Abstract title:
The effect of high humidity culture conditions over embryo development: a continuous embryo monitoring assessment

Study question:
Since in vivo culture, culture conditions are humid. Our aim was to know how high humidity might affect embryo development comparing to dry conditions by using single step culture media in a continuous embryo monitoring incubator (CEM).

Summary answer:
Our results suggested that culture conditions with high humidity atmosphere did not affect embryo development but increase the pregnancy and implantation rate.

What is known already:
Oil overlay has supported successful use of a dry incubator for culture human embryos, preventing changes in the pH, temperature. However, dry conditions may affect the osmolality due to the evaporation of culture media. Therefore, the use of humid conditions avoid osmolality changes. In a previous study we found statistical differences in terms of blastocyst rate. Pregnancy and implantation rate was affected but remained not significantly.

Study design, size, duration:
A total of 7544 embryos from 1043 patients, from ovum donation programme and own oocytes and that were culture on a time lapse incubator system (Geri genea biomedix, Australia) were included in a retrospective and multicentric study (three IVF units and 5 CEM incubators) from 2016 to 2018.

Participants/materials, setting, methods:
This CEM incubator has 6 separated small incubators. Three of them works in a dry atmosphere (DC) and the other 3 in humid conditions (HC). In the dry chambers, the embryos from 478 patients were cultured and under HC a total of 558. Retrospectively, blastocyst, good morphology blastocyst rate, pregnancy, implantation and miscarriage rate were evaluated.

Main results and the role of chance:
In a previous study we found that culture conditions with high humidity atmosphere promoted embryo development and reproductive outcome. This time, with increased sample size, we didn´t found any differences in embryo development. By performing an stratified analysis, humid conditions were equally distributed by clinic and treatment (multivariable analysis). We had very similar blastocyst rate when the embryos were culture under high HC (71.3% vs 71.0% DC). Also the proportion of blastocyst with good morphology was very similar 38.1% in HC vs 37.7% and DC. The ongoing pregnancy rate (OPR) was higher in HC vs DC (52.5% vs 47.7% respectively), additionally the implantation rate slightly better in HC 54.85 vs 52.7 but but remained not significant.

Limitations, reasons for caution:
The retrospective nature of the study may limit the conclusion although sample size is remarkable. A prospective reandomize study may solve the remaining questions surrounded this topic.

Wider implications of the findings:
Our results would suggest that HC may increase the reproductive outcome of our patients when using single step media in CEM incubators although effects are limited and still need to be confirmed with a
larger sample and improved designs.

**Trial registration number:**
NA

**Keywords:**
Humid conditions
culture
blastocyst
osmolarity
one step