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We are not alone!
MICROBIOTA IN THE TIMES OF COVID

INFERTILITY,
a psychological challenge

WHAT IS THE IMPLANTATION RATE OF MY EMBRYO?

Editorial

Dr. José Jesús López Gálvez
CEO of the UR Group

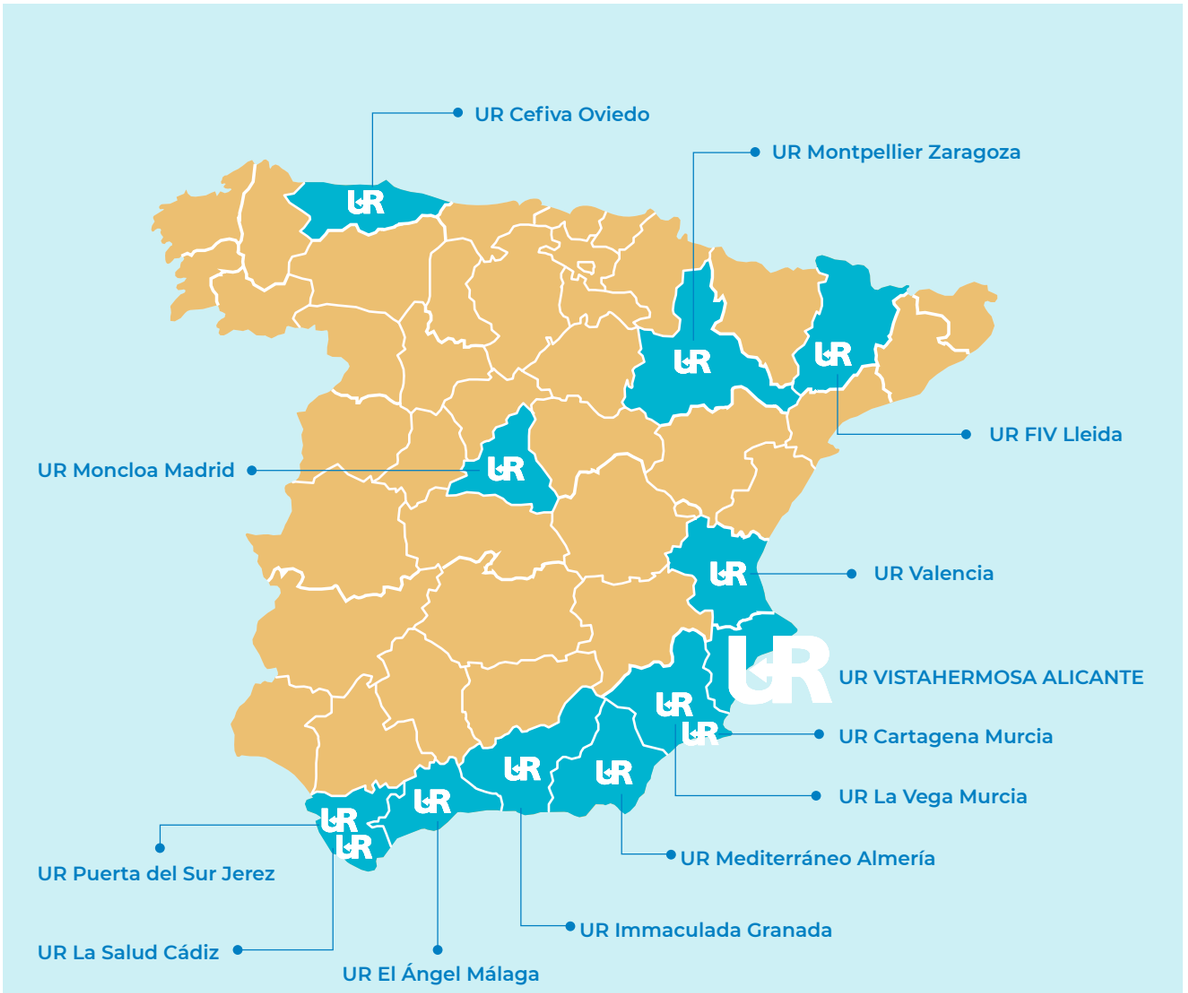
Dear Readers, once again we are pleased to share with all of you the most interesting issues in the world of fertility and the main initiatives carried out in UR Group and all its reproduction units in this last issue of the magazine at the end of 2021.

The first thing I'd like to highlight and let you know about is **Grupo Internacional UR's III Seminars** held last October in Madrid. At the seminar, we were able to reflect on these new post-COVID times we are living in and what they mean for us and for our patients.

Firstly, as we are still living with the pandemic, we continue to apply all the biological safety standards that we are already used to following in each of our clinics, units and centres. We are encouraging **on-line interactions** with our patients (and internally as well, among each unit's own team) and applying new financing advantages adapted in the best way possible to the current economic situation, so that most of our patients and contacts can realize their dream: having a healthy baby at home. We continue to seek the opportunity of achieving the healthiest embryo possible by applying new technologies, always in the most personalised and best way possible for each case. This is because we aspire only to fulfil what has become the motto for each of our units: our guarantee is your pregnancy.

