

The in vitro fertilization (IVF) laboratory:

THE EGGS REACH THE LABORATORY

How do the eggs get to the Laboratory?

Following oocyte retrieval, the eggs surrounded by their cumulus cells are transferred to the laboratory in sterile tubes. They eggs are then identified using a microscope, cleaned and kept in culture medium in an incubator at 37°C.

Are all the eggs collected in a given cycle of the same quality?

The quality of the eggs is a property that is unique to each woman and to each cycle. Therefore, not all the eggs obtained after retrieval are of the same quality. Usually, 80% of the oocytes are mature and the remaining 20% are immature. Immature eggs are usually not suitable for in vitro fertilization.

THE SPERM REACH THE LABORATORY

When should the semen sample be collected?

After oocyte retrieval, the male is asked to produce a semen sample. The sample is collected by masturbation into a sterile container labeled with his name. The semen sample is then transferred to the laboratory where it will be processed for the IVF procedure.

Can a semen sample be frozen during the first visit?

Yes. We strongly recommend to freeze a semen sample during the first visit. In this way, the couple will not have to come to our Center the day of the retrieval. Only when the embryos are ready to be transferred.

What procedure is followed when donor semen is required?

Our team will allocate a suitable sperm donor for the couple or, in its case, for a single woman or a lesbian couple. Frozen donor sperm samples are usually obtained from our own sperm bank. If the required semen samples were not available, they will be ordered from another sperm bank.

On the same day of the egg retrieval, the sperm from the selected donor will be processed for the IVF procedure.

Does the semen require any special preparation?

Yes. Semen samples, either obtained from the male partner by masturbation or, in its case, from the sperm bank, need to be processed by density gradient centrifugation before they are used for IVF.

THE EGG AND THE SPERM ARE INTRODUCED TO EACH OTHER: THE FERTILIZATION PROCESS

After several hours in culture, the eggs are brought into contact with the sperm in order to achieve fertilization.

What does conventional In Vitro Fertilisation entail?

IVF is one of the most frequently used techniques in assisted reproduction. It consists of bringing the egg and the sperm together to ensure that both genomes combine. When the semen sample is normal and there is a history of earlier fertilization, "conventional" insemination is performed. This consists in placing the eggs in contact with a specific concentration of processed sperm (one hundred thousand sperm with good motility) in culture medium and incubating them at 37°C until fertilization is observed.

How is intracytoplasmic sperm injection performed? When is it necessary?

When the sperm sample is of low quality or there is no prior history of fertilization, the insemination technique used is Intracytoplasmic Sperm Injection (ICSI).

ICSI is a highly sophisticated technique, requiring a great degree of precision, expertise and experience by the embryologist. It is the introduction by injection of a "live" sperm into the cytoplasm of the egg.

In the case of the Egg Donor Program, ICSI is absolutely necessary, regardless of the quality of the semen, in order to ensure a high rate of fertilization.

ONE DAY AFTER IN VITRO FERTILISATION

What do we see the day after the insemination?

Seventeen to twenty hours after insemination, the eggs are observed under an inverted microscope at 400x magnification to see whether or not fertilization has occurred. A fertilized egg has two pronuclei: the female pronucleus and the male pronucleus.

We can see eggs which have been correctly fertilized, eggs that did not fertilize and eggs which have fertilized in an anomalous way.

Do all eggs fertilize after in vitro fertilization?

No. Under normal conditions, using sperm from the ejaculate, the rate of fertilization both in IVF and IVF-ICSI is about 70%.

The first cycle of IVF can be considered as diagnostic and allows the medical staff to determine the couple's level of fertility. In some cases, we can find low levels of

fertilization (5-20%) or even complete fertilization failure.

Are chromosomal embryo abnormalities more frequent in IVF than in natural conception cycles?

No. Although the average age of patients that undergo their first IVF treatment is higher than in the general population, and, therefore, the risk of embryo abnormalities higher, the rate of genetic abnormalities in children born after IVF treatment is similar to that found in natural conception.

TWO DAYS AFTER IN VITRO FERTILISATION

What do we see two days after in vitro fertilization?

The first embryo division (an embryo with two cells or blastomeres) usually occurs 25 hours after fertilization. Forty eight hours after fertilization, the second embryo division takes place (embryo with four blastomeres). However, at this point, since not all embryos grow at the same rate, some embryos may have 2, 3, 4 or 5 cells, with different degree of fragmentation between the blastomeres.

THREE DAYS AFTER IN VITRO FERTILISATION

What do the embryos look like three days after fertilization?

The embryos must have doubled the number of cells they had on the second day. By the third day of culture, the embryos display between 7 to 9 blastomeres with different degree of fragmentation. Some embryos may become blocked and their development halt between the second and third day.

Are all embryos the same?

Just like people, no two embryos are exactly alike. Each one has its own genetic and morphological features. Not all the embryos from the same cycle display the same quality.

The embryos are carefully analyzed with an inverted microscope under 400x magnification and are classified on the basis of their morphological features (rate of cellular division, equal or unequal size of cells and percentage of cellular fragmentation).

The embryos are rated from 1 to 10, where 10 is an embryo with 4 equal-sized, unfragmented cells on the second day of culture and between 7 and 9 equal-sized, unfragmented cells on the third day.

What happens to the surplus embryos that are not transferred? Can they be frozen?

Yes, "surplus" embryos which are not transferred to the uterus, are cryopreserved

in liquid nitrogen at -196°C , provided that they are of good morphology. Otherwise, they will not be frozen and will be discarded.

How long can the embryos remain frozen and still be implanted later?

If the storage conditions of the embryos in liquid nitrogen containers are adequate, based on theoretical grounds, they can be cryopreserved indefinitely. At present, we have data of embryos that have been cryopreserved for 13 years and that resulted in the birth of a healthy child.

How long does the law allow the embryos to remain frozen?

The time of storage of embryos depends on the specific legislation of each country. With regard to how long embryos can be cryopreserved, the current legislation of assisted reproduction in Spain states:

"Surplus embryos obtained after in vitro fertilization, that are not transferred to the woman in a given reproductive cycle, can be cryopreserved in authorized embryo banks. The cryopreservation of surplus embryos can be prolonged until the physicians responsible, with a favorable report from an independent specialist from another center, consider appropriate that the recipient meets the required criteria for the application of the assisted reproductive technique selected".

What is the destiny of frozen embryos allowed by the legislation?

The current Spanish legislation of assisted reproduction contemplates four possible options: (i) the embryos can be used for personal use in future transfers; (ii) the embryos can be left for donation after signing the corresponding Consent Form; (iii) embryos can be donated for research; and (iv) embryos can be discarded. It should be pointed out that a maintenance fee is required to keep the embryos cryopreserved. Failure to pay this maintenance fee or lack of response to a written notification the embryos will be automatically assigned to our Embryo Donation Program.