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Implementation of embryo laser biopsy method in an assisted reproduction laboratory

Ana Cardoso Prada nº22207342

Dissertação defendida em provas públicas para a obtenção do grau de Mestre em Embriologia e Reprodução Humana.

Orientador: Prof. Dr.ª Miriam Iglesias

Coorientador: Prof. Dr. Ignacio Santiago Alvarez Miguel

Lisboa, 2024

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Versão Final

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Vogais: Prof. Dr.ª Rocio Nuñez Colagne – arguente

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Resumo

A complexidade dos ciclos realizados em laboratórios de reprodução assistida têm vindo a aumentar com decorrer o tempo. É o caso da biopsia embrionária para a realização de testes genéticos pré-implantação, cada vez mais necessária em casos clínicos. Este aumento faz com que os laboratórios tenham a necessidade de evoluir e adaptar sem prejudicar o seu bom funcionamento, procurando acompanhar as necessidades dos pacientes.

Neste trabalho foi analisado o processo de implantação da biópsia embrionária recorrendo a técnica de laser no laboratório de reprodução assistida do Hospital Quirónsalud Madrid. Aproveitando o estágio curricular realizado neste laboratório para observar a forma como este processo foi gerido.

Não tendo sido possível abordar todas as perspetivas da implementação desta técnica, este trabalho teve como foco a preparação do laboratório para acolher esta técnica, o treino e a validação das embriologistas. Estes aspetos foram considerados fundamentais na gestão deste processo. Através de um questionário foi possível obter a opinião das embriologistas relativamente ao seu treino, validação e de que forma sentiram o seu trabalho afetado pela introdução da nova técnica.

Foi possível concluir que a gestão de um processo de implementação de novas técnicas num laboratório de reprodução assistida é desafiante. Uma tarefa com diversos detalhes cruciais, como o conforto dos embriologistas durante o processo, de modo que se minimize a possibilidade de erros gerados por stress ou ansiedade.

Palavras-chave: Tecnologia de reprodução assistida (ART), gestão laboratorial, biópsia embrionária, formação, validação.

Abstract

The complexity of the cycles performed in assisted reproduction laboratories has increased with time. An example is the case of embryo biopsy, used for preimplantation genetic testing, which is becoming more common to use in clinical cases. This growth means laboratories must evolve and adapt without compromising their proper functioning to seek patient's needs.

In this work, the implementation process of the embryo biopsy laser technique was analysed in the assisted reproduction laboratory of Hospital Quirónsalud Madrid. Observed through a curricular internship, allowing the author to perceive the laboratory management needed for this process.

As it was not possible to address the full extent of the implementation of this technique in this work, this work focused on the preparation of the laboratory for this technique and the training and validation of the embryologists. These aspects were considered fundamental in the management of this process. With a questionnaire, it was possible to obtain the embryologist's opinion regarding their training and validation, as well as how their work was affected by the introduction of the new technique.

It was possible to conclude that the management of an implementation process of new techniques in an assisted reproduction laboratory is challenging. A task with several crucial details, such as the comfort of embryologists during the process to minimize the possibility of errors generated by stress or anxiety.

Keywords: Assisted reproductive technology (ART), laboratory management, embryo biopsy, training, validation.

Abbreviatures and Acronyms

APA - American Psychological Association

AI - Artificial Intelligence

ART - Assisted Reproductive Technology

COVID - Coronavirus Disease

DNA - Deoxyribonucleic Acid

ESHRE - European Society of Human Reproduction and Embryology

ICSI - Intra Cytoplasmic Sperm Injection

PGT - Preimplantation Genetic Testing

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1. Introduction

In assisted reproductive technology (ART), preimplantation genetic testing (PGT) is a test to analyse the DNA of oocytes or embryos. Recommended in cases of advanced maternal age, recurrent problems in the implantation of embryos in the uterus or the carry of a pregnancy to terms, presence of known chromosomal abnormalities from the intended parent/s or hereditary genetic disease. Allowing analysis of embryos created with ART and selecting for transfer only the ones with the highest probability of developing a healthy child (Davidson *et al.*, 2019).

To perform a PGT, the first step is to perform an embryo biopsy on the created embryos of the clinical case in the study.

Depending on the embryo stage when the biopsy is performed, there are three embryo biopsy types: polar body biopsy on day 1, cleavage stage biopsy on day 3 and blastocyst biopsy on days 5 through 7. Represented in Figure 1 and being only the last two types of biopsies the focus of this thesis.

