύνη μας,
μα δυστυχώς δεν υπάρχει
άλλη λέξη ...
Ευχαριστούμε για όλα

eugonia

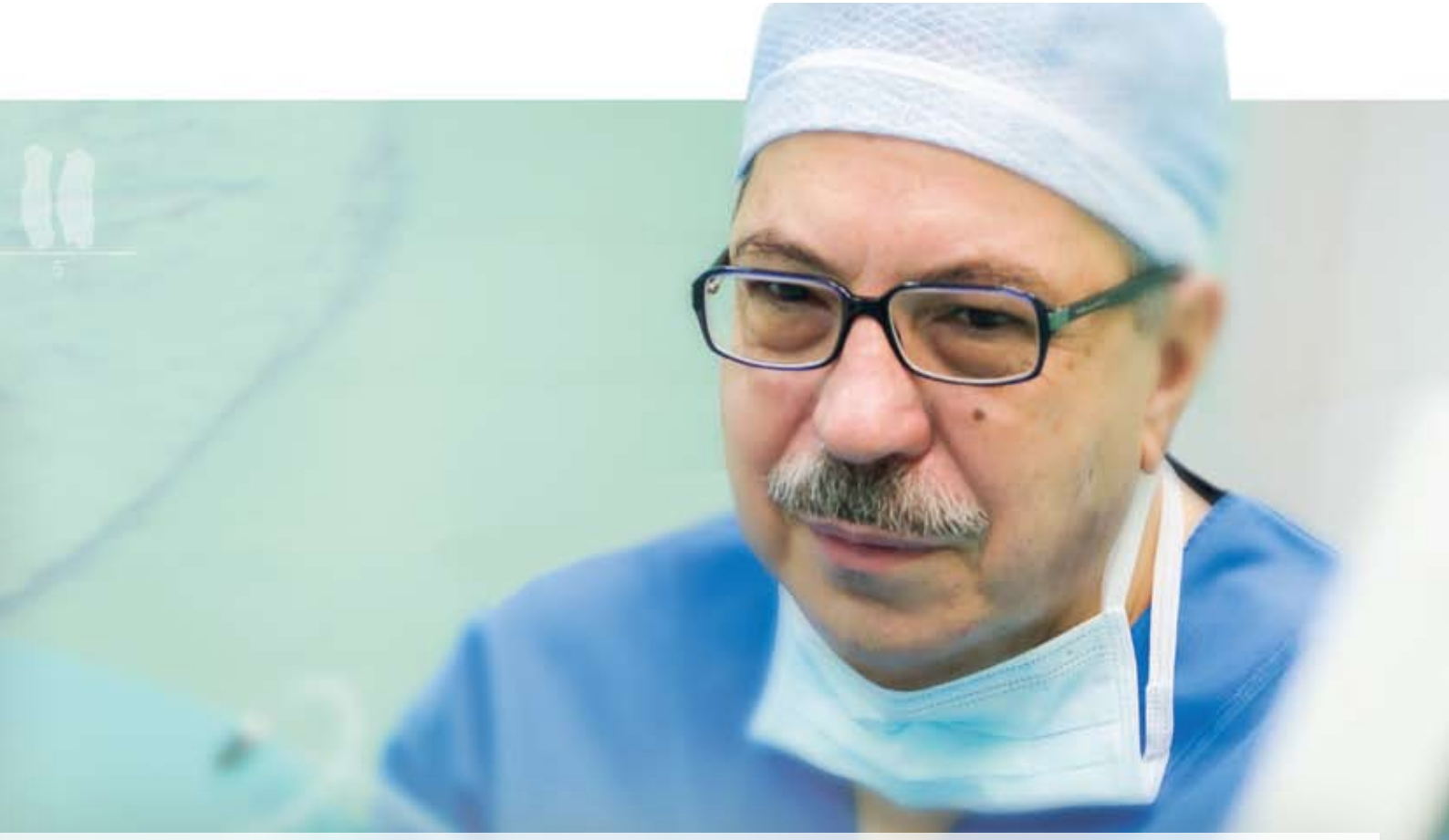
www.eugonia.gr

Dear friends,

It is our principle that all women have a right to motherhood, if they wish so. Infertility, although more frequently today than in the past, is no longer an unsurpassable obstacle. What is needed is detailed information of the couple, individualized treatment and the use of the latest scientific methods of assisted reproduction technology (ART).

Our Unit is not a mere observer of scientific developments, but is a pioneer in research and contributes actively with the publication, in international journals, of original studies that increase the efficiency and safety of IVF. The results of our studies guarantee top pregnancy rates, which in some cases are similar or even higher than several renowned IVF Units in Europe and USA. Part of our contribution is to inform clinicians but also the general public with the publication of our book, which is the only scientific edition on IVF in Greece today.

It is our choice to develop personal relationships with every couple who seeks our help. We are aware that psychological support is very important during the fragile situation of waiting for a positive outcome. We do not agree with strong interventions that do not take into account the couple's personal circumstances; on the contrary we take advantage of these circumstances in order to help nature do its work freely.



I often tell my associates that each couple that comes through our doorstep places their hope in our hands. This does not happen very often in every day relationships and is not included in common clinical practice. This is a deeply human moment that does not allow us to operate as “cold” professionals, but gives us the courage to make everything possible to respond to your trust.

With the present booklet, but also our website **www.eugonia.gr** we attempt to make your choice easier, offering basic knowledge and sound scientific information on matter related directly or indirectly with infertility and ART. So that you are aware of the steps and procedures of IVF, the drugs, the safety of the method for you and your child.

Trifon G Lainas
PhD, Reproductive Gynecologist
Director of Assisted Reproduction Unit
Eugonia

Basic knowledge

- A couple is described as “infertile” when systematically attempting to conceive for over a year without success, while being at reproductive age.

- IVF is safe and efficient solution, that has given the joy of parenthood to thousands of infertile couples.

- IVF is the fertilization outside the body (fertilization in the glass as the term describes). Therefore, the interaction of eggs and sperm and subsequent fertilization are achieved in the laboratory instead of taking place naturally in the fallopian tubes.This is a deviation which overcomes certain barriers that prevent fertilization from occurring naturally within the body. However, in most cases, the eggs are the woman’s and the sperm are the man’s, so the resulting embryos are genetically theirs.

- Fertilization takes place in the embryology laboratory by clinical embryologists and the embryos are transferred back to the uterus by a specialized gynecologist. If pregnancy occurs, its course is identical as after natural conception, and the children born are similarly healthy.

- The use of ICSI (intracytoplasmic sperm injection) gives a solution to nearly all cases of male infertility. Thanks to ICSI, men with severe problems in sperm numbers and motility can become fathers, while in the past they had no hope of fatherhood. All that is required is a few motile spermatozoa for the fertilization of an equal number of eggs.

- Laser laparoscopic and hysteroscopic surgery is the method of choice for infertility-related conditions, but is also a modern safe and effective method to treat the majority of benign gynecological conditions.

- The rapid developments that occur daily in the physiology of reproduction, embryology, reproductive endocrinology, laparoscopic and hysteroscopic surgery offer new means to treat infertility. At the same time, the evolution of technological equipment and the groundbreaking progress in their application have lead to a dramatic increase of pregnancy rates worldwide in state-of-the-art IVF Units.

Reasons to choose Eugonia

Selection criteria

The selection of an ART Unit is a very important decision, and it is your decision. The selection criteria can be objective or subjective. They are related to the efficiency and the specifications of the Unit, but also the personal relation of trust that develop with its staff.

We offer you all the information you need and all the reasons to choose Eugonia:

- Our pregnancy rates are among the highest internationally
- We achieve a successful pregnancy in the first IVF attempt for the majority of women
- We offer a solution to previous failed IVF attempts
- We formulate an individualized and friendly treatment for each couple.

Eugonia...with international recognition

- We contribute to the international scientific developments, with our research activity and by publishing original studies in high-impact scientific journals.
- We have deep knowledge and experience due to continuous scientific update and the principles of evidence-based medicine.
- We design optimal protocols of ovarian stimulation for women with poor response and women with polycystic ovaries
- We treat severe OHSS safely and efficiently.

“Eugonia”...
with knowledge, high specifications and technology (state of the art?)

- We have:

a) Quality management certification ISO 9001

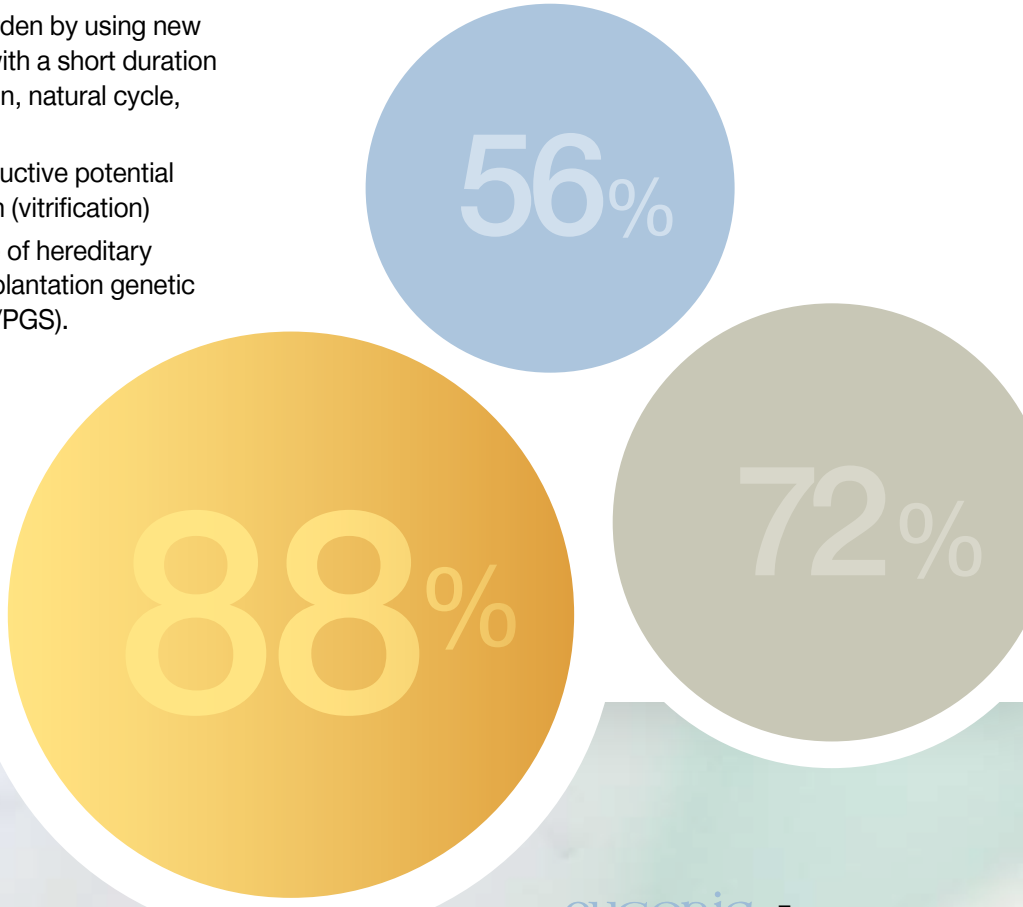
b) Modern technical equipment

c) A detailed and extensive database, which supplies us with statistical figures, useful in everyday practice but also for the publication of original studies

- We reduce the physical burden by using new patient-friendly protocols with a short duration (antagonists, single injection, natural cycle, mild stimulation protocols)
- We preserve female reproductive potential by oocyte cryopreservation (vitrification)
- We avoid the transmittance of hereditary genetic diseases by preimplantation genetic diagnosis/screening (PGD/PGS).

“Eugonia”...
with responsibility and sensitivity

- We ensure that the couples are fully informed about each phase of the treatment, with the option of professional psychological support.
- We adhere to strict principles of morale and bioethics.



Pregnancy rates at “Eugonia”

Pregnancy rates

The quality of services of an IVF Unit are judged by the end result. We present the pregnancy rates of Eugonia (expressed per embryo transfer). There was no patient selection in terms of cause and duration of infertility, number of previous attempts and method of fertilization.

National register

The national register of pregnancy rates of all IVF Units in Greece would provide with candidate couples with a reliable selection criterion. This already happens in several countries (USA, UK, France, Germany, Australia). In Greece, the function of the independent authority of Assisted Reproduction has been temporarily withheld. One of its duties was to collate and publish pregnancy rates of ART Units in Greece.It is anticipated that this nationar register will soon come into force and will become a formal conformation of success rates for each Unit so that candidate couples can compare Greek and foreign ART Units.

Our research profile leads to increased pregnancy rates

Every day, new knowledge is accumulated from scientists worldwide in order to increase pregnancy rates. The publication of a study in a medical journal contributes, apart from the recognition of the researchers, to better and sounder knowledge of the subject. Exposure of a manuscript to peer review requires constant update and knowledge of the literature, and also an extensive database for the statistical analysis of data. These are part of evidence-based medicine but are not practiced by everyone.

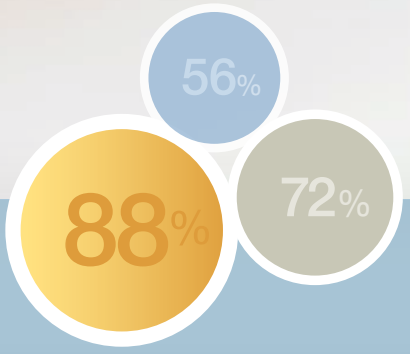
What they depend on

Pregnancy rates depend on many factors. Some are obvious (quality and number of embryos, age, sperm quality, cause of infertility) and some not so obvious (e.g. the knowledge and skill of gynecologists, embryologists, nurses and other scientists involved in the treatment, infrastructure and quality control of the laboratory). Sound knowledge, experience and use of modern methods affect significantly the success of an IVF attempt. Not all gynecologists, embryologists and nurses are the same.

There are differences. Knowledge and experience should not be taken for granted. These differences cannot work miracles of course, but can maximize the chance for a positive result.

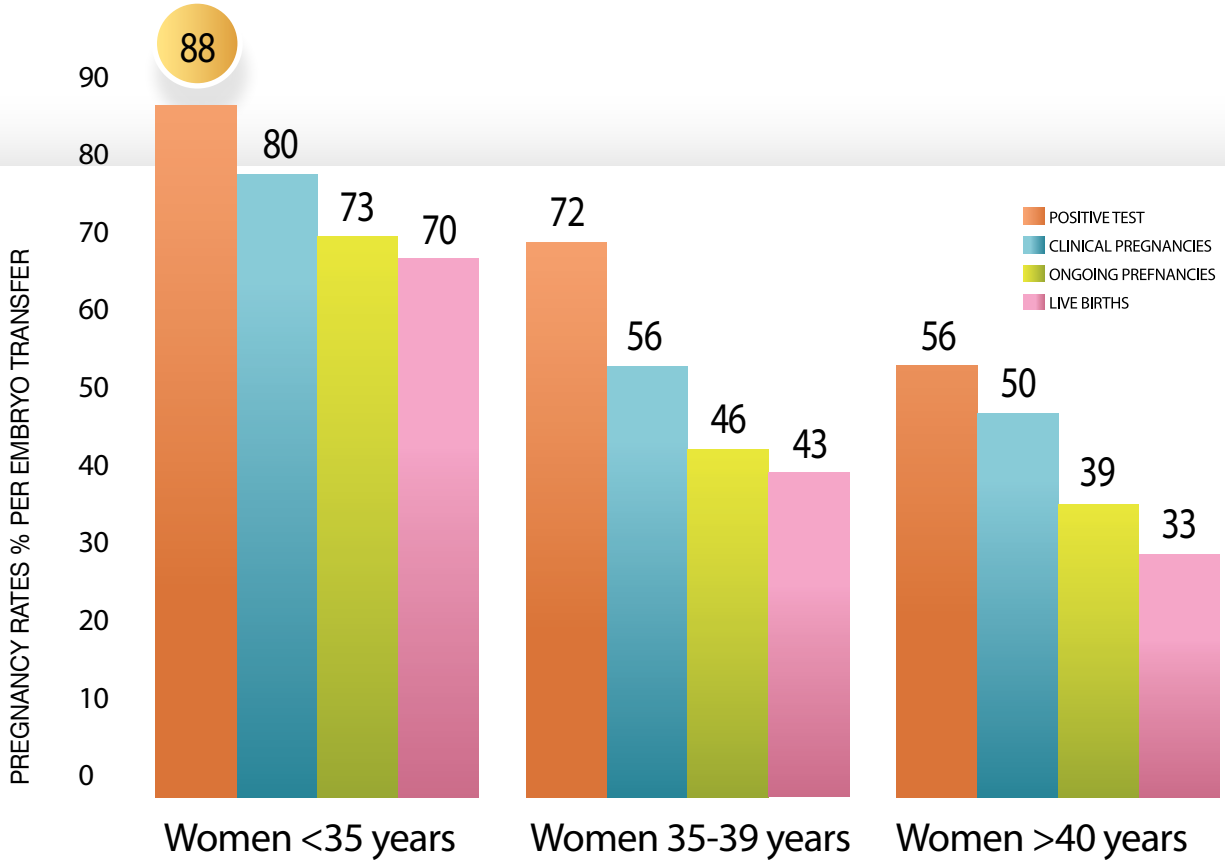
Pioneers of success internationally

Eugonia is an ART Unit with stable and top pregnancy rates.
These rates put us in the highest positions worldwide, regarding live births and also positive pregnancy tests.



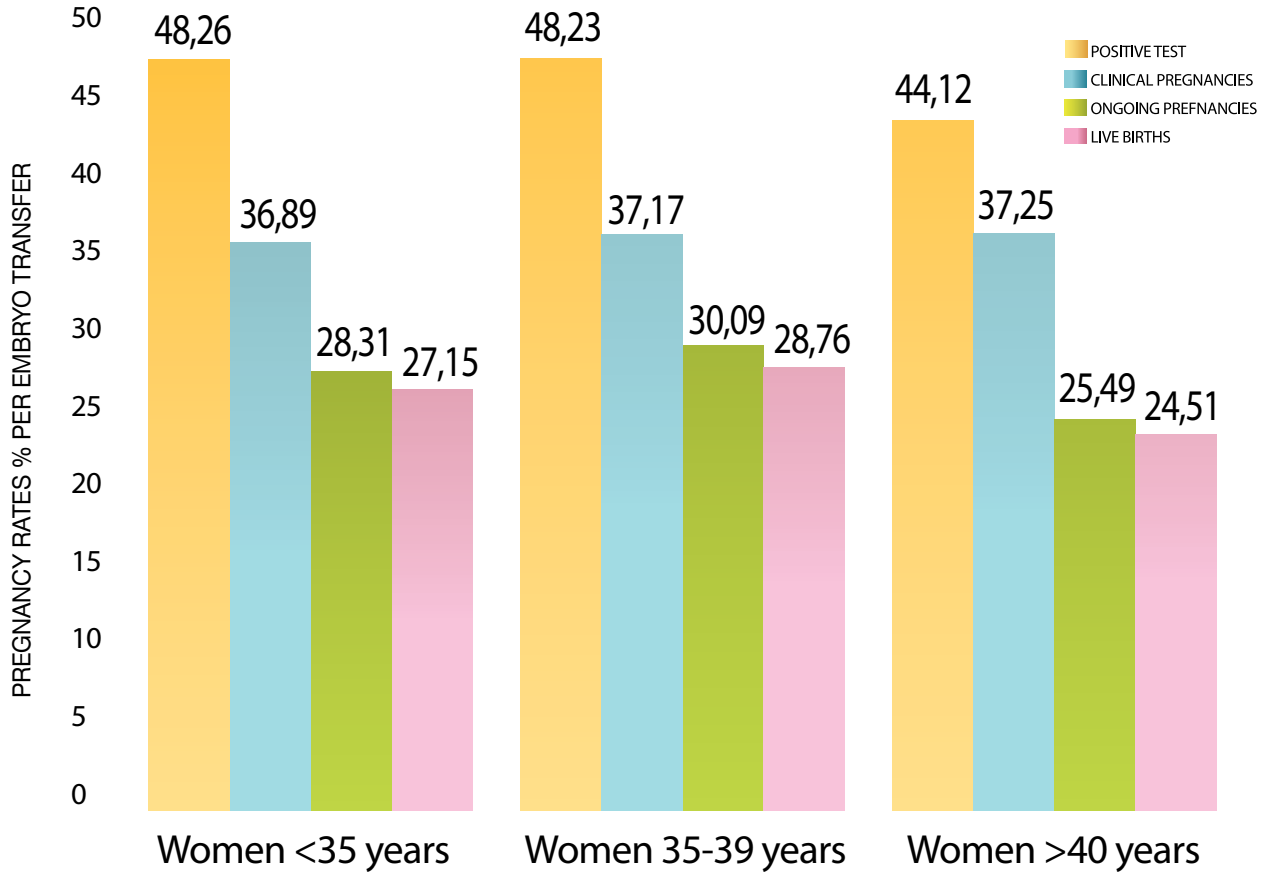
Pregnancy rates “Eugonia”

Chart 1 : Pregnancy rates in fresh blastocysts 2010 - 2011



Note: The table refers specifically to women whose embryos reached blastocyst stage during treatment cycles in 2010-2011.

