I want to get pregnant now!

Dr Marisa López-Teijón

I want to get pregnant now!

The essential guide to fertility

Amat editorial

Amat Editorial is an imprint of Profit Editorial I., S.L.,
Travessera de Gràcia, 18; 6o 2a; Barcelona, 08021

Translation: Ailish Holly

Photographs: Shutterstock

Cover design and drawings: XicArt

Layout: Eximpre SL

ISBN: 978-84-17208-78-3

National book catalogue number: B-1.090-2019

First edition: January, 2019

Printed by: Gráficas Rey

Printed in Spain

Dedicated to all those women who fight with all their love to achieve the dream of having a child, and also to the professionals who make it possible.

I hope this book comes as an embrace of energy and affection to comfort us in each of our moments of despair.

Index

1

I want to get pregnant now!

You have decided that you want to get pregnant; that now is the moment you have been thinking about for so long. Now you feel that you have everything you thought you needed to be a mother. Maybe your desire to have a baby is so great that you have decided to sacrifice some of the things that you thought were essential in your life. Or maybe you have fallen head over heels in love with a man and you feel that he has to be the father of your children.

The probability of this moment arriving increases as you get older. The current trend of women in societies with a more advanced socio-cultural and economic level is to have few children and to postpone the age at which they become mothers. This situation can be seen every day in our surroundings and the statistics prove it.

Elisabeth is one of these women who really wants to have a baby. She cannot believe her luck: in the same month she has been promoted to the position of branch manager in her bank and her in-laws have given her enough money to pay off the outstanding mortgage on the apartment! Unbelievable luck! She is the eldest of three sisters. Since she was a young girl she has dreamed about having children; however, for her

that dream could only come true after finishing her studies, finding a good job, a stable partner and a three-bed apartment. Achieving all of this was not easy, and when this situation finally arose, she carefully gauged how much a baby could set all of the above off keel. I am referring to combining being a mother with having a career, the risks of losing out on promotions in work, of having no free time… And aware of all these factors, she has decided that it is worth it. She is convinced that a child is more important.

It is Saturday and **Marta** has been stood up. Her boyfriend tells her that he can't meet her for lunch. She sits down in the park beside the restaurant feeling exhausted, thinking: "What am I going to do now?" She is immersed in her thoughts when a girl approaches her. "Would you mind helping me?" she asks her as she places a baby in her arms before even getting a response and takes another one out of the buggy. Marta holds him –she couldn't do anything else–, smiles at him, and feels something indescribable. She is overcome by an uncontrollable desire to hug him, but she doesn't dare. She can't remember ever having held a baby before.

Marta always imagined her future with children, but she still saw it as something distant despite being 37. At that precise moment, she feels like her time has come: she really wants a baby although she is not sure that her boyfriend will want the same. And although only a few months ago this would all have seemed impossible to her, now she is willing to leave her partner and have a child on her own, if necessary.

Gynaecologists and paediatricians are surprised that nearly all couples reach the birth without ever having changed a nappy or knowing what the umbilical cord is. We wonder: "Have they never even minded a baby? Have they never wondered what their own belly button is? What have they been thinking about for the past nine months?"

Additionally, often they are couples who know a lot about everything: they conscientiously prepare for any task, they read up on the film they're going to see before going to the

cinema, they study guide books before embarking on a trip… Nevertheless, they have no idea about many of the details related to this tiny person who is going to change their life considerably more than a book or a holiday. This situation has a logical explanation. Knowing how to look after a child has always been something considered to be implicit in a woman's essence. Until rather recently, the woman looked after her younger siblings until she married, and a year later she started her own cycle of pregnancies and births.

And suddenly, without an intermediary generation, we are realising that the instinct of knowing how to look after a child has not remained impregnated in the genes of the females of our species, and that all those skills that were presumed to be innate and instinctive, have to be learned.

In this information society of master's degrees and online courses, with the most educated generation of young people in history, in which we study and sit exams for everything, it is taken for granted that without any preparation, parents will be experts in childcare; that we will know how to feed this new-born, and how to get it to stop crying inconsolably.

The most intense moment, the moment of greatest fulfilment, fear and expectation, is the day the new family is discharged from hospital after the first birth. Suddenly, the parents realise that they don't know how to look after the baby and they don't want to leave. They ask to be told about all of this immediately! If we could film it, it would be a comedy well worth seeing.

How many babies have you looked after?

As we have mentioned above, today, when the first child is born, often the parents have never looked after a baby. It is rare that anyone today has a much younger sibling. Their nieces and nephews, if they have any, are far away. There is no time to have an intense relationship with their friends' babies and, in general, the busy society in which we live has made us lose our passion for small children.

Rarely do we stop to admire how beautiful the baby next to us is. In shops, we get looked at if our children touch anything and hear things such as: "Be careful! We don't want them to get hurt". There are hotels that do not allow children (or dogs), and now there is a flight company that is going to promote child-free flights…

So the arrival home with the first child is a really difficult moment because babies do not come with an instruction manual.

Now I will tell you about **Rose**, who has just abandoned the group of young people who don't study or work, because she has got her first job: she's a gym instructor in Barcelona. Her salary is not enough for her to move out of her parents' house, but she can afford to stay on top of all her beauty whims. She has an incredible figure, she is pretty and wears all the accessories that her favourite blogger has flagged for this season. In the gym bar she has met a famous football player from the Barça team and they've gone on three dates. Today she has arranged to meet him again and she is nervous because she has decided to stop taking the pill and to see what happens… She is crazy about him and she wants absolutely everything with this man. Beforehand she had never even thought about whether or not she would like to have children, she doesn't even like children. When she told Lourdes, her best friend, what she was doing, she told her that she didn't understand why she wanted to get pregnant: "to me it seems like you're just looking for that guy's money and social position", she told her. And that is what it looked like. But Rose really was in love, with everything that word entails.

What requirements do each of us ask of a man so that he can be a candidate for us to fall in love with and become the father of our children? Could he be a man much younger than you, much less educated or of a different race?

It is an entirely personal choice based on our principles, education, motivations of which we are not even aware, and our anthropological history. For many women, when they meet a man who they feel confident could raise their pack,

that feminine instinct is aroused. On the contrary, men, when they fall in love, tend to feel the instinct to have sex with the woman, but not to raise children... They have been spreading their sperm for millions of years; however, raising children was not up to them.

I love Luis Huete's book *Construye tu sueño*. I highly recommend it because it describes the reasons that drive us. Each person has a different percentage of each motivation, and none is good or bad; it depends on how we manage it. Traditionally it is thought that the driving forces in the world are money, sex, and power. However, the author assures us that this is not the case and that these are the arms we use to achieve what we really want.

What drives you? How far would you be willing to go to achieve your greatest desire? Let us see what our protagonists do.

2

Everyone wants to get pregnant in the first month

(Looking for the positive…from desire to reality)

The most frequent current profile of women who want to get pregnant is: 30-35 years old, huge expectations, a daily folic acid tablet and a calendar. She stops taking the pill hoping to get pregnant that same month. But a year later only half of these women will have conceived, and one in five will only conceive with medical assistance.

How long will it take you to get pregnant?

The monthly fertility rate among young women having regular intercourse is 20% per cycle, while among women aged 40 it is 5% per cycle. The possibilities of pregnancy depend on:

- the woman's age,
- the quality of the semen,
- the degree of fertility of each of them.

My clinical experience with young egg and semen donors has made me see that the **degree of fertility is a personal, innate characteristic** and that all degrees exist. So, when two very

fertile people meet, pregnancy will probably occur in the first months, while, when a woman and a man with low fertility meet, despite having a complete bill of health and with nothing preventing them from getting pregnant, it will take a lot longer.

We see 25-year-old women who barely respond to the medicine to donate eggs and we have to rule them out, others who develop a lot of eggs, but only a small proportion of them mature; others whose eggs are not easily fertilised or the embryos they produce do not have a great capacity of evolving. On the contrary, other women come and "conceive with anyone".

At 35 the ovaries have aged

At the age of 35, 10% of the ova remain and the lower our ovarian reserve, the lower the quality of our ova. In general, menstruation has been considered as a test of the capacity to have children, but this is not the case.

Women are born with a set number of oocytes which gradually disappear through a phenomenon called "atresia", so that at puberty approximately 300,000 ova remain in the ovaries. During each menstrual cycle approximately 1,000 oocytes develop, but only one will reach ovulation; the rest are lost. Oocytes are the only female cells with 23 chromosomes, that is, half that of other cells. The process of reducing them by half is called "meiosis" and this is completed in the hours following ovulation.

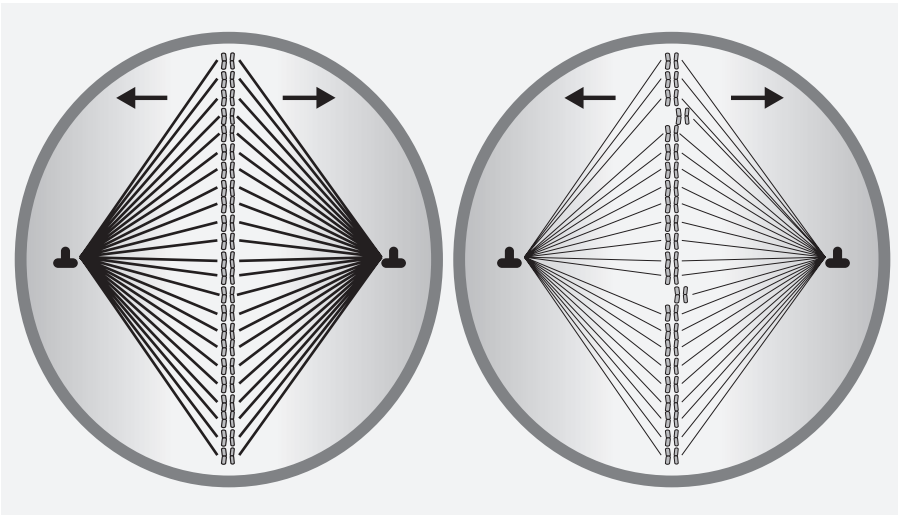
Oocyte meiosis

Every person has 23 pairs of chromosomes in all their cells (46 chromosomes in total), two of which determine the sex: XX for women and XY for men.

The 46 chromosomes face each other to separate, releasing the filaments that unite them until 23 remain. As a woman ages, alterations occur in this process. In young women the rods are resistant and the chromosomes separate correctly,

while over time these become weaker, like the filaments in a lightbulb, and they can break.

Thus, extra chromosomes (if it is 21 it will give rise to Down Syndrome) or fewer chromosomes can remain, leading to failure at fertilisation or giving rise to embryos with chromosome alterations which in their majority result in early miscarriages.



Oocyte meiosis

Often women aged between 38 and 40 with normal periods have run out of the stock of ova capable of giving rise to a healthy child: we call this "hidden ovarian failure". From 37 onwards, the risk of presenting chromosome anomalies increases significantly and, from 40 onwards, if the women needs assisted reproduction technology, the possibilities of success using her own eggs are low.

Elisabeth is 40 and **Jack** is 42. She has regular cycles. This is confirmed by the calendar on her mobile phone, where she rigorously takes note of the day her period arrives each month. She has gone for gynaecological check-ups every year and everything appears normal. They have intercourse when they are not exhausted from work, so, on Thursdays and

Saturdays. From what you already know, each month she has a 5% chance of getting pregnant. And after a year, a bit less than 40%.

Marta wants to get pregnant, but, at the moment, her partner doesn't know. So her possibilities of getting pregnant are uncertain. Edward, aged 56, is Marta's boss. He has two children from a previous marriage and has no desire to become a father again. She is wondering how she should tell him that she wants a child. It's not easy. In previous conversations he made it clear and, if he eventually accepts, it will probably only be to not lose her. Marta is frightened; however, she has made a decision.

Rose has only gone to the gynaecologist once to be prescribed the pill. She and her partner are both very young. She has moved in with him and they have started to have intercourse every day, unless he is playing outside of Barcelona. In theory, her possibilities of getting pregnant are high, although they will vary according to the quality of his semen. Later on you will discover that this factor is closely related to the place where he grew up. Since the stories are all true I won't mention our protagonist's name, so you cannot yet know where he is from.

The importance of the age at which people have children is well known in women and little known in men. However, they also have a biological clock.

The father's age can have repercussions on fertility

Ageing of the male reproductive apparatus can manifest in three aspects:

- In the **seminogram** a progressive reduction of the volume of semen ejaculated occurs. This is due to atrophy of the seminal

vesicles, which produce the liquid that transports the sperm produced in the testicle.

- The passing of time can lead to **oxidation of the sperm** and thereby reduce the fertilising capacity of the semen. A DNA chain is similar to the Christmas tree decoration that has small presents joined together by a cord. To fit inside the nucleus of the sperm they coil up. This is called the "packaging phenomenon".

  When it reaches the inside of the oocyte they open to unite their 23 packages with those of the ovum. Imagine that you tidied those decorations away brusquely and when you take them out the following Christmas the cord has broken in several places. This happens in some males through oxidation phenomena: the cords break and all the chromosomes are inside the oocyte, loose, and they cannot find their female equivalent. This problem is similar to the breaking of the oocyte filaments that we mentioned previously.

  We study the degree of DNA fragmentation of a man in a semen sample. Although this may be high it can nearly always be solved with anti-oxidant tablets. We help the testicle to package things up more carefully!

Furthermore, damage in the genetic material contained in the sperm increases with age, since time means there are more possibilities of mutations happening in our cells, which can lead to diseases in our children. **Thus, a gene's risk of spontaneous mutations is 5 times higher in a father aged 45 than in one aged 20.** 10% of occurrences of Down Syndrome and up to 40% of cases of Klinefelter Syndrome are calculated to be related to the father's age (over 55). The same occurs with diseases such as achondroplasia and other illnesses such as schizophrenia and autism. Late motherhood and fatherhood increase the risk of having a child with an autism spectrum disorder: for every 5 years the parents age, the probability increases by 18%.

Nevertheless, men produce sperm throughout their life and preserve their fertility. Female friends of mine often say to me: "That 77-year-old actor is going to have a child with a 40-year-old

woman…It must be semen from a sperm bank!" I think it is the opposite: his semen and donor eggs.

The most suitable biological age to have children is from 20 to 30, and today we are having them from 30 to 40. Should we be considering motherhood at a younger age? Or, on the contrary, accept the medical consequences derived from having children later?

As a specialist in reproduction I can tell you that the average age of the patients we see for the first time is 37. Some are concerned about their age because they were not in a position to start trying before. Or because they have undergone several treatments to no success and time is ticking. Others feel like young girls; what they do not understand is that they are young in many ways, but maybe their ovaries are not. Women aged 40 often come for advice on freezing their eggs because they are starting to consider motherhood …

It is true that life expectancy and quality of life has increased, and socially this enables women to have children much later. Medically, it may mean resorting to egg donation or even giving up on pregnancy on account of the risks it entails: from 50 onwards we no longer provide treatments.

My personal opinion is that there is no turning back in the cultural, financial and emotional independence of women. The discourse that we should have our children between 20 and 30 is obsolete, unrealistic and paternalistic. Fortunately, today's medicine enables us to help you achieve your objective.

Oocyte cryopreservation. An alternative to delay fertility

When performed correctly, oocyte cryopreservation (ultra-rapid freezing) is an incredible breakthrough. It must be performed at the appropriate moment, in other words, when the oocytes are good quality, and in an entirely trustworthy laboratory so that we can be assured that it will be performed using the correct method. Ova and oocytes are the same thing.

The objective is to keep some ova to have the option of delaying motherhood without having to resort to donor eggs. It is important to note that it is a possibility, and that a complete guarantee cannot be given, since many other factors come into play, such as the quality of the semen used to fertilise these ova and our body's ability to implant embryos…

Consequently, we do not know the exact number of oocytes we are going to obtain. **In general we advise keeping 10-12 oocytes, which is like freezing a year's eggs.** In a healthy woman aged 35 or under, the monthly possibility of getting pregnant with normal semen is approximately 20%, and in a woman aged 40, 5%.

Ideally, ova should be frozen when the woman is aged between 30 and 36 years, because it is not the same to preserve 35 year-old eggs and 40 year-old eggs. In any case, the ovarian age does not always correspond with the biological age. Generally this varies by approximately three years. It can be determined by studying the anti-Müllerian hormone in the blood and decided upon according to the analysis result. In the clinic, many women who are interested in freezing their eggs do it late and, for ethical reasons, we advise against it. We do not want to give them a false sense of peace of mind.

The majority of cases in which we freeze eggs in our centre have a very similar profile: women aged between 35 and 38, with a high socioeconomic and cultural level, who want to have children, but do not have a partner. They hope to build a family and get pregnant naturally, but they want to have some eggs frozen in case their fertility decreases. A cycle of egg-freezing costs 3,000 Euros and the annual maintenance fee until they are used is 400 Euros / year.

I would like to share some reflections with you on the measures taken by Apple and Facebook regarding paying the costs of freezing the eggs of those female employees who opt to postpone their motherhood to prioritise their professional career. Although the initiative of these companies may appear as good news for the female employees of these two companies who considered freezing their eggs, in my opinion it can be seen as a way of rejecting motherhood. Below I lay out some arguments:

1. In such a company, who would dare say that she does not want to postpone motherhood and that she is pregnant?
2. Apparently, the companies offer it as part of a pack of special incentives to attract very talented individuals. They make it very clear that they want intelligent women, but women who are postponing motherhood. They want young people. When a few years pass, and they have children, the company will decide if they still want them or if they decide to dismiss them and they will have their children in another company.
3. As I have said, women usually freeze their eggs for personal, not professional reasons. In general, by 30, people have completed their professional training. From that age onwards, when working, situations may arise such as a special professional project or a post in another country for a year, but they are nearly always temporary situations that force the woman to delay pregnancy by a year or two, but not to freeze her eggs.

Almost at the same time as this piece of news, a study was published concluding that women are more productive throughout their professional career if they have children. They perform better than women who do not have children, and they excel compared to men. What is your opinion? What do you think about Apple and Facebook financially helping their female employees to freeze their eggs? I have heard many different answers, and nearly all of them are well reasoned.

3

What can you do?

Myths and truths about what can help you to conceive

1. It goes without saying that to get pregnant you have to have **sexual intercourse on your fertile days**. If your menstrual cycle is regular, in other words, if you have your period every month, you are ovulating. Women who do not ovulate do not menstruate or they menstruate every few months and have irregular cycles.

   To know when you are ovulating you simply have to take note of the usual length of your cycle. We know that there are 14 days between ovulation and the next period. So, if your cycle is 28 days, you probably ovulate on day 14 of the cycle. If your cycle is 27 days, on day 13. It is not always exact, but you do not need ovulation tests. It is more practical to have intercourse during the three days before and on the day of ovulation. We know that sperm can wait for the egg and survive for three days in the fallopian tubes and that the egg can be fertilised for 24 hours; it degenerates one day after ovulation. Ideally, intercourse should take place on these days, but it does not have to happen every day. Although you have a mission…let's try to keep the romance alive!

   Sperm move from the vagina to the fallopian tube at a speed of 2-3 mm / minute. Thus, 45-60 minutes pass between

ejaculation and reaching the egg. You can be pregnant just a few hours after intercourse!

2. **You must take** folic acid. The neural tube of the embryo begins to develop very early, before many women even know they are pregnant. Folic acid helps to prevent serious alterations of the spinal cord (such as spina bifida) and the brain (anencephaly) as well as other defects such as cleft lip. During pregnancy the folic acid requirements increase; that is why it is important to start taking it a month beforehand and to continue taking it throughout the pregnancy. In doing so the risk of these alterations is reduced by 70%.

   The main food sources of folates are vegetables. But they are very sensitive to all cooking processes and the majority of their content stays in the cooking water, the steam or the oil. Eating them raw is not a solution either, because there are individual differences in the degree of absorption of folic acid in the diet, and the pharmacological format is absorbed better than the food source.

3. Now is the time to go for a gynaecological check-up and find out what your **ovarian reserve** is.

   The ovarian age is not the same as the age on our identity card. When we talk about the probability of getting pregnant in each cycle, doctors are referring to the ovarian age. It can be a year older or younger than your chronological age, and it is important to know. Today, the best indicator is the level of the **anti-Müllerian hormone** in the blood. The **basal antral follicle count in the ultrasound** is also a good indicator. The number of eco-negative (black) images in your ovaries is counted. As you know, we are born with a predetermined number of eggs, and from puberty onwards, eggs are lost every month. If you take the pill, and even during pregnancy, this process does not stop.

   Some women are born with a lower number of eggs, or lose more than is habitual each cycle. This is part of the innate fertility of each woman. It is partly genetic, but I also think that the level of toxins accumulated in the mother has an influence.

In advanced societies, women want to have children later; however, the toxic setting of industrialisation works against them, since this can mean that fewer egg precursor cells form. These cells are called "oogonia".

4. It is important **not to neglect your relationship with your partner** and continue with the same sexual habits. It is very possible that you are orienting your sex life exclusively towards your goal: for sperm to reach your prized egg that month. And the days that you are not fertile your libido reduces or even disappears. Today we know that a few days' abstinence is not required to improve the quality of the semen: it can optimise the number of sperm in the ejaculate, but it worsens other parameters such as DNA fragmentation. Ideally, try to maintain the same frequency as always, making sure to have intercourse on the days around ovulation.

   Although you might not think so, your partner has probably noticed, and probably does not like it. They feel that some days you reject sex and other days you want it, and they never know how you will react. They think you have changed from having sex for pleasure to having sex as a chore. I know that you have an objective…But surely you can combine it with your ability to seduce and make him be the one to be pursuing you those days, so that it looks like he is the one ovulating.

   Every time you get your period it is a drama, and the hormone drop associated with it makes you feel more sensitive. I think it is a good idea to allow yourself to get upset, and then quickly focus again on your action plan. With the information you have at hand, you can decide the time you think is reasonable to wait before considering that there may be something preventing you from getting pregnant. The maximum time you wait should be one year. In the meantime, enjoy your mission; I'm sure you have plenty of less thrilling ones.

What you should not do. False myths

Bingo! **Rose's** period hasn't come. She stopped taking the pill last month and now she's three days late. She is feeling a mixture of emotions: this is what she wanted, but everything has happened so quickly that "she hasn't found the moment" to tell her partner that she is no longer taking the pill. How will he react? He hadn't asked her anything either… and if he didn't want to have a baby he would have worn a condom. She wants to get pregnant, but now she is thinking that it will end in a baby and, at 25, she can't imagine breastfeeding, going to the playground with a buggy…How scary!

She goes straight to the chemist and does a pregnancy test that is negative. She repeats it five "unbearable" days later and…it is negative again. Three days later she gets her period. Her friend Patricia had told her that when you've **taken the pill for a long time**, you have to wait a few months to get pregnant. But that is not true. You can start trying to get pregnant as soon as you stop taking it. I suppose this false belief has come about because when you have not ovulated for several cycles the first ovulation can be delayed. This is what happened to Rose.

In the clinic we continually hear false beliefs that I would like to mention. A lot of people think that **each month we ovulate from one ovary** and this is not the case, but I have even seen it written in school text books. The egg is a cell that is inside a follicle and both have to develop. The follicles pass through the following phases: primordial, primary, secondary and tertiary. It is in this fourth phase when they are mature. The primordial follicles lie inside the ovary, and three months before the cycle in which the ovary ovulates, many start to grow. Tens of primordial follicles become primary follicles in a process known as "recruitment". But, gradually, they are left behind, they degenerate. Thus, few become secondary follicles and only one becomes tertiary (it measures 20-30mm). If there are two finalists in this cycle it could

lead to twins. The dominant follicle produces substances that prevent the others from developing.

This all occurs in the ovarian tissue. It does not matter if it is in the right or left ovary. When a woman only has one ovary she always ovulates from this ovary. If she has both, the process is random; she can ovulate for several months in a row from the same ovary.

Unfertilised eggs are microscopic cells that are eliminated by a type of white blood cell called "macrophage" which is part of our cellular cleansing system. They are not expelled with menstruation! They are collected by lymphocytes and taken to the bloodstream.

The age at which a woman has her first period has no relation with the age of menopause. It is very common to hear: "I got my first period very early, so I will have a late menopause". This is not true.

Marta, before telling Edward that she wants to get pregnant, starts to prepare herself and read up on the internet about "the best advice to make it happen quickly". She presumes that if he agrees, she should conceive quickly; she is afraid that if it takes too long or if she needs some sort of treatment, he might regret it. So she starts a list of healthy lifestyle changes: eat salads, fruit and vegetables, lose 3kg, go to the gym, stop drinking beer and gin and tonics, avoid being around smokers… Oh! And avoid stress and anxiety! She also decides to practise new positions in bed and to stay in a position with her legs bent so that the semen does not come out. Of course, all of this is very healthy, but I can assure you that it is not going to affect her possibilities of conceiving.

Elisabeth does not need the internet to know that she has to take folic acid. Actually, she started taking it three months ago, just in case. She also told Jack that he must immediately stop smoking and drinking alcohol, and she has bought him new boxer shorts so that he will stop wearing those underpants which restrict his testicles.

I would now like to tell you some of the conclusions of the semen quality studies that I had the honour to direct. The goal was to analyse the characteristics of the semen of Spanish men and compare the results with those of men from other countries and other provinces and try to determine the implications of environment and lifestyle.

105,525 data of both sociological and medical interest were processed and the conclusions call into question the influence of lifestyle habits traditionally associated with infertility and demonstrated that the **quality of semen is mainly determined by the environmental pollution of the place where this man grew up**.

Moderate **alcohol** consumption does not affect the quality of semen, but in men who drink more than three glasses of liqueur per day the concentration and motility of the sperm decreases. This amount is considered alcoholism.

Smokers do not have poorer quality semen than non-smokers. The seminal parameters do not vary according to the number of cigarettes smoked per day. However, separate to these results, it is important to highlight that multiple scientific works have demonstrated that some substances contained in tobacco (such as benzopyrene, cotinine and pesticides used on the plant leaves) reduce fertility. They can affect the functions of the sperm membrane and alter penetration of the egg. Other studies observe that tobacco causes oxidative stress, damaging the DNA (genetic code) of the sperm.

The consumption of **drugs** *apparently* does not lead to poorer quality semen. Men who habitually take drugs presented better seminogram parameters. This is surprising, but it can be explained by the fact that these substances activate the spermatic function and that of the sperm, although at the end, on their way to the egg, their energy diminishes and therefore, so does their ability to fertilise.

Men who suffer from **stress** have a lower concentration of sperm. No other seminal parameter is affected and there is no variation depending on the level of stress. Those who experience stress have the same ejaculation frequency as those who do not. To date, on a medical level, the action mechanism via which stress can cause this has not been determined.

Men hoping to conceive ejaculate less. The feeling of a "chore" blocks the male desire and the woman orients sexual intercourse around her fertile days.

The frequency reduces as the desire to conceive increases:

- Men who are not trying to conceive: 3.2 ejaculations/week.
- Men who have been trying to conceive for one year: 2.6 ejaculations/week.
- Men who have been trying to conceive for two years: 1.2 ejaculations/week.

Semen quality increases with ejaculations

The quality of semen is significantly better in men with a higher number of ejaculations, and it increases in direct proportion to a greater frequency. It is good stimulation for the formation of sperm in the testicles. This is logical; function trains the organ, just as occurs with muscles.

Testicular injuries do not have any repercussions on the fertility of men affected. Nor does carrying their mobile phone in their trouser pocket.

It has been calculated that only half of women who do not manage to get pregnant seek help, and only 22% receive medical treatment.

The main reasons are economic, moral and religious, which lead them to accept the situation or reject the treatments, a lack of information or inadequate information, not having family support or simply preferring to hide it.

When they seek help often it is only for **alternative treatments to increase fertility**, such as homeopathy, medicinal herbs, acupuncture, repositioning of the uterus, mud baths, etc. All of this may reduce anxiety, but there is no scientific or statistical basis that confirms their effectiveness.

Additionally, they may delay the age at which they access medical treatment, and this can have consequences.

As we have mentioned, the degree of fertility is a characteristic of each person and therefore the possibility of conceiving depends on the fertility of the couple. Some couples have a low suc-

cess rate (for example because only a small proportion of their embryos are good) and they may think that anything they did during the months prior to the pregnancy was the solution. I think this is also what happens when conception occurs after adopting a child.

4

The desire to have a baby and new family models

The desire to have a baby and to go to unforeseen measures to make it happen is something that has not changed in the history of humanity. What have changed are the motivations, and the ways of making it happen. Reproduction specialists are meeting new profiles of patients which reflect the changes in our society, a new social horizon characterised by:

The waxing woman, the waning man

"Man longs for a woman who no longer exists, while woman seeks a man who has still not arrived". In this regard, the psychologist Jack Urra means that "Don Juan" retired a long time ago. Men's identity was based on power and on the world of work, but with the incorporation of women, the roles have become blurred, and men are waiting to find their place again. Male values have decreased in value and need to be redefined. A revolution like that led by women awaits men. We have moved from retrosexual to technosexual, metrosexual and ubersexual (virile and refined)... Now there is talk of a new type of man, the emotional-sexual type.

Men today feel out of place. And in the meantime, women are bearing a double load. Nearly all women find more support in their mother than in their own partner. Less than half state that at home the household chores are shared. They feel that what was sold to us, the so-called "women's liberation" was a bad deal. As the writer Vincente Verdú says, "now it is time for a model of a couple in which the woman tends to be right and the man tends to be guilty".

