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Foreword

This guide provides simple and essential information about the complex world of Assisted Reproductive Technology (ART), which includes various techniques designed to help couples overcome infertility issues.

Having preliminary information about medical procedures can help you face the various stages of treatment with greater awareness and reduce anxiety and stress¹ (ESHRE, 2015).

The National ART Register, established by Law No. 40/2004 at the National Institute of Health, collects data on all the ART cycles performed in Italy each year. This surveillance system collects "aggregate data" from each ART center, providing information on the accessibility, effectiveness, and safety of ART application.

The data presented in this brochure regard ART treatments carried out in 2020. They are the most recent data currently available, considering the observation time elapsing from the ART treatment to pregnancy onset, term, and the birth of a child.

Since data on ART application and its results change slightly each year, the figures provided can be considered up-to-date and effective in depicting the overall scenario.

¹ Gameiro S, Boivin J, Dancet E, de Klerk C, Emery M, Lewis-Jones C, Thorn P, Van den Broeck U, Venetis C, Verhaak CM, Wischmann T, Vermeulen N. ESHRE guideline: routine psychosocial care in infertility and medically assisted reproduction-a guide for fertility staff. Hum Reprod 2015 Nov;30(11):2476-85. doi: 10.1093/humrep/dev177. Epub 2015 Sep 7.

1. Who can access ART treatments?

In our country, ART is regulated by Law 40/2004, its Guidelines and by the subsequent amendments introduced by four rulings of the Constitutional Court.

Access to ART is restricted to couples who are adults, of the opposite sex, either married or cohabiting, of childbearing age and with living partners.

Access to ART is possible in the following cases:

- When it has been ascertained that it is impossible to remove the causes preventing procreation by any other means.
- In cases of infertility or sterility resulting from a cause that has been ascertained and certified by a medical report.
- In cases where a fertile couple carries transmissible genetic diseases that require preimplantation genetic diagnosis of the embryo (see Constitutional Court ruling no. 96/2015).
- In cases where one of the partners in a fertile couple is a carrier of sexually transmitted viral diseases such as AIDS or hepatitis B and C, in order to prevent transmission to their partner.



2. What is allowed and what is not allowed in Italy?



- Performing ART with the couple's own gametes (sperm and egg).
- Performing ART cycles with donor gametes, either female (oocytes) or male (semen), or with double donation, meaning both donor gametes in the same treatment cycle.
- Performing preimplantation genetic diagnosis.
- In certain specific cases, performing cycles with cryopreservation of embryos. Surplus embryos can be frozen for later use if their transfer is not conducive to procreation or is not in the best interest of the patient's health (Constitutional Court Ruling no. 151/2009).



NOT allowed

- Applying ART techniques to same-sex couples or single individuals.
- Carrying a pregnancy on behalf of others (surrogacy).
- Donating embryos.
- Fertilizing oocytes with sperm of a deceased partner.

3. What is ART and how does it work?

Assisted Reproductive Technology (ART) refers to a set of highly specialized and complex fertility treatments that can help couples who have difficulty conceiving. ART can offer a solution to a couple's infertility problem:

- in cases where pregnancy is not established after a given time (after 12 months or even before for women over 35 years of age);
- when treatment alternatives have failed or are not feasible.

N.B. The experience of infertility has an impact on the psychophysical health of individuals and couples, in terms of anxiety, psychosocial stress, frustration, marital difficulties, etc. Resorting to ART may be a demanding process, so it would be advisable to seek psychological support while undergoing treatments. To access this type of service, ask your ART Center's staff for information at the beginning of your journey.

In Italy, the application of ART is regulated by Law 40/2004, which classifies the techniques used into three levels: I, II and III. Level I techniques are simpler and less invasive, as fertilization (the union of the female egg with the male sperm) takes place within the female genital tract. They are not considered as assisted reproductive techniques in other countries. Level II and III techniques, on the other hand, are more complex, as fertilization takes place *in vitro*, that is, outside the female reproductive system.



