



Trabajo Original

Clinical outcomes in elective vitrification of embryos

Resultados clínicos en el programa de vitrificación electiva de embriones

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Abstract

Objective: To compare the clinical outcomes of an elective vitrification program with those of a fresh embryo transfer program including vitrification of the remaining embryos.

Material and methods: Retrospective study of 99 cycles from the elective vitrification program (Group A) and 150 cycles from the nonelective vitrification program (Group B) carried out from January 2014 to December 2015 in Instituto Bernabeu, Alicante, Spain. In both groups, the embryos were from the patient's own oocytes. The variables evaluated in group A were clinical indication, endometrial preparation protocols for frozen embryo transfer, percentage of embryo survival after thawing, and day of embryo vitrification. The main clinical indication (54.5% of cases) in Group A was to avoid ovarian hyperstimulation syndrome.

Outcomes: The percentage of embryo implantation (35.2% vs. 27%), the percentage of positive pregnancies with β -hCG (58.5% vs. 42.9%), and the percentage of clinical pregnancy (41.5% vs. 32.5%) were superior in Group A when we transferred embryos of types A and/or B according to the ASEBIR classification, although no statistically significant differences were found ($p = 0.230$, $p = 0.082$, and $p = 0.360$, respectively).

Conclusions: A "freeze-all" strategy is the procedure of choice for avoiding ovarian hyperstimulation syndrome or possible embryo-endometrium asynchrony at the time of the transfer. It also provides clinical results that are at least comparable to those obtained with fresh embryo transfer.

Key words:

In vitro fertilization. embryo elective vitrification. embryo selection. frozen embryo transfer. fresh embryo transfer

Resumen

Objetivo: Comprobar los resultados clínicos del programa de vitrificación electiva de embriones frente al de transferencia en fresco y congelación de los embriones restantes.

Material y métodos: Se han estudiado de forma retrospectiva 99 ciclos de vitrificación electiva (Grupo A) y 150 ciclos de vitrificación no electiva (Grupo B) realizados entre enero de 2014 y diciembre de 2015 en el Instituto Bernabeu de Alicante. En ambos grupos los embriones obtenidos provenían de ovocito propio. En el grupo A se valoraron las indicaciones clínicas, los protocolos de preparación endometrial para la criotransferencia (CT), el porcentaje de supervivencia embrionaria a la descongelación y el día de vitrificación embrionaria. La indicación clínica mayoritaria (54.5% de los casos) en el grupo A fue evitar el Síndrome de Hiperestimulación Ovárica (SHO).

Resultados: El porcentaje de implantación embrionaria (35,2% vs. 27%), el de embarazo positivo con β (58.5% vs. 42,9%) y el de embarazo clínico (41,5% vs. 32,5%) fue superior en el grupo A cuando se transfirieron embriones de categoría A y/o B según los criterios de la Asociación Española para el Estudio de la Biología de la Reproducción (ASEBIR), aunque no se alcanzaron diferencias estadísticamente significativas ($p = 0,230$, $p = 0,082$ y $p = 0,360$, respectivamente).

Conclusiones: La vitrificación electiva de embriones nos ha permitido por un lado evitar complicaciones como el SHO y por otro, obtener resultados clínicos cuanto menos comparables a los ofrecidos con transferencia embrionaria en fresco.

Palabras clave:

Fecundación in vitro. Vitrificación electiva de embriones. Selección embrionaria. Criotransferencia. Transferencia en fresco

Recibido: 17/09/2016
Aceptado: 07/03/2017

Sellers Gil R, Ten Morro J, Tió Marquina MC, Sellers López F, Bernabeu Pérez R. Clinical outcomes in elective vitrification of embryos. Prog Obstet Ginecol 2018;61(5):438-443

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INTRODUCTION

Increased efficiency in stimulation techniques in recent years and improved embryo culture conditions in the *in vitro* fertilization (IVF) laboratory have made it possible to obtain several transferable embryos. Nevertheless, the current tendency is to transfer a single embryo in order to reduce the frequency of multiple pregnancies and their complications. The success rates of single-embryo transfer are similar to those of double-embryo transfer (1).

In the last few years, elective freezing has been proposed in order to avoid some of the complications of assisted reproduction cycles and improve the efficiency of cryopreservation of a whole embryo cohort using vitrification (2-4). Frozen embryos are transferred in a subsequent cycle, with appropriate preparation of the endometrium following current protocols or the natural cycle.

The number of transfers of cryopreserved embryos (frozen embryo transfer [FET]) has recently increased thanks to the advantages offered by vitrification of embryos (5, 6). Since high-quality embryos are generally cryopreserved, they usually guarantee appropriate results after FET (7).

Embryos can be frozen at any stage of their development, as the percentage of survival is similar for all of them. Consequently, there is no consensus on the best stage for vitrification.

Embryo cryopreservation by vitrification makes it possible to defer transfer for various reasons, mainly to avoid ovarian hyperstimulation syndrome (OHS), to obtain an appropriate endometrium for embryo nesting, or to allow for preimplantation genetic diagnosis. Other reasons for this approach are to synchronize slow growing embryos with the requirements of the endometrium or to defer transfer because of the contraindication for pregnancy in patients who have to undergo immediate treatment of cancer.

The objective of this study was to collect clinical data from an elective embryo vitrification program between January 2014 and December 2015 and compare them with data from the fresh embryo transfer program.

MATERIAL AND METHODS

Study population

We performed a retrospective study at Instituto Bernabeu, Alicante, Spain.

We included 99 elective vitrification cycles with embryos in their third or fifth day of development. The number of cycles is greater than the number of patients because some patients attempted more than 1 FET during the data collection period.

The data collected for group A cycles were clinical indica-

tion for embryo freezing, day of vitrification, endometrial preparation protocol for FET, embryo survival on thawing, and grading of the embryo according to developmental stage based on the criteria of Asociación Española para el Estudio de la Biología de la Reproducción (ASEBIR [Spanish Association for the Study of Reproductive Biology]) (8).

Group B included 150 cycles in which embryos were transferred fresh, with the spare embryos in the cycle undergoing vitrification. As in Group A, only embryos from their own oocyte were taken into consideration.

Embryo vitrification cycles for preimplantation genetic diagnosis or complete chromosomal screening were not included, since they could introduce confounding factors such as partner with advanced age, history of recurrent miscarriage, and being a carrier of mutations or chromosomal abnormalities.

All patients were informed about the procedures and provided their corresponding informed consent.

Ovarian stimulation and oocyte collection

Ovarian stimulation was performed on an individual basis depending on the characteristics of the patient using 2 standard protocols: a short protocol (with antagonists) and a long protocol (with agonists).

The process finished 36 hours after induction of ovulation with ultrasound-guided transvaginal ovarian drilling under sedation.

IVF, intracytoplasmic sperm injection, and embryo development

Once all of the cumulus-oocyte complexes were recovered using ovarian drilling, they were washed using a buffered medium (G-MOPS Plus™; Vitrolife). The cells of the cumulus and of the corona radiata surrounding the oocyte were denuded using hyaluronidase and then aspirated gently with a Pasteur pipette.

In clinical cases with completely normal male factor, we decided to perform conventional IVF, whereas in cases with severe abnormalities of the male factor, we used intracytoplasmic sperm injection (ICSI), which was performed between 3 and 5 hours after oocyte collection.

After 16-18 hours, fertilization was verified based on the presence of 2 pronuclei and 2 polar bodies. The zygotes obtained were cultured individually in 30- μ L microdrops in cleavage medium (CM, Cook Medical, Bloomington, USA) until day 3 of embryo culture. In cycles with a long culture, the embryos were passed to CCM medium (Vitrolife, Göteborg, Sweden) on day 3 until the blastocyst phase was reached on day 5 or 6 of development. Culture was performed under conditions of hypoxia using a mixture of 7% oxygen and 7% CO₂. The embryos were periodically

observed to evaluate their development and grade them according to the criteria of ASEBIR.

Embryo vitrification and devitrification protocols

Embryos selected for elective vitrification according to clinical criteria and the embryos remaining from fresh transfer cycles were cryopreserved following the protocol established by IrvineScientific®. A closed HSV straw (Crio-BioSystem Group IMV Technologies) was used for storage in liquid nitrogen at -196°C . The same protocol was used for embryo devitrification.

Embryo transfer

The embryo was transferred in both groups using abdominal ultrasound with a flexible catheter (Rocket Medical, UK).

In the case of embryo transfer in the same stimulation cycle, the most appropriate time point in embryo development was chosen, and the corresponding progesterone support was provided.

FET was based on 2 strategies:

- Natural cycle, based on ultrasound ovulation criteria. This was sometimes modified with administration of Ovitrelle®.
- Artificial cycle, based on administration of estradiol in transdermal patches (Evopad®; Janssen) or orally (Meriestra®; Novartis) from the first day of the cycle, at increasing doses, and with ultrasound verification of appropriate endometrial development. Transvaginal progesterone (600 mg daily) was subsequently added (Utrogestan®; Seid).

If a suitable endometrium was not obtained, stimulation was with Letrozole (Femara®; Novartis).

Statistical analysis

Data were collected using IBM® SPSS Statistics for Windows, Version 20.0.

Data were described using mean (SD) or percentages depending on the type of variable. Quantitative variables were compared using the t test; categorical variables were compared using the Fisher exact test or χ^2 test.

RESULTS

With respect to the 99 cycles in Group A, the main clinical indication (54.5%) was avoidance of ovarian hyperstimulation, which was achieved in all cases. In the remaining cycles, the indications were as follows: presence of elevat-

ed progesterone in blood on the day of the ovulation trigger (14.1%), unsuitable endometrium (15.2%), slow-growing embryos (10.1%), and personal reasons (6.1%). As for the endometrial preparation protocols for embryo transfer, cycles involving estrogen replacement accounted for 89.9% of cases, whereas natural preparation stimulated with Ovitrelle® represented 9.1%. There was only 1 case of endometrial preparation with a cycle stimulated with Letrozole.

Table I shows the overall clinical results for both groups. The groups were homogeneous in terms of patient age, endometrial thickness, and number of embryos transferred. No statistically significant differences were observed for any of the parameters studied, with the exception of the number of embryos thawed ($p = 0.006$), those that survived ($p = 0.007$), and those that were transferred ($p = 0.003$), which was always higher in Group A.

Figure 1 shows all the cycles in both groups depending on the day of development when vitrification was performed. In most cases in Group B, it was decided to vitrify the remaining embryos on the fifth day of development, whereas in Group A, the cases were more widely distributed.

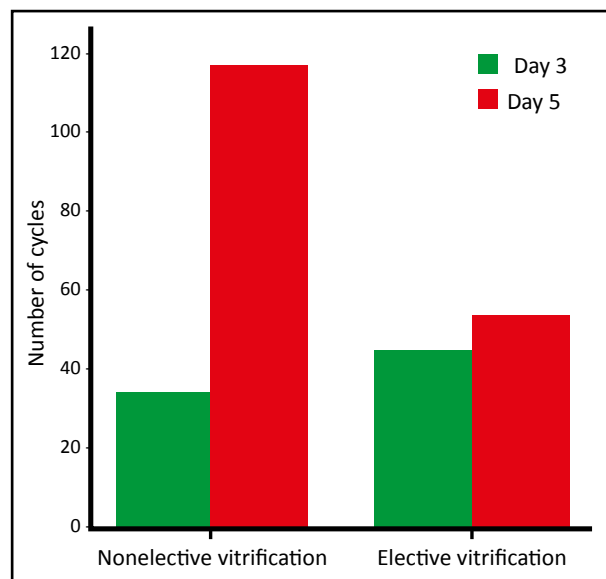


Figure 1.

As for the percentage of implantation, and to avoid possible bias resulting from embryo quality, a more in-depth study was made by comparing data when embryos are grouped in category A and/or B of ASEBIR (Table II). The main result in this respect was the greater percentage of implantation in Group A by transferring embryos of the same grade. While the difference was not statistically significant ($p = 0.230$), the percentage of implantation tended to be higher when elective vitrification was used.

Table 3 shows the clinical results obtained in both groups according to embryo quality. The most revealing result is the percentage of positive pregnancies with β -hCG (β -hCG > 6 mIU/mL), which is higher in Group A, although the difference is not statistically significant ($p = 0.082$). However, elective vitrification does tend to be associated with this clinical parameter. Similarly, the clinical pregnancy rate was higher in Group A, even though the difference between the groups was not statistically significant ($p = 0.360$).

Table I.

Overall clinical outcomes in both groups

	Group A (elective vitrification)	Group B (non-elective vitrification)	p
Patient's age	35.03 \pm 3.905	34.87 \pm 3.830	0.76
Endometrial thickness (mm)	8.69 \pm 1.648	8.88 \pm 1.600	0.39
No. of frozen embryos	2.09 \pm 0.801	1.79 \pm 0.838	0.006
No. of embryos that survive	1.97 \pm 0.680	1.69 \pm 0.843	0.007
No. of embryos transferred	1.77 \pm 0.533	1.55 \pm 0.563	0.003
No. of embryos transferred (A and/or B)	1.10 \pm 0.843	0.95 \pm 0.777	0.15
No. of embryos transferred (C)	0.56 \pm 0.747	0.57 \pm 0.757	0.89
Percentage of embryos surviving	94.1	94.4	0.98
Percentage of positive pregnancies with β	45.5	47.3	0.80
Percentage of clinical pregnancies	32.3	35.4	0.68
No. of amniotic sacs	1.24 \pm 0.43	1.17 \pm 0.38	0.47
Percentage of clinical abortions	27.27	15.1	0.18
Percentage of implantations	23.7	26.8	0.90

Note: Values are shown as mean \pm SD. A p value < 0.05 indicates that the difference between the groups is statistically significant.

Table II.

Percentage of implantation in both groups according to embryo quality

	Group A (n = 88)		Group B (n = 122)		p
	No implantation	Implantation	No implantation	Implantation	
A and/or B	57 (64.8%)	31 (35.2%)	89 (73%)	33 (27%)	0.230

Table II.

Clinical results in both groups according to embryo quality

	Group A	Group B	p
Percentage of positive pregnancies with β	58.5	42.9	0.082
Percentage of clinical pregnancies	41.5	32.5	0.360
Percentage of medical abortions	21.7	17.9	0.739

DISCUSSION

The present study evaluated the clinical outcomes of an elective embryo vitrification program by comparing them with those of fresh transfer and freezing of the remaining embryos.

The results obtained suggest that elective vitrification, or a segmented strategy, has advantages in the various IVF cycles where it was applied, since it prevented ovarian hyperstimulation and transfer of slow-growing embryos without compromising a patient's ability to become pregnant. Furthermore, the effectiveness of assisted reproduction techniques can be improved when embryo transfer is deferred and the embryos for transfer are frozen (9).

Cryopreservation could not be applied using vitrification without recent advances in cryobiology (10,11). Similarly, vitrification has led to a high percentage of embryo survival on thawing compared with other techniques such as slow freezing (11), resulting in a greater percentage of implantation and subsequent pregnancy per FET performed (12-14). It is also possible that the thawing process selects the embryos to be transferred in each FET on a numerical and morphological basis (15,16). Successful FET makes it possible to transfer a limited number of embryos, which in most cases are of optimal quality, and to prevent subsequent complications associated with multiple pregnancy (17).

Elective vitrification can also be implemented as an alternative for preventing the harmful effects of ovarian stimulation on embryo-endometrial synchrony (15-18), since some stimulations have been shown to compromise suitable receptivity of the endometrium once FET has been carried out (19,20). This strategy makes it possible to transfer embryos at the time of highest endometrial receptivity.

Ovarian hyperstimulation is the most severe complication in any ovarian stimulation process, since it prevents transfer of the embryo during the stimulation cycle. In addition, deferring transfer using elective vitrification proves that not only does the risk of ovarian hyperstimulation decrease, but the percentage of pregnancies increases with respect to the cycles where the transfer was fresh (21-25). Similarly, some studies support the thesis that elective vitrification cycles have higher percentages

of pregnancies than those where the transfer was fresh (26,27), whereas in others this affirmation is refuted (28).

Another study found the percentage of clinical pregnancy to be 80% for embryos that were thawed after elective vitrification and 65% for those from fresh cycles (16). Although we found no statistically significant differences, our clinical results were similar in both groups. However, when both groups are given embryos of the same grade (A and/or B according to the criteria of ASEBIR), the outcomes of pregnancy and embryo implantation tended to be better in cases of elective vitrification. Nevertheless, in order to really compare the efficacy of elective vitrification with fresh transfer, we must take into account the cumulative pregnancy rate with respect to all attempts made until pregnancy is achieved. We are currently collecting information for a future review of this subject.

There may be some reservations about the health of children born after application of assisted reproduction techniques, and, more specifically, after elective freezing (29-1). One study compared results from 3 different groups: those born from fresh transferred embryos, those born from frozen embryos, and those born after spontaneous pregnancies. Embryo freezing did not affect perinatal outcomes compared with fresh embryo transfer, although the overall perinatal outcomes of both assisted reproduction techniques were worse than those of spontaneous pregnancies (30).

As for the possibility of malformations, one study analyzed children born from spontaneous pregnancies and those born from singleton pregnancies after IVF or ICSI with fresh transfer and elective vitrification (31). Again, the authors concluded that neonatal outcomes for children born from singleton pregnancies after embryo freezing are better than those for children born from fresh transfer, although worse than those of children born from spontaneous pregnancies. In any case, no significant differences were observed in the percentages of congenital malformations.

Other reviews (32,33) have shown that the birth weight of babies conceived using freezing is greater than that of those born using fresh transfer, whereas it is no different from babies born after a spontaneous pregnancy. These studies investigate the uncertainty surrounding the epigenetics of embryo freezing in children conceived using this approach.

Lastly, we were unable to confirm the status of the newborns after elective vitrification, since the information was not available from the patients. Nevertheless, this information is being collected, together with the cumulative pregnancy rates, for purposes of a future review.

In conclusion, elective vitrification of embryos is not only the procedure of choice for addressing ovarian hyperstimulation or possible embryo-endometrium asynchrony at transfer, but it also yields clinical results that are at least similar to those obtained after fresh transfer.

Conclusions: Elective vitrification of embryos has enabled us to prevent complications such as ovarian hyperstimulation and obtain clinical results that are at least similar to those obtained with fresh embryo transfer.

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