## Vitrification Of Blastocysts In CBS Straws: Comparison Of Two Methods

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**Objective:** To decide if a difference exists between two methods of blastocyst vitrification in CBS Straws.

**Design:** A retrospective study comparing viability, implantation and ongoing pregnancy rates of contrasting vitrification methods. Data from 82 FET cycles (69 patients) performed between September 24, 2009, and February 22, 2011, were analyzed.

**Materials and Methods:** Embryos were cultured in Sage media in 5% $O_2$. Blastocysts of freezable quality were vitrified on Day 5 or Day 6. Freezable quality is defined as a blastocyst with an expanded blastocoel cavity, a well organized and compacted ICM, and a trophectoderm comprised of a medium to large number of cohesive cells. All blastocysts were vitrified and thawed using $S^3$ Vitrification and Thaw media with equivalent exposure times for both procedures.

Direct Straw Method: A single blastocyst was pulled directly into a 0.3mL CBS High Security Embryo Straw (CBS straw) in 200uL medium followed by an air pocket. The straw was immediately heat sealed on both ends and placed in vapors in a Planar Controlled Rate Freezer at -100ºC for 5 minutes before plunging into $LN_2$. To thaw blastocysts, the straw was removed from $LN_2$ and exposed to room temperature air for ten seconds followed by ten seconds in a 30ºC water bath. The blastocyst was then expelled into a 400uL pool of the first thaw dilution and allowed to acclimate for 30 seconds before being transferred to a fresh drop of the same medium. The blastocyst was then moved through subsequent dilutions.

MicroSecure Vitrification (µS-VTF; Schiewe): Medium containing the blastocyst was drawn into a shortened Stripper Tip. The Stripper Tip was then immediately placed into the CBS straw, heat sealed at both ends and plunged directly into $LN_2$. To thaw these blastocysts, the CBS straw was removed from $LN_2$, the end immediately cut and the Stripper Tip dropped into a 60mm culture dish containing 13mL of 37ºC 1M Sucrose/HTF-HEPES so that the end containing the blastocyst was submerged. The blastocyst was then expelled into the first thaw dilution and moved through all remaining dilutions.

**Statistics:**
Poisson Distribution: Viability and Implantation Rate
Logistic Regression: Ongoing Pregnancy rate

**Results:**

| Method | Viability | Implantation Rate (Ongoing Heartbeats/# Blastocysts Transferred) | Ongoing Pregnancy/Transfer |
|---|---|---|---|
| Direct Straw Method | 56.3% (80/142)* | 32.9% (27/82) | 42.8% (21/49) |
| µS-VTF | 91.7% (44/48)* | 36.4% (16/44) | 50.0% (12/24) |

*P=<0.001

- The viability of blastocysts vitrified by the µS-VTF method is significantly higher than the viability of blastocysts vitrified by the Direct Straw Method.
- For blastocysts that survive the thaw, the implantation rate and ongoing pregnancy rate are the same for the two vitrification methods.

**Conclusion:**
The µS-VTF vitrification method results in better outcomes than the Direct Straw Method because of the significant improvement of blastocyst viability.

**References**:
Stachecki JJ, Cohen J. $S^3$ vitrification system: A novel approach to Blastocyst Freezing.The Journal of Clinical Embryology 2008; 11:5-14.
Schiewe M. MicroSecure Vitrification (µS-VTF) Procedure: Optimum simplicity, security, cost-savings & effectiveness combining FDA-approved products. The Journal of Clinical Embryology 2010; 13:33-51.

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## What Is The Perceived "Gold Standard" For Sperm Counting Chambers? A Survey

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**Objective:** In the andrology field there is a great demand for accurate tools to help men that have fertility problems. Semen analysis has been a crucial tool for evaluation of the male partner in many situations which looks at the quantity of quality of the sperm. These situations include: post-vasectomy analysis, infertility evaluations, as well as sperm banking. The method(s) of semen analysis

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