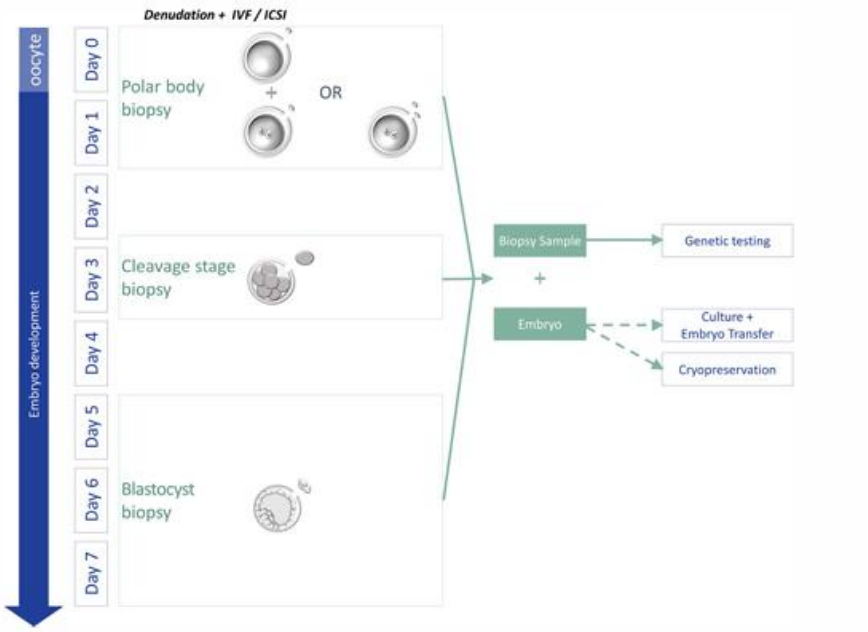


Figure 1: Procedure of oocyte and embryo biopsy (Kokkali et al.*, 2020).*

In cleavage stage biopsy, performed on day 3 of embryo development, the embryo is in the cleavage stage. Consists of the embryo being a group of identical cells called blastomeres inside the zona pellucida. This biopsy, also called blastomere biopsy, involves the extraction of a few blastomeres after the creation of a hole in the zona pellucida, shown in Figure 2 (Davidson *et al.*, 2019).

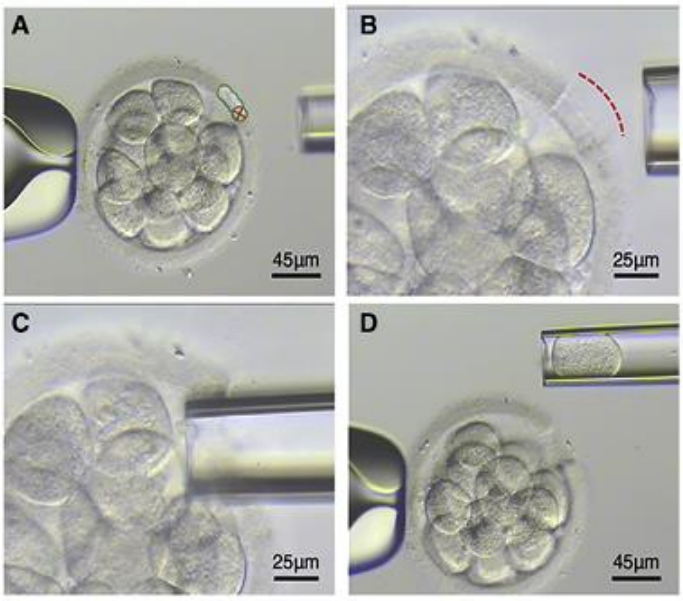


Figure 2: Laser assisted blastomere biopsy. Four distinct stages of the process. (Davidson et al.*, 2019)*

As the name states, in blastocyst biopsy, possible to be performed from day 5 until day 7 of embryo development, the embryo is in the blastocyst stage. The embryo must present the structures of this state that consist of two different cell types inside the zona pellucida: trophectoderm cells that will form the future placenta and inner cell mass cells that will form the embryo. This biopsy involves extracting a few cells from the trophectoderm after creating a hole in the zona pellucida. This biopsy can also be referred to as trophectoderm biopsy and possible to be observed in Figure 3 (Davidson *et al.*, 2019).

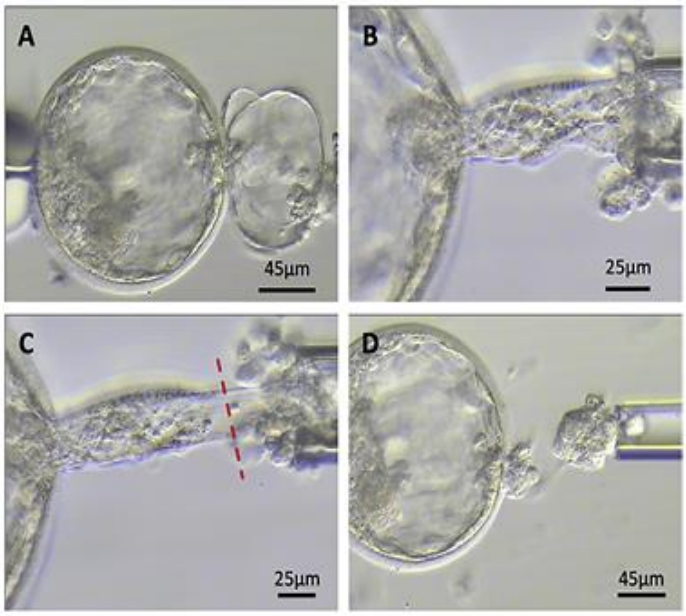


Figure 3: Laser assisted blastocyst biopsy. Four distinct stages of the process. (Davidson et al.*, 2019)*

As referred to, a major step needed in the embryo biopsy is the creation of a hole in the zona pellucida. Nowadays, the laser is the most used technique in clinical practice in this procedure, applicable in other parts of the embryo biopsy. In a laser-assisted biopsy, the hole in the zona pellucida is formed by laser pulses. Subsequently, the sample is extracted using a micropipette proper for biopsy. During the biopsy, the embryo is immobilized using a holding micropipette (Davidson *et al.*, 2019; Kokkali *et al.*, 2020).

While in the cleavage stage biopsy, the sample is easily removed from the embryo after aspiration with the biopsy micropipette, in the trophectoderm biopsy sample detachment can be done with two different techniques, 'pulling' and 'flicking'. The choice depends on the behaviour of the embryo on the biopsy, ensuring the minimal damage possible to the biopsied embryo and sample cells. In the pulling method, the blastocyst remains immobilized by the holding micropipette, while the biopsy micropipette separates the sample with a pull movement. The flicking method starts with laser pulses fired into the junctions between the biopsied embryo and sample cells. Separation is complete with a mechanical movement of the biopsy pipette against the holding pipette (Benavent *et al.*, 2019).

After the execution of the embryo biopsy, the sample needs to be carefully introduced into a tube, a process referred to as 'tubing'. This way, it is possible to send the sample to a laboratory for the genetic testing of the cells extracted. Meanwhile, the biopsied embryo recuperates and is kept cryopreserved until the results. If the genetic diagnosis is an inconclusive result, or there is any type of failure in the embryo biopsy, it is possible to do a re-biopsy. A procedure consisting of repeating the embryo biopsy described earlier after thawing the embryo (Kokkali *et al.*, 2020).

Introducing the embryo biopsy process in an ART laboratory requires multiple adaptations in the materials, equipment, routines and so forth. The aim must be to provide this technique with the best quality and prevent the risks for the embryo and cells of the biopsy. These purposes enhance the importance of ART laboratory management and quality control.

According to the European Society of Human Reproduction and Embryology (ESHRE) recommended guidelines, the preparation for an embryo biopsy starts with cleaning, decontaminating all the surfaces corresponding to those used in this technique and preventing contamination with protective clothing. Which provides an environment

without contaminations of DNA that can put the integrity of the sample and corresponding results into question. The equipment must be ensured that it is correctly installed and equilibrated, materials used sterilized and biopsy dishes equilibrated and identified. All the procedures and their preparation must be according to written protocol (Kokkali *et al.*, 2020; Carvalho *et al.*, 2020).

In an ART laboratory that performs embryo biopsies, it is recommended for staff who handle the biopsy to be competent and trained according to the legislation of the country in question. The training to perform an embryo biopsy must be able to guarantee a certification and consist in preclinical training and, afterwards, supervised clinical training. Training is also needed in cases of prolonged periods without performing the technique, to assure the capacities of the staff (Kokkali *et al.*, 2020; Carvalho *et al.*, 2020).

The importance of training represents one of the major details when introducing the embryo biopsy technique in an ART laboratory, reassuring that managing the implementation of a new technique in laboratory comes with many responsibilities and challenges.

This thesis intends to demonstrate the study of the implementation process of the embryo biopsy in the Hospital Quirónsalud Madrid ART laboratory, possible to observe due to the realization of an internship between the 16th of January 2024 and the 30th of April 2024.

The organization of the thesis aims to mimic the order of decisions and events during the introduction of the technique referred to in the laboratory. Information used in this work was given by the Hospital Quirónsalud Madrid ART laboratory or obtained through observation during the referred internship. Other sources of information appear indicated according to the APA norms of citation.

2. Implementation Process

2.1. Decision Making

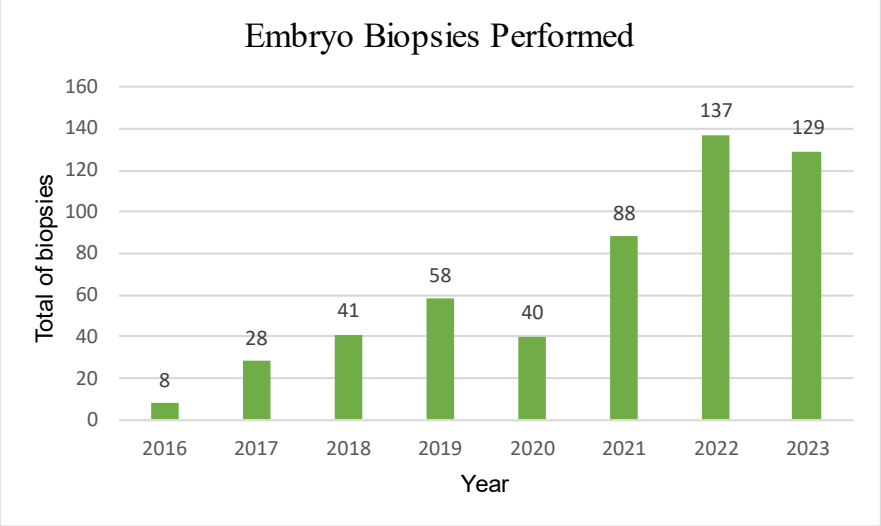
Before owning an inverted microscope of microinjection with laser technology incorporated in the laboratory, the ART centre of the Hospital Quirónsalud Madrid still would offer the possibility of embryo biopsy to patients indicated for this analysis. The process consisted of hiring personnel belonging to external groups of laboratories to carry out the technique. Meanwhile, the biopsy was performed by placing a portable laser from

this company in the usual microinjection microscope, used daily for the intracytoplasmic sperm injection (ICSI) technique.

In Table 1 and Graph 1, it is possible to observe the number of biopsies performed from 2016 until 2023, always performed by external laboratory groups. As shown, the number of cases of this technique increased significantly through the years, being the year 2020 an exception due to the COVID pandemic. The growth in the number of biopsy cases became one of the principal justifications for performing this technique independently.

Table 1: Embryo Biopsies Performed. Number of embryo biopsies performed in the Hospital Quirónsalud Madrid ART laboratory from 2016 to 2023.

Year	Total of biopsies
2016	8
2017	28
2018	41
2019	58
2020	40
2021	88
2022	137
2023	129



Graphic 1: Embryo Biopsies Performed. Number of embryo biopsies performed in the Hospital Quirónsalud Madrid ART laboratory from 2016 to 2023.

In order to make the decision of turning the embryo biopsy an independent technique of this laboratory, it was necessary to weigh in the advantages and disadvantages.

Advantages identified:

- Being independent allows the laboratory to proceed with an embryo biopsy in times when external embryologists cannot, such as on holidays;
- Since the laboratory's embryologists are responsible for embryo biopsies, the technique could be performed at the most opportune time during the daily work, not being dependent on the external embryologist's schedule;
- Not needing an Igenomix embryologist for embryo biopsies has economic advantages, becoming more affordable.

Disadvantages identified:

- Introducing this technique involves having more embryologists than usual for the shifts in this laboratory;
- Committing to independently performing the embryo biopsy means that this technique will possibly need to be executed during holiday breaks when fewer embryologists are working;
- The implantation process gives a responsibility to the laboratory to perform the technique independently, even with the uncertainty of being able to do it efficiently;
- The introduction of new techniques with an elevated level of complexity as the embryo biopsy in the laboratory elevates the stress level of the embryologists.

After analysing the positive and negative outcomes of doing the embryo biopsy independently, the decision was to proceed with the idea. The implementation process in the Hospital Quirónsalud Madrid ART laboratory started in 2023, with training and validation by Igenomix group, maintaining the biopsy performed by the same group until termination.

It was also necessary to search for other laboratories to perform the results of embryo biopsies after validation by Igenomix. To have a safety option in cases of incapacity of the first group or cases of monogenic disease analysis. The chosen group was Sistemas Genómicos by SYNLAB, an old partnership with the Hospital Quirónsalud Madrid ART laboratory to perform embryo biopsies.

2.2. Elected Equipment, Materials and Methods

The start of the embryo biopsy implementation process is dependent on the installation of the necessary equipment: an inverted microscope of microinjection with laser technology, micromanipulators and microinjectors (Kokkali *et al.*, 2020).

In the case of study, the decision of installing the necessary equipment was held by the Hospital administration and Quirónsalud group. That way the laboratory was able to obtain the equipment in question when possible.

The laboratory already had two inverted microscopes of microinjection before the implementation process, adapting one of them to perform the embryo biopsies, the ECLIPSE Ti2-U from Nikon. It was only necessary to purchase the laser technology to install in the microscope. The choice of brand and model was based on information collection from other laboratories, ending with the decision to purchase the Octax LaserShot and NaviLase, controlled with EyeWare Software, all by Vitrolife. In Figure 4, it is possible to observe the organization of these two equipment.

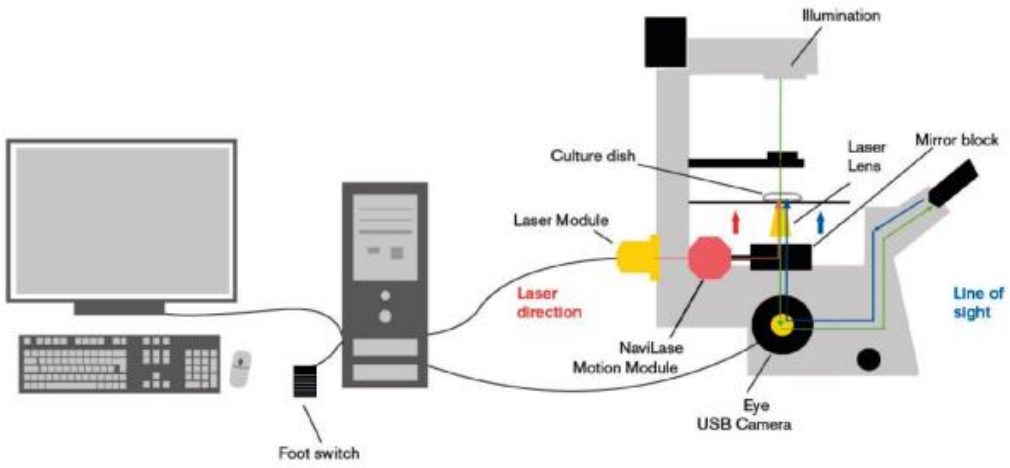


*Figure 4: Illustration of an inverted microscope equipped with laser technology. In this case, the Octax LaserShot and NaviLase laser technology (*Octax Laser Shot MTM Octax Navi Lase® User Manual*, 2023).*

The laser technology of Octax LaserShot and NaviLase consists of an infrared laser beam emitted along the microscope's optical axis by a diode. The laser pulse is controlled and activated with the EyeWare software. Trigger the laser pulse is possible through the computer´s mouse or a foot switch. Eye safety is guaranteed to the operator under the correct use of the equipment (*Octax Laser Shot MTM Octax Navi Lase® User Manual*, 2023).

The microinjectors and micromanipulators were also purchased before the implementation process, consisting of different models from the brand NARISHIGE already incorporated in the microscope that was adapted to perform embryo biopsies. On both sides of the microscope, one for holding another for the biopsy, the microinjector is the IM-11-2, a pneumatic microinjector, having better precision and practicability than an oil hydraulic injector. An MM-92 motor drive manipulator is also installed on both sides, allowing an easy adjustment of the micropipettes before biopsy using the three axes of movement. Another micromanipulator is used, only on the biopsy side of the microscope, with the format of a hanging joystick, allowing an even more precise movement with the biopsy micropipette.

In an embryo biopsy, it is necessary to have specific materials available. As referred, the micropipettes are an essential material for this technique. That brings the responsibility of choosing one type for holding the embryo and another to perform the biopsy. This choice was made by testing different brands and models of micropipettes during the time of training and validation of the embryologists of the laboratory, allowing to weigh in the opinion.

The methods adopted for the embryo biopsy in the ART laboratory in question were the ones learned in an Igenomix training session, suffering adaptation depending on the protocols of other external laboratories that also process embryo biopsy samples.

2.3. Chronology

The implementation process started with installing an inverted microscope of microinjection with laser technology incorporated in the laboratory on 23 October 2023. However, there were issues with the calibration and operation of the equipment, leading to repairments and reinstallation on 13 December 2023 and again on 11 January 2023.

The training of the laboratory biologists initiated after the microscope and laser technologies were well-functioning and in their proper settings. The technicians made two groups for the training session dates, both theoretical and practical, on 15 and 19 January 2024.

After the training sessions, the laboratory biologists started to practice the embryo biopsy technique with discarded embryos, allowing them to collect samples to send to the Igenomix laboratory for validation on 8 February 2024. The validation consists of the

analysis of five samples per biologist. However, due to Igenomix issues, on 16 February 2024, the laboratory received the information that new samples had to be sent for validation. The new shipment occurred on 14 March 2024.

The results of the second validation were received on 26 March 2024. Allowing the embryologists to start performing embryo biopsies on clinical cases with the supervision of Igenomix embryologists on 27 March 2024. Cases without supervision started on 28 April 2024.

After the end of Igenomix validation, the partnership with Sistemas Genómicos was implemented. Validations samples were sent on 16 April 2024, results received on 22 April 2024, starting cases autonomously for this group on 24 April 2024.

2.4. Qualification

To be qualified to handle the embryo biopsy independently, an ART laboratory must undertake training for this technique. The process must consist of preclinical training and supervised clinical training. However, different approaches to these two steps are possible depending on the laboratory group providing the training and the previous experience of the laboratory's embryologists undergoing training.

According to ESHRE, it is recommended to practice the embryo biopsy technique in 50 embryos minimum during the preclinical training and more 20 embryos for the supervised clinical training stage. However, more embryos are recommended to be biopsied with supervision when the trainee does not have prior experience in the technique. The damage and survival of the biopsied embryos must be monitored, and all processes of the training and parameters should be documented and compared with standard values (Kokkali et al., 2020).

The qualification process of the Hospital Quirónsalud Madrid ART laboratory followed the Igenomix protocol. The process was initiated with theoretical and practical training of the laboratory’s embryologists. The training consisted of a working day divided equally between theoretical and practical training. To not disturb the daily work in the laboratory, the training was on two days, each one for a different half of the laboratory’s embryologists, while the other would handle the laboratory’s tasks.

After the training, the embryologists started the collection of samples for validation, following the Igenomix criteria.

2.4.1. Validation

For a new clinic to perform an embryo biopsy and tubing of the sample to Igenomix analysis, it is necessary to perform first a 'validation' or 'dry run'. Five biopsied samples are collected by the embryologist of the enrolling laboratory, using the best possible quality embryos discarded. Validation allows to minimise errors in the results of future clinical cases due to difficulties in the biopsy and tubing by embryologists involved (*IGENOMIX LABORATORY USER MANUAL*, 2024; *Embryologist Validation-Instructions and Biopsy Sheet*, 2022).

Validation samples sent must fulfil specific requirements to be analysed, similar to the ones for clinical cases. The samples should consist of 5 to 8 embryo cells, and the lid of the tube correctly labelled with the initials of the embryologist and the number of the validation sample. For validation samples, there must also exist a blank tube without embryo cells but with the washing medium used during the tubing. The blanks must also be correctly labelled, using the same method as the validation samples with an addition of a 'B' after (*Embryologist Validation-Instructions and Biopsy Sheet*, 2022).

The validation samples and respective blanks must be sent to the Igenomix laboratory with the *Embryologist Validation-Instructions and Biopsy Sheet*, Appendix 1, where the embryologist on validation must fill in correctly the details of their sample's details (*Embryologist Validation-Instructions and Biopsy Sheet*, 2022).

In the Hospital Quirónsalud Madrid ART laboratory, the validation process was carried out by all the embryologists. The samples were obtained during working time, managing time between the daily tasks and validation until the shipment of all the validation samples from the embryologists.

The analysis of the validation samples is equivalent to the PGT tests, consisting of the DNA amplification of the biopsied cells. As referred, the validation samples are obtained through discarded embryos, which can lead to amplification errors due to the cell's lack of quality. However, in the validation samples of Hospital Quirónsalud Madrid ART laboratory, the error was not due to the quality of the cells but rather issues of the Igenomix laboratory. This occurrence led to the necessity of collecting new validation samples for all the embryologists in the laboratory.

2.4.2. After Validation

The results of the second group of validation samples allowed the embryologists to be qualified to perform the embryo biopsy technique independently. However, after the results, this method was still executed with the supervision of Igenomix on the first clinical cases. The supervision allowed a smooth transition between the training and the responsibility of performing the technique in an actual clinical case.

During the end of the Igenomix validation process, it was crucial to develop another partnership with an external laboratory able to process embryo biopsy samples. As referred, the choice was Sistemas Genómicos by SYNLAB. Being qualified with Igenomix made the enrolment in Sistemas Genómicos easier, only needing one validation sample per embryologist to get qualified to perform embryo biopsy for this laboratory. In this new partnership, although the biopsy protocol was significantly different, being more complex in the washing and tubing of the samples, there were no incidents, and the results were positive.

3. Analysis of the Implementation Process

The implementation of a new technique in a laboratory proved to be challenging to manage, having multiple and diverse details to care for. One of them is the comfort of the embryologists in the laboratory, for example, the choice of the type of micropipettes to purchase. Following this detail, a questionnaire was formulated by the author of this thesis using Google forms. This questionnaire, in Appendix 2, was aimed to analyse the opinion of all the embryologists in the Hospital Quirónsalud Madrid ART laboratory through nine questions. The questions were all regarding the implementation process of the embryo biopsy that they experienced, approaching different steps of their qualification process and an overview of the whole process.

For all questions, the choice was the raking question type with a scale from 1 to 5. The meaning of the scale varies according to the question. In the questions “How do you feel about the training time?”, “How do you feel about the theoretical training time?”; “How do you feel about the practical training time?” and “How do you feel about the validation time?”; the number 1 in the scale represents the answer “Too short”, and the number 5 represents the answer “Too long”. For the questions “Were you comfortable after the training to start the validation?” and “After validation, were you comfortable to

perform an embryo biopsy?", the meaning of the number 1 is "Very uncomfortable", and the number 5 is "Very comfortable". In the case of "How do you feel about the quantity of samples sent to validation?", the number 1 in the scale represents the answer "Too little", and the number 5 the answer "Too much". For "How would you evaluate your embryo biopsy technique at the moment?", the number 1 means "Insufficient", and the number 5 means "Perfect". In the last question, "How did the implementation process of the embryo biopsy affect your daily work in the laboratory?" the number 1 represents "Affected nothing", and the number 5 represents "Affected a lot". The results obtained with this questionnaire are in Table 2, showing the number of answers from the five embryologists for each number of the scale in the different questions.

With the results of the first three questions, it is possible to deduce that in this implementation process, the timing of training could have been longer. The opinion of the embryologists showed that they missed more training time, being the practical training where this shortage was more noticed. This insecurity with the quantity of practice time can be a reason for the results obtained in the fourth question. Most of the embryologists, 80%, answered that they were in the middle of being "Very uncomfortable" and "Very comfortable" to start the validation.

Regarding the validation process, the conclusions are that the time given to the sample collection could have been longer, and more samples could have been analysed for validation. Most of the embryologists, 60%, responded that the time to collect validation samples was between "Too short" and "Too long". However, one embryologist answered with 1, "Too short", and another with 2. For the quantity of validation samples question, there was also an answer with 1, "Too little", and most of the answers, 60%, were the 2. Only one answer ensured the number of samples was positive, responding with number 4.

With the results for question number seven, it is possible to infer that the majority of the embryologists have not felt comfortable after receiving the validation to start performing embryo biopsies on clinical cases. There was an answer with 1, "Very uncomfortable", and two with 2. The remaining answers showed comfort with the situation. Motives that can justify this discomfort can be the perception of a lack of practice or the number of validation samples previously mentioned. Another reason can be the normal stress response to the responsibility of performing a new technique with an elevated grade of difficulty and risks.

Table 2: Questionnaire Results.

Question	Answer										Total
	1		2		3		4		5		
	n	%	n	%	n	%	n	%	n	%	
1. How do you feel about the training time?	1	20	2	40	1	20	1	20	0	0	5
2. How do you feel about the theoretical training time?	0	0	2	40	2	40	1	20	0	0	5
3. How do you feel about the practical training time?	1	20	2	40	1	20	0	0	1	20	5
4. Were you comfortable after the training to start the validation?	0	0	0	0	4	80	0	0	1	20	5
5. How do you feel about the validation time?	1	20	1	20	3	60	0	0	0	0	5
6. How do you feel about the quantity of samples sent to validation?	1	20	3	60	0	0	1	20	0	0	5
7. After validation, were you comfortable to perform an embryo biopsy?	1	20	2	40	0	0	1	20	1	20	5
8. How would you evaluate your embryo biopsy technique at the moment?	0	0	2	40	2	40	1	20	0	0	5
9. How did the implementation process of the embryo biopsy affect your daily work in the laboratory?	0	0	1	20	0	0	2	40	2	40	5

Observing the answers given to question number eight, asking for an auto evaluation on the technique of the embryo biopsy, most embryologists showed to evaluate their technique as sufficient or good. Although one embryologist responded with 4, the remaining answers were divided between 2 and 3. For the author, who observed all the training and most of the embryologist's embryo biopsies, this auto-evaluation was slightly off than expected, considering the technique was generally good. The timing of execution of this questionnaire in question, the early stage of biopsy on clinical cases with the supervision of Igenomix, can defend the observation made. By asking this question during this period, it was possible that stress and insecurities still existed since the implementation process could have been considered not finished yet.

With the last question results, it is possible to conclude that the implementation process significantly affected the daily work in the laboratory for the embryologists. Two responded with 5, “Affected a lot”, two with 4 and the remaining answer consisted of a 2. These results were expected since the implementation process was done during working time, leading to more responsibilities and tasks, along with all the stress already mentioned.

It has been concluded that the increment of complexity in an ART laboratory provides a route to longer cycles, and the principal consequence is the more working time required from embryologists. That can also harm the comfort of the embryologists in the laboratory. More complexity leads to more probability of errors, which in turn, can be mentally exhausting. It is a must in the management of a laboratory to take care of the timings and other requirements of the embryologists. Assuring a safe working environment reduces the consequences of a complex laboratory environment (Alikani *et al*., 2014).

In the case of Hospital Quirónsalud Madrid ART laboratory, although the timings were not the most comfortable for the embryologists, it was not a problem of management but rather a problem of Igenomix methodology that could have been more compatible. The management of the implementation process of the embryo biopsy laser technique proved to be challenging, for example, the occurrence of setbacks such as calibration errors on the equipment. Nevertheless, the management of this case can be considered successful and well thought out. As shown through the search for other partnerships after validation with Igenomix, even though a new partnership brings more managing challenges.

In the future, complexity of managing an ART laboratory should continue, being important to find strategies to make this task easier. With the evolution of technology, new ways to help the managing of an ART laboratory will appear. Existing already AI warning systems that can evaluate embryo culture conditions and embryologist performance (Bormann *et al.*, 2021).

4. Conclusion

The implementation of a new technique in an ART laboratory involves different details and problems to resolve. With this thesis, it was possible to study the example of the implementation process of the embryo biopsy laser technique in the Hospital Quirónsalud Madrid ART laboratory. It is possible to conclude for the author that more knowledge was acquired with this work, opening a route to a better understanding the complexity of managing an ART laboratory.

