

# Objectives

MicroSecure vitrification ( $\mu$ S-VTF) evolved as a non-commercial system integrating FDA-approved devices: sterile flexipettes (300 $\mu$ m ID; Cook Medical) into CBS™ embryo straws. The mean cooling rate (1391°C/min) and warming rate (6233°C/min) of this closed, aseptic VTF system were verified by Dr. Greg Fahy (Schiewe et al., 2015). In 2014, CBS removed traditional hydrophobic plugged embryo straws from the worldwide marketplace, being replaced by 0.3ml semen straws. In turn, a modification of our  $\mu$ S-VTF procedure (Schiewe et al., 2017) was necessary to prevent wicking of flexipette contents. The goal of this study was to clinically validate the overall performance of our  $\mu$ S-VTF technique for human blastocysts over a four-year duration.

## Design

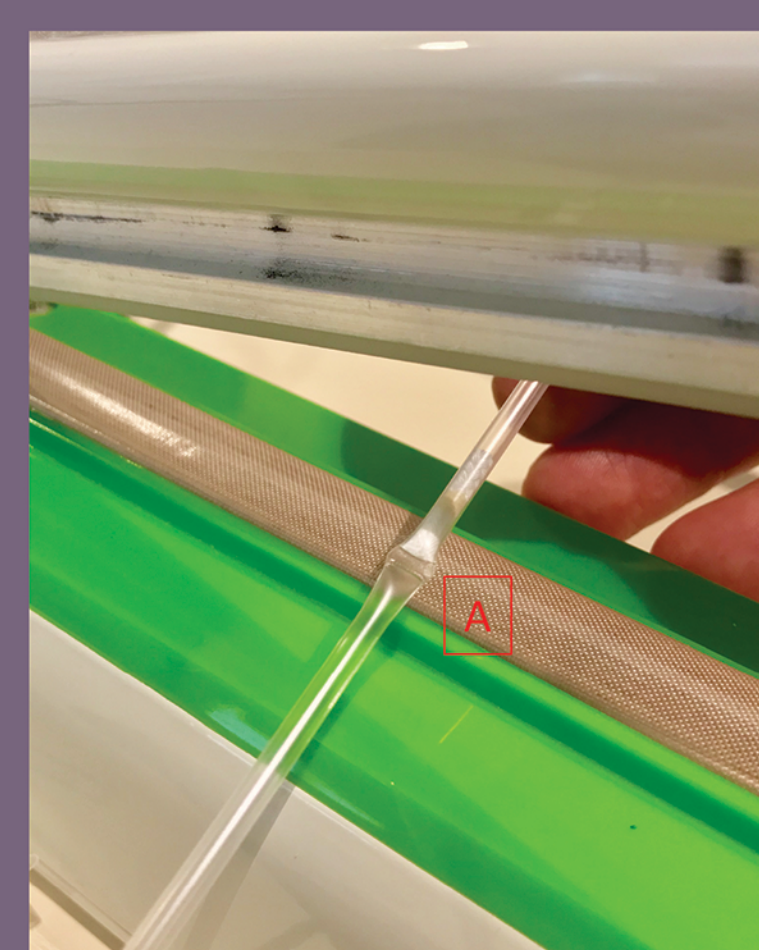
Retrospective analysis of  $\mu$ S-VTF application with an a priori arrangement of FET cycle years (2015-2018) and the number of euploid embryos transferred (1 or 2) were contrasted by Chi-square analysis ( $p < 0.05$ )