3.1 Level I treatment: intrauterine insemination (IUI)

Intrauterine Insemination (IUI) is the simplest technique of Assisted Reproduction. It is a procedure that increases the chances of conception by introducing previously treated semen into the female body to facilitate the encounter between sperm and oocyte. IUI can be performed during a natural menstrual cycle, without pharmacological treatment or with moderate hormonal stimulation. In the latter case, the woman begins hormone therapy with subcutaneous injections to stimulate the ovaries to produce a greater number of follicles (future oocytes). After a few days, the woman undergoes a series of ultrasound checks and blood tests to monitor the progress of ovulation during treatment and measure the concentration of certain hormones. When the follicles (the "containers" of the oocytes) have reached the optimal number and size, insemination occurs. A sample of semen is collected, treated in the laboratory, and then deposited into the woman's uterus with a soft and small catheter, introduced through the vagina.

Intrauterine insemination is performed on an outpatient basis and does not require anesthesia.

After the insemination, a blood test is typically done after about fifteen days to verify whether a pregnancy has been achieved (measurement of the Beta fraction of chorionic gonadotropin "Beta hCG", which increases in the presence of pregnancy). This technique is especially useful when sperms have difficulty reaching the Fallopian tubes. It requires that the tubes connecting the uterus to the ovaries are accessible, which can be verified beforehand with a hysterosalpingography, and that the seminal fluid has good characteristics.

In Italy, intrauterine insemination can be performed using either the partner's semen or of an anonymous donor.







3.2 Level II and III treatments

In Level II and III treatments, the meeting between the oocyte and the sperm, or fertilization, takes place outside the woman's body in the laboratory. This is why the process is called *in vitro* fertilization. The main difference between the two levels lies in the type of anesthesia required. Level II techniques are procedures that can be performed with local anesthesia and/or deep sedation while level III technologies require general anesthesia with intubation.

Here are the main phases of these treatments

A. Ovarian stimulation

During this phase, the woman undergoes a personalized hormonal stimulation treatment, which is determined by the medical team based on her specific clinical condition. Daily subcutaneous injections of stimulation drugs are administered with the aim of maturing multiple follicles in the ovary (normally only one is produced in a spontaneous cycle) to obtain more oocytes for fertilization. The growth of the follicles is monitored through ultrasound scans and blood tests to measure hormone levels until the presumed ovulation is reached and the egg retrieval procedure can be planned.



B. Egg retrieval (pick-up) and in vitro fertilization

Once the follicles have reached an optimal number and size, they are surgically retrieved from the ovary by needle aspiration. This procedure is performed while the woman is under local anesthesia and/or deep sedation. Using ultrasound guidance, a very thin needle is inserted through the vagina to reach the ovaries. In the meantime, the male partner's seminal fluid is collected.



In the presence of serious factors of male infertility, where there is not an adequate number of spermatozoa in the seminal fluid, the technology may include the procedures for their recovery. These procedures can be surgical (TESE), microsurgical (MESA; m-TESE) or performed by needle aspiration (TESA, TEFNA, PESA).

• **TESE**: is a biopsy procedure of testicular tissue that retrieves spermatozoa from a small, surgically removed piece of tissue from the testicle. When performed with microsurgical technique, it is referred to as m-TESE.

- **MESA**: is a microsurgical procedure that retrieves spermatozoa through microaspiration from the epididymis, a channel connected to the testicle.
- **TESA e TeFNA**: involve sperm aspiration from the testicle through the use of a thin needle connected to a syringe.
- **PESA**: is a transcutaneous needle aspiration procedure that retrieves spermatozoa from the epididymis.

These procedures can be performed under local anesthesia or deep sedation.

Once the female oocytes and male spermatozoa have been obtained, there are two options for fertilization in the laboratory, with ICSI used exclusively for severe male infertility:

• **IVF** (*In Vitro Fertilization with Embryo-Transfer*). Viable oocytes and sperm obtained following treatment are placed together in a culture broth or liquid medium in a Petri dish to allow them to meet and the sperm to spontaneously penetrate the female gamete to enable fertilization. They are then left in an incubator for several hours and are constantly monitored for cell replication and development, which can result in the creation of one or more embryos.

• ICSI (Intra Cytoplasmatic Sperm Injection). This method is indicated for severe male infertility, previous IVF failures, or cases with a limited number of oocytes available for insemination. In these cases, chances of fertilization are improved, as the sperm is directly injected into the oocyte using a small needle. Following this, the fertilized oocytes can develop into embryos over the next 2-3 days and can then be transferred to the uterus.