Consequently, women believe that they no longer need a man at their side to live a fulfilled life, and they are considering motherhood on their own. They replace love for a man with love for a child. Furthermore, today it is very socially accepted: 92% of women do not criticise women who decide to have a child without a stable partner. And nor do men.

My father was a typical man of his time. I have two sisters and on one occasion, when they were very small, they said they were going to marry engineers. My father smiled at them. I always wanted to be a doctor, but at that moment I stated: "I'll be an engineer". He looked at me and answered: "A woman without a man is nothing". I remember being very upset, especially because I thought that there were only two engineers and that my sisters had already taken them. When my father was 80 he asked me to help a friend of his to have children. She was a very competent 42-year-old lawyer in Ponferrada. I was surprised by the evolution of his thinking: he explained to me that his friend did not want a husband, she wanted a child and she thought that it was better not to deceive anyone to get pregnant.

We are seeing it in the clinic: we are attending a growing number of single women seeking treatment with donor sperm because they cannot find the ideal man with whom they would like to conceive. In four years, the number of single women seeking assisted reproductive technology (ART) has multiplied fourfold. Some have visited with their boyfriends, but they wait outside because they want to remain completely separate…They are *satellite boyfriends* who will be *satellite fathers* who give up on fatherhood before they even try it…

Lesbian women who want to have a baby with their female partner also visit our clinic. They decide who will carry the baby

(gestational mother) and who will give their eggs (biological mother), but the baby is both of theirs.

We also see women who want to freeze their eggs because they have not yet met their ideal man, and they do not know if they ever will. They do not want to get frustrated every birthday, thinking about their eggs aging.

The well-known delayed motherhood

In more advanced countries, women tend to have few children and to increasingly postpone the age at which they have them. However, the desire to have children of women of reproductive age is the same as that of their mothers: 2.5 children. The difference is that in their mothers' day, they had them, and today they do not get to have them.

To contribute some data, I have selected a survey from the Spanish National Research Council (CSIC) carried out on 10,000 women aged between 18 and 65 called "Fertility and values in 21st-century Spain". The birth rate in the European Community is 1.46 children per woman. **Spanish women hold two world records: that of having only 1.2 children per woman and that of having them at the oldest age**. 31.5 years is the average age at which a woman has her first child in Spain.

This delay in motherhood and the decrease in the quality of semen means that 15% of couples and 18% of Spanish women require medical assistance to be able to be mothers.

Why do we have children later?

A. Contraceptive methods

Thanks to contraception, women can decide about their maternity, and this has become a right rather than a duty.

B. Cultural change: study and work

Women now study and are an integral part of the labour market.

The age at which a woman has her first child is later the higher her educational level: the average age for a woman to have her first child in women with higher studies is 33.5.

Women do not reject maternity, but they want to combine it with their career. This is impossible with a high number of children or with maternity before 30.

They know the huge effort involved in working hard in the home and out, looking after children and their partner and, in many cases, considering their age, their older family members too.

Before having children, 60% of women believe that maternity will be an obstacle for their professional life. Only 4% believe this circumstance is negative for men.

But what do they think when they have children?

- 81% think that having children hinders their professional life.
- 28% reduced their professional activity.
- 17% stopped working definitively. They were forced to choose between work and children.
- 28% left their job for at least one year.
- 8% mentioned that they suffered discrimination on the grounds of their maternity.

C. Socioeconomic changes

The cost of housing is a key factor: 30% of under-30s still live with their parents, and they marry later. 80% of women now aged between 30 and 34 have lived with their partner before marrying.

Women share the household expenses and the mortgage. They are aware of the divorce rate and they want to be financially independent.

Beforehand, women went from financially depending on their father to financially depending on their husband, and today they know that a husband might not be forever and are aware of the need to be financially independent. Moreover, the majority of families make ends meet thanks to both salaries.

There are huge differences between countries as regards the laws surrounding the reconciliation of professional and family life

and the money allocated to social grants for maternity. Having seen the statistics of the number of children per woman in countries with a similar culture, it is clear that this is a very important factor.

The cult of eternal youth and delayed ageing

We see this in our clinic: women on the cusp of 50 who are absolutely desperate to become mothers with a new younger partner. It is a common phenomenon in Anglo-Saxon countries. They feel rejuvenated, and they do not understand that everything about them is marvellous except their ovaries. They beg us to accept them as patients, and we insist that it would not be ethical to do so, on account of the risks entailed in such a late pregnancy.

The British author Agatha Christie used to say that "an archaeologist is the best husband a woman can have, the older she gets, the more interested he is in her". In Spain, we have not yet observed this phenomenon. Rather, we see what the psychologist Walter Riso called the principle of Tarzan: "men don't let go of one liana until they are holding on to another younger one".

All of these social changes are reflected in the media; they are normalised and they fuel each other.

Men are beginning to affirm their right to be single fathers

The current feminisation of the world is enabling men to affirm their right to be sensitive and, like women, to become single fathers. We have provided treatment to friends and work colleagues who decide to have a child together.

We receive requests from homosexual and heterosexual men seeking information about having a baby with a surrogate mother in countries where it is legal. This is also fostered by the difficulty in adopting children.

Men are also going to unforeseen extremes to become fathers. We are asked for in vitro fertilisation by "suspicious" couples, in other words a European man aged 50-60 and a Vietnamese or Ethiopian woman aged 30 who do not understand each other in any language. We do not know up to what point we should intervene morally, but it seems clear that they are agreements made online.

It would appear that maternity and paternity are beginning to become dissociated from the emotional commitment. Reproduction is becoming dissociated from life as a couple and from sex. Having a child is beginning to be considered more as a personal project than a family one.

The appearance of new families

The philosopher José Antonio Marina says that: "the family was very stable while it was an economic institution necessary for survival. In poor societies single people do not survive. When the economic situation changes, the emotional purposes of family come first, and greater expectations, and at the same time, greater possibilities of failing, appear".

Today relationships begin and end, life expectancy is increasing so much that monogamy is becoming difficult. Divorce and second marriages mean that a considerable proportion of people want to have children with their new partner. A lot of the time the man had had a vasectomy because he did not want any more children and now whether he wants to or not, he feels "obliged" because his new wife has not had children.

The writer Eduard Punset explains it well. Beforehand we used to die at 40. Now at this age many have completed their life project: this is their house, their job, their wife, their children, their environment, and…now they find themselves wondering, "another 40 years like this?" Often they lose their lust for life and they seek out changes that motivate them.

It would appear that the old motto "*until death do us part*" has become obsolete.

There is a whole catalogue of family models that visit us in the clinic:

- **Simple families:** two spouses without children.
- **Two-parent families**: two spouses with children.
- **Single-parent families**: one person, with children. It tends to be the woman. The father may be a sperm donor. It could also be a *deceased father* who passed away before conception and left frozen semen for his widow.
- **Reconstituted or blended families**: they bring together children from various relationships.
- **Same-sex parent families**: a homosexual couple with or without children. According to the laws of each country, maternity between lesbians and paternity between homosexuals is recognised or not.
- **Families "living apart but together"**: a new phenomenon, they live apart but are together, each partner in their own house.
- And we have even invented the … **"unipersonal family units"**: families of one.

Are we as specialists bringing about these social changes or are we simply responding to them?

5

HELP! I can't get pregnant

Rose is angry with the world, and very concerned: she is sure there is something wrong with her. This month she had even snuck into the hotel where the FC Barcelona players were staying for the Manchester match and, during their break, when James's room-mate wasn't there, they had sex. She had managed to get in by pretending to be a waitress. She did it because they are crazy about each other, because she knew he'd love the surprise and because she had bought an ovulation test that told her that that day was the right day.

She rings her friend and screams at her that her period has come. Lourdes asks her if she has sanitary towels…She thinks that that's all she needs! What sort of friend is she? She just doesn't understand her.

Right then her partner arrives home and she hugs him and tells him everything. To her absolute surprise, he cries out of the emotion and tells her that he wants everything with her too, a baby and more, and tells her to look for the best clinic to check if there is anything wrong.

Marta doesn't know how to tell Edward that she wants a baby. Since she decided she hasn't found the right moment to tell him because his teenage daughters and his ex-wife are unbearable and are the topic of conversation every day. Finally, she has prepared the scene: a weekend at the beach, dressed in new clothes and with a glass of wine in her hand she tells him that she's in love with him, that she'd like for only the two of them to exist when they're together and that she can't imagine her life without a little one that looks like him running around the house.

Edward doesn't say a word. His eyes are like saucers and he makes faces of surprise, amazement, fear…Finally he talks, avoiding Marta's eyes, and he tells her that he loves her too, that he also would like no one else to exist when they are together, and that that is why he doesn't need "anyone else".

On Sunday Edward leaves her in her apartment and says goodbye to her as if nothing had happened, as if the discussion was over.

Marta spends the night crying, but on Monday she gets up with determination and calls her friend Margaret because she knows that she donated eggs. They have a meal together, and Margaret tells her that whatever treatment she has, it is a straightforward process. She encourages her and tells her that she will go to whatever clinic she wants with her. Marta joins a forum of single mothers and considering her finances, chooses a low cost centre that puts on special offers for single women who take on maternity on their own.

Elisabeth has a tampon in one hand, and a handful of tissues in the other, and on the shelf of the bathroom a pregnancy test that she was going to do tomorrow. Her face is streaked with mascara.

She hasn't told anyone that she and Jack are trying to conceive and now, at this precise moment, she wants to talk to someone. Someone other than Jack because the last five

times her period came he said the same thing: "Don't worry darling, you'll get pregnant another month".

First she needs some consolation, someone to cry with, someone who tells her that she is doing everything she can to conceive and then that she needs to look for solutions.

But even still, she prefers not to tell anyone. She could talk to her mother, but…

What if Jack is the problem? She would be betraying his privacy. She could mention it to her friends Nuria or Patricia…No, she had better not. Because they got pregnant quickly. They don't know how she feels in this situation and what's more, she's the efficient one in the group.

She decides to pull herself together and act. She doesn't know whether to ring her gynaecologist or to look up reproduction clinics on the internet. She has a good relationship with her gynaecologist who has done her check-ups for years, but she'd prefer to see an expert directly. She wants the fastest and most effective solution. So she cheers up, logs onto the internet and prepares an Excel sheet with a table comparing what she considers to be the pros and cons of the Barcelona clinics that come up in the first pages of her search. They all have the best success rates, they all speak wonders of themselves and all the feedback that appears is fantastic…She doesn't know which one to choose. She is also surprised by the difference in prices. Thinking about it she remembers that her cousin Carmen, a rheumatologist, had twins through in vitro fertilisation (IVF). She calls her because she thinks that as a doctor in Barcelona she would surely have made the best choice. "What should I consider when choosing a good fertility clinic", she asks her?

—"Choose a centre that treats you well, because they will probably treat your embryos the same way. It is also important to assess the degree of continuous innovation because assisted reproduction is a science that is making continuous breakthroughs and you deserve the best", Carmen told her.

Choosing a clinic

Some people might think that the worst thing that can happen to them is that their pregnancy test is negative. This is not so; there are worse things.

I am increasingly concerned that I am seeing a growing number of patients who have had badly oriented and poorly performed previous reproduction treatments. The reason for this is the proliferation of low cost fertility centres. Some are new, and others used to work well, but during the recession found themselves obliged to lower their prices and, consequently, the quality of their service.

Why are some reproduction centres more expensive than others? I would like to tell you my view of the topic, as a doctor and also as the managing director of the *Institut Marquès*, with the information that I have about the financial management of medical centres.

In reproduction clinics, the majority of the budget is allocated to staff. The most outstanding doctors and biologists work in the best centres. This happens in many other fields: the most skilled Formula 1 drivers are on the best motor-racing teams, the most talented football and basketball players have contracts with first division teams. The best professionals seek the highest salaries, and the prestige and guarantee of a good brand.

Health professionals seek, in addition, ethics, scope, innovation, to be able to work with state-of-the-art technology, attend conferences that help us to keep our knowledge up-to-date, belong to a team in which there are excellent specialists in all areas and to avail of all the time we might need to look after our patients.

This means a higher economic amount, not only because of the salaries, but because the centres that have teams of this level need to have more staff members so that they can devote part of their time to producing scientific works, to attending conferences and seminars, to participating in projects, etc.

On the contrary, lower budget centres hire doctors and biologists with less experience or a lower professional capacity. All that

is valued is the number of patients that can be seen in each session and that the administrative guidelines outlined by the company are followed. Nothing else is demanded. In general, they are looking for very short-term profitability, because in many cases the company is owned by a capital risk type investment company. It gains profit and sells regardless of the quality or the number of complaints or accusations. In three or four years they will have gone.

Other economic areas may be allocated a greater or lower amount from the budget and the level of quality also depends on these. I am referring to the facilities, the number of people looking after the patient, the sanitary material, the educational training, the continuous research and innovation and having all the services internally, although these are not profitable.

How does all of this affect the patients?

The consequences of all of this affect the patients in the indication of the suitable treatment for their particular case, in the results of their cycle, in the safety during the laboratory process and in the choice of their egg or semen donor.

In low cost centres, when indicating the suitable reproduction treatment, the doctors and biologists do what they can, and it is not always the best option for the patient.

We see cases in which an assessment by andrologists or geneticists was required and it was not provided because the centres did not have these specialists on the team. 40-year-old patients arrive to our clinic after having performed in vitro fertilisation; they had 8 embryos, 4 embryo transfers were performed and there was a pregnancy in the last one which was interrupted by a chromosome anomaly that was detected when they performed amniocentesis. All of this could have been avoided with a genetic analysis, but in the centre they were not told this because they did not have an internal service. If they were to request it from an external laboratory, their income would be much lower, since they would have to pay this external centre, and consequently they would no longer earn money off all those cycles of transferring frozen embryos.

The patients who we receive for the first time have an average of four previous failed treatments. In many cases these cycles have been performed very well, but we are increasingly encountering treatments that had practically no possibility of succeeding and the couple was made lose their time, hopes and money. In particular I am referring to in vitro fertilisation punctures with only one follicle and/or hormonal analyses that reveal very low egg quality.

The success of the cycle depends in many cases on the complimentary treatments indicated: DGP, IMSI, EmbryoScope, PolarAide…, which we will discuss later. In those centres that do not have them or do not know how to perform them, these are simply not indicated, with everything this entails. Additionally, in good centres, clinical sessions are held in which the different specialists participate, helping to manage the cases more efficiently. The doctors are not pressurised to perform cycles regardless of the prognosis.

The results of reproduction treatments

The results of each centre depend on the skill of the biologists and on whether there are specialised biologists in each technique. In low cost centres the same biologist has to do everything. They also depend on the culture media and all the material used in the laboratory, which of course can vary considerably in price.

Ovaries and embryos do not take into account bank holidays, and neither do good centres. Their activity is the same every day. When centres try to avoid medical and laboratory activity at weekends (fewer shifts to pay) this has considerable oscillations in the results. I know of centres that only collect eggs on certain days of the week!

The results also depend on each biologist's back-up team. All the processes with eggs and embryos need to follow very precise times. If one day there are many cases, or for example, many eggs are taken in the ovarian puncture, more biologists will be needed. If there are none, there will be delays.

Another important aspect is the quality of the laboratory installations and the monitoring carried out on the environmental

conditions of same (temperature, gases, pH of the culture media…). An IVF laboratory can be set up with very little money and it can work, but not in the same way as one with the best technology. The embryos do not sleep as well in a one-star hotel as in a five-star one. This requires continuous investment and in economic terms it is not profitable. Additionally, the patients cannot see it. That is why, in order to ensure that quality standards are being complied with, it is important to demand that the selected centre has official certificates supporting its correct operation. There are certificates of different levels; the highest assisted reproduction laboratory certificate is the UNE-179007.

The importance of safety in in vitro fertilisation laboratories

As I have mentioned, some people might think that the worst thing that can happen to them is that their pregnancy test is negative. This is not so. To ensure that each egg is fertilised with the corresponding semen or to transfer each patient's embryos without any potential confusion, in high level centres the staff work in pairs. Each biologist must monitor another. A biologist is not allowed to work alone on any task, not even at the weekend; there is always someone else supervising. This is expensive, but it reduces the risk of error.

Embryos are very sensitive to the physical conditions of their surroundings. Although there are obligatory controls to detect contamination in the laboratory installations, many other control measures are needed as regards the quality of the air, temperature, humidity…Inevitable accidents can always occur; for example, a tube of water breaking or contaminated material entering, but what differentiates one laboratory from another is the ability to detect it and resolve it without it affecting the embryos that are being cultured that day.

I recall that I had to confront colleagues from my team when I decided that we needed two laboratories, separated by a reinforced door, because the investment and maintenance was double the cost (for example, two exterior air renovation and filter

machines instead of one). Sometime later we had a problem with the environmental humidity levels and thanks to this measure we could move the culture plates with the embryos to the reserve laboratory and nothing happened. As the saying goes, sometimes you get what you pay for.

The security level of the embryo and highly-prized semen tanks is also very important (for example, freezing before performing chemotherapy) to avoid robberies, sabotages, misconduct…

If you are sure that you are doing your job well you can be transparent (good restaurants show their kitchens). In my centre, patients can see their embryos from home; they can watch the EmbryoScope video live. This practice is not very widespread and many centres are amazed that we do this, and ask us if we do not have legal repercussions. No we do not. We have never had any. On the contrary, our patients value and appreciate it because it is an exercise of transparency, of sharing information and emotion, and because we do not have anything to hide.

There is a very worrying statistic: in the cheapest centres, cycles are not ruled out if they have a poor response, and embryo transfer always takes place, even if these are of poor quality.

Investment in safety is expensive and is proportional to the price of the cycle. All of these extra safety measures are not obligatory. What I have mentioned here can occur despite complying with the measures set out by the health authorities in any country.

Choice of egg and semen donors

When donor semen is required, gynaecologists tell their patient that they will assign her one with very similar characteristics to those of her husband, or if it is a woman without a male partner, that they will assign the one that they consider the most suitable. If the centre has its own bank, the gynaecologist will see all of the donor's characteristics, even his photo, and they can choose from many. They have invested a lot of effort and resources in the selection of these donors. Think that for example, in our sperm bank, only 4% of candidates are admitted. On the contrary, if the centre does not have its

own sperm bank, the gynaecologist cannot choose and will request the donor semen from an external bank, and of course will not see photos.

If we are only talking money, a sperm bank is not profitable. Egg donors prefer to go to those centres that they consider better, more luxurious. According to the quality level, each centre rules out a greater or lesser number of male or female candidates. They rule out short-sighted people, people of short stature, people with a lower standard of education, people who have consumed drugs, etc. The donors who are ruled out go to other centres with less strict selection criteria.

Donor assignation can be performed in many ways, but it is performed best if as a doctor you can choose from many, and dedicate it time and enthusiasm. And all the better if your centre allows you to assign two donors per patient, in case one of these has to cancel (due to low response, flu, personal reasons…).

Knowing the importance of donor selection, at times I wonder how even for this treatment, some people choose the cheapest centre. If it does not go well, what can you do afterwards? I think that egg donation treatments should be performed in a clinic you fully trust, since, as you can see, there are many aspects that you cannot control.

I believe that medical centres must organise themselves in such a way as to have the most skilled professionals, the best facilities and the highest standard equipment with the sole aim of looking after and treating patients. They should not be a business in which some groups invest money simply to gain profit in the short term. I am horrified to see how they try to attract people with few resources to achieve their greatest dream, and they are deceitful when they present the results and the quotes. Often, people tolerate the fact that cheap places provide lower quality, but we should bear in mind that in this place, all the services will be of the same level. If when you have a doubt or a problem nobody is able to look after you well…the same thing will happen with your embryos. As a doctor, all of this makes me feel ashamed.

6

The first visit to a fertility clinic. Objectives and key tests

Objectives

The goals of this first visit are:

1. To write up a clinical history.
2. To discuss and request the first study tests.
3. To establish a good doctor-patient relationship.

The clinical history is written taking into consideration aspects related to the couple's fertility. The most important are:

- The age of both partners.
- History of previous pregnancies. This also includes previous pregnancies in the man's past relationships.

It is important to know if the pregnancies evolved or ended in miscarriage, since there are alterations which manifest as a difficulty to achieve gestation and, if eventually there is a pregnancy, a miscarriage occurs. "Sterility" is the inability to conceive, and "infertility" is the difficulty to bring the pregnancy to term, in other words, having miscarriages. What I would like to highlight is that the cause of sterility and infertility is at times the same. **It is impor-**

tant to perform a complete diagnosis of the origin of the problem before providing treatment; I cannot explain how sad and frequent it is to receive patients who when they finally conceive realise that it will not evolve.

Other factors to assess are:

- Possible hereditary diseases.
- A history of gynaecological illness such as pelvic infection, fibroids, endometriosis…or surgery on the uterus or ovaries.
- Characteristics of menstruation.
- Weekly frequency of intercourse. My colleagues and I tend to ask this question looking at one member of the couple because interestingly they tend not to provide the same response. And of course this is not due to sexual relationships with other people. If she replies, she may only refer to the ovulation week, thinking that it is what most interests us, and say every day.
- Male's profession: since some jobs may be a risk for fertility; medicines and urological history or infections.

How much time needs to pass to consider that a couple has fertility problems?

Although traditionally a year is considered, it is best to adapt this time to each couple's situation and to personalise each case. There are situations in which it is best to start from the moment at which the couple expresses their desire to achieve pregnancy. For example, if the patient has alterations in her menstrual cycle, a history of endometriosis, pelvic operations or infections, if she is more than 35 years old or if the male has had genital or urinary disease.

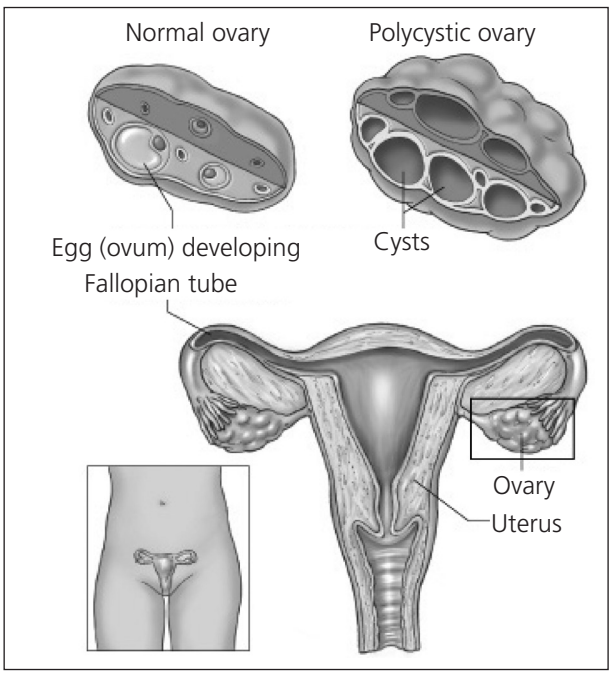
Another of the aims of this first visit is to discuss and request the first study tests.

The first study phase revolves around ruling out the most usual causes. In order for pregnancy to occur the following is necessary:

1. Ovulation must occur and the oocytes must be apt, in other words, capable of giving rise to an evolving embryo

ARE YOU OVULATING?

If you have normal menstrual cycles we can presume that you are ovulating. Only if you have alterations in your cycle such as menstruating every few months could there be a possible ovulation problem.



An ultrasound and hormonal analysis help us to make a diagnosis. The ultrasound allows us to look at the appearance of the ovaries. Some women have delays in their periods every month because their ovaries are polycystic and they do not ovulate regularly. In this examination, we also count the tiny follicles as an indication of the ovarian reserve.

In other cases, alterations in periods and ovulation are due to high levels of prolactin in the blood. This hormone is produced when a woman is breastfeeding, but a functional disorder of this hormone is common and the patient finds herself in a situation similar to that of breastfeeding.

ARE YOUR EGGS GOOD QUALITY?

Since chapter two, you have become an expert, so now you know that the quantity and quality of the ovarian reserve decreases with age, and that the ovarian age does not always coincide with the biological age. The follicular total is established at birth, and as the years go by, the number of eggs that are susceptible to being fertilised and to giving rise to a full-term pregnancy reduces.

To determine the egg quality, a **blood test** is requested, called the "basal hormone analysis". This must be performed on the second or third day of menstruation, in order to assess the levels of FSH, the hormone that stimulates the development of the follicles, LH, the hormone required to induce ovulation, Oestradiol, the hormone produced by the follicles that are growing, and Prolactin.

It is important to know your ovarian age from the very beginning, and in women aged over 35 we also analyse the anti-Müllerian hormone.

Anti-Müllerian Hormone
Normal values according to the woman's age

Woman's age	Quantity of mg/dL
20-24	3.97 (3.55-4.33)
25-29	3.34 (3.03-3.87)
30-34	2.76 (2.34-3.55)
35-39	2.05 (1.76-3.24)
40-44	1.06 (0.76-2.13)
45-50	0.22 (0.12-0.49)

2. The semen must be fertile

To determine whether the semen is fertile or not, a seminogram is requested.

An ejaculate must contain more than 2 ml, more than 20 million sperm/ml; 25% of them must be able to move well, and 4% of them must have a normal shape.

When the seminogram diagnosis is **Normozoospermia** it means that the sperm are normal.

It is interesting that the day a patient analyses their semen for the first time is a very important day. They want to do whatever they can to make sure it is a success.

The patient receives the following **Instructions for collecting a semen sample:**

1. The sample can be collected at home or in a special collection room in a clinic.
2. Remain sexually abstinent for three to five days. This means no semen loss whether through intercourse, masturbation or any other circumstance, during said days.
3. When you hand in your sample you will be asked for your National Identity Document and the corresponding request form. Without these documents no sample will be accepted.

HOW TO PROCEED:

1. A sterile bottle is needed. This can be bought in a chemist.
   No other container will be permitted. A commercial condom will not be accepted. However, if necessary, a condom without spermicide can be requested in the centre, and placed inside the sterile bottle once the sample has been collected.
2. You will be given a label which must be filled in with the name, date and time at which the sample was collected. The label must be placed on the external part of the container. Also write the days of abstinence and if the sample is complete.

3. It is advisable to pass urine before collecting the sample. The skin of the penis must then be washed with soap and water, ensuring that no soap remains on the skin which could alter the result of the sample. The sample is collected through masturbation. It is important to collect all the semen. If some drops are lost or if it spills it may interfere with the result and it must be labelled as an incomplete sample.
4. The bottle with the semen sample must be handed in within one hour of being collected. It needs to be processed as quickly as possible to separate the sperm from the seminal liquid.

   The adventure begins with the request form and information. How to collect the sample seems obvious, but perhaps we need to explain it more to avoid surprises, since sometimes we receive samples that look like a murder has taken place: all the sperm are dead after having used a condom.

How should I deliver the container?

Many people want to make sure that the sperm do not escape from the container, and they place cellophane on the top of the bottle. The most fearful ones use surgical tape. Presentation of the treasure is also important and here we see a lot of variety: from wrapping it in tin foil, to the most original bags from the different shops where they have bought food and clothes, etc. When we have performed population studies with young people, some even brought their samples in jam jars and very narrow plastic tubes (I don't know how they managed to aim properly!)

How should I transport the bottle?

If people are delivering the bottle by car, they ask us should the air conditioning or heating be put on. This does not affect it. If they are delivering it by motorbike we ask them to make sure it is held correctly. One patient had it in his jacket pocket and it fell out; his semen ended up spattered all over the window of the car behind him.

How can I take it on time?

The men ejaculate and run out to deliver the sample within an hour. When they leave it in the clinic they are surprised that the person who collects it does not also run to deliver it to the laboratory, as if it were a relay race. What they do appreciate at this moment is that we do not look at the bottle because they always think that a small quantity has come out. It is true; 2.5 ml takes up very little room! The question "should I have filled it more?" is very common. The answer is that the quality of the semen does not depend on the volume. The liquid is produced in the seminal vesicles and in the prostate and it is only a vehicle for the sperm. What we do know is that good sexual stimulation will lead to a better sample.

How can sample confusions be avoided?

This concerns patients a lot, and it concerns us too. We always check in front of the patient that the bottle is correctly labelled with their name, and in our centre, his photo is checked on our computer system to ensure that it is the same man. If his wife delivers the sample, she has to present a document signed by the man. Strict controls are continued in the laboratory.

Lastly, I would like to mention a couple of things in relation to the days of sexual abstinence prior to collecting the sample. For a seminogram, three to five days are indicated as standard, but it is simply a reflection of reality, since this is the most usual frequency of intercourse between couples. But when the semen sample delivered is to use in the treatment, we increasingly give less importance to the previous abstinence, because if the man has not ejaculated for one day, there is more semen volume, but old and dead sperm also gather and worsen the DNA fragmentation. Additionally, to perform sperm micro-injection (ICSI), very few sperm are needed.

Thus, when the adventure comes to an end with a report that says "Normozoospermia", it is a weight off the man's shoulders! When the report from the first seminogram performed by a man presents alterations, before giving a definitive diagnosis, another analysis must be performed two weeks later because there are

great physiological variations in the quality of the semen. If the man gets sick and has a temperature on the day he was to give the sample, he thinks that it is better not to do it because the sperm might not give good results. However, the cycle to form the sperm is two and a half months; therefore, if the result is not good, it will be later on. An ejaculate may be worse than usual after having a temperature and not remembering it.