And with all these challenges, difficulties and the new post-COVID reality, the group has continued seek excellence and the best care; in the last year, it has strengthened its position with **new units** and with growth in all areas, facilities and services. Our patients have found even **better answers** to their fertility treatment with more research, more teaching, more technological resources, more experts in each area of reproduction, experience and quality in processes, greater personalised attention and better results and success rates for each case.

I'd just like to close this year by saying that our response to this current enormous challenge is the total commitment of the company and of all the people who are part of it to solve our patients' fertility problems.



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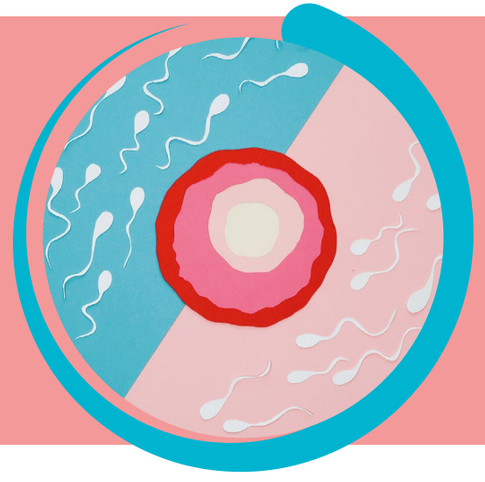
Index

DNA fragmentation in sperm



The importance of semen analysis

Arlén Hernández
Embryologist
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The initial assessment of infertile couples must include a semen analysis, since the male factor accounts for half of infertility cases. A semen analysis looks mainly at the macroscopic, microscopic and biochemical characteristics of the ejaculate in accordance with World Health Organization recommendations.

The total number of sperm in the semen is a reflection of their production in the testicles and the permeability of the post-testicular ducts. The microscopic features analysed include the **concentration, motility and morphology** of the sperm. Nonetheless, none of these parameters is, on its own, a powerful predictor of infertility.

Efforts to find the ideal marker to differentiate infertile men from fertile men and to predict the possibility of achieving pregnancy have currently intensified.

Despite this, the cause of infertility cannot be determined in **30-50%** of infertile males, who are classified as men with idiopathic infertility. Clinical evidence in assisted reproduction suggests that failure in fertilisation or embryo development may be the result of an alteration in the sperm components mentioned above. However, these elements are not assessed in the conventional semen analysis. Sperm that are damaged or have DNA fragmentation are able to fertilise, and that damage may persist even after fertilisation takes place.

During the in-vivo fertilisation process, the female reproductive apparatus has **mechanisms against the selection** of DNA-damaged sperm. This feature does not exist with in vitro fertilisation (IVF) or intracytoplasmic sperm injection.

It has been proposed that the main origin of the fragmentation of sperm DNA is **oxidative stress**. This molecular event occurs when there is an imbalance between the formation of reactive oxygen species and the antioxidant defence. Several research projects have shown the relationship between a **lower antioxidant capacity and high levels of oxidative stress** in semen samples. In addition, other studies point to this relationship with DNA-damaged sperm.

This examination consists of finding the breaks or injuries in the genetic material of the sperm. The higher the number of injuries, the lower the integrity of the genetic material, thus reducing the pregnancy rate.

Scientific evidence points to the fact that the fragmentation of sperm DNA has a significant impact on natural and assisted reproduction. There is also a strong association between the fragmentation of sperm DNA and early pregnancy loss. Therefore, the **analysis of the integrity of sperm DNA** should be included in the male fertility assessment protocol.

The sperm DNA fragmentation test is recommended in the following cases:

- Infertility of unknown (**idiopathic**) cause.
- **After repeated failures** in assisted reproduction techniques
- Cases where **poor embryo quality** has been observed
- Patients who have suffered **repeated miscarriages**.
- **Varicocele**.
- Cases of **frozen semen** (a check is made to see
- whether the frozen sample has acceptable levels of fragmentation)
- **Episodes of fever** in the past three months.

With regard to assisted reproduction treatments, it has been shown that sperm capacitation techniques – especially density gradients – are effective in elimination many of the DNA-damaged sperm.

If the fragmentation index is low, **the egg may be able to repair this damage** on its own after fertilisation. We do not have a clear understanding of this mechanism, but it does depend largely on the type of injuries and on the quality of the egg.

Some antioxidants that improve semen quality include **vitamins C, E and A**. These are naturally found in fruits, vegetables, vegetable oils, brown rice, soybeans, chocolate, oregano, tea, etc. Treatment with antioxidants for two or three months may reduce the fragmentation of sperm DNA by up to **20%**.

We are not alone!