In vitro fertilization can be performed using freshly removed oocytes in most cycles ("fresh" procedures), or with previously collected and cryopreserved oocytes ("freeze and thaw" procedures).





C. Embryo transfer to the uterus

This is a simple, quick, and painless procedure. The gynecologist inserts a thin catheter though the vagina to deposit the embryos into the uterus. At this point, the embryos can implant themselves into the endometrial wall, the inner lining of the uterus. After approximately two weeks, a blood test is performed to measure the levels of Beta hCG to determine whether the embryo has implanted and confirm pregnancy.



Embryo formation using level II and III technology can be carried out with various options such as:

- Fertilization without gamete donation by using the oocytes and seminal fluid of the couple's partners .
- Fertilization with egg donation using the oocytes of a donor and seminal fluid of the male partner.
- Fertilization with sperm donation using the oocytes of the female partner and seminal fluid of a donor.
- Fertilization with double donation using oocytes and seminal fluid from two donors.

These transfers can be carried out using either newly formed embryos in "fresh" cycles or cryopreserved embryos that have undergone a previous freezing process at very low temperatures, usually at 196 ° C, known as "freeze and thaw" cycles









The cryopreservation of embryos is a technique developed to optimize ART processes, increasing the chances of pregnancy in treatment cycles while avoiding the need for repeated ovarian stimulation and related oocyte retrieval surgeries.

FER (*Frozen Embryo Replacement*): involves the transfer of cryopreserved embryos to the uterus. The embryos are cryopreserved in liquid nitrogen and transferred to the uterus after a fresh cycle if pregnancy is not achieved, or they are used as a therapeutic option by deciding not to transfer any fresh embryos and postponing the transfer to a later cycle (known as the "*freeze all*" strategy). Subsequent transfers with cryopreserved embryos do not require further ovarian stimulation or egg retrieval procedures for the woman.



FO (*Frozen Oocyte*): involves the transfer of embryos obtained from cryopreserved oocytes. The oocytes are frozen after collection and can be used for future fertilization attempts without the need for additional hormonal stimulation. This technique is less commonly used today.



Cryopreservation of seminal fluid is a technique used to freeze and store spermatozoa while maintaining their viability. This technique is highly effective and has been perfected over the years.







3.4 Preimplantation Genetic Testing (PGT)

For couples who may be at risk of transmitting a genetic disease or chromosomal abnormality, or for those who have experienced multiple implantation failures or are of advanced maternal age, genetic testing can be performed on embryos before they are transferred to the uterus. This is allowed under Article 14, paragraph 5 of Law 40/2004, which permits couples to be informed about the health status of their embryos produced through *in vitro* fertilization. Preimplantation genetic testing can detect the presence of hereditary genetic diseases or chromosomal abnormalities in embryos at very early stages of development before they are transferred to the uterus.

There are different types of preimplantation genetic testing available:

- **PGT-M**, which detects single-gene disorders or specific genetic diseases that may affect or be carried by the intended parents.
- **PGT-A**, which detects numerical chromosomal abnormalities (aneuploidies).
- **PGT-SR**, which detects structural abnormalities in the embryo's chromosomes. It is typically recommended when one or both of the intended parents are carriers of a chromosomal translocation.



4. Where can I undergo ART treatments in Italy and how can I find the services?

In all of Italy, special centers called ART centers provide Assisted Reproductive Technology services. You can find a list of authorized ART centers on the website of the ART National Register of the National Institute of Health: <u>www.iss.it/rpma-centri-italiani</u>. By selecting the region of your interest, you can get the list with all up-to-date information about the centers active in your area.

There are three different levels of centers, depending on the techniques applied.

- Level I centers only use Intrauterine Insemination (IUI) with gametes from the couple or from a donor. They also offer the option of cryopreserving male gametes.
- Level II and III centers provide more advanced techniques with in vitro fertilization protocols (IVF and ICSI) in addition to IUI technology. These centers also offer surgical sperm retrieval techniques and cryopreservation of male and female gametes (FO) and embryos (FER). The techniques can be performed with gametes from the couple or from donors. Level II and III centers differ in the type of anesthesia they use during the procedures.

