Universitat de les Illes Balears

BACHELOR'S THESIS

RETROSPECTIVE STUDY OF THE CUMULATIVE PREGNANCY RATE PER TRANSFERENCE, PER CYCLE AND PER PATIENT COMPARING DIFFERENT PROCEDURES

Francina Arrom Mayol

Degree in Biology

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Key words:

Pregnancy rate, IVF, assisted reproduction, **β**-HCG, freeze-all technique, cryopreservation, fresh transference

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Abstract

Since the groundbreaking Assisted Reproduction Techniques investigations became important in the medical and biological practices, improvements of the treatment of infertility have emerged in an exponential way. In Invitro Fertilization, one way to evaluate the outcome of the technique is the calculation of the pregnancy rate (PR). In this thesis, the evaluation of the IVF laboratory of Son Espases University Hospital (HUSE) was done by calculating the PR in three levels: per patient, per cycle and per transference, comparing different procedures at the different levels. At transference level, the comparation was done between fresh transferences and cryotransferences; at a cycle level, it was done differentiating the type of cycle (freeze-all, fresh only or combined cycle), comparing the number of cycle and separating the patients per number of cryopreserved embryos obtained and number of cryotransferences performed. Finally, at a patient level, the comparation was done between patients who ended the process with a pregnancy and those ones who didn't. Results show a good quality of the laboratory, with more patients getting pregnant. Furthermore, at cycle level, the obtention of 3 embryos to cryopreservate and its future transference seems to be beneficious for the patient, being a sign of good prognosis. For freeze-all techniques more studies need to be done before select the groups of patients that can be beneficiated with this technique.

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Introduction

In the present days, a 15% of the couples in developed countries have problems in conceiving a child. Currently, in Europe, the 2,6% of babies are conceived by assisted reproduction techniques (ARTs) and in Spain this rate increases up to 9%[17]. We define infertility as the incapacity of getting pregnant after 12 months, or more, of having sexual relationships without any contraceptive method or the obstacle of a person's capacity to reproduce either as an individual or with his or her partner[25]. At this point, we refer to primary infertility if the couple have not become ever pregnant, while secondary infertility refers to couples who have been able to get pregnant at least once[25]. Refer to the origin of infertility, in the 35% of the cases, infertility comes from a feminine factor, r 35% from the masculine factor, a 20% from both and finally, the 10% from an unknown factor[9]. However, infertility in some cases can be prevented by taking healthy habits. But in some cases, the cause of it lies in, for example, from hormonals, genetic or anatomic issues[9]. Since assisted reproduction techniques (ART) appeared decades ago, those couple's probability of having a healthy new-born at home have increased over the years due to the quick improvement of the assisted reproduction knowledge.

The terminology of assisted reproduction techniques englobe all the medical procedures focused on address infertility. Nowadays, many techniques are offered; from drug therapies and surgical repair to artificial insemination and in vitro fecundation. We can find in this list the directed coitus, in which the gynaecologist induces the ovulation in order to identify the fertile moment of the women; Artificial Insemination (AI), where the semen from the couple or from a donor is introduced into the cervix or the uterine cavity of the woman; fertility preservation (normally in oncologic patients or with an aggressive treatment), that consists in freezing the gametes in order to have the possibility to have a biological child in the future; and Preimplantational Genetic Diagnosis (PGD) in which a biopsy of the embryo is performed in order to identify the embryos that carry hereditary diseases. We can find two more techniques, In Vitro Fertilization (IVF) and Intracytoplasmatic Sperm Injection. These techniques are the ones the study is focused in and they will be explained below.

Historical precedent

When the human assisted reproduction appeared, its purpose was to increase the probabilities of pregnancy, but while its techniques have been improved, the main objective has become to have a single healthy new-born in order to optimize resources and reduce the risks involved. Assisted reproduction has suffered an exponential growth. The first baby conceived by assisted reproduction techniques was born in 1978, and only 6 years after there was the first birth of a cryopreserved embryo by slow freeze method. With the apparition of the vitrification method, the cryopreservation method was improved, and in 1990 there was the first live birth of a vitrificated embryo.

With the improvement of embryo incubators and the apparition of *trigas* incubators and *time-lapse* technology, the embryo culture can be extended up to day 5, what gives the chance to differentiate the best embryos to transfer.

All improvements through the years let change the main objective of assisted reproduction techniques. Multiple pregnancies are described as a complication, but at the beginning, pregnancy rates were so low that with a multiple embryo transference the chances increased. Thanks to the upgrades of ARTs, in 2002 the European Society of Human Reproduction and Embryology (ESHRE) concluded that the aim of ART was to obtain a single healthy baby to reduce multiple pregnancy complications.

