

## Sodium Hyaluronate Supports High Survival and Clinical Pregnancy Rates of Blastocysts using a microSecure, DMSO-free Vitrification System

### Background

The success of blastocyst vitrification (BL-VTF) has led to an increase in SET and reduction of multiples. Hyaluronate (HA) is a known extracellular adhesion molecule that stabilizes membranes and promotes growth, proliferation, and embryonic development. HA acts to increase solution viscosity and promote vitrification. In 2012, we prospectively vitrified Day 6 PGS-BLs with (+) or without (-) HA and determined there was no difference ( $P \geq 0.2$ ) in implantation or clinical pregnancy rates (+HA: 94.1%; -HA: 84.6%).

### Objective

In 2013, we prospectively applied HA to all BL-VTF, and evaluated its effectiveness on cellular integrity and embryonic viability in PGS and non-PGS cycles.

### Materials and Methods

All BLs were vitrified in HA-enriched I.C.E. VTF media. Aseptic microSecure VTF was used (flexipettes sealed inside 0.3ml CBS embryo straws). Warming cycles were conducted 1-3 hr prior to ET, using a gradual 5-step elution. BL were cultured in Life Global medium + 7.5% LGPS and 1% HA under Ovoil in MCO-5M tri-gas incubators. Chi-squared analysis was performed to determine differences ( $P < 0.05$ ) in survival, implantation, and pregnancy outcomes.

### Results

Table 1: VFET Clinical results for BLs vitrified in HA-enriched I.C.E. vitrification media.

Group	Age	Survival	Patients	Implantation	Clin. Preg Rate
NPGS	<38	78/80 (97.5%)	41	54.5% <sup>b</sup>	73.2% <sup>b</sup>
	38-42	19/20 (95%)	10	30.4% <sup>c</sup>	60.0% <sup>c</sup>
w/PGS	<38	67/67 (100%)	55	79.1% <sup>a</sup>	85.5% <sup>a</sup>
	38-42	36/36 (100%)	27	85.7% <sup>a</sup>	88.9% <sup>a</sup>
Fresh	Donor	(2010-11) N/A	48	76.0% <sup>a</sup>	93.5% <sup>a</sup>
	<38	N/A	92	88/175 (50.3%) <sup>b</sup>	64.1% <sup>bc</sup>
	38-42	N/A	61	25/165 (12.7%) <sup>d</sup>	32.8% <sup>d</sup>

HA supplemented VTF media supported high rates of survival and pregnancy across all groups.

Implantation and pregnancy outcomes were significantly higher in the PGS groups compared to non-PGS, but similar to our fresh 2010-11 Donor Egg ET rates, which are among the highest in the country (2011 SART). Furthermore, there has been no difference between the fresh ET and VFET-nonPGS groups for patients <38 y.o. (64.1% vs. 73.2%). However, VFET pregnancy outcomes were higher than the fresh ET group for patients 38-42. VFET-PGS success is independent of age when one or two euploid BLs are transferred.

### Conclusion

The addition of a large macromolecule like HA can offer dual support by increasing both the viscosity of the VTF solution and providing for cellular adhesion/membrane stabilization. Using I.C.E. media and  $\mu$ S-VTF we could optimize pregnancy success, with BLs maintaining full developmental competence. The data show that BLs vitrified in HA enriched media are equally effective, if not improved, in producing pregnancy outcomes comparable to fresh BLs. In fact, older women (38-42 yo) appear to benefit from VFET involving an unstimulated uterus, thus supporting the trend toward VFET only cycles.

### Support

None.