Microbiota in the times of COVID

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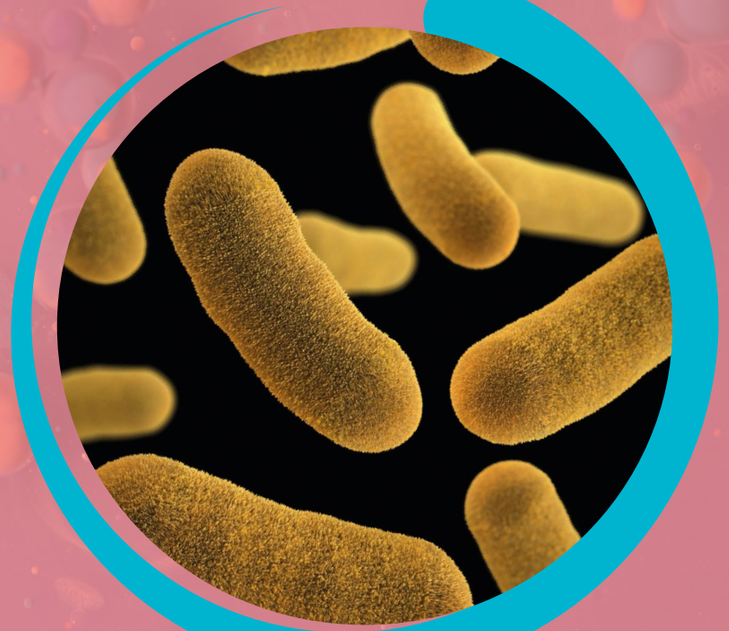

My mother's advice during my childhood is now coming to mind. She told me to avoid washing my face with soap because – she said – it would ruin my skin because it wiped away the "natural protective substances" our skin had. That really stuck with me, and, ever since, I have followed this rule almost to the letter. And who knows if, thanks to that, I have a few less wrinkles!

This position had its merit at a time when all germs were considered to be an enemy of the body and should be eliminated because they were potentially pathogenic. So, logically, they had to be wiped out, even using antibiotics, the discovery of which was one of humanity's great milestones.

Today, things have changed, as was shown a few years ago with the techniques of massive microbial DNA sequencing (NGS techniques). We now know that no, we are not alone!

> This is so because there are as many bacterial cells existing in our bodies as the number of cells we have. In other words, 50% of our body's cells are microbes – one bacterium per cell, approximately – and this means that between **one and three per cent** of our weight is bacteria. In fact, we carry around a kilo or more of bacteria without even realising it.

We have known for many years that animals and humans are carriers of micro-organisms. Until very recently, these have been treated with great indifference. In fact, it was believed that it was important for health that they did not exist because they posed a threat of causing infections when our defences are low. Now, let's not fall into the COVID trap and return to the old theory of exterminating all "living bugs" audacious enough to get near us.

The microbiota is the set of living microorganisms (bacteria, viruses, fungi and others) that reside symbiotically in our body. These microbial ecosystems are found in the gastrointestinal, genitourinary and reproductive, and respiratory systems, the oral and nasopharyngeal cavity, and the skin.

Until the beginning of this century, we had accepted the dogma that babies were born sterile, and that some organs – such as the uterus – were also sterile. However, using molecular mass sequencing techniques, we now know that the

foetus in the uterus already has microbes. Logically, they come from the mother.

The uterine cavity was classically considered a sterile place, but this dogma has also been disproven in recent years. Now, we are learning more and more about the importance of the **endometrial microbiota** in embryo implantation. The endometrial microbiota is, by the way, different to the vaginal microbiota, which has been studied the most widely due to its easy accessibility. Studies of endometrial microbiota show that uterine implantation is clearly favoured when it is composed of more than **90% Lactobacillus**. This is why we advise a systematic study of it in patients with implantation failure. The trend is to expand the study to more cases and to create personalised treatments.

This means that the endometrial microbiopsy is done via a Pipelle in the office. It is easy to do and there is very little discomfort, and makes it possible to determine the intrauterine microbial DNA using modern NGS techniques.

How does the microbiota act in our bodies?

The most obvious way is the nutritional role of intestinal microbes that are fundamental to the metabolism of multiple substances. The defensive role of certain microorganisms, such as Lactobacillus, in colonising an organ and occupying the space to prevent the proliferation of pathogenic organisms is also becoming more widely known.

The microbiota also acts by modulating the immune system, which is specific to and different in each person. It also has a crucial role in the development of neurons and cognitive functions, through complex communication between the products of the intestinal microbiota and the central nervous system by means of what has been called the "**gut-brain axis**". This means that neurotransmitters produced in the intestine by bacteria, such as GABA, noradrenaline, acetylcholine, etc. can travel through the vagus nerve.

It is becoming increasingly known that changes in the microbiota influence the emergence and development of diseases, such as diarrhoea due to Clostridium difficile, colon cancer, metabolic and neurological diseases, oral health and changes in the genitourinary and reproductive apparatus.

How can the microbiota be manipulated to improve health?

There are two types of strategies, with some modulating the existing microbiota and others aimed at adding new germs to colonise the organism.

1. Strategies that modulate the microbiota:

Dietary changes change the microbiota temporarily, but prolonged personalised dietary changes could be maintained to achieve permanent changes. Avoid the indiscriminate use of antibiotics: they alter the microbiota, so they should be used only in clearly necessary cases in order to prevent resistance. Lastly, **prebiotics**, which are nutrients not digestible by the human digestive system but rather stimulate and encourage the growth and activity of intestinal bacteria. Thus, they do not contain living microorganisms.

