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Pregnancy rates from transfer of embryos cryopreserved on day 5 versus day 3 with post-thaw culture

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Abstract
Context: Debate persists around the success rates of cleavage stage versus blastocyst transfers in fresh cycles. Freeze all and embryo pooling protocols have made vitrification and thaw cycles indispensable, introducing another angle to the decision for the best stage to cryopreserve embryos.
Aims: To compare the pregnancy rates of embryos vitrified and transferred post-thaw on day 5 (FOD5) against that of embryos vitrified on day 3 and transferred post-thaw after culture as day 4 morulas or day 5 blastocysts (FOD3).
Settings and Design: Retrospective study in a private clinic.
Methods and Material: A total of 163 freeze-thaw transfer cycles in two groups. One group involved 76 cycles whose embryos were vitrified on day 3 (FOD3) and transferred either after culture to day 4 morulas or day 5 blastocysts. Another group was of 86 cycles in which blastocysts were cryopreserved on day 5 (FOD5) and transferred after a 2-hour incubation period post-thaw.
Statistical analysis used: [i] Odds ratio of success for dichotomous outcomes [ii] the Chi-squared method.
Results: 37 of the 76 FOD3 cycles (48.68%) and 41 of the 86 FOD5 cycles (47.67%) had a positive outcome.
Conclusions: No statistical difference in pregnancy rates between embryos cryopreserved at cleavage stage on day 3 followed by a minimum of 1 day of culture post-thaw versus those cryopreserved and transferred at the blastocyst stage.

Keywords: cryopreservation, freeze-thaw, blastocyst, embryo, vitrification, freeze-all

Introduction

Debate revolving around the optimal day of transfer in Assisted Reproduction Technique (ART) is ongoing, often leaving clinicians and embryologists confused about the best option for their patients. There have been claims of better pregnancy rates with blastocyst transfers compared to cleavage stage transfers and a reduction in multiple pregnancies. The temptation of taking embryos to day five *in vitro* may, however, lead to cycle cancellations. While some may consider such cancellations to be useful with the argument that only the fittest embryos survive culture, others may disagree on the grounds that the environment in the incubator does not replicate that of the uterus in entirety and would, therefore, prefer to proceed with the transfer or cryopreservation of cleavage stage embryos.

In addition, protocols such as freeze all and embryo pooling have made vitrification indispensable, increasing the importance of identifying the most promising day to freeze and to transfer.

Our goal in this study was to compare the pregnancy rates of embryos frozen, thawed and transferred on day 5 (FOD5) with those frozen on day 3 (FOD3) but transferred after being cultured for at least one day after being thawed.

Subjects and Methods

This retrospective study included a total of 159 thaw embryo transfers over a period of 7 years at a private fertility clinic. All the embryos were cultured using Vitromed's V-Onestep for continuous embryo culture from fertilization to blastocyst and for embryo transfer. Vitrification was performed using Cryotech's vitrification kit, thawing with Cryotech's warming kit, and the embryo transfers were performed with Cook's embryo transfer catheters. All patients were prepared with oral estradiol valerate starting from the second day of the period, after ruling out

the presence of any residual follicular cyst on ultrasonography. Intramuscular progesterone (100mg) was initiated once the endometrial thickness reached a minimum of 8mm and had a triple layer pattern. A transfer was subsequently performed after the exact number of days of progesterone corresponding to the age of the embryos on the day of transfer. (Fig. 1 and Fig. 2). Only patients who had at least two grade A embryos available to thaw were included in the FOD3 group. All patients were continued on estradiol valerate, progesterone and aspirin (75mg) once a day until the day of beta hCG (human chorionic gonadotropin).

A test was considered positive if the beta hCG level was equal to or above 50mIU/mL on the eleventh day post embryo transfer and doubled after 48 hours. On the other hand, a test was considered negative if the beta hCG was less than 50mIU/mL. None of the patients with beta hCG levels less than 50mIU/mL in our study had rising beta hCG levels.