In Vitro Fertilization / Intracytoplasmic Sperm Injection

Among all the ARTs explained above, convectional In Vitro Fertilization (IVF) and intracytoplasmic Sperm Injection (ICSI) play an important role. Both procedures involve an extracorporeal fertilization of the gametes performed in the laboratory. The difference is that in the case of ICSI, one single spermatozoon is directly injected into the oocyte, while in IVF both gametes are incubated in favourable conditions to obtain a fecundation [25]. In the case that more than 1 good quality embryo is obtained, there is the possibility to carry out the cryopreservation of the others so that they can be transferred in the future. Different steps are needed to conclude the treatment with a successful labour. The ensemble of this steps set up a cycle that begins with the ovarian stimulation, go through the obtention of oocytes and spermatozoids,

fertilization, embryo culture and embryo's preservation and transference. If after all there is not a pregnancy, the cycle starts again (Figure 1).

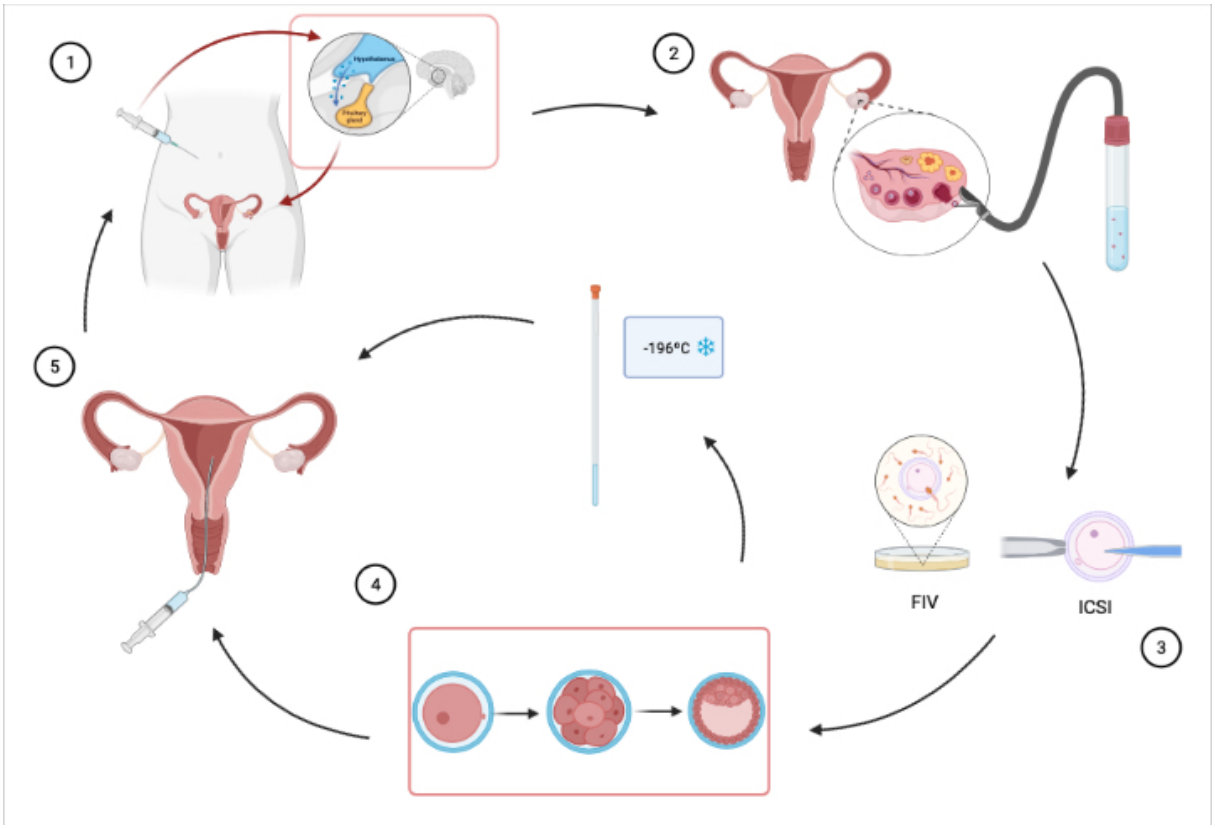

Figure 1. In vitro fertilization cycle. Numbers indicates the different steps: (1) Ovarian stimulation, (2) ovarian punction, (3) Fertilization with FIV or ICSI, (4) Embryo culture, (5) Embryo transference. Created with BioRender.com

1. **Ovarian stimulation**

   The main objective of the first step of the cycle is to obtain as much as mature follicles as possible and this is done with "fertility drugs"[9]. With the administration of agonists or antagonists of Gonadotropin-Releasing Hormone (GnRH), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Progesterone in different moments of the menstrual cycle, a Controlled Ovarian Stimulation (COS) is carried out[3].

2. **Ovarian punction**

When follicles are big enough, with a needle guided by ultrasound, enlarged follicles are punctured and oocyte pick-up is done. Then, mature oocytes with good quality will be selected for fertilization[9]. Since the oocytes are obtained from the follicular liquid, they are cultured in *trigas* incubators. This is the way to simulate them as much as possible, as the conditions inside the fallopian tube are prone to be successful. In order to guarantee the success, those incubators are at a constant temperature (37ºC) and controlled pH of 7,2-7,4[17]. *Trigas* incubators are called like this because they use CO_2, O_2 and N_2 to maintain pH and create a hypoxia environment like the inner feminine reproductive system[17].