2. Strategies for adding new microorganisms:

Such as **probiotics** – food supplements that contain strains of bacteria and living yeasts aimed at colonising the affected organ – that leave no room for pathogens and even annihilate them by carrying bioactive molecules harmful to them. Others are **symbiotics**, which are food supplements that contain a combination of a microorganism (probiotic) along with a carbohydrate (prebiotic) that benefits microbiota growth.

Lastly, another, more novel, strategy is the **faecal microbiota transplant**. This attempts to replace a patient's intestinal microbiota with that from healthy donors with healthy cultures, or even autotransplantation.

However, restoring the microbiota in the event of a disease is much more complicated than we could imagine. This is because millions of interactions between the organism's bacteria and cells take place in the **microbiota**. At the moment, the only treatment that seems truly effective is faecal transplant for treating recurrent Clostridium difficile infections. Some newborns are benefitting from it when other, prior, treatments were ineffective.

The treatment of women with altered endometrial microbiota is also beginning to bear fruit in cases of implantation failure in which a uterine microbiota with a content of less than **90% lactobacillus** has been detected. In these cases, the uterine flora must be replenished by the administration of Lactobacillus of various strains, both orally and vaginally, beginning one or two months before embryo transfer. By the way, we are already beginning to hear about the "endometrial virome" in these COVID times.

In short:

The importance of the endometrial microbiota in embryo implantation

Implantation is clearly favoured when it is composed of more than 90% Lactobacillus

The microbiota is the set of living microorganisms that reside symbiotically in our body.

Fundamental to the metabolism, acts by modulating the immune system and it has a crucial role in the development of neurons

Strategies for manipulating the Microbiota

- *Prolonged personalised dietary changes*
- *Avoid the indiscriminate use of antibiotics*
- *Adding prebiotics, probiotics y symbiotics*
- *Faecal microbiota transplant*

Infertility

A psychological challenge

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Infertility is defined as a disease of the reproductive system characterised by the failure to achieve a clinical pregnancy after **12 or more months** of regular sexual relations without contraception. It creates a **significant psychological burden**, since having a child is one of the key tasks in adult life. This may lead to an emotional crisis rooted in frustration and, in many cases, depression, that may go unnoticed.

It has become increasingly prevalent and now affects **one of every six couples** worldwide, according to data from the European Society of Human Reproduction and Embryology (**ESHRE**). Several studies (Gameiro et al., 2014; Vikstrom, Josefsson, Bladh, and Sydsjo, 2015) have described the psychological impact and the impact on the quality of life of infertility and assisted reproduction techniques.

They have shown a greater incidence of psychological problems decades after infertility treatments such as in **vitro fertilization** (IVF), which are sometimes minimally invasive medical procedures. In addition, psychological factors such as pre-conception stress may increase the risk of infertility. Special mention must be made of women **aged 42 or over** who have had to use techniques with donated eggs due to the fact that the quantity and quality of their eggs decrease with age. Treatment with donated gametes raises concerns in future parents about the implications that the lack of genetic inheritance may have on the parent-child relationship.

Women with a history of depression have a **higher risk of infertility** and are less likely to undergo assisted reproduction treatment. **The psychological state** of patients with infertility **should be assessed**, ideally through questionnaires validated by a mental health professional. In particular, women with a history of anxiety and/or depression should be carefully evaluated before treatment begins, since the level of stress in patients with infertility **tends to increase** as treatment intensifies and as the process continues.

Therefore, patients with in vitro fertilization (IVF) often experience more stress than women who are at the beginning of their infertility assessment.

Many report depressive symptoms before their cycle begins. This is likely to reflect the impact of repeated, fruitless and less invasive forms of treatment, but it may also reflect a previous history of mood/anxiety disorders that are unrelated to infertility.

Most IVF patients report symptoms of **depression, anxiety, anger and isolation** after a failed treatment. Many of these symptoms persist for prolonged periods. Infertility specialists have traditionally assumed that patients leave treatment for only two reasons: **active censorship**, that is, their doctor advises the couple to stop treatment due to poor prognosis, and **economics**, since the cost of the procedure is often not covered by insurance. Nevertheless, this assumption has been questioned, since most patients covered by insurance voluntarily end treatment before completing their allocation of covered cycles, and the main reason for abandonment seems to be the psychological burden of the procedures.

In its guide for mental health professionals, the European Society of Human Reproduction and Embryology (**ESHRE**) highlights the importance of the psychological evaluation in choosing the type of help to provide couples with infertility. It is essential to perform an individualised psychological assessment before the assisted reproduction treatment. This will make it possible to identify those who have a psychological disorder or show emerging symptoms in order to prevent their mental health from further declining.

There are a large number of questionnaires for **assessing the psychological well-being** of patients in general. One of the instruments designed and validated for the population with fertility problems aimed at assessing quality of life is the **Fertility Quality of Life tool** (FertiQol), which has been translated into 38 languages and validated in a number countries.

