Embryo stage of development is not decisive for reproductive outcomes in frozen-thawed embryo transfer cycles

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ABSTRACT

Objective: To evaluate if the outcomes of IVF/ICSI in frozen-thawed embryo transfer and fresh embryo transfer cycles differ in relation to cleavage and blastocyst stages.

Methods: Retrospective cohort study to compare IVF/ICSI outcomes between fresh embryo transfer and frozen-thawed embryo transfer cycles, according to the stage of embryo development. Analysis was carried out on 443 consecutive embryo transfer cycles performed between January 1st and December 31st, 2014. Women aged up to 38 and submitted to embryo transfer cycles with fresh (n = 309) or frozen-thawed (n = 134) embryos at a private center for assistance in human reproduction were considered for analysis. Results in each group were stratified according to the stage of embryo development: cleavage stage and blastocyst stage. Main outcome measures were implantation rate, clinical pregnancy rate, ongoing pregnancy rate and live birth rate per cycle.

Results: In the fresh embryo transfer group, for cleavage stage versus blastocyst stage, respectively, implantation rates were 22% and 47% (p = 0.0005); clinical pregnancy rates were 34% and 64% (p = 0.0057); the ongoing pregnancy rates were 30% and 61% (p = 0.0046) and live birth rates were 28% and 55% (p = 0.0148). There were no significant differences in the rates between cleavage and blastocyst stages in the frozen-thawed group, neither between fresh and frozen-thawed cleavage embryo transfers nor between fresh and frozen-thawed blastocyst transfers.

Conclusion: Our results confirm that blastocyst transfer is better than cleavage stage in fresh embryo transfer cycles. In frozen-thawed cycles, cleavage or blastocyst stages seem to offer similar reproductive outcomes.

Keywords: Embryo development, reproductive outcomes, frozen-thawed embryo transfer, blastocyst

INTRODUCTION

In Assisted Reproductive Techniques (ART), successful implantation depends not only on embryo quality, but also on endometrial receptivity (Evans et al., 2014). Traditionally, embryo transfers (ET) were performed on the cleavage stage, because the uterus was supposed to be the best environment for embryo survival (Laverge et al., 2001). On the other hand, understanding embryo metabolism and development of sequential culture media enabled embryo transfers on blastocyst stage (Gardner et al., 1998 a,b).

Theoretically, blastocysts present a higher implantation potential, since in vivo embryos reach the uterus at least on day 4 of fertilization (Gardner et al., 1996). There is also evidence that uterine contractility decreases at the time of blastocyst transfer, thus reducing the chance of embryo expulsion (Fanchin et al., 2001). Finally, after controlled ovarian stimulation, supraphysiological levels of estrogen may alter endometrial receptivity; blastocyst transfer would prevent premature exposure to an altered uterine environment (Gardner et al., 1998a,b; Valbuena et al., 2001; Fatemi & Popovic-Todorovic, 2013; Roque et al., 2015).

This is also the reason for performing frozen-thawed ET. Since endometrial development is more precisely controlled in those cycles, it would be possible to prevent an embryo-endometrium asynchrony (Roque, 2015). However, clinical pregnancy rates (CPR) and live birth rates (LBR) do not seem to differ between frozen-thawed ET and fresh ET cycles, mainly if blastocysts are transferred.

The aim of this study was to evaluate if the outcomes of IVF/ICSI in frozen-thawed embryo transfer and fresh embryo transfer cycles differ in relation to the stage of embryo development.

MATERIALS AND METHODS

This was a retrospective cohort study to compare IVF/ICSI outcomes between fresh ET and frozen-thawed ET, according to the stage of embryo development (cleavage versus blastocyst). The analysis was carried out on 443 consecutive ET cycles performed between January 1st and December 31st, 2014. The main outcomes were implantation rate, clinical pregnancy rate, ongoing pregnancy rate and live birth rate per cycle.

We studied the women aged up to 38 and submitted to ET cycles with fresh (n = 309) or frozen-thawed (n = 134) embryos at a private center for assistance in human reproduction. Results in each group were stratified according to the stage of embryo development: cleavage stage (from day 2 to day 4) and blastocyst stage (days 5 and 6). Table 1 presents patients’ characteristics per treatment group. The evaluated outcomes were biochemical pregnancy, clinical pregnancy, ongoing pregnancy and live birth rates.

The Institutional Clinical Committee approved the study. Patients gave written consent for assisted reproduction technology treatment, and an oral consent was obtained for confidential data use for research purposes. A specific written informed consent was not considered necessary for this study, since the data was obtained exclusively from patient files, respecting anonymity.

The statistical analysis was performed using GraphPad Prism software, version 5.00 (GraphPad Software, Inc., 2007). Fisher’s exact test was used to compare rates between groups. The level of significance was set at p < 0.05 in all analyses.

RESULTS

In the fresh ET group, for cleavage stage versus blastocyst stage, the implantation rates were 22% and 47% (p = 0.0005), respectively; clinical pregnancy rates were 34% and 64% (p = 0.0057), ongoing pregnancy rates
Table 1. Patients’ characteristics per treatment group.

| Outcomes                  | Cleavage | Blastocyst | p value* |
|---------------------------|----------|------------|----------|
| **Fresh ET**              |          |            |          |
| Age, years                | 33.9 ± 3.25 | 33.03 ± 2.26 | NS |
| Embryos transferred, n    | 2.04 ± 0.52 | 1.94 ± 0.35 | NS |
| **Frozen-thawed ET**      |          |            |          |
| Age, years                | 32.9 ± 3.96 | 31.64 ± 4.02 | NS |
| Embryos transferred, n    | 2.05 ± 0.49 | 1.85 ± 0.42 | 0.0486 |

The data is expressed as means ± standard deviations.

ET = embryo transfer; NS = not significant.

*Statistical analysis performed by unpaired t-test or Mann-Whitney test or Fisher’s exact test.

Table 2. Comparison of reproductive outcomes from fresh and frozen-thawed embryo transfers, according to embryo development stage.

| Outcomes                  | Cleavage (%) | Blastocyst (%) | p value a | OR       | 95% CI     |
|---------------------------|--------------|----------------|-----------|----------|------------|
| **Fresh ET**              |              |                |           |          |            |
| Implantation              | 22           | 47             | 0.0005    | 0.3209   | 0.1712-0.6018 |
| Clinical pregnancy        | 34           | 64             | 0.0057    | 0.2918   | 0.1231-0.6918 |
| Ongoing pregnancy         | 30           | 61             | 0.0046    | 0.2730   | 0.1150-0.6482 |
| Live births               | 28           | 55             | 0.0154    | 0.3268   | 0.1385-0.7712 |
| **Frozen-thawed ET**      |              |                |           |          |            |
| Implantation              | 27           | 39             | NS        | 0.5691   | 0.2991-1.083  |
| Clinical pregnancy        | 43           | 53             | NS        | 0.66     | 0.2855-1.526  |
| Ongoing pregnancy         | 36           | 45             | NS        | 0.6878   | 0.2928-1.616  |
| Live births               | 36           | 40             | NS        | 0.8187   | 0.3467-1.933  |

Outcomes presented: Implantation: the ratio of the number of fetal hearts to the number of embryos transferred; Clinical pregnancy: an ultrasound confirmed fetal heart after the 6th gestational week; Ongoing pregnancy: pregnancy continued after the 14th gestational week ET = embryo transfer; NS = not significant; OR = odds ratio; CI = confidence interval.

*Statistical analysis performed by unpaired t-test or Mann-Whitney test or Fisher’s exact test.

There were 30% and 61% ($p = 0.0046$) and live birth rates were 28% and 55% ($p = 0.0148$). There were no significant differences in rates between cleavage and blastocyst stages in the frozen-thawed ET group (Table 2).

**DISCUSSION**

Our results confirm that blastocyst transfer is better than cleavage stage ET in fresh cycles. Our findings are in agreement with both systematic reviews assessing this comparison, which favored blastocyst culture for improving live birth rates (Papanikolaou et al., 2008; Glujovsky et al., 2012). The most likely explanation is that blastocysts may be able to overcome the negative impact by controlled ovarian stimulation on endometrial receptivity (Gardner et al., 1998a,b; Valbuena et al., 2001). Besides, it seems that there is a natural selection through embryonic development, which does not allow most of the chromosomally abnormal embryos to reach higher developmental stages, and ultimately to implant (Gardner et al., 1998a,b; Papanikolaou et al., 2008).

On the other hand, in frozen-thawed ET cycles, cleavage or blastocyst stages seemed to offer similar reproductive outcomes. This was probably because the non-stimulated endometrium (or minimally stimulated) in those cycles may be highly effective in equalizing the results for embryos at any stage of development.

There are several factors that explain why supraphysiological steroid levels impair embryo implantation. First of all, controlled ovarian stimulation (COS) leads to advancement of pinopodes appearance and histological features linked to implantation, resulting in an embryo-endometrium asynchrony (Mirkin et al., 2004). There is also a progesterone receptor down-regulation in glandular and stromal cells, leading to a desynchrony, where glandular and stromal maturation do not match the same day (Valbuena et al., 2001; Mirkin et al., 2004).

Another point is the evidence that COS causes deregulation of more than 200 genes related to...
implantation, in comparison to natural cycles. The altered gene expression profile leads to an aberrant endometrium, which probably impairs the ideal intrauterine microenvironment for embryo implantation (Horcajadas et al., 2005; Labarta et al., 2011).

Moreover, elevated estrogen concentration may increase uterine contractions. This effect is more important for cleavage ET, since the contractility decreases progressively, reaching a nearly quiescent status at the time of the blastocyst transfer (Fanchin et al., 2001).

Based on the obtained data, we were not able to find significant differences in rates between fresh and frozen-thawed cleavage ET or between fresh and frozen-thawed blastocyst ET. Previous studies have demonstrated similar results for cleavage stage embryos (Bdolah et al., 2015), but conflicting results have been published regarding blastocyst transfers (Feng et al., 2012; Doherty et al., 2014; Özgür et al., 2015; Gomaa et al., 2016).

The existence of biases related to the retrospective design of our study cannot be excluded and our results must be considered with caution. Statistical analysis demonstrated a small but significant difference in the numbers of embryos transferred between frozen-thawed cleavage and blastocyst transfers; thus, implantation rates may be misleading (Griesinger, 2016). Also, our study did not explore the number or even the existence of top quality embryos at the freezing, thawing and transfer times, which are supposed to be factors that may negatively influence results. There is recent evidence that transferring one top quality embryo may be more important to improve the chance of a live birth than endometrial function in frozen-thawed cycles (Veleva et al., 2013), and it may be related to a higher PR (Salumets et al., 2006).

Besides, our study design limited exploration of other factors that may influence reproductive outcomes, such as body mass index, etiopathogenic diagnosis of infertility, primary versus secondary infertility, pregnancy in a previous fresh cycle, type of luteal support, precocious progesterone elevation.

In conclusion, our study suggests that blastocysts are the best option for fresh ET. However, in frozen-thawed ET, no significant differences in reproductive outcomes were found between cleavage and blastocyst stages.

CONFLICT OF INTERESTS

The authors declare there were no potential conflicts of interests of financial or other nature, which may be deemed to influence the objectivity of the manuscript. Also, there was no funding received for this study from any organization.

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