The male factor (male sterility) is associated with 50% of conjugal sterility; in 30% of cases as the only responsible factor, and in the remaining 20%, in combination with a female factor.

3. A uterus that is capable of receiving an embryo

We perform a vaginal ultrasound to rule out any uterine or ovarian disease such as fibroids, polyps (in both cases, if they are small and do not obstruct an orifice of the fallopian tubes it has been shown that they do not affect the womb and they do not need to be removed), endometriomas…We also assess the morphology of the endometrial cavity. The endometrium is the internal layer of the uterus. It develops every month in preparation for an embryo to implant. If this does not occur, it breaks away and leaves the body, in what is known as "menstruation".

4. The fallopian tubes must be permeable

What does it mean for the fallopian tubes to be permeable? It means that they allow the sperm to arrive from the uterus and meet the egg, that they are able to collect the egg that one ovary has released, that they provide the necessary nutrients to the gametes (the eggs and the sperm) and to the embryo during the days it has these in its interior. Additionally, after the egg has been fertilised, the embryo must be transported to the uterus, where it will arrive three days later. When the fallopian tube allows everything apart from the last stage, the embryo remains inside it and an ectopic pregnancy occurs.

The test that studies the permeability of the fallopian tubes is called a "hysterosalpingography". It consists of placing the patient in the gynaecological position and introducing a contrast material

into the cervix. Radiologically it can be observed how it fills the uterine cavity and flows through the tubes. If the appropriate preparation is performed with antibiotics and antispasmodics it is a risk-free and straightforward procedure.

If the hysterosalpingography is normal we will consider the tubes to be permeable, but it is not a guarantee that they are performing all their functions of nutrition and transport correctly. If there are alterations such as obstruction when the contrast is flowing or dilatations (known as "hydrosalpinx"), the treatment is in vitro fertilisation. The gametes will meet in the laboratory.

If both tubes are found to be blocked, a tubal factor can be definitively diagnosed.

What happens if one fallopian tube appears to be correct and the other is pathological? This is debatable, but if the patient has no history of surgery, and we know that the most frequent cause of tubal affectation is infectious, it is hard to imagine that only one of them has been affected. One was probably more affected than the other but neither of them will be operating correctly.

The main way in which the tubes are affected is through a sexually transmitted infection caused by germs that reach them from the vagina. Usually it presents no symptoms. It has no treatment. The tubes do not recover, but in these cases endometrial cultures of Chlamydia, Ureaplasma and Mycoplasma are recommended. If these are positive, antibiotics are prescribed to treat the endometritis (inflammation of the endometrium), since it could hinder the implantation of embryos. The man must also take the antibiotic to prevent reinfections.

When I have to tell a patient that her tubes are not healthy I try to be positive and immediately explain that she could have children via IVF, but often they insist on determining the origin of the infection. Since it does not tend to present symptoms it is possible that it happened many years ago. I try to encourage them not to start looking for guilty parties, I mean a list of their previous sexual partners or those of their husband. However, I don't know what must happen when they get home …

I have put the fallopian tube test last because, considering the high number of males with poor quality semen, it is better to assess the seminogram before requesting this test. Likewise, if a problem

requiring IVF is diagnosed through the ultrasound or the hormone analyses, this test will not be necessary either.

In the past, a surgical intervention called a diagnostic laparoscopy used to be performed to directly observe, from inside the abdomen, the uterus, the ovaries and the functionality of the fallopian tubes, but today this practice is no longer in use.

With these first phase studies the cause of sterility is determined in the majority of cases.

If all of these tests are normal, the couple pass onto the second phase, since we still need to determine whether fertilisation is happening correctly or not. This phase consists of implanting the embryos and the quality of these.

5. Establish a good doctor-patient relationship

Another of the key points in the first visit is to establish a good relationship with the patient. It is very important that you find a doctor in whom you have absolute trust, who is concerned for you and demonstrates it. But it is also essential that this doctor is part of a large team on account of everything I explained in the previous chapter.

Elisabeth and Jack arrive to the clinic on time for their first visit. They turn off their mobile phones and take out their file with the dates of the last menstruations and the exact days on which they had intercourse. They prepared it together and they present it to me, laminated.

—"We have been trying for nearly a year and we are not conceiving. How can that be?" they ask me.

—"Well, there might not be anything wrong at all. After a year, only 40% of couples your age have conceived. Another year later, another 30%, and one in five will only achieve it with medical help. I don't know why you have not managed to get pregnant but we will start a sterility study."

—"What do you mean sterility?" Elisabeth asks, looking outraged and surprised.

—"I know that it is a shock to find out that after a year of not conceiving we talk about sterility. It is a horrible word that provokes very negative feelings."

—"I never thought that this could happen to me, that you could tell me I'm infertile"…-Elisabeth complains.

—"Elisabeth, we will look for the cause and we will find a solution. But today, as well as asking you for some tests, I want to talk about how you are feeling, and we are going to draw up a plan so that all of this becomes a good experience."

—"Well, so far the experience is bad. My husband tells me that some days I reject him and others I look for sex, and that he never knows how I am going to react. But of course…I am focusing our intercourse around ovulation. He says that I don't enjoy sex anymore and he's partially right"— Elisabeth confesses.

—"Ideally, you should try to maintain the same frequency you have always had, making sure to have intercourse in your case on day 13 of your cycle. I also suggest that you talk about this topic among yourselves for a maximum of 15 minutes per day."

—"He's going to be very happy if I listen to you, and what's more, you have said that he only needs to do the seminogram. I have to do the hormonal analyses, the ultrasound and I don't know how many other things."

Jack takes her by the hand and tells her that he will do everything he needs to do, and that they will be together for all the tests.

Marta enters the office with Margaret. She is very confident and serious. She sits down, looks at me and says: "Doctor, I want to do an insemination with donor sperm".

I smile while I think that, from the way she is speaking, more than a desire, it sounds like a sentence. I explain that I need an ultrasound, the fallopian tube test and an anti-Müllerian hormone test to determine her ovarian age. If all of these tests come back correct, we can perform the inseminations.

I also ask her to think about which aspects of the whole process concern her the most, how she is going to explain it to her family and friends, and I ask her to tell me in our next visit when we discuss treatment options.

She tells me that she wants to have a baby on her own, and later, when I ask her if Margaret is her partner, she laughs and explains that she has a male partner, but that he does not want to have any more children, and that he is "out of the picture". When **Marta** told Edward that she didn't want to end up without children and what her decision was, they didn't speak for two weeks. Then one day he waited for her outside work and repeated to her that he didn't want to start again with the responsibility of having children, but that he didn't want to lose her. Eventually, they came to an agreement to try to share what they could; in other words, he would continue living on his own, and see her at the weekends when he didn't have his children, and financially support Marta's pregnancy.

She has put her feelings for him and his attitude to the side because at the moment the idea of getting pregnant is fulfilling her. She comes to my centre because Edward's wife had her babies here and he is going to pay the costs. His involvement is very strange: when Marta arrived with Margaret, the nurse said that they were waiting for her, and she met Edward in the waiting room.

Rose rang to make an appointment and to request absolute confidentiality because her "famous boyfriend", we'll call him James, does not want to be seen in a fertility clinic. They arrive all lovey-dovey; he is wearing sports clothes and she is wearing a lot of make-up and stiletto heels, dressed up to the nines for the occasion. At the beginning of the visit James is more interested in his mobile phone than in what I am saying, but he raises his head when I mention the seminogram as a necessary test. He is very nice, and with a tone of humour and irony he tells me that he was worried about where he had to "collect the sample". But when I give him the instructions, he starts to laugh and says that he was

thinking of doing it at home in case he was recognised, but that after reading the instructions, and finding out what he will find in the "collection room", he is not going to miss out on that experience.

7

Semen quality. Factors related to male fertility

When we ask a man for a sample for a *seminogram* or for other studies, there does not tend to be any problem. He collects the sample at home, and brings it into us. But when it is time to give a sample for in vitro fertilisation, things change. In general, they are nervous, rendered oversensitive by the responsibility of having to do it properly. They are afraid they will not be able to reach the necessary arousal in an unfamiliar place, with a clinical environment, at the specific time they are asked and often with their wife in surgery having her eggs retrieved.

If on top of this the cause of sterility is theirs, their involvement increases even more on account of a certain feeling of guilt. The doctors ask them if they think they are going to have problems that day. The majority say no, others prefer to bring it from their home or hotel, or to freeze it, fearing that there may be difficulties. They really appreciate being able to say this to the doctors and to see that it is very common because they cannot complain to their wives or reveal their fears, since the answer is usually "well, darling, it's the only thing you have to do…"

The "no problem" men tend to leave the collection room with a "mission accomplished" expression on their face, but fear that the sample is of less quantity or poorer quality than other times.

The quantity of semen (volume of ejaculate), the quality of this and the level of orgasm depend on the degree of sexual excitement attained previously. This is better the higher the intensity and duration of arousal. This is because there is increased vascularisation of the seminal vesicles and of the prostate which are the glands that produce the seminal liquid and thereby lead to a full ejaculation.

When arousal is lower, as tends to happen on that day, often the ejaculate is incomplete and the man notices. Sometimes they get a mental block and they are not able to do it, and it becomes a traumatic experience.

In these situations, we ask them if they cannot get an erection or if they have managed to become aroused but cannot ejaculate. In the former case we give them a tablet which induces an erection. But it is more frequent to not be able to ejaculate since this reflex is blocked psychologically. In these cases we give them an anxiolytic.

We ask them all to relax and sit down or to walk for an hour, and we explain that, if they cannot do it, they do not need to worry because we can extract the sperm using a very simple technique. Although they imagine that this simple procedure is puncturing a testicle they do not get scared, it takes the pressure off them. It is exceptional to have to resort to this plan B.

The wives of these men with mental blocks are surprised by the protagonism that their partner suddenly acquires, when she is the one leaving the operating theatre! What is certain is that in IVF cycles the man is very much forgotten about, and we have decided to help him.

In my experience, I think that the collection rooms need to be changed so that it is not like a clinic. In addition, the men need to be told how to emotionally support their wife during the cycle and especially in the days prior to the pregnancy test.

With this in mind I have spent months asking men how they would like the collection room to look. I have not gathered much information because each of them has completely different tastes.

Below are the results of my research:

Men share a desire for privacy, to be on their own to be able to focus on sexual arousal. They also value hygiene and previously knowing the place because they feel more secure.

As I was explaining, there is great individual variation as regards what arouses them and which position they prefer for ejaculation. This also happens in the position they adopt during sexual intercourse; some positions impede ejaculation. Thus, according to my statistics, the majority like to masturbate seated on a comfortable sofa, but others prefer to lie down and some prefer to stand.

In order to provide all possible sexual stimuli I prepared a selection that they would encounter in our collection rooms, and then they can choose.

On the internet and in sex shops the amount of sex toys for women is remarkable compared to the small amount there is for heterosexual men. Finally, the selection was as follows:

- The traditional pornographic magazine.
- A video screen with all types of topics and sequences to choose from. Secretly, let me tell you that I tried to remove those of women dressed as nurses but my team did not let me. They are the most popular!
- Virtual reality glasses with a device to watch adult films.
- A rubber vagina. They can use it during arousal.
- A gift pack with virtual reality glasses and a rubber vagina so that afterwards they can share their experience with you.

This video shows how the semen collection rooms look in the Institut Marquès:

https://institutomarques.com/en/assisted-reproduction/special-techniques/erotic-personal-system/

Is male fertility reducing?

For evident reasons, women are attracted by sperm, but, in my case, the fact that I work in sterility means that this curiosity is healthy. It is one of my favourite topics and this has meant that for years my team has been researching the causes of male sterility.

According to the WHO, up to 1985, the normal number of sperm in an ejaculate was 100 million/cc. As normality is established according to the average of the analyses performed, these had to be reduced, in 1986 to 60 million /cc., in 1992 to 20 million, and in 2010 to 15 million/cc.

In 2003, we began to study the quality of semen among Spanish men. We performed population studies, comparing the quality of semen according to the different autonomous regions and according to age. We are happy to have contributed toward convincing the scientific community that the traditionally attributed causes (stress, tight trousers, alcohol…) are a myth, and that the reality of the problem is the result of chemical toxins. Industrial pollution is playing a key role.

The toxins to which we are referring are chemical substances produced by humans over recent decades and used in industry, agriculture and in the home. They are called "endocrine disruptors", and they comprise a long list of compounds which behave like oestrogens in the woman's organism. They are highly resistant to biodegradation, they are present in our food and in the atmosphere, they accumulate in the body, especially in fat, and humans and animals are not designed to eliminate them. The first contact with these chemical toxins begins at the beginning of life. They travel from the mother's blood, through the placenta, to the embryo. The type of toxins and the quantity of each will depend on the mother's levels.

At present, there is a lot of talk about diet during pregnancy, but with regard to these substances, it is not what the mother eats during pregnancy, but what that woman has lived with since her childhood, since her intrauterine life. They reach us and act like oestrogens, in other words, female hormones. During the development of the foetus's testicles, at two to three months of pregnancy, the action of testosterone, the male hormone, is very important. These false oestrogens compete with the testosterone and do not allow it to correctly perform its function. Fewer sperm producing cells form, and in the most serious cases, chromosome (genetic) alterations occur in them.

This worsening of semen quality is occurring in industrialised areas and in rural areas exposed to pesticides, and consequently,

there are great geographical variations. According to our data, the ejaculate of a man from Galicia has almost twice as many sperm as that of a Catalan man. Additionally, in areas of greater contamination, younger men are worse than older men, since these were not in contact with the toxins in their childhood because they did not exist.

In adults, this causes subfertility in the general population. It does not mean that they are sterile, but that they will take longer than others to achieve pregnancy, depending on the age and fertility of their partner. On an individual level, it causes sterility or repeated miscarriages. **We have made paramount progress in many aspects of our health, but reproductive health, in industrialised areas, has been reduced to less than half of that of our grandparents' generation**.

These chemical substances and materials created by humans in the last decades: pesticides, plastics, paints, varnishes, detergents…, nature does not know how to metabolise them or break them down. Imagine plastic in the sea or the synthetic microfibers that detach from fleece jumpers when washed; they remain forever and the components accumulate in fish that we then eat. Animals and humans are not able to metabolise methacrylate either.

In my opinion, these substances were created for us to live better. Sofa upholstery, for example, has flame retardants that reduce the risk of fire, but the effect of these was not known. Nobody is guilty. But from the moment the harmful effects of these substances are known, change must be imposed. This change must materialise in global environmental policies and in all of us. It is of no use that the European Union prohibits the use of a substance, if we then buy something produced by countries that do allow it. In a world of globalised trade, if a certain pesticide is vetoed and then we eat fruit from another place, the problem is not being corrected.

We must also be informed of what we are consuming. We have the duty and the right to know what is contained in foods and cosmetics or in bottles with Bisphenol-A, a substance that is given off when heated…Because there are alternatives. Imagine the care you are trying to give your baby, and without knowing it, you are doing this.

Today, an increasing number of experts are joining forces to denounce the effects of chemical substances on the health of citizens. I belong to the group of specialists in endocrine disruptors promoted by the *Fundación Vivo Sano*, and with them I collaborate in different activities, such as, for example, the production of the documentary "*La letra pequeña*"[1] (The Small Print). Although it is true that since the recession they are paying us less attention; it is considered a more remote problem.

In the great majority of healthy men with an altered seminogram, there is no acquired medical reason, but there are some medical illnesses that can affect fertility. The most frequent are sexually transmitted illnesses, prostatitis and varicocele.

What is varicocele?

Varicocele is dilatation of the veins leaving the testicle. It occurs more frequently in the left testicle. In the majority of cases varicocele is asymptomatic. It tends to be found during the genital examination of men who come with sterility problems or non-specific testicular pain or discomfort (symptomatic varicocele). It is caused by the absence or failure of the valves in the veins. Acting as lock gates, these prevent the blood from returning to the testicle after having left the testicle in an upward direction via the spermatic vein and coming out at the level of the renal vein. Dilatation of the veins in the testicle can produce an increase in the temperature of the testicle, a lack of oxygenation of same and obstruction through compression of the beginning of the seminal conduct. In addition, waste elements from the kidney or the adrenal gland can reach the testicle and act as toxins. All of these effects can lead to a decrease in the production and/or quality of the sperm. Data from the World Health Organization (WHO) clearly indicate that varicocele is related with seminal anomalies, a smaller testicular volume and deterioration in the function of the Leydig cells, responsible for producing testosterone. **Varicocele is present in 11% of adult males and in 25% of those presenting al-**

1. You can watch it at www.vivosano.org.

tered seminograms. Treatment is seldom required and consists of tying the veins of the testicle at the groin (varicocelectomy). The procedure is performed on an outpatient basis under local anaesthetic.

Now you are also going to become an expert in **seminogram assessments**. The table below shows the normal parameters according to the WHO.

	4th edition (1999)	5th edition (2010)
Liquefaction	Complete	Complete
Volume	2ml	1.5ml
Colour	Opalescent white	Opalescent white
pH	7.2-7.8	>7.1
Concentration (ml)	20 million	15 million
Progressive motility	50% of a+b type sperm	32%
Vitality	75%	58%
Morphology	15%	4%
Leucocytes (ml)	< 1 million	< 1 million

The different diagnoses can be:

Normozoospermia: This means that the semen meets the normality parameters established by the World Health Organization.

Hypospermia: Volume less than 1.5 ml. On many occasions there is no pathology and it is related with the sample collection conditions. Losing some of the sample at the moment of collection, insufficient days of abstinence, poor sexual stimulation or short duration of same…However, if it is confirmed we must rule out an obstruction in the seminal conduct. This requires an assessment by experts in andrology.

Hyperspermia: Volume above 6 ml. Usually, it does not mean any type of pathology, since it is related to the size of the seminal vesicles. These glands are responsible for practically 70% of the total seminal volume.

Azoospermia: Lack of sperm in the semen. It affects approximately 2% of men. It may be due to sperm not being produced in the testicle or to an obstruction in the conducts of the seminal conduct which prevents them from ejaculating. This is a similar situation to men who have had a vasectomy. These cases also require a visit to the andrologist.

Oligozoospermia: When the concentration of sperm is less than 20 million per ml, or when in the total of the ejaculate there are less than 40 million sperm. This can present in different degrees. In many cases the cause is not determined or there is no treatment for it. It requires assessment by an andrologist, since in 19% of men it is associated with alterations in the chromosomes of the sperm (pathological meiosis). If it does not improve, the appropriate assisted reproduction technology is used according to the average sperm recovery test (SRT), in other words, intrauterine inseminations or in vitro fertilisation.

Polyzoospermia: When there are more than 200 million sperm per ml. It is questionable whether it could be a cause of sterility, because such a high concentration of sperm could hinder the progressive motility of these. In many cases it is associated with a reduction in the ejaculate volume. In practice, it is solved by preparing the semen to perform intrauterine inseminations.

Astenozoospermia: This refers to the reduction in the number of mobile sperm. It is the most frequent seminal alteration. It can be due to several causes. If it is mild astenozoospermia, we do not give it much importance; when it is moderate or severe it requires an andrological study.

"Type A motility" refers to when the sperm move in a fast, progressive and linear fashion. "Type B motility" is when these parameters are reduced. "Type C motility" is when there is only vibratory movement. If this does not improve after the andrology consultation, the relevant assisted reproduction procedure according to the SRT test is used, in other words, intrauterine inseminations or in vitro fertilisation.

Teratozoospermia: A sperm has to have a head, a middle piece and a tail. And so many millions of sperm are needed to fertilise just one egg because the majority have altered shapes. There is no need to feel scared if they have a double tail, a double head, or because

they do not even look like a sperm. In fact, if 14% of the sperm present a normal shape, it is sufficient. Teratozoospermia does not have to give rise to miscarriages or to chromosome anomalies, nor does it affect fertility, since the sperm that do not have an appropriate morphology will not be able to fertilise the egg. We have to worry if the teratozoospermia is severe, in other words: if less than 4% are normally shaped, additional procedures may be prescribed.

I have focused on talking to you about the dangers of disruptors in male fertility causing miscarriages, genital deformations and testicular cancer, but these also affect women's fertility: they cause early puberty and some types of cancer, especially of the thyroid and breast. Over time, we are discovering more nocuous effects.

My conclusions are that:

- The deterioration of male fertility is mainly caused by environmental pollution and, therefore, presents great geographical variations.
- The key time for being affected may be during the development of the foetal testicles.
- As a result of oestrogenic disruptors from the food chain, a pregnant woman can pass high levels of oestrogens to her male foetus.

8

Diagnosis. Why I'm not getting pregnant

One month later, **Elisabeth** comes back to the clinic with Jack, who as soon as he arrives wants to know the results of the seminogram.

—"The semen study is normal. What stands out is that the ovarian age is older than expected. In the analyses we see that your anti-Müllerian hormone is 0.6 ng/ml, which corresponds to 40 years of age, lowering your fertility" —I explain to Elisabeth—. "The majority of your eggs are not separating their chromosomes properly, and this means that probably only one of every five embryos can evolve correctly. If we do not do anything, your chance of becoming pregnant each month is approximately 4%, and we still have to consider a high percentage of miscarriage. We can wait the time that you need, but I advise you to do a cycle of in vitro fertilisation. From the result of your analyses we know that you still have apt eggs, but in a few months, when the anti-Müllerian hormone drops to 0.3 ng/ml, the possibilities will be remote. We have to stimulate the ovaries to obtain a lot of follicles in a cycle and hope to be fortunate enough that your best eggs will develop."

—"We'll do it because I've no other option, but it all seems very forced to me. It's like going against nature. I have two sisters: Fátima is 32 and Mireia is 36. She took two years to get pregnant and she thinks I should wait in case I get pregnant without in vitro fertilisation", she answers.

—"You're right, but nature planned for women to have babies before 30, and with men with excellent semen. It is very important that you know that during the time that goes by between when we decide we want to conceive and when we achieve pregnancy the relationship with our loved ones can change a lot. Your sister loves you, but she doesn't know how hurtful her comments can be. Does your family know?"

—"My mother kept saying: 'You only talk about travelling and going out with your friends', and it really hurt me. That is why I've told my parents and sisters, and now they are giving us constant advice."

—"It's good that your close family know, but it is best to ask them to stay quiet. Do not let them talk to you about anything related to fertility treatments, or tell you what you should or shouldn't do. You should also avoid, for the moment, going out with friends who have babies or with pregnant friends. It is normal that you might have felt uncomfortable during your sister's pregnancy. I'm sure she only talked about that and now she only talks to you about her baby. It will happen to you too, but for the moment it is best to be with friends without children or pregnancies."

—"Doctor, I'm calm and I know that we are going to achieve this, but I don't like seeing Elisabeth this nervous. How can I help her?" asks Jack.

—"You have to be more united than ever and support each other regardless of what happens. In this situation each person will react according to their nature, just as you do with any problems in life. I suggest that you look at it as a challenge, as a process that can unite you if you nourish it with affection, romance and irony."

—"And what can I do to stop myself thinking about this all day long?" asks Elisabeth.

—"I think you need to keep yourself busy, with your job, your hobbies… Try to fill your time. And only dedicate a few minutes of each day to thinking about this, about your goal, about how you are going to feel when you have your baby in your arms. Before explaining what in vitro fertilisation consists of, I want you to have serious and updated information about this process so that we can organise the treatment cycle in the next visit. Please take a look at these links on our webpage and come to a biology interview to find out about the most relevant complimentary techniques for your case. It is important to avoid reading inappropriate or outdated texts on the internet. It is important to filter the information because you can end up getting hurt or being misguided."

Marta arrives with Margaret and with the reports of the tests I asked her to do. The fallopian tube test and the anti-Müllerian tests are normal. An adequate antral follicular count is observed in the ultrasound, but a 2-cm cyst has also been detected on her right ovary.

—"Doctor, I've had that cyst for the past three or four annual check-ups, and it has not grown. My gynaecologist is not concerned about it", she tells me.

—"Marta, it could be an endometrial cyst. It shouldn't affect your possibilities of getting pregnant, but I can't be sure. It is not serious and it cannot degenerate into anything bad, and it does not have to be removed. However, we need to assess if we can perform an insemination or an in vitro fertilisation. I think we should do three cycles of inseminations with donor semen and, if we are not successful, move onto IVF."

—"I'm worried now. **What is endometriosis?"**

—"It is a disease that consists of the presence of endometrial tissue, which grows inside the uterus for the embryo to implant, in other places. It can appear in different places,

but the most frequent are the ovaries, the fallopian tubes, and the interior of the abdomen."

—"And how did I contract that?"

—"Women can be born with these implants or they can occur through what we call 'retrograde menstruation', in other words, when the uterus contracts during menstruation to expel the superficial layers of the endometrium, fragments may move into the fallopian tubes and nest in these, in the ovaries or in the layer that covers the interior of the abdomen (peritoneum). As you know, the hormones produced by the ovaries make the endometrium grow, and if an embryo does not implant, this breaks up and a period occurs. Well, that tissue responds in the same way, and every month bleeding occurs. This bleeding inside an ovary produces a blood cyst, also known as a chocolate cyst because the accumulated and oxidised blood looks like chocolate."

—"But, can it hinder pregnancy?"

—"It might not affect fertility, but these cysts are big or if there are a lot of small sources they can damage the healthy ovarian tissue. The endometrium that nests inside the tubes can damage the internal layer and cause adhesions which prevent the transport of the eggs or embryos and can also damage the cells that produce the nutrients which maintain them during the days they are in them. The implants in the interior of the abdomen can make the tubes get stuck and consequently lose their mobility, preventing them from catching the eggs. They can also secrete substances that damage the egg."

—"I understand. Knowing that I think it is a good idea to start with the inseminations. I am also concerned about the donor."

—"The donor…we'll talk about him later."

Rose and James are the youngest and must fun couple I have seen today. They are holding hands, and each one is sporting their own style: she is dressed for a cocktail party and

he is wearing a tracksuit. I tell them that Rose's tests are fine and that I am happy because we know where the problem is, and that now there are solutions for nearly everything.

The seminogram highlights that there are only 5 million sperm per millilitre; that the motility is very low, and that 98% of them have an abnormal appearance. James reacts well, although he can't stop moving his legs and I start imagining what he must be like with a ball between his feet…

— "We have to repeat the seminogram to check this diagnosis and you will have an appointment with a doctor from the andrology team. We need to know what is causing this and if it can be cured with treatment."

—"And what if they can't find the cause or there is no treatment and I can't have children?" asks James.

Smiling, I tell him that a lot of the in vitro fertilisation treatments that we perform are due to male fertility issues and that in the worst case scenario, we will do that.

ANDROLOGY APPOINTMENT STUDY OF MALE INFERTILITY

Dr Ferrán García describes the appointment:

James and Rose arrive at my clinic late on a Thursday evening. At first sight, they look like two teenagers in love. They are young, enviably young. James is athletic in appearance and I calculate that he is about 1.85m tall. He has dark skin, mahogany eyes and a groomed beard as black as his hair which tries, unsuccessfully, to make him look older than his childlike face reflects. He is dressed as if he has just come from training with a short-sleeved yellow polo shirt, navy blue tracksuit bottoms and blue runners, all from the same sports brand. When I reach out my hand I see the Maori tribal tattoo that covers his right forearm and arm, and hides under the sleeve.

Stuck to his side, and holding onto his left arm as if she were afraid of losing him, is Rose. She looks like she has just

stepped off the catwalk: She is wearing a fitted pink sleeveless dress, from a very well-known brand, a double string of natural pearls around her neck, and earrings to match. Her light tan highlights the sky blue colour of her eyes and her long jet black hair gives her a certain exotic air.

After the usual presentations, we sit down opposite each other, with James on the left, and Rose on the right. James cannot hide his nerves and he compulsively starts to tap the fingers of his right hand on the table surface, until she takes his hand and holds it softly.

Dr López-Teijón, aware of me being a Barça fan, had told me that a famous footballer was going to visit me. I try to empathise with him talking to him about his team's great season. At first it seems he has forgotten what has brought him to my clinic and even starts to relax, but suddenly he changes the topic brusquely and asks me:

—"How did the second seminogram turn out?"

—"Very similar to the first one. There are two million more sperm than in the first, but the motility and morphology are very low. We could say that the probabilities of achieving pregnancy naturally are the same in both ejaculates. Although pregnancy is possible, the probabilities of it happening are approximately 1%. This means that if we are not able to significantly improve the quality of the semen, we will have to resort to assisted reproduction techniques."

—"The result from the first semen analysis was a hard blow for me. I wasn't expecting it, but today I'm more prepared" -James explains-. "How can this happen to me? I have never smoked, I don't like alcohol, I've been monitored by doctors, dieticians, etc. for my whole life… I don't understand!" he complains.

—"Although it might not make you feel much better, this is the most frequent stereotype that we find. A young, healthy man, without any toxic habits,…Not all of them are professional athletes like you, although a lot of them play

sport regularly and, nevertheless, their reproductive capacity is reduced as a consequence of alterations in the semen."

—"What could be the reason?"