This questionnaire has been shown to be very capable of identifying aspects of quality of life directly related to infertility. Thus, detecting and preventing psychological dysfunctions and improving the quality of life is part of the multidisciplinary treatment of infertility.

Endometrial preparation

Studies and types of endometrial preparation

In the 21st century

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UR Cartagena

Reproductive techniques have changed greatly since their inception over 30 years ago. We have embryo vitrification available to us, and this has revolutionised our way of working in a very positive way. It is a very safe technique with survival rates that vary from **80 to 95%** depending, primarily, on embryo quality. This technique allows us to do the transfer during the patient's next cycle or whenever she chooses for personal reasons. Once the patient decides to have the embryos transferred, several endometrial preparation options may be suggested.

The objective is to transfer the embryo into the endometrial cavity when the patient's **endometrium is receptive**. This will depend on the embryo's days of life, since embryos can only be implanted on a few specific days. This period is called the "**embryo implantation window**".

Numerous studies have been conducted on which type of preparation is better, but none has been shown any superiority over another. We currently have two types of endometrial preparation: natural cycle and replaced cycle.

The modified natural cycle

Is based on monitoring the patient from the first days of her period by doing various ultrasounds until it is shown that she has a follicle of the right size. From there, ovulation is induced by **medical treatment** (B-hCG or pregnancy hormone) so that, a few days later, vaginal progesterone is started and the endometrium is transformed until the day the embryo transfer is carried out.

This form of preparation is recommended only for patients who have **regular cycles** that are not too long and who do not wish for any medical treatment.

Within the natural cycle, there is a variant that consists of doing exactly the same thing as the modified cycle, but without inducing ovulation by using medical treatment. The patient is monitored only with ultrasounds until we confirm there is a good-sized follicle. From there on, the patient must perform several urine ovulation tests. It is hardly used nowadays, due to the high cancellation rates and the difficulty it has in some patients with irregular cycles.

The substituted cycle

Is based on **monitoring the endometrium** in patients who are treated using low doses, either orally or topically, in the form of ointments or patches. This type of preparation is commonly used due to its high safety, convenience and good results, especially in **egg donation cycles**. This is because of the irregularity and poorer quality of menstrual cycles in women of advanced maternal age.

We have mainly two variables:

The replaced without agonist variable:

Consists of giving the patient low daily doses within the first days of her period and checking via ultrasound whether the thickness and type of endometrium are correct. Once this has been confirmed, **progesterone** is administered and embryo transfer is carried out a few days later.

The substituted with GnRH agonist variable:

Is based on having a substituted cycle with low-doses.

With this variable, it is administered either in the luteal phase of the previous cycle as single agonist dose, or with **five to seven antagonist doses** from the day the patient begins taking the oestrogens.

After **10-14 days of oestrogens**, the endometrium is checked to see whether the endometrium is right.

If so, progesterone administration and embryo transfer are carried depending on the day of life of the embryo, just as in the cycle without an agonist.

Non-invasive preimplantation genetic test:

Can we forget about an embryo biopsy?

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Geneticist
UR HLA Vistahermosa

In recent years, a great deal of progress has been made in the field of reproductive medicine with new strategies emerging aimed at a common goal: to achieve the birth of a healthy baby. These emerging techniques in the biomedical field include the non-invasive **preimplantation genetic test (PGT)**. Its purpose is to obtain genetic information from the embryo in the least harmful way possible before it is transferred to the maternal uterus.

To do this, the first step is to carry out the technique of in vitro fertilisation (IVF), which consists of combining the oocyte and the sperm in the laboratory. This treatment is indicated in various cases, such as advanced maternal age, low sperm count, ovulation problems, or when other, simpler, assisted reproduction techniques have failed.

If the fertilisation process is successful, the fertilised egg will begin to be divide and will result in an embryo. The embryos are cultivated in special incubators in the embryology laboratory. Here, embryologists regularly monitor their development, selecting the highest quality embryos for transfer to the maternal uterus or for vitrification for later use.

Until a few years ago, embryo selection was carried out exclusively on the basis of embryo morphology, which takes into account parameters such as the number of cells, their size and the percentage of fragmentation. However, morphology is not always related to the embryo's chromosome endowment.

Human cells are made up of **46 chromosomes**, structures that contain our genetic information, our DNA. Half of them are inherited from our father, and the other half from our mother. We say that an embryo is **euploid** when all its cells contain 46 chromosomes, while an **aneuploid** embryo is the one has too many or too few chromosome copies.

Today, we know that aneuploid embryos are common and that it depends on – among other factors – maternal age. For instance, some **25%** of embryos from **30-year-old** women are aneuploid. This proportion increases up to **50%** at **40 years** (Franasiak JM et al., Fertil Steril, 2014).

Aneuploid embryos are not compatible with life; the vast majority of them end up in miscarriages or do not implant. We can select the best embryo based on its morphology during in vitro fertilisation techniques, but it may be aneuploid. This means that there will be a greater risk of implantation failure, a miscarriage or even having a child with serious abnormalities.

