THE LO2 TRIAL, PHASE I: A PAIRED RANDOMIZED CONTROLLED TRIAL (RCT) COMPARING BLASTULATION RATE IN ULTRA-LOW (2%) VS. LOW (5%) OXYGEN IN EXTENDED CULTURE (EC)

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OBJECTIVE: In mammals, the oxygen (O_2) tension in the uterus is lower (2%) than the oviduct (5-7%). This fits with a shift in the metabolic strategy of the embryo after compaction and may have implications for EC in clinical IVF. Indeed, in a recent study utilizing discarded human embryos, blastulation rates were superior when cultured in 2% compared to 5% after day (d) 3. However, it is exceedingly difficult to control for the myriad factors impacting blastulation when employing discarded material. Thus, this study sought to gain further insight into the impact of ultra-low O_2 tension using a prospective, split cohort design with human embryos destined for clinical use. This provides the most rigorous control for patient specific variables.

DESIGN: Paired RCT

MATERIALS AND METHODS: Management prior to d3 was unchanged from standard practice, including culture at 5% O_2. On d3 changeover, at lower power magnification, half of all patients' embryos were randomized to EC in 2% O_2 and half at 5%. Standard blastocyst (blast) morphology evaluation was made on d5 and 6. Embryologists were blinded to randomization arm for each embryo. The primary outcome was the number (#) of clinically usable blasts (UB) per randomized embryo ($\geq$4CC by modified Gardner criteria). Secondary outcomes were overall blastulation rate, good quality blast rate (SART criteria), and ploidy status. Wilcoxon-signed rank and chi-squared tests were utilized. The sample size was calculated with an anticipated 15% difference in UB rate (α=0.05, β=0.20). Mitochondrial DNA copy number (MtDNA CN) analysis was also compared between the conditions.

RESULTS: 670 d3 embryos from 57 patients were included. The conversion rates to UB in 2% and 5% were 59% (196/332) and 54.4% (184/338), respectively (p=0.26). Conversion rate to any blast was higher in 2% (74% [243/332] v. 66% [224/338], p<0.05). On paired analysis, there was no difference in conversion to UB between the groups. However, more patients had a greater # of embryos blastulate in 2% than 5% (Table). Aneuploidy and morphology data were no different. Within patient cohorts, MtDNA CN clustered according to O_2 conditions.

CONCLUSIONS: Reduction in O_2 tension to 2% after d3 did not significantly improve the # of UBs available per patient. Blast quality and ploidy status were also equivalent. However, fewer embryos in the 2% group arrested at the cleavage or morula stage, resulting in a higher overall blastulation rate. Furthermore, differences in MtDNA CN suggest possible changes in metabolic fitness. Clinical outcomes remain to be elucidated.

Paired comparison of 57 patients whose embryos were split between 2 and 5% oxygen after day 3				
	Superior in 2%	Superior in 5%	Equivalent	p-value
Overall Blastulation	30	17	10	0.04
Usable Blastocyst	25	20	12	0.26
SART Good Quality Blasts	16	9	32	0.74
Comprehensive chromosomal screening results of clinically usable blastocysts				
	2%	5%	p-value	
Aneupoidy Rate	38.8% (76/196)	35.9% (66/184)	0.63	
Incidence of Mosaicism	7.7% (15/196)	7.1% (13/184)	0.99	