—"Generally speaking, we could say that there are four main causes. Firstly, there are congenital causes. The fertile potential of each man begins to be established during the embryonic development of the testicle. Imagine that the testicle is a factory. Well, at birth, each man has a certain number of machines, some more, some less, some none at all, depending on genetic, hormonal, environmental factors, etc. which alter the embryonic development of the testicle. Then there are acquired causes: during our life we can have testicular diseases, infections, injuries and varicose veins, abuse of toxic substances, drugs, environmental pollution, etc., which can reduce the fertile potential that we have at birth. Thirdly, there are genetic causes, which represent at least 15% of cases. Finally, there are unknown causes, which make up approximately one third of the total."

After listing the causes of infertility, I ask him about any diseases he may have had that could be related to the reduction of his fertile potential, although without the slightest hope of finding anything, knowing the strict medical monitoring to which he has been subjected as an elite athlete. As was to be expected, James responded no to all of my questions about possibly having had a disease.

"I have never had anything" James assures me. "Not even an injury that put me in surgery" he states, forming a smile.

When I finish the questions about his medical and surgical background, I ask him to join me to perform a physical examination. As we cross the mere five metres of corridor separating my office from the examination room, he asks me:

—"Doctor, do all your patients have to do this?"

—"Of course", I answer him, "the examination is an important part of the first andrological visit which often provides us with information that helps us to determine the cause or causes of infertility. In addition, it is not only infertility, we may also find diseases that affect the patient's health, such as varicocele, which are varicose veins in the testicle, cysts or tumours, among other diseases. For example, testicular cancer is up to 20 times more frequent in sterile men with altered seminograms."

—"Don't scare me!" he exclaims.

—"That is not my intention. Don't worry, you'll see, everything will work out fine. If you had something, with all the tests they do on you, they would have already found it", I tell him to put his mind at ease.

—"You're right, doctor."

When we finish the examination, we return to the office. As he enters the room James gives Rose, who is sitting down, a look of complicity, and both of them smile.

—"Well, only one thing stands out in the examination. The testicular size is smaller than normal, approximately half of what it should be."

—"And what does that mean?" asks Rose.

—"What is the problem we have in the semen?" I respond with a question.

—"That I have a low sperm count and the majority of them are malformed", James hurries to clarify.

—"Correct. As we said before, the testicle is a factory. If the size of the factory is smaller, there are fewer machines inside to make sperm. Consequently, fewer sperm are produced and usually when this happens, motility is also low, and the morphology is altered."

—"And can it be treated?"

—"No, unfortunately we do not have a treatment that can make machines appear where there are none. Considering there is no known cause, it is highly likely that it is congenital.

As we said at the beginning, the probabilities of achieving pregnancy naturally are very low. It is possible, because if there are sperm that possibility exists. However, it is unlikely to happen. If we want to increase the probability we will have to resort to in vitro fertilisation."

—"Yes, the doctor told us that already", Rose intervenes.

—"But before performing in vitro fertilisation the study needs to be completed to rule out any genetic anomalies in the sperm"- I tell them while I take a pen and draw on a white sheet of paper-. "Imagine that the spermatozoon is a vehicle which is used to transport the man's genetic load, in other words, the chromosomes, to the egg. We have said that the problem we have is that there are few vehicles and as a result there is a low probability of achieving pregnancy. Well, that stops being a problem because we put in a crane to take them to the egg."

—"A crane?" James asks, smiling.

—"The crane is the sperm microinjection, or ICSI."

I take the pen again to back up my explanation with a drawing: "We take a sperm with a micro-pipette, we transport it and introduce it into the egg. Beforehand we will select the sperm with a normal shape. We use a technique called IMSI, which magnifies the image. It is like looking at the sperm on a computer screen and zooming in to see it up to 20 times bigger than we were seeing it. This allows us to select those that present the best morphology.

—Are you following me?" I ask.

— "Yes", they reply in unison.

—"Therefore, the problem that you have a low number moving and that they are malformed is no longer a problem thanks to the application of these techniques."

—"That means that there could be another problem", adds James.

—"Indeed. The problem could be in what transports the sperm, in the number of chromosomes. Depending on the

chromosome content, in the human body we can differentiate between two cell types, the somatic ones, which have 46 chromosomes, and the reproductive ones, the egg and sperm, which have 23, because when they unite they create a cell with 46 chromosomes, which will be the embryo. All men, including fertile men, produce a percentage of sperm that do not have a normal number of chromosomes. But this percentage is very low, approximately 6%, and as a result, it normally does not have consequences on reproduction. However, some men with sterility problems produce a very high percentage of sperm with an altered number of chromosomes. This will have reproductive consequences, in the form of not achieving pregnancy, miscarriages or increasing the risk of having a child with a chromosome alteration."

—"So, I might have that problem?"

—"It is unlikely, but it is better to rule it out before doing the IVF."

—"And what do we have to do?" asks James.

—"We said that the sperm have 23 chromosomes. However, the machines that make sperm have 46. This means that in the production line, the genetic load is reduced by half. This is thanks to a process called meiosis which is a type of cell division. Meiosis can be altered, especially in those men who produce fewer sperm. When this happens, more chromosomally altered sperm than normal are produced."

—"And how is meiosis studied?" asks James.

—"There is a direct way that consists of taking sperm precursor cells from the testicle and analysing them when meiosis is taking place. At this point, I am not considering this study, since, as the cells are in the testicle, a small intervention called a testicular biopsy needs to be performed. It is performed with local anaesthetic on an outpatient basis and it takes 20 minutes. A 1cm incision is made on the scrotum skin, we access the testicle, and we take a lentil-sized sample

which will contain the cells that we want to study. Then we stitch it up and you can go home."

—"If you think it's necessary, I am willing to do the biopsy."

—"No, at this point, I don't think it is necessary. We will use an indirect way of studying meiosis. Instead of studying these precursor cells we will study the end product, which is the sperm. We will use a technique called FISH, which serves to study the end product of the meiosis, in other words, the sperm. The advantage of this technique is that it can be performed on the ejaculate, although the disadvantage is that we cannot study the 23 chromosomes. Usually the five chromosomes that most frequently present anomalies are studied: X, Y, 13, 18 and 21. FISH is the acronym for 'Fluorescence in situ hybridization'. It is as if we had a specific magnet for each of the 23 chromosomes, and we put a fluorescent substance, yellow for example, on this magnet. The magnet will look for its chromosome and colour it yellow. Normally, we should only see one yellow dot on each sperm head, since there should only be one chromosome for each of the 23. If we see two yellow dots, it means that there are two of the same chromosomes, when there should only be one. We compare the percentage of sperm that present anomalies in the chromosomes studied with that of fertile men. If there is an increase, and additionally, if this increase is statistically significant, the test result is altered."

—"Do you think it might be altered?" asks James.

—"It could be; but I think that it is unlikely. Additionally, since you have to give a semen sample anyway, we will also complete the study with the DNA fragmentation test of the sperm."

—"What is that?" James asks in a surprised tone.

—"The DNA chains that make up the chromosomes can suffer from tears, usually due to what is called oxidative stress, which has different causes. In theory, a sperm with fragmented DNA which fertilises an egg will lead to a non-

viable embryo, unless the egg repairs the DNA fragmentation. Since the repair capacity is higher the younger the woman, and Rose is very young, I don't think that the fragmentation, should it be altered, will be an obstacle to conceiving. Do you have any questions?"

—"When can we do the tests?" Rose asks.

—"When James has been abstinent for three days."

They both burst out laughing.

I give them the request for the tests and we say goodbye. I confirm that when the results come in I will phone them to tell them. Three weeks later the FISH results and the fragmentation results arrive. Both are normal. I ring James's phone, but he doesn't pick up, so I call Rose.

—"Rose? Good morning. It's Dr García from the *Instituto Marquès*. I am ringing to give you the results."

— "Is everything okay, doctor?" asks Rose.

—"Yes. The two tests we performed on James came back normal."

—"That is wonderful news, doctor! Now what do we have to do?"

—"Now you need to make an appointment with the doctor so that she informs you of the steps you need to take to perform the IVF.

—You'll see, you'll get pregnant on the first go!"

— "Thank you, doctor. I hope so. Have a nice day!"

—"You too. Regards to James."

9

Egg and semen donors

I would like to invite you to think for a moment about what you most value in a person, and what you most value in yourself. Write it down before you continue reading. When you have finished, you will see that the traits you have written down can be encompassed in three options:

- **character** (positive attitude, predisposition to help others, friendliness, pleasantness, strength, fighting spirit, involvement…),
- **intellectual capacity** (intelligence, mental sharpness…)
- **and physical appearance** (beauty, style, elegance…).

Now imagine that you need donor eggs or semen to have children. How would you like that person to be? Would you reply the same as before?

When donor eggs or sperm from a sperm bank are needed, how are they assigned? **What do our patients most value?** We have analysed a survey that we conducted with hundreds of patients from our centre. We asked them to list the aspects they most value in their egg or semen donor in order of importance. It turns out that what most patients value in their donor is their physical appearance –in 51% of cases– followed by character –in 31%– and educational level –in 18% of cases–.

The responses do not vary according to the nationalities of the patients, or whether they refer to egg or sperm donors. Men and women both respond the same, and there are no significant differences in the replies from women without male partners who have an insemination or opt for in vitro fertilisation with semen from a sperm bank. Are you surprised by the responses? What do you think?

I understand that above all, they give importance to the physical appearance because it is what the majority of people think we inherit. Let us see if that is really the case.

What traits do we inherit and what traits do we acquire after birth?

It is true that a person's physical appearance has a large hereditary element, although it is also true that genetics are capricious and myriad combinations arise. Height, hair and eye colour, facial features…depend on genetic inheritance, but apparently so does the percentage of symmetry of both sides of the face which can make someone more or less beautiful or ugly.

Physical appearance is also influenced by the environment the child has and has had. The way they move, look, laugh; in other words, gestures are learned. Children copy the people around them. What is more, attitude is very important and this depends on personality. A person's beauty and attractiveness go far beyond measurements and a face. There are people who know how to dress well, wear make-up, sport a good hairstyle, look other people in the eyes and walk through life gracefully with a smile and with their shoulders relaxed. Victor Kuppers says that some people are like lit lightbulbs, and others are always burnt out.

Look at the photographs by the North American photographer Gracie Hagen: they reflect how one same body can look attractive or not according to the person's attitude.
http://www.graciehagen.com/Illusions-of-the-body/

Intellectual capacity. Until recently it was believed that 50% of intelligence was inherited, according to studies on the intellectual coefficient performed on children. With the advances made in genetic development analysis (which studies the effect of genes during a person's whole life), it has been observed that the genetic contribution towards intelligence manifests over time and reaches 80% at adult age.

Studies on identical twins raised in different families demonstrate this too. They show a high degree of intellectual concordance between the twins, despite the different environments in which they have lived.

Character, emotional and social intelligence, in other words, how a person feels, acts, thinks and relates. There has always been great debate about what percentage of a certain character trait is innate and what percentage is acquired. Today, the trend is to believe that some traits are determined genetically, others are deter-

mined by the environment, and others for which there is a biological predisposition, but which will manifest or not according to the family environment, the culture of the group, education and circumstances.

The five personality traits that are considered to be innate are:

- The degree of introversion – extroversion.
- The degree of emotional stability. The tendency towards being bad-tempered, depressed, anxious, angry…and on the contrary, the control of the emotions.
- The degree of interest in new experiences, curiosity, imagination, creativity.
- The degree of interest in others, altruism, pleasantness, empathy.
- The degree of self-discipline, responsibility, the ability to follow rules, organisation, meticulousness.

Twins have a more similar character than siblings, and identical twins much more so, even if they are raised in different places. But from birth, identical twins present differences in character, since some individual experiences are apparently acquired during pregnancy.

In view of the above, how do we assign our donors? As regards the physical appearance, we know them perfectly, and we study their traits in detail according to those of the patients who are going to receive these gametes. As regards intellectual level, we carry out information campaigns exclusively in universities, not because it is necessary (if a friend who does not go to university comes we accept her too), but because we know that there are more male and female candidates there since the men and women who donate tend to have a high educational level (in environments with lower educational levels, donation is not well-accepted).

Lastly, as regards character, our psychologists interview the male and female donors and perform tests on them to rule out any potential diseases. We cannot determine what character they have, if a donor is sociable or timid or many other things. But we can establish their lifestyle: we know that they all have very de-

fined traits of commitment, emotional balance and great courage. And apparently, these traits are hereditary!

For us, assigning a male or female donor is an act of great importance and we experience it with a sense of responsibility and honour for the trust our patients have placed in us.

Why do some women donate eggs?

For many years I have helped women who can only become mothers with donors' eggs, listening to them and to the women who want to donate. When we ask them why they want to undergo treatment to be a donor they tend to reply that:

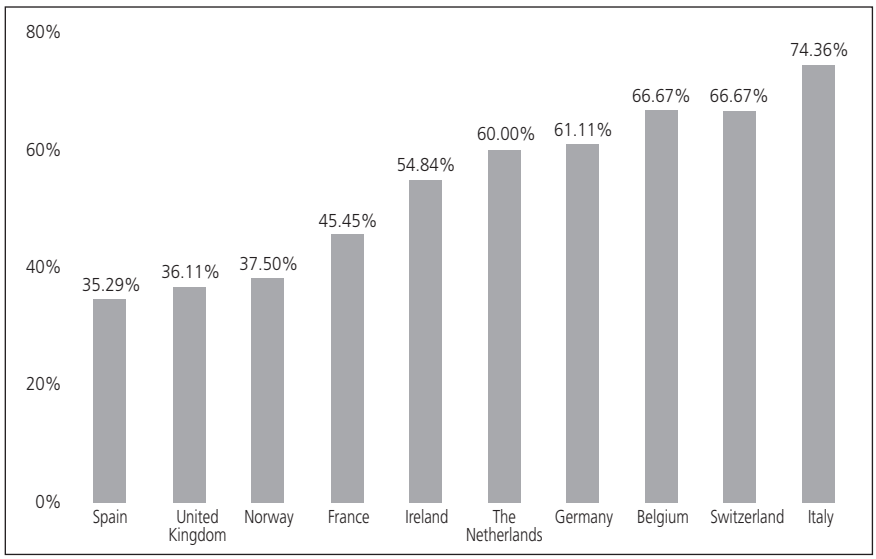
- A friend did it and it was a good experience of helping others.
- They saw an advertisement in university and they are interested in the economic compensation. When they are asked what they are going to do with the money (900 Euros), the majority reply that they will pay their enrolment fees for the course, because it is a problem for their parents. I also think it is a way for them to help their family.
- They have a relative with fertility problems and they are aware that they might also need help in the future.
- They had to abort previously and they want to help the baby that they were not able to have to be born. In these cases they constantly ask how the receiver's cycle is going and if it ends in pregnancy they feel comforted.

Legal differences across countries regarding egg and semen donation

The social consideration of sterility is linked to each country's cultural and religious tradition and is also conditioned by the legislative framework. In a congress held by the European Fertility Society we presented a study carried out in 10 countries in Europe which consisted of questions about how sterility is considered so-

cially in their country, and with whom they have shared their problem: friends, family, work colleagues…

Do you think that reproduction treatments are still taboo in your country?



As you can observe in this table of results, Italians are the worst off: 74% of women believe that sterility is taboo in their country and they experience it in an environment of social rejection. In this vein, each country has different legislation. There are countries in which donation is prohibited, while in others the donors cannot be anonymous and therefore there are very few of them. In others economic compensation is not allowed. This leads to suffering and many people end up childless, especially those with few economic resources. It also creates a need to travel to other countries to be able to perform the treatment.

According to the WHO, sterility is a disease. It is not a whim. My personal opinion is that behind all of these prohibitions lies an economic reason. Governments prefer to say that they do not consider it ethical than have to cover the costs of these treatments. It is difficult to understand, for example, that in France, the country of "*liberté*", women who are not legally married are not allowed

to have an insemination with donor sperm. I think it would be more honourable to permit it even though the costs are not covered by public funds.

Catalonia is the area in the world with the highest donation rate: blood, bone marrow, corneas, kidneys…, and also semen and egg donors. I find it very difficult to understand the different social consideration that exists when a person donates any other tissue and when they donate eggs.

I would like to share this letter with you, which we received from a patient who achieved pregnancy thanks to an egg donor:

"Dear donor, thanks to your help, our dream has come true, and we have been blessed with Lorena, an adorable little girl. After many years of pain and suffering, all of our hopes and dreams have come true with the most beautiful little girl we could ever have imagined. We will never be able to thank you enough for sharing the miracle of life with us, and I assure you that we will always treasure you. Thank you from the bottom of our hearts, you are a very special person who has completed our existence. Thanks to your effort, I was able to give life to our little Lorena".

10

In vitro fertilisation and complimentary treatments

When the solution to your sterility problem is IVF, it is very important to know how it will be performed in order to have the highest probability of success. Today, we have many complimentary techniques at our disposal which can increase the percentage of pregnancy twofold in one treatment cycle.

It is also essential to know the causes of sterility, because this will indicate the suitability of one or another technique.

Your IVF follows a series of processes. The first is:

Egg retrieval

The retrieval of eggs takes place in surgery. It is performed on an outpatient basis, it does not require staying overnight in the clinic, and, in the majority of cases, it causes minimal discomfort.

Under general anaesthetic, the follicles are aspired through the vagina and ultrasound monitoring is used. This process is brief: it takes approximately eight to ten minutes, and when you wake up you will be told how many eggs were retrieved. At that moment the number is known, but not all of them will be mature.

We calculate that those follicles measuring more than 14mm in diameter on the day the stimulation phase finishes can give rise to a mature egg. If this is not the case, the maturation process is completed in the laboratory. Soon afterwards, you can leave the clinic, but you must go home to rest.

This is an oocyte. Oocyte and egg are the same thing. They are similar to a hen's egg (not in size!). They reach the laboratory a few minutes after being retrieved.

Oocyte

The in vitro fertilisation laboratory

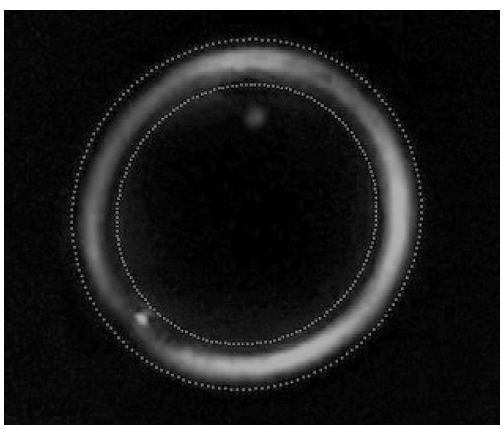
The IVF laboratory is a key element for success in an assisted reproduction programme. The location and the design of the laboratory, the equipment, and the culture media are of vital importance for the strict requirements of the embryos grown in vitro. An in vitro fertilisation laboratory should be like a giant uterus.

Imagine what a uterus looks like inside…How much light is there? Almost none; so the laboratory must be dark. What does it smell like? There are no odours. Atmosphere control means that the biologists cannot wear cosmetics or perfume. They can wear deodorant but it must not contain alcohol or perfume. What temperature is it? 37 degrees centigrade. There is a constant temperature control system for the incubators and, for example, the surfaces where the culture dishes containing the embryos are heated. What is inside the uterus? The internal layer is called the endometrium and during the menstrual cycle it changes, preparing its surface and producing secretions which encourage an embryo to implant. Today there is a fantastic way of simulating this with the culture media.

They contain the substances that the endometrium produces and every day different cultures have to be added because their requirements vary. What is there not in the uterus? There is no contamination of any type. This is hard work! Having a sterile atmosphere involves having a lot of technology and going to a huge effort. But it is essential to avoid germ-type pollutants from entering as well as our "special enemies": the volatile organic compounds. They are chemical substances that are emitted by paints, solvents, lacquers, cosmetics…and they remain in the atmosphere in the form of vapours. They tend to deposit in fatty media. The culture media contain oils and these compounds are toxic for embryos.

To enter the laboratory you need to put on a clean uniform every time, as well as a hat and shoes. No accessories such as watches, or make-up can be worn. When you open the door the positive pressure will surprise you; you will feel wind coming towards you which serves to prevent air from outside from coming in. The ground is covered in a surface that can discharge static electricity and your feet will stick to the ground in the first section because there are adhesive mats. The ceiling has absolute and activated charcoal filters to maintain the air purity.

The operating rooms have a higher CO_2 and humidity concentration that usual: the embryos like that. And the tables are hydraulic. That way, when you lean on the table to work, vibrations will not be transmitted to the embryos. Oh! Also, there are no mobile phones in the uterus. So inside the laboratory there are no mobile phones to avoid possible damage from radiofrequencies.



Polarizer

The eggs arrive to the laboratory immersed in the follicular liquid in sterile tubes. They are located using a microscope, cleaned and classified according to their maturity. Then they are

stored in the [Polarizer] incubator with the culture medium at 37ºC until they are inseminated.

The quality of the eggs is an intrinsic characteristic of every woman.

If at this point we adapt a special optical system called "polarAIDE" (a light polarizer) to the microscope, we will be able to see the eggs much better.

The external layer of the egg is called the pellucid membrane and when it has high birefringence it is a sign of quality. The coloured zone inside is the meiotic spindle, in other words, the system that is created inside the nucleus to separate the chromosomes. This is where the genetic material is stored. After ovulation, the separation phases of the genetic code are completed. You already know that we have 46 chromosomes and that the egg must reduce them to 23, to half, so that when they join the other 23 contributed by the sperm, a new being is created.

These images enable us to see the stage of maturity of the egg. This is important to tell us the exact time at which we should perform the ICSI. Additionally, having located the nucleus, accidental punctures are avoided during the process of introducing the sperm into the egg, thereby avoiding the risk of damaging the genetic code.

The polarAIDE is especially indicated in those cases of "high value eggs". That is what we call the eggs of patients who develop few eggs.

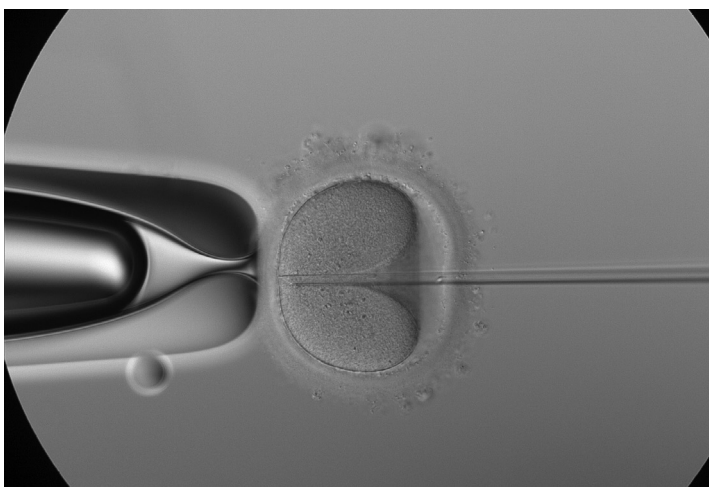
The meeting between the eggs and the sperm

The eggs can be fertilised using two techniques: the conventional in vitro fertilisation or the sperm microinjection or ICSI. In the **conventional in vitro fertilisation**, each egg is inseminated with 100,000 mobile sperm. One of these, at random, will penetrate the membrane of the egg and a reaction will occur which will prevent others from entering. They are put in a culture medium and incubated at 37ºC.

The **sperm microinjection or ICSI** consists of introducing one single sperm inside the egg's cytoplasm:

https://institutomarques.com/en/assisted-reproduction/treatments/the-ivf-laboratory/

ICSI is performed when the amount of motile sperm is low, but if we are in the first cycle of IVF, and especially if it is a case of sterility with unknown causes, ICSI is recommended at least in some of the eggs to try to ensure that fertilisation occurs and thereby avoid failures.



ICSI

One day later: fertilisation

Inseminated eggs are kept in the incubator and 17 to 20 hours later it can be observed whether fertilisation has occurred. If this is the case, two spheres can be seen in the egg: the two pronuclei corresponding to the male and female genetic codes.

The first cycle of IVF is also diagnostic and serves to observe the fertility of each couple. We can encounter low fertilisation rates or even a total failure in fertilisation. On average, 75% of mature eggs should be fertilised. In some cases fertilisation does

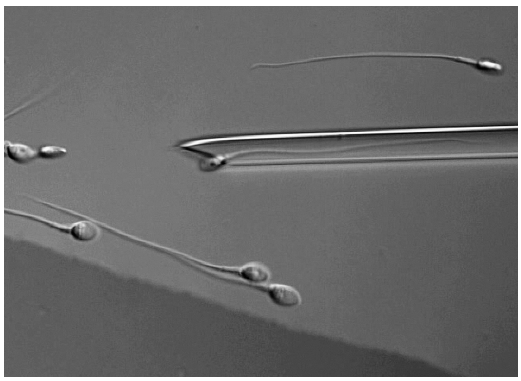
not occur or is very low because the membrane of the egg hinders it or because the substances contained in the heads of the sperm to perforate the membrane have been altered.

Can we do anything to make as many as possible be fertilised? Yes, choose the best sperm! In IVF with ICSI the sperm are selected with a microscope with a magnification of 400. But there is a technique called IMSI that uses a magnification of 16,000.

https://institutomarques.com/en/assisted-reproduction/special-techniques/imsi/

We consider it normal to only have 4% of sperm with a normal appearance; the large majority have pathological characteristics such as a double tail, a round head, vacuoles, etc., and now we know that the best looking ones are the ones that have the highest probability of carrying the genetic load well and, therefore, a better potential of fertilisation and of leading to evolving embryos. It is a long process because if biologists only consider a small percentage as good with a conventional microscope, imagine when they see it that well… The best are selected one by one, as many as we have mature eggs.

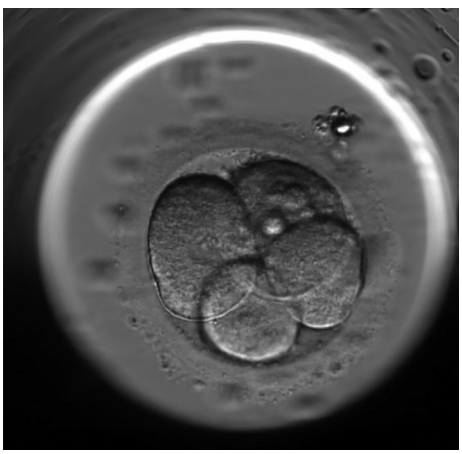
IMSI is indicated in men with teratozoospermia, in other words, those in whom fewer than 4% of sperm have a normal morphology, in patients with alterations in the DNA fragmentation, and in difficult cases due to sterility with a long history or failures in previous cycles. In these cases, the success rates increase considerably.



IMSI

Two days later: first embryos

The fertilised eggs then begin the first cellular divisions. From this point on, every 12-15 hours we will observe how the number of cells increases.



Four-cell embryo

Each embryo has its own characteristics and at this point it may be divided into two, three, four or five cells and each one has its own nucleus. In one same cycle each embryo evolves at its own rhythm.

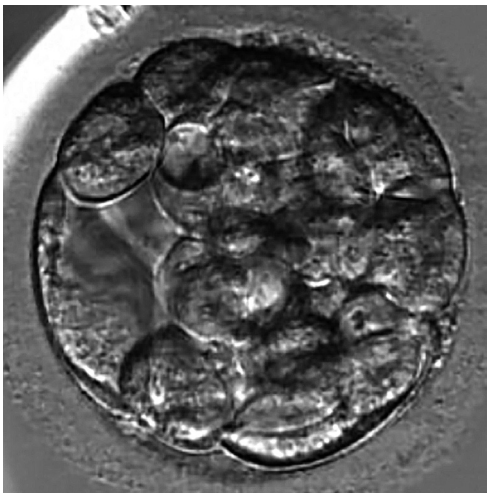
Three days later: embryo development

The following day, the embryos need to have duplicated the number of cells that they had on the second day. Usually they present 8 cells.

Eight-cell embryo

Four days later: morula

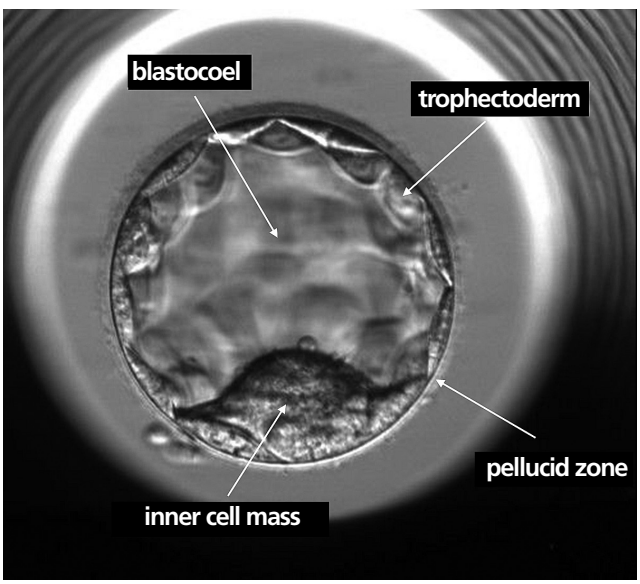
When there are 16 cells, these join together, forming a structure that is called a morula because it resembles a blackberry (*morum/mora* in Latin). The cells of the centre of the morula form the inner cell mass and will give rise to the embryo's tissues. The cells on the periphery will form the placenta.



Morula

Five days later: blastocyst

At this moment, liquid enters and a cavity forms, known as the blastocoel, which increases in size and moves the cells to one side. At this stage the embryo is called a blastocyst.