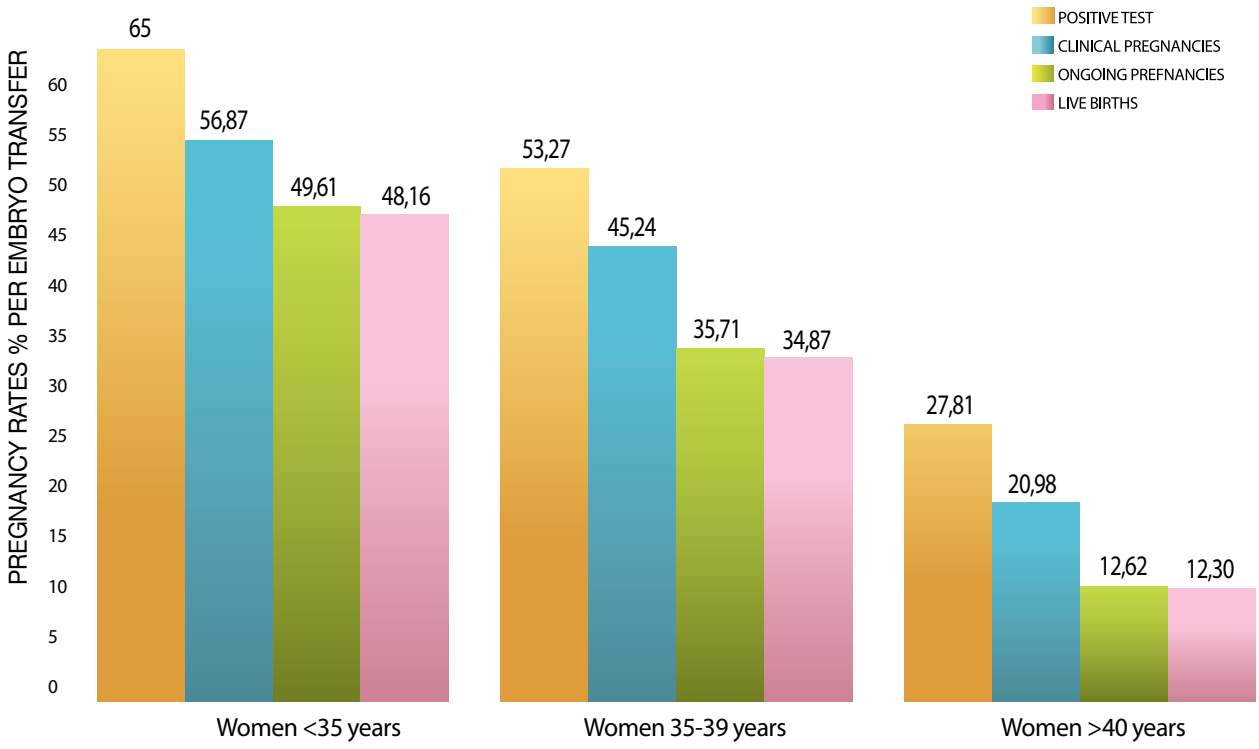
Chart 2 : **Pregnancy rates in frozen cycles 1997-2011**



Frozen

This refers to treatment cycles with cryopreserved embryos of Day 2, 3 and 5-6 during 1997-2011.
We have strict criteria regarding the selection of embryos for cryopreservation.
Our usual practice during the last few years is to culture supernumerous embryos up to the blastocyst stage and proceed to cryopreservation according to specific quality criteria.

Chart 3: Pregnancy rates in fresh cycles 1997- 2011



Fresh

** Note: The table refers to the overall treatment program. The results include transfer of Day 2,3 and 5-6 embryos (blastocysts) and it excludes frozen embryo transfer.*
CLINICAL PREGNANCY: intra-uterus pregnancies > 7 weeks with (a) positive heart beat(s).
ONGOING PREGNANCIES: > 12 weeks with (a) positive heart beat(s).

1. Infertility

Epidemiology 1.1

The problem of infertility occurs frequent and is constant. Internationally, 15% of couples have difficulty in conceiving in general, or in conceiving the desired number of children, according to estimations of the World Health Organization (WHO).
The probability of conception by a couple of reproductive age who have regular unprotected sexual intercourse with no use of contraceptives is 20% per month. If we add the monthly conception probability (20%) for 12 months, the percentage of conception reaches statistically 93%, instead of a simple addition that would result in 240%.
The infertility percentage remains stable through the centuries.
It had been recorded that in a region of England in the 19th century, 1 out of 6 marriages was not productive (16%).

Terminology 1.2

Subfertility is the failure to conceive after 12 months of sexual intercourse without protection or use of contraceptives.

Infertility is the complete inability to conceive.

Monthly Fertility Rate (MFR) is the probability of conception in a menstrual cycle.
The human is not a particularly fertile mammal. It is estimated that in humans the MFR is 20%, while in baboons it is 80% and in rabbits 90%.

Causes of infertility 1.3

The most common causes of subfertility include:

- Sperm-relates deficiencies (concerning the concentration, motility and morphology of spermatozoa)
- Ovulation disorders
- Hostile behavior of cervical mucus and cervical disorders
- Uterine disorders
- Abnormalities of the fallopian tubes (obstruction or adhesions)
- Endometriosis
- Increased female age
- Lifestyle (smoking, alcohol con sumption, working environment)
- Unexplained sub/infertility, which occurs in a considerable percent age (25-30%) of infertility cases

When should I visit the doctor? 1.4

Before the onset of investigation and treatment of infertility we should answer an important question: has the frequency of sexual intercourse been adequate, despite which no pregnancy has occurred?

If the answer is yes and no conception has been achieved in a period of one year, then it is necessary to investigate the causes of subfertility in order to select the suitable treatment.

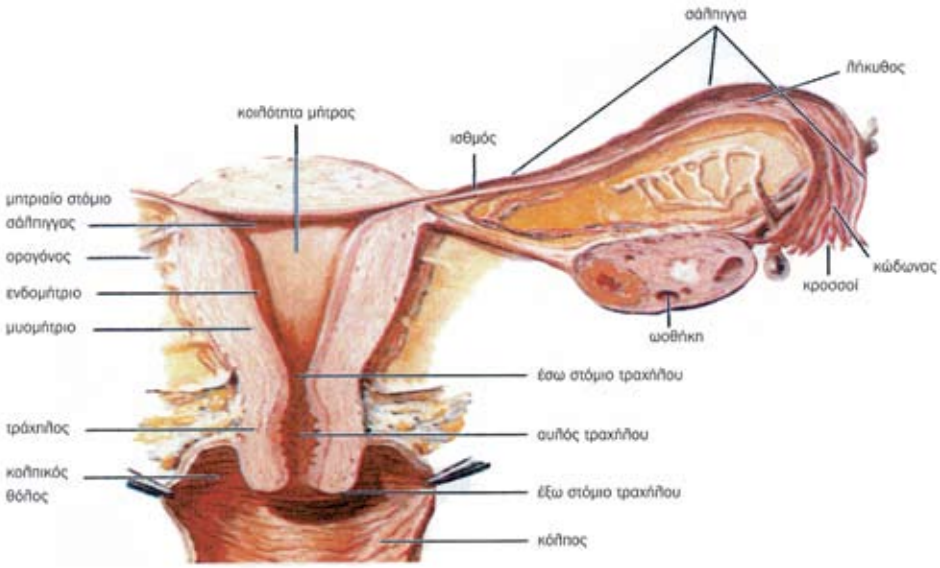
The philosophy of dealing with subfertility may include:
a) a “wait and see” period
b) undergoing tests to explore the causes of infertility in order to select the suitable treatment
c) time course of treatment (intrauterine insemination, hysteroscopy-laparoscopy etc)
d) progress directly to IVF

2. Learning about natural conception

Terminology of anatomy and physiology 2.1

The knowledge of the anatomy and the function of the female reproductive system, and the understanding of the process of conception can help us comprehend the causes of subfertility better.

The following information is useful in understanding the process of IVF, the reasons for the use of drugs and the importance of hormone tests and ultrasounds during the treatment cycle.



Female inner reproductive organs

• Ovaries
They are the female reproductive glands. There are two ovaries (right and left ovary) and they have a dual function: production of eggs and hormones. They have an almond shape, a size of 30x20x20 mm and are located inside the pelvic cavity, in direct contact with the fallopian tubes. The ovary is a store of follicles (small cysts containing the eggs) of various sizes and developmental stages.

• Follicles
Small cysts filled with fluid and sized approximately 8 - 20 mm, which develop within the ovary and contain the eggs. Each follicle contains a single egg. Their number is estimated to be around 2.000.000 at the time of birth, 300 - 400.000 at puberty, 25.000 at the age of 38 and 1000 at menopause.
Of all the follicles, only about 400 reach the stage of a mature fully grown follicle (Graafian) in the entire duration of a woman's reproductive life.

• Ovulation
The rupture of the mature follicle and the release of the egg.
It is triggered by a sudden rise (surge) of the hormone LH.

• Oocytes (eggs)
They are the female germ cells (gametes) that always carry the sex chromosome X. They grow and mature within the ovarian follicles, from which they are released at ovulation.

• Fallopian tubes
Organs in a shape of slender ducts that connect the uterine cavity with the abdomen. They consist of 4 segments: interstitial, isthmus, ampulla and infundibulum with fimbria. Their inner cavity is the site of fertilization and embryo transport to the uterus.

• Uterus
A hollow muscular organ in a shape of a pear, located in the pelvic cavity in which the embryo implants and develops. Also called womb. The two higher ends (horns) contain the uterine openings of the fallopian tubes. The lower end communicates with the cervix.

• Cervix
It is the outer end of the uterus, has a cylindrical shape and length of about 4 cm, containing a canal (endocervix). It connects the uterine cavity with the vagina.

• Cervical mucus
Secretions of the glands of the endocervix, with varying composition and thickness. It offers a friendly environment for the passage of spermatozoa during the fertile days of the cycle.

• **Vagina**
A fibrous muscular canal with a length of approximately 7.5 cm, which receives the penis during sexual intercourse. The inner end of the vagina, called tholus, communicates with the cervix, while the outer end leads to the opening of the vulva.

• **Endometrium**
It is the tissue (mucosa) lining the inner cavity of the uterus and is discharged along with blood during menstruation (period).

• **Corpus luteum** (yellow body)
A mass of cells that forms from a Graafian follicle after the release of a mature egg at ovulation. It produces mainly progesterone which acts on the endometrium making it receptive to the embryo.

• **Menstrual cycle**
It is the time between thefirst day of the period (menstruation) and the first day of the next period.

• **Hypothalamus**
The part of the brain that regulates reproductive function by secreting gonadotrophin releasing hormone (GnRH), which acts on the pituitary and triggers the production of gonadotrophins (FSH and LH).

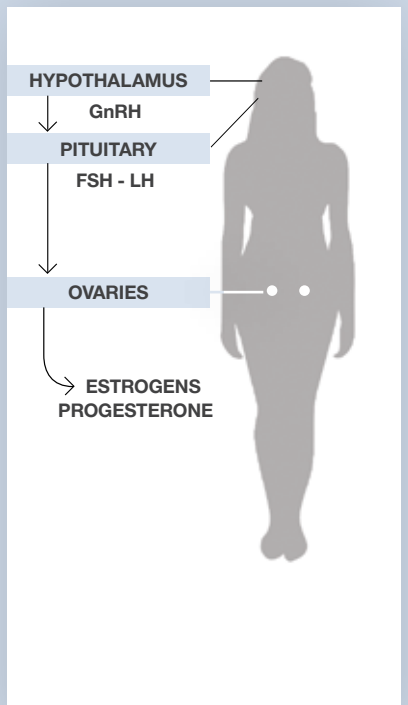
• **Pituitary (hypophysis)**
A small endocrine gland of central importance as it secretes hormones regulating the function of the ovaries, testes, adrenal gland (epinephros), thyroid etc.

• **Testes**
The male reproductive glands. There are two testes (right and left testis) residing in the scrotum, a sac made of skin underneath the penis. The testes have a dual role: production of spermatozoa and hormones.

• **Semen**
Biological fluid of complex composition discharged at ejaculation and containing spermatozoa (comprising 7% of the total volume) and secretions of the prostate, seminal vesicle and other glands.

• **Spermatozoa**
The male germ cells (gametes) that carry either one of the two sex chromosomes (X or Y) and decide the gender of the embryo (female and male respectively). They are produced by the testes and their number normally reaches tens of millions at each ejaculation.

• **Fertilization**
It is the fusion of a spermatozoon with an egg to create an embryo. Fertilization marks the creation of a new organism and decides the gender, depending on the chromosome composition of the gametes: if an egg (X) is fertilised by a female spermatozoon (X) it will give rise to a girl (XX), while if fertilised by a male spermatozoon will result in a boy (XY).



The hypothalamus-pituitary-ovarian axis and reproductive hormones (provided by C. Cazlaris)

Which hormones are secreted during a menstrual cycle? 2.2

During the menstrual cycle hormones are secreted from the hypothalamus, the pituitary and the ovaries, causing the maturation and finally the release of the egg from the ovary. These hormones interact with each other via feedback mechanisms, creating the right environment for conception to occur.

At the beginning of a natural menstrual cycle the hypothalamus-released GnRH activates another gland, the pituitary, to produce follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH promotes the growth and development of the follicles and LH is responsible for their final maturation and ovulation.

Important events of the menstrual cycle 2.3

In every natural cycle, 8-12 follicles start to grow under the control of the FSH. One of these follicles dominates, grows faster than the rest and is called the "dominant" follicle. This is the follicle that will reach the final maturation stage. The rest of the follicles will become atretic, meaning that they degenerate via the mechanism of atresia.

With the action of the LH on the dominant follicle, ovulation takes place and the mature egg is released.

The developing follicle, which constitutes the main functional and reproductive unit of the ovary, produces the hormone eostradiol (E2) in increasing quantities, which acts on the endometrial epithelium (endometrium) and increases its thickness.

High E2 levels result in LH secretion. The sudden rise ("surge") of LH results in the final oocyte maturation and the release of the egg with ovulation. This phase of the menstrual cycle represents the fertile days. After ovulation, the follicle develops into a structure called the corpus luteum, which mainly produces the hormone progesterone. Progesterone, in combination with oestrogens (oestradiol), prepares the endometrium to receive the embryo and support its growth.

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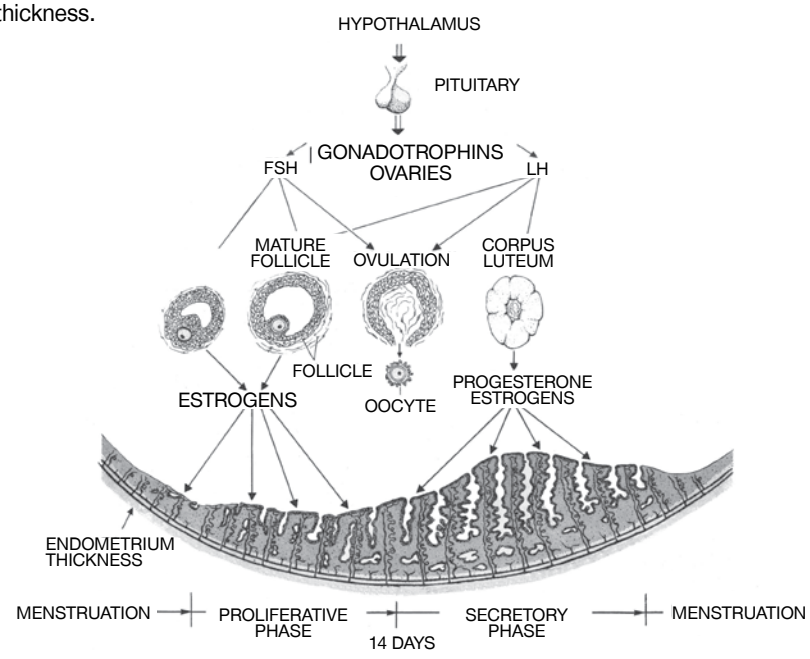


The relationship between hypothalamus- hypophysis (pituitary) - ovary and the effect of ovarian hormones in the endometrium during the menstrual cycle.

The Process of fertilization 2.4

Human reproduction is a result of the union of a sperm with an egg. Eggs are produced in the ovaries and sperm in the testes.
The union of the sperm with the egg, will result in the embryo that will grow in the uterus so that the child is born.
If there is intercourse during the “fertile days”, the sperm will concentrate in the vagina wall.
The sperm will pass through the external cervical os, which is the gateway between the vagina and the internal sex organs and is open during the “fertile days”. The sperm will then go through the cervical canal towards the uterine cavity and through the proximal tubal ostium to reach the fallopian tubes.

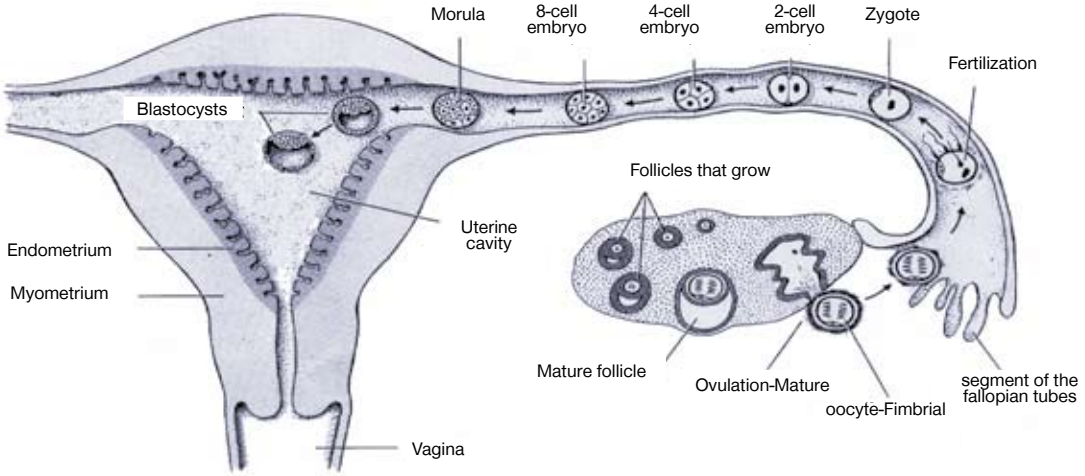
The fallopian tube will then receive the egg that was recently released with ovulation, via its fimbria.
Sperm and egg meet in the ampulla of the fallopian tube and this is where the egg will be fertilised by only one sperm. The fertilised egg (zygote) will remain in the fallopian tube for 5-6 days, where it divides into 2, 4, 8 cells and then becomes a morula and a blastocyst.
As a blastocyst (an embryo with around 6-120 cells) it will then migrate towards the uterine cavity and implant by invading the endometrium where it continues to develop.

Requirements of fertilization 2.5

- The tubes to be open and functional in order to receive the egg from the corresponding ovary and allow the sperm to reach the egg.
- Sperm with normal parameters, meaning that the spermatozoa should be in satisfactory numbers and have a good progressive motility so that some of them will manage to bypass any obstacles and reach the egg.
- Transport of spermatozoa from the cervix to the tubes must be unobstructed and environment of cervical mucus should be friendly
- Ovulation to take place, so that the mature egg is released from the Graafian follicle.
- Intercourse during the fertile days.

It is also known that:

- Endometriosis can greatly reduce fertility.
- In around 25-30% of all subfertility cases the cause is unexplained.



Schematic representation of the ovarian cycle, fertilization of the oocyte in the fallopian tubes, embryo cleavage and transport of the embryo to the uterine cavity.

3. In vitro fertilization

What is it?
3.1

In vitro fertilization (IVF) is the most commonly used ART method. It was first successfully used in the human in 1978.