The National ART Register maintains a list of all public, private, and private affiliated centers authorized by the Regions to conduct ART procedures, and it collects data on all the treatments performed, their effectiveness, safety, and outcomes.



By accessing the National ART Register website <u>www.iss.it/rpma</u>, you can see which techniques are offered at each center (IUI, IVF, ICSI) and whether preimplantation genetic testing is performed. Additionally, you can find out if these centers provide treatments with donor gametes, cryopreservation of oocytes (FO), spermatozoa and embryos (FER) as well as services for couples who are HIV-positive or HbsAg and HCV-positive. Please note that this information is self-declared by ART centers and therefore subject to variations.

Access criteria for ART cycles reimbursed by the National Health Service (SSN) can differ slightly across the regions based on the age of the woman receiving treatment and the number of treatments provided. Currently, the availability of ART treatments varies from one region to another. Figure 1 below shows the number of cycles per million inhabitants performed in 2020, which can help evaluate the supply of ART cycles in a particular region. The different colors show that some regions offer an adequate number of cycles (>1.500 per million inhabitants), while others offer an insufficient number of cycles.

In case you wish to receive ART treatments covered by the NHS at public or NHS-contracted private centers outside your region of residence you need to:

- ensure that you meet the requirements established by the current regional legislation, such as not exceeding the age limit and the number of ARTs reimbursable by the NHS.
- contact your Local Health Authority to obtain a permit that guarantees financial coverage for the treatment.

Figure 1. Number of cycles performed per million inhabitants in the single Italian Regions. 2020 data.







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5. How can I access ART treatments covered by the National Health Service (NHS)?

Pursuant to the Prime Ministerial Decree (DPCM) of 12 January 2017, all services related to ART have been included in the LEA (Essential Levels of Assistance) provided by the National Health System.

There are differences among the Italian regions regarding the following aspects of ART services:

- Age limits for accessing benefits.
- Number of services that are reimbursable.
- Waiting times.
- Costs incurred by the couple.
- Procedures for the provision of services.

For cycles with gamete donation

ART with gamete donation was legalized in Italy in 2014 after a Constitutional Court ruling (no. 164)².

Regarding the costs of ART services with donor gametes, almost all the Regions have adopted the conventional rate established by the State-Regions-Autonomous Provinces Conference of Trento and Bolzano 14/121 / CR7c / C7 of 25 September 2014.

²Constitutional Court Judgment no. 162 Year 2014. Gazzetta Ufficiale Serie Speciale n. 26 del 18/06/2014)

To learn more about the costs and methods for accessing ART treatments with or without gamete donation, we recommend that you contact your Local Health Authority and/or the Health Department of your region of residence.

6. What are the main outcomes of ART in Italy and how can we interpret them?

When we look at the process of assisted reproductive technology (ART) and its outcomes, including the potential situations couples may experience from the start of treatment to the moment they bring a child home, we are considering a repeated series of procedures involving the use of technology with fresh embryos and cryopreserved embryos. Thanks to advancements in cryopreservation technology and its efficiency, couples can undergo multiple cycles of ART treatments with a single ovarian stimulation and egg retrieval. They can start a with a fresh embryo transfer during the first cycle and then use cryopreserved embryos for subsequent cycles. The number of pregnancies resulting from all treatment cycles is expressed as the cumulative pregnancy rate. In order to maximize the likelihood of success, it is important to continue the treatments until all the biological material generated (oocytes and/or embryos) is utilized.

Young age is a determining factor in the success of an Assisted Reproduction Technology cycle.



In Italy in 2020, the average age of women undergoing fresh ART cycles was 36.9 years. This figure has steadily grown over the years and is the highest compared to other European countries. When starting with a fresh cycle, the percentage of successful pregnancies varies from 18.1% for women under 34 years of age to 4.5% for those aged 43 years or older.

If a sufficient number of oocytes are obtained through ovarian stimulation (which is heavily influenced by the woman's age), they can be cryopreserved and / or fertilized to create embryos for immediate transfer or to be cryopreserved for subsequent cycles. Cryopreservation allows for a new cycle to be started without undergoing further ovarian stimulation and egg retrieval. In this case, by considering not only fresh cycles but also freeze and thaw cycles, the likelihood of achieving a pregnancy (expressed as a percentage) increases compared to only using a fresh cycle. This percentage, known the cumulative pregnancy rate, takes into account all the pregnancies achieved from both fresh cycles and thawed cycles.