Blastocyst

A blastocyst is a mass of cells with a central cavity full of liquid, called the blastocoel, and it is surrounded by two different cellular layers. The external layer (trophectoderm) will give rise to the placenta, and the internal layer will give rise to the embryo.

The cellular content continues multiplying and the blastocyst expands until the external membrane breaks: this is the hatching process. The cellular content leaves and it implants in the maternal uterus.

Long culture and transfer during blastocyst stage

Transferring embryos in the blastocyst stage means the best embryos can be chosen and improves the synchrony between the embryonic stage and the uterine environment, thereby increasing the implantation rates.

It is the most suitable technique for those patients who want to transfer one single embryo to avoid multiple gestations or for patients who have undergone various treatments and have not achieved pregnancy.

If the laboratory conditions are optimal and the appropriate culture media are used, 60% of embryos will reach the blastocyst stage.

In an IVF treatment, for example, of a 35-year-old woman from whom ten eggs were retrieved, eight of which were mature, it is highly possible that seven will be fertilised and that three days later six embryos will have divided correctly. We can transfer, shall we say, two embryos that day, or leave them to reach the blastocyst stage. Two or three will probably reach it, but we have avoided transferring those that were going to come to a standstill. In that way, the probabilities of transferring the best embryos increase.

Every day the biologists take the embryos out to examine them under the microscope. Today there are incubators called "EmbryoScopes" which have a built-in camera that continuously takes images of the embryonic development…It makes a film

of the beginning of life! In this EmbryoScope video you can observe the development of a human embryo from fertilisation of the egg to five days later with one of the songs we play in the incubators.

https://institutomarques.com/en/assisted-reproduction/music-and-fertilisation/

This new technology increases the pregnancy rates in two ways. On the one hand, it helps to avoid removing the embryos from the incubator to take them to the microscope every day to be examined, with the temperature and light changes that this involves.

On the other hand, it facilitates a better selection of the embryos with the highest implantation potential and thus precisely these ones are transferred to the mother. We will see what the cellular division rhythm was, how the cells are structured, and how they change over time. For example, an embryo that is divided into four cells 40 hours after in vitro fertilisation would be given the highest score in terms of cellular division. However, the EmbryoScope video reveals that sometime after the examination, suddenly one cell fuses with another and is absorbed! And it ends up with three cells. If you had not seen this, the next day, when examined, this embryo might be divided into eight cells, it would get a high score again and it could be selected as one of the most suitable for transfer.

It is incredible how an embryo can change its characteristics in minutes. For example, if we want to choose the best student in the class, it is best to ask the teacher who knows them all year round, than to look at a picture.

This is only one of the many examples I can give you, but we know that the embryos that do not follow the correct steps have fewer possibilities of developing because they are associated with alterations in their chromosomes and probably with other

negative aspects that we still may not know, but that we are trying to discover.

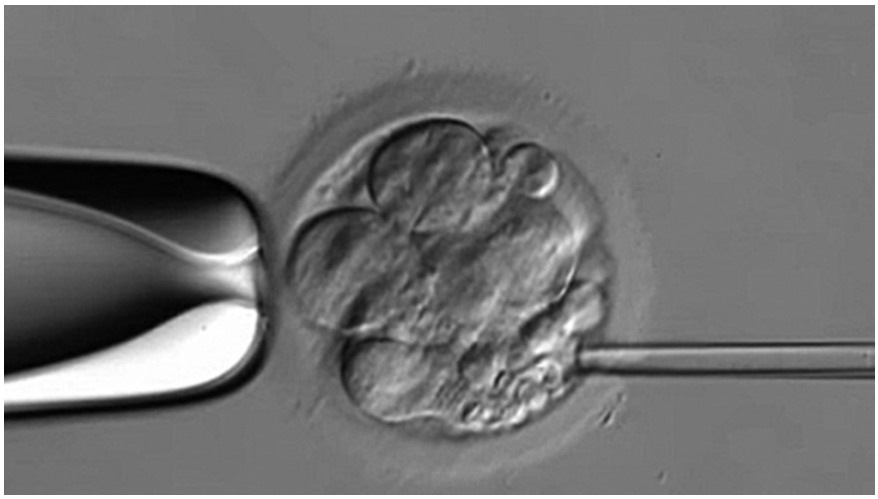
You already know that it is incredible that there are no two people physically the same apart from identical twins (they are the result of an embryo dividing in two). But, did you know that no two embryos are the same? That from the very instant of fertilisation we have always had our exclusive characteristics which have made us unique from that very moment? Every embryo, every foetus, every child, every person is an unrepeatable miracle of nature. The number of possible genetic combinations is infinite and the probability of them being repeated is practically impossible. The human genome contains 3,200 million nucleotides, which are the subunits that make up DNA and that are combined in a different way in each person.

The EmbryoScope is indicated in all cases, but especially when the embryos are going to be in the laboratory for longer, for example, if they are going to be transferred to the mother five or six days after fertilisation to perform a genetic analysis of the chromosomes or to bring them to the blastocyst stage, which is what we call a long culture. A mark is put on each embryo every day, like at school. The highest mark is 10 or, according to other classifications, the best is A.

When you are given a mark for your embryos I want you to know that it is graded according to the following aspects.

- The number of cells should be adequate for that day of development.
- The cells should look similar to each other.
- There should be no fragments between the cells (impurities from the division process).
- And each cell should only have one nucleus.

Thus, an embryo can score a low mark on account of having many impurities in its cells, bits of cellular membranes, broken away in the division process. These fragments hinder the cells from becoming compact, in other words, they stop the cells from joining together to create all the structures of the embryo, they hinder its development, and can make its evolution come to a standstill.



Fragment removal

In these cases, we try to remove as many fragments as possible and thereby improve its potential.

https://institutomarques.com/en/assisted-reproduction/special-techniques/microsurgical-fragment-removal/

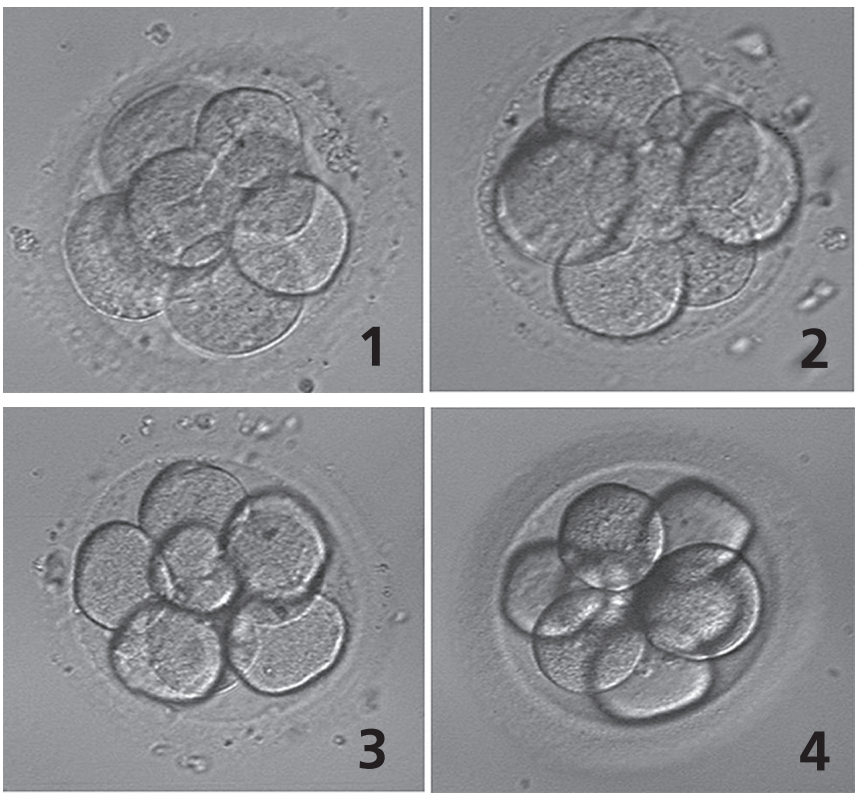
Another way of improving the embryo's potential is assisted hatching.

https://institutomarques.com/en/assisted-reproduction/special-techniques/assisted-hatching/

I will explain what it consists of. When we break an egg and the shell is hard, it means it was laid by an old hen. When the shell is soft, it is from a young hen. The same thing happens with a woman's eggs.

As you know, the embryo develops inside the egg and after five to seven days the pressure of the growing cells breaks the pellucid membrane in what is known as hatching. It breaks the shell and implants inside the uterus. When it looks thick under the microscope, holes can be made in it so that it breaks with greater ease. As you can see, we look after our embryos like small patients, and we do everything we can to help them be born.

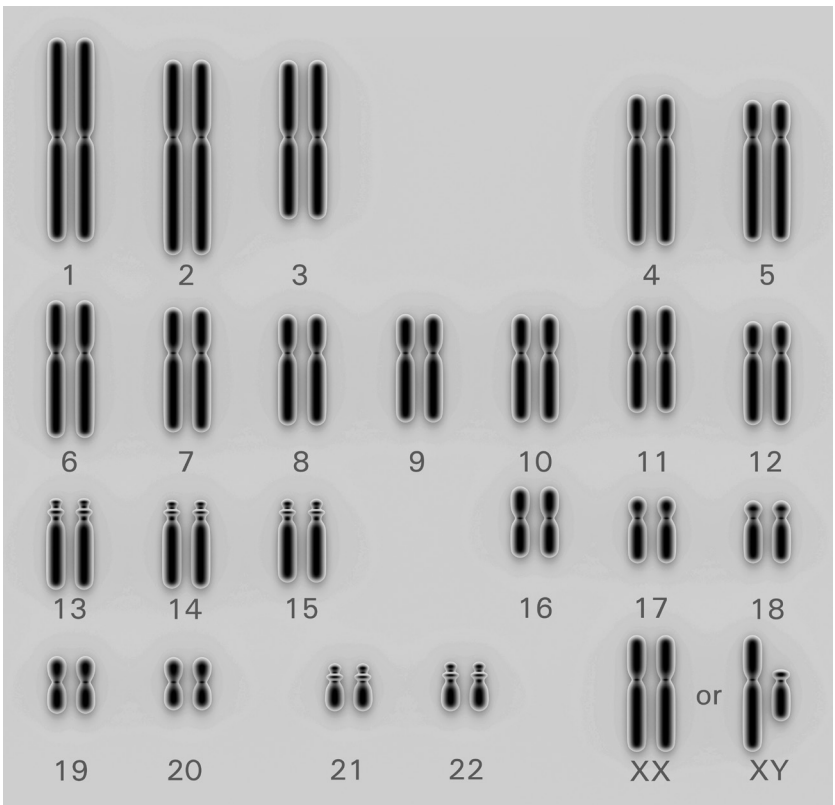
On the third day after IVF, the embryos can be transferred to the patient or be left in the culture for two more days. For the transfer, we choose the best looking embryos; in other words, those with a higher score based exclusively on their morphological appearance, but we do not know if their chromosomes are correct. This cannot be seen with a microscope.



1: Trisomy 21 (Down Syndrome); 2: Trisomy 18 (Edwards Syndrome); 3: Chromosomally normal; 4: Trisomy X, 18, Monosomy 22.

But a morphologically good quality embryo can be a carrier of chromosome anomalies. Above are four embryos belonging to a patient. All of them were morphologically suitable for transfer. At a quick glance, they look the same, but only embryo 3 could really give rise to a healthy evolving pregnancy. Embryo 1 has Down Syndrome, embryo 2 has a syndrome with malformations that are incompatible with life, and embryo 4 does not have the potential to continue dividing for many more days.

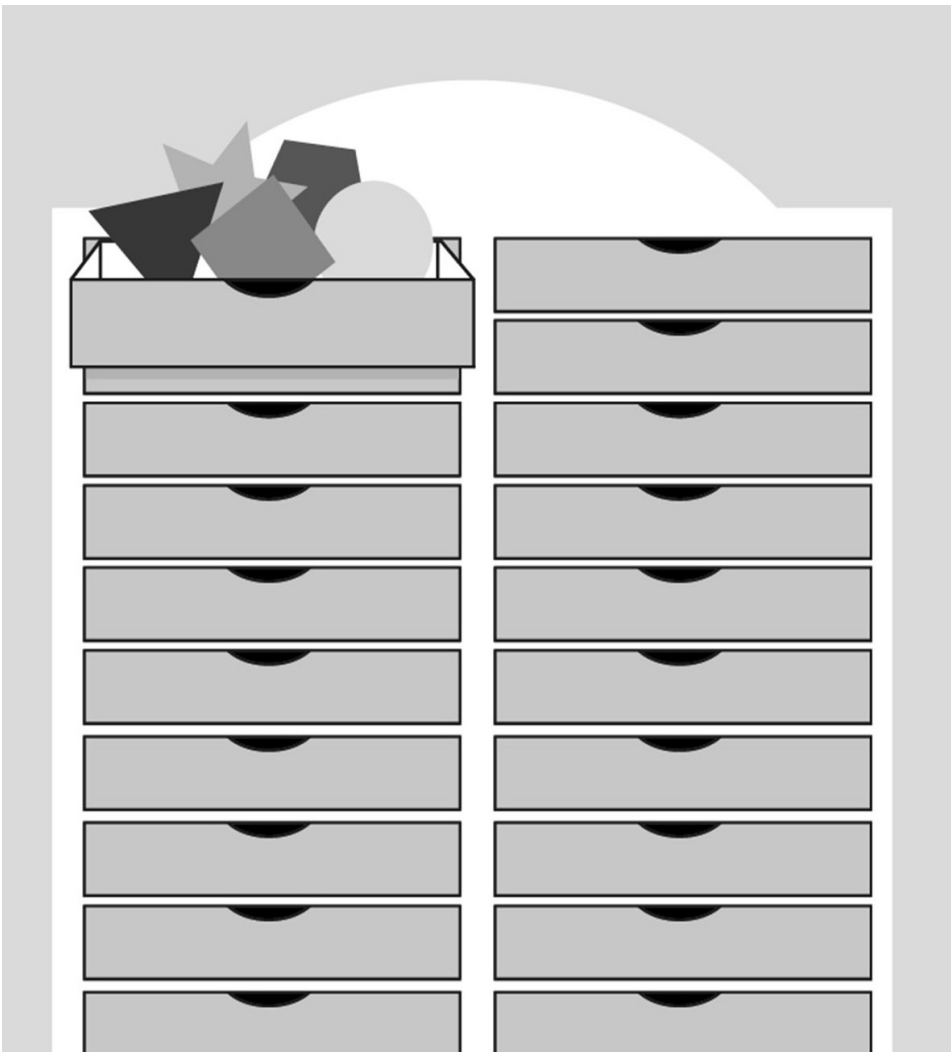
Before continuing, it is useful for you to know about some very important concepts. I want you to be an expert in genetics when you finish this book. The diagnostic test that studies the number and structure of a person's chromosomes is called a **karyotype** and it is performed via a blood test.



Normal karyotype

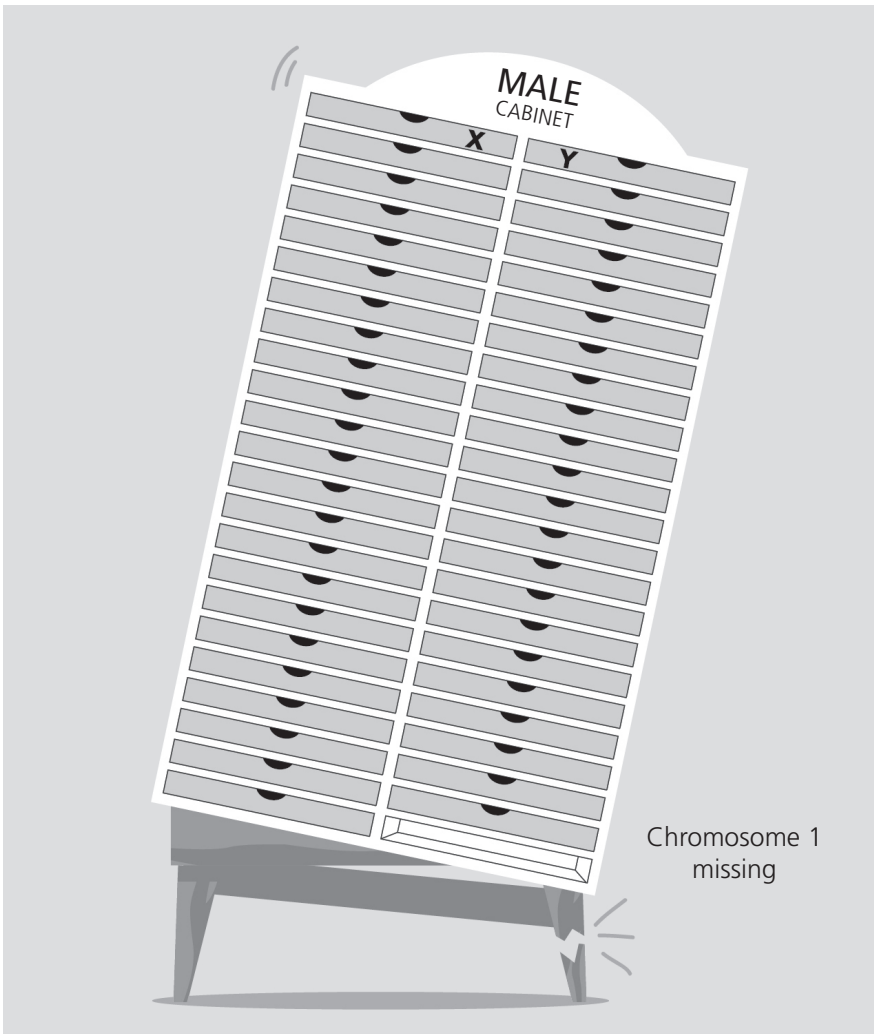
I imagine **chromosomes** like a cabinet with 23 drawers.

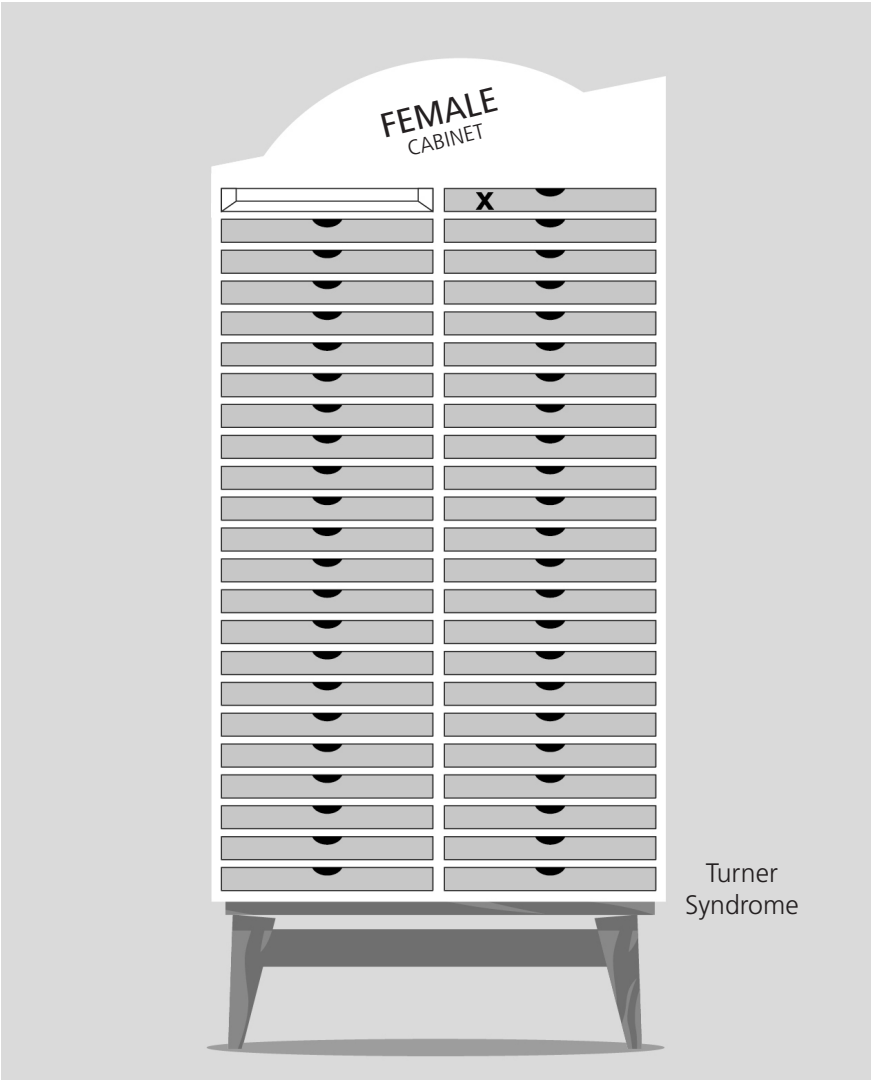
Each drawer of this cabinet contains genes. The genes are structures that determine the characteristic traits of each person. Each gene has a specific mission, for example, it determines height, eye colour, organ function, predisposition to diseases… Each chromosome contains between 200 and 3,000 genes which are in charge of different functions.



Are all the chromosomes necessary?

In order for an embryo to be able to evolve and be born it needs to have all the chromosomes. A missing chromosome hinders the embryonic development in the early phases, leading to miscarriage.

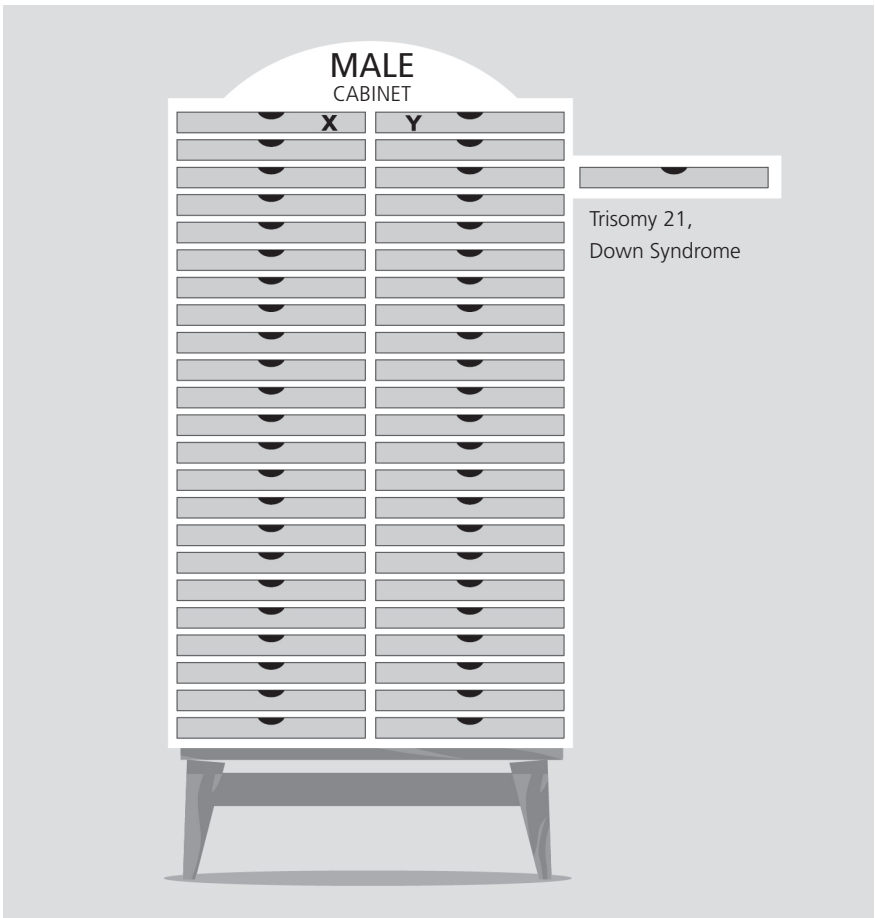




There is only one exception: the absence of one of the sex chromosomes, which gives rise to Turner Syndrome 45 XO. Women who have this disorder look like children and suffer from infertility, since they do not develop sexually. Today we treat them to achieve fertility, and they can become mothers through egg donation.

Can there be extra chromosomes?

The majority of embryos with extra chromosomes cannot develop and in the earliest stages, they do not manage to implant, or they cause miscarriages during the first trimester of pregnancy. However, others with trisomies can be born and give rise to more frequent genetic diseases, such as Trisomy 21 or Down Syndrome.



- Klinefelter 47 XXY Syndrome manifests with an abnormal body constitution, very long legs and a disproportionate body. Individuals with this disorder present sterility and testicular hypotrophy (smaller testicles).

- Triple X Syndrome, 47 XXX. They are girls with psychological and behavioural problems, and they also tend to be tall in stature. It affects one in 1,000 girls born and is often not diagnosed.
- Syndrome 47 XYY is not a syndrome that entails anomalies but the violence rate and tendency to take drugs is higher than usual among these men.

A **mutation** is a change in the information carried by a gene. You probably associate the word mutation with something very bad, but this is not always the case. Genetic mutations are necessary changes for the species to evolve and to adapt to our environment. For example, dark skin colour in hotter places. But there are also errors of nature: anomalous mutations can take place in a group of cells, and give rise to a tumour, or mutations in the reproductive cells which lead to the birth of children with diseases that would additionally be hereditary.

As the maternal age increases, the ovarian reserve ages, resulting in an increase in the number of embryos with chromosome alterations. Thus, the risk of having a child affected by some chromosome anomaly in the general population is 1/385 at 30 years of age, 1/179 at 35 years of age, 1/63 at 40 years of age, and 1/19 at 45 years of age.

This table shows the percentages of chromosome anomalies according to maternal age and, as can be observed, from 37 years onwards, it is considerable.

Percentage of chromosome anomalies in embryos according to maternal age.

Maternal age	% of altered embryos*
35-37	42.3%
38-39	68.6%
40-42	79.2%

*Estimated data from the *Institut Marquès*

When we analyse embryos from donors, in other words, from young and healthy people, 33% are altered. As you can see, even in the best case scenario, human reproduction is not perfect, and that is why gestation does not occur in all cycles. We humbly acknowledge that none of the existing treatments today can impede the physiological involution of female fertility.

The analysis of the chromosomes of embryos is called "pre-implantation genetic diagnosis", **PGD**. It is also called pre-implantation genetic screening, **PGS**.

https://institutomarques.com/en/assisted-reproduction/special-techniques/pgd-preimplantational-genetic-diagnosis/

I will explain how it is performed. When an embryo is on its third day of life, it has six or eight cells, and at that point, all the cells are the same. We can extract one to analyse it. Nothing happens to the embryo (like a lizard's tail); it is regenerated in a few hours. It sounds like science fiction, but today, in 48 hours, we know its karyotype, its chromosome map.

According to studies conducted in our centre, in the best situations, in other words, in embryos from egg and semen donors, one third present chromosome anomalies. This seems like a lot, but human reproduction is not very efficient.

The percentage of anomalous embryos increases with the woman's age, with 78% of embryos of patients over 40 being altered. The DGP enables us to transfer embryos which, in addition to looking correct, are healthy.

It is not performed systematically because it is still an expensive procedure and because not all centres are able to do it; although the clinics that do not have this technology can send the cell to another centre to be analysed.

Some say that the embryo can be damaged, but in expert hands this is not the case. At the beginning of ICSI people also said that it was better not to do it, that the biologists could break the egg during the process…

The embryos are transferred two days later, five days after IVF. Thus, by choosing the chromosomally normal embryos for transfer, the possibilities of pregnancy increase, the risk of spontaneous abortion reduces, as does the possibility of reaching the amniocentesis stage with a problem that could have been detected previously.

Sometimes there are cycles in which all the embryos present anomalies and of course…none are transferred. We even see this in patients from whom we analyse ten embryos! If this patient had not been analysed, two or three fresh embryos would have been transferred, seven would have been frozen and two or three freezing cycles with everything that entails…Hopes, money, trips, absenteeism from work, etc.

As we have mentioned, embryo chromosome anomalies are the hidden reason for many failures to achieve an evolving pregnancy with in vitro fertilisation. DGP is of great diagnostic value. It means that only normal embryos with analysed chromosomes are transferred and avoids the freezing of altered embryos. When the couple has a high percentage of anomalous embryos we have to analyse if the origin is female, male, or both. For example, if the woman is 41 years old this is probably the reason, and so, we will look at the possibility of using an egg donor.

The analysis of embryo chromosomes is especially indicated in:

- Patients aged 38 or more.
- Men with low semen quality, because the lower the seminal quality the higher the risk of chromosome anomalies in the sperm.
- Repeated failures in in vitro fertilisation.
- Patients with a history of miscarriages.

In order for an embryo to implant after in vitro fertilisation and to give rise to an evolving pregnancy, the uterus needs to be ready to receive it, the embryo must have a normal morphology and number of chromosomes as well as strong energy (this is provided by the mitochondria and is less intense as the ovaries age), the embryo and the endometrium must come to an agree-

ment so that implantation occurs and there is no immunological rejection.

But despite all of this, and despite the fact that each child is a miracle, we have managed to reach more possibilities of success than failure every time we transfer embryos. The fertilisation and evolution rates of the embryos depend on the conditions of every laboratory and they are best in large laboratories, with well-trained biologists equipped with state-of-the-art technology.

In short, we can perform standard in vitro fertilisation or we can apply personalised treatments to each of our tiny patients. This is what we call "embryo medicine".