Genetic study of the embryo

The only way we currently have to minimise the risk of transferring aneuploid embryos is to perform a genetic study that will determine the number of embryo chromosomes in the preimplantation phase; in other words, before it is transferred to the maternal uterus. This study is called **PGT-A** (Preimplantation Genetic Testing for Aneuploidy). It is used particularly in cases of advanced maternal age, repeated miscarriages and recurrent implantation failures, among others.

This genetic test is performed from **three to five days after fertilisation**. First, it is necessary to perform an **embryo biopsy**, that is, to extract one or more cells from each embryo. This material is then analysed in the laboratory to identify the embryos with correct chromosome loads. Therefore, they have a greater chance of resulting in a normal pregnancy.

Embryo biopsy

The embryo biopsy is a crucial step during the PGT. It can be performed on the third day after fertilisation, when the embryo has six to eight cells. In this case, a small opening is made in the so-called "**zona pellucida**" (the membrane surrounding the embryo), where a cell is extracted for analysis. However, embryo biopsy is currently usually performed on Day 5 or 6 after fertilisation. This phase is called the **blastocyst** phase, and the embryo is made up of more than one hundred cells. In this case, a small group of cells is extracted from the trophectoderm, the outer cell layer of the blastocyst that will become the placenta.

The blastocyst stage biopsy offers important advantages compared to the biopsy on Day 3. On the one hand, we can obtain a greater quantity of genetic material, since we extract a larger number of cells; on the other, since the embryo contains more cells, it is less harmful. It also allows embryo mosaicism (embryos made of euplid and aneuplid cell lines) to be detected.

Advantages and limitations

Like all techniques, the PGT-A has some advantages and some limitations. Compared to the IVF cycles where the PGT has not been performed, the technique has benefits that include:

- Improving implementation rates
- Reducing miscarriage rates
- Improving pregnancy by transfer rates
- Reducing the risk of having a baby with a chromosome anomaly

On the other hand, it does have some limitations:

- It is an **invasive method** in which the vast majority of embryos survive the biopsy, although there is a small possibility that the embryo will not develop.
- It is a **complex technique** that requires specific equipment and personnel with extensive experience.
- There is a possibility of misdiagnosis due to **embryo mosaicism** as only one of the embryo cell lines is detected.

Non-invasive PGT

In 2016, the detection of free DNA in the middle of blastocyst culture was published and suggested as a tool for determining the embryo chromosome load in a non-invasive manner. This DNA is released by the embryo into the culture medium during its in vitro development by mechanisms that are not yet entirely clear. In recent years, several studies have shown the ability to detect, extract and amplify DNA from the embryo culture medium, especially in the blastocyst phase, and its potential clinical application for non-invasive PGT has been evaluated.

Currently, there is some controversy about the usefulness of the non-invasive PGT. However, the latest publications indicate that, when DNA contamination from maternal cells is eliminated and the procedures in the embryology laboratory are optimised, the DNA released by the embryo is a good indicator of its chromosome load (Huang et al., Proc Natl Acad Sci USA, 2019; Chen et al., Front Cell Dev Biol, 2021).

How is the non-invasive PGT done?

The protocol is much simpler than the traditional PGT, as there is no need to perform an embryo biopsy. The basic steps are as follows:

- On Day 3 or 4 after fertilisation, each embryo is washed and transferred to a **new culture medium**, where it is incubated until Day 5 or Day 6.
- During this period of time, the embryo releases free DNA into the medium.
- On Day 6, the embryos **are vitrified** and the culture medium is collected and analysed to detect the free DNA and identify possible aneuploidy.

The **non-invasive PGT** has several advantages over the traditional PGT:

- The risk of potential embryo damage after the biopsy is eliminated.
- The protocol is much simpler.
- The cost of specific equipment is reduced.

However, it also has some limitations:

- The origin of the free DNA of the cells is still unclear. There is still **some controversy** as to whether the free DNA released to the culture medium is representative or not of the chromosome composition of the entire embryo.
- It is necessary to **minimise the risk of contamination** with maternal DNA, which may lead to a misdiagnosis.
- It should be **validated in each laboratory**. This entails the optimisation and standardisation of the culture conditions and the protocols for recovering it from the culture medium to obtain a sufficient quantity of free DNA and to avoid contamination with maternal DNA.

Despite the limitations of the technique, the information provided by the non-invasive PGT can be used as a system for prioritising the identification of the embryos with the greatest possibility of being euploid, thereby increasing the likelihood of achieving a normal pregnancy.

> The non-invasive PGT is a technique with **great potential** and with some limitations that are expected to be resolved in the near future.

What is the implantation rate of my embryo?

Sofía Sánchez
Embryologist
UR HLA Montpellier

This may be one of the most common questions that we gynaecologists and embryologists face in our daily practice. This very reasonable question – posed just before the much-desired transfer, and after all that our patients have gone through during the assisted reproduction treatment – is, however, one of the most difficult to answer.

According to the studies published and our clinical experience, we can indicate a percentage of success in general, but that percentage will always vary according to the case at hand. It is this that prevents us from giving a specific and individualised answer.

