



# CREANDO

## FAMILIAS

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### **Oocyte vitrification**

preserves the fertility potential associated with the age of the woman at the time of cryopreservation



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### **Sperm capacitation**

The process that selects the sperm that has the highest fertilizing potential



**Editorial**  
**Dr. José Jesús**  
**López Gálvez**  
CEO of the UR Group

Once again, I am delighted to greet you in an edition that is special for all of us. In your hands is our last magazine of 2020, Issue No. 3 of Creating Families. This is an exciting project that we continue to consolidate because - as we explained at the start - it was created from our desire to disseminate knowledge about assisted reproduction and to share the best information from the field of reproductive with society.

To this end, we continue to enjoy extensive collaboration from the professionals who make up the Reproduction Departments at Grupo Internacional UR, along with the commitment from another of our dissemination and training projects, the Chair of Reproductive Biomedicine.

As I write these lines to you, I'm deeply aware of the difficult times we are living through because of the Covid-19 pandemic. For us, however, it is just another reason why we must not lower our guard, redoubling our efforts to continue the constant enhancement of our service, protocols, processes and care by providing all our departments with the best conditions and creating a safe environment, all aimed at offering our patients confidence and peace of mind so they can realise their dream: to have a child.

What's more, we're aware that this situation has also become very difficult both socially and economically. In response, we have created different aid programmes so that nothing prevents starting the path that leads to having a healthy baby at home.

I don't want to sign off without leaving a line and a wish filled with hope for 2021, with the conviction that we will leave these days of worry and uncertainty behind us as soon as possible, along with a message of encouragement that, in the coming months, joy will return to our lives and your reproductive project will be successful.



## 6-7

Sperm capacitation,  
The process that selects  
the sperm that has the highest  
fertilizing potential

---

*Miguel Ángel López Martín*

## 10-11

Recurrent miscarriages,  
Comprehensive study and  
solutions for the possible causes

---

*Bárbara Romero*

## 14

Reproduction in Lesbians  
What is and what is not allowed

---

*José Félix García España*

## 17

Endometriosis,  
The disease that can  
compromise female fertility

---

*Valeria Sotelo*

## 8-9

The genetic quality of sperm,  
decisive for the success of the  
pregnancy

---

*Teresa Rubio*

## 12-13

Oocyte vitrification preserves  
the fertility potential associated  
with the age of the woman at the  
time of cryopreservation

---

*Salvador García Aguirre*

## 15-16

The abuse of anabolic steroids  
affects the male and female  
reproductive system causing  
infertility

---

*Bernardo Fernández Martos*

## 18-19

Time lapse incubation,  
Pioneering technology to  
optimize embryo selection

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*Rocío López Sánchez*

# SPERM CAPACITATION

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The process that selects the sperm that has the highest fertilizing potential



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**Miguel Ángel López Martín**

Embryologist

UR HLA Jerez Puerta Del Sur

The sperm quality of 21st century men has suffered a decline compared to their ancestors'. The function of sperm is to penetrate an egg and release its DNA so that fertilization can occur. In the event that the genetic material is damaged, there will be less chance of success.

**Sedentary life, obesity, and toxic habits** are very harmful to the production of sperm with good fertile capacity. Numerous studies show that males who **smoke** have a 13% decrease in sperm mobility, a reduction of up to 23% in concentration, and an increase in DNA fragmentation, as well as a 28% decrease in the likelihood of pregnancy.

**Excessive alcohol consumption** is linked to teratozoospermia in males—less than 4% of sperm have normal morphology—and oligozoospermia—semen with low sperm count—decreased libido, and erectile dysfunction. Additionally, diseases such as **diabetes or prostatitis**—a bacterial infection of the prostate gland—may also damage the sperm.

In the various assisted reproduction techniques (IA, IVF or ICSI), sperm capacitation processes are routinely used as part of the process of assessment of sperm quality through the **spermiogram with MSC (Motile Sperm Count)**.

Under natural conditions, sperm undergo a quantitative and qualitative selection process, so that only the best reach the egg and fertilize it. Natural capacitation occurs after ejaculation, once the sperm come into contact with the female genital tract. This capacitation process intended to fertilize the egg entails a number of changes. Mobile sperm are separated from the immobile ones, which are left behind, while the seminal plasma is eliminated and the sperm are accelerated. This activation generates changes in the movement pattern and in the acrosome, and a part of the head of the sperm containing enzymes, which when released will allow it to penetrate the cells surrounding the egg until it becomes fertilized.