The EmbryoScope from home

Every morning, when the biologists arrive at the in vitro fertilisation laboratory, they marvel at the videos of the evolution of each embryo. They are such impressive images that we thought the patients should be able to see them too and get excited about them…I thought it was a pity that they were missing out on something so beautiful, so we designed a system to remedy it. In 2012, the *Institut Marquès* designed a system, to date unique in the world, which enables our patients to observe how their embryos are developing via the internet. Using their computer or their mobile phone they can "enter" the in vitro fertilisation laboratory and observe their embryos live.

How do the patients feel when they observe the development of their embryos, before they are transferred to the woman's uterus? What emotions are triggered by contemplating a small number of cells that may become their future baby?

Over these past few years we have heard their comments and we have carried out several surveys. And they value it very positively, since it makes them feel like they are participating in the process. One of the main concerns of couples who do in vitro fertilisation is knowing how the embryos are in the laboratory and if they will evolve correctly or not. Often they reach the embryo transfer stage without knowing how they are. Observing

what is happening in the laboratory increases the feeling of control and reduces the uncertainty: being able to gain access and see how their embryos are developing reduces the anxiety in this phase of the cycle. A small percentage of patients prefer not to continue watching them out of fear/respect…It affects them too much.

When we ask them: which embryo do you think is the most suitable for transfer? 26% of cases answer this question, and the embryo mentioned by the patients is one of the transferred embryos in 86% of the cases. It is incredible how well they understand the images! Beauty is symmetry and a lack of imperfections. The couples' concern for the embryos that were not able to evolve is remarkable; from such an early stage their protective instinct has already been awakened.

The most frequent comments when contemplating the embryos are: it is an exciting, impressive, surprising, unique experience, seeing the cells moving rapidly during division, it is like an explosion of life! It is like an "embryonic Big Brother".

Embryomobile

One of my favourite emails is one I received from a patient who, from the mine in which he worked in Australia, used to climb up to the surface every day to look at his embryos. His semen had been frozen in Barcelona and his wife was travelling to Spain to transfer the embryos created with donor eggs. They could share these images.

Opening the laboratory up online to our patients is an example of the empowerment that has emerged in recent years: a new profile of patient who actively participates in the management of their health. The internet has brought about a change in this doctor-patient relationship, shortening the distances. Open and shared knowledge on the internet means that an increasing number of people have access to the information they need to manage their treatment. What is more, it is a prime example of transparency, and the video is the best report of how the cycle has gone.

11

Beginning treatment

The in vitro fertilisation cycle begins

Elisabeth and Jack have just left the biology interview and cannot wait to start the cycle. I begin by explaining to them what **ovarian stimulation** consists of.

To perform in vitro fertilisation, we try to help several follicles develop to complete maturity and we do this by administering a subcutaneous hormonal treatment, and sometimes a nasal treatment.

In each menstrual cycle, hundreds of follicles begin their growth, but when one of them outgrows the others, the development of the other follicles comes to a standstill. This is a physiological process so that we have children one by one. When two grow, there is a possibility of having twins. The type of medication and the dose are customised for each patient, based on her age, the morphology of her ovaries, the hormonal analysis, the body mass, the response to stimulation in previous cycles if there have been any, and our experience.

Ovarian stimulation lasts approximately eight to ten days, during which tests are performed to check how the ovaries are responding. As part of these tests a blood test is performed to determine the levels of Oestradiol (the hormone produced by the

growing follicles) and a vaginal ultrasound to observe how many follicles are developing in each ovary, and the size of each. According to these tests, the medication dose is modified and the date for the next test is given, one or two days later.

When the follicles have reached approximately 18-20 millimetres in diameter, and the Oestradiol levels are adequate, another medication to induce ovulation is administered.

This injection is the most important, since an error in the time at which it is given or in the way it is administered can result in a failure in the retrieval of the eggs. In some cases, when the response is not as required, the cycle can be cancelled and recommenced with another stimulation protocol.

—"In your case, Elisabeth, you need a high dose. The medication is very simple to administer and before you leave we will show you how to prepare it so that you can inject yourself into your abdominal flesh. That will give you some autonomy. The IVF process is simple. Think that young women who donate eggs go through it, and if it were painful or risky, we would not do it. You can and you must live a normal life, combining it with your work. You just need to rest on the day your eggs are retrieved."

—"Doctor, with everything you have said about how easy the process is I am thinking about telling my sister Fátima to freeze her eggs. What do you think?" Elisabeth suggests.

—"I think it's a very good idea. Especially because the degree of fertility is a characteristic individual to each person and it has a family element. You have an older ovarian age than expected, so your sister could too."

—"We have read all the legal information that you gave us, but we have some doubts about how we should fill in the informed consent forms concerning what happens to the frozen embryos" asks Jack.

—"Later we will talk about that, but first I want to discuss the number of embryos that we are going to transfer. My advice is to only transfer one because that way we avoid the risks of a twin pregnancy and because currently, in good

laboratories, the embryos do not lose implantation potential, even if they have been frozen. Think about it among yourselves.

Now we have to design your IVF cycle."

—"From what the biologist told me in the interview, it is as if IVF were a car to which we have to add the extras we want."

—"You're spot on. IVF was initially invented for problems in the fallopian tubes and the 'test' performed its function, but today it is applied in all types of fertility problems. There are many complimentary treatments and in each case there are some that are not indicated, others that are necessary and some that are optional, according to what each couple wants."

—"We want to analyse the embryos to prevent miscarriages."

—"Yes, considering your age, we will do a pre-implantation genetic diagnosis. Now, with your next period you can start the injections and we will meet again in the next analysis test and ultrasound."

Beginning of the artificial insemination cycle

Marta asks me:

—"How is an intrauterine insemination cycle performed?"

—"The first thing we will do is stimulate the follicular development with medication. In inseminations, we give a very low dose because we want one or two follicles to grow. That way we avoid the risks of a multiple pregnancy. You will start with 75 IU (international units) of subcutaneous Gonadotropin, which is the hormone that stimulates the ovaries, FSH (follicle stimulating hormone) and LH (luteinizing hormone). The growing follicles produce Oestradiol and when the levels exceed 300 pg/ml the hormonal circuit that induces ovulation is triggered.

In a treatment cycle we have to inhibit this process to prevent you from ovulating before time. That is why we need to add another medication. We can choose between GnRH antagonists which are administered subcutaneously once the largest follicle has reach 14 mm in diameter, or subcutaneous or nasal spray GnRH agonists from the first day of your period."

—"I would prefer the nasal spray. But, can we not do it without medication?" Marta asks me.

—"Yes, we can, but the possibilities of pregnancy are lower and many more tests are required. With the medication we know exactly when you are going to ovulate."

—"What risks do the medications carry?"

—"Many studies have been conducted about possible diseases and risks of cancer after having received these drugs. However, there is no scientific evidence that they cause any posterior problem. When a woman is pregnant for nine months, she has much higher hormone levels than these.

If an excessive response occurs, it can give rise to **ovarian hyperstimulation syndrome**, but with the doses indicated for insemination and the tests performed, this is exceptional. This risk appears in stimulations for in vitro fertilisation in women with polycystic ovaries. In these cases, we must induce ovulation with medications which after ovulation cause menstruation, freeze the embryos and perform the transfer in the following cycle. That way we avoid risks, and today the results in terms of pregnancy are the same with fresh and frozen embryos."

—"How many days of injections?"

—"Eight to nine days. When the follicles have reached approximately 18-20 mm diameter, the HCG injection is administered. This hormone provokes the last changes of maturity and ovulation. In other words, the external membrane of the follicle breaks and the egg is released 36 hours after being injected."

—"What happens on the day of insemination?" Marta asks.

—"I will tell you what happens in the laboratory and what happens in the clinic. When the partner's semen is used, the man collects a semen sample at home and delivers it to the centre within one hour.

In the semen laboratory the semen is prepared using a gradient technique that separates the seminal plasma, and strengthens and concentrates the motile sperm. If donor semen is being used, the patient is placed in the gynaecological position and a thin cannula is inserted via the cervix, through which the sperm will be deposited in the uterus. A few minutes later they will have reached the fallopian tubes where the egg is waiting for them. It is not uncomfortable, it takes a couple of minutes and you do not need to rest afterwards."

—"How many inseminations are performed in each cycle?"

—"If the cycle is performed with treatment, only one per cycle is needed. The insemination needs to be synchronised with ovulation (only a few hours should have passed). If 24 hours have passed since ovulation or the semen arrives 24 hours before this, it is unlikely that we will achieve our aim."

—"What happens after insemination?"

—"I will prescribe you a treatment with one pessary (vaginal suppository) of progesterone every 12 hours for 11 days. The aim is to prepare the endometrium optimally to receive the embryo. You can lead a completely normal life.

Once the insemination has been completed, you will not need to have any more tests. You just have to wait. As you get closer to the day of the pregnancy test, you will feel increasingly nervous. You need to be psychologically prepared for these days."

—"I saw on the internet that you can buy semen from European centres and have it sent to your home. You can even choose them" Marta tells me.

—"I do not recommend 'home inseminations'. The thawed semen placed in your vagina with a syringe on the day you think you are ovulating has very low possibilities of success.

During the freezing and thawing processes, although the semen may be of very good quality, it loses motility. That is why the results are much better if it is strengthened after thawing and deposited inside the uterus."

—"What are my chances of getting pregnant?"

—"The probabilities of pregnancy depend mainly on the age of the patient and the number of follicles that develop in each cycle. With ovarian stimulation and intrauterine insemination, the probabilities of pregnancy per cycle are 20-25%. Once pregnancy has been achieved, the risk of miscarriage depends on the woman's age, and the risk of ectopic pregnancy, which is when the embryo implants outside the uterine cavity, is like in any spontaneous pregnancy, and has an incidence of 2%. This also happens in pregnancies after in vitro fertilisation."

—"Can inseminations be performed every month?"

—"Yes. They can be done one after another; there is no need to rest between one treatment cycle and the next. Before beginning each cycle we will assess if any variations need to be introduced in the ovarian stimulation."

—"How many insemination cycles can be performed?"

—"According to the statistical data, 90% of patients who achieve pregnancy do so in the first four cycles. Therefore, if it has not been successful after three or four cycles, the procedure may need to be changed. Other sterility factors may exist that cannot be corrected with inseminations.

—In your case, if you do not get pregnant in three cycles, we will perform in vitro fertilisation."

—"You have already explained it to me, but, could you tell me again who the sperm donors are and if tests are done to ensure they are healthy?"

—"They are young and healthy men who want to help people who need it. The majority of them are university students. They undergo a rigorous medical and analytical check-up which, for example, in our centre includes the FISH study in sperm, sperm DNA fragmentation and the 50 most frequent mutations for cystic fibrosis. The blood tests to de-

tect sexually transmitted illnesses are repeated regularly. A family clinical history is also performed, to rule out hereditary diseases."

—"I'd like to know who my donor is. Have you already selected him?"

—"Yes. I chose from many donors from our sperm bank. I have seen his face, his physical characteristics and the number of children he has. There is a maximum number of children they can have in each country. This is determined by law to avoid siblings meeting each other and potentially getting into a relationship with high genetic risk due to consanguinity."

—"My friend Margaret tells me that she is going to give me the injections. I can do it on my own but I'd prefer her to do it. Then I can see her and talk."

I don't tell her, but Marta appears sad to me. Often women who take on maternity on their own are happy and excited and this enthusiasm spreads to the people accompanying them. They tend to come with their mother or close friend and they feel secure and supported. I don't know how to help her…

12

Twin pregnancy

Rose and James are over the moon. All of James's tests came back correct and they can start the treatment. In fact, they want to do it "today". I can't help but laugh as I tell them we will start with her period, in one week, and that seems too late to them!! They need to know the exact day the eggs will be retrieved because James is constantly moving around for matches. I tell them that it will be 11-14 days after the beginning of her period, but we cannot know the exact day until two or three days before, and that it is better to have a frozen semen sample in case it coincides with him being out of Barcelona.

He is not sure. He wants to be with Rose that day. She gets emotional and starts crying as James lifts her off the chair and hugs her. They stay like that for a while, as if I weren't there, while I prepare the course of medication, consent forms, etc.

Since the dramatic scene doesn't come to an end, I interrupt telling them that they will experience the process together because they can look at the EmbryoScope from home.

Now I suggest that they transfer only one embryo in the blastocyst stage. I explain that the results in terms of pregnancy rates are the same as for two embryos on the third day of evolution, and we avoid a twin pregnancy. But they don't pay me any heed. They would be over the moon to have twins and they want to transfer two blastocysts. I explain that although Rose is young, healthy and tall, a twin pregnancy is a high risk pregnancy and I ask them to reflect on this decision.

Do you want to have twins?

In 2011 we conducted a survey with patients who attend our centre for the first time with fertility issues and who have no children. 900 patients from 31 countries were asked if they would prefer to have twins, one child or if they were indifferent. The results indicate that the majority of couples who perform reproduction treatments would prefer to have twins.

52% would prefer to have twins, 30% would prefer one child and 18% do not mind as long as they achieve pregnancy. There are great differences between countries. In Spain, Italy and some Scandinavian countries such as Norway, more than 60% of couples state that they would like twins. On the contrary, in countries such as Germany and France, this percentage reduces by half and only 30 and 40% of couples respectively would like to see themselves pushing a double buggy.

Fertility specialists are not surprised by the results of this survey. Every day we experience the conflict between what the patients want and what we the doctors advise, because it is difficult to explain the risks of a twin pregnancy to women who are afraid they will never be mothers.

When we discuss the possibility of having twins, the majority imagine that they will be doubly happy and that their family will be complete. Coks Feenstra, expert in twin studies, after interviewing tens of adult twins says: "Being a twin is having twice the joy and half the pain".

Do you know what causes a twin pregnancy?

On the one hand, **dizygotic or fraternal twins** come from two different embryos and resemble each other like any other sibling. Spontaneously, it is due to the genetic predisposition to ovulate from two follicles. A double ovulation occurs and a different sperm fertilises each egg. This heredity is transmitted both by men and women. Women who have twins in their family have a higher chance of having them themselves. If the man carries the heredity, it will be expressed in his daughters (men do not ovulate). The myth that it skips a generation is not true, but we see it frequently because of what we have just mentioned.

If a woman has sexual intercourse during ovulation with more than one man there is a possibility of having a twin pregnancy from two different fathers. The **natural incidence of twin pregnancies** is 0.8%. It varies according to race. In Asia it is lower and in some African countries such as Nigeria it is double (one in every 40 births). We also know that it increases with the age of the woman and the more children she has had previously.

In countries with access to assisted reproduction techniques, 1.6% of births are twin births. After fertility treatments, twins occur because we frequently transfer two or three embryos to increase the possibilities of pregnancy. But the trend is fewer multiple pregnancies, and triplets are exceptional.

Monozygotic or identical twins come from one same embryo. A zygote is a cell formed by the union of an egg and a sperm. It is divided and produces two physically identical people. Despite advances in genetics, the real reasons causing this spontaneous embryonic division are unknown. Regardless of the treatments applied to the embryo, it increases with any reproduction technique, even if it is not IVF.

It occurs in the first days of embryonic life, and depending on when it happens, the twins can share the same placenta and even the same sac of amniotic fluid. Spontaneous monozygotic twins are rare: they only represent 1% of twin pregnancies and 3% of those resulting from treatments. These children have a much higher risk of not being born because the circulation of the blood

may not be distributed evenly between them, or their umbilical cords can become tangled.

Triplets

Today, in 2016, we should under no circumstances transfer three embryos. The possibilities of an embryo implanting have increased as IVF techniques have improved, and it is such a high risk for the woman and the babies that it does not make sense. Frequently, transferring more embryos stemmed from avoiding the loss of vitality after freezing processes but today, with the excellent results achieved with vitrification, this has changed.

In 1999 I had the opportunity to witness a unique experience. After an in vitro fertilisation cycle, one of the embryos divided into three, and three identical triplets were born. Fortunately, thanks to excellent care, they were born healthy. Now they are very good-looking, clever and athletic boys. If you look up "Identical triplets Institut Marquès" on the internet, you can even watch the Caesarean section. When I see the statements I made to the press at the time I am shocked: things have changed so much!

At the time, ultrasounds were poorer quality, and in the first ultrasound we saw one vesicle and an incipient embryo; a week later we saw two embryos, and a week later three embryos inside the same gestational vesicle. During the first year of life, we followed their development along with scientists and paediatricians. These children provide the possibility to study the traits that are determined genetically and those that are independent of the genetic code.

The weight evolution curves, cephalic perimeter, height and dentition…were all completely identical. But their character was already different in the incubator, and identical twins have different fingerprints and footprints. The pattern of the fingerprints is formed at three months of pregnancy. The skin of the palms, soles and fingers is especially fine and sensitive. The movement of the amniotic fluid around the foetus draws the lines; similar to the marks left by waves on the sand. They are random.

According to the conclusions of the police, in the first few days of life, fingerprints often do not leave enough of an imprint to enable posterior identification. They do not change, but they are really valuable from three months onwards. Footprints provide a more efficient identification at birth, but they lose their value when the child starts to walk. Thus, fingerprints are different in all human beings.

A twin pregnancy by definition is a high risk pregnancy

For a woman's body it is a great effort to adapt to this extra load and the following can occur:

- High blood pressure in 15-20% of women expecting twins.
- Diabetes in 5-10%.
- Moderate-severe anaemia in 40% of cases. 5% require post-partum transfusion.
- Caesarean section in 50-85% of cases, according to the centres.

These risks increase with the age of the mother, and women who have conceived after assisted reproduction techniques are older. For the children, the risks mainly stem from prematurity and delayed intrauterine growth. Twins who are born before 35 weeks gestation are considered premature.

- 19% of twins are premature.
- Delayed growth of one twin occurs in 30% of pregnancies and of both in 15% of cases.

Perinatal mortality (death between five months' gestation and the first week of life) in single pregnancies is 5 in 10,000 and in twins is 30 in 10,000, in other words, six times higher.

13

The life and fate of frozen embryos

From what my patients tell me, there is great ignorance surrounding **how frozen embryos live**. Some imagine entire walls filled with cabinets with a type of mini bunk-bed. Others think they are in rooms like huge fridges, others in capsules placed in panels of cells like bees… But, in actual fact, they are microscopic and take up very little room. In a container measuring one metre high and 70 cm wide, there is enough room for 10,000 embryos.

The residents of the cold nursery live in tanks with compartments in which siblings are placed together, in plastic beakers, and each family has a different colour. Inside the beakers, they sleep in white plastic straws and each of these can contain one, two or three embryos. These straws also contain a rod of a particular colour, labelled with the name.

We have special printers that print miniscule labels with all the identification codes. Thus, each family has its combination of colours and codes, and their address within the residence-igloo is recorded on the computers, in order to be able to locate them.

Blastocyst hatching

They can be frozen at different stages of development: from the day of fertilisation, when it is still only one cell, to five or six days later, as a blastocyst, when it contains many cells but is still the same size. During the first seven days of life the embryo grows inside the egg's external membrane, just as chicks grow within the shell of an egg…Let us not forget that an egg is a hen's oocyte! When the cells break this membrane, it is called blastocyst hatching. They leave and immediately implant in the internal layer of the uterus. That is why they can only be frozen up to this point.

The embryo freezing process takes a few hours. They are placed in a cryoprotectant solution that helps to prevent the formation of ice crystals and they are gently sucked into the straws. These are placed in a freezing machine in which the temperature is gradually reduced, and after 90 minutes they are at -196º centigrade. The straws are then introduced into a container filled with liquid nitrogen. 30 years ago the first boy who had lived as a frozen embryo using this system was born.

However, today we prefer to use another system called vitrification. The difference is that the time in which they go from 37º to -196º is several minutes and they are turned into a solid material similar to glass.

Vitrification was developed because traditional freezing was not working with eggs. Like at home: we can freeze chicken, but

not eggs because they contain a large quantity of water which forms ice crystals when frozen; these break the delicate structures that they contain. In addition, if during the thawing process some of the embryonic cells are damaged and disappear, an embryo, for example frozen as four cells, can end up with two, but it has the capacity to continue dividing and in several hours it will have four again. However, an egg is only one cell, so if it is damaged it cannot be repaired. Vitrification is an extremely laborious procedure and it requires very experienced hands, but pregnancy rates have greatly improved, since the percentage of embryos that survive is close to 100%.

There is probably no time limit for the life of a frozen embryo. In 2006, at the conference held by the Spanish Fertility Society, we presented the record published in this respect, as part of our Embryo Adoption Programme: a boy who had been frozen for 13 and a half years was born. He has two biological siblings, but he was born into another family and he will never meet them.

In all laboratories, the tanks containing semen, eggs and embryos are white or steel, very serious and boring. But ours our very pretty; we have put toys in them…

Fresh or frozen embryos. What is best?

In 2014, Dr Esther Velilla, our laboratory director, presented the latest results, and I was amazed because the pregnancy rates via transfer were the same with embryos that were transferred fresh and those that were transferred frozen. Even with embryos that had been frozen twice! This situation occurs when more embryos are thawed than will eventually be transferred. In these cases, they are left in culture and, if they reach the blastocyst stage, they are frozen again. Sincerely, I thought that I would never witness anything like that in my professional life.
We are witnessing an enormous medical breakthrough because it means:

- Greater possibilities of pregnancy per cycle. By cycle we mean each time eggs are retrieved. This means that in many

cases we do not need to perform another puncture to attempt a second pregnancy.
- Reduction in the number of multiple pregnancies. Despite preferring a single pregnancy, patients often ask us to transfer more embryos to prevent them from deteriorating in quality by being frozen.
- If following the retrieval of eggs the patient cannot continue the treatment for medical or family reasons, it will not matter if the transfer is not performed fresh. This is very important in women with polycystic ovaries because they run the risk of hyperstimulation.

Several studies have been published revealing that the results with frozen embryos may even be better, since the preparation of the endometrium is more physiological. In fact, they propose freezing them all and not performing fresh transfers. I do not expect that much. What is more, it would delay the process and make it more costly.

To date, patients do not completely believe it. A very nice husband of a client said to me one day: "How could it be the same? You can even tell the difference with hake!"

Let's look into it.

The great challenge of freezing blastocysts

I have already told you how frozen embryos live. What biologists have found hardest is managing to freeze blastocysts. Blastocysts are embryos that are five or six days old. They have a mass of some 200 cells which will give rise to all the structures of the embryo, called the inner cell mass. The other cells are called the trophectoderm and this will become the placenta. In addition, they have a very large lagoon of water known as the blastocoel and an external membrane that is at the point of breaking, since the blastocyst is the stage prior to implanting in the uterus.

The difficulty in freezing blastocysts is mainly due to the quantity of water they contain.

To successfully freeze blastocysts, water is extracted from them, whether by piercing them or by surrounding them with a chemical solution that absorbs the liquid. First they are left without water, and then they are rehydrated to thaw. And not a bother on them!

In this video you can observe how this process is performed. The images have been accelerated, since these processes take hours: https://institutomarques.com/en/assisted-reproduction/treatments/in-vitro-fertilization/blastocyst/

The fate of frozen embryos

If you did not want any more children, what would you do with your frozen embryos? I would like you to please consider the following situation: you have two children who were born after IVF and you do not want any more, but there are three remaining frozen embryos from the treatment cycle. Let us also suppose that, as this has happened in Spain, you can choose any of the possible outcomes for them. What would you decide?

I will tell you exactly what the law establishes and our experience with patients.

When an IVF cycle begins, an informed consent form is signed, choosing the fate of the embryos that are not transferred. At this time, nearly all patients decide to keep them, thinking that perhaps they will not achieve pregnancy with the fresh embryos or that they might want more children in the future. Every year they receive a letter from the centre to validate or change this decision.

The law in this respect differs in every country.

According to the Spanish Law on Assisted Reproduction, Law 14/2006. Chapter III. Article 11:

> *"The different possible fates of cryopreserved preembryos, as well as, where relevant, cryopreserved semen, eggs and ovarian tissue, are":*

a. Their use by the woman herself or her spouse

> *"This is possible until the woman's fertility circumstances are exhausted and she can no longer receive an embryo for medical reasons".*

As the law does not establish a specific age limit, or specify the medical pathologies that may contraindicate a pregnancy, it is difficult to know when this stipulation ends. Thus, **38.8%** of patients continue to keep their embryos for their own use. It is important to note than **91%** of these patients have completed their reproductive plans and do not want to have any more children, but they prefer to keep them because they cannot decide on any of the other options.

b. Donation for reproductive purposes

In other words, donating them to other patients. In our centre, only **4.7%** of the patients who send us letters have chosen this option. When I ask them why they do not want others who are in the same situation as them to receive them, they tell me that they are afraid their children will meet their siblings.

I tell them that our assignment system does not allow this because they go to another country. However, this does not change their minds. They do not choose this option with the intention of protecting their children. Some couples do choose it, but afterwards the centres cannot carry out their wish because their embryos do not meet the requirements to be "suitable for donation", for example, when the woman is aged over 35.

c. Donation for research purposes

> *"Patients must receive and sign a letter from the Centre specifying the research project to which the embryos will be donated and they must renounce economic compensations derived from said research".*

3.9% of patients donate their embryos to research. This option terrifies them; they imagine terrible things. We have still never donated an embryo to research because there are very few lines of research with embryonic stem cells.

d. Terminating their preservation without any other use

Only **4.7%** of patients choose to destroy the embryos. They tell us that they feel sad about getting rid of them.

This means the receiving woman's fertile period has to have ended, and this must be accredited by a medical report from professionals who do not work in the centre. This requirement means that only one third of these embryos have been destroyed, since we do not receive these reports, which have to be organised by the patients themselves.

The majority of patients, 47.9%, do not respond to the letters they receive from the centre.

It is not that they wash their hands of the matter; it is that this decision is very important to them, and it causes them emotional conflict.

Thus, despite having a law that permits all the possible options of fate, the majority of the embryos are abandoned. According to the legal regulations, in this case they become custody of the medical centre.

In 2004, the *Institut Marquès* implemented the first "Embryo Adoption Programme" in the world, and since then, we have sent all the embryos that meet the medical requirements to be "suitable for donation" to the Adoption Programme (embryos from young and healthy parents; woman aged less than 35; entire medical history of ancestors and children born from the IVF cycle; their physical characteristics, blood group and Rh, etc.).

Thus, for the first time in the world, we coined the term "embryo adoption". We believed that the fate of the embryos could not only be the decision of our team, and we decided to share this responsibility with the whole of society, providing these embryos with the possibility of living, helping them to find a mother.

Embryo adoption

While adopting a child means welcoming a human being as your child, something the child's biological parents were not able to do, adopting embryos means performing a treatment to become

pregnant with embryos that have been left with no fate. The difference between embryo adoption and donation is only legal. In the "Donation Programme", the embryos are from couples who expressly donate them in writing to other people.

In the "Adoption Programme" the ancestors have not chosen the fate. Therefore, they are "abandoned" embryos and the centre becomes the legal custodian. I beg of you not to think that the people who do not respond to our letters are "abandoning their remaining embryos". This is not true. The large majority tell us "I trusted you to create a family and I still do. Decide what you think is best".

No procedure is required. In both cases, all that is required is the informed consent form for this assisted reproduction procedure. In both cases both spouses have to sign if they are legally married. If it is a de facto couple and the man signs the consent form, it means that at that moment he acknowledges the potential paternity. If it is a woman on her own she signs it. In all-women couples it is the same as in mixed couples. On a medical level, the procedures are the same for the two programmes.

Since the beginning of the programme the response has been exciting on account of the great social acceptance and the support obtained from different groups, as well as the fact that women from all social classes, races and nationalities have come to adopt them. Only one week after starting our Embryo Adoption Programme, the news had reached nearly every country in the world, and we received letters from groups of all trends and colours, from environmentalist associations to religious associations to lend us their support and congratulate us on the initiative. Today we continue to be the centre in the world where the most embryo donations and adoptions take place.

How are embryos assigned?

When patients choose embryo donation/adoption in our centre, they sign a consent form in which they state that they know and accept that in the assignment, race will be considered, and nothing else, not even the blood group, although this is not medically important.

We ensure that the embryos are donated to different autonomous communities or countries to prevent consanguinity or siblings from meeting.

Who adopts embryos?

Every day my colleagues and I continue to mention how special each and every one of the embryo transfers in this programme is. It is exciting to think of the origin of each embryo and how fate has made it reach this woman; the love, dreams and hope that can be felt in this moment. Behind each case there is a story either of many failed previous treatments or of having given up on the possibility of being parents because morally they do not contemplate it for different reasons, and this has appeared as a new opportunity. They are the nicest transfers, brimming with hope!

For every child that is born, a book of love, devotion and appreciation for life could be written. What is more, the chapters about their biological origin, of when they were embryos, of chance and destiny, would be fascinating.

The receivers are, in half of the cases, couples with continuous failures in assisted reproduction techniques with an average of 4.1 years wanting a baby and 4.4 failed attempts. Many have already given up on the possibility of having a child and this programme opens up a new opportunity for them.

But the other half of the cases correspond to situations with a considerable social component: women without a male partner, individuals who had started the procedures to adopt a child and couples who had not contemplated reproduction treatments on account of their religious or ethical beliefs.

Spain has a more liberal assisted reproduction law than that of many other states. Thus, the patients who we have treated come from 28 different countries.