IVF is the fertilization outside the body (fertilization in the glass as the term describes). Therefore, the interaction of eggs and sperm and subsequent fertilization are achieved in the laboratory instead of taking place naturally in the fallopian tubes.

This is a deviation which overcomes certain barriers that prevent fertilization from occurring naturally within the body. However, in most cases, the eggs are the woman's and the sperm are the man's, so the resulting embryos are genetically theirs.

How is it done?
3.2

The eggs are recovered from the follicles that have developed in the woman's ovaries by the procedure of egg collection. In the laboratory, the eggs come in contact with the sperm inside special dishes with culture medium, in order for fertilization to occur. The fertilized eggs (zygotes) are placed inside an incubator and are cultured under special conditions for 2-6 days, so that the embryos can achieve their first stages of development.

Instead of reaching the uterus naturally, i.e. via the fallopian tube, the embryos are transferred to the uterus by a specialise gynecologist using a thin catheter into which the embryos have been previously loaded by the embryologist. The embryos implant in the endometrium by themselves just like in natural conception. If implantation occurs then a pregnancy will follow.

When to choose IVF 3.3

There are absolute and relative indications concerning one or both partners.

Absolute indications are:
- Lack (following removal) or obstruction of fallopian tubes centrally or peripherally (hydrosalpinges).
- Lack of spermatozoa (azoospermia) requiring surgical retrieval.
- Very low number of motile normal spermatozoa (severe oligo-asteno-teratozoospermia).

Relative indications include:
- Long period of infertility
- Age
- Severe sperm-related problems (severe oligo-asteno-teratozoospermia)
- Unexplained infertility
- Failure of other methods (i.e. ovulation induction for timed intercourse or IUI)
- Severe endometriosis
- Preimplantation genetic diagnosis (PGD) for β-thalassaemia, sex-linked diseases, special inheritable diseases*
- Prior to chemotherapy*
- Donation of eggs, sperm or embryos*
- Surrogacy *

** Rare indications. See sections "Preimplantation genetic diagnosis" – "Alternative ART methods – Donations".*

Is IVF unnatural?
3.4

Conventional IVF does not violate the laws of natural conception. Despite certain phobias of the public, IVF takes place in the laboratory without further intervention in the interaction of the gametes, while embryo implantation occurs unaided.

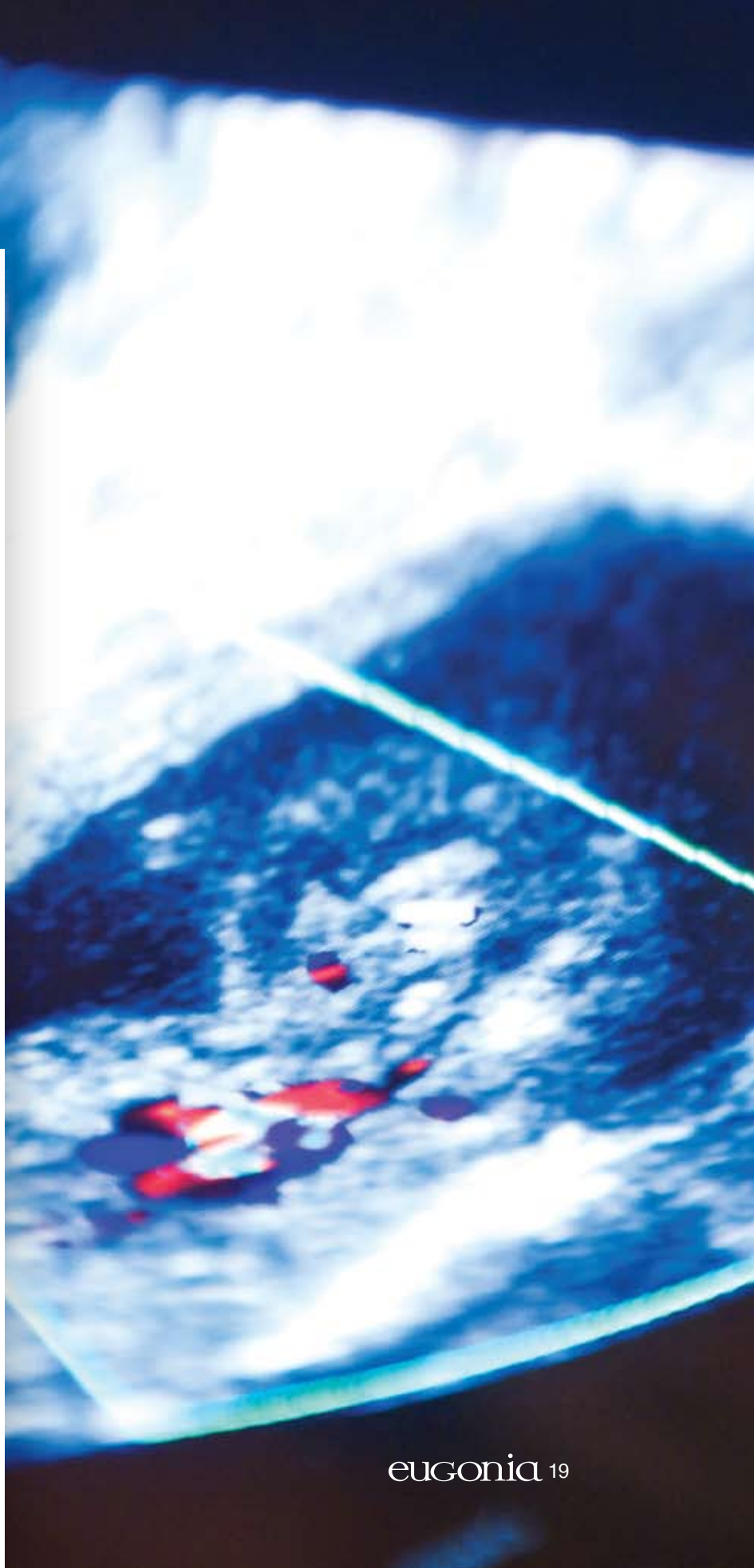
Other versions of conventional IVF, such as ICSI, are indeed intrusive methods, but are used to overcome certain problems, most often related to sperm.

In all cases however, if a pregnancy is achieved, it progresses in exactly the same way as following a natural conception.

Will my child be healthy?
3.5

IVF children are as healthy and normal as children conceived naturally, and do not present a significant increase in related and chromosome abnormalities, as shown by several large scale epidemiological studies.

This is confirmed by the fact that more than 1.5 million children have been born to date following IVF, and some of them have already had children of their own.



4. Beginning your treatment

Stages of IVF treatment 4.1

The IVF treatment cycle includes the following stages:

- Investigation and diagnosis of infertility.
- Preliminary tests.
- Stimulation of the ovary with drugs (12-14 days) for the development of multiple follicles.
- Monitoring using a series of ultra-sounds and hormone tests.
- Induction of final oocyte maturation (a single evening injection).
- Egg collection.
- Fertilization and culture outside the woman's body (2 - 6 days).
- Embryo transfer.
- Pregnancy test (13 days after the embryo transfer, measurement of blood levels of β-human chorionic gonadotrophin (β-hCG).
- Test of clinical pregnancy using trans-vaginal ultrasound (4 weeks after embryo transfer)*.
- Test of ongoing pregnancy on 12th week of gestation (10 weeks after embryo transfer)*.

* *These tests are performed by your treating physician, as the work of the IVF Unit has been completed.*

What must I do first?
4.2

You must first book an appointment for a consultation either yourselves or via one of our collaborating doctors who referred you to our unit. It is preferable that your partner is present at the consultation. It is also useful that you gather any previous related tests (semen analysis, hysterosalpingography, blood tests, hormone levels, photographs or videos from previous hysteroscopies or laparoscopies), reports from previous IVF attempts, operations, laparoscopies etc.

Which tests are necessary? 4.3

The preliminary tests before the onset of an IVF programme include:

- Sperm tests (semen analysis, sperm preparation, semen culture)
- Hormone profiling and transvaginal ultrasound on the 3rd day of the cycle.
- Blood tests for hepatitis, HIV I-II, VDRL (necessary).
- Pap-test, breast examination in women over 35, heart check up.
- Special examinations, such as laparoscopy or hysteroscopy, which may be recommended by the Medical Director of the unit, based on the assessment of the medical history and other tests (information on laparoscopy-hysteroscopy can be found in a following section).

The tests of prenatal diagnosis are also recommended.

Where can I have my tests? 4.4

Eugonia covers the entire spectrum of the necessary examinations at special reduced prices.

It is necessary that the ultrasound, hormone tests and sperm tests are performed in our unit so that the results can be assessed by the scientific team of Eugonia and be compared with similar other cases. This provides an additional level of quality control of the whole process.

However, if this is not possible, these tests can be performed elsewhere and be brought or sent to our unit. For patients living outside Athens, some tests can be done at their place of residence.

It is preferable that all the tests have been completed before the start of the treatment programme.

Patient monitoring
4.5

The monitoring is performed in our unit and involves a series of ultrasounds and hormone tests. You may find a detailed description in the section: “Treatment course”.

5. Coming to our Unit

Your first visit at Eugonia 5.1

At your first appointment, you will be welcomed by the coordinator of the unit (sister or midwife). The consultation/interview will be conducted in three phases:

First, the midwife will take a detailed record of your medical history and information from any previous attempts.

Then, you will meet the Medical Director of the unit, with whom you will discuss extensively the diagnostic and therapeutic strategy recommended. The Doctor will note in your history sheet all the necessary tests that need to be done, the treatment protocol, doses of drugs and details about the treatment programme you will follow.

In a third phase, the midwife will explain and give you written instructions for any further tests, if necessary, as well as details about when and how these will be conducted. You will also be given the prescriptions for the necessary drugs, instructions about the treatment protocol, dosage, time and manner to take your drugs, and also medical notes, forms and instructions about your next appointment with us.

You must remember that this first appointment may last a while, depending on the information that need to be recorded and the extent to which you wish to discuss your treatment with the Doctor (usually 1 - 2 hours).

Finally, you will be given official information brochures and consent forms required by the law, so you have enough time to peruse them. Consent forms must have been signed and filed in your medical folder before the onset of the treatment.

Your visits to the unit during the treatment programme 5.2

You will be informed about your visits to the unit during the treatment for hormone tests and ultrasounds. Should you have any questions or problems feel free to contact the units midwife, who, in turn, will inform the Doctor.

In each phase of the programme, the results of your tests will be assessed by the Medical Director and his colleagues, aiming to decide on the way forward regarding your drug dosage and treatment continuation.

Each organism responds differently to the treatment so each case is judged on its own merits. Perhaps it may be possible to re-assess the dosage of the drugs, or modify some timing aspects of the programme. Our principle in your monitoring is always the best outcome of your attempt and your final success.

The scientific team of “Eugonia” will be by your side, for every aspect of your treatment, with respect and scientific commitment

6. Fertility drugs

The drugs selected for treatment increase the chances for achieving a pregnancy without posing any health risks

Why we use drugs
6.1

It has been shown that pregnancy success rates following IVF increase when more than one good quality embryos are transferred to the uterus.
However, embryos formed after IVF do always have the desirable quality. Therefore, in order to have the ability of selection, we need several embryos, which will derive from the fertilization of several eggs. The eggs, in turn, will be retrieved from several follicles.
We therefore use pharmaceutical protocols of controlled ovarian stimulation to promote the development of multiple follicles.

Which drugs are used 6.2

The main drugs used are:

A. GnRH analogues

Pharmaceutical analogues of the hormone GnRH intend to inhibit the premature rise of LH that causes ovulation. In this way we are able to prevent the undesirable rupture of follicles before the egg collection. In the past, before the use of GnRH analoguesabout 20-33% of cycles were cancelled due to premature ovulation.
GnRH analogues are classified in two categories:

a) GnRH agonists (trade names: Arvecap, Daronda, Suprefact, Gonapeptyl) and
b) GnRH antagonists (trade names: Orgalutran, Cetrotide)

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B. Gonadotrophins

They are synthetic pituitary gonadotrophins and are applied in order to stimulate the development and maturation of multiple follicles. They are classified in the following categories:

a) Recombined gonadotrophins (recFSH, recLH)
- Puregon (recombined FSH)
- Gonal-F (recombined FSH)
- Pergoveris (mixture of recombinant FSH, recombinant LH)

b) Urinary gonadotrophins (hMG)
- Altermon (purified FSH)
- Metrodin-HP (purified FSH) and
- Merional – Bravelle (hMG with FSH-LH mixture)

c) Corifollitropin- α (corifollitropin alpha, with commercial name Elonva)

C. Drugs for triggering final oocyte maturation

a) Human chorionic gonadotrophin (hCG). This is the last drug to inject during your treatment. It is applied once at a specific time (36 -38 hours before egg collection), when the maturity of the follicles is adequate. Drugs: Ovitrelle (recombined hCG) and Pregnyl, Profasi (urinary hCG).
β) GnRH agonists (Arvecap, Gonapeptyl, Daronda). It can be used instead of hCG (36-38 hours before oocyte collection) in patients pretreated with antagonist protocols or natural cycles.

These drugs are available in several forms:

1) ready to use injectable solution
2) powder that is mixed with a special diluent to produce the final solution
3) pre-filled cartridge or pen-like syringe

Injections are given subcutaneously or intramuscularly, depending on the instructions given. The pen is graduated in international units so you can apply the drug yourselves with precision and safety.

D. Progesterone

It is usually used after the embryo transfer to hormonally support the luteal phase and therefore the environment of the uterus that will receive the embryo. It is available as a vaginal cream (Crinone) or pills (Ultrogestan).

Other medicines like estrogens, cortisone, antibiotics and aspirin may be used if deemed necessary. In some cases contraceptive pills are used before the start of the treatment programme.

E. Other drugs, such as estrogen, antibiotics, aspirin, heparin may be used if it is deemed necessary.

Why are drugs necessary? 6.3

The drugs used are analogues of natural hormones in order to create a pharmaceutically controlled reproductive cycle.

They aim at the following:

- The recruitment and maturation of multiple follicles
- To prevent untimely ovulation and loss of eggs due to early rise of LH. The drugs temporarily disrupt the communication between the pituitary and the ovaries leadin to downregulation of the glands (using GnRH agonists or antagonists).
- The selection of the ideal time of ovulation (with the application of hCG or GnRH agonist).
- To support the uterine environment to receive embryo (with the use of progesterone).

Do the drugs reduce my ovarian reserve? 6.4

No, as the drugs rescue and promote the maturation of follicles that would otherwise degenerate through atresia. Atresia is the degeneration of follicles initially recruited in a single menstrual cycle.

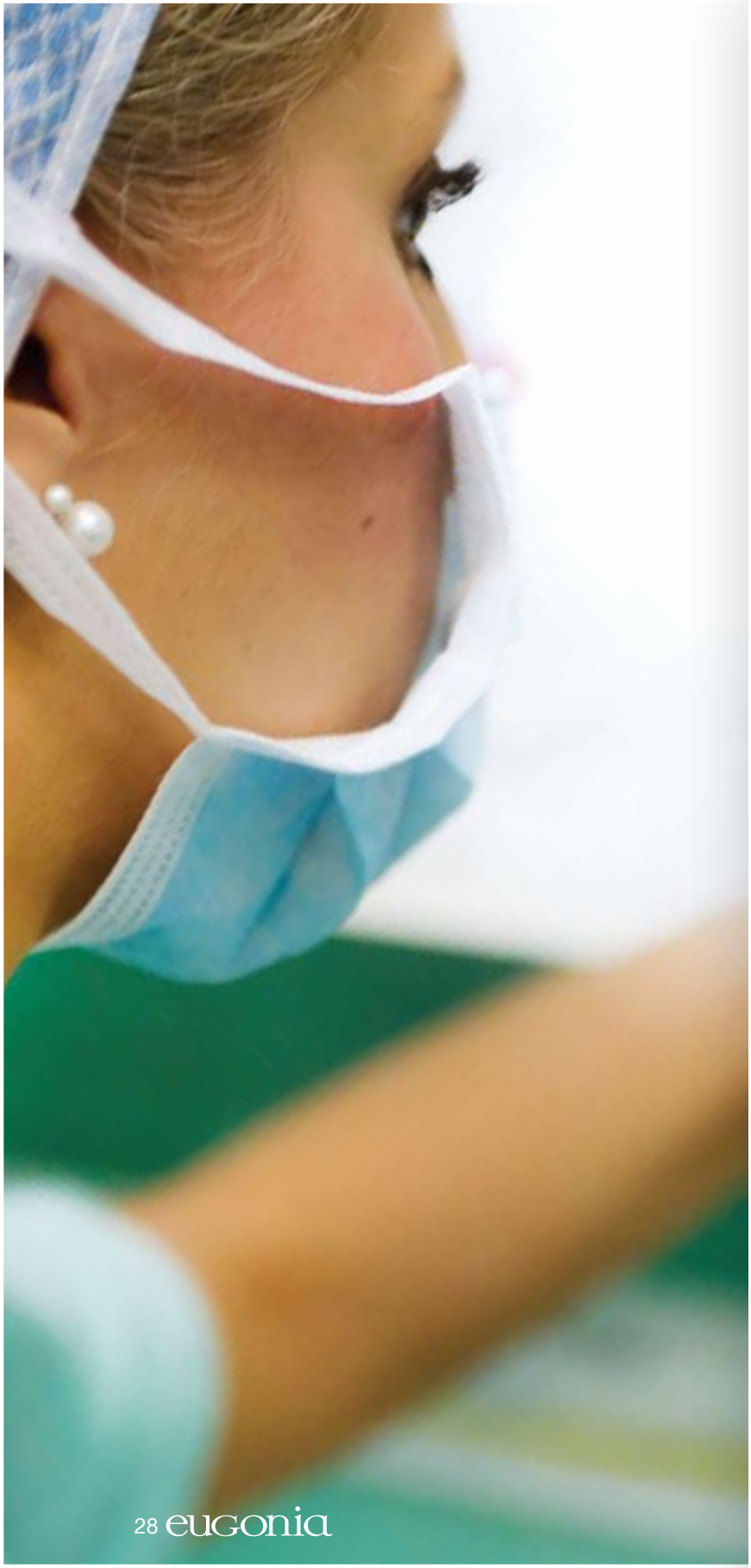
It is worthwhile noting that the ovary contains approximately 300-400.000 follicles, of which only 400 mature during a woman's reproductive life (the rest degenerate via atresia, i.e they become atretic).

Possible side effects of the drugs 6.5

Mild symptoms from certain drugs and rare allergic reactions (hot flushes, headaches, sweating, blocked nose) are practically insignificant and can be easily overcome.

Ovarian hyperstimulation syndrome (OHSS) is a side effect of controlled ovarian stimulation. It occurs in a small percentage of women beginning treatment. In the majority of cases it can be prevented and when it occurs it can be treated (see relevant section, and also our website **www.eugonia.gr**).

The danger of subsequent ovarian, uterine or breast cancer is similar with the general population as demonstrated by large epidemiological studies *(for more information see the section "Complications of IVF").*



Controlled ovarian stimulation (COS)

Controlled ovarian stimulation (COS) is the main reason for the increase of pregnancy rates in IVF during the last three decades. COS entailes the design of pharmaceutical protocols (combination of drugs) in order to achieve multiple follicle development and to prevent early follicle rupture due to the surge in LH and loss of oocytes. In the past, premature follicle rupture occurred in at least 25% of IVF cycles. After 1984 with the use of the long protocol using GnRH agonist, the interest of researchers and the pharmaceutical industry was focused on developing more patient friendly protocols, with immediate downregulation of LH and with shorter duration. In 2000 the new friendly GnRH antagonist protocols were presented.

Common protocols of COS 7.1

The most common controlled ovarian stimulation protocols are: antagonist protocols, the single-injection protocol, long protocol, natural or modified natural cycle, short protocol, and mild stimulation protocols. The choice of protocol depends on the cycle characteristics of each women, ovarian response in previous attempts, age and other factors.
However, these protocols are flexible in their use, because each individual responds differently to the drugs. An important part of our work is to select the optimal protocol and the optimal dosage for each patient separately.

7. Controlled ovarian stimulation (COS)

Long protocol
7.2

The long protocol has been in use for quite some time. The first publication was in Lancet scientific journal by Porter and colleagues in 1984 and it is also known as the GnRH agonist down regulation protocol. The basis of this protocol is the down regulation of the pituitary and therefore the prevention of a premature LH surge. It is well known, that a premature LH surge would result in follicle rupture prior to the egg collection and thus in loss of the oocytes.
Pituitary down regulation is achieved with the continuous administration of GnRH agonist analogues. Having established a continuous suppression of the pituitary, the stimulation of the ovaries whose main goal is the recruitment and development of multiple follicles then follows. This means that the communication between the pituitary and the ovaries is cut off and that we are the ones to take over.

The long protocol involves two phases:

1st phase: Downregulation using GnRH agonists (Arvekap, Daronda, Suprefact, Gonapeptyl). Duration approx. 10 -14 days. Initiation of agonist can be done a) on day 21 of the menstrual cycle in a regular cycle of 28 days, b) on day 2 of the menstrual cycle, c) three days before the end of OCP treatment. At the end of the first phase we confirm downregulation of pituitary and ovarian function.

2nd phase: Ovarian stimulation using gonadotrophins (Puregon, Gonal-F, Altermon, Pergoveris, Merional, Menopur, Bravelle) during continuous downregulation of the pituitary. Duration approx. 10-14 days.

The total duration of the long protocol is about 1 month.

Natural cycle – Modified natural cycle (MNC) 7.3

During a natural cycle, no drugs are used for the stimulation of the ovaries. The development of the single leading follicle and the maturation of the endometrium are monitored with a series of ultrasounds and hormone tests. Due to a high rate of premature ovulation and loss of the oocyte, the natural cycle has been replaced by the modified natural cycle.
During a modified natural cycle (MNC), antagonist and minimal doses of gonadotrophins are administered in the last days of the follicular phase. In this way premature ovulation is avoided.
The advantages of natural cycles are no/minimal drugs, no side effects or complications, short duration, low cost etc.
The disadvantage is that from a single follicle we must retrieve a single oocyte, which must be mature, get fertilized, divide into a good quality, chromosomaly normal embryo with high developmental competence, capable of implanting in the uterus and result in pregnancy.
Indications for natural/modified natural cycle include poor ovarian response, several previous IVF attempts, the desire to avoid drug administration, and some rare contraindications of drug stimulation.

Antagonist protocols

Antagonist protocols
7.4

In this protocol, gonadotrophic stimulation begins on the 2nd or 3rd day of the cycle, while the downregulation using the antagonist GnRH analogue follows.

The duration of the antagonist protocol is approximately 8 -12 days.

The antagonist administration can start either blindly on the 5th-6th or more rarely the 1st day of stimulation with gonadotrophins (fixed antagonist protocol), or based on ultrasound and hormone criteria (flexible antagonist protocol, Lainas et al 2005).

The late domination of antagonist protocols

The idea of the researchers and the pharmaceutical industry to create patient friendly protocols with short duration and smaller physical distress was not easily accepted by the scientific community.

The reasons for the slow acceptance of antagonists over the traditional long protocol include:

- Difficult learning curve
- Initial use in difficult cases or previous failed attempts
- Initial meta-analyses (Al Inany 2002) showing increased clinical pregnancy rates in favour of the long protocol and mislead international opinion.

The change begins to take place in 2006 by Kolibianakis et al, and is further supported in 2011 in the latest Cochrane review by Al Inany et al, who conclude with the phrase "...move away from the long protocol".

After 12 years, antagonist protocols tend to be the first choice internationally. We feel justified to have supported the antagonist protocol from the early stages with our research work and our publications..

Advantages of antagonist protocols

Antagonist protocols are patients friendly for the following reasons:

- They have fewer injections
- Shorter treatment duration (at least 12-15 days less when compared to the long protocol)
- Smaller total gonadotropin dosage
- Smaller chance of complications like OHSS (statistically significant)
- Shorter duration of analogue
- Less side effects (hot flashes, night sweats, nervousness, insomnia etc.)
- Are suitable for modified natural cycles (M.N.C.)
- Are suitable for the application of mild stimulation protocols.

The contribution of Eugonia in antagonist protocols

The scientific team of Eugonia has published numerous studies on women undergoing assisted reproduction using the antagonist protocol in internationally renowned scientific journals. These studies include the optimization and new stimulation protocols, offer a novel treatment to IVF complications, and two of them are the larger studies in the bibliography. Our studies show that GnRH antagonist protocols:

1. Reduce the chance of OHSS* development by 20% in women with polycyctic ovaries compared to the long protocol *(Lainas et al 2010, 2007).*
2. Increase pregnancy rates in women with poor ovarian response, *(Lainas et al 2008).*
3. Flexible antagonist admioistration based on individualized criteria is related with increased pregnancy rates *(Lainas et al 2005).*
4. Antagonist administration in the luteal phase is a novel treatment of established severe OHSS, without the need for hospitalization *(Lainas et al 2012, 2009a, 2009b, 2007).*

** OHSS= ovarian hyperstimulation syndrome*

Short protocol 7.5

The short (or flare up) protocol is used mainly for women with poor ovarian response.

In the short protocol:
- Administration of GnRH agonist begins usually on the 1st or 2nd day of the cycle and administration of gonadotrophins on the 2nd - 3rd day.
- There are no distinct phases (downregulation-stimulation) and its duration is 10 -15 days.

Mild stimulation protocols 7.6

According to the revised terminology of the International Society for Mild Approaches in Assisted Reproduction (ISMAAR), mild stimulation protocols include:
a) the natural cycle,
b) modified natural cycle, and
c) mild stimulation.

Mild protocols

Mild ovarian stimulation involves the administration of a low dose of FSH (up to 150 IU/day) for a shorter duration, aiming at the development of fewer follicles and the collection of up to 7 oocytes. Mild stimulation protocols mainly use GnRH antagonists.

However, the use of mild stimulation protocols remains low, mainly because they require a sound knowledge of the GnRH antagonist protocol and optimal laboratory conditions.


ΤΡΙΚΗ
ΕΓΚΥΚΛΟΠΑΙΔΕΙΑ
εξωσωματική γονιμοποίηση

Corifolitropin a (single injection) 7.7

Corifollitropin-a (Elonva) is a new recombinant gonadotrophin (FSH) designed for ovarian stimulation. The drugs used in ovarian stimulation are of vital importance for the development of multiple follicles and oocytes, aiming at increasing pregnancy rates. The protocols of ovarian stimulation currently used, include daily gonadotrophin injections, combined with GnRH agonist or antagonist, depending on the protocol. Daily injections cause a physical burden in women undergoing IVF treatment.
Elonva is administsred once as a single injection at the beginning of the follicular phase and can sustain multiple follicle development for up to 7 days. From Day 5 of stimulation we start administration of GnRH antagonist to prevent a premature LH surge.

Two large clinical trials have evaluated positively the effectiveness and safety of corifollitropin-a. These studies showed that following a single injection of corifollitropin-a, ongoing pregnancy rates, the number of oocytes retrieved, and side-effects were similar to traditional protocols (i.e. using daily injections). Therefore, a single injection of corifollitropin-a is more patient-friendly and can substitute the unwanted daily injections for 7 days.
Corifollitropin-a is mainly used in a GnRH antagonist protocol. Therefore, knowledge and experience on the use of this protocol is a prerequisite for the administration of corifollitropin-a.

The scientific team of Eugonia has extensive knowledge and experience, as well as a series of published papers on the use of GnRH antagonist protocols.
We now participate in an international study to evaluate corifollitropin-a. The first results are encouraging and a significant amount of experience has been accumulated.


eutovia

8. Course of IVF treatment

Monitoring during treatment 8.1

Examination during ovarian stimulation includes:

- Follicle development and the increasing thickness of the endometrium (using transvaginal ultrasound)
- Hormone levels, such as E2, LH, progesterone (using blood samples)

These will require 4-6 morning visits to our Unit.

The Director in collaboration with other doctors and midwives of our Unit assess the results and compare them to previous measurements and your medical history. They will decide on the dose of drugs, and other instructions that will be added to your personal file. Our midwives will inform you extensively and responsibly about the course of your treatment programme, drug dosage and the date for the repeat of checks.

Catheter test 8.2

It is a painless procedure that mimics the embryo transfer. It allows us to observe the characteristics of the cervix in detail so we can be best prepared for the day of the embryo transfer.

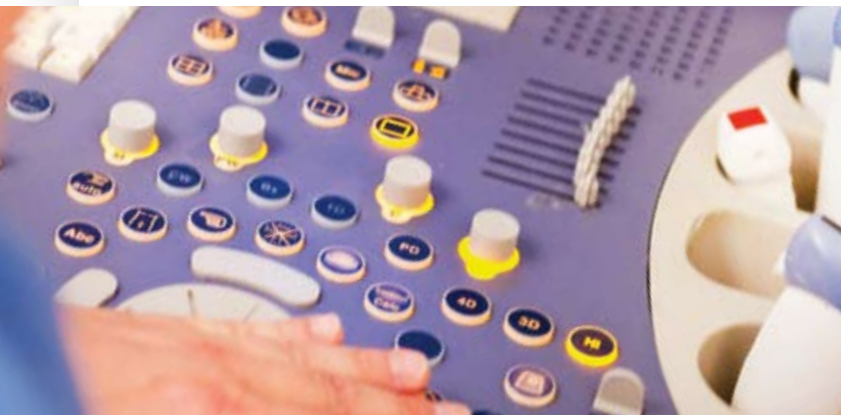
Triggering final oocyte maturation 8.3

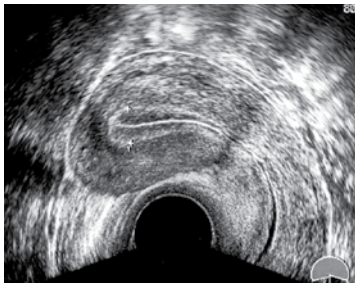
The day when sufficient follicle maturation is observed you will be informed of the exact time for your last injection which will induce final oocyte maturation and ovulation (Pregnyl or Profasi or Ovitrelle) as well as the dose that must be used. This injection is human chorionic gonadotrophin which mimics the LH action and induces final oocyte maturation and ovulation. Alternatively, in patients at high risk for ovarian hyperstimulation syndrome, hCG can be replaced by GnRH agonist (Arvecap, Gonapeptyl, Daronda), provided that the patient has been treated with a GnRH antagonist protocol.

The injection is administered late in the evening or after midnight. Ovulation is expected about 36 hours later.

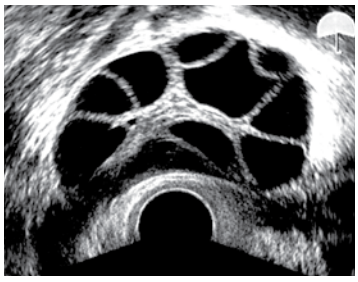
This last injection concludes the painful phase of daily injections and marks the end of GnRH analogue and gonadotrophin administration. The following day is a day to rest and prepare for the egg collection. This last injection is very particular in that it must be done at a very specific time, which will be the deciding factor for the time of egg collection. If we attempt to retrieve the eggs before they develop completely, i.e. much earlier than 36 hours after the injection, we shall collect immature eggs.
On the contrary, if the injection is done before the programmed time there is a danger that ovulation will occur prior to the egg collection and the eggs will be lost.

If the injection is not done, the egg collection will have to be cancelled or postponed. The eggs will not be mature and it will not be possible to recover them from the ovary. If the injection is not done you must inform our Unit the following morning so your egg collection is re-programmed and the entire cycle is not cancelled.





Ultrasound monitoring of endometrium thickness, during multiple follicle development treatment. The picture demonstrates a triple-line pattern on day 8 of follicular development. *("Eugonia" archive).*



Multiple follicle development on day 8 of ovarian stimulation *("Eugonia" archive).*

Your lifestyle during treatment 8.4

During the drug treatment you do not have to change your way of life or modify your habits concerning work, exercise, diet or sex. If deemed necessary from the results of the preliminary tests, the male partner may have to take antibiotics at the same time as the onset of your ovarian stimulation.

If it is necessary after the preliminary results, your husband gets preventive antibiotic treatment, at the beginning of your own drug treatment.

Psychological support 8.5

For some couples, the problem of infertility has psychological effects. Emotional distress is usually greater after previous failed attempts or when IVF is regarded as the last chance of having a baby.

Eugonia gives you the option of talking to our collaborating specialised psychologist. It has been proven that psychological support reduces the emotional load. We therefore encourage you to contact our psychologist, who will positively contribute to your IVF attempt

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Cycle cancellation 8.6

It is possible that a small percentage of women undergoing ovarian stimulation for fertility treatments may have to be cancelled. Cancellation of a treatment cycle is recommended when the response of the ovaries to the drugs is very poor, contrary to our expectations. Our aim is to always give patients the best chances of success in each cycle started and to eliminate the already minimal risk of possible complications.

9. Oocyte retrieval

What is it and how is it done? 9.1

The egg collection is the recovery of eggs from the ovaries. It is performed in a special room in our Unit, under sterile conditions, at a specific time, usually 35-36 hours after the last injection (Pregnyl, Profasi, Ovitrelle).

The egg collection is performed by the doctor transvaginally, under constant ultrasound guidance, which facilitates the doctor's precise manoeuvres. The follicles are punctured one after another using a special needle that traverses the vaginal wall.

The contents of each follicle are aspirated into special tubes and are passed on to the embryology laboratory. The embryologist locates the eggs and places them in dishes containing culture medium.

All the equipment and consumables used fulfill special requirements, ensuring that they are not potentially toxic to the gametes and the embryos.

Is it painful? 9.2

The procedure of egg collection is practically painless as it is done under intravenous analgesia (sedation) administered by the anaesthetist. Another reason why the egg collection should be done under sedation is to avoid any involuntary movement by the patient that could momentarily move the egg collection needle and cause damage to the ovary or other structures close to the ovary (uterus, intestine, a large blood vessel etc.)

An egg collection is usually a quick procedure, lasting about 10-30 minutes, depending on the number of the follicles, the degree of difficulty of the aspiration etc. The patient usually needs to stay in the recovery room for a further 30 minutes to 1 hour to rest and allow the staff to ensure that they are well enough to leave the Unit.

Before leaving the Unit, the patient is informed about the number of the eggs retrieved and will receive further instructions for the continuation of the treatment.

What preparations are necessary? 9.3

A light dinner the evening before the egg collection, shaving and cleansing of the outer genitals.

On the day of egg collection: you must refrain from eating or drinking, and must not have polished nails or perfume. It is advisable that your schedule is free for the morning of the egg collection.
You must arrive at the Unit at the specified time along with your partner, who will provide the sperm

Is it dangerous? 9.4

There is a chance of trauma or inflammation of the internal organs, but it is estimated internationally to be minimal. In the hands of an experienced doctor this possibility is practically negligible or even zero.

10. Sperm collection

Instructions for the couple 10.1

At the same time as the egg collection, or straight after, the male partner will produce the sperm, preferably by masturbation. It is very important for the entire sample to be collected. If not all the sample is collected or if the sample does not contain enough spermatozoa, the male partner may be asked to provide a second sample a few hours after the first one. The couple will be informed about the day of egg collection, at which time the male partner must have 2-5 days abstinence from ejaculation.

Sperm preparation 10.2

After the sperm production, the sample is processed (preparation-condensation) in order to select the motile and morphologically normal sperm. Following this preparation, the spermatozoa are kept in the laboratory under sterile culture conditions until placed together with the eggs.

Facing ejaculation problems 10.3

These are identified during the preliminary examination of the couple and ways of overcoming them are explored.

In the case of retrograde ejaculation (backward release of semen into the bladder) the male partner needs to drink a sodium bicarbonate solution (to alkalinize the urine) before he collects a urine sample, and the spermatozoa are recovered following a special preparation process.

In patients with ejaculation deficiency, observed in cases of spine injury (paraplegic-tetraplegic), diabetes and neurological conditions, sperm can be collected by induced ejaculation using a special device (electroejaculation).

Surgical sperm retrieval 10.4

Sperm can be recovered directly from the testes either by needle aspiration (FNA) from the testis or epididymis, or via a surgical biopsy of small testicular pieces (TESE).

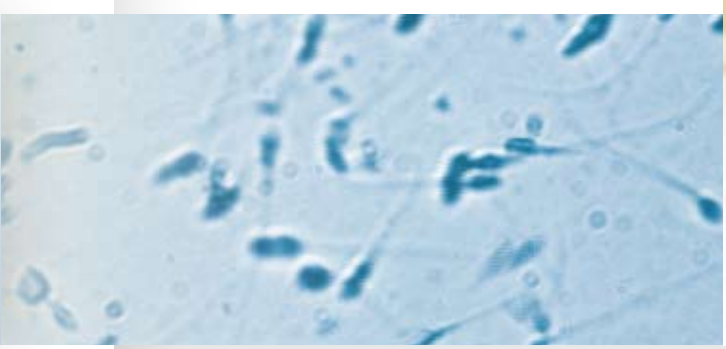
Surgical sperm recovery is recommended in cases of azoospermia, inability to collect a semen sample on the day of egg collection or failure of electroejaculation.
The procedure can be performed on the day of the egg collection under intravenous or local analgesia. The testicular tissue excised is processed by the embryologist in order to retrieve any sperm. Once motile sperm have been isolated, the mature eggs can be fertilized using intracytoplasmic sperm injection (ICSI). Any excess motile sperm can be cryopreserved for future use.

Azoospermia 10.5

Azoospermia is the absence of spermatozoa in the ejaculate, and it is classified as obstructive or non-obstructive. In cases of non-obstructive azoospermia the sperm is recovered surgically.
In obstructive azoospermia, sperm production is normal but no sperm appear in the ejaculate due to an obstruction of the male reproductive tract.

The obstructive aetiology includes obstruction of the vas deferens, congenital absence of the vas, or vasectomy. In obstructive azoospermia sperm can be easily obtained surgically using aspiration (FNA) or testicular biopsy (TESE).
In non-obstructive azoospermia there is no production of spermatozoa in the testicles. This complete lack of production or minimal production of sperm (oligoasthenoteratospermia, which is practically as severe as azoospermia) suggests testicular failure.

The condition can be idiopathic or be attributed to lack of testicular descent, injury, inflammation, contractible diseases (such as mumps), radiation, chemotherapy or chromosomal abnormalities.



Spermatozoa
("Eugonia" archive)

11. Laboratory stage

Conventional in vitro fertilization (IVF) 11.1

This represents the main laboratory stage of your attempt.
A few hours following the egg collection the embryologist places a specific number of motile spermatozoa in the culture dishes containing the eggs.
In conventional IVF there is no further intervention.
The spermatozoa approach the egg on their own and one of them penetrates it and fertilizes it.

Intracytoplasmic sperm injection (ICSI) 11.2

ICSI is applied when the sperm is incapable of fertilising the egg on its own (as previously described). Using highly specialised equipment, the embryologist deposits a single spermatozoon inside the egg to induce activation and fertilisation of the egg. Only one motile spermatozoon is required for each egg.

The method has been successfully used since 1992 and overcomes almost all the barriers that cause male infertility.

Thanks to ICSI, men with severely compromised sperm quality (reduced number, low motility, bad morphology or ejaculation problems) can now father a child of their own; something that was impossible in the not so distant past.

Until today, thousands of babies have been born from ICSI, with no indication that the method presents a danger for embryo quality and child health (according to a large scale epidemiological study from Belgium).

Except the way that fertilization occurs, the procedure is the same as conventional IVF as far as the man and woman are concerned

.

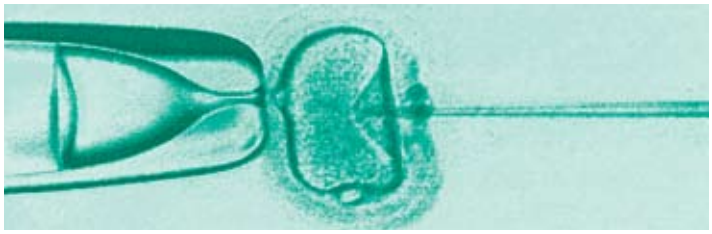
Fertilization assessment 11.3

The following morning after oocyte retrieval, i.e. 16-20 hours after insemination the fertilization check is performed. The embryologists check under the microscope how many oocytes have been fertilized normally and isolate unfertilized or abnormally fertilized oocytes (eg polyspermic). Especially polyspermic oocytes should not be transferred because they are related with pregnancy complications like miscarriages. Normally fertilized oocytes are placed back in culture and continue to develop. During their stay in the laboratory (usually 2-5 days) their development is checked regularly.

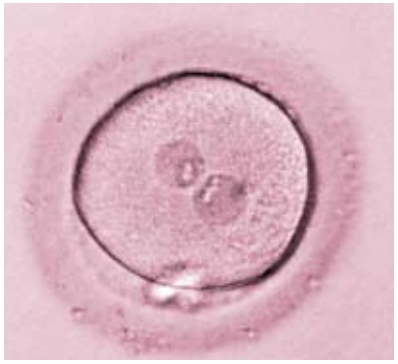
Embryo cleavage 11.4

Embryo development begins a few hours after the formation of the zygote. The zygote divides in two cells that are called blastomeres. The next divisions follow, giving rise to more and smaller blastomeres. This process is called cleavage.

Two days after oocyte retrieval cleavage has started and embryos have undergone 2 cell divisions. Cleavage rates are high, about 95%. Typically, the first division occurs about 16 hours after fertilization (2-cell embryo). The second division happens 12 hours later (4-cell embryo) and divisions continue in an increasing rate. Between 4 and 8-cell stage the embryonic genome is activated. The next stages are the morula (16-32 cells) and the blastocyst stage.



The injection pipette which contains the spermatozoon has penetrated the zona pellucida of the oocye *("Eugonia" archive).*



Normally fertilized oocyte.
The two pronuclei can be seen very clearly *("Eugonia" archive).*

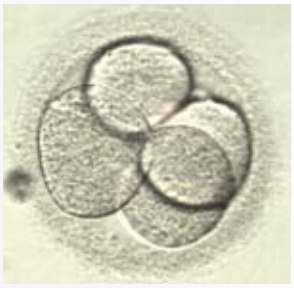
Embryo evaluation 11.5

The achievement of a successful pregnancy largely depends on the number and quality of embryos transferred to the uterus. Therefore, we need to evaluate a patient's embryos during their development and select for transfer those with the highest morphological characteristics. Evaluation of day 2 and day 3 embryos is based on two characteristics: the number of cell divisions (cleavage) and morphological appearance.

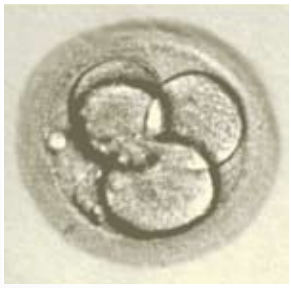
Cleavage: On Day 2 after oocyte retrieval the embryos must have 2-4 cells, with optimal stage the 4-cell stage. On Day 3, the embryos must have 5-8 cells, with 8-cell embryos being the best developmentally.

Morphology: Embryos are categorized into four grades (I-IV) based on size and shape of blastomeres and percentage of fragmentation. Grade I embryos are the best morphologically, without fragmentation, normal size and shape of blastomeres. On the contrary, Grade IV includes poor quality embryos with total fragmentation or degenerated blastomeres. Intermediate Grades are II and III. Ideally, on day 2 we want to have 4-cell embryos with grades I or II. On day 3, best embryos are regarded 8-cell embryos with grades I or II

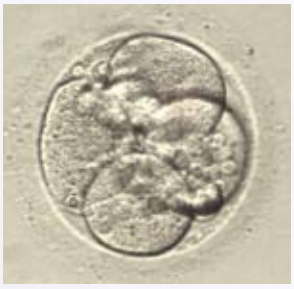
.



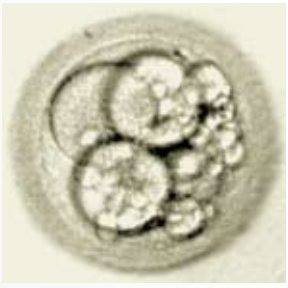
A) 4-cell embryo,Grade I.



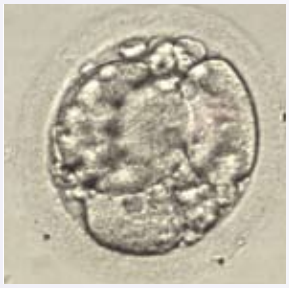
B) 4-cell embryo,Grade I-II.



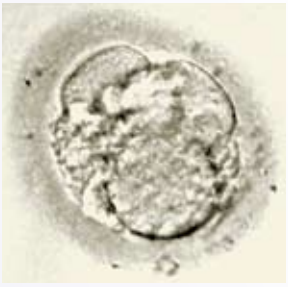
C) 4-cell embryo,Grade II.



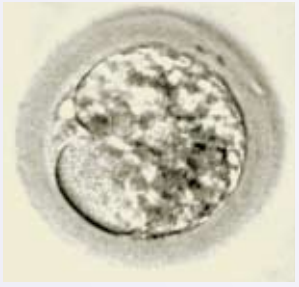
D) 4-cell embryo,Grade II-III.



E) 4-cell embryo,Grade III.



F) 4-cell embryo,Grade III-IV.



G) Embryo with increased fragmentation that does not allow the evaluation of the cell number, Grade IV.

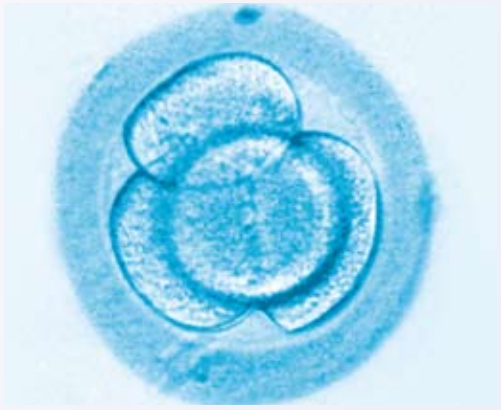
Evaluation of embryos with 4 cells.The photos show embryos ranging from Grade I to Grade IV, as evaluated by the embryologists (A, B, C, D, E, F, G) *("Eugonia" archive).*

** For more embryo photos, please refer to the "Eugonia" website:* ***www.eugonia.gr***

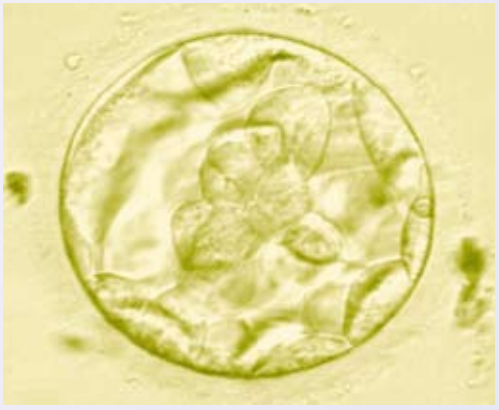
Preimplantation embryo Development

2-cell embryo, grade I-II (Day 2),
*(**"Eugonia"** archive).*

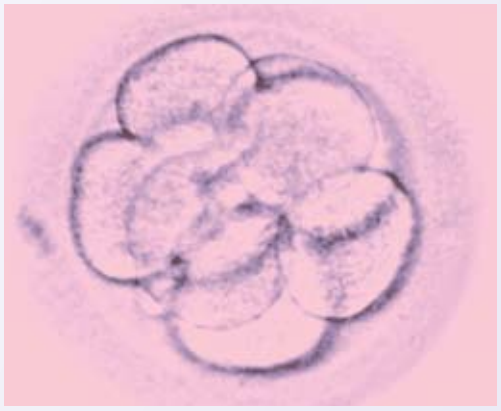
Morula (Day 4),
("Eugonia" archive).



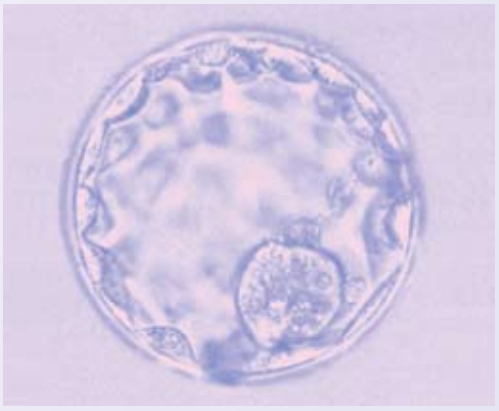
4-cell embryo, grade I (Day 2),
("Eugonia" archive).



Early blastocyst (Day 5),
("Eugonia" archive).



8-cell embryo, grade I (Day 3),
("Eugonia" archive).



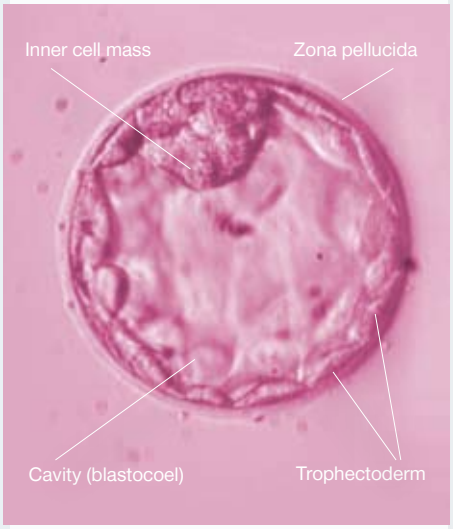
Expanded blastocyst (Day 5-6),
("Eugonia" archive).

Pregnancy success rates at ‘Eugonia’ Unit for blastocysts transfer are over 80%

Morula stage 11.6

The cleavage stages are followed by the Morula stage, which is observed on day 4 and is formed after the compaction and fusion of blastomeres of the dividing embryos.

As the morula continues to develop, fluid starts to accumulate forming a cavity, the blastocoel, giving rise to the blastocyst.



Fully expanded blastocyst
(“Eugonia” archive).

Blastocyst stage 11.7

The blastocyst 11.7.1

In natural conception, the blastocyst is the last preimplantation stage in embryonic development before implantation and establishment of pregnancy. In IVF, embryos that are transferred on days 2 or 3 do not implant in the endometrium immediately, but only when they reach the blastocyst stage. This method differs only in the day of the embryo transfer, which takes place on day 5 or 6. Until then, the embryos develop in the laboratory under culture conditions.

Blastocyst culture

Theoretically, growth to the blastocyst stage is an indication of normal embryo development. In addition, it has been proven that embryos reaching the stage of hatching blastocyst have approximately a two-fold increase of implantation chances.

The fully grown blastocyst is characterized by expansion, thinning of the zona pellucida and the accumulation of fluid among the cells. At this stage, the embryo contains 60 - 120 cells that form two distinct groups. The outer cell mass (trophoblast) that will give rise to the placenta, and the inner cell mass that will form the embryo.

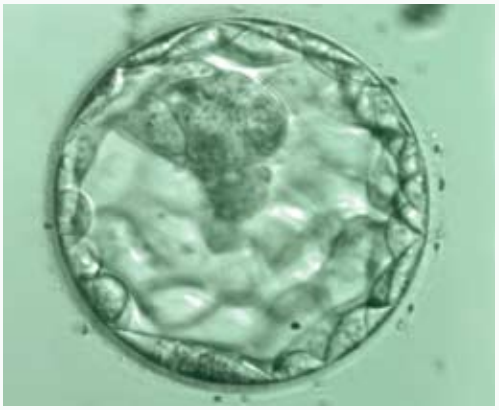
Blastocyst grading 11.7.2

For blastocysts there is a different grading system in place than the one for day 2 and day 3 embryos. This grading system is based on the combined assessment of the different characteristics of a blastocyst which are the degree of expansion, the progression of hatching and the quality of the inner cell mass and the trophoectoderm.

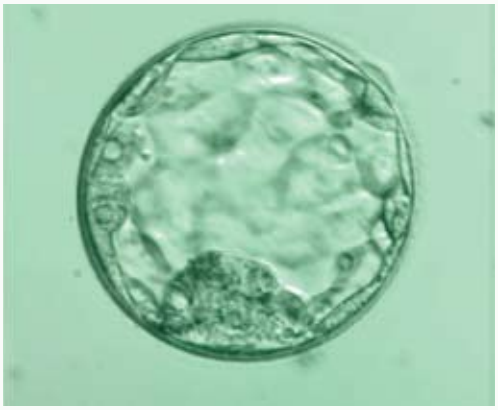
Expansion in graded with numbers 1 to 6. Inner cell mass and trophectoderm are graded with the letters A, B or C. Thus, on day 5, the ideal blastocyst is rated as 4AA (expanded blastocyst with excellent inner cell mass and trophoectoderm), whilst on day 6 the ideal blastocyst is rated as 5AA or 6AA (hatching or fully hatched blastocyst with excellent inner cell mass and trophoectoderm).
The process of blastocyst transfer (5th or 6th day following egg collection) is similar to the embryo transfer on day 2 or 3, with the only difference being the number of the transferred embryos. Up to 3 embryos are transferred on day 2 and 3, while only one or two blastocyst are selected transfer.

For further information visit our website ***www.eugonia.gr***

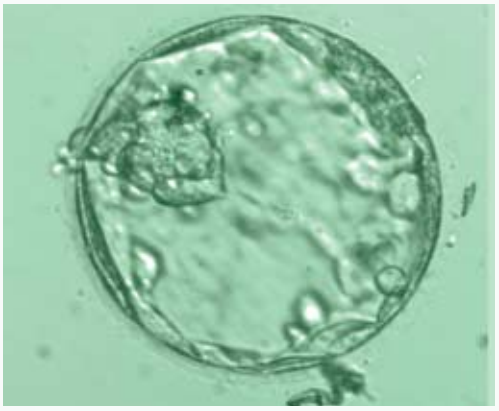
Blastocyst evaluation



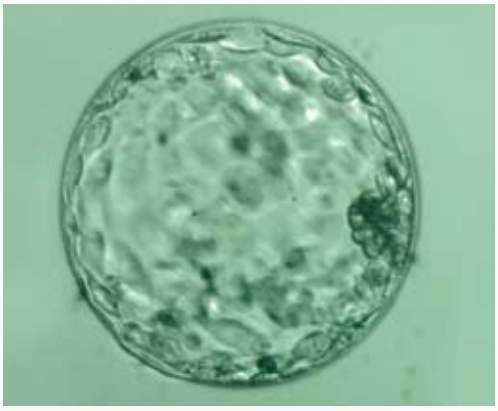
Fully expanded blastocyst 4AA *("Eugonia" archive)*



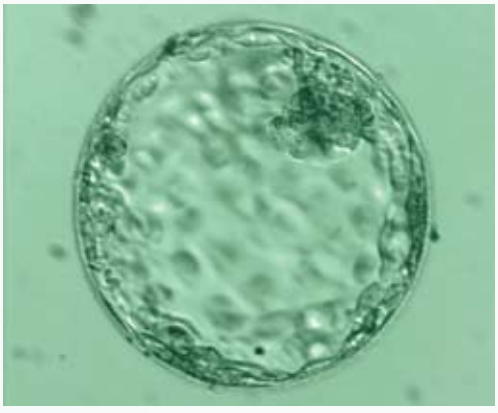
Fully expanded blastocyst 4AB *("Eugonia" archive)*



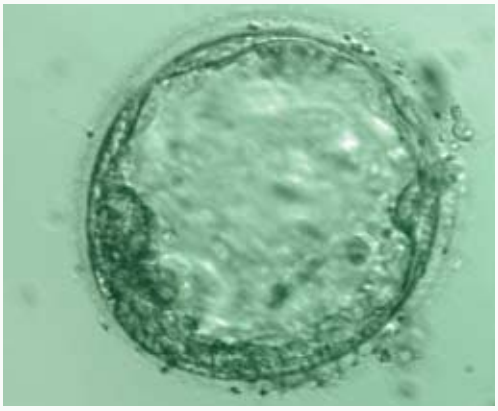
Fully expanded blastocyst 4AC *("Eugonia" archive)*



Fully expanded blastocyst 4BB *("Eugonia" archive)*



Fully expanded blastocyst 4BC *("Eugonia" archive)*



Fully expanded blastocyst 4CC *("Eugonia" archive)*

Blastocyst 11.7

Blastocyst culture leads to increased pregnancy rates 11.7.3

Blastocyst culture offers a strong selection method of the best embryos for transfer. It is already know that not all embryos can reach the blastocyst stage during culture in the laboratory. At Eugonia Unit, in average, 50% of fertilised oocytes will reach the blastocyst stage. Thus, the embryos that reach the blastocyst stage on the 5th day of their culture have already proven their dynamic for further development and they have a higher chance of implantation. Nonetheless, blastocyst culture requires the use of specially designed culture media and a modern laboratory with experienced embryologist and optimal culture conditions.

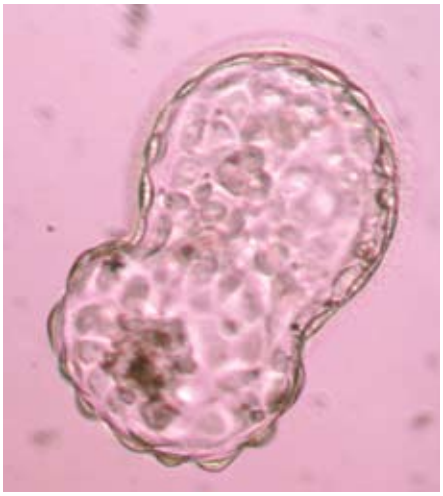
The advantages for blastocyst transfer include:

- Better selection of the embryos that have the highest potential for transfer
- Increased pregnancy rates
- Improved synchronisation with the endometrium
- The possibility of transferring 1 or 2 embryos (in comparison to 3 embryos that are usually transferred on day 3) and thus the reduction in multiple pregnancy rates
- The chance of monitoring for ovarian hyperstimulation syndrome (OHSS) and a safer embryo transfer as long as severe OHSS has not developed

Blastocyst culture is not suggested in all cases, as the availability of enough good quality embryos is a necessary requirement.

This culture method can be applied to women with polycystic ovaries that had many eggs collected, to women with an increased chance of developing severe OHSS, in cases of severe oligo-astheno-teratozoospermia or testicular biopsy, in cases of previously failed attempts (when transferring embryos of day 2 or day 3), in cases of preimplantation genetic diagnosis, etc.

At Eugonia, there is a successful programme of culture and transfer of blastocysts, with specific internationally acceptable criteria. The scientists, doctors and nurses of the Unit have the necessary knowledge and experience to inform and guide the patients in this matter.



Blastocyst during advanced stages of hatching. *("Eugonia" archive)*

Pregnancy Rates 11.7.4

Pregnancy success rates at Eugonia Unit following culture and transfer of blastocysts are over 80% per embryo transfer for women of all ages. More specifically, for women <35 years of age, the positive pregnancy test has reached an impressive 88% per embryo transfer over the last couple of years (2010-2011). Pregnancy rates for women 35-39 years of age have also increased (72%), as well as for women over 40 years of age (56%). (See our blastocyst pregnancy rates analytically on page 9).

Preimplantation genetic diagnosis/screening 11.8

Preimplantation genetic diagnosis (PGD)
11.8.1

PGD allows the identifications of genetic abnormalities of the embryo while it develops in culture. The method detects certain genetic abnormalities in the embryo, which are responsible for known congenital or hereditary diseases.

Embryos that undergo PGD are biopsied on Day 3 after oocyte retrieval. The embryos are first placed in a special solution to loosen the intercellular bonds. Then a small opening is created on the zona pelucida using laser, and using a special micropipette one cell (blastomere) is removed from each embryo. The biopsied cell will undergo genetic analysis, while the remaining embryo will continue to develop in culture. It has been observed that the removal of a single blastomere at this stage of embryonic development does not adversely affect embryo viability.

With PGD, we identify specific gene mutations (using polymerase chain reaction, PCR), and structural or numerical chromosome abnormalities (using FISH, or array-CGH).

The abnormal embryos are therefore identified and excluded from the embryo transfer. Only healthy embryos are selected for transfer in the uterus, at the blastocyst stage on day 5 or 6. If the gene causing a genetic disease is located on a sex chromosome, then sex selection is required to avoid development of the disease in the embryo. This is the only case when sex selection is performed.

Cases that require PGD include: thalassaemia, cystic fibrosis, Down syndrome, etc.

Thanks to the continuous evolution of genetic analysis technology, an increasing number of genes responsible for hereditary diseases can be identified, helping us to avoid even more genetic diseases, even some forms of cancer.

PGD has an advantage over conventional prenatal diagnosis methods, ie amnioparacentisis and trophoblast biopsy, as it can avoid potential abortion if the diagnosis is positive for a certain abnormality. It must be clear that PGD searches for specific abnormalities and does not preclude the birth of a child with a different genetic disease.

It is necessary that all couples who are candidates for PGD seek genetic consultation by a geneticist. PGD must always be followed by prenatal diagnosis in case of pregnancy.