In the lab, several techniques are used with the same purpose and work with a sample to achieve the

best possible fertile conditions. Within the sperm capacitation methods, **density gradients** are the most used, where sperm, through the force generated by a centrifuge, cross through several layers of a viscous solution, generated with silicone particles, which selects them based on their density, thus obtaining a sample with better mobility, morphology and maturity. It also allows to effectively eliminate viruses and bacteria present in seminal plasma.

Another alternative is the one called **Swim Up**, a seminal sample spinning washing technique that concentrates sperm at the bottom of the work tube in a specific culture medium and then leaves them as long as necessary at a 45-degree angle. During this time only the sperm with the best mobility will colonize the top of the medium and are the ones that will be selected. The advantage of this technique is that the selection is more physiological than the previous one.

Both techniques are simple to use; we may choose one or the other depending on the characteristics of the sample and the technique in which it will be applied.



# THE GENETIC QUALITY OF SPERM

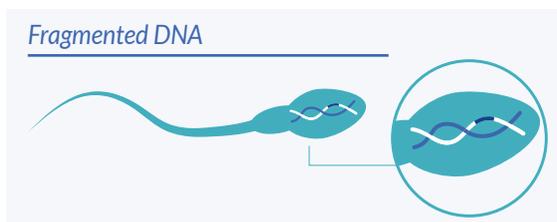
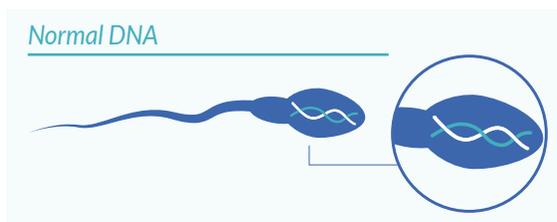
## decisive for the success of the pregnancy

**Teresa Rubio**

Embryologist

UR HLA La Vega

Approximately 40% of the causes of infertility are due to a male factor. The genetic quality of sperm is one of the factors that determine a healthy and successful pregnancy. **Sperm DNA fragmentation** occurs when there are breaks in the genetic material within the sperm. These breaks can be single-strand or double-strand. These abnormalities have been associated with reduced fertility and pregnancy rates, low embryonic quality, and an increase in miscarriage rates.



Causes may be **internal**, caused by processes within the body: **Incorrect maturation** of sperm during production. Failures in **apoptosis (programmed cell death)** that prevent damaged sperm from being naturally eliminated prior to ejaculation and excessive production of free radicals (ROS) during the transport of sperm through the testicle and in the ejaculation.

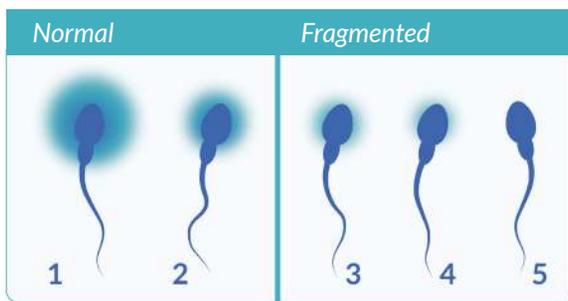
**There are also external factors:** Exposure to environmental factors, toxic industrial chemicals, pesticides, tobacco, alcohol, drugs..., scrotum varicose veins, damage induced by radiation therapy or chemotherapy, exposure of the testicle to high temperatures and advanced age in males (> 40 years).

The routine study of the infertile male consists of a basic study of the semen that includes a spermogram and sperm capacitation. Many clinicians agree on the limitations of this **conventional semen analysis**, as the seminal parameters do not actually reflect the **integrity of the DNA or the fertilizing capacity of the sperm**. A basic semen analysis should be considered only the starting point, to identify men whose chance of achieving a natural pregnancy is

reduced.

Approximately 15% of infertile males have a normal spermiogram, but normal semen parameters do not mean that there is a normal fragmentation. The fragmentation percentage may be considered an **indicator of semen quality**.