Focused on studying different details of the management of the laboratory through this type of situation, observation and presence in the laboratory were essential. The execution of the questionnaire also had a positive outcome. Allowing the author to comprehend the impact of the changes suffered on the daily work and the general opinion of the embryologists of the laboratory in study.

In this thesis, it was not possible to do a study of the before and after of the implementation process on the laboratory clinical results. Would be interesting another perspective on how this process affected the laboratory, for example, the quality of the laboratory environment.

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6. Appendices

- **Appendix 1:** Embryologist Validation-Instructions and Biopsy Sheet
- **Appendix 2:** Embryo Biopsy Implementation Process Questionnaire

Appendix 1

Embryologist Validation- Instructions and Biopsy Sheet

The fields marked with * are mandatory to perform the test

CLINICIAN INFORMATION

*Clinic/Hospital/Medical Practice: _____

IVF Lab Manager: _____

*Email address for delivery of results: _____

DETAILS OF EMBRYOLOGIST BEING VALIDATED

*Embryologist Name: _____

*Embryologist Initials: _____

*Previous tubing experience (Y/N) – Details: _____

Instructions

A validation run (dummy/dry run) must be performed for each new clinic and all its embryologists that will be performing the tubing process. Any embryologist joining the clinic post initial validation and/or have not gone through the validation process must be validated before performing tubing on clinical samples. The quality of the embryos can affect the quality of the results; therefore, it is recommended to use good quality embryos for the validation runs (if available).

Requirements for multiple cell/clump biopsies (i.e. trophectoderm cells from blastocyst embryos):

- A minimum of 5 biopsied samples (clumps) are required.
- Samples/clumps should contain 5-8 cells.
- Include one blank per sample/clump: After "tubing" the biopsied sample/clump inside the 0.2ml microcentrifuge tube, from the last drop used for washing retrieve ~1-2µl of "washing/tubing" buffer and release in a new 0.2ml microcentrifuge tube designated as blank for that sample/clump.
- Label the lid of the sample/clump with the **initials of the embryologist followed by a number in a consecutive manner**. Record this in the table below in addition to the tube's unique ID number (provided by Igenomix UK and on the side wall of the tube). For example: SD1, SD2
- "Blanks" to that sample/clump are labelled the same way as in step 4 and adding the letter "B". For example: SD1B, SD2B
- For more detailed instructions on tubing of biopsied samples, follow the instructions detailed in the "Washing Tubing Instructions" document.

Igenomix UK Ltd, Surrey Technology Centre, 40 Occam Road, Guildford, GU2 7YG. Email: info.uk@igenomix.com. Tel: +44(0)2080688176. www.igenomix.co.uk

Embryologist Validation- Instructions and Biopsy Sheet

*SAMPLE DETAILS & BIOPSY WORKSHEET

Lot no. of wash/tubing buffer used:_____

Embryo ID		Day of Biopsy	Embryologist initials		Cells seen in tube[2]	Comments[3]
Tube label Embryologist's Initials+EMB #	Tube ID[1]	D5/D6/D7	Biopsy	Tubing	Yes/ No	

(1) This the number on the side of the tube provided by Igenomix.
(2) Note if the cells were seen while being expelled from the capillary into the tube
(3) Note any other observations that might be relevant, e.g., bad quality of cells, cells lysed while tubing etc.

FOR IGENOMIX USE ONLY

Received by:	Date/Time:
Sample accepted/rejected (provide reason if rejected):	

Igenomix UK Ltd, Surrey Technology Centre, 40 Occam Road, Guildford, GU2 7YG. Email: info.uk@igenomix.com. Tel: +44(0)2080688176. www.igenomix.co.uk

Appendix 2

Embryo Biopsy Implementation Process

This anonymous questionnaire aims to collect opinions about the implementation process of the embryo biopsy method in your laboratory. The information obtained will only be used by Ana Prada, in the thesis: "Implementation of embryo laser biopsy method in an assisted reproduction laboratory".

* Indica uma pergunta obrigatória

How do you feel about the training time? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Too short	○	○	○	○	○	Too long

How do you feel about the theoretical training time? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Too short	○	○	○	○	○	Too long

How do you feel about the practical training time? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Too short	○	○	○	○	○	Too long

Were you confortable after the training to start the validation? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Very uncomfortable	○	○	○	○	○	Very comfortable

How do you feel about the validation time? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Too short	○	○	○	○	○	Too long

How do you feel about the quantity of samples sent to validation? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Too little	○	○	○	○	○	Too much

After validation, were you comfortable to perform an embryo biopsy? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Very uncomfortable	○	○	○	○	○	Very comfortable

How would you evaluate your embryo biopsy technique at the moment? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Insufficient	○	○	○	○	○	Perfect

How did the implementation process of the embryo biopsy affect your daily work in the laboratory? *

Choose from 1 to 5 the option that best corresponds with your experience.

	1	2	3	4	5	
Affected nothing	○	○	○	○	○	Affected a lot

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