The main risks associated with the application of Level II and III ART are basically linked to the occurrence of twin or multiple pregnancies, which increase the likelihood of complications for both the mother (gestosis, diabetes, premature detachment of the placenta, etc.) and the child (prematurity and low birth weight, respiratory failure, delayed psychomotor development). Thus, reducing the rate of the twin and multiple pregnancies with ART is a crucial strategy for protecting the health of both the mother and child. To this end, the ART operator evaluates the number of embryos to be transferred to the uterus in order to increase the chances of a successful pregnancy while balancing the risk of twin or multiple pregnancies. Typically, the number of embryos to be transferred is determined based several factors, such as the couple's characteristics, the type of infertility, the woman's age (with younger women having a higher risk of twin pregnancies according to the number of embryos transferred), the quality of the gametes and embryos, and the number of previous treatments. In most European Union countries, transferring a single embryo represents the preferred approach in order to achieve safe and satisfactory success rates and thus meet the primary goal of ART: the birth of a single healthy child³ (ESHRE, 2021).

Moreover, some studies from the international literature suggest that individuals born from ART may have a higher risk of abnormalities, malformations, and neonatal pathologies compared to those born in the general population^{4,5} (ESHRE, 2015; Sutcliffe & Ludwig, 2007).

³ ESHRE Clinic PI Working Group, Veljko Vlaisavljevic, Susanna Apter, Antonio Capalbo, Arianna D'Angelo, Luca Gianaroli, Georg Griesinger, Efstratios M Kolibianakis, George Lainas, Tonko Mardesic, Tatjana Motrenko, Sari Pelkonen, Daniela Romualdi, Nathalie Vermeulen, Kelly Tilleman, The Maribor consensus: report of an expert meeting on the development of performance indicators for clinical practice in ART. Human Reproduction Open 2021;2021(3):hoab022, https://doi.org/10.1093/hropen/hoab022

⁴ ESHRE Capri Workshop Group. Birth defects and congenital health risks in children conceived through assisted reproduction technology (ART): a meeting report. J Assist Reprod Genet. 2014 Aug;31(8):947-58. doi: 10.1007/s10815-014-0255-7. Epub 2014 May 29. Erratum in: J Assist Reprod Genet. 2015 Sep;32(9):1429. PMID: 24870703; PMCID: PMC4130939.

⁵ Sutcliffe AG, Ludwig M. Outcome of assisted reproduction. Lancet. 2007 Jul 28; 370(9584):351-9. doi: 10.1016/S0140-6736(07)60456-5. PMID: 17662884.



Assessing the risk of abnormalities in children born from ART is a very complex process that takes several factors into account, including maternal age older than the average of the general population and presence of infertility-related pathologies in one or both partners. The international literature currently lacks clarity on whether the increased risk found in some studies is linked to the specific population of infertile couples or the application of ART procedures. Some investigations suggest that children conceived with the ICSI technique have a higher risk of birth defects and chromosomal abnormalities when compared to those conceived spontaneously or through IVF, but this is more likely due to the altered quality of seminal parameters, particularly in cases of severe male infertility, rather than to the ICSI technique itself⁶.

N.B. To properly interpret the results, it is important to understand that pregnancy rates can be calculated at different stages of the ART process, such as at the start of treatment, during egg retrieval, or at the time of embryo transfer. As a result, the pregnancy rates will vary across the different stages of the process and will typically be higher at the end of the process, that is, at the time of embryo transfer.

⁶ Lacamara C, Ortega C, Villa S, Pommer R, Schwarze JE. Are children born from singleton pregnancies conceived by ICSI at increased risk for congenital malformations when compared to children conceived naturally? A systematic review and meta-analysis. JBRA Assisted Reproduction 2017;21(3):251–259. <u>https://doi.org/10.5935/1518-0557.20170047</u>

6.1 Results of ART with gametes from the couple





















6.2 Results of ART with donor gametes

In Italy, the ban on the use of gamete donation techniques mandated by Law no. 40/2004 was eliminated by Ruling no. 162 of the Constitutional Court⁷ in 2014, thereby introducing the option of using ART with egg donation, sperm donation, and double donation (eggs and sperm). Among these techniques, egg donation is the most commonly used approach in the context of ART with gamete donation.