There are different tests that measure sperm DNA fragmentation: the TUNEL assay, the Comet assay, the SCSA. These detect double-strand or single-strand damage but are expensive techniques and require special instruments. The most widely used technique within assisted reproduction labs for its simple methodology is the **SCD (Sperm Chromatin Dispersion)**. It estimates the level of indirect fragmentation by measuring the amount of nuclear halo dispersion in the healthy sperm. It detects only **single-strand damage**.



*DNA Fragmentation Index (DFI) set at a limit of 30%*

## What can be done to reduce sperm fragmentation?

We know that **DNA damage can be repaired by the oocyte after fertilization**. This depends mainly on the **quality of the oocyte**, which is directly linked to the woman's age and the level of damage in the DNA strands of the sperm that produced fertilization, as the oocyte can only repair single-strand damage.

In cases of sperm DNA damage, there are several alternatives that may reduce the percentage of fragmented sperm. The most commonly used techniques within reproduction clinics are:

### Use of oral antioxidants

There are several studies that show the benefit of this type of treatment in males, which decreases fragmentation and increases the pregnancy rate.

### MACS ART Annexin V System (annexin columns)

In this case the damaged sperm will bind to annexin V, and the non-apoptotic cells with intact membrane will pass freely.

### But there are also others, like:

- **IMSI:** Sperm are selected by high powered microscope.
- **PICSI:** Sperm are selected by binding them to hyaluronic acid, a more physiological selection.
- **TESA (Testicular Sperm Aspiration):** It takes sperm directly from the testicle thus preventing them from passing through the epididymis, which may increase DNA fragmentation levels.

A good preventive measure is for the man to perform periodic ejaculations and reduce the number of days of sexual abstinence to one day before in vitro fertilization, thus reducing the number of fragmented sperm in the ejaculate and improving its quality.



# RECURRENT MISCARRIAGES

Comprehensive study and solutions  
for the possible causes

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**Bárbara Romero**  
Gynecologist  
UR HLA Inmaculada

“ We want a child...  
Will we be able to have one?  
Can we avoid having another  
miscarriage? ”

Recurrent miscarriages have a strong impact on a couple's emotional state and are a disappointing situation for medical professionals, as well as a challenge. Miscarriage is the most common complication in pregnancies, as **15 to 20% of all pregnancies** end in miscarriage. However, the likelihood that a couple will have two or more repeated miscarriages is less than 5%, and this requires a study of the related and predictive factors of a new miscarriage.

Many factors influence recurrent miscarriages, but a woman's age is critical, doubling the initial risk starting at 40 years, nearing 40 to 80%. In other words, more than half the pregnancies in women over 40 may end in a miscarriage.

The recommendation is to initiate a miscarriage study after two have occurred, but we must individualize each case, considering the age, personal and family history, circumstances surrounding gestational losses, and the couple's emotional state.

Possible causes of miscarriage include genetics, immune, uterine disorders, thrombophilia, microbiome alteration, infections, endometritis, etc., and treatments vary depending on the condition.

### Alterations in the embryo

Up to 70% of embryos in patients with recurrent miscarriages have chromosomal alterations. In vitro fertilization with preimplantation genetic testing (PGT-A) carries out a chromosome analysis of embryos before transfer, which helps prevent miscarriages.

### Endocrine diseases: thyroid disorders or diabetes

Their treatment, together with healthy lifestyle habits, may improve chances of completing the period of gestation.

### Alterations in coagulation: thrombophilia testing

When confirmed, it may be treated with heparin.

### Immune causes:

#### Am I compatible with my partner?

These are difficult to prove, but may be treated with corticosteroids or the transfer of a single embryo.

### Alterations of the uterine cavity

A hysteroscopy is the best way to assess the uterine cavity to confirm a pathology and treat it at the same time. It also allows us to take samples for an endometritis study, for example.

### Alterations in the sperm

A link between sperm DNA fragmentation and miscarriage has been established. In this regard, antioxidants and treatments such as annexin columns or MACS allow us to select the best sperm. In other cases, the result of the sperm FISH analysis is not within range and performing preimplantation embryonic genetic testing (PGT-A) may decrease the likelihood of miscarriage.

### Psychological factor

Psychological support and care during the first few months of pregnancy are very important in these couples.