So, what can cause the implantation rate vary from case to case?

The simple fact is that embryo implantation is a very complex event that is conditioned by many factors. The implantation occurs when the embryo in the blastocyst stage (Day 5 or Day 6 of development) and attaches to the endometrium (a mucous layer that covers the inside of the uterus), penetrating it in order to continue developing inside the uterus.

This whole event has to take place in the correct uterine environment, thus obtaining synchronisation between the development of the embryo and that of the endometrium.

The definition of embryo implantation already clearly identifies two of the most important factors that will influence the implantation rate: embryo and endometrium.

The embryology laboratory is tasked with assessing and selecting the optimal embryo to transfer. The assessment of **embryo quality** ranges from the gametes (oocytes

and sperm) to the last day of cultivation, and it includes its morphological and kinetic characteristics.

Therefore, during these days of development, we will be studying the **factors related to the embryo** that will influence the implantation rate.

Oocyte quality

The oocyte has the most important role in the early days of embryo development. The **patient's age** will determine the oocyte quality and will be one of the main factors that affect embryo quality and, thus, the implantation rate.

The latest results published by the Spanish Fertility Society (SEF) show that the pregnancy rate with the mother's own oocytes decreases as the patient's age increases: **45%** (<35 years), **35.9%** (35-39 years) and **22.5%** (≥40 years).

Sperm quality

The ICSI technique has made it possible for us to solve cases with a serious male factor, in addition to allowing us to make a better sperm selection by choosing the sperm with the best **motility and morphology**. We also have other, complementary, techniques that help us make a better sperm selection when there are alterations in the sperm's genetic material that will affect the correct fertilisation of the oocyte or the subsequent development of the embryo.

Cultivation system

Due to the new time-lapse incubation system and the advances and improvements in embryo culture media, the trend in laboratories is to bring the embryo to the **blastocyst** stage (Day 5 or Day 6 of cultivation). The best quality embryos are the ones that will reach the blastocyst stage, while those of lesser quality will not develop correctly or will simply cease to develop. Thus, we will obtain more information on embryo development, meaning that we will do a better embryo selection in order to transfer the best quality embryo.

Embryo genetics

Being considered a good quality embryo does not mean it is genetically normal. This is something we have to remind people of continuously. An embryo with chromosome alterations that are not compatible with life will result in implantation failures or a subsequent miscarriage. To do this, the **PGD (Preimplantation Genetic Diagnosis)** will allow us to select "healthy" – that is, normal – embryos. This tool, along with the morphological evaluation of the embryo, will allow us to select the healthy embryo with the greatest implantation potential.

As we have already commented, synchronisation between embryo and endometrium development is crucial for implantation. If the endometrium is not prepared on the day of the transfer – regardless of however good the embryo quality may be – it will never be implanted correctly. Therefore, a **receptive endometrium** is another crucial factor for implantation to occur.

The main factors related to the endometrium that may influence the implantation rate are:

Endometrial thickness

Most research concludes that implantation rates are better when a **trilaminar endometrium** is achieved that is at least 7 mm thick. This thickness will be attained by administering external hormones such as oestrogens and progesterones.

Implantation window

This is the time period that the endometrium is receptive and allows the embryo to be implanted. This window may change, which results in the transfer of the embryo and the implantation window not being synchronised. This factor is the most difficult to control, although there are currently molecular diagnostic tests that can study it.

In addition to the quality of the embryo and the endometrium, there are **other factors that may affect the implantation rate:**

Uterine factors

Infections, polyps, myomas, and uterine malformations will affect the implantation and development of the embryo in the endometrium.

Immune factors

Immune system disorders that cause the mother's immune system to damage the embryo by identifying it as a foreign body. This causes a failure in implantation or a subsequent miscarriage.

Thrombophilias

These are blood clotting disorders related to implantation failures and repeated miscarriages. Treatment with aspirin and heparin is indicated for these cases.

Endometriosis

This heterogeneous illness may – in addition to affecting the ovarian reserve – cause pelvic adhesions and changes in hormone production. This may lead to ovulatory dysfunction and changes in endometrial receptivity, thereby reducing the rate of embryo implantation.

Polycystic ovary syndrome (POS)

This may make implantation difficult due to a change in the production of sex hormones that may affect endometrial receptivity, including oestrogens and progesterone.

Other factors that will also influence the implantation process include:

Tobacco use affecting the quality of the gametes; changes in the BMI (obesity and low weight) or stress that will influence the woman's normal hormonal function. As we can see, the implantation is a complex event involving a multitude of variables. We must take these variables into account when determining an individualised implantation rate. The entire team will try to study and control the maximum possible variables and attain the best possible implantation rate based on the case.

> We must always remember that the success that really matters is not implantation or pregnancy in our patients, but the birth of a healthy baby.

We cannot ignore the fact that there is a considerable percentage of **spontaneous miscarriages**. That is why we will always try to explain the rates as clearly as possible, so that we can help our patients to reconcile the hopes provided by statistics with the emotional cost of the whole process.

Questions and answers

The expert responds...

