COMPARABLE LIVE BIRTH AND CLINICAL OUTCOMES FROM BLASTOCYSTS VITRIFIED WITH A SHORTENED 3-MINUTE PROTOCOL VERSUS A STANDARD METHOD

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Background:

The vitrification of blastocysts is one of the most critical steps in in-vitro fertilization (IVF), ensuring optimal embryo survival and viability throughout cryopreservation and warming. The standard vitrification (SV) protocol involves 6-10 minutes in equilibration solution (ES) followed by 30-60 seconds in vitrification solution (VS). A recent study introduced a shortened 3-minute vitrification (3MV) protocol, which reduces the ES exposure to 2 minutes while maintaining the same VS exposure, with the goal of streamlining laboratory processes without compromising embryo viability or pregnancy outcomes (Kaskar et al., 2023). While the initial results demonstrated the success of the 3MV protocol in terms of blastocyst survival - pregnancy rates, live birth outcomes were not evaluated. Our study builds upon these findings by comparing live birth and clinical outcomes between the 3MV protocol and the standard method, providing a comprehensive assessment of this new approach.

Objective:

To compare the live birth rate following frozen embryo transfer (FET) of blastocysts vitrified with a 3-minute vitrification (3MV) protocol compared to a standard vitrification (SV) protocol.

Materials and Methods:

Design: Retrospective cohort
Setting: Academic fertility center

Subjects:

- FET cycles started between January 2022 to December 2023
- 3MV Group: 138 FETs (160 embryos transferred, avg 1.2 embryos per transfer)
- SV Group: 1015 FETs (1176 embryos transferred, avg 1.2 embryos per transfer)

Protocol Overview:

- Standard Vitrification (SV): 6-10 minutes in ES + 30-60 seconds in VS
- Shortened 3MV Protocol: 2-10 minutes in ES + 30-60 seconds in VS

Procedure: All blastocysts were collapsed using a laser immediately before being placed into equilibration medium. Both groups used identical rapid warming processes, with blastocysts

warmed for 1 minute in thaw solution (TS) at 37°C, followed by incubation and transfer after brief exposure to culture medium.

Outcomes and Statistical Analysis:

The primary outcome was live birth rate. Secondary outcomes included positive β-HCG rates, biochemical pregnancy, ectopic pregnancy, clinical pregnancy, and miscarriage rates. Parametric and non-parametric tests were performed, with linear and logistic regression models controlling for PGT-A status, day of transfer, embryo number, endometrial preparation, oocyte age, and infertility diagnosis.

Results:

Outcome	3MV Group (138 FETs)	SV Group (1015 FETs)	p-value
Live Birth Rate	42.8%	42.4%	0.931
Positive β-HCG Rate	59.4%	58.4%	0.824
Biochemical Pregnancy Rate	9.4%	8%	0.562
Clinical Pregnancy Rate	48.6%	50.2%	0.709

Blastocyst Survival:

- Both groups showed a **100% survival rate** post-thaw.

After adjustment for confounding variables, no statistically significant differences were found between the protocols for primary or secondary outcomes.

Conclusions:

The shortened 3MV protocol for vitrified and warmed blastocyst-stage embryos is as effective as the SV protocol, demonstrating no negative impact on live birth or secondary outcomes. This protocol's flexibility—allowing embryos to remain in ES for up to 10 minutes—enables embryologists to efficiently stagger vitrification of multiple embryos, streamlining operations in busy IVF laboratories.

Adopting the shortened 3MV protocol can enhance IVF lab efficiency without compromising clinical outcomes. This approach offers a scalable solution for meeting the rising demand for reproductive services while ensuring high-quality patient care.

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References:

1. Kaskar, K., Sieren, K., Brabner, V., Huneke, B., VerMilyea, L., VerMilyea, T., & Silverberg, K. (2023). Effect of reducing the vitrification and warming times on blastocyst survival and pregnancy rates. *Fertility and Sterility, 120*(4), e143. https://doi.org/10.1016/j.fertnstert.2023.07.765