Embryo culture conditions under high humidity significantly enhances blastocysts formation and quality according to an automatic time-lapse algorithm

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Study question:
Is blastulation and quality rate conditioned by morphokinetics at early stages of embryo development? Is the humidity in an incubator chamber affecting morphokinetics and blastocyst rates?

Summary answer:
There is a correlation between blastulation rates and quality with the Xtend-classification and a higher proportion of embryos with better Xtend scores in high humidity.

What is known already:
Blastulation may be predicted by early stages of embryo development although controversial results still are present in the literature. The optimal incubator humidity is still a controversial topic in IVF laboratories. Even though the in vivo condition is humid, the possible growth of microorganisms under these conditions are higher than with dry incubators.

However recent studies have shown better results in humidified chambers probably due to more stable culture conditions regardless oil overlays in culture dishes (Fawzy et al Fert Ster 2017).

Study design, size, duration:
University-affiliated infertility clinic. Retrospective cohort study in ovum donation program from January 2018 to October 2018. In the first part of the study, we analyzed 3001 embryos from 361 patients. In the second part, 116 patients with 1016 embryos were randomly distributed under high or low humidity in a continuous embryo monitoring incubator.

Participants/materials, setting, methods:
Embryos were generated by ICSI and incubated in a Time-lapse incubator (Geri, Genea, Australia) that used an automatic cell-tracking software. An improved version of this algorithm was developed taking into the values of P2 and P3, egg age, number of cells on day3 and a texture image analysis correlated with fragmentation.

In the second part, the embryos were randomly distributed in chambers with or without humidity.

Main results and the role of chance:
The Xtend classification was directly correlated with higher blastocyst rates and better good quality embryos. With an average blastulation rate of 73.6%, we studied rates in each category (1:91%; 2:86.30%; 3:79.7%; 4:63%; 5:38.3%). We studied the percentage of good-quality blastocysts (A/B ASEBIR morphological classification) in each Xtend category (1:57%; 2:47%; 3:36%; 4:25%; 5:11%)(p<0.001).

A total of 1016 embryos were incubated in the Geri system under different humidity conditions, 561 in chambers with humidity and 455 in chambers without humidity. All of them were classified according to the Xtend algorithm. In the humidity chamber, 23.5% were classified as 1, 22.1% as 2, 17.6% as 3, 18.2% as 4 and 18.5% as 5.

In the dry chambers 26.2% were classified as 1; 18.9% as 2, 12.7% as 3, 17.6% as 4 and 24.6% as 5. We assembled the Xtend categories in two groups(1-2-3 vs 4-5)and compared the proportion of embryos in
each group (humid vs dry incubation) showing more embryos classified as 1-3 in humid (63.30%) compared with dry incubation (57.26%), even though differences were not statistically significant (p=0.075).

Moreover, blastocyst rate in humidity chambers was 77.2% versus 70.9% in dry chambers and percentage of good quality blastocysts 42.8% (humidity) vs 35.8% (dry) (p=0.022).

**Limitations, reasons for caution:**
The retrospective nature of this study may be a reason for caution. The classification system itself has some errors due to difficulties in cell tracking generating “none result”, however we only included cases where classifications were provided. A further analysis could include different humidity measurements to establish the optimal value.

**Wider implications of the findings:**
This study correlates Xtend categories with blastulation rates, demonstrating a direct link with the diagnostic test. According to humidity, results obtained show a higher proportion of good-quality embryos (classified as 1-2-3) as well as higher blastocyst rates under humidity conditions.

Therefore, incubation under high humidity might improve the final outcome of the cycle.

**Trial registration number:**
Not applicable.

**Keywords:**
Embryo
time-lapse
blastocysts
Humidity
Algorithm