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THE SCIENCE OF INCUBATOR HUMIDITY: DOES IT REALLY MATTER FOR IVF OUTCOMES?

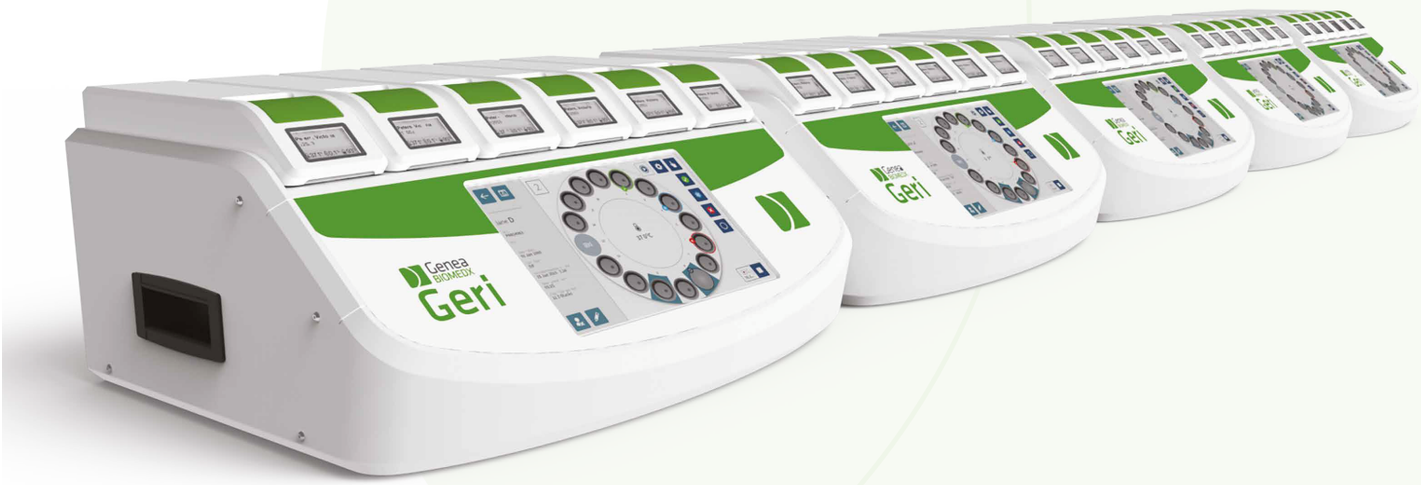
Authored by Lei Tan Ph.D, Teija Peura Ph.D and Shehua Shen MD, ELD (ABB), July 2025

1. Dry vs. Humid Incubator: why does it matter?

• IVF laboratories have increasingly adopted extended embryo culture and benchtop incubators, including those with time-lapse modules, many of which operate under dry conditions[1].While dry incubators offer benefits such as faster recovery times after door openings and reduced contamination risk due to the absence of standing water, their growing use has reignited concerns about media evaporation and the resulting increase in osmolality during extended culture[1].

• Although oil overlays can help buffer against pH and temperature fluctuations, they do not fully prevent evaporation-related osmolality drift in dry environments[2]. In contrast, humidified incubators mitigate this risk by stabilising osmolality, thereby creating conditions more closely aligned with the in vivo environment[1].

• Mammalian embryos thrive within a narrow osmolality range (255-295 mOsm/kg[3]). Deviations from this range can impair cell function, alter developmental timing and reduce viability[3].This white paper synthesises evidence from basic research through clinical studies to highlight the critical role of humidity control in optimising embryo culture conditions.



2. Basic Research: Humidified Incubators Minimise Osmolality Increase Across Culture Conditions

As summarised in Table 1, basic research across a wide variety of culture systems, including different media types, oils, dish designs and incubator models, consistently shows that dry incubators lead to osmolality increases of 20-40 mOsm/kg over 5-7 days of continuous culture.

In contrast, humidified systems maintain stable osmolality levels. These findings persist across diverse experimental variables, emphasising evaporation as a universal challenge in dry cultures.While modifications such as optimising oil type, dish configuration and media-to-oil ratios can help mitigate evaporation, none match the consistency of humidified conditions in stabilising osmolality.

