Post-hatching versus pre-hatching blastocyst biopsy for preimplantation genetic testing (PGT) is unexpectedly associated with reduced implantation

Said T Daneshmand, MD[1], Kevin S Richter, PhD[2], Valerie A Flores, MD[1], Shannon Kokjohn, MS[1]

[1] **San Diego Fertility Center**, San Diego, California, USA, and [2] **Fertility Science Consulting**, Silver Spring, Maryland, USA

Background

Single blastocyst transfer only recently became the norm for *in vitro* fertilization (IVF) treatment performed in the United States. Before 2016, most IVF transfers were of multiple embryos. However, there has been steady progression toward fewer embryos per transfer over time. By 2020, for the first time, multiple embryos were transferred in fewer than 20% of all reported US IVF cycles. Unlike multiple embryo transfer, single embryo transfer allows for complete and unambiguous data regarding outcomes of every transferred embryo, from initial uterine implantation (confirmed by positive maternal serum hCG test) to live birth and beyond. The newly standard practice of single embryo transfer thus provides unprecedented ability to reevaluate factors associated with successful embryo implantation.

Objective

To evaluate embryo implantation rates according to oocyte source (patient or donor), blastocyst morphology, and cryosurvival among transfers of single vitrified blastocysts confirmed as euploid by preimplantation genetic testing (PGT).

Materials & Methods

All transfers of single vitrified blastocyst-stage embryos, previously diagnosed as chromosomally normal (euploid) by next-generation sequencing (NGS) PGT, performed at a single private fertility center during 2021 through 2023 were reviewed. Analysis was limited to embryos biopsied on Day-6 after oocyte retrieval, which constituted the bulk of transfers (76%), to eliminate any variability in outcome associated with blastulation day. Blastocyst morphology was graded by experienced embryologists according to Gardner's standard three-component scoring system. Survival following vitrification and warming was subjectively graded as excellent, good, fair, or poor. The primary treatment outcome was clinical pregnancy, defined as ultrasound confirmation of an intrauterine gestational sac with heartbeat. Treatment outcome was evaluated according to potential predictive factors using logistic regression analysis. Error bars in illustrative figures indicate 95% binomial confidence intervals.

Results

Mean patient age at oocyte retrieval was nearly identical (35.2 versus 35.2 years, p=.84, student's t test) between pregnant (n=595) and non-pregnant (n=533) patients using their own oocytes. Clinical pregnancy rates per transfer also did not differ significantly between embryos derived from donor oocytes versus patients' own oocytes (n=1371 and 1128, 55.7% versus 52.7%, p=.14, χ^2). These groups were therefore pooled in subsequent analyses of embryo morphology and cryosurvival.

Complete morphological grades of transferred blastocysts were available for 2491 of these 2499 embryo transfer cycles (99.7%). Most blastocysts (79.5%) underwent trophectoderm biopsy for PGT at stages 3 or 4 (expanding/expanded), while the remaining 20.5% were biopsied at stages 5 or 6 (hatching/hatched). The inner cell mass and trophectoderm were more often graded A (58% and 57%) versus B (42% and 43%). Multiple logistic regression analysis indicated a clear but modest association between these three components of blastocyst morphology and clinical pregnancy outcomes (model p<.001, McFadden's R^2=.01, area under the ROC curve =.546). Developmental stage (expanding/expanded versus hatching/hatched) was most predictive of pregnancy (β=.33, p=.001) followed by trophectoderm grade (β=.18, p=.033); inner cell mass grade was not significantly associated with pregnancy after accounting for developmental stage and trophectoderm grade in this multivariable analysis (β=.12, p=.16) (Figure 1). Figure 2 illustrates the additive combined effects of developmental stage and trophectoderm quality on pregnancy outcomes.

Cryosurvival of most (88%) of the transferred blastocysts was graded as excellent. Among the remaining embryos, pregnancy dropped substantially with declining cryosurvival (57% for excellent, 40% for good, 24% for fair, and 0% for poor cryosurvival, respectively, p<.0001).

Figure 1

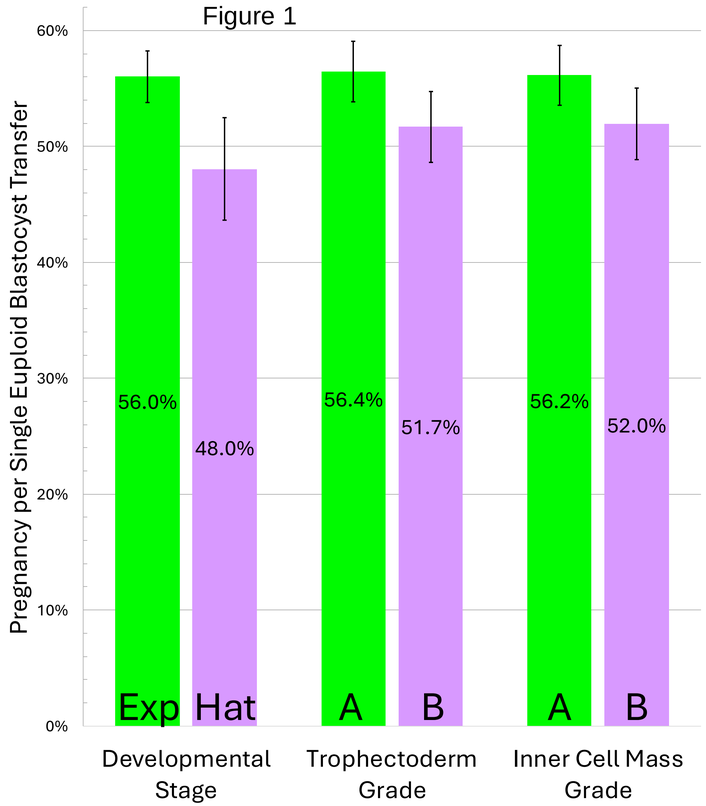
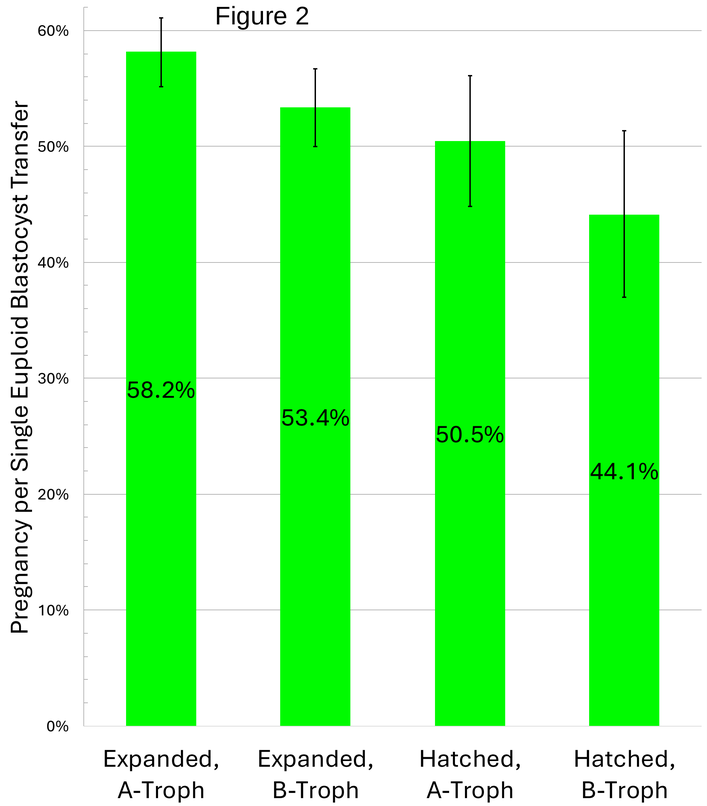


Figure 2



Conclusions

This analysis, restricted to single euploid blastocyst transfers, confirms that standard inner cell mass and trophectoderm grades have similar predictive value as reported for transfers of non-biopsied embryos [1,2]. Our results suggest that trophectoderm grade is more predictive of outcome than inner cell mass grade.

Unexpectedly, we also found that pregnancy rates were significantly lower (48% versus 56%, relative decrease = 16%) among spontaneously hatching or hatched blastocysts compared to blastocysts biopsied and cryopreserved prior to hatching. These results are in direct opposition to studies of non-biopsied blastocysts, whether transferred fresh [3] or following cryopreservation [4], which report higher success rates with transfer of hatching or hatched blastocysts. These unanticipated results suggest that performing trophectoderm biopsy for PGT after rather than before spontaneous blastocyst hatching has begun may be substantially more damaging to the embryo and may significantly reduce its viability and implantation potential. Therefore, forgoing planned biopsy after natural hatching has begun may be warranted.

References

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SAN DIEGO Fertility Center