3. **Fertilization**

Fertilization is done in *in vitro* conditions, however, depending on spermatozoa's and oocyte's quality and also taking into account the infertility cause, ICSI or FIV will be realized.

a. IVF

The main advantage of IVF conventional is that it is less invasive, and the spermatozoon fecundates the oocyte on its own. This technique is recommended in case of good quality of semen. While oocytes are obtained and selected, sperm is analysed according to OMS criteria and trained with *swim up* technique[20]. Each oocyte is cultured with 50,000 – 100,000 mobile spermatozoa for 17-20 hours, during this time, fecundation is expected[20].

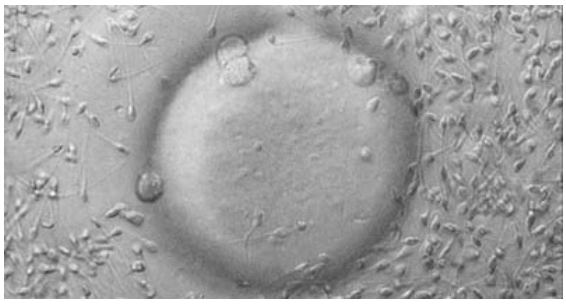

Figure 2. Conventional FIV fertilization seen in an electronic microscope. Google images

b. ICSI

When semen's quality is not so good or other factors are involved as PGD, oncologic patients, cryopreserved oocytes or failed IVF, ICSI is performed. This

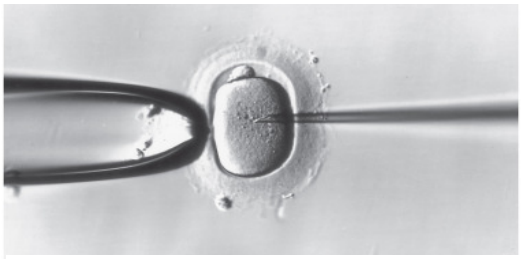

Figure 3. ICSI fertilization seen in an electronic microscope[3]

technique is more aggressive than IVF considering that a single spermatozoon is directly injected into the oocyte,[20] but the probabilities of fecundation increase. With ICSI technique, oocytes are denudated from the exterior layers, granulosa cells, with the reaction to hyaluronidase[CN!4]. It is important to maintain as much as possible the temperature and pH of the gametes because every single fluctuation can affect the chromosome distribution[20].

4. **Embryo culture**

   Once the fecundation is done, embryos are cultivated again into *trigas* incubators. The quality of the embryos on day 3 is checked following cleavage-stage embryo classification[12,15]. This classification takes into account the number of cells and the size of the blastomeres, the percentage of fragmentation and the presence of multinucleated blastomeres. Most viable embryos are those with 6-8 equal blastomere cells, with less than a 10% of fragmentation and without multinucleation[15]. In some cases, embryos can get blocked, but in case they are not, on day 5 blastocysts are also evaluated in order to transfer the most viable one. Quality of blastocysts is evaluated according to its packed inner cell mass (ICM), quality of trophectoderm and the degree of expansion of the blastocele being excellent blastocysts those ones with high quantity of packed ICM and cohesive epithelium formed[22].

5. **Embryo transference**

   Depending on the patient, after evaluating embryo's quality, the transference is done in day 3 or day 5. Over the years, the trend has become to transfer, when it's possible, one single embryo in day 5, to obtain a better synchrony with the endometrium and a lower risk of multiple pregnancy[20]. Two types of embryo transference can be distinguished: fresh or frozen.

Cryopreservation

Cryopreservation of embryos is the process of the storage of embryos in liquid nitrogen for a future transference[21]. It is used in two occasions: if after embryo culture there is more than one viable embryo, the ones that are not transferred are vitrificated, or if

there is a high risk of ovarian hyperstimulation and/or progesterone levels up 1,5 ng/ml that can compromise the implantation of the embryo in the uterus[7]. Cryopreservation of the embryos consists in preserving the cellular viability stacking the chemical reactions and the diffusions between the cells and their environment. This process is done by taking the embryos with cryoprotectant agents (CPAs) at -196ºC. CPAs are substances that avoid cell damages by dehydrating intracellular space protecting the cells from the creation of ice and keeping the intracellular salt concentration low. There are two types of CPAs: permeating and nonpermeating. In one hand, permeating CPAs can penetrate the cell, displacing intracellular water[21]. Dimethyl sulphoxide (DMSO), ethylene glycol (EG) and propaneidiol (PROH) are examples of permeating CPAs. On the other hand, nonpenetrating CPAs stay outside the cell and take out the water by osmosis. One example of nonpermeating CPAs is sucrose[21]. Cryopreservation methods can be distinguished into slow freeze or vitrification, the difference between them resides in the cooling and warming rates and the concentration to CPAs. Vitrification method has been extended and currently is the most used because of the simplicity of its protocol and the better results obtained[21].