⁷Constitutional Court Judgment no. 162 Year 2014. Gazzetta Ufficiale Serie Speciale n. 26 del 18/06/2014

While most European countries allow the use of ART with gamete donation, there are discrepancies in the types of donation that are permitted across different countries. These differences may be influenced by various factors, such as financial issues relating to the healthcare system (e.g., accessibility and treatment expenses) or cultural considerations (social acceptance, customary laws, and religious beliefs within the community)

ART procedures involving gamete donation may include:

- fertilization with egg donation, which involves the use of oocytes from a donor and seminal fluid from the partner.
- fertilization with sperm donation, which involves the use of oocytes from the partner and seminal fluid from a donor.
- fertilization with double donation, which utilizes oocytes and seminal fluid from two separate donors

Currently, most European countries allow sperm donation, with the exceptions of Bosnia and Herzegovina and Turkey. Egg donation is permitted in almost all European countries, with the exceptions of Bosnia and Herzegovina, Germany, Turkey, and Switzerland (although the law is presently being amended in the latter case). Double sperm and egg donation is not allowed in countries where egg donation is prohibited and also in Armenia, Croatia, France, Montenegro, Slovenia, and Sweden.











PERCENTAGE OF PRETERM BABIES (BORN BEFORE 37 WEEKS OF GESTATION) PER TYPE OF PREGNANCY ACHIEVED THROUGH ART WITH DONOR GAMETES IN 2020





7. What is the commitment of the National ART Register for the near future?

The National ART Register is committed to enhancing its ways of communicating ART outcomes to couples seeking to have a child to help them make informed decisions. The Register will strengthen its data collection system and improve the processing and presentation of results achieved by Italian ART centers. As a result, there will be a better understanding of ART and couples with specific reproductive problems will receive targeted information.

To achieve this goal, the National ART Register is working in collaboration with the Ministry of Health on the project "Enhancement of data collection on single cycles of Assisted Reproduction Technology (ART) in order to consolidate the surveillance system of the National ART Register (Ministry of Health- National Institute of Health, National ART Register)".

By enhancing its data collection system, the National ART Register will be able to provide more detailed and transparent information as well as guidance to both patients and healthcare professionals. It will also be able to conduct investigations that have a high epidemiological impact, and it will play a greater role in the dissemination of accurate and specific information for the study of infertility and its treatments. The detailed information that can be obtained by implementing the current data collection system is shown below through a simple example.

On the left-hand side of Figure 2 below, you can see the information obtained today regarding the use of the FER technique (*Frozen Embryo Replacement* - Transfer of cryopreserved embryo into the uterus) in women aged between 36 and 40 years. The pregnancy rate calculated based on the transfers performed is 37%.

On the right-hand side of Figure 2, you can see that by collecting data on each treatment cycle, specific pregnancy rates can be obtained for each age group based on the transfers performed. By including also other pertinent information, such as the ovarian reserve of a woman, it is possible to evaluate the extent to which that factor may affect her chances of getting pregnant. As can be seen in the second line, for a 37-year-old woman with diminished ovarian reserve, the pregnancy rate drops from 38% to 33%.

By entering certain data of the couple such as age, infertility factors, ovarian reserve evaluation, previous pregnancies and children, number of attempts with ART, and the time spent trying to get pregnant, the probability of achieving a successful pregnancy can be accurately calculated for that specific couple.





Figure 2. Pregnancy rates calculated with the current data collection system vs pregnancy rates calculated with the new single-cycle data collection system for women aged 36 to 40 years undergoing FER (*Frozen Embryo Replacement*)





National ART Register of the National Institute of Health

website www.iss.it/rpma



Ministry of Health website

www.salute.gov.it



ESHRE (European Society of Human Reproduction and Embryology) website

www.eshre.eu







ICMART (International Committee for Monitoring Assisted Reproductive Technologies) website

www.icmartivf.org



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