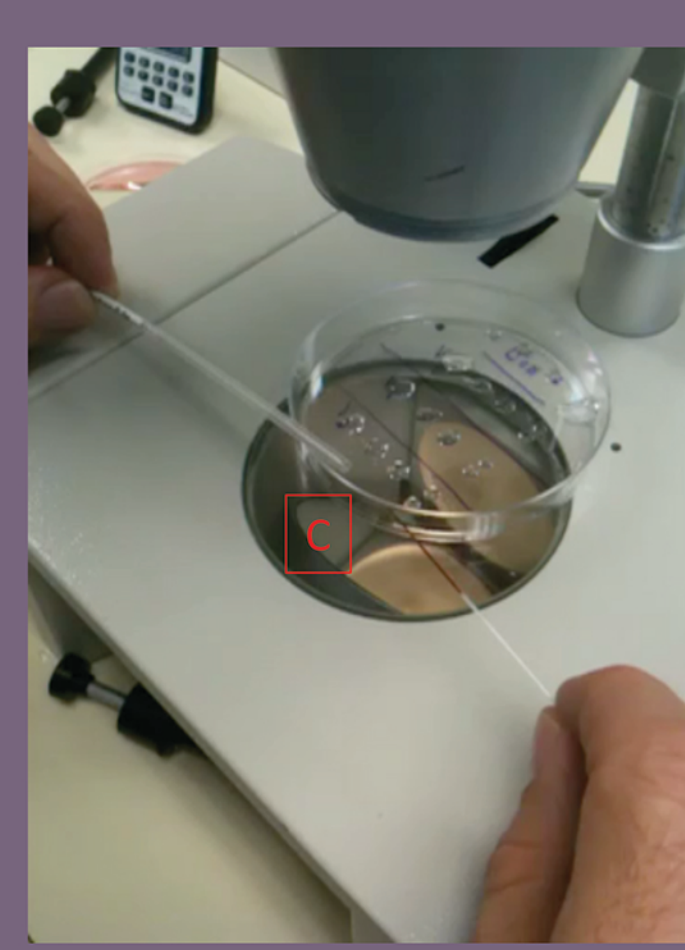
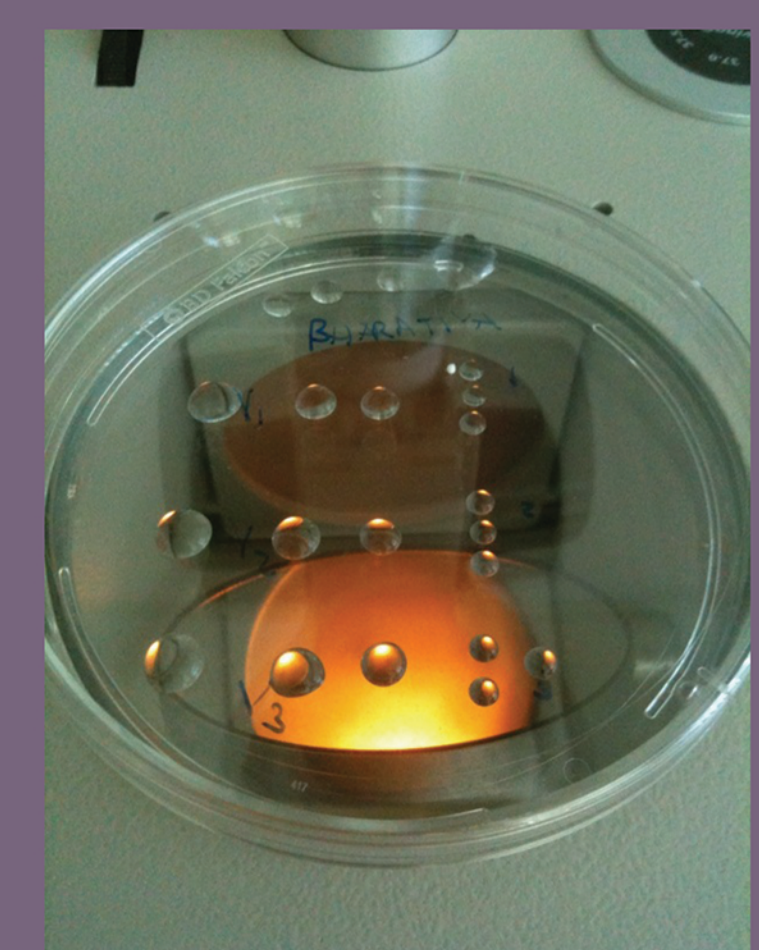
## Modified microSecure VTF procedures:

See Video Link:

<https://www.jove.com/video/54871>



To prevent the wicking potential of the cotton-PVA plug in semen straws, we have added an inner weld seal (A) to our device system, as well as the weld seal on the end which secures our colorized label and weighted rod (B). The VTF tip is then inserted and sealed closed (C).