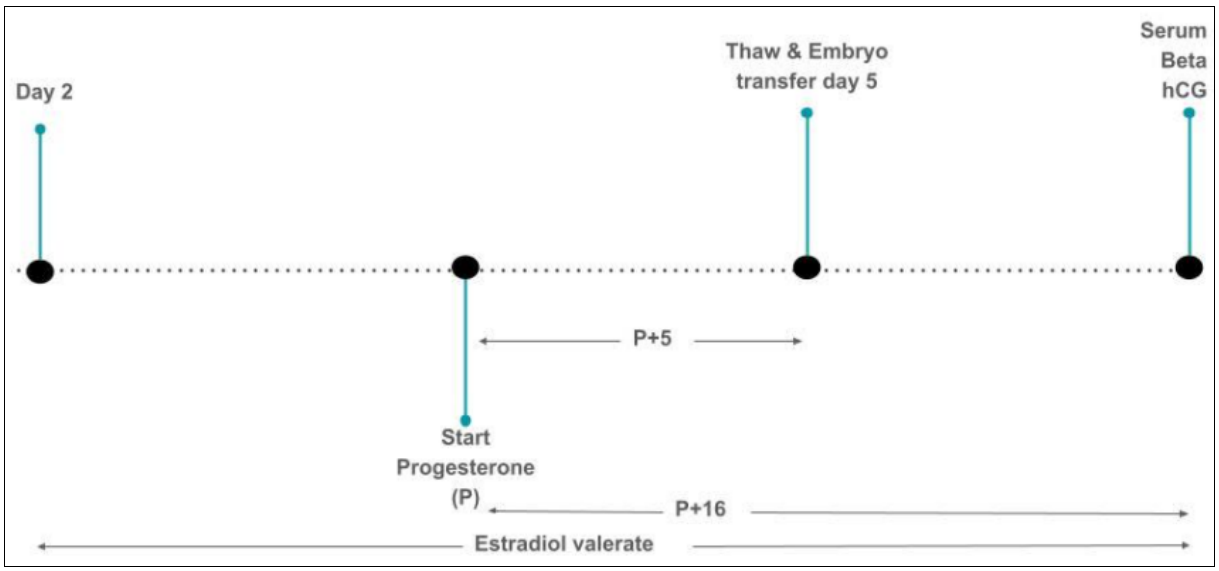

Fig 1: Time line for the FOD 3 group

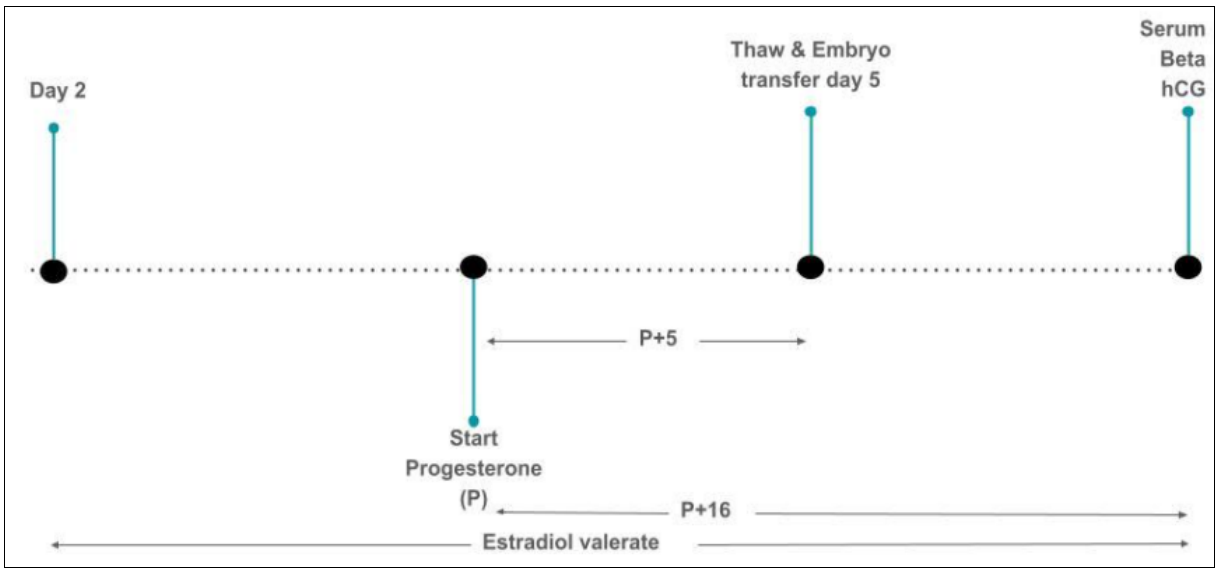

Fig 2: Time line for the FOD5 group

Results

The average age of the patients and average embryos per transfer are tabulated below (Table 1). The decision to do a day 3 freeze (FOD3) versus a day 5 freeze (FOD5) for any given cycle was with no embryological preferential bias for choosing one method over the other. There were no cycle cancellations amongst the 76 thaws from the FOD3 group in our study. For the purpose of statistical analysis, we therefore, have dichotomous outcomes (pregnancy or not) from two independent samples, FOD3 and FOD5. Thirty-seven of the 76 FOD3 cycles and 41 of the 86 FOD5 cycles had a positive outcome, yielding a pregnancy rate (PR) of 48.68% and 47.67% respectively that were not statistically significant (OR=1.0413; 95% CI 0.5615 to 1.9310; p=0.90). Similarly, the Chi-square test revealed a test statistic value of 0.0077 and p value of 0.93, further confirming no significant difference in pregnancy rates between the two groups.

Table 1: Demography of FOD3 and FOD5

	FOD3	FOD5
Average Age	33.37	32.45
SD	4.43	3.99
Median	33	32
Avg. no. of embryos transferred	1.83	1.79

Discussion

One of the perpetual questions in ART is the optimal day for embryo transfer, inviting numerous Cochrane reviews and their updates over the past two decades. The latest update suggests that clinical pregnancy rates are higher with fresh blastocyst stage transfers compared to fresh cleavage stage transfer, but concludes that more quality studies are required to determine a preference between the groups as far as cumulative pregnancy rates are concerned [1]. Our preference for the transfer of embryos beyond the cleavage stage at our center is driven by this review. Keeping this review in mind, day 5 blastocysts have an advantage over cleavage stage embryos in fresh transfers; but what about cryopreserved embryos and thaw transfers? The impact of some of the factors involved in cryopreservation on outcomes are discussed below:

Optimal stage for cryopreservation

Higher ongoing pregnancy, implantation and survival rates post-thaw have been demonstrated with the cryopreservation and transfer of blastocysts as compared with cleaved embryos by Anderson *et al* [2]. However, unlike our study in which we cultured the cleavage stage embryos for a minimum of one day post-thaw, this particular study transferred them on the same day. It is possible that the culture of cleavage stage embryos post-thaw may play a role in raising the pregnancy rates to that from the transfer of thawed blastocysts, but this is at the risk of failure of growth and cycle cancellation. In our study we had no cycle cancellations in the FOD3 group, probably due to our patient selection criteria of having at least two grade a embryos to thaw.

Going beyond pregnancy and implantation rates, a systematic review of perinatal outcome of singleton pregnancies with respect to the stage of cryopreservation was presented by Alviggi *et al* [3]. They revealed a similar risk of preterm and very preterm birth after blastocyst and cleavage stage transfers in frozen cycles. On the other hand, blastocyst transfers resulted in a higher incidence of large for gestational age (LGA) babies, and possibly an increase in perinatal mortality as indicated by only one of the studies in their systematic review, when compared with cleavage stage transfers in frozen cycles.

In another study about perinatal outcomes, Wang *et al* [4] compared relative likelihoods of having a 'healthy baby' (a single baby born live at term, weighing > or = 2500g, surviving for at least 28 days postpartum and not having congenital anomalies) for different stages of cryopreservation. In their study involving 150,376 cycles, they concluded that the likelihood was descending in the following order: transfer of fresh blastocysts > blastocysts from thawed cleavage embryos > fresh cleavage embryos > thawed blastocysts > thawed cleavage embryos. Being a referral unit for Obstetricians and Gynaecologists, with no perinatal outcome follow up, we are unable to comment on the perinatal outcomes of the patients of our study.

In addition to cleavage stage embryos and blastocysts, vitrification of embryos at the bipronuclear stage has also been performed in various parts of the world. Shapiro *et al* [5] compared outcomes for patients randomized to have all embryos cryopreserved at the blastocyst stage versus those that were frozen in the bipronuclear stage with subsequent post-thaw culture to blastocysts. They inferred that there were no differences in the implantation rate, ongoing pregnancy rate per thaw and ongoing pregnancy rate per transfer, and concluded that cryopreservation at the blastocyst stage and at the bipronuclear stage have similar results and that the choice between them was based on logistical factors.

Our contribution to the literature has been two-fold. We add to the body of empirical evidence in the cleavage versus blastocyst stage cryopreservation debate. We also present evidence for comparing blastocyst preservation with cleavage stage where the latter is cultured for a period after the thaw.

Post thaw recovery

Self-evidently, post-thaw recovery of structure and function of cryopreserved embryos is critical for success. Various studies have suggested a decline in pregnancy and live birth rates with an increase in the percentage of blastomere loss on thawing of embryos [6, 7]. One of the disadvantages of cryopreserving the highly cellular blastocyst is the inability to accurately determine the number of blastomeres lost on thaw. In addition to structural loss, in our experience, it is difficult to assess the functional capability of blastocysts as with cleavage stage embryos in which mitosis can be demonstrated by extending their culture post-thaw. Keeping the comparable pregnancy rates per transfer in mind, day 3 post-thaw culture provides the opportunity to prevent the transfer of those embryos that do not resume mitosis. Even though the resulting cycle cancellation is disappointing for the couple and the physician, we feel that it saves the patient from administering two weeks of unnecessary luteal support.

Post-thaw interval

Regarding the post-thaw duration of the culture of cleavage stage embryos, Wang *et al* [8] demonstrated no benefit of an overnight culture of 'optimal' day 3 embryos (embryos with 7 to 8 equally sized mononucleated blastomeres and <10% fragmentation) compared to a 2-hour wait before transfer. However, cycles with an overnight culture of their 'suboptimal' embryos had a higher incidence of cancellation than those that had at least one 'optimal' embryo. Whether these cancellations may have avoided unnecessary transfers is controversial.

In contrast, Rato *et al* [9] established an inverse relationship between the implantation and live birth rate per embryo transferred with the duration of the post-thaw culture. Their study concluded better outcomes with short (2–5 h) culture as compared with long (18–24 h) post-thaw culture of cleavage stage embryos.

At our center, we prefer to culture all embryos for at least 24 hours to demonstrate mitosis before transfer after counselling the couple regarding the possibility of failure to transfer in the event of lack of growth on culture. In our study, only one cycle was cancelled from the FOD3 group due to failure of further cleavage.

Cumulative live birth rate (CLBR)

With an increase in the utilisation of the freeze-all strategy for OHSS-free (Ovarian Hyperstimulation Syndrome) clinics, CLBR - even though difficult to calculate - has emerged as an important determinant to decision making in ART [10]. One of the few studies on CLBR that compared results of fresh versus freeze-all cycles concluded that the outcomes were similar in both groups when cleavage stage embryos were cryopreserved and transferred. However, the CLBR was significantly higher in the freeze-all group when embryos were cryopreserved and transferred at the blastocyst stage. This indicates that cryopreservation followed by thaw transfer at the blastocyst stage may be a more effective approach in achieving parenthood in the long run for patients undergoing the freeze-all protocol. Nevertheless, more studies in this area are required to support this inference.

Conclusion

The decision regarding cryopreservation and subsequent embryo transfer for a couple depends on multiple variables - clinical, embryological and logistical. Of these, logistical reasons are often considered 'not as important' as the former two. However, if there was a way of making decisions on the basis of logistical reasons without compromising the outcome purely based on clinical and embryological factors, it would ease the 'guilt' of the physician of deciding on the basis of logistics. We believe that our results allow us to advise clinicians that they relax the cryopreservation date constraint by having flexibility between cleavage stage or blastocyst stage. The slack generated by this flexible approach can then better accommodate the preparation of the patient's endometrium for implantation. It would also allow the clinician to accommodate other practical factors that might include the quality and number of the embryos available at the cleavage stage, facilities available in the laboratory, expertise of the embryologist and willingness of the patient to accept cycle cancellation.

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