## At the UR HLA Inmaculada, we conduct a comprehensive study of miscarriages

We advise and offer solutions for each of the possible causes, to achieve the best uterine receptivity and chromosomally tested embryos, thus lowering the likelihood of a miscarriage and increasing your chances of bringing a healthy child home.

# OOCYTE VITRIFICATION

preserves the fertility potential associated with the age of the woman at the time of cryopreservation



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**Salvador García Aguirre**  
Medical Director of UR Montpellier

In the last few years news coming from the other side of the Atlantic has put the vitrification technique in the spotlight. The companies Apple and Facebook give their employees the opportunity to freeze their eggs if they want to. These companies pay for this treatment because, they claim, it enables women to freeze their eggs in their fertile years that, often, coincide with their most productive professional period.

In Spain and neighboring countries it is a technique that has been growing in popularity for quite some time now, seeing as more women are joining the workforce which, among other factors, means that they think about preserving their fertility and oocyte vitrification in the period of their lives when they have high quality eggs, so that they have good chances of conceiving in the future with guaranteed success, when they decide to have a family and want to thaw them to get pregnant.

The purpose of preserving fertility is to cryopreserve female gametes to postpone childbearing. By doing so **a woman aged 45 can be a mother whose biological age is 30**. That is what will happen to her body as long as she has opted for oocyte vitrification. It enables women to plan motherhood and still have good chances of conceiving using oocytes that are the age of when they were cryopreserved.

Women's fertility starts to decline at the age of 35, when both the oocyte quality and the quantity decrease and therefore assisted reproduction techniques are needed more for the desired pregnancy. The advantage of a woman using her own vitrified oocytes for an IVF cycle at an advanced age is that it will be more effective to use vitrified oocytes that are younger than those that the woman currently has. Another advantage here is the genetic bond that is not acquired with donated oocytes.

In our European society oocyte vitrification is not as well-established or as free of controversy as in the USA, although it is catching on gradually and it is starting to be a normal technique that is recommended.

## Fertility preservation methods:

### Oocyte vitrification

It is the method that has been most widely accepted recently, ever since, this ultra-rapid freezing technique, was refined enough to ensure a high survival rate of the egg cells after thawing.

### Embryo vitrification

In this case, it isn't the eggs cells that are vitrified but rather the embryos, which requires a stable partner. If this is possible, this method is as good as or better than the former method, in terms of the likelihood of conceiving after thawing.



In view of the aforementioned, childbearing should not be postponed beyond a certain age and if a woman does want to leave it to another more appropriate time, bear in mind that oocyte vitrification is the best option to preserve fertility.

# REPRODUCTION IN LESBIANS

## What is and what is not allowed

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**José Félix García España**

*Medical Director of UR El Ángel*

The "new" family model is increasingly present in our reproduction units. The presence of lesbian couples who desire a pregnancy may raise questions that sometimes make us doubt whether they are within what is legally permitted by the law. Insemination of one of the partners through the use of a sperm bank or IVF does not pose any problems, as there is no difference either technically or legally from the procedures that single women undergo.

Something that is completely different is the use of the **ROPA (Reception of Oocytes from the Partner) Method**, though which in a lesbian couple, one of the women undergoes the stimulation of her eggs and these, once fertilized, are transferred to her partner, who will carry out the pregnancy.

This initially raised some issues. The law states that eggs must be donated from an anonymous donor, and since this is clearly NOT an anonymous donation, would it even be legally possible? The detail that makes this technique possible is the fact that the two people involved are in a relationship. In this case, a homosexual couple has the same rights as a heterosexual couple, in which there is an exchange of gametes. This opened the door to lesbian couples to exchange their own through the use of the ROPA Method.

But these couples present situations that take us one step further. What if both wish to undergo an IVF with the same donation, and we "swap" the resulting embryos so that they both become pregnant with their partner's eggs, using the same donor for both?



First of all, the law states that previously frozen embryos may be used by the woman herself or her spouse. There is nothing that prevents them legally from being stimulated simultaneously, provided that the embryos of both have a certain and immediate reproductive destiny.