What does the treatment consist of?

It is very straightforward and painless. The patient comes to the centre one day for the first visit and another day to transfer the em-

bryos. Just two days. In the first visit the woman's health is checked, to ensure she can carry the pregnancy, an ultrasound is performed as well as a catheter test which will be used to transfer the embryos via the cervix.

We can divide the treatment into five phases:

1. The treatment consists of oestrogen patches applied to the skin from the first day of menstruation. In a few days the uterus will be ready to receive the embryos.
2. An ultrasound is performed to check the endometrial morphology and thickness on day seven to nine of the cycle. The patient does this where she lives.
3. The transfer is scheduled for a few days later. The results are best this way. Before the transfer, a vaginal progesterone treatment is applied.
4. One to three embryos can be transferred, depending on the patient's wishes, but usually we transfer one or two. The patient does not need to stay overnight, and we do not think rest is required either. The patient can travel after leaving the clinic.
5. After 14 days, if the pregnancy test is positive, the oestrogen and progesterone doses are increased and must be maintained for two months, since ovulation has not occurred. It is a hormone replacement cycle.

It is a normal gestation. The pregnancy is monitored by the patient's usual gynaecologist.

Posthumous assisted reproduction

Some people die and leave frozen semen or embryos. At times they are a "genetic inheritance".

A man, for example, can freeze his semen before treatments such as chemotherapy which could affect the function of the testicle, or freeze it for in vitro fertilisation because he foresees that the day the eggs are retrieved it will be difficult for him to take his semen to the

reproduction centre because he will be away, lives far from the clinic or due to difficulties collecting same.

It can also happen that embryos are frozen, since an in vitro fertilisation often generates more embryos than those transferred to the woman and "leftover frozen embryos" remain.

Reproduction centres systematically take this into account. To proceed to semen or embryo freezing, the patient must first sign a consent form stating what they want the fate of these to be in the event of death: destruction or use, and in this case, the name of the woman who can use them.

The woman who receives this potential inheritance and wants to have children after the man has passed away, finds herself in a legal situation that varies according to countries.

In Spain the woman does not need to be the legal spouse of the deceased person, but she has a maximum period of 12 months to become pregnant. After this time, the notary must definitively know who are the heirs of the material assets of the deceased individual.

In my work, every day I witness the face of surprise of patients when they read the documents. I have experienced special situations such as treating a recently widowed woman in Ireland who asked us to implant her husband's embryos which had been transferred from there because in her country she could not do it; or parents who are desperate to try to have a descendent of a dead son and not be able to because in the consent form, the latter had not left any name, or the widow simply did not want to.

It is curious that we the professionals who are so much in contact with the start of life have to ask our patients every day what their wishes are for the end of same.

14

Embryo transfer and waiting for the pregnancy test

Rose, as she is young and slim, has taken a stimulation prescription with 200 units of gonadotrophins, the hormones that stimulate ovulation. At first, she said that she didn't want to inject herself, but Anabel, my ever reliable nurse, insisted on showing her and she did it perfectly. What's more, she is happy to have overcome another challenge.

She did not think she would tell anyone that she was having treatment. However, her first test was on a Saturday, when James was playing away. She couldn't hold it in anymore and she told her friend Lourdes and her parents. That day they all came to the ultrasound!

Jordi, her father, told me that now young people do things backwards. The logical thing would be to get married, and then have a baby. He didn't understand why they would be having in vitro fertilisation before the wedding but….they would probably get married soon. Her mother's eyes were still on stalks after hearing the surprising news and seeing how fast everything was happening, and the shock left her speechless. Lourdes, after realising that her advice had not worked, supported her because she had never seen Rose so happy.

During the cycle, 11 follicles developed and she was lucky because her eggs were retrieved on a Tuesday and they could both come to the clinic.

Nine mature eggs were retrieved, and the next day I called her to tell her that six had been fertilised. On day +2 all of the embryos were divided and had been given scores: 10, 10, 9, 9, 7, 6. On day +3 all the embryos were divided and had been given scores: 10, 10, 9, 7, 6, 6. I tell them that on day +4 I will not ring them because they cannot be assessed. However, since she insists, I tell her that at that time all the cells join to send information to each other and to agree on their differentiation and on the tissue that each one is going to form. Under the microscope we can only see what we call the morula. Therefore, we have to wait until the next day to see if the embryos are capable of performing this function correctly until a blastocyst is formed.

Since James was away at times, they talked every night on Skype and discussed the images of the embryos in culture.

On the day of the transfer, Rose and James are nervous when they arrive, but once they see me smiling they relax. I tell them that I am very happy because we have three blastocysts with a very good appearance and, that according to their wishes we are going to transfer two and freeze one.

Despite everything we had told them in the biology visit, they find it hard to understand that it is normal that three of the six embryos did not evolve correctly.

After leaving the office to go to the transfer room, James tells Nuria, my secretary, that if we had put the FC Barcelona anthem on instead of Antonio Orozco, maybe all the embryos would have evolved.

The embryo transfer is the last step in the in vitro fertilisation procedure and it is of decisive importance to the end result. It consists of depositing the embryos as delicately as possible inside the uterus. Technically, the embryo transfer occurs vaginally, without anaesthesia and on an outpatient basis. With ultrasound monitoring, a catheter is gently introduced into the cervix and the embryos are placed inside the

uterine cavity. The patient's bladder must be full in order to be able to see the catheter. Afterwards, she rests for a few minutes and can carry on with normal life.

Assisted reproduction processes may generate levels of anxiety. The most stressful moments are during the fertilisation and evolution of the embryo in the laboratory and after implantation, while the patient waits for the pregnancy test.

Rest after embryo transfer

In 2012 we presented a wonderful piece of work which shows how the uterus moves the embryos so that they implant correctly. We conducted a study which reveals what happens when the embryos arrive into the uterus, both naturally, through a fallopian tube, and following in vitro fertilisation through the cervix.

The endometrium (internal layer of the uterus) makes movements and, when the embryo arrives, its function probably consists of keeping it in the uterine cavity, preventing it from being expelled and from implanting in unsuitable places.

After the embryo transfer many patients feel guilty for not resting enough, especially if the cycle does not finish successfully. We tell our patients that they do not need to rest immediately after the embryo transfer or in the following days, but they are surprised by this information and they do not seem to pay much heed to it. They are scared that if they stand up or strain at all, such as going to the toilet, the embryos "will fall out". They think they need to be consciously trying to keep them inside the uterus. This creates anxiety and adds to the stress of not being able to rest as much as they would like. In addition, it coincides with the phase of the cycle in which they are at their most labile and nervous, since they are waiting for the pregnancy test.

These findings play down the importance of rest and support the early mobilisation of patients after embryo transfer. It is very helpful to know that the implantation does not depend on anything you do or stop doing and that, once the embryos have reached the uterus, our endometrium takes charge of everything else… **It is as if it were rocking them so that they implant in the right place!**

It is a waiting period brimming with desire and hope that the embryos will implant, but also filled with fear of failure and with our emotions running high.

What can be done to encourage implantation? What can be done to prevent anxiety?

All our patients ask us this. My advice is to live life as normally as possible, and to keep your mind busy. Women who decide to stop working spend their days wondering what is going to happen, and this creates even more anxiety. We often mention that they can have sexual intercourse, while avoiding very vigorous penetrations in the first few days, but we say this out of habit; it has no real basis. In any case, patients tend to avoid sex because they don't feel like it at all…

There are continuous breakthroughs in in vitro fertilisation laboratories to improve the quality of the embryos, but there are very few to improve the implantation of the embryo. **As the Cochrane reviews state today, all the treatments implemented have not been able to scientifically prove their efficacy: treatments such as for example endometrial scratching (performing a biopsy on the endometrium in the previous cycle, to activate it), surgery on the uterine cavity (to correct heart-shaped uteruses), removal of the fallopian tubes with hydrosalpinx, analysis of the endometrial receptivity of the cycle, the anti-parental antibodies or the use of diverse medicine such as anticoagulants (aspirin, heparin).**

Each time an IVF cycle is performed, there are new opportunities of pregnancy. If prior to this, the patient has had some of these treatments, it is logical to think that it was due to that, but this is not so. It tends to simply be due to the fact that this embryo implanted. Pure statistics.

I think that it is important to spend these days with positivity and hope, and to avoid anxiety. In this respect, I have done my bit in two aspects:

Since the lack of control or information is one of the triggers of stress during the days of IVF, being able to see the evolution of

their embryos live means parents are much calmer when they reach the transfer date.

At present, we are conducting a study to analyse the effect of musical vibrations through the vagina on implantation. It is a clinical trial that includes 900 patients who place a Babypod (vaginal device that sends music into the mother's womb) in their vagina for 20 minutes every morning and every night from the day of transfer until the pregnancy test. We also ask them to write a phrase every day about how they feel and to fill out a survey on their mood. We want to see if the stimulation of the endometrium using music can improve the receptivity of the endometrium. I am looking forward to completing the study to be able to tell you the conclusions regarding the success of the cycle. However, today, with more than 500 surveys completed, more than 80% of the patients who have participated in the study state that, using the device, they feel calmer and even more optimistic, because it gives them the feeling that they are doing something useful to contribute towards the pregnancy prospering.

The majority of them had already transferred embryos previously and they recount that the use of the device reduced their anxiety. In clinical trials, control groups must also be established to compare the results, so some of the patients who agreed to participate did not use the device, and out of those who did use it, half of them had devices with no speakers –but the patients do not know this, they hear the music through earphones–, to verify if there is a placebo effect.

Since I calculate that when this book is published the study will have been completed, I will tell you that anxiety is reduced in those who used the Babypod, whether it emitted music or not.

Marta has done the insemination with donor semen and she is feeling so-so. She feels uncertain not knowing whether she is going to get pregnant or not; hopeful, because it is something that she wholeheartedly wants, and fearful about how people around her are going to react when she tells them. In her case the nervousness is greater than usual because she cannot accept "not knowing who

the donor is". On the day of insemination we had the following conversation:

—"Can I choose the semen donor?" Marta asks me.

—"No. Spanish law says that the donor's identity cannot be revealed. It has to be anonymous. In other countries it is permitted."

—"But, can I at least know what he looks like?"

—"You can find out his physical characteristics and any other information except any data considered personal which could reveal his identity. Your donor is 20 years old, he has fair skin, dark brown eyes, light brown hair, he is 1.81m tall and weighs 72 kg. His blood group is A Rh+. I have seen his photo and he is very handsome. You have to trust me and my good taste."

—"I know that you do all kinds of medical tests. But, what will I do when the paediatrician asks me about his medical background? My friends Luis and Ana have a lot of problems with that because their son is adopted and they weren't given information about his biological parents."

—"We do not accept people with important medical problems as donors, but it can occur that these are discovered after donation. People often ring us saying, for example, that their grandfather has colon cancer and that their descendants are encouraged to have special tests done. In these cases, we transfer this information to our patients."

—"And what if he needs a bone marrow or kidney transplant?"

—"For some transplants such as kidney, the blood group and the RH need to match, so it can be any relative or friend. For a bone marrow transplant immunological compatibility is essential. In these cases 25% of siblings are compatible. Parents are never compatible because the child only has half of their genetic code. The parents are not an option. If you want a second child, we would advise you to leave a sample from the same donor in your name so that your children will be biological siblings. But if they were not, this would

not be discovered either, because when you have an immunological compatibility analysis only that is looked at. A diagnosis that says: 'They are not siblings' or 'he is not the father' is not given."

—"And if I wanted a specific donor?"

—"If you insist on that, there are American and Danish banks where you can choose your donor on the internet. We do the ovarian stimulation here and you travel to our centre in Ireland on the day of insemination. Many other women give great importance to this. You are not the only one."

—"No, no. What a mess! And what about ordering semen online?"

—"It's a possibility. I know a lot of my colleagues are against it because a lot of laws are overlooked and because they think it is very risky. I personally think they are serious semen banks. We also buy semen samples from them with particular Asian or black race characteristics. I think that individual liberty must be above the laws of the country where you live. The problem I see is the bad results in terms of pregnancies achieved."

Marta finally asks me what her donor does for a living and I tell her he is studying business studies. This seems to give her some peace of mind.

Elisabeth has responded to the medication with eight follicles. Eight eggs were retrieved, six of which were mature. Five were fertilised and on the third day of evolution four were suitable for biopsy. I talk to her and her husband and I tell them that we are doing well, and that now we just need a bit of luck that one of them will be chromosomally healthy.

On the day of the transfer they are quite calm because they have seen on their mobile that three embryos are in the blastocyst stage, perfect for the fifth day of development. They have read up a lot and they have followed the development of their embryos step by step.

I receive the report just in time for our appointment. In the past 48 hours all the chromosomes have been analysed and we can transfer the embryos fresh.

But, they are all altered.

Effectively, the morphological appearance of three of them is very good, but one has trisomy 21 and 13, and the others have two other anomalies in more than three chromosomes. When they see my face, before I say anything, they both start to cry. It is a very sad situation; for me as well, and for all the doctors. Often we would like another person to come in and console us all…

I tell them the results and the sadness gives way to tenderness. **Jack** hugs Elisabeth while telling her that it doesn't matter, that they will try again. **Elisabeth** replies that she wants to do it as soon as possible and I tell them that they have done everything perfectly and that I am with them on this. We will do another cycle in two months.

Ten points to help you survive the emotional roller coaster

Prior to an infertility diagnosis, feelings of sadness, denial, anger and anxiety appear as well as a fear of building hopes and a fear of not achieving pregnancy. We have put together these ten points in an attempt to ensure that the desire to have a baby is stronger than the inconveniences and to not lose hope during the process.

1. Recognise and accept the feelings. It is common to feel discouragement, denial, guilt, anxiety, fear of building hopes, fear of failing in the attempts, tiredness and…many other emotions, but they are transitory.
2. Keep a positive attitude, take the process as a challenge and avoid feeling like a victim.
3. Visualise your objective by imagining yourself hugging your baby.

4. Try to prevent the treatment from affecting your relationship with your partner by only talking about the topic for a few minutes every day.
5. Keep yourself busy. Keep working without taking any unnecessary breaks. Time goes by more quickly when we are distracted.
6. Tell the people around you that you are undergoing treatment, but you decide when and how to discuss the topic. Ask family members and friends to be discrete and respectful.
7. Try to temporarily avoid pregnant women and babies; it is normal to feel rejection or sadness around them.
8. Consider the process as a really romantic way of having children.
9. Remember that with current breakthroughs, 95% of women who perform reproduction treatment become mothers.
10. Do not forget to fight to the end. Wars are not always won in the first battle.

Should you feel overwhelmed by the situation or need help, seek psychological assistance.

15

Egg donation

Elisabeth, in her first cycle, had responded well in terms of follicular development and the appearance of her eggs under the microscope, but all of the embryos were altered. In this aspect, the cycle was worse than expected. To rule out a possible male contribution, before repeating the treatment we did a FISH study on Jack's sperm. It was normal.

In the second cycle, we changed the stimulation programme and **Elisabeth** responded to the medication with nine follicles. The nine eggs were retrieved, seven of which were mature. Five were fertilised and on the third day of evolution three were suitable for biopsy. But again, the three embryos had chromosome alterations.

This time the scene on the day of the transfer was different. Disbelief gave way to anger and shall we say, to asking me for explanations….me and Carolina Castelló (the head of the IVF laboratory with whom they had had the biology visit). Afterwards, when things were calmer, we talked about how to continue; whether to try again or to move on to donor eggs.

On the one hand, **Elisabeth** wanted to try again, but on the other hand, she wanted to get pregnant as quickly as

possible. I told them that the level of anti-Müllerian hormone and her ovarian response make us think that there were ovules capable of giving rise to a healthy embryo, but that bad luck, or some other ovarian factor that we cannot diagnose, could make the same result reoccur. I asked them to take a few days to reflect.

According to my experience, when a woman is told she needs donor eggs, **emotionally** she goes through the following phases:

1. Disbelief: *This cannot be happening to me, these analyses are wrong, this doctor doesn't know anything…*
2. Quest for a guilty party: *Why did my gynaecologist not tell me before? It's my partner's fault, he wanted to wait…*
3. Give up on the idea: *If it can't happen with my eggs then I'll leave it.*
4. Reflect on what they have been told: that she can be a mother, that she will become pregnant and give birth… And of course, that the child will be hers.
5. Accept it with hope and gratitude towards the donor.

Some women get stuck in one of these phases and others pass through them in more or less time. It depends on the circumstances, the character, the way they usually tackle problems and how much they want to be a mother.

Once they have accepted it and made a decision, the first thing they tend to think about is who the egg donor will be. When the gynaecologist tells them that such a low level of anti-Müllerian hormone or such a high level of FSH hormone indicates that we are only going to be successful with donor eggs, we are always referring to statistics of IVF results. This patient could become pregnant naturally and have a miscarriage or a healthy baby. But if they require assisted reproduction techniques, we need a specific ovarian reserve to be able to perform it successfully.

When I deliver the bad news that the analyses indicate ovarian failure, I always explain to them how I think they must be feeling about the "genetic mourning". I think that I have to do it because it helps them. In fact, later they are very appreciative. However,

they nearly always leave that visit looking at me badly should we say, for two reasons: one, because they are in the phase of disbelief, wanting to repeat the analyses again in another laboratory, or wanting to consult it on the internet with another doctor; or two, they do not like what I am saying to their partners.

Let me explain. You will already have realised that I am a feminist, but I am also aware of the great differences between men and women regarding the way we relate to our surroundings and to ourselves.

I tell the patient that the decision about accepting an egg donation is hers, without talking to her husband. She must assess it alone; gather herself and her thoughts and feelings, and then tell her partner what she wants to do. Once she has made the decision she must ask her partner to support her. When this happens, the large majority of men agree with the option chosen by their wife.

The usual conversation tends to be:

Her: Well, honey, if it isn't to be, it isn't to be. Let's leave it.

Him: Whatever you say. I love you either way and we don't need to have children at any cost.

Her: Of course! Because the problem isn't yours! We won't have kids because of me, and you won't forgive me.

Him: Well then, let's think about it. We didn't want IVF either and we accepted it. No one has to know. I don't want you to give up on something you want so much.

Her: As easy as that! Since you're not the one who needs a donor…

The man becomes a wall against which she bounces the anger of this phase and they don't know how to manage it. I tell the men that they need to be in the presence of a lawyer when they answer her. And they don't like that I say that to their partners. It is logical.

Elisabeth and Jack have reflected on it and they are sitting before me. They are sweethearts and they have come with their homework done. Smiling, I ask her in which emotional phase she is.

—"I think I'm between the fourth and fifth. I have a load of questions and doubts. I wonder should I tell anyone."

—"On a medical level, we think that your gynaecologist should know because the tests to screen chromosome anomalies in the embryo are based on chronological age and, therefore, they have to be performed bearing in mind the age of the donor.

Regarding whether to tell your children or not is your own decision. The majority choose not to tell them. Additionally, since it is an anonymous donation, they will not be able to look for the donor. But it is a personal matter between each couple. I recommend a book called: *Revelaciones, filiaciones y biotecnología*, by Maria Isobel Jociles, which talks about that in depth."

—"What does the treatment consist of?"

—"In vitro fertilisation with donor eggs is a very simple treatment that will not be uncomfortable for you at all because the donor goes through the stimulation phase and the ovarian puncture.

You will apply some oestrogen patches on your skin from the first day of menstruation. In a few days the uterus will be ready to receive the embryos. After seven or eight days we will perform an ultrasound to determine the endometrial morphology and thickness: it should have the appearance of three lines and measure more than 5 mm.

The retrieval of your donor's eggs, with which we have synchronised your cycle, tends to be a few days later. That is the day we will perform the in vitro fertilisation. We ensure we have at least six eggs of optimal quality. If after observation under the microscope this circumstance has not arisen, you will be reassigned the reserve donor.

The transfer is scheduled for a few days later and before the transfer a vaginal progesterone treatment is applied. If the pregnancy test is positive, the oestrogen and progesterone dose is increased and must be maintained for two months, since there has been no ovulation. It is a hormonal replacement cycle."

—"What are my chances of becoming pregnant?"

—"IVF with donor eggs is the reproduction technique with the greatest possibility of success per cycle. The embryos have a great potential of implanting because they come from eggs from a young woman with no fertility problems, and the uterus is preparing itself hormonally to receive them in the best way possible."

—"Do some women regret it?"

—"Before doing the treatment we see patients who give up because they cannot accept it. But once treatment has started, during the pregnancy and after the baby has been born, the acceptance is absolute and they tend to forget it. They feel it is their baby in absolutely every aspect. They often tell us that they trust everything they have gone through because this baby was who they were waiting for, and it could not be anyone else. **Jack**, how are you?"

—"I am very happy with my wife's decision to move directly onto a treatment with many more possibilities of success. She is going to give someone the gift of life and she will be the best mother in the world."

16

It's never going to happen!

When in vitro fertilisation fails over and over again

Why does in vitro fertilisation fail?

I'm surprised to see what I have just written. I have devoted more than 30 years to assisted reproduction and I often think the opposite. The question I ask myself is: how is it possible that this works? I still think that each child is a miracle!

IVF can fail because the embryos were not good, or because the uterus was not suitably prepared to receive them. But even if both are in good condition, all embryos do not implant, because the endometrium and the embryo do not always agree. It is a complex process that can have three types of failure:

1. Failures that are part of our nature. Human reproduction is imperfect.
2. Failures in the procedure at any stage in the process.
   For example: if there is blood in the uterus it will hinder implantation. This blood can arise from a difficult transfer, from the endometrium eroding with the catheter during transfer or from the abdomen after an egg retrieval procedure during which the patient bled.

3. Assisted reproduction is a science that has evolved hugely in recent years, but there are still a multitude of circumstances and processes that we do not know.
   For example, beautiful and chromosomally normal embryos from a 41-year-old woman might not implant because their mitochondria lack capacity. Mitochondria are the cellular organelles that produce the energy required to divide. Their strength reduces over time, so the ability to continue dividing an embryo reduces with age. But we do not know how to analyse it previously.
   I would love to know who and where the "orchestra conductor" who marks the rhythm of cell division in an embryo is and how these cells are differentiated to each form a different tissue.

On a practical level, in my experience, I can say that the majority of failures are due to the fact that the cause of infertility was not correctly diagnosed before doing the cycle. This is putting the cart before the horse. Following the norms of medicine and logic, diagnosis comes first, and then treatment.

With a standard IVF treatment a lot of women become pregnant, but if the cause of the infertility has not been correctly diagnosed there is a possibility that it will not be effective and that unsuccessful cycles will be repeated. It is a pity that the new diagnostic techniques are not applied.

Many of the patients we receive in the leading centres have a long history of infertility; couples who have performed an average of several cycles of previous unsuccessful in vitro fertilisations. The main problem is an incomplete or incorrect diagnosis. For example, ten years of infertility and they resort to IVF with donor eggs because the woman is older and, nevertheless, the cause of infertility is male. If the man is not studied correctly, the procedure will not be successful with donor eggs either.

How can we know if the embryos are healthy?

When an evolving pregnancy is not achieved, the cause may be embryonic. The IVF can fail because the percentage of embryos with chromosome anomalies is higher than expected, due to an ovarian age that is older than the chronological age or genetic anomalies in the sperm. We conducted a study with 700 patients who on their first visit reported infertility of more than eight years, with an average of 4.7 cycles of IVF without pregnancy or ending in miscarriage. In the majority of these cases, 71%, the cause was the poor quality of the embryos due to the male factor. In many cases, it was a hidden male factor that had not been previously diagnosed. Embryonic medicine enables us to genetically analyse the embryos using Pre-implantation Genetic Diagnosis. When this technique is applied in patients with failed in vitro fertilisation, it is observed that many of these embryos have alterations in the chromosomes, and we also know that these alterations increase as the woman ages, and in men with poor quality semen.

Embryonic medicine also serves to determine if the cause of these alterations is male, through tests that analyse the chromosome separation process in the testicle (meiosis), the chromosome composition of the sperm (FISH in sperm) and the study of sperm DNA fragmentation. All of these techniques enable us to determine why previous attempts at pregnancy have failed, and thus foster the possibility of healthy babies being born.

How can I know if I have implantation problems?

If you have not achieved pregnancy and the embryos had the correct appearance, an implantation study is recommended. We know that there are multiple substances and factors involved in the process that allow the embryo to cling on and penetrate the endometrium to implant, but the majority of them are still unknown.

The tests we have to perform the study of endometrial receptivity are: hysteroscopy, endometrium biopsy, Chlamydia,

Ureaplasma and Mycoplasma cultures, and colour Doppler ultrasound. These are performed to rule out anomalies in the uterine cavity (for example, adhesions following a curettage for abortion), infections (these endometritis do not tend to produce symptoms) and vascularisation alterations. The majority have a straightforward and effective treatment.

Why is there such long-term sterility? Why can a couple take so long to become parents?

When faced with sterility, some people accept it and do not do any medical treatments, others stop treatments after losing hope, and others never stop fighting to achieve it. Reproduction medicine still does not have an explanation for all cases of sterility or repeated miscarriages, but it is one of the specialities that has been making the most progress in recent years.

New diagnosis techniques and complimentary treatments in IVF improve the results and mean an increasing number of people can now become parents. But it does not always happen.

At present, if the couple wants and has the possibility of accessing correct medical treatment, nearly all cases are successful, although in some cases they will have to renounce their genetics.

For doctors it is also exasperating to see that we are not achieving the goal and often **empirical treatments** are chosen. These are indicated without having a certain diagnosis. For example, giving patients with repeated miscarriages infant aspirin, even though the analyses do not show anti embryonic tissue antibodies. Every season, one of these becomes a trend. If we do not openly explain it to patients, it is not ethical and it creates false hopes. Some of these treatments are expensive and painful, and we do not know their subsequent risks.

Men are responsible for 66% of conjugal infertility cases. In 33% of cases, he is the only person responsible, and in 33% it is associated with a female factor. Despite this, the majority of reproduction centres do not have an andrologist, a doctor

exclusively working in this new and constantly evolving discipline, and as such, the study of the male factor is insufficient or non-existent.

The fundamental pillar on which the diagnosis of the male factor is based is the seminogram. However, a normal seminogram is not a guarantee of fertility, considering approximately 15% of sterile men present normal seminograms. This circumstance means that from the outset, the man is ruled out as responsible for the conjugal sterility, simply because the seminogram is normal.

However, there is a hidden sperm pathology that cannot be detected through a normal semen analysis. Defects in the sperm membrane, alteration of the acrosome reaction (the acrosome is a small sac situated in the head of the sperm carrying substances to perforate the external membrane of the egg to enter and fertilise it), fragmentation of the sperm DNA chains, defects in the centrosome, or transporting an altered number of chromosomes, are some of the sperm anomalies that cannot be detected in the routine seminogram.

Why is the male factor not studied in depth in a lot of centres?

On the one hand, when the ICSI sperm microinjection became generalised, it looked like all male sterility problems were going to be solved, and sperm research came to a considerable halt.

On the other hand, in countries with laws that prohibit PGD and the donation of gametes, these diagnostic methods are not indicated on account of not being able to offer other treatment alternatives to the patients.

Causes of miscarriage

Miscarriages can have different causes. Thanks to the breakthroughs in assisted reproduction techniques, today there is more complete information about some of the causes of miscarriages that up to now were little known by gynaecologists not specialised in the subject.

The traditional tests seek to rule out problems of hormonal, infectious, anatomical or immunological origin. It is important to remember that 60% of repeated miscarriages have a genetic cause.

When a patient has had two miscarriages or more, and the problem has not been diagnosed or treated, the probabilities of repetition are high, and increase in direct proportion with the number of previous miscarriages.

Today we have other tests that seek to study the embryo itself and the ability of the woman to allow implantation and to carry a pregnancy to full term. These causes of miscarriage are often the same that cause repeated failures in in vitro fertilisation and therefore must be studied and treated in a similar manner.

In addition to the medical problem, a woman who has had miscarriages is faced with a difficult emotional situation. The feeling of frustration is amplified by having to tell her family and friends, and by the fear that it will reoccur. The hope of having a baby comes up against the possibility of a repeated failure and the woman experiences this situation with great uncertainty and anxiety. In these situations, the psychological support provided by specialists in the area is essential.

For how long should I try?

It is very hard to answer this question because many factors come into play. Even if the woman wants it wholeheartedly, there are circumstances that can make it very difficult: financial and moral reasons, the laws in her country, the possibility of accessing reputable centres, little support from her partner, or simply feeling unable to accept another failure.

I also wanted to get pregnant the first month. I was like Elisabeth. I decided it was time in the final year of my specialisation studies, and since I didn't get pregnant in the first month, I gave myself a deadline of another month, and I had bought medicine to induce ovulation if necessary. I didn't need to take it in the end because the egg from that month is called Borja, and the egg from two years later is called Bruno.

I had it easy, but I was willing to do ANYTHING to become a mother. I'll even admit that I had nightmares in which I went to a shopping centre with a nursery and robbed a baby…

I adore children, of all ages, even those that only have two

cells. I wanted to have a lot but I realised that the passion for my profession, medicine, impeded me and now I have three passions: my two boys and my work. What is more, every morning when I see the pregnancy tests, I feel like I am helping lots of children be born and I don't have to be responsible for taking them to school.