# Efficacy of a Modified microSecure Vitrification ( $\mu$ S-VTF) Procedure With DMSO-free Solutions Applied to a Dedicated Blastocyst Biopsy/Vitrification-all/PGT-A Program: Optimizing Single Healthy Term Live Births



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# Methods

The  $\mu$ S technique modification of semen straws involved creating a mid-straw weld seal in front of the inner PVA plug, thus eliminating any contact with the fluid/embryo-filled flexipette tip once loaded prior to sealing the ends and LN<sub>2</sub> immersion. Human blastocysts (>2CC grade), subject to trophectoderm biopsy on Day 5 to 7, were vitrified by  $\mu$ S-VTF using a glycerol/EG-based solution ( $\geq 7.9$ M; Innovative CryoEnterprises, NJ). Straw-enclosed, individual embryos contained in an open flexipette (3 $\mu$ l volume) were warmed in a 37°C 0.5M Sucrose media bath (60 $\mu$ m dish, >15ml).

# Results

OVATION FERTILITY	2015	2016	2017	2018	Total	Mean Values
# IVF cycles	540	558	597	594	2289	
# Oocytes	7914	8604	8703	8238	33459	14.4/cycle
# Mature Eggs	6503	7039	7045	6667	27254	81.50%
# 2PN (%)	{82.2%}	{81.8%}	{80.9%}	{80.9%}	{81.5%}	
	4971	5513	5454	5117	21055	77.30%
	{76.4%}	{78.3%}	{77.4%}	{76.8%}	{77.3%}	
# BL ET	4	3	3	1	11	0.50%
# VTF cycles	533	509	555	542	2139	94.17% cryo
# Vitrified BL	2975	3284	3249	3055	12563	5.7 BL/cycle
						(59.7% BL rate)
# VFET cycles		587	662	615	1864	88% PGT-A
# PGD cycles	444	533	483	508	1968	
# BL Warm	612	656	720	672	2660	
# BL Survived	611	651	713	665	2640	99.00%
	{99.8%}	{99.2%}	{99.0%}	{99%}		
# BL ET	518	650	711	659	2538	
# SET	436	526	594	574	2130	
# + Pregnancy	334	410	470	451	1665	
# CPR (%)	309	359	416	436	1520	
	{70.9%}	{68.3%}	{70.0%}	{76%}		
# Live Births (%)	289	331	368	407	1395	65.50%
	{66.3%}	{62.9%}	{62.0%}	{70.9%}		
# Implanted (%)	293	351	419	437	1500	70.40%
	{67.2%}	{66.6%}	{70.5%}	{76.1%}		
# MZ Twins (%)	1	3	4	5	13	0.61%
# SABs (%)	20	28	48	29	125	7.50%
	{6%}	{6.8%}	{10.2%}	{6.4%}		
# Multiple ET	81	61	54	41	237	
# BL transferred	168	127	117	85	497	
Mean # Embryos/ET	2.1	2	2.2	2.1	8.4	2.1 BL/VFET
# + Pregnancy	69	47	45	35	196	
# CPR (%)	59	46	39	32	176	
	{72.8%}	{75.4%}	{72.2%}	{78%}		
# Live Births (%)	53	41	34	31	159	67.3%
	{65.4%}	{67.2%}	{63.0%}	{75.6%}		
# Implanted (%)	81	72	59	48	260	52.3%
	{48.2%}	{58.1%}	{50.4%}	{56.5%}		
# Multiple Births (%)	23	25	16	12	76	38.80%
	{33.3%}	{53.2%}	{35.50%}	{34.30%}		
# SABs (%)	6	5	5	1	17	8.70%
	{8.7%}	{10.6%}	{11.1%}	{2.9%}		

# Conclusions

- Our modified  $\mu$ S-VTF procedure continues to be a safe, secure, simple and inexpensive technique, which has proved to be highly effective.
- Vitrified ET (VFET) cycles have become our clinical norm based on the validation of a reliable VTF method that has eliminated procedural variability. Over 99% complete survival is attained regularly.
- The commercially forced modification made has added an additional preparation step requiring careful quality control checks and balances.