Freeze-all policy consist in cryopreserve the entire cohort of good quality embryos without doing any fresh transference and transferring them when the patient has a more physiological endometrium[18]. Until now, many IFV laboratories have done this technique only in cases where the patients have a risk of pregnancy complications such as Ovarian Hyperstimulation Syndrome (OHS) or a Preimplantational Genetic Diagnosis[25]. However, the improvements of the vitrification technique offer the possibility to some patients to access to this policy, having a better synchrony with the endometrium and increasing the possibilities of getting pregnant[8].

Preimplantational Genetic Diagnosis (PGD)

In case there are familiar precedents of inherited disorders, PGC can be performed in order to identify the embryos that carry some diseases and prevent de birth of affected offsprings[5]. It is also indicated in couples that are carriers of chromosomal anomalies due to embryos that are more predisposed to failure[5].

Parameters affecting successful rates

During the IVF/ICSI cycle, different factors can affect the successful outcome. Some cases of infertility have better prognosis than others. One of those most implicated factors is the age of the woman. There is an inverted correlation between age and probability of pregnancy[14]. At this point, every step of the cycle is important: number of cycles performed, number and quality of oocytes obtained, fecundation rate, quality of the embryos obtained, and day and type of embryo transferred.

Complications in Assisted Reproduction Techniques.

Nowadays, ARTs are not perfect, and some risks are taken. Some complications related with assisted reproduction are ectopic pregnancy, multiple pregnancy or Ovarian Hyperstimulation Syndrome (OHS). At the beginning, more than one embryo was transferred in order to obtain a successful cycle, nowadays it's done with a maximum of 3 embryos following the actual Spanish law *"Ley 14/2006, sobre técnicas de reproducción humana asistida"*[10]. With ART improvement through the years, a multiple embryo transference and in consequence multiple pregnancy, iatrogenic complication has become the most frequent in assisted reproduction; increasing mortality and morbidity rates[13]. Ectopic pregnancy is another well-known pregnancy complication in which the embryo attaches outside the uterus with associated complications as haemorrhagic shock[11]. Although ectopic pregnancy occurs in natural conceptions with an incidence of 2%, in clinical pregnancies this rate increases up to 8%[6].

The physiopathology of OHS can appear due to an excessive answer to ovulation inductors during the ovarian stimulation, usually with gonadotropins. There is a liberation of vasoactive substances to the systemic circulation that increase the capillary permeability inducing third-spacing when liquid from intravascular space moves through the interstitial space, decreasing intravascular volume. Those substances are liberated after the human chorionic gonadotropin provokes a massive luteinization of the follicles[2]. There are different grades of OHS, but it can cause lethal complications. In those cases, the embryo transfer is cancelled and the embryos are cryopreserved. The patient waits until hormonal levels are normal again and the mother

is out of risk, after that there is a specific endometrium preparation to obtain a better synchrony between the embryo and the endometrium. The frozen embryo is thawed and transferred when the endometrium has growing up correctly.

Hypothesis

The obtention of embryos available to freeze, and its subsequent transference increase the pregnancy rate.

The execution of the freeze-all technique has benefits on the patients and increases its pregnancy rate.

Objectives

The main objective of this thesis is to compare the pregnancy rate (PR) for patient and for cycle of patients of the Human Reproduction Unit of Son Espases University Hospital. This will be performed taking into account the type of embryo transference (fresh or frozen) in order to evaluate and discuss the effectiveness of the IVF/ICSI laboratory.

Materials and methods

Patient selection and description.

For this study, the 309 patients who have been treated by the Human Reproduction Unit from Son Espases University Hospital (HUSE) between January 2019 to December 2020 were selected. However, 51 patients who didn't have any transference were dismissed, resulting a final population of 258 patients.

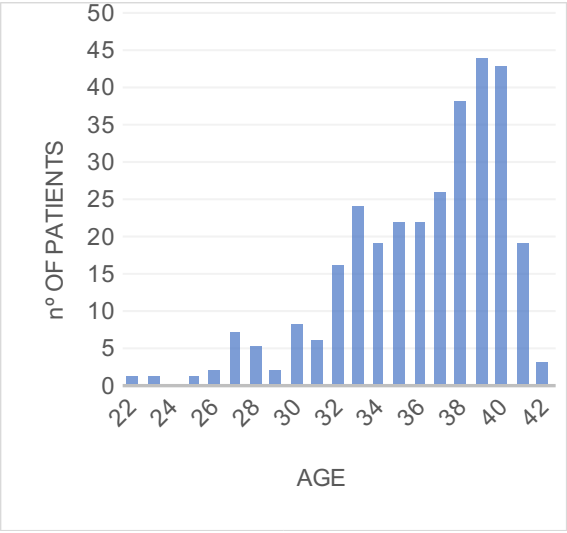

Figure 4. number of patients included in the study separated by age

Each patient completed a maximum of 3 cycles, following internal protocols. During this period, 352 cycles, with at least one transference, were done: 184 1st cycle, 104 2nd cycle and 64 3rd cycle. From all the cycles, 117 (33,2%) had only cryotransferences; 204 (58%) had only a fresh transference and 31 (8,8%) had fresh and frozen transference.