On a medical level it is also difficult to advise somebody when they should stop having treatments because the techniques are constantly improving and because we are living in "Google" times, with access to information and the possibility of changing country to access all types of treatments. Thus, even those patients who cannot achieve pregnancy through any method, can opt for surrogacy.

Surrogacy

Surrogate maternity is a very important and controversial topic that can be looked at in many ways.

When there is something new in reproduction I tend to get phone calls from journalists to ask me about it. Sometimes I read things that I told them ten years ago and I am surprised by how much my criteria have changed. In this regard, I have gone from thinking that surrogacy was horrible, even a way of using the body of a woman in a vulnerable situation, to helping whoever asks me to achieve it.

This evolution has come about after experiencing very sad situations, such as that of a woman whose son died at birth and who had to have her womb removed after haemorrhaging, and after seeing the devotion and love of women who have got pregnant knowing that they are only going to look after that child during the months prior to the birth. I think that a lot of the criticism applies to egg donation.

Where is it legal?

Legally, in the majority of countries the filiation of the child is determined by birth, and therefore, the baby belongs to the woman who gives birth to him/her and the law prohibits uterine rental.

In some countries such as England, Ireland, Portugal and South Africa, it is only permitted in cases in which the woman has an illness which renders her incapable of carrying a pregnancy, as long as there is no financial agreement and the women are from the same country. In some countries there must be a family link between the two women.

Surrogacy with financial agreement is permitted in some states in the United States, Canada, Russia, Ukraine and others, but the majority of treatments today take place in these four countries.

Until relatively recently, there were many cases in India, Thailand and Mexico, but the law was changed and these have been interrupted.

This legal scenario is current in 2016, but it changes every year.

Who might need a surrogate womb?

We continuously see people who ask us for information on how and where they can do it. On the one hand, couples or single women who are medically incapable of carrying a pregnancy because their womb has been removed, they were born with uterine malformations, they have to take medication that is incompatible with pregnancy or they have a disease that contraindicates pregnancy. Also, women who have had many unsuccessful IVF treatments.

On the other hand, gay men, whether single or in a relationship, and also single heterosexual men. Men are increasingly claiming their right to single fatherhood. I'm sure you are aware of the famous men who have done it.

Why would a woman offer her womb for surrogacy?

Which women are capable of going through a reproduction treatment, pregnancy and labour to then hand over the new-born baby to other people? In an international conference a study was presented that shows that surrogate mothers do not present psychological consequences and that the reasons for which they

do it are in 91% of cases to help, although in actual fact it is to help their children; in 8% of cases it is for the pleasure of being pregnant –our experience as gynaecologists tells us that being pregnant is not a pleasure; what is more, in the majority of cases, it is the absolute contrary, the pleasure is having the baby- and only 1%, exclusively for money.

From what I have seen, the majority of surrogate mothers do it to obtain resources to maintain their children. They are proud to do it and they experience it as "I help you to raise your child and you help me to raise mine".

A child conceived in this way can have three mothers: the biological mother (who gives her eggs), the gestational mother (who carries the pregnancy) and the legal mother (who will look after him/her forever). It can also be a legal father.

The woman who carries the pregnancy is the gestational mother, but she is usually not the biological mother. The eggs belong to the legal mother or to an egg donor. In addition, they always have their own children, among other things to avoid the risk of becoming sterile due to complications during birth.

What relationship is established between the woman and the parents of the child?

The relationship between the legal parents and the woman subrogating her uterus is very different according to countries and cultures.

In the US and Canada they can meet and have the relationship that they have decided to establish. Continuous communication via the internet is common, including occasional visits and presents, but anonymity can also be chosen. In these countries, the surrogate mother lays down the rules. She can even select the legal parents.

After the birth, a fast trial is held in which all three are present and in which they sign the finalisation of the contract they had established as "temporary adoptive mother". In other words, someone who looked after the child during a period in which the legal parents were unable to do so: pregnancy. I know patients

who, after leaving this trial, a week after the birth, all went to a barbeque in the gestational mother's house.

Emotional support is necessary in these cases, and in these countries it is provided by surrogate agencies and the legal parents.

In the Ukraine and Russia, the centre takes charge of everything. Usually there is no type of communication with the legal parents. After the birth there are procedures with the courts and the police. In many cases they leave the country with the child appearing as the child of the surrogate mother and the man, and once in Europe the man's wife adopts the child.

Financial aspects

A surrogacy process is paid for in phases: there are fees for each part of the process, which are paid as the pregnancy evolves, with a scale of extras such as amniocentesis, twin pregnancy, etc.

The amount the surrogate mother receives is much less than you might think. The majority of the money goes to lawyers, agencies, medical expenses for the reproduction treatment and pregnancy, birth and incubator if required, insurance and travel expenses. For information purposes, a surrogacy process, if everything goes well on the first attempt, can cost approximately 60,000 Euros in the Ukraine, double that in the US and intermediate prices in the other countries.

Obviously, there are many people who, despite wanting it wholeheartedly, cannot afford it financially.

What needs to be considered?

A lawyer specialised in this area in the parents' country of residence is essential. A reproduction specialist who has experience of these cases and is up-to-date on the medical aspects of the clinics is also advisable. I know of a lot of patients who organised it privately on the internet and fell victim to a scam, as Paco Rego explains in his article published in the newspaper *El Mundo: "El timo del bombo"*.

After accompanying patients on this adventure I have had the opportunity to see and share all types of experiences: negative experiences due to repeated failures or because upon arrival, for example, in India, to start the process, they were rejected by the staff in the centre because the husband was in a wheelchair (in their culture, the idea of illness as a divine punishment is still quite widespread); and other fantastic experiences when they finally have their baby in their arms.

Questions for the debate

Undoubtedly, scientific and medical breakthroughs such as surrogacy generate a social, cultural and legal debate. Of course not everything that is technically possible is morally acceptable.

What is your ethical opinion of surrogacy?

Do you think the discomfort and risks of pregnancy can be paid for with money? Or, on the contrary, should it be looked at as an exchange of ways of helping? How would you make the law in your country? Would you allow surrogacy in all cases, just in some, or never? Would you be a surrogate mother for your sister?

One day I had a small discussion with Carmen Garijo, a wonderful woman who is the subdirector of the magazine Glamour. She told me that famous models and artists wanted to avoid the physical effects of pregnancy and tended to opt for surrogacy. She also told me people were afraid that "à la carte children" are being made in reproduction centres, choosing parents according to specific characteristics and not others. All of this is a myth. The truth is that all those women who want to have a baby want to feel it inside them and they ask us for the same as the rest: to help them to have a healthy baby as quickly as possible. Just because their image might seem frivolous does not mean that they are, at least in this matter.

As regards us making designer children, the truth is I don't know what they are talking about. Those of us who have devoted our life to helping others to achieve their dream of being parents do not do strange things in the laboratory, and nor are we asked to.

17

Music and the beginning of life

What is music for?

A chapter devoted to music in a book for women who want to get pregnant might seem unusual, but I will show you that it makes absolute sense: music is very much related with the origin of life. I can affirm this because we have a very important current line of research on the effects of music on people from when we are a cell, an oocyte, to the moment of birth.

To understand the meaning of some of the discoveries we have made in this field, I had to read the theories on the origins of life. I want to share the summary of conclusions I have drawn with you. Bear in mind that they are only my personal suggestions after asking myself what music is for. Why has it always been present throughout history?

Music to communicate

Music is the most ancestral form of communication between humans. Communication through sounds, gestures and dances came before the spoken word. The first language was musical

more than verbal, and this is still the case; instinctively we speak to babies in a high tone and melody because we know that that is how they best understand us, and realise that we want to communicate with them.

Natural selection meant that the humans who best developed communication skills survived, since these are essential for increasingly large, and therefore, safer groups: looking for food, protecting themselves from animals, raising children, etc.

Thus, biology strengthened human beings' skill at singing; musical ability was an adaptation tool, it became established as a method to improve survival.

Music is the greatest stimulus we humans have for communication; greater than language. The reason why is because it triggers emotion, it enables us to communicate a message with an emotional load.

Music to feel moved

In addition, music is a universal language of emotions. The ear is sensitive to tones and melodies that provoke specific moods that are the same for all. The human perception of specific musical sounds causes the same feelings of happiness, sadness or fear in everyone. An Asian person feels happy when listening to rising tones of Spanish guitar music and sadness when listening to falling strains.

This ability to induce a mood shared by all encourages relationships, cohesion and social links.

Why do we put on the team's anthem before a match? Whenever humans gather for a reason, music is present: at weddings, funerals, university graduations, military parades, sports events, prayers, romantic meals, or when a mother rocks her baby…

Today, in advanced societies, we listen to music and appreciate dance, but we rarely sing or dance. I think that we have replaced music as a means of communication with writing, the telephone, emails, etc. and now we use it as an element for pleasure, as well as for culture and art.

Music for learning

Some theories state that music also developed because it stimulates learning. It stimulates the attention and memory systems; we all know that it is easier to learn the multiplication tables with music and how we always remember the lyrics of a song even if we have not heard it for years.

Apparently, without the ability to hear musically, we could not learn a language.

Music to meet our mate

As verbal communication in the human species evolved, sexual courtship changed from fighting to non-violent persuasion through songs or advantages in physical appearance.

The function of music in sexual selection also exists in other species such as birds; the males are capable of composing new melodies, and the longer and more elaborate the repertoire, the quicker the female ovulates.

Music serves to exhibit sexual aptitudes. Tribal dances are based on good muscular structure and energy (physical health), on rhythm (mental health) and song (creativity). Anthropologically, these were the male attributes that attracted women because they were indicators of protection and food for her children. Miller, from the University of California, states that women preserve these sexual tastes in their genes and has conducted studies in which it is demonstrated that women during ovulation have the same preferences as prehistoric women.

There are studies that affirm that the men with most success in terms of the number of mates are rock singers, even if they are ugly. Women want to have sexual relations with them but not marry them. Since sex has been separated from maternity, women want them for sex, but not as fathers of their children. Today, we have other social and cultural conditioning factors, and according to authors such as Miller, the choice of the father is associated more with the man's economic capacity.

Music as a courting method is especially evident in adolescence. When our body is getting ready for reproduction these instincts are more evident.

Music for pleasure

Without a doubt, music is also a stimulus of pleasure. The "nucleus accumbens" is made up of a group of neurons in the brain. When stimulated it produces dopamine, a substance that makes us experience wellbeing. This nucleus is the hub of pleasure from sex, food, cocaine, heroin, nicotine, addiction to games and, as demonstrated by Valorie Salimpoor in 2013, the pleasure of listening to music.

Anthropologically, sexual pleasure (which only exists in humans and dolphins) can be explained as an incentive for reproduction. In other words, if the primitive man, who did not have much food, had not obtained pleasure from sexual relations, he would have avoided the energy expenditure this entailed, thereby endangering the evolution of the species.

The same can be said of the woman: if it were not for the pleasure of sex, she would have avoided having children. That is what I mean by sexual pleasure being an incentive for reproduction. What's more, it generates a desire for more!

I suppose that to reinforce all the actions that are important for survival, our brains developed mechanisms to foster and reward these behaviours.

Humans have learned to bypass the original activities and we now access the reward systems directly. We can eat food with no nutritional value for pleasure, have sex without procreating, listen to music on our own, etc., but the pleasure centres of the limbic system do not know how to differentiate between them.

Thus, it is possible that all the theories of the origin of music are correct. That in evolutionary terms it has remained as an instigator of sexual courting and as an element of communication between tribe members. And as time passed, we no longer needed it for this, but we have continued using it to stimulate the pleasure receptors in our brain.

While music plays an important role in communicating, choosing a partner and mating, it is also very present in the creation of life.

Music improves in vitro fertilisation

Since the beginning of assisted reproduction, an attempt has been made to ensure that the conditions of embryos in the laboratory are similar to those in the fallopian tubes and the uterus, both on a physical level and ensuring that the development of the culture media contains the same nutrients.

Thus, thinking that the IVF laboratory should be like a giant uterus, I began to reflect on the embryos' surrounding atmosphere and I wondered: what can be heard in the uterus?

That is how, in collaboration with a sound engineering consultancy, we came to design a system that is capable of emitting music inside the embryos' incubators for 24 hours every day at 80 decibels.

We conducted a study that demonstrates that music vibrations increase the possibilities of the sperm fertilising the egg; in other words, that music improves in vitro fertilisation. We presented it at the Fertility Europe Congress, in July 2013 in London.

We analysed 985 eggs that had been fertilised in vitro from 114 patients. Each patient's eggs were randomly divided into two groups cultured in two different incubators: one equipped with a speaker system and another conventional one. The results show that those cultured with music presented a statistically higher fertilisation rate, 4.8% more.

We chose three different musical styles -pop, heavy and classical music- to measure potential variations according to the type of frequency, but we did not detect any significant differences between one and another. Of course, embryos do not have the sense of sound, they cannot hear anything. So how does music improve the fertilisation rate?

In natural conditions, the eggs and embryos travel though the fallopian tubes towards the uterus in conditions of continuous movements which move them, but also surround them with the

nutrients they need and remove them from the waste products generated during the cell division.

These cell movements promote the exchange of substances, but in the laboratory they remain static in the culture media and as a result, the toxic products they release –free radicals or ammonia– are stored in the culture medium itself.

Our hypothesis is that the micro-vibrations stir the culture media in which the egg is bathing, produce a more homogeneous distribution of the nutrients it needs and disperse the toxic products, preventing them from accumulating.

The effect of the musical vibrations on the cell growth in vivo and in vitro has been studied in various fields, but it is the first time the effect of music on in vitro fertilised eggs has been studied.

Since the beginning of IVF there have been continuous breakthroughs improving the success of the treatments, and although it may seem like little, an almost 5% increase in the fertilisation rate is a great achievement, especially for a leading centre like ours.

IVF to the beat of music. A concert for embryos

Since discovering that the musical vibrations improve the fertilisation rate we have systematically built it into all of our incubators. The songs are selected according to the biologists' music taste because they hear them constantly when they open the incubators and are at a very high volume.

Every so often the type of music changes although we have observed that there is no difference. At the moment it is pretty lively in there, the music is heavy metal.

A few months before writing this book, Antonio Orozco played a private concert for 380 lucky individuals. The lucky individuals were the embryos developing at that time in the in vitro fertilisation laboratory.

Soon afterwards, after receiving the Ig Nobel Prize in the University of Harvard, we celebrated it with a new duo between Sharon Corr and Álex Ubago, in our clinic in Barcelona. It was broadcast to our laboratories all across the world via streaming.

Embryos cannot hear, but foetuses can. An embryo is up to 12 weeks' gestation; from then on it is called a foetus. So we began to study it and we discovered foetal hearing.

What can foetuses hear in the mother's womb?

The first thing we did was put speakers on the abdomens of pregnant women at a very high intensity (the equivalent of an airplane taking off) and, when we performed the ultrasound, we saw that the foetus did not react. In fact, in ultrasounds, gynaecologists had never observed changes in the foetus as a result of external noises or because the mother speaks.

So we thought that perhaps they could not hear, that in the same way that foetuses can see but they do not see because there is not enough light, surely they can hear but they do not hear because the sound does not reach them. And so our objective appeared: we had to take the source of sound closer to them…**we had to put a music system in the uterus**. And then it occurred to me that we should try it by placing a speaker in the vagina of pregnant women.

We repeated the experiment with more patients, and t**his is what we discovered: that when foetuses hear music they react by moving their mouth and their tongue…they try to vocalise**.

https://institutomarques.com/en/scientific-area/response-fetus-vaginal-music-2/

But the only way the sounds can reach them as we hear them is by emitting them vaginally.

We designed the scientific study. We recruited patients from our centre who were between 14 and 39 weeks pregnant. In each case we studied the response before, during and after the foetal musical stimulation in three consecutive periods of five minutes' duration each. We determined three different groups of patients:

- With music emitted abdominally at an intensity of 98 decibels (level equivalent to the noise generated when a train passes).
- With a vibrator, because we needed to know if the foetal responses were due to the sound vibrations or the music. The vaginal vibrator emitted a noise at an intensity of 68 decibels, equivalent to a conversation at a high tone. Don't worry! Pregnant women can use vibrators.
- With music vaginally at 54 decibels (the level of a conversation at a normal tone).

Over a three-month period we evaluated 106 patients, without any type of medical incident and without observing any negative effect of the use of the vaginal device. Moreover, we were pleasantly surprised to see the parents' excitement during the ultrasound sessions as they watched the spectacular images of their babies' face, mouth and tongue movements.

In less than one year we had included more than 1,000 patients in parallel studies on foetal hearing through the vagina.

This was despite how uncomfortable the initial prototype was! But they didn't mind. They loved it! On one occasion, when with a patient from the vibrator group, I had the remote control in my hand and accidentally increased the intensity to the maximum…When she left she said to the nurse: "You do strange studies in here!"

During our professional life, when we do ultrasounds, the foetus is a passive subject, which we observe and measure. But in these ultrasounds the fact that we are able to waken the baby, elicit a response from them and, in short, communicate with them, is really remarkable.

In many of these ultrasounds, the gynaecologists are very much affected by the parents' emotions to such an intense experience.

Results

In ultrasounds prior to the beginning of the stimuli we saw that 45% of foetuses moved their head and limbs and 30% made movements with their mouth and tongue. This is what they usually do when they are awake.

When the **musical stimulation was applied vaginally using Babypod**, almost 90% of the foetuses woke up, reacted with non-specific head and limb movements and with specific movements of the mouth and tongue that significantly reduced when they stopped hearing the music.

Almost 80% of the foetuses reacted with a very notable movement, opening their jaw wide and sticking out their tongue as much as possible.

During the **abdominal music stimulation with earphones** no increase in the specific mouth or tongue movements was observed. The same occurred in the group of **vaginal vibrator stimulation**.

These results prove that, if they do not respond to music through the abdomen, it is because they do not hear it. And if we emit music through the vagina they respond because they do hear it.

Foetuses do not react to the vaginal vibrator. This means that they respond to music and not to sound vibrations. This is very important when diagnosing deafness. People who are deaf perceive vibrations, but not music.

We saw that the response is produced from week 16 of gestation! Can you imagine the spectacle it would be to see a tiny 11-centimetre and 100 gram foetus reacting to hearing music? It is incredible. That is how we discovered that the foetus hears and responds from much earlier than what was initially thought.

Foetuses present a different response in each exploration and the time they take to react varies greatly. The type of movement, the number and intensity of same, and the amount of time it takes them to stop making them after the stimulus has stopped is also different. But as the pregnancy progresses, there is a greater response of facial movements. We did not observe differences between boys and girls.

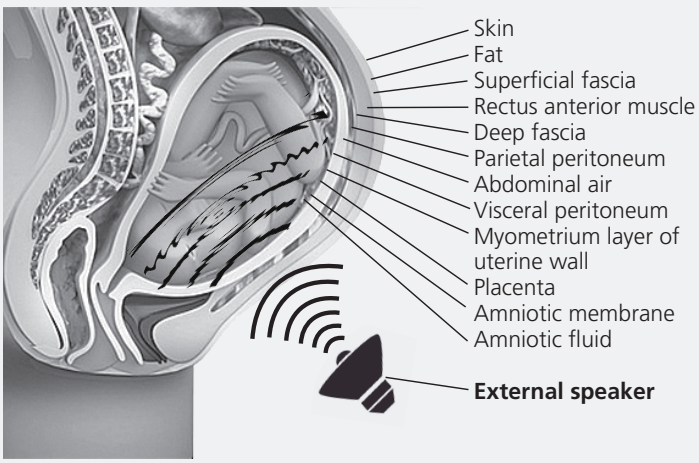
From the outset I was very interested in knowing what would happen with twins, and it is incredible, because they respond in

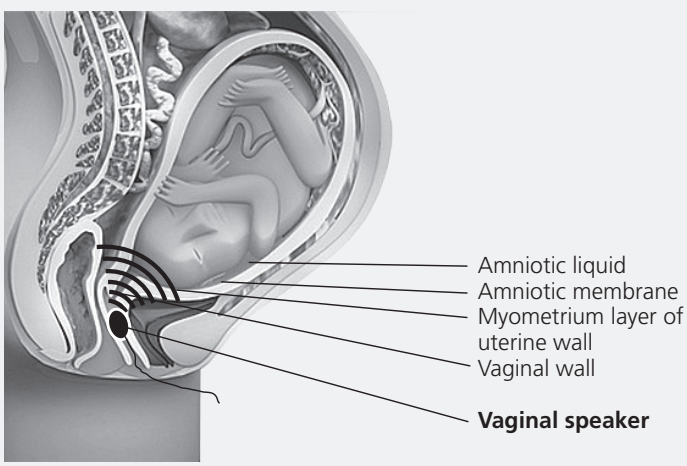
the same way! At this point we had discovered very important things. But then, what can really be heard in the uterus?

Why do sounds not reach them through the abdomen, but they do through the vagina?

The foetus receives sounds from inside their mother's body: her heartbeat, breathing and intestinal movements. They also hear sounds coming from what the mother is doing, when she talks, or when she walks wearing high heels, as well as hearing noises from the outside.

But the foetus lives in a soundproofed environment. The mother's organs and the abdominal wall isolate it from sound.





From the abdomen, part of the sound is reflected externally and another part is absorbed and distorted by the different layers of soft tissue that make up the abdominal wall. The foetus only receives the transmitted part and it is of a lower intensity and clarity than at the point from where it is emitted.

Studies on sheep with intrauterine microphones calculate that the majority of sounds reach them as whispers, at an intensity of 30 decibels. Furthermore, the words uttered externally and recorded from within the uterus are only intelligible in approximately 50% of cases. They reach them distorted, with modifications in the pitch and the tone.

It is as if the foetus were surrounded by pillows. When we hear someone talking in the room next door, although we can hear them, we understand less.

Thus, we can say that the sonorous atmosphere of the uterus is like the background noise in a forest. Furthermore, the majority of the sounds are very repetitive; they become used to them and do not react to them. They do not prevent them from sleeping.

In order for them to hear the same as us, the only way is vaginally.

The vagina is a closed space, and therefore, the sound is not dispersed. Moreover, the layers of soft tissue that separate the foetus from the sound emitter are fewer: only a vaginal and uterine wall. We remove the barrier of the abdominal wall.

By placing a speaker in the vagina, the foetus can hear at nearly the same intensity at which the sound is emitted. Thus, thanks to the vaginal device designed and developed for this study, **we have discovered the formula for foetuses to hear like us**; for the sound to reach them effectively, intensely and without distortions.

You can imagine the number of women who say to me "I spent so much time talking to the baby and playing music not knowing that they couldn't hear it…". And the number of works published about whether the new-born baby recognised their mother's voice. They cannot recognise it because they have not heard it like they hear it at birth.

What practical applications can this discovery have?

Firstly, it enables us to communicate with foetuses and stimulate them neurologically. We already know that early sensorial stimulation is good for children.

It also helps us to rule out foetal deafness: if the foetus responds to the music it means that he/she is not deaf.

Moreover, using this device in ultrasounds, means that these **are easier and quicker**. By inducing foetal movements, the sonographer can see the foetus's structures better.

There are also other further applications: we can reduce the anxiety of pregnant women who do not notice foetal movements, because by emitting music we can make the foetus respond and the mother feels it.

It is a unique experience for parents and their future child: together they can share the pleasure of music.

And lastly, scientifically this breakthrough opens a passionate line of completely pioneer pre and post-natal research.

Our hypothesis suggests that music causes a response of vocalisation movements since it activates brain circuits of language stimulation and communication. In other words, learning starts in the mother's womb.

To perform the study, a special vaginal device was designed which was subsequently marketed with the name Babypod® and is used by women all over the world.

18

The pregnancy test

Rose did not pay much heed to me. On the day of the transfer she sat down on the sofa and only got up to go to the bathroom. James went out to buy her flowers. Lourdes came to keep her company and show her online shops of baby clothes on her mobile. Her parents rang her hundreds of times.

She decided that her job as an aerobics instructor was incompatible with her situation and she asked for medical leave.

Over the next few days she remained in a situation similar to isolation. At the beginning she coped quite well, but then she started to feel anxious. She realised that everyone was taking it for granted that she was already pregnant but she couldn't feel anything.

She called the emergency telephone number of the clinic because she couldn't feel anything. The doctor told her that it was normal and told her to stay calm because there was a high chance of success. That was the last straw; a high chance meant that it was possible that she would not get pregnant and her fear of failure was horrible, especially failure in front of others.

Her anxiety alternated with sadness because she was convinced that there would be an engagement ring with the flowers, but there was not.

The days preceding the pregnancy test were eternal. She went to the bathroom to look at her panty liner ten times an hour…but finally the moment came.

14 days after her eggs had been retrieved she arrived to the clinic with the first urine of the morning in a container.

They walk into the office and when I say "congratulations, the test result is positive", Rose bursts out crying and James starts a ritual dance like he does when he scores a goal. It is such an emotional and beautiful scene that I leave them in the office on their own.

Marta did not get pregnant with the first or second insemination. In the last ovulation test for the third cycle she came with Edward. When I begin to give them instructions I notice they are very serious and I ask them what is wrong.

She tells me that she cannot stand the way Edward is behaving. That she has told him that she does not want to keep seeing him because it hurts her, and now he has turned up here, to accompany her…as if nothing had happened.

He says that he came to pick her up because he wants to talk to her after the visit.

On the street he tells her that he loves her, that he has no right to ask her not to have children, that he was selfish. He asks her to forgive him and tells her that he wants the nest to be full again, and that if she will let him, he would like the insemination to be natural.

Edward's two first children came soon after starting to try to conceive. He must have good semen, because Marta gets pregnant the day after that conversation.

Elisabeth has done a cycle of egg donation and it went very well. Eight eggs were retrieved, six of which were mature and five were fertilised. Three days later there were three divided embryos and two days later one evolved into a blastocyst.

They were glued to the images of their embryos in the incubator. They watched the EmbryoScope video every few

hours, and they rang each other at work to discuss them. As fewer than predicted were evolving they were very disheartened and the days before the pregnancy test were horrible, in this case for both of them.

But the pregnancy test was positive! We barely got a chance to speak because they were gobsmacked, they just wanted to hug each other and cry. But Elisabeth sent me this email the following day:

"We thought that this was not for us. That we were destined to be happy, but in a "different way".

My husband and I came to the clinic to have a pregnancy test like someone who goes to a routine medical check-up for a cataract.

No nerves, not expecting any news that would surprise us.

In the few minutes spent waiting from when we arrived and sat down on the sofa at the end of the hall, I visualised what would happen a few minutes later. We would go into an office and a member of our doctor's medical team would tell us as tactfully and openly as possible that they were very sorry but that it hadn't worked this time either.

My heart didn't skip a beat with this thought. I felt that this was what was going to happen and I expected it with resignation and serenity. The long amount of time and the repeated failures had already affected me without me even being aware of it.

Suddenly, we see our doctor appear in the hall and greet us with the warmest smile and open her arms to hug us. She says "you are pregnant, congratulations". And I, feeling sceptical, hug her, like someone who has not understood what has just been said. Like when you hear something in a hushed tone and you freeze and need it to be repeated to you to make sure you heard it correctly.

I think I say to her: "but, how? This can't be true". And she, without stopping smiling, says; "Yes, you are pregnant. The test was positive. Let's go into the office". Suddenly, while she hugs my husband, my inner voice says: "This can't be. You didn't come to be told that you are pregnant. You came for them to tell you that we would try again".

On our way to the office we meet nearly everybody: the nurse who accompanied us on the day of the transfer, Anabel, the doctor's nurse who we trust and hold in high esteem after the time spent together, the

women at reception who are always interested in how things are going. It is inevitable that they find out that we're expecting!

We go into the office, still in absolute astonishment. I don't know where I leave my jacket and I sit down on one of the chairs, beside my husband.

The doctor tells us in more detail about how she and her team started the day on such a positive note, when they saw the positive urine test result.

"It was about time!" she tells me, "you had waited long enough".

My husband and I look at each other and I say to her again: "But are you sure I am? It couldn't be that the medication I am taking has given a positive result?" As soon as I finish the sentence I feel ridiculous.

The doctor, without losing an inch of her smile, tells me: "You are pregnant. The levels were very high. There is no doubt".

"I can't believe it", I tell her. "I've been feeling menstrual pains for days", I tell her again. She answers: "Those are spasms in the uterus as it expands so that the embryo can grow".

Incredible, we still can't believe it. For days I had been telling my husband that I could feel what I felt every month: my body getting itself ready for menstruation. My husband is still absolutely astonished but he tells me at that moment that he had never lost hope.

The doctor asks us to do a blood test, not only to confirm the pregnancy, but to check my levels. And we go home to wait for the results.

The first song we hear on the radio is "Happy" by Pharrell Williams. It feels like fate is talking to us and saying "wake up from your nightmare. Your time has come. You have been granted what you have wanted for so long and thought you would never have".

Life had not been easy since we began to consider the possibility of being parents. A lot of emotion and money was lost, leaving us on the emotional and financial edge, and without any short or medium term escape. In a few words, we weren't able to take much more in any sense.

And suddenly it was as if Santa had come and we had won the lottery all at once.

My husband and I are living a dream. It is very early to know how everything will turn out, as we still have most of the pregnancy ahead of us. But without a doubt, I think that we have been returned that feeling that moves each and every one of us every day, which is to fight for what we love, for what we want, and which we, more me than my husband, had lost: HOPE".

I have included this email because it is a love story.