When is it advisable to carry out Preimplantation Genetic Testing (PGT-A)?

Dr. Antonio Urbano
Geneticist at UR HLA Inmaculada, PGT-A specialist

Today, we know that embryos with chromosomal alterations (aneuploidy) are frequent and that this depends on – among other factors – maternal age. Aneuploid embryos are not compatible with life; most of them end up as a miscarriage or do not implant. The only way we currently have to minimise the risk of transferring aneuploid embryos is to carry out a genetic study to determine the **number of the embryo's chromosomes** during the pre-implantation phase, i.e., before transferring it to the maternal uterus. This study is called PGT-A (Preimplantation Genetic Testing for Aneuploidy) and is especially used in cases of a**dvanced maternal age**, repeated miscarriages and recurring implantation failure, among others.

This genetic test is carried out between Day 3 and Day 5 after fertilisation. First, it is necessary to carry out a **biopsy of the embryo**, i.e., extract one or more cells from each embryo. Next, this material is analysed in the laboratory to identify the embryos with a correct chromosomal load as these are more likely to lead to a successful pregnancy.

Compared to IVF cycles done without the PGT, the benefits of this genetic embryo selection include, among others, improved implantation rates, lower spontaneous miscarriage rates, improved embryo transfer pregnancy rates and lower risks of having a baby with a chromosomal anomaly.

Is it possible to improve fertility by changing food and lifestyle habits?

Nuria Santamaría
Embryologist at UR HLA Mediterráneo

Although a woman's age continues to be one of the most important factors in fertility problems, it is not the only one. Diet and lifestyle are considered to be increasingly influential factors on the fertility levels of both men and women. Healthy, balanced diets and a reduction in the consumption of "trans" fats (industrial pastries and fried foods) and the monitoring of animal protein consumption (mainly red meat and processed meats) in favour of plant-based proteins (legumes, nuts, soy products, etc.) are healthy habits that can help us on the way towards becoming pregnant.

It is also advisable to take some **vitamin supplements** containing **folic acid, iodine and DHA** – these are considered nutrients beneficial to fertility – but always under the supervision of a specialist. In addition, moderate and regular physical activity is beneficial, as it maintains body weight, thereby improving reproductive health. It is advisable to diet in case of being overweight, since obesity and being overweight hinder fertile capacity; to **avoid stress**, which may cause a reduction in fertility; **give up tobacco**, as its large amounts of toxic components affect semen quality; and **reduce caffeine** consumption, because high levels may affect fertilisation ability.

What does the new ovarian rejuvenation technique consist of?

Dr. Manuel Lloret Ferrándiz
Head of UR HLA La Vega

The passage of years is a fundamental factor in women's fertility, because the ovarian reserve, i.e., its fertile potential, begins to be affected beginning at the **age of 35** and falls drastically after 40. Advanced maternal age and low ovarian reserve are seen nearly every day in the clinic. This is what moves us assisted reproduction specialists to investigate techniques that reverse ovarian ageing.

The goal of the innovative ovarian rejuvenation technique is to reverse primary ovarian ageing. We can divide it into two groups: the first uses **stem cells**, and the second uses a technique to activate follicles that are asleep (latent) especially in patients with a low ovarian reserve that have residual follicles. The technique used is to obtain **platelet-enriched plasma** as a regenerative treatment that offers women the possibility of being mothers using their own eggs.

The treatment is very simple and only one blood extraction is necessary. This is centrifuged in the laboratory to obtain platelet-enriched plasma, which has a large amount of growth factors. This plasma is introduced into both ovaries through **transvaginal ovarian needle drilling**, and in vitro fertilisation is then done two or three months later.

This treatment is a reproductive option that will increase the chances of achieving pregnancy by natural means; it also raises the success rate of in vitro fertilisation, and serves as a preventive treatment to maintain ovarian and hormonal activity longer, as well as menstruation in the event of perimenopause.

What is endometriosis and how does it affect women's fertility?

Dr. Valeria Sotelo
Gynaecologist at UR Vistahermosa.

Endometriosis is a benign, but chronic, problem. We speak of endometriosis when the endometrium – the layer that covers the uterine cavity – appears and grows outside of it, affecting a woman's reproductive capacity.

Women suspected of having endometriosis must be counselled during the gynaecological appointment on the risks involved by this pathology with regard to current or future reproductive desires.

Today, there is no consensus in reproductive medicine on how to address the issue of women who are suffering from this disease. In general, if it is mild, and the patient is young, **artificial insemination** or simply **ovarian stimulation** may be carried out. However, the most appropriate technique and the one that offers the greatest probabilities when the pathology is more invasive and **affects the Fallopian tubes** is vitro fertilisation. When the degree of endometriosis is very severe and it is impossible to access the ovaries to carry out needle drilling and obtain the oocytes, donor eggs will have to be used to achieve pregnancy, so **egg donation treatment** will be required.

We recommend that women with this disease go to a specialist so that each case can be addressed personally, and an assessment made – based on the stage of the endometriosis, symptoms and patient age – of the best type of intervention to achieve pregnancy.

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