Regarding to patient's characteristics (Table 1, Figure 4) patient's age include from 22 to 42 years old with a mean of 36 years old. About oocytes the maximum of oocytes obtained was 43, however the mean was 8,8. The mean of mature and fertilized oocytes were 4,5 in both cases, and their maximums, 15. Only embryos in blastocyst stage (day 5) were cryopreserved. However, some exceptions were done if patient's conditions demand it. Few embryos could be cryopreserved, and with a mean of 1 embryo approximately per patient, the maximum of cryopreserved embryos per cycle was 7.

Variable	Mean	SD
Age	36,2	3,8
nº of oocytes obtained	8,8	6,2
nº of mature oocytes	4,5	3,8
nº of fertilized oocytes	4,5	3,8
nº of cryopreserved embryos	1,2	1,9

Table 1. Clinical characteristics of women included in the study

IVF treatment

Depending on the fertility cause, the number of oocytes obtained, its quality and the number of cycles already performed, oocytes were fertilized with IVF or ICSI techniques and in some cases, both were used in the same cycle. As far as it is possible, the less invasive technique was used. Embryos were cultured in a tri-gas incubator until transference and/or cryopreservation. Embryo's culture until day 5 and single-embryo transference were prioritized as far as the patient's diagnosis allow it. Cryopreservation was done principally with Origio (Barcelona, Spain) and in some cases Kitazato (Yanagishima, Japan) mediums and preserved in liquid nitrogen tanks.

Pregnancy rate measurement

Success will be measured through pregnancy rates. In this case, a successful IVF/ICSI cycle is considered when β -HCG (Human Corionic Gonadotropin) is detected, since is a proof of early pregnancy. The β -HCG is positive when the value is upon 100±20

mUI/ml and there is confirmation with ultrasound the presence of an intrauterine pregnancy, in early pregnancy (5 week pregnant, counting from the oocyte pick up or theoretical oocyte pick up in cryotransfer cases).

Pregnancy rates per transference, per cycle and per patient were calculated using the following general formula:

$$\frac{\beta - HCG}{N}\,(\%)$$

N represents the total size of the diagnostic group, and β *-HCG* represents the number of patients with a positive β -HCG registered after embryo transference. Pregnancy rates were calculated using Excel (Microsoft 365).

Per transference

At a transference level, simple rate was calculated considering the percentage of transferences that finished in a pregnancy. Fresh transferences were compared with cryotransferences. Results were also compared with the Spanish Fertility Society (SEF) and the European Society of Human Reproduction and Embryology (ESHRE) reference values, as a quality indicator of the laboratory (Table 2).

Per cycle

At a cycle level, all transferences of the same IVF cycle, independently of the type of transference, were assembled and the cumulative pregnancy rates were calculated (Table 3). However, PR was also calculated differentiating the type of cycle: freeze-all (all transferences done in the cycle were cryotransferences), fresh only (all the transferences of the cycle were fresh transferences) and combined cycle (in the same cycle there were fresh transferences and cryotransferences) (Figure 5).

Taking into consideration if there was a fresh transference or not, pregnancy rate were calculated separating the patients per number of cryopreserved embryos (Figure 6).

PR per type of cycle was calculated separating the patients depending on the number of cryotransferences that were performed (Figure 7).

Per patient

Finally, the cumulative pregnancy rate per patient was calculated taking into account all transferences and all cycles the patient did, observing if after all the procedures the patient ended the infertility treatment with a pregnancy.

Statistical analysis

Pregnancy rates were analysed through the statistic package RStudio (1.2.5001 version, Boston, USA). Chi-squared test was done to compare the results using a signification level of 0,05.

Results

Pregnancy rate per transference

General pregnancy rate per transference was calculated, resulting 42,5% compared to ESHRE's value that is a minimum of 35%. Furthermore, the ESHRE also offer an aspirational of 60%[8]. When results are calculated according to the type of transference (Table 2), fresh transference obtains a pregnancy rate of 41,3%, while cryotransferences have a pregnancy rate of 43,78%. Statistical analysis does not reveal differences between the type of transference (p-value= 0,673).

	PREGNANCY RATE (%)	**SEF (%)**	**ESHRE (%)**
Per transference	42,5	-	35
Fresh transference	41,3	43,5	-
Criotransference	43,78	52,3	-

Table 2. Pregnancy rates per transference obtained in Son Espases Hospital between 2019 and 2020 compared with Spanish Fertility Society (SEF) and European Society of Human Reproduction and Embryology (ESHRE) referent values.

Pregnancy rate per cycle

	PREGNANCY RATE (%)
Per cycle	49,02
Cicle 1	50,54
Cicle 2	42,72
Cicle 3	52,3
P – value	0,356

Table 1. Pregnancy rates per cycle obtained in Son Espases between the years 2019 and 2020.

Pregnancy rates per cycle were analyzed differentiating the number of cycle and the type of cycle. Moreover, general pregnancy rate per cycle was calculated (Table 3). The results show a general rate of 49,02% of pregnancies after a cycle. When pregnancy rate is calculated for each cycle, the results are: 50,54% for the first cycle, 42,72% for the second cycle and 52,3% for the third cycle. A decrease in the second cycle and an increase in the third cycle can be seen. However, no significative difference is shown between the number of cycles.

When the type of cycle is compared, pregnancy rates results are: 57,26% for freeze-all cycle; 44,12% for cycles with only fresh transferences, and 61,29% for cycles with fresh transferences and cryotransferences (combined cycle). In this case, statistics show a significative difference (p-value= 0,032) between fresh only cycles and only fresh and combined cycles (Figure 5).

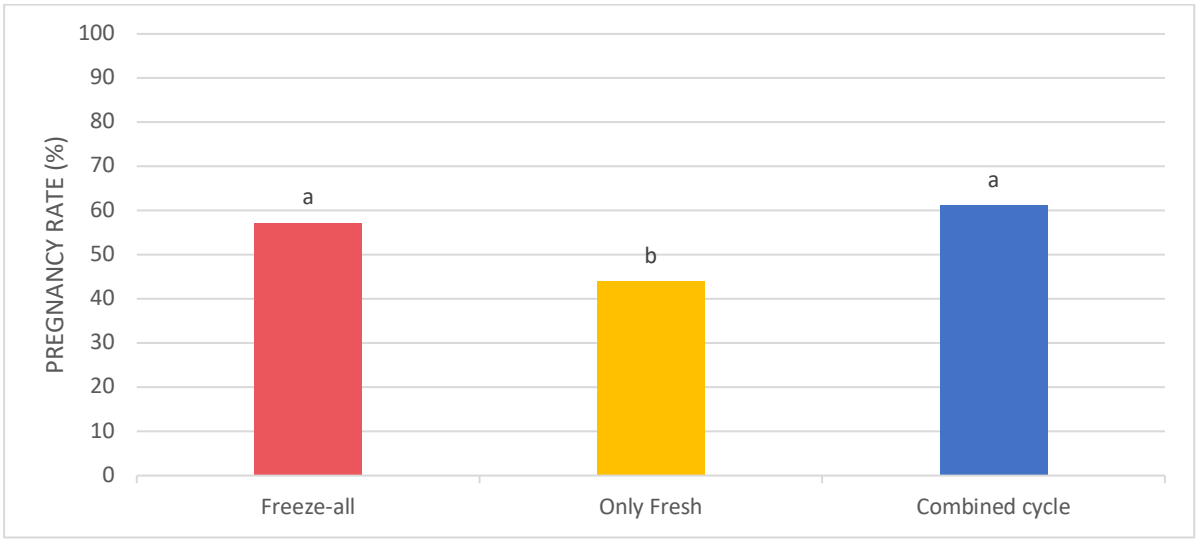

Figure 5. Pregnancy rates obtained in Son Espases Hospital between 2019 and 2020, differentiating the type of cycle: freeze-all, only fresh or combined cycle. Different letters above bars distinguish statistical differences.

Pregnancy rate was also analyzed separating transferences by number of cryopreserved embryos and the realization of a fresh transference or not within the same cycle (Figure 6). With a fresh transference, the highest pregnancy rate was archived when the patient obtained 2 embryos for cryopreservation (78,57%), while when the patient didn't have a fresh transference, the highest pregnancy rate was archieved with 3 embryos (83,33%). P-value for 6a (with fresh transference) was $3{,}71 \times 10^{-6}$, while P-value for 6b (without fresh transference) was 0,0059.

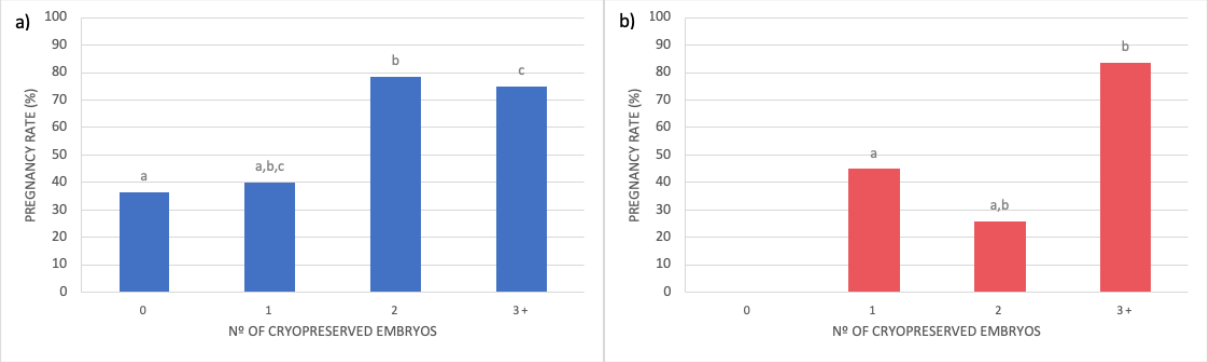

Figure 6. Pregnancy rates obtained considering the realization of a fresh transference (a) or not (b) in a cycle, and the number of cryopreserved embryos. Different lleters mean significative difference

Differentiating the three types of cycle performed, pregnancy rate was also calculated distinguishing between the number of cryotransferences performed in the cycle (Figure 7). In combined cycles the best pregnancy rate was obtained with a single cryotransference (66,7%), while in freeze-all cycles the best one was with 3 or more cryotransferences (80%).

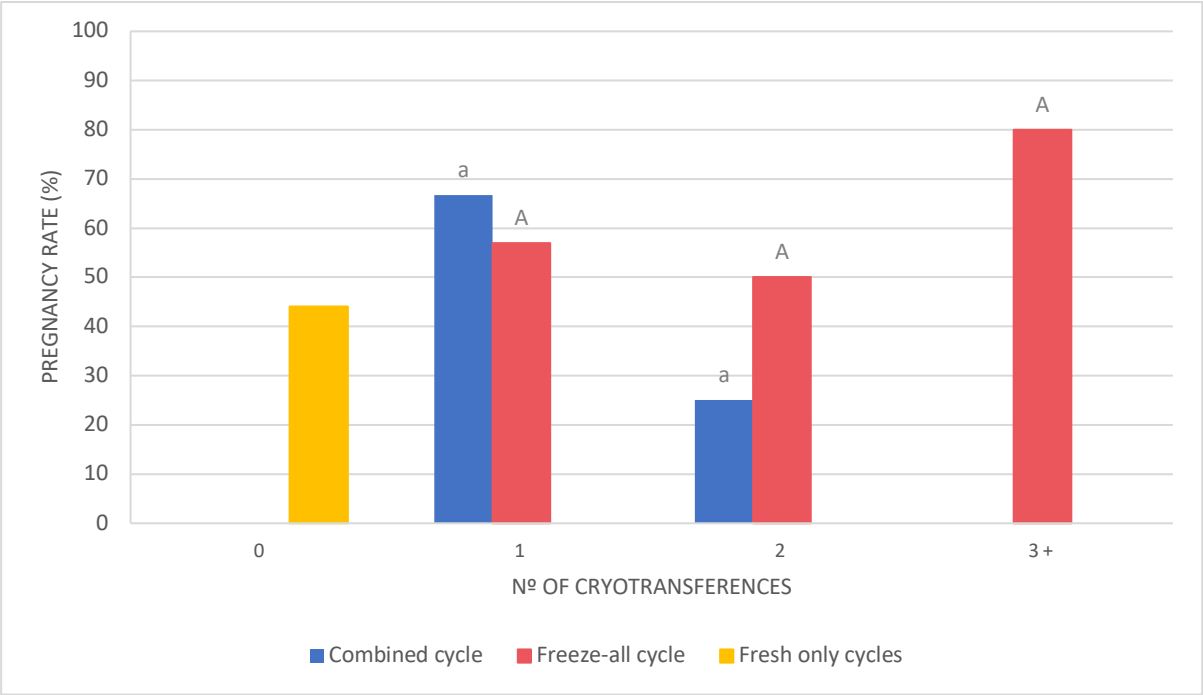

Figure 7 Pregnancy rate obtained considering the type of cycle (yellow fresh only cycle, blue combined cycle and red freeze-all cycle) taking in account the number of cryotransferences done in the cycle. Small letters and capital letters differentiated different statistic test

Pregnancy rate per patient

Per patient, from the 258 patients who passed for the hospital, 166 ended the process with a β -HCG positive. This makes a pregnancy rate of 64,34%. There is a significant difference (p-value < 0,05) between patients with a positive β -HCG after the cycle or cycles and patients without it.

Discussion

Into the IVF laboratory many things need to work, each step of the process can influence the outcome of the assisted reproduction. For that reason, quality controls need to be taken. In this case, general pregnancy rate it is higher than the minimums established by the ESHRE, 42,5% over the 35%. However, the aspirational pregnancy rate indicated is the 60%[8] (Table 2). This result represents how assisted reproduction works well, but at the same time, there is an ambition to continue improving the rates. Comparing the SEF referent values to the pregnancy rate per fresh transference, the

results are slightly lower than the referent, but they are still located into the optimal range stablished (39,1% - 54,9%)[1]. Regarding to PR per cryotransference, the SEF stablished an optimal rate in 52,3%. In this case, this study obtained a rate of 43,78% implying that is located between the optimal and the ideal category[1]. Quality indicators for IVF laboratory of Son Espases Hospital reflect a good quality, even though they can improve, especially in cryotransferences.

Starting with the first hypothesis, the results after calculating the pregnancy rates indicate that the obtention of good quality embryos available to cryopreserve increases the pregnancy rate in IVF patients. Years ago, cryopreservation of embryos didn't work as well as nowadays, as different studies report[13]. But with the apparition of vitrification, the survival rate of the embryos passed from a 60% up to a 78-100%. For that reason, in 2015, pregnancy rates of cryotransferred embryos were slightly lower than fresh transferences[17]. With the improving in ART's as the introduction of vitrification, cryotransferences doesn't mean less chances of getting pregnant and even outcomes rates have become equal[16]. This situation also happened in this study. The p-value between fresh transference and cryotransferences was 0,673 (Table 2), indicating a lack of significative difference between the two treatments. In this case, the IVF laboratory of Son Espases have a good rate in cryotransferences compared with fresh transferences.

At a cycle level, before analyse the different types of cycles, it is necessary to discuss the cumulative PR general per cycle. Table 3 indicates a general cumulative PR per cycle of 49,02%. Zhang *et al*. (2019) presented a PR per cycle between 32,6% and 45,05% in patients with an average age of 37. Considering this, cumulative general PR per cycle in Son Espases corresponds with the results presented by Zhang *et al.* The difference can be for the age of the patients, because in this study, the age average is of 36. It is demonstrated for many studies that the age is a conditioning factor in IVF that decrease the outcomes rates over the years[19].

Regarding to the PR per number of cycles, comparing the results obtained in 2009 for Beth *et al.*[12], the fact that IVF increases in an exponential way is proved as the IVF laboratory of Son Espases reported a cumulative PR of 32,9% in first cycle, 29,1% in second cycle and 30,2 in the third cycle. Furthermore, in this study a cumulative PR of

50,54% was obtained in the first cycle, 42,72% in the second and 52,31% in the third one.

However, as we can observe in Table 3, the same trend is observed in both cases: a decrease of the second cycle's PR over the first one, and an increase of the PR in the third cycle. This can be explained by considering, that in the first cycle, patients with good prognosis as for example, young patients with a good response to ovarian stimulation that have good quality embryos, will get pregnant soon. When these patients are discharged, patients with a worst prognosis stay in the process. But then, when they arrive to the third cycle, PR increases again. This happens due to the learning of the patient over the process. In each cycle, the patient's response to medication or the type of fertilization (FIV/ICSI) is observed, and through the cycles, these factors can be adjusted, increasing the probabilities of getting pregnant.

When the types of cycles are compared (Figure 5), statistics show a significative difference between cycles with cryotransferences (combined and freeze-all) and only fresh transferences over the cycle. This graphic, together with the PR obtained per transference comparing fresh ones and frozen ones, reasserts the first hypothesis presented in this thesis. When a patient obtains good quality embryo's for cryopreservation, is a sign that patients have a better prognosis. When fewer oocytes (<4) are obtained, probabilities of the embryo's failure increase. For that reason, in these cases, transferences are done in day 3 (embryo stage) and not day 5 (blastocyst stage) and probably no embryos will be suitable for cryopreservation since only good quality ones will be vitrificated[22]. For that reason, pregnancy rate in cycles with only fresh transferences are lower, because probable patients have a lower number of embryos obtained or they are not suitable for cryopreservation.

Even that, no significative difference between freeze-all cycles and combined cycles was notified with a PR of 57,26% and 61,29 respectively (Figure 5). Right now, freeze-all cycles are performed in Son Espases only in case fresh transference is contraindicated, as high risk of OHS or PGD. However, even more studies need to be done[4] in order to identify the situations in which fresh transference is indicated and freeze-all cycles mustn't be performed. Freeze-all seems to be a good option for improving outcomes rates in IVF due to the better synchrony with the uterus. In Figure

7, in freeze-all cycles we can observe that the introduction of cryotransferences in the cycle improve the PR (combined cycles with 1 cryotransference have a PR of 66,7%) and arriving to the maximum in freeze-all cycles (PR of 80%) when 3 cryotransferences are done. For these reasons, second hypothesis of the thesis can't be proved.

In Figure 6, when patients are separated by number of cryopreserved embryos even if they had a fresh transference or all the transferences were frozen, the highest pregnancy rate obtained, with statistical difference was with the total use of 3 embryos. This fact also supports the first hypothesis. Even though, not always quantity and quality go together, because when more oocytes are produced, its quality can be compromised.

Finally, the cumulative pregnancy rate per patient (64,34%) indicates that assisted reproduction techniques, in special IVF, are working and more patients have ended the process with a pregnancy.

Conclusion

The results have demonstrated that IVF laboratory of Son Espases University Hospital (HUSE) has a good quality. IVF is working well without differences between fresh or frozen cryotransference and with the 64,34% of the patients ending the process with a pregnancy. The obtention of 3 embryos to cryopreservate seems to be a good prognosis indicator and the future transference of this embryos will increase the pregnancy rate. However, even freeze-all technique is useful in cases where there are complications, more studies need to be done to determinate which types of patients without bad prognosis could beneficiate from this technique.

Acknowledgments

I want to thank Dra. Laura Peralta, for accepting, teaching and guide me through this adventure; it has been a pleasure learn by your side. I also thank Dra. Laura Torres for being there when I needed. Thanks, Aina Rosa Millan for the help and the advice in statistics. I also appreciate the treatment received in the Human Reproduction Unit of Son Espases, specially from Raquel. I'm *obrigada* to Gabriela Rodrigues and Augusta Gama for discovering me the fascinating area of the assisted reproduction. I can't end this thesis without thanking my parents for the questionless support and Aina, for being always my point in the world. Adrià, thank you for staying by my side in bad moments, and celebrate with me the good ones. Finally, thanks to all people who shared with me these 5 years, you are the best part of the degree.

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