TECHNICAL REPORT
03 | 2022

pH equilibration times in 2 different embryo culture dishes

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Multi-well dishes such as the Minitube 5-well dish or the 4-well dishes of other brands are common in assisted reproduction procedures. They are frequently used for oocyte collection, insemination of oocyte-cumulus complexes during conventional IVF or for embryo culture.

Apart from media composition and osmolarity, adjustment and maintenance of a physiological pH value is of crucial importance for normal development of embryos in vitro and finally for treatment success.

When preparing a culture dish, its wells are filled with medium which is covered with a layer of pharmaceutical-grade paraffin or mineral oil. The dish is placed in an incubator where the medium gradually reaches the target temperature and a stable pH value during the so-called equilibration time. Physiological pH is adjusting as an equilibrium between the incubators´ CO_2 atmosphere and the buffer system of the culture medium.

Among others, the equilibration time needed to reach a stable pH depends on the physical properties of the culture dish like the gap between the lid and the bottom part or the diameter of the wells filled with media which determines the contact surface between gas atmosphere and medium. In practice, keeping equilibration time short can be an advantage.

This study was aiming at a comparison of pH equilibration times between the Minitube 5-well dish and a third-party 4-well dish.

Materials and methods

A „pH-1 micro“ pH meter (PreSens Precision Sensing GmbH, Regensburg / Germany) was used for continuous, contact-free pH monitoring from culture medium. pH sensor spots were integrated into the wells of the dishes to be tested, allowing for fluorometric pH measurement at an accuracy of approx. ± 0.03 pH. The dishes were resterilized after modification.

One well each of the culture dishes was filled with 700 µl of HTF medium supplemented with 10 % (Vol/Vol) Serum Substitute Supplement (FUJIFILM Irvine Scientific, Santa Ana / USA). The open dishes without oil overlay of the medium were exposed to ambient atmosphere for 20 minutes in order to raise media pH. Subsequently, the medium was covered by 400 µl of sterile mineral oil (Oil for Embryo Culture, FUJIFILM Irvine Scientific).

pH monitoring from culture medium was initiated right after placing the dishes in the incubator, taking readings every 5 minutes over a period of up to 20 hours. Three culture dishes of each type were tested in identical replicates. pH monitoring was performed in a 14 litre incubator (Galaxy 14 S, Eppendorf, Hamburg / Germany) at 37°C and 5.3 % CO_2.

Minitube 5-well culture dish

Results

The Minitube 5-well dishes reached a stable media pH within 5 hours after preparing the culture dishes whereas the third-party 4-well dishes needed an average time to pH equilibration of 10 hours (see graph).

Most probably, the significantly shorter equilibration time of the Minitube dishes is directly related to their larger well diameter (19 instead of 16 mm). The contact surface between culture medium and the CO_2 atmosphere inside the incubator is 30 % larger in the 5-well dishes as compared to 4-well dishes.

Conclusion

Minitube 5-well dishes need significantly less pre-incubation time to be ready for use at appropriate media pH and temperature than 4-well dishes of other brands. Similarly, a reduced pH recovery time after transient removal of the dish from an incubator and exposure against ambient atmosphere can be expected.