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**Title:**

THE DURATION OF STORAGE OF BIOPSIED EMBRYOS NEITHER IMPACTS  
IMPLANTATION POTENTIAL NOR SURROGATE MARKERS OF PLACENTATION

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**Objective:**

Blastocyst vitrification facilitates embryo banking for successive single embryo transfers (SET) and has become an integral part of IVF treatment. Although storage at  $-196^{\circ}\text{C}$  is thought to halt metabolism, the molecular structure of vitrified cells might be dynamic and sensitive to storage temperature variations and impacted by long-term cryostorage. Previous studies of the effect of cryostorage duration on clinical outcome focused on embryos slow-frozen at the zygote or cleavage stage; and no study to date has assessed whether blastocysts which have undergone trophectoderm biopsy are vulnerable to impaired implantation or placentation as a result of long-term cryostorage. This study examined whether the duration of cryostorage of euploid blastocysts adversely impacts reproductive potential or perinatal outcome.

**Design:**

Retrospective, observational study

**Materials and Methods:**

The study included patients that underwent autologous IVF and aneuploidy screening of blastocysts by preimplantation genetic testing (PGT), followed by subsequent vitrification, warming and SET, from July 2011 to November 2016. Donor oocyte cycles were excluded. Cryostorage duration was calculated as the interval (days) elapsed from when the blastocyst was



vitrified to when it was warmed and transferred. Multivariate logistic and linear regression analyses were performed. Main outcomes included implantation, clinical pregnancy, live birth and early pregnancy loss. Secondary outcomes included infant birthweight and gestational age at delivery.

### **Results:**

Vitrified-warmed, euploid blastocysts, derived from autologous IVF, were transferred in 2,320 SET cycles. The average duration of cryostorage was 4 months, with a maximum duration of up to 4.9 years (mean  $126.4 \pm 197.3$  days; range 21-1794 days). Controlling for patient age at IVF and embryo transfer, BMI, endometrial thickness and day of embryo biopsy, the duration of cryostorage did not impact the odds of implantation (OR 1.0 [95% CI 0.99-1.01]), clinical pregnancy (OR 1.0 [95% CI 0.998-1.004]), live birth (OR 0.998 [95% CI 0.99-1.003]), biochemical pregnancy loss (OR 0.998 [95% CI 0.99-1.002]), and clinical pregnancy loss (OR 1.01 [95% CI 0.98-1.05]). The duration of cryostorage did not significantly impact birthweight ( $\beta = -0.01$ ,  $p=0.96$ ) or gestational age at delivery ( $\beta = -0.22$ ,  $p=0.51$ ).

### **Conclusion:**

Modern advances in cryopreservation and PGT have facilitated the opportunity for patients to bank embryos with confidence. This emerging treatment strategy will result in the increased storage of biopsied embryos for longer durations. This study is the first to demonstrate that long-term cryostorage of vitrified, biopsied blastocysts does not adversely affect reproductive potential, fetal growth or the likelihood of preterm delivery. Based on these findings, patients can be reassured regarding the efficacy, efficiency and safety of embryo banking.