The obstacle may come in the choice of the male donor, as it is necessary to seek the greatest phenotypic and immunological similarity possible to the donor. This means that both women should have some physical similarities, so that the reproduction lab may choose sperm that would adapt to both of their phenotypes. My intention was to present an example of the challenges that new family models pose in our day-to-day work and that without the help of a legal team to advise us, it would be difficult to make the right decisions. More than technical problems, these are legal questions we occasionally have to face.

# THE ABUSE OF ANABOLIC STEROIDS

affects the male and female reproductive system  
causing infertility



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## **Bernardo Fernández Martos**

*Head of Nursing of the UR Group  
UR HLA Vistahermosa*

Anabolic steroid abuse has been subject to increasing interest, especially after certain studies conducted in the USA that showed a frequent consumption of these substances for non-medical purposes.

The anabolic steroid prototype is the **masculine testosterone hormone**. There are many other synthetic analogues, such as mesterolone, methenolone, nandrolone, and stanozolol. The development of new anabolic steroids has been aimed at obtaining drugs that can be administered orally and

to attempt to separate anabolic and androgenic actions. Although it has been possible to obtain oral drugs, separating the androgenic and anabolic functions has not been possible.

One of the main non-medical uses of anabolic steroids is in the sports world to increase physical performance. However, its consumption has spread among those non-professional athletes who wanted to increase their physical performance, have more muscle mass or simply improve their body image. This is not limited to men, since the use of these sub-

stances for these purposes has also been observed among women, who use them to reduce body fat or improve their body image.

Anabolic steroids are administered orally or by injection, and athletes often take them in cycles of weeks or months, rather than continuously, following patterns called “cyclical use”. This use consists of taking several doses of steroids over a certain period, stopping for some time and starting again. Additionally, different types of steroids are often combined to maximize their effectiveness while minimizing adverse effects. This process is known as stacking.

The systemic side effects that can occur from anabolic steroid abuse include:

- **Modification of sexual characteristics:** testicular atrophy and increase in breasts in men, as well as decreased breast size, increased clitoris size, voice changes, and the growth of facial hair in women.
- For adolescents: **Stunted growth** due to premature skeletal maturation and accelerated changes in puberty.
- In men, **sterility** can be caused due to a decrease in seminal quality; while women have shown an **alteration in menstrual cycles**.

The abuse of anabolic steroids alters the hormonal production involved in reproduction and may have either reversible or irreversible changes in the subjects, depending on the dosage and the duration of their consumption.

**In women**, they have shown to have menstrual cycle alterations, having cases of amenorrhea (lack of periods), which combined with a hyper-androgenic environment may have effects on oocyte quality, and generate infertility issues due to anovulation.

**In men**, changes to their spermogram have been



observed, with a drop in the number, mobility, and morphology of sperm cells, which also results in infertility issues. The administration of exogenous androgens causes a drastic reduction in the production of testosterone. As normal spermatogenesis requires an adequate concentration of intratesticular testosterone, men who use steroids have shown to have significantly lower sperm production and azoospermia (absence of sperm) can often be observed. The scope and reversibility of the side effects on sperm production depend on the dose and the duration of the use of steroids, which could result in a permanent problem.

Therefore, it is necessary to make a serious assessment, mainly because the consumption of anabolic steroids by athletes is frequently based on self-designed treatment schemes, while some are advised by a partner, their trainer, or these are guided by recommendations published in generalist magazines that lack scientific rigor. Participants in this sort of training and who consume these substances to improve their performance and body image, are not aware of the dangers it may entail for their overall health and, in particular, the changes to their reproductive function, which can lead to infertility, depending on the dose and duration of their consumption.

# ENDOMETRIOSIS

## The disease that can compromise female fertility

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**Valeria Sotelo**

Gynecologist

UR HLA Vistahermosa

Endometriosis is a benign but chronic problem. Endometriosis is when the endometrium, which is the layer of tissue that covers the uterine cavity, appears and grows outside the uterus, which affects the female reproductive capacity. It is the innermost tissue and it should only and exclusively be located in the uterus. Although it is most frequently found in the ovaries, it can also adhere to the fallopian tubes, even the bladder or the intestines. This unusual growth might lead to the irritation of the surrounding structures, which causes pain and adhesions that cause scar tissue in them.