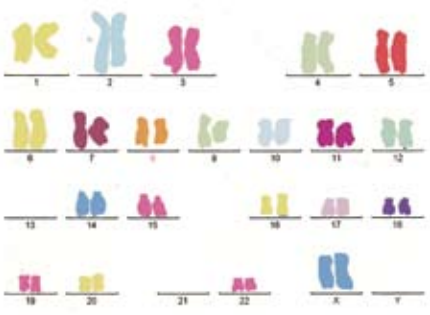
The scientific team at “Eugonia” has the required training and expertise to offer PGD for the prevention of genetic diseases in collaboration with renowned Genetics Centres.

Preimplantation genetic screening (PGS)
11.8.2

In cases of repeated failed IVF attempts, especially for women of advanced age, in couples with normal karyotype and severe male infertility, or in cases of repeated early pregnancy loss, the embryos may undergo preimplantation genetic screening (PGS).

Embryos are biopsied and isolated blastomeres are tested for the presence of structural or numerical chromosome abnormalities using special cytogenetic methods (FISH).

However, this method can only identify up to 9 chromosomes, and therefore is associated with a high degree of misleading results..



Coloured karyotype *(K. Pagkalos archive).*

Comparative genomic hybridization (CGH)

11.8.3

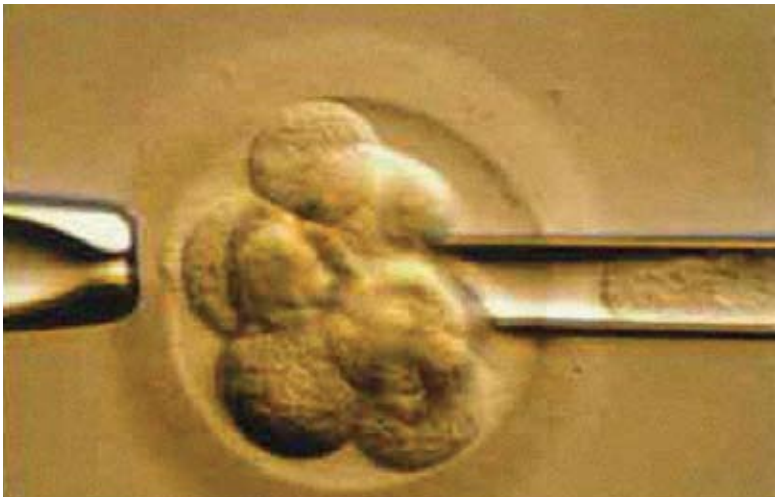
The development of array Comparative genomic hybridization (a-CGH) for preimplantation genetic diagnosis (PGD) provides useful information on the frequency and type of aneuploidies in gametes and embryos.

The technique allows screeing of all the chromosomes and can be applied at the stage of the mature oocyte (biopsy of 1st polar body), zygote (biopsy of 1st and 2nd polar bodies), day-3 embryo (biopsy of one blastomere) or blastocyst (trophectoderm biopsy).

a-CGH gives us the ability to screen all chromosomes, contrary to the technique of FISH, which evaluated only a limited number of chromosomes (usually X, Y, 13, 16, 18, 21, 2).

a-CGH showed that 20-30% of oocytes are aneuploid in women aged 20-30, while aneuploid oocytes from women over 40 are 50-80%.

Therefore, PGS using a-CGH is an important development of molecular genetics. The technique can lead to increased pregnancy rates because it can detect chromosome abnormalities with high accuracy, resulting in the transfer of healthy embryos.



Blastomere biopsy for preimplantation genetic diagnosis.

Other laboratory developments 11.9

The "omics' in assisted reproduction 11.9.1

During the last years, molecular techniques that offer additional information for the viability and developmental potential of embryos have shown a great advancement in the assisted reproduction field. These techniques, due to their name, have been included in the omics group.

They are about the study of the entire profile of the embryo activity in various levels of expression (DNA, RNA, proteins or metabolites). Thus, the study of the entire genome is called genomics, the study of RNA transcription is called transcriptomics, the study protein translation is called proteomics and the study of the metabolism is called metabolomics.

These techniques, which are either invasive (genomics, transcriptomics) or non-invasive (proteomics, metabolomics) form the spearhead of research and some of them are already applied in the embryology laboratory.

These techniques are expected to play a pivotal role in objectively determining the developmental potential of each embryo so as to select one healthy embryo for transfer and increase pregnancy rates.

New culture media 11.9.2

New generation culture media contain improved and complex components that excellently support the embryo during its pre-implantation development in the laboratory.

More specifically, for the prolonged culture for 5 or 6 days, up to the blastocyst stage, the so called sequential culture media are used. These sequential media have different components that reflect the continuously changing nutritional and metabolic requirements of the developing embryo.

Recently, new culture media have developed that contain special growth factors (e.g. GM-CSF) which seem to significantly benefit patients with previous miscarriages and possibly patients with previous failed assisted reproduction cycle attempts, poor sperm quality, etc.

The advances in the culture media field have contributed significantly to the increase in pregnancy rates

Continuous monitoring of embryonic development 11.9.3

With the use a special camera that is placed inside the incubator and the use of specialised software, it is possible to have a continuous monitoring of the embryo development (time lapse monitoring). In this way, the embryologist can have access to important information regarding the start of the first cleavage, the development and synchronisation of the divisions of each embryo, the appearance of fragmentation, as well as the presence of any abnormalities such as multinucleation in one or all the cells of an embryo.

With the use of this technology, we can pinpoint the healthy embryos with good morphology and a normal development pattern with a greater accuracy and we can also exclude from transfer embryos of poor quality and decreased potential, thus increasing the chance of achieving a healthy pregnancy with a normal development

.

Assisted hatching

11.9.4

The initial excitement over the usefulness of assisted hatching in increasing implantation is now under question. However, indications for the use of the method include thick or hardened zona pellucida, frozen-thawed embryos, eggs from women of increased age, high basak FSH, etc.

The embryologist can assist blastocyst hatching by opening a small hole on the zona pellucida (assisted hatching).

Eugonia offers the assisted hatching method with the use of laser, in the cases that is has been deemed necessary by our scientific team.

The ideal embryology laboratory 11.10

The embryology laboratory is where the collection and fertilisation of the oocytes takes place and where the embryos remain under culture conditions until the day of embryo transfer. It is a place of high specialisation and sterility, with advanced technology equipment, that under the guidance of an experienced and well-trained embryologist can have a substantial effect in the pregnancy rates of a Unit.

The ideal embryology laboratory, as the one in Eugonia Unit, is characterised by:

- Highly trained embryologists that are up to date with the latest scientific advances in the assisted reproduction field.
- Cleanliness of the environment with the use of special filters and air-exchange systems
- Clear and detailed protocols for every procedure taking place
- Quality assurance with continuous monitoring of all factors that may affect the embryos and the gametes (monitoring of temperature and culture conditions, as the continuous recording of culture media pH with the pH-online system, etc.)
- Maintenance of a database with detailed clinical information
- External quality control and ISO accreditation from an official government authority
- Research work that leads to publications in internationally renowned scientific journals.

All the above describe the embryology laboratory at Eugonia and reflect the high pregnancy rates in our Unit.

12. Embryo transfer

What is it? 12.1

Embryo transfer is the procedure of transferring the embryos back in the woman's uterus. It is a painless procedure that last for about 5-10 minutes.

It is performed using a special flexible thin catheter which is inserted through the cervix into the cavity of the uterus under ultrasound guidance. The embryologist selects the best quality embryos for transfer, based on certain morphological characteristics and aspirates the selected embryos inside the catheter along with minimal volume of culture medium.

The embryo transfer can be performed at the Unit 2 to 6 days after oocyte retrieval.

The value of ultrasound guidance 12.2

After loading the embryos into the transfer catheter, the gynecologist inserts the catheter into the uterine cavity and deposits the embryos with very gentle and precise movements.

The exact location of the catheter is constantly checked using ultrasound.

Therefore, a careful and atraumatic embryo transfer in skilled and experienced hands can maximize the chances of pregnancy.

At Eugonia, the procedure of embryo transfer reflects our attention to detail and is always performed under meticulous ultrasound guidance.

Does it need preparation? 12.3

The only kind of preparation required is to drink 4 glasses of water without urinating before arriving at the Unit, as the embryo transfer is performed under pelvic ultrasound.

You should refrain from using perfume as it may be toxic to the embryos. Remember to bring your Ultragestan pills with you. You can eat normally.

How many embryos will be transferred? 12.4

An important decision that must be taken after discussion with the couple involves the number of embryos transferred to the uterus. We will inform you about the quality of your embryos before the embryo transfer.

According to national legislation the number of embryos transferred must not exceed three for women below the age of 40, and four for women over 40 years of age. However, this number can be further reduced in special cases (e.g. young woman with good quality embryos) without impairing the outcome. The factors that must be taken in consideration include embryo quality, age of the woman and medical history.

The choice of number of embryos transferred must meet a fine balance between the increasing pregnancy chances, which are usually enhanced by increasing the number of embryos, and at the same time reducing the chances of a multiple pregnancy, which is achieved by reducing the number of embryos.

Internationally, there is a tendency to reduce the number of embryos for transfer to one, as multiple pregnancy is not regarded as a successful achievement any more, but an undesirable side effect of IVF.

What happens next? 12.5

After the embryo transfer you will remain in our Unit for about an hour. Together with instructions for drugs (usually progesterone pills or cream) we shall give you a photograph of the embryos transferred to your womb.

Usually, the first pregnancy test is performed 13 days later (blood levels of -hCG).

What precautions should I take in the next few days? 12.6

The embryos will usually implant to your uterus 4 - 5 days after a day 2 or day 3 transfer, and 1- 2 days after a blastocyst transfer.

Therefore, during these days, it is advisable to avoid physical or psychological tension and sexual intercourse. It is not necessary to skip work, as long as it is not overly tiring. Dietary habits can remain the same

.

Two 4AA blastocysts that have been selected for embryo transfer *("Eugonia" archive).*

13. Cryopreservation

Embryo cryopreservation 13.1

During an IVF cycle it is very common to have surplus embryos of good quality after the performance of the embryo transfer. These embryos can be frozen and stored in liquid nitrogen (-196°C) in a well-equipped IVF laboratory, for 5 or more years.

Use of frozen embryos and pregnancy rates 13.1.1

According to Greek legislation, frozen embryos can be stored for up to 5 years, with a possibility of extension for a further 5 years following application by the couple.

The couple may use the cryopreserved embryos for a future pregnancy without the need of repeated ovarian stimulation, egg/ sperm collection and IVF, but only monitoring and embryo transfer. Frozen embryos may be used if the couple's fresh cycle has succeeded and they now wish to have another child, or if the fresh cycle has not resulted in a pregnancy. In both cases, frozen embryos can be thawed and transferred to the uterus following patient monitoring.

International pregnancy rates using frozen-thawed embryos are slightly lower than fresh embryos.

This is due to the fact that the best quality embryos have already been selected and transferred at the initial fresh cycle, and also because some of the embryos may be partially or totally destroyed upon thawing.

However, transfer of frozen embryos increases cumulative pregnancy rates from a single egg collection (fresh and frozen embryos), making embryo freezing a very useful method, as it also eases the economical and psychological burden of the couple.

Does it require drugs? 13.1.2

The transfer of frozen embryos is scheduled so that the uterine environment is favorable to receive the embryo, and have an optimal implantation period. The preparation of the endometrium happens either during a natural cycle with monitoring of ovulation, or a pharmaceutical cycle. The embryos are usually thawed a few hours before the embryo transfer.

After thawing, the number of embryos and blastomeres that have survived are evaluated. Embryo quality is assessed and the number of embryos for transfer is then decided.

Is it safe for my child? 13.1.3

The procedure of cryopreservation is considered to be safe for children born. All relevant epidemiological studies on thousands of babies born from cryopreserved embryos are reported to be healthy without a statistically important increase of congenital abnormalities.

Sperm cryopreservation - When should it be performed? 13.2

Theoretically, sperm can stay frozen indefinitely. Recently, a pregnancy has been reported in the literature using sperm which had been frozen for 20 years.
Sperm cryopreservation should be advised:

- When there is a risk of reproductive ability loss (testis removal, chemotherapy, radiotherapy.)
- When the male partner will be absent on the day of oocyte retrieval or on the day of the intrauterine insemination.
- In cases of surgical sperm recovery, when there are motile spermatozoa left over following ICSI, so as to avoid repeating the surgical sperm recovery in a future treatment cycle.
- In cases of sperm recovery using electroejaculation.
- In cases of ejaculation problems due to psychological factors.
- In cases of progressively declining sperm quality.
- Prior to sterilization by vasectomy.

Vitrification

Oocyte cryopreservation
13.3

Until recently, oocyte cryopreservation remained a challenge in human IVF, because the low survival rates of oocytes lead to low pregnancy rates. Recently however, the method of vitrification has revolutionized oocyte freezing and it is associated with high rates of oocyte survival, fertilization, embryo quality and pregnancy rates. The method can be also applied on cleavage stage embryos and blastocysts.

Vitrification is a method of ultra-rapid freezing. The oocytes are placed in special cryoprotectant solutions and then are plunger directly in liquid nitrogen, where they will be stored until later use. In this way, the interior of the oocytes reverts to a form of glass, avoiding the formation of ice crystals that are detrimental to oocyte viability.

Since vitrification was introduced to IVF relatively recently, statistical data regarding the success and safety of the method do not allow solid conclusions. However, all available studies in the literature report encouraging results regarding the health of children born following the method.

Vitrification can be successfully applied in the cryopreservation of fertilized oocytes, cleavage-stage embryos and blastocysts

.

Applications of vitrification 13.3.1

Oocyte cryopreservation is performed during an IVF cycle following ovarian stimulation. It is a method of storage of genetic material for women who wish to postpone having a child until later in life, as well as in cases of premature ovarian failure or lack of spermatozoa in the male partner's semen sample (azoospermia) or following testicular biopsy on the day of oocyte retrieval..

The experience of "Eugonia" 13.3.2

In Eugonia, we use vitrification for oocyte cryopreservation, following the international scientific developments, informing the patients about the method's advantages and disadvantages.

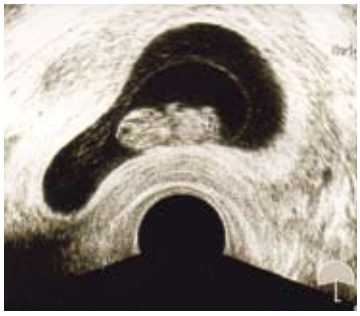
In Eugonia we already have several successful pregnancies and live births following oocyte vitrification.

14. Pregnancy test

When will I know if I am pregnant? 14.1

When the blastocyst has settled (implanted) in the uterus, the developing placenta (trophoblast) produces a hormone called b-human chorionic gonadotrophin (b-hCG). This hormone is detectable in the blood 10 days after the embryo transfer and in the urine of the pregnant woman some days later (pharmacy pregnancy test kit).

The test requires a blood sample and the result is obtained three hours later. If the test is positive it is repeated after 2 days.
The multiplication of the initial value indicates normal progression of pregnancy, which is termed 'biochemical' at this stage (as it is only demonstrated by the biochemical detection of hCG).



Intra-uterine clinical pregnancy of 8 weeks. The embryo can be seen during a transvaginal scan *("Eugonia" archive).*

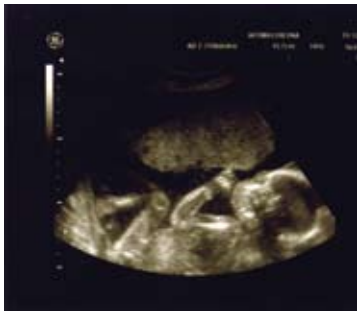
Clinical pregnancy
14.2

Fifteen days after the positive pregnancy test, a transvaginal ultrasound confirms the clinical pregnancy by examining the endometrium, the number of sacs, the presence of one or more embryos and the heart beat.

The task of our Unit has now been completed. Once a pregnancy has been achieved its progression does not differ to a natural pregnancy and you can address your personal obstetrician for your further monitoring.

Ongoing pregnancy 14.3

Normal pregnancy progression and embryo development as verified by ultrasound scan after the 12th week mark the ongoing pregnancy.



Ongoing pregnancy.
Ultrasound imaging of a 12 week embryo *("Eugonia" archive).*

Is there is chance of miscarriage? 14.4

Miscarriage rates following IVF are similar compared to pregnancies from natural conception. The chance of embryo loss after a positive pregnancy test is 15 - 17% of biochemical pregnancies.

Usually the causes are related to physical malfunctions or chromosomal abnormalities of the embryo, causes that are not possible to predict during the process of IVF. Increased age of the woman is another important factor, among others, responsible for this unfortunate result. We remind you that the quality of embryos transferred is defined using only morphological criteria, which are not able to depict possible chromosomal abnormalities, with the exception of PGD (*see section 11.8).*

The chance of a clinical pregnancy not resulting in a live birth is small and statistically similar to natural conception. From the stage of ongoing pregnancy until birth the chance of embryo loss is estimated at around 1%

.

Negative pregnancy test
14.5

If the measurements of b-hCG are found negative (b-hCG levels < 5 IU), the drugs are discontinued following instructions from the doctors and menstruation (period) will follow a few days later.

We are aware of the unpleasant feelings that stem from a failed attempt. The only way to deal with this situation is to examine, together with the couple, all the parameters that may have lead to a negative result. The only right way to deal with this situation if to examine and analyze all the parameters which may have lead to a negative result. This information will help us to redesign the couple's therapy aiming to achieve a successful pregnancy in the next IVF attempt. The clinical and scientific staff of Eugonia have the knowledge to provide answers and a solution to a previous failed attempt.

The interval between two consecutive IVF cycles must not be smaller than 3 months to give the ovaries time to recover, and to yourselves time to recuperate psychologically.

It is important not to lose hope in the face of failure. We are here to help and support you in this difficult phase.

15. Complications after IVF

Ovarian hyperstimulation syndrome (OHSS) 15.1

What is OHSS? 15.1.1

OHSS is the most serious complication of IVF. It is distinguished in mild, moderate or severe from, depending on the severity of symptoms.

Symptoms include abdominal distension, stomach ache, vomiting, increase of body weight and reduced urination. Moderate OHSS occurs in 3-6%, while severe OHSS occurs in 0.2-2% of women undergoing IVF. Mild OHSS lacks clinical significance, moderate OHSS requires monitoring because of the risk of progression to a severe form. Severe OHSS includes fluid accumulation in the third space, increase of ovarian volume, hematocrit, white blood cells and liver indices.

More rarely in critical forms there might be breathing difficulty, fainting and abnormal hematological and biochemical markers. In this case hospitalization is necessary.

OHSS develops 3-7 days after oocyte retrieval (early onset), or can be pregnancy-induced, 12-17 days after oocyte retrieval (late onset-pregnancy induced).

A subsequent pregnancy is not threatened by OHSS. But pregnancy can further induce the symptoms of OHSS

.

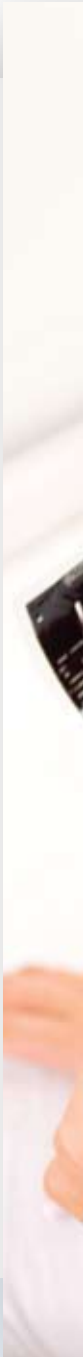
Risk factors 15.1.2

Risk factors for the development of OHSS include young age, low body weight, high gonadotrophin doses, previous OHSS history, high dose of hCG and polycystic ovaries.

Women with polycystic ovaries are at high risk for developing OHSS. Especially the risk of severe OHSS ranges from 9-38%.
This variation is mainly due to the lack of a universal classification system of OHSS.

OHSS is caused by the exogenous hCG administration in the presence of a large number of follicles (more than 20).

Our recent published study using strict criteria showed that the incidence of severe OHSS in women with PCO is 11.3%.



The contribution of "Eugonia" in the prevention and treatment of OHSS 15.1.3

1. Administration of GnRH antagonist in the luteal phase

This innovative method is described in a large study of high risk patients, proposing a radical change of practice in everyday clinical practice for the management of severe OHSS. Our method offers a treatment and avoids hospitalization, that would otherwise include symptomatic therapy with paracentisis, albumin administration and possible admission to intensive care units for several days. Using our novel treatment, the patient is rapidly relieved from the symptoms and all that is required is a small number of visits to our Unit for monitoring.

The treatment includes subcutaneous administration once a day for 4 days of GnRH antagonist (Orgalutran, Cetrotide) which is the exact same drug used during ovarian stimulation in antagonist protocols, and at the same dose of 0,25 mg.

Our approach minimizes the physical, psychological and financial burden associated with hospitalization. It should be noted that symptomatic treatment described above is, unit today, the routine practice in the case of severe OHSS.

