CULTURING IN A NON-HUMIDIFIED INCUBATOR? IMPROVED BLASTOCYST DEVELOPMENT WITH LIFEGUARD OIL AND DAY 3 MEDIA REFRESHMENT

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Background: Non-humidified incubators increase the evaporation rate from culture droplets, raising the media osmolality and pH over extended incubation periods [1]. “Heavy” oils can slow this detrimental evaporation [2], but which heavy oil is best for a non-humidified incubator?

Continuous, uninterrupted culture can produce comparable or improved blastocyst development compared to frequent media refreshment [1]. However, in non-humidified incubators, media dehydration from uninterrupted culturing may act as stressors that can delay or impair blastocyst development [1].

In this prospective, randomized study, our objective was to optimize culture conditions within our non-humidified benchtop incubator (K-System, CooperSurgical) by determining which set of culture environments yielded the highest quality blastocysts among sibling embryos.

Objective: To conduct two studies to evaluate the effects of different heavy oil overlays – LifeGuard (CooperSurgical) vs. OVOIL HEAVY (VitroLife) – and media refreshment on day 3 vs. continuous, uninterrupted culture until day 5, on quality blastocyst development.

Materials and Methods: Embryos were group-cultured (3 per 30 µL drop; Global Total (CooperSurgical); 35mm dishes; 4 mL of oil overlay). Dishes were pre-equilibrated 18-22 h prior to use. “Quality blastocysts” were defined as 3BB or better.

Oil overlay: Sibling 2PN zygotes were split randomly into dishes with either OVOIL Heavy or LifeGuard oil overlay. On day 3, embryos were graded, hatched (laser-assisted) and transferred to a new culture dish with the same oil overlay. On day 5, embryos were transferred to a new culture dish with the same oil overlay.

Day 3 media refreshment vs. continuous culture: Sibling 2PN zygotes were split randomly. Embryos in one dish remained undisturbed in the non-humidified incubator until day 5; embryos in another dish were changed over to a fresh, pre-equilibrated dish on day 3. No laser assisted hatching or embryo grading occurred on day 3. All dishes were prepared with LifeGuard oil (based on our results from the oil overlay experiment). All embryos were transferred into a fresh culture dish on day 5.

Results: Quality blastocysts were observed in 62.0% (18/29) of embryos cultured with LifeGuard oil vs. 37.0% (10/27) of embryos cultured with OVOIL Heavy; more embryos arrested with OVOIL Heavy (χ^2, $p = 0.04$) (Table 1). Quality blastocyst rates with and without a day 3 dish change were comparable (33/67 vs. 29/63 respectively). However, a day 3 dish change yielded significantly more AA, AB or BA graded blastocysts (78.7% vs 51.7%) and fewer BB graded blastocysts compared to embryos cultured uninterrupted until day 5 (χ^2, $p = 0.02$) (Table 1).

Conclusions: In our non-humidified incubator, blastocyst quantity or quality improved significantly with culturing protocols that minimize media dehydration. Lifeguard oil is denser and more viscous than OVOIL Heavy [3]; higher density oil has been shown to reduce evaporation from the underlying media [2]. Likewise, fresh culture media following a day 3

changeover provides a more optimal osmolality and pH during compaction and early blastocyst development. Blastocysts from culture dishes that remained in the non-humidified incubators for up to 114 hours were generally of a poorer quality.

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References:

[1] Swain, J. Controversies in ART: considerations and risks for uninterrupted embryo culture. Reprod Biomed Online. 2019 Jul;39(1):19-26.

[2] Swain, J. Different mineral oils used for embryo culture microdrop overlay differentially impact media evaporation. Fertil. Steril. 2018; 109: e53.

[3] Gunasegaran et al. Thick and viscous or light and easy: An oil overlay comparison. Fertility & Reproduction 2022 04:03n04, 211.

Table 1. Outcomes of sibling embryos cultured with different treatments in a non-humidified benchtop incubator.

Study	Day 3 Media Refreshment?		Oil Overlay	
	YES	**NO**	**LifeGuard**	**OVOIL HEAVY**
Total number of 2PN embryos cultured	67	63	29	27
Total number of				
QUALITY BLASTOCYSTS	33	29	18^{+}	10^{+}
→ *Graded AA, AB or BA*	*26**	*15**	*14*	*8*
→ *Graded BB*	*7**	*14**	*4*	*2*
POOR QUALITY BLASTOCYSTS	19	13	5	5
ARRESTED EMBRYOS (at multicell, compaction or morula stage)	15	21	6^{+}	12^{+}

Groups marked with * or $^{+}$ have a $p < 0.05$ (chi-squared analysis).