Table 1. Basic research studies of osmolality changes in dry vs. humidified incubators

STUDY	DRY INCUBATOR	HUMID INCUBATOR	CULTURE MEDIA	MEDIA VOLUME	OIL	OIL VOLUME	STUDY LENGTH	RESULTS
Swain et al., 2016#[2]	K-system G185	Sanyo MCO-18MIC	specifics unknown	25µL	Vitrolife unwashed oil	3.5mL	7 days	Osmolality remained stable in humidified incubator but increased 30 mOsm/kg in dry incubator
Swain et al., 2018#[4]	K-system G185	Not used	Sage cleavage media + 10%SPS	20-60µL	Ovoil; light oil; heavy oil	3-5mL	6 days	Osmolality could increase 35 mOsm/kg in dry incubator
Holmes et al., 2018#[5]	K-system G210	K-system G210 with water dish	Sage cleavage media	500µL	not specified	1mL	6 days	Osmolality and pH significantly increased in both dry incubator with or without water dish, but water dish condition had less increase.
Yumoto et al., 2019[6]	Naka compact; K-system G185	Cook K- MINC-1000	Naka onestep; Global total; CSC complete with HSA	50-200µL	Naka washed oil; Naka light oil; Naka heavy oil; Kitazato heavy oil	3mL	5 days	Osmolality remained stable in humidified incubator but increased 20 mOsm/kg in dry incubator
Mestres et al., 2021[7]	Astec mini MN-2; Astec CUBE AD-3100	Astec mini MN-2; Astec CUBE AD- 3100	embryoMax KSOM	20µL	Cook Sydney IVF Culture oil (light)	4mL	7 days	Osmolality remained stable in humidified incubator but increased 30 mOsm/kg in dry incubator
Bossi et al., 2023[8]	K-system G185	Forma 4130	Irvine scientific CSCM-C; Cook Sydney IVF cleavage	25µL	Ovoil; Irvine light oil	3mL	7 days	Osmolality was relatively stable in humidified incubator but increase of osmolality could be up to 41 mOsm/kg in dry incubator

3. Clinical Evidence: Retrospective Studies Suggest Better Outcomes with Humidified Culture

Retrospective studies conducted in IVF clinics suggest that humidified incubation is associated with improved embryo development and/or clinical outcomes, including higher blastocyst formation rates, improved embryo quality and better pregnancy and implantation outcomes. Although not all differences reach statistical significance, the overall trend observed across various culture platforms supports the benefit of humidification. Notably, the only study that did not observe differences used sequential media with a Day 3 medium change, a practice that may mitigate the risks associated with single-step, uninterrupted culture in dry incubators[9]. See Table 2 for detailed results and comparisons.

Table 2. Retrospective clinical studies comparing dry vs. humidified incubation

STUDY	DRY VS. HUMID CONDITION	STUDY SIZE	MEDIA	KEY RESULTS	CONCLUSION
Perez Albala et al, 2019#[19]	Geri dry vs. humid chamber	116 patients with 1016 embryos (561 humid vs. 455 dry)	unspecified	Blastocyst rate 77.2% (humid) vs 70.9% (dry); Good quality blastocyst rate* 42.8% (humid) vs 35.8% (dry) (p=0.022)	Humidified culture condition enhanced blastocyst formation and quality.
De Los Santos Molina et al., 2019#[11]	Geri dry vs. humid chamber	7544 embryos from 1043 patients (558 humid vs. 478 dry)	unspecified	Blastocyst rate 71.3% (humid) vs 71.0% (dry); Good quality blastocyst rate 38.1% (humid) vs 37.7% (dry); Ongoing pregnancy* 52.5% (humid) vs 47.7% (dry); Implantation* 54.8% (humid) vs 52.7% (dry)	Humidified culture condition was associated with better pregnancy and implantation.
Albert Rodriguez et al., 2020#[10]	Geri dry vs. humid chamber	5755 embryos (3125 humid vs. 2630 dry)	Single step media	Blastocyst rate 52.8% (humid) vs 53.1% (dry); High quality blastocyst rate* 9.9% (humid) vs. 1.6% (dry); Pregnancy rate* 62.4% (humid) vs 55.0% (dry); In PGT embryos, euploid rate 39.6% (humid) vs 39.2% (dry)	Humidified culture condition was associated with better reproductive outcome when used with single step media and uninterrupted culture.
Chi et al., 2020[12]	ESCO Miri with (humid) or without (dry) outer well media	796 IVF cycles in 673 patients	G1/G2 media (Vitrolife), media change on D3	D3 cycles (539 humid vs. 156 dry): Clinical pregnancy 51.0% vs. 46.2%; Ongoing pregnancy*: 49.5% vs. 37.8%; Pregnancy loss*: 1.5% vs. 8.3%. D5 cycles (89 humid vs. 12 dry): Clinical pregnancy 59.5% vs. 50.0%; Ongoing pregnancy: 57.3% vs. 25.0%; Pregnancy loss* 2.2% vs. 25.0%;	Humidified condition by supplying outer well with media resulted in improved pregnancy outcomes.
Valera et al., 2022[13]	Geri dry vs. humid chamber	1627 ICSI cycles (833 humid vs. 794 dry, propensity-matched)	Gems Geri single-step, no media change	No significant difference in embryo development outcome. All cycles clinical pregnancy 64.9% (humid) vs. 60.6% (dry); PGT-A cycles clinical pregnancy* 64.5% (humid) vs. 52.6% (dry); Univariable logistic regression analysis revealed that embryos cultured in humid conditions had higher odds of clinical pregnancy* than those cultured in dry conditions (OR = 1.236, p = 0.041)	Embryo culture under conditions of high relative humidity contributes to optimise clinical results in undisturbed culture in a time-lapse incubator with single-step medium.

**indicates statistical significance; # indicates conference abstracts from ESHRE or ASRM*

4. Clinical Evidence: Randomised Controlled Trials (RCT) and Sibling Oocyte Studies Reproducibly Favour Humidified Culture

Randomised controlled trials and sibling oocyte studies (summarised in Table 3) offer compelling evidence that humidified incubation improves embryological and clinical outcomes.These study designs minimise confounding by controlling for inter-patient variability, and consistently demonstrate higher rates of blastocyst development, better embryo quality and increased pregnancy success in humidified environments. Such studies provide the strongest evidence base in support of humidified culture systems.

Table 3. Randomised clinical studies comparing dry vs. humidified incubation

STUDY	TYPE	DRY VS. HUMID CONDITION	STUDY SIZE	MEDIA	KEY RESULTS	CONCLUSION
Fawzy et al., 2017[14]	RCT	G185 incubator with (humid) or without (dry) water dish	297 patients randomized to dry (142) or humid (145)	Global Total	All embryo development outcomes* better in the humid group Positive β-hCG: 61% (humid) vs. 49% (dry) Clinical pregnancy*: 57% (humid) vs. 43% (dry) Ongoing pregnancy*: 52% (humid) vs. 37% (dry) Implantation*: 36% (humid) vs. 27% (dry) Pregnancy loss: 9% (humid) vs. 12% (dry)	Human embryos cultured in a dry incubator had statistically significantly decreased implantation and clinical and ongoing pregnancy rates.
Del Gallego et al., 2018#[15]	RCT	Geri dry vs. humid chamber	176 patients randomized to dry (93) or humid (83)	Geri and Gems media	Blastocyst rate*: 74.5% (humid) vs. 69.2% (dry) Pregnancy: 83.3% (humid) vs. 66.7% (dry) Pregnancy loss: 16% (humid) vs. 26.5% (dry)	Results suggest a clear improvement on embryo development and subsequent IVF outcome when embryos are cultured in a humidified atmosphere.
Holmes et al., 2019#[16]	Sibling oocyte	Geri dry vs. humid chamber	16 patients (215 embryos, 110 dry vs. 105 humid)	Sage sequential (media change D1, 3, 5)	Fertilization rate: 79.0% (humid) vs. 81.8% (dry) Good cleavage rate: 79.5% (humid) vs. 76.7% (dry) D5 blastocysts: 48.2% (humid) vs. 43.3% (dry) D5 blastocysts ≥3BB*: 24.1% (humid) vs. 14.4% (dry) D6 blastocysts: 62.7% (humid) vs. 54.4% (dry) D6 blastocysts ≥3BB*: 49.4% (humid) vs. 25.6% (dry) D7 blastocysts: 62.7% (humid) vs. 60.0% (dry) D7 blastocysts ≥3BB*: 50.6% (humid) vs. 31.1% (dry)	Humidified culture yielded more good quality blastocysts.
Mauri Lopez et al, 2024#[17]	Sibling oocyte	Geri dry vs. humid chamber	379 MII oocytes from 30 patients	G-TL single step (uninterrupted in humid while media change D3 in dry)	Usable blastocyst rate: 53.1% (humid) vs. 48.6% (dry) A-grade D5 blastocyst rate*: 32.3% (humid) vs. 19.9% (dry), OR = 1.87 A-grade rate among all usable blastocysts*: 60.9% (humid) vs. 40.8% (dry)	Humidified culture significantly enhanced top quality blastocyst formation. These data highlight that Day 3 medium renewal doesn't equalise conditions to humid culture.

**indicates statistical significance; # indicates conference abstracts from ESHRE or ASRM*

CONCLUSION AND TAKEAWAYS

- Evidence from basic science, retrospective clinical studies and randomised controlled trials converges on a single conclusion: humidified culture environments support superior embryo development and clinical outcomes in IVF.
- This finding is particularly relevant in light of the increasing adoption of extended, uninterrupted culture using single-step media, where osmolality stability becomes a critical factor.
- This conclusion aligns with the Cairo Consensus Guidelines on IVF Culture Conditions[18], which state:"embryologists should consider humidification as an obvious choice for culture, but only if incubator manufacturers recommend the use of humidification."
- Given the consistent results across a range of systems and methodologies, IVF laboratories should critically assess their incubator configurations.
- Strategies to mitigate osmolality drift in dry incubators include the use of outer well media, water dishes, optimisation of oil and media types and volumes, and scheduled media renewal.
- However, selecting an incubator that supports a fully humidified culture environment remains the most reliable approach to maintaining optimal conditions and improving IVF success.

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