In addition, our study proposes a new OHSS classification system based on numerical parameters, which facilitate direct comparisons of studies. Free access to the full text of the study published in the Journal of Reproductive Biology and Endocrinology, can be found via our website.

Three small studies by our team have also been published in Reproductive Biomedicine Online

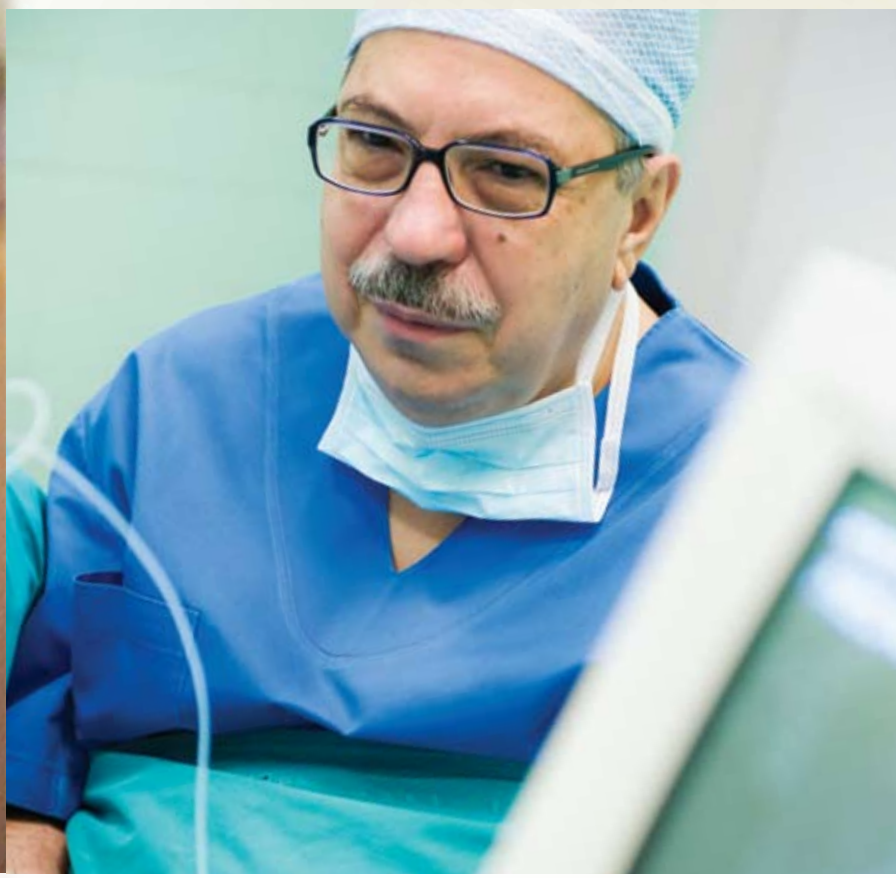
.

2. Methylprednizolone administration

This is a prevention method and our first publication on OHSS in 2002. It reflects our attempt to avoid hospitalization 10 years ago.

3. GnRH antagonist protocol in women at high risk for OHSS

These studies show that the antagonist protocol is associated with a significantly lower incidence of OHSS compared to the traditional long protocol *(Lainas et al 2005, 2007).*



What causes OHSS? 15.1.4

OHSS is triggered by administration of exogenous hCG (the last injection of the programme) and involves a production of a large amount of follicles (>20) during ovarian stimulation.

The pathophysiological cascade of events is as follows: Ovarian stimulation using exogenous gonadotrophins is followed by multiple follicle development. Administration of hCG to trigger final oocyte maturation causes massive luteinization. Secretion of angiogenic factors, such as VEGF, by the multiple corpora lutea leads to increased permeability of blood vessels and shift of fluid to the third space.

The clinical evidence of OHSS is the presence of ascites.

.

Prevention of OHSS 15.1.5

Several method have been published for the prevention of OHSS, including coasting, intravenous albumin administration, embryo cryopreservation, cabergoline, cortisone, and more recently the use of GnRH agonist for triggering final oocyte maturation.

GnRH antagonist protocols significantly reduce the chance of OHSS development by 20% in women with PCOS (high-risk patients) compared to the traditional long protocol, while maintaining similar pregnancy rates. This is shown by our study *(Lainas et al 2010)* which is the largest study in the literature and was published in the journal Human Reproduction.

For a full list of our publications, please visit our website

Management of severe OHSS 15.1.6

We developed and we use a novel treatment approach for established severe OHSS. Using this method, we managed to cause the rapid regression of the syndrome and avoid hospital admission. Based on this strategy, which is a worldwide innovation of Eugonia, all patients can proceed at least to oocyte retrieval, avoiding cycle cancellation.

In addition, using this method we achieved the first births worldwide after blastocyst transfer in patients with severe OHSS receiving GnRH antagonist for only a few days.

Ectopic pregnancy
15.2

Ectopic pregnancy is the implantation of the embryo to an extra-uterine cavity, most usually in the fallopian tubes, or the cervix. It can happen after natural conception at a rate of 1-1.5%. The rate is slightly higher in IVF programmes.

It is diagnosed at early stages (6th week of gestation) as the first ultrasound that is performed 14 days after the positive pregnancy test can accurately identify the location of embryo development.

Ectopic pregnancies are treated using laparoscopic surgery.

For more information visit our website ***www.eugonia.gr***

Multiple pregnancies
15.3

Assisted reproduction cycles are associated with higher rates of multiple pregnancies.

Usually, twin pregnancies do not face any problems, provided that there is careful patient monitoring. However, triplet and more high-order pregnancies are more difficult and are associated with more problems and complications, regarding the health of the mother and the chance of premature labour. The most serious complications of premature labour are brain hemorrhages that lead to paralysis. Lately, the is a clear tendency in Europe to reduce the number of embryos transferred. As a result, triplet pregnancy rates is 1%, while twin pregnancies occur in 19.6% *(source: ESHRE 2012)*

Trauma or hemorrhage 15.4

In IVF, there is a minimal risk of traumatizing internal organs or major blood vessels during oocyte retrieval. However, this risk practically negligible in experienced hands and when all clinical precautions have been taken. Also, small hemorrhage is possible to be caused by the passing of the oocyte retrieval needle through the vaginal wall.

However, it is small, harmless and stops after the oocyte retrieval without complications.

Are there long term effects on my health?

15.5

There are no long term effects for the health of women undergoing IVF. Public worries (usually based on insufficient information), are understandable but are practically have no basis.

All the large international epidemiological studies conclude that the risks of developing ovarian, uterine or breast cancer are comparable to the general population.

These large studies from Australia on 29700 women *(Lancet 1999)*, from Great Britain on 5556 women *(Human Reproduction 2002)*, from France on 9255 women *(Human Reproduction 2004)*, from Denmark on 54362 women *(Jensen et al, 2009, BMJ)* as well as other meta-analyses *(Nes et al 2002; Kashyap et al 2004, Berit Jul Mosgaard et al 1997, Mahdavi et al 2006, Brinton et al 2004, Burkman et al 2003)* showed that there is not a statistical difference between women who received fertility drugs and developed ovarian, uterine or breast cancer, and women who did not take fertility drugs and developed cancer.

In addition, there is no evidence of increased risk for cancer in children born from IVF, compared to children conceived naturally *(study on 17000 vs 30364 children born after IVF; Klip et al 2001*).

As a precaution, all women should have a pap-test and a breast examination before the onset of IVF treatment. A mammogram is recommended for women over 35 years old.
In cases with breast family history, women should consult a mastologist irrespective of their age

16. Donations

Donor sperm 16.1

The application of ICSI offers a solution to the majority of severe male infertility cases. However, in some rare cases there is total absence of sperm (non-obstructive azoospermia). In these cases the couple may use donor sperm to achieve a pregnancy.

Donor sperm is obtained from specially organized sperm banks. International guidelines state that donor sperm can only be available in frozen state from an anonymous donor, who has been thoroughly tested for sexually transmitted and genetic diseases. The selection of the donor is made by the couple under the guidance of an embryologist of Eugonia, based on blood group and other characteristics, like colour of hair and eyes, height etc.

Eugonia collaborates with approved international sperm banks that conform to strict regulations, according to European and international legislation

.

Oocyte donation 16.2

Some women have lost their ability to produce their own oocytes due to ovariectomy, following chemotherapy or radiotherapy, premature menopause, or poor responders. For these women, the only solution to have a baby is oocyte donation. This means they have to undergo IVF using their husband's sperm and oocytes from an anonymous donor.

Oocyte donors may become:

- Women who offer all their oocytes to be used by another woman
- Women with many oocytes who already participate in an IVF programme and who wish to donate a proportion of these oocytes to another woman (egg sharing).

Oocyte donation must be anonymous according to Greek legislation, and the age of the donor must be less than 35 years. The anonymity of the donation is fully protected. In addition, the law also states that oocyte recipients can be women less than 50 years of age.

At Eugonia, it is mandatory policy to ask for written and signed consent of both donors and recipients of genetic material. These documents are kept in a classified file. All the relevant legal regulations are strictly adhered to.

We are at your disposal to inform you about the medical criteria and requirements of oocyte donation.

The existing programme of donation of genetic material at Eugonia reflects the strict moral and legal principles of our scientific staff.

Embryo donation
16.3

The indications for embryo donation concern couples with lack of reproductive capability. In these cases frozen embryos can be used, which the parents decide to donate instead of having them destroyed.

This is a highly altruistic act. The decision lies solely on the donor couple. Should they decide to donate, they both sign a special consent stating that they give up any right and that they anonymously donate the embryos for no financial return to anonymous recipients.

Surrogacy
16.4

Surrogacy applies to couples with normal eggs and sperm but the woman has a non functional uterus or is unfit to gestate for medical reasons.

Fertilization occurs in the embryology laboratory. The gestation of a couple's embryo by a third woman is allowed by judicial permission according to article 1458 of N.3089/02, provided that there is a documented and unselfish agreement between the couple and the surrogate (and her husband if the latter is married).

Terms of donation
16.5

According to Greek legislation, an oocyte/embryo donation program must operate under specific terms:

- Protection of recipient health
- Anonymity of donation
- Age
- Ethical issues of donation
- Consent of donors and recipients

Donations are last resort options aiming at the joy of parenthood. We believe that a couple who decides either to donate or receive oocytes or embryos, must be fully informed and must consent to undergo the process. For this reason, we provide the necessary documentation from the very early stages of the programme.

Intrauterine insemination (IUI)

IUI is the oldest method of assisted reproduction. It is a simple procedure whereby the prepared sperm of the male partner is placed in the uterine cavity. Fertilization occurs naturally within the fallopian tubes without any other intervention. A prerequisite for the use of IUI is unblocked fallopian tubes.

IUI can be the initial choice in young women with unblocked tubes when the male partner has mild or moderate sperm problems.

The method is also applied using donor sperm.

17. Future prospects

In vitro maturation of oocytes 17.1

In vitro maturation (IVM) aims at achieving the final maturation of eggs under optimal culture conditions. The immature eggs are obtained following minimal or no use of drugs for ovarian stimulation. The ultimate goal is the production of fully mature fertilizable eggs by puncturing follicles of small diameter. A similar method involves in vitro maturation of primordial follicles derived from frozen-thawed strips of ovarian tissue, in order to isolate and mature the enclosed oocytes.

The method is quite promising in the field of ART, especially for women wishing minimal or no ovarian stimulation using hormones (risk of OHSS, risk of cancer).

For now, the method is still at an experimental stage and its efficacy is limited. The main interests of IVM research groups are the enhancement of collection of immature egg from small follicles, improvement of culture conditions and guarantee that in vitro matured eggs are healthy and normal.

.

Cryopreservation and transplantation of ovarian tissue 17.2

The method intends to preserve a woman's reproductive ability prior to aggressive treatments for malignant conditions, aiming to restore normal ovarian function in the future.

A small part of the ovary which contains several immature follicles is removed by laparoscopic surgery or laparotomy and is frozen in Cryopreservation banks.

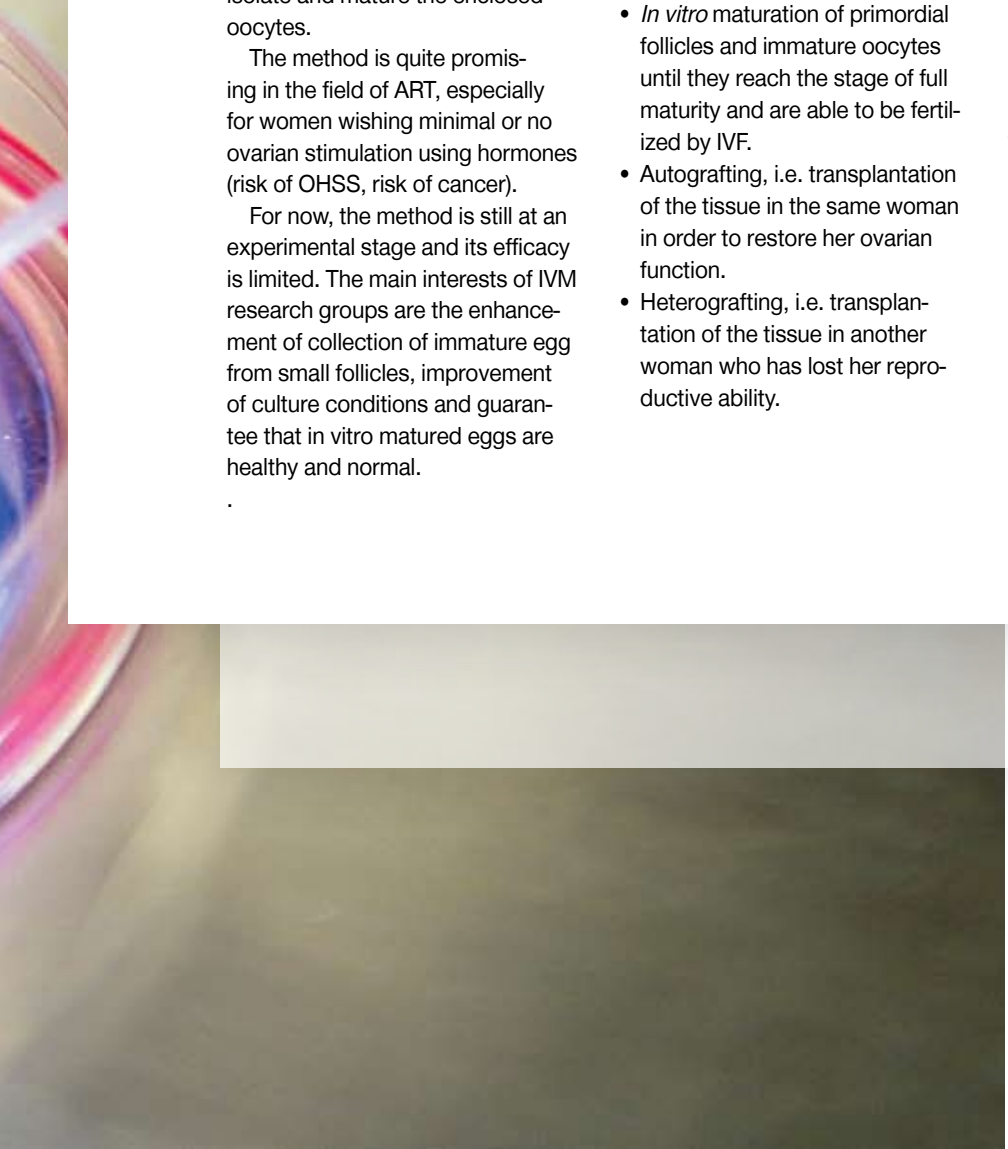
This confers the following future prospects:

- *In vitro* maturation of primordial follicles and immature oocytes until they reach the stage of full maturity and are able to be fertilized by IVF.
- Autografting, i.e. transplantation of the tissue in the same woman in order to restore her ovarian function.
- Heterografting, i.e. transplantation of the tissue in another woman who has lost her reproductive ability.

Depending on the location of the graft, transplantation may be:

- Orthotopic: transplantation in the anatomic location of the ovary
- Heterotopic: transplantation in a more convenient anatomic position (e.g. under the skin) which facilitates oocyte recovery from follicles developing under drug stimulation

Cryopreservation and restora-tion of ovarian function following transplantation are still under research. Experimental data have shown restoration of ovarian function for a limited period. Researchers investigate the factors that are responsible for the re-establishment of the function of the transplanted ovarian tissue and the restoration of the woman's reproductive ability.



18. The role of hysteroscopy - laparoscopy

Endoscopy - Endoscopic surgery 18.1

Endoscopy (laparoscopy and hysteroscopy) are valuable tools for the diagnosis and treatment of female infertility. Endoscopic (hysteroscopic/laparoscopic) surgery, by widening its range of application, is now the treatment of choice for certain infertility cases, and tends to minimize the need for traditional laparotomy.

Hysteroscopy allows us to view the cavity of the uterus, i.e. the location that will receive the developing embryo.

Laparoscopy is useful for examining the internal female reproductive organs (i.e. uterus, fallopian tubes and ovaries).

Some pathological conditions of female genital organs cannot be diagnosed with any other method but by hysteroscopy (e.g. endomitritis) or laparoscopy (e.g. peritoneal endometriosis or adhesions). It is possible that these conditions remain undiagnosed for a long time period in a significant percentage of infertile women. Many of these conditions can be identified and treated during the same operating procedure under anesthesia.

Hysteroscopic and laparoscopic surgery represent a technological revolution in surgery. The surgeon and his colleagues operate by viewing the image on the monitor via a camera attached to the laparoscopy probe. Laser beams, electrosurgery units, numerous special tools and endoscopes comprise the technological equipment used. As well as specialized scientific knowledge and experience, the surgeons must have a sound technical knowledge of the special equipment.

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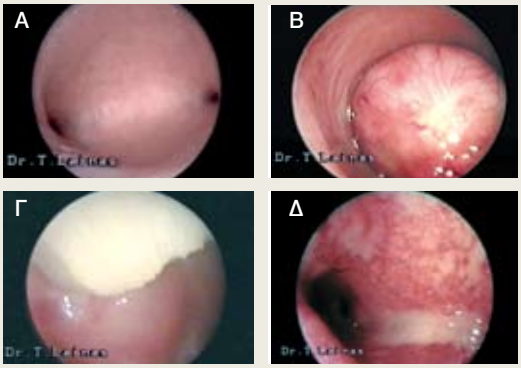


A. Normal uterine cavity.
B. Submucusal fibroid.
C. Osseous metaplasia. Rare hysteroscopic finding.
D. Endometritis

(hysteroscopic images).

Advantages 18.2

Endoscopic operations have an advantage over older methods of open surgery because::

- Hospitalization lasts for one day only
- Laparotomy is avoided (open surgery)
- The danger of development of post-operational lesions is avoided
- Post-operational pain is minimal
- There are no post-operational scars on the skin of the belly
- The duration of the operation is significantly smaller compared to laparotomy
- The opening of the uterine cavity is avoided in hysteroscopic surgery
- The return to everyday life and work is quick.

Hysteroscopy in assisted reproduction 18.3

What it is and how it is done 18.3.1

It is a special examination that allows us to observe the uterine cavity, the endometrium, the uterine openings of the fallopian tubes and the canal of the cervix.
It is almost painless and last a few minutes. In few cases of highly stressed patient we can administer mild anesthesia (sedation).

The uterine cavity is approached via the vagina and cervix, without a section or injury. This is achieved using a hysteroscope, a kind of telescope of small diameter 2.9 mm.

A special camera of small weight is attached to the hysteroscope for the imaging and recording of the operation.

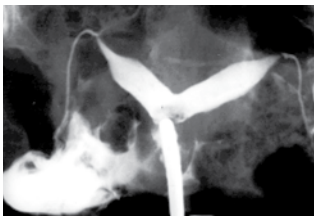
What is it useful for? 18.3.2

Hysteroscopy offers the possibility to directly observe the uterine cavity for an accurate diagnosis. Using hysteroscopy we can examine:

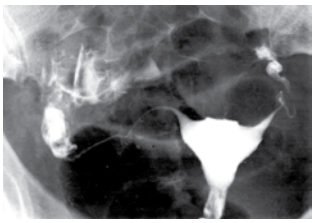
- The morphology (size and shape) of the uterine cavity *(Image 2)*
- The uterine openings of the fallopian tubes *(Image 3)*
- The structure and development of the endometrium
- The cervical tube (endocervix) *(Image 3)*

Hysteroscopy has an advantage over hysterosalpingography in sensitivity and specialization in investigating infertility and repeated miscarriages.

Hysteroscopy is a valuable tool for the diagnosis and treatment of infertility, as 62% of infertile women has been found to display endometrial cavity pathology.



Hysterosalpingographic image of the uterine septum prior to section *("Eugonia" archive).*



Hysterosalpingographic image of the uterine septum following section *("Eugonia" archive).*

When is it recommended? 18.3.3

Hysteroscopy is recommended:

- For the specification of hysterosalpingography or ultrasound findings concerning adhesions, polyp, fibroids, congenital abnormality.
- After repeated miscarriages.
- After two consecutive unsuccessful IVF cycles.
- In history of miscarriages, abortions and operations to the uterine cavity.
- To investigate unexplained infertility combined with laparoscopy.
- As a routine method before IVF (it is recommended by several specialists), especially in the absence of hysterosalpingography.

A significant percentage of women with normal hysterosalpingography demonstrate endometrial cavity pathology when subjected to hysteroscopy.

Hysteroscopic surgery 18.4

It is a modern, safe and fast method of surgically treating benign pathological conditions of the uterus.

It is harmless and effective when executed by experienced surgical teams with cutting-edge technical knowledge. It is a method of choice in infertility cases related to these conditions

What is it useful for? 18.4.1

Hysteroscopic surgery is applied:

- For the lysis of endometrial adhesions
- For the removal of endometrial or cervical polyps
- For the resection of the uterine septum
- For the removal of submucosal fibroids
- For the removal of foreign bodies (e.g. intrauterine contraceptive coils)

The advantages are similar to those of laparoscopic surgery.

For more information please visit our website www.eugonia.gr

Laparoscopy 18.5

What it is and how it is done 18.5.1

It is a special examination performed in a hospital under general anesthesia.Using a special organ, the laparoscope, we can examine the inside of the abdomen and the inner genital organs of the woman from a small 1 cm opening on the umbilicus..

What is it useful for? 18.5.2

Laparoscopy offers valuable help in the diagnosis of several gynecological conditions related to infertility. Many of them are not possible to diagnose otherwise (such as using ultrasound or hysterosalpingography).
Using laparoscopy we can examine:

- The size and morphology of the uterus, fallopian tubes and ovaries.
- The permeability of the fallopian tubes which is ensured by introducing a special dye (methylene blue) through the cervix and expecting it to come out from the other end of the tube (infundibulum and fimbria).
- The ovarian-fallopian tube relation, i.e. optimal contact of infundibulum and fimbria of each tube with the respective ovary.
- The pathology of inner genital organs, particularly endometriosis and adhesions

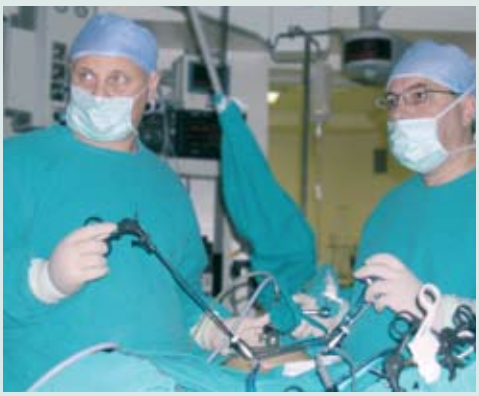
.

Which conditions are diagnosed? 18.5.3

- Endometriosis (peritoneal*, ovarian, rectovaginal septum)
- Adhesions* (tubal, ovarian etc)
- Hydrosalpinges (extended tubes with obstructed infundibulum)
- Congenital abnormalities of the uterus (bicornuate*, bipartite, unicornuate)
- Uterine fibroids
- Ovarian –paraovarian cysts
- Ectopic pregnancy

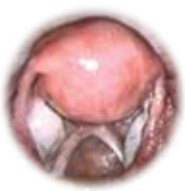
**These conditions can be diagnosed onlylaparoscopically*

The doctors at “Eugonia” operate under internationally accepted and pioneering laser laparoscopic surgery protocols with strict requirements and complete respect to the tissues and organs



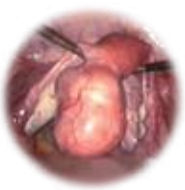
Laparoscopic surgery with the use of CO_2. laser. Surgeons Dr T. Lainas and Dr I. Zorzovilis perform the operation from the image that appears on the monitor. *Photograph taken in the operating theatre.*

Laparoscopic images

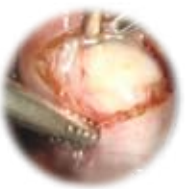


The uterus, the fallopian tubes and the ovaries. Normal laparoscopic image.

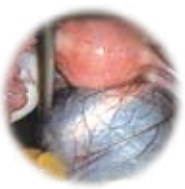
Endometriotic cyst of the left ovary.



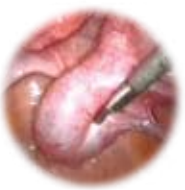
Pedunculated submuscosal fibroids of the uterus.



Removal of a uterine fibroid with laser.



Paraovarian right cyst.



Right hydrosalpinx.

Laser laparoscopic surgery 18.6

Advantages 18.6.1

It is performed without the classic 10-20 cm section (laparotomy), with four small holes (three holes of 0.5 cm diameter each at the level of the pubic region and one main 1 cm hole on the umbilicus).
The procedure is performed using a laparoscope connected to a laser CO2 unit and special tools.

The use of CO2 laser beams confers accuracy in the section and vaporization (i.e. conversion of the excised tissue from solid to gas), qualities that are considered ideal for the treatment of infertility-related conditions.

A necessary requirement is the excellent technical knowledge of laser laparoscopy of the surgical team.

Which operations can be done laparoscopically 18.6.2

The technique can be applied in the entire range of gynecological surgical operations:

- Treatment of endometriosis – vaporization of chocolate cysts
- Fallopian tube plastic surgery, fallopian tube opening
- Adhesion lysis
- Removal of ovarian or paraovarian cysts
- Ectopic pregnancy with retention or removal of the fallopian tube
- Removal of uterine fibroids
- Treatment of chronic pelvic pain using L.U.N.A.

Vaporization using SwiftLase has an advantage over diathermy. With the addition of SwiftLase, the depth of vaporization is smaller, known and very particular from histological studies, and does not leave carbon residues. On the contrary, in other methods (e.g. diathermy) it is impossible to specify the depth of damage to the ovarian tissue.

Endometriosis and laser laparoscopic surgery 18.6.3

In endometriosis, the endometrium (i.e. the tissue that lines the inner cavity of the uterus and is discharged with period blood) develops and functions outside the uterus (i.e. in an ectopic location).

Endometriosis is the second most common gynecological condition after fibroids. It is associated with pelvic pain, chocolate ovarian cysts, dysmenorrheal (painful period), and dyspareunia (painful sexual intercourse) etc.

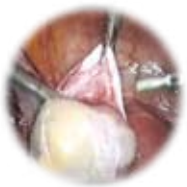
Depending on its localization in the pelvis, endometriosis can be classified as peritoneal, ovarian (endometrioma – chocolate cycts) and endometriosis (or adenomyosis) of the rectovaginal septum.

Vaporization of peritoneal endometriosis using laser CO_2 is the ideal treatment method.

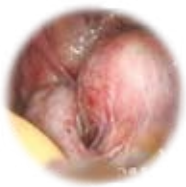
The precision of the method allows the selective removal of the damage and protects neighbouring vital organs and healthy ovarian tissue. Vaporization of peritoneal endometriosis using CO_2 laser is the ideal method of treatment.

Vaporization using SwiftLase has an advantage over laparoscopic cyst removal because it allows larger reserves to remain in the ovary as it removes no healthy ovarian tissue.

For more information you refer to our published study *(Treatment of peritoneal and ovarian endometriosis by using CO_2 laser with or without "SwiftLase", T. Lainas, G. Petsas, I. Stathopoulos, N. Bournas, S. Eliadis, 4th Congress of the European Society for Gynaecological Endoscopy (ESGE) Brussels, Belgium, 6 - 9 Dec 1995)*



Removal of dermoid cyst with laser laparoscopic surgery.



Endometriosis of the rectovaginal septum.

19. Selecting an ART Unit

Objective selection criteria of an ART Unit

A couple's wish to have a baby is an important prerequisite in order to undergo IVF treatment. However, the wish is not enough. It very important that the couples choose the IVF Unit based on objective quality criteria. Which may these criteria be?

The quality characteristics, as well as the criteria the couple use to select an IVF Unit (see diagram), have been published in journals of scientific societies (ESHRE, ASRM), and have been the topic of international congress lectures.

According to the scientific team of "Eugonia", the selection criteria must include:

Scientific excellence

- High pregnancy rates
- High quality and range of services
- Research activity and published studies in international scientific journals of high impact
- Continuous update on recent developments and use of modern methods based on the principles of evidence-based medicine
- Reduction of physical burden by using new protocols of short duration

State of the art infrastructure of staff and equipment

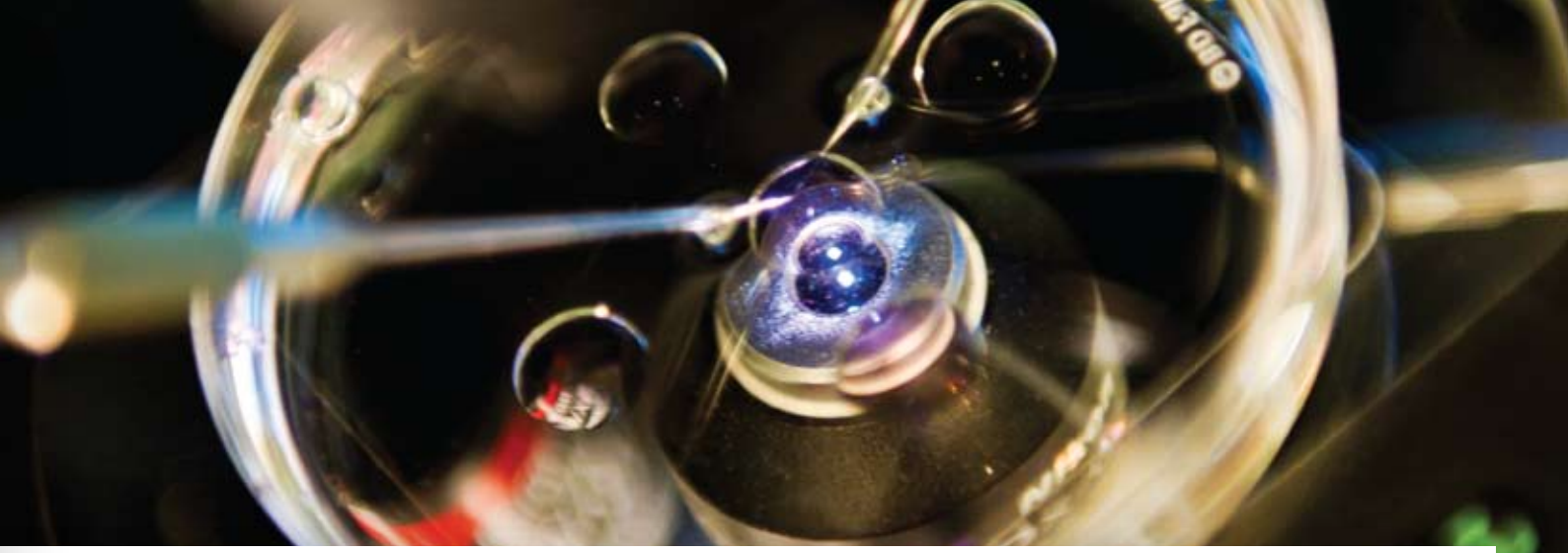
- Scientific staff with deep knowledge and experience,
- Embryology laboratory with modern equipment, adherence to strict cleaning procedures, detailed protocols, and certification by a government authority.
- Creation and constant update of a database
- Quality management certification ISO 9001

Human-centered approach

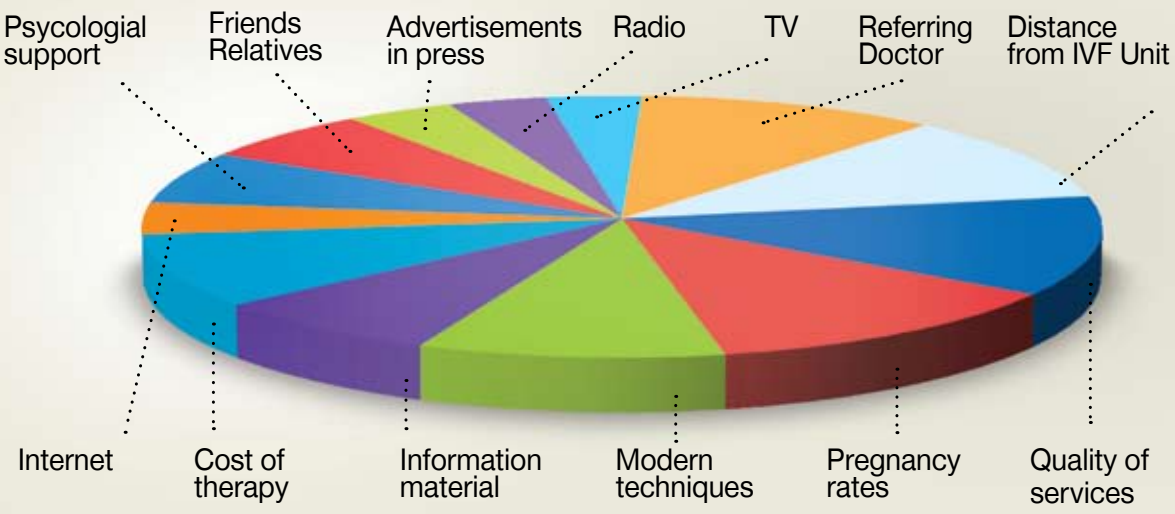
- Individualized treatment
- Direct communication
- Friendly and warm approach
- Psychological support
- Reduction of stress associated with IVF treatment
- Rapid review of the patient's history and initiation of a new treatment cycle in case of a negative result

Reduction of the cost of IVF treatment

At "Eugonia", our attempt to adhere to all the above criteria reflects our high pregnancy rates.



Criteria for IVF Init selection



Data taken from: Marcus 2005, Fertility & Sterility, 83:779

20. Our team

The mission of our scientific and research work is a contribution to the happiness of our patients

Trifon G. Lainas

Obstetrician - Gynecologist
MD, PhD University of Athens

Creator and Director of
"Eugonia" ART Unit

TG Lainas graduated from the University of Athens, and during the 80's served as Senior Registrar at Elena Venizelou maternity hospital.

Following a long path of experience and knowledge, he has developed – as well as a medical- a rich teaching, research and publishing work, with worldwide recognition.

He actively participates to international conferences either with submitted studies or as invited speaker.

Restless spirit, observer, but also creator of scientific developments in his field, he holds a long list of original studies and publications in international scientific journals, and has also written a book entitled "Human Reproduction and In Vitro Fertilization"(in Greek language).

The spectrum of his research work includes protocol optimization in reproductive endocrinology and reproductive surgery.

He is member of international and national scientific societies (ESHRE, ASRM, ESGE, ΕΜΓΕ, ΕΕΓΣ, etc) and has organized and chaired the International Seminar of Reproductive Biology (1992, Athens Megaron Mousikis).

"Eugonia" is the outcome of his scientific path, the Unit with the top pregnancy rates, for couples with infertility problems.

Our research work

The scientific team of "Eugonia", lead by Dr T Lainas, has a rich research work, in the form of published papers in renowned scientific journals or free communications in international and national conferences. A large part of our work is at the stage of preparation or writing.

Published studies in international scientific journals by the team of Eugonia:

Lainas, T., Petsas, G., Stavropoulou, G., Alexopoulou, E., Iliadis, G. & Minaretzis, D. (2002) **Administration of methylprednisolone to prevent severe ovarian hyperstimulation syndrome in patients undergoing in vitro fertilization.** *Fertil Steril 78, 529-33.*

Lainas, T., Zorzovilis, I., Petsas, G., Alexopoulou, E., Lainas, G. & Ioakimidis, T. (2004) **Osseous metaplasia: case report and review.** *Fertil Steril 82, 1433-5.*

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Lainas, T. G., Sfontouris, I. A., Zorzovilis, I. Z., Petsas, G. K., Lainas, G. T. & Kolibianakis, E. M. (2007b) **Management of severe early ovarian hyperstimulation syndrome by re-initiation of GnRH antagonist.** *Reprod Biomed Online 15, 408-12.*

Lainas, T. G., Sfontouris, I. A., Papanikolaou, E. G., Zorzovilis, J. Z., Petsas, G. K., Lainas, G. T. & Kolibianakis, E. M. (2008) **Flexible GnRH antagonist versus flare-up GnRH agonist protocol in poor responders treated by IVF: a randomized controlled trial.** *Hum Reprod 23, 1355-8.*

Lainas, T. G., Sfontouris, I. A., Zorzovilis, I. Z., Petsas, G. K., Lainas, G. T., Alexopoulou, E. & Kolibianakis, E. M. (2009a) **Live births after management of severe OHSS by GnRH antagonist administration in the luteal phase.** *Reprod Biomed Online 19, 789-95.*

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Lainas, T. G., Sfontouris, I. A., Zorzovilis, I. Z., Petsas, G. K., Lainas, G. T., Alexopoulou, E. & Kolibianakis, E. M. (2010) **Flexible GnRH antagonist protocol versus GnRH agonist long protocol in patients with polycystic ovary syndrome treated for IVF: a prospective randomised controlled trial (RCT).** *Hum Reprod 25, 683-9.*

Lainas, G., Kolibianakis, E., Sfontouris, I., Zorzovilis, I., Petsas, G., Tarlatzi, T., Tarlatzis, B. & Lainas, T. (2012) **Outpatient management of severe early OHSS by administration of GnRH antagonist in the luteal phase: an observational cohort study.** *Reproductive Biology and Endocrinology 10, 69.*

Meet our team 20.1

Eugonia is based on the scientific soundness and effectiveness of its team, but also on the personal scientific soundness of its staff of all specialties.

The scientific staff is not e mere observer of modern scientific developments. They participate in their creation, with their research work and their published original studies in international scientific journals.

Eugonia executes its mission with complete and full services at all levels.

For this reason, Eugonia is staffed by:

- Clinicians-gynecologists with long experience in all fields of investigation and treatment of infertility, specializing in IVF, reproductive endocrinology and surgery, hysteroscopic and laser laparoscopic surgery.
- Embryologists with long experience at the best IVF Units abroad, and specialized in new techniques.
- Nurses specialized in IVF and long experience in the field.
- Experienced anesthetists
- Psychologist with postgraduate training
- Administration staff and receptionists with a high level of professionalism
- Our staff speak English fluently, but also other languages, like Russian, French, Italian.

Trifon G. Lainas
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Director of the Unit
Doctorate University of Athens

Ioannis Zorzovilis
Obstetrician-Gynecologist
MD

George Petsas
Obstetrician-Gynecologist
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Konstantina Anagnostara
Clinical Embryologist
M.Med.Sci

Georgia Stavropoulou
Head nurse

Ioanna Voulgari
Midwife

Marina Panagopoulou
Midwife

Elvira Giouvani
Midwife

Kaiti Mavragani
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Argyro Carousou
Administration,
Reception

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20.1 Meet our team

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ΔΕΞΙΟΥ ΔΕΙΚΤΗ ΜΗΤΕΡΑΣ

ΑΠΟΤΥΠΩΜΑ
ΔΕΞΙΟΥ ΠΕΛΜΑΤΟΣ ΝΕΟΓΝΟΥ

ΕΙΔΟΣ ΤΟΚΕΤΟΥ

ΝΕΟΓΝΟ
ΦΥΛΟ

ΜΑΙΕΥΤΗΡΑΣ

ΟΝΟΜΑΤΕΠΩΝΥΜΟ

ΗΜΕΡΟΜΗΝΙΑ ΓΕΝΝΗΣΗΣ

ΩΡΑ

ΣΥΜΠΛΗΡΩΝΕΤΑΙ ΣΤΗ Μ.Ε.Ν.Ν.

ΥΠΟΓΡΑΦΗ
ΚΑΙ ΟΛΟΓΡΑΦΟ ΟΝΟΜΑ
ΠΟΥ ΠΑΡΙΣΤΑΤΟ ΣΤΟΝ ΤΟΚΕΤΟ
ΚΑΙ ΠΗΡΕ ΤΑ ΑΠΟΤΥΠΩΜΑΤΑ