The therapeutic approach and management of endometriosis affecting women is significantly different depending on whether the objective of the treatment is to alleviate the pain or deal with infertility problems. **Hormone therapy and radical surgery** might be a good option to treat pain, although these strategies often make it more difficult to conceive, seeing as fertility treatments are based on the maximum preservation of the ovarian function, which is frequently an obstacle for pain treatment.

Women who suspect they have endometriosis or have been diagnosed with it, should seek advice from their gynecologist about the implicit risks of this disease with regards to their current or future desires to become mothers. The clinical symptoms of this disease can vary a lot, some women are asymptomatic, and others require surgical intervention to remove the endometrial tissue and peritoneal adhesions. Moreover, there are four stages of the disease, the first is minimal and the fourth is severe.

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## Assisted reproduction techniques to conceive

At the moment, in reproductive medicine, there is no consensus on how to deal with infertility in women who are affected by this disease. In general, if the disease is in the minimum or mild stage (stage I or even II) and the patient is young, artificial insemination or simply ovarian stimulation can be used. However, when the disease is more invasive and affects the fallopian tubes, the most suitable technique that has the highest chances of conceiving, is in vitro fertilization.

When the endometriosis is very severe (III or IV) and access to the ovaries by ovarian puncture is impossible due to multiple adhesions, or if the ovarian reserve is very low, ova from a donor will have to be used to conceive, therefore egg donation fertility treatment is required.

For all these reasons, if you have this disease it is best to visit a specialist to address each case personally and assess what sort of intervention is more favorable to achieve pregnancy depending on the state of the endometriosis, the patient's symptoms and her age. Laparoscopic surgery is an appropriate option for severe endometriosis, since it offers great possibilities of pregnancy in these patients.

# TIME LAPSE INCUBATION

Pioneering technology to optimize embryo selection

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**Rocío López Sánchez**

Embryologist

UR HLA Vistahermosa



After undergoing ovarian puncture and harvesting oocytes, embryologists proceed to fertilize them. About 18.5 hours post-fertilization, those eggs that have been properly fertilized are left to incubate for a period of 3 to 5-6 days, this way we can observe and assess the different changes that take place in the embryonic development, which allows us to classify them by their implantation potential and, find the best options when vitrifying the embryos or carrying out embryonic transfer.

To preserve an optimal culture medium for the development of the embryos, incubators are used that maintain the necessary conditions for their evolution, similar to the physiological conditions: low O<sub>2</sub> and CO<sub>2</sub> concentrations, a temperature of 37°C, means of culture that maintain the levels of glucose, pyruvate, and other nutrients needed for embryonic growth.

For the successful embryonic transfer it is essential to observe embryonic evolution in different phases. However, the frequency of this attention generates stress in the embryonic metabolism when the culture is done in a conventional incubator, due to the environmental changes that take place when they are taken out of their medium each time that they need to be observed.

This circumstance has derived in the development of a new embryonic observation method. **Time lapse system (TLS)** is a solution to this problem, since it enables the observation of the growth and development of the embryos non-invasively by means of a video recording system that is attached to an inverted microscope, like a movie of sorts.

TLS works by combining an incubator, an optical microscope and a software program. The integration of these elements enables constant surveillance of the embryos in real time to ensure that they maintain stable culture conditions.

This sophisticated technology allows embryologists to comprehend better the morphological mechanisms involved in fertilization, which considerably improves embryonic selection, granting

*Conventional incubator*



*Time lapse incubator (GERI)*



more information for the patient, while allowing us to provide teaching material for the embryologists to improve knowledge on embryonic development. Thus we can identify those with higher implementation potential and reduce the number of embryos to be transferred.

The former conclusions do not indicate that traditional incubators do not operate correctly, in fact, we obtain very good results by using them, since the culture conditions are appropriate and we strive to make external readings as quickly as possible so that the impact on the embryos is minimal, but in my point of view, if we have the chance of using a time lapse system, we shouldn't hesitate to do so.



### **HLA EL ÁNGEL - MÁLAGA**

HLA El Ángel - Málaga is in the TOP10 Private Hospitals according to Monitor de Reputación Sanitaria (MRS)



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“ There’s nothing more rewarding  
than helping you ensure a healthy pregnancy  
and a healthy baby. ”

Dr. José López Gálvez

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