

Brief Communication

How long does it take for microbes to initiate growth in sealed and sterile bottles of embryo culture and handling media?

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Abstract

The need for asepsis in IVF practice is well-recognized by the entire IVF fraternity. Its critical importance in both the clinical and laboratory IVF practice needs no emphasis. The potential for the embryo culture, gamete handling and other IVF specialty media (CMs) to harbour microorganisms and damage the culture conditions is one of the main concerns of IVF workers. The question is how long would it take for microbes to begin colonising CMs in sealed unopened containers left in storage? The objective of this study was to answer this question and to determine the quality and efficacy of the manufacturer in producing quality CM. The CMs used in this study were purchased in the years 2013 and 2014. These CMs include the bicarbonate-buffered embryo culture, HEPES-buffered gamete handling and IUI media. The CMs were custom manufactured by FertiPro NV, Beernem, Belgium for Cellcura® ASA, Oslo, Norway. Expired CMs were stored at room temperatures for 12 to 13 years. They were routinely checked for microbial growth twice a year. In 2026 the study was terminated. The test CMs were investigated for microbial growth using standard ATCC protocol with minor differences. No microbial growth was noted in all CMs tested. The results of this study indicated that microbial growth will not occur in CMs if manufactured under stringent manufacturing conditions compliant with ISO 13485 guidelines even after years of storage in conditions that normally would have encouraged growth of microbes in fluids produced under less stringent manufacturing condition. In conclusion, this study confirmed the efficacy and capability of the manufacturers of human embryo culture, and handling media in producing CMs of very high quality completely devoid of microorganisms including possibly microbial spores. This is a testimony to the excellent production standards practiced by manufacturers of human IVF CMs. This is the first report of its kind.

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Introduction

Aseptic technology at the workplace is the hallmark of assisted reproduction practice. The need for asepsis is an undisputed fact well recognized by the entire IVF fraternity. Its critical importance in both the clinical and laboratory IVF practice needs no emphasis. Hygiene and cleanliness in IVF practice is crucial for favorable treatment outcome. In the ART laboratory, the culture of gametes and embryos requires stringent culture conditions free of microbial contamination. In particular, the sterility of the culture medium is considered of

paramount importance to ascertain the development of quality and viable embryos (Bezar et al, 2025).

Microbial contaminations in embryo culture systems have been reported with mixed outcomes. In general, however, the fraternity is convinced microbial contamination is harmful to the culture conditions and possibly the embryos (Bezar et al., 2025; Borges et al., 2020; Cottell et al., 1996; Elder et al., 2005; Pelzer and Allan, 2012; Zheng et al., 2023). It is therefore a norm

and the practice of majority of ART laboratories to use culture media containing antibiotics to minimize the risks of microbial growth (Pelzer and Allan, 2012; Kastrop et al., 2007; Zheng et al., 2023; Cottell et al., 1996).

Considerable effort both by relevant authorities as well as individual workers has been made to implement appropriate standards of practice to ascertain workers comply with aseptic technology in routine IVF practice (ASRM 2022; Borges et al., 2020; ESHRE, 2026; Magli et al., 2002; Pelzer and Allan, 2012).

The potential for the embryo culture and handling media to harbour microorganisms is one of the main concerns of IVF workers. Although unproven, the mindset of IVF workers with regards to the efficacy of CM manufacturing practices appear to have resulted in a fastidious predilection to purchase only CMs manufactured in advanced nations based on the unproven presumption media production in less advanced nations may not comply with the stringent requirements for media production, and therefore the quality of production is suspect. Consequently, the proliferation and development of the culture media industry in some newly developed or developing nations appear to be severely negated unless it is managed by principals based in advanced nations (unpublished observations).

The question is how long would it take for microbes to begin colonising CM in unopened sealed containers left in storage? The objective of this study was to answer this question and to determine the quality and efficacy of the manufacturer in producing quality CM.

Materials and methods

The CMs used in this study were purchased in the years 2013 and 2014. These CMs include the bicarbonate-buffered embryo culture, HEPES-buffered gamete handling and IUI media. The CMs were customised manufactured by FertiPro NV, Beernem, Belgium for Cellcura® ASA, Oslo, Norway. Once the media passed their expiry dates, they were placed in storage at room temperature. These were 50ml bottles and their caps remained sealed throughout the study period. The usual day time temperatures in

Shah Alam were between 31 to 35°C and the nighttime temperatures ranged from 24 to 25°C. The clarity of the test CMs were checked monthly in the first year of storage and then twice annually between 2013 to 2026 (12-13 years).

If the media remains completely clear, they are considered as being sterile and devoid of microbial growth. However, if they appear turbid, microbial growth is suspected and the affected CMs will be tested to detect bacterial growth. The protocol applied for the detection of bacterial contamination of the CMs are similar, with minor differences, to the ATCC Guidelines (www.atcc.org/resources/culture-guides/bacteriology-culture-guide; accessed 13 July 2026).

One day before the experiment, the required set of agar plates and nutrient broth in tubes are placed in the air incubator held at 37°C. At 24 hours if no microbial growth were noted, these agar plates and broth tubes will then be inoculated with the test and control CMs. However, they will be discarded if these plates and tubes were contaminated with bacterial growth.

Using a sterile graduated 1ml pipet 0.5ml of the medium is aseptically dispensed into the dish and then 0.5ml of the same medium is likewise dispensed into nutrient broth tubes. A drop of the medium is placed and streaked across the glass slide and dried in air. Using the sterile applicator the medium is spread over the surface of the agar dish. The nutrient broth vortexed gently in an aseptic manner. The nutrient culture dish and broth are then placed in the air incubator at 37°C for 24hrs.

The glass slide is stained and microscopically examined for detection of bacteria. The total number of replicates were 4 per medium. Two sets of agar plates and nutrient broth tubes served as uninoculated blank controls. At 24hours of incubation, the agar plates and broth tubes were examined for microbial growth.

The experiments, identical to the aforementioned, are repeated three weeks later on the same media bottles which were previously opened during the first set of experiments. This was intended to determine whether microbial growth occurred during the three weeks since the bottles were last opened.

Results

None of the bottles of CMs showed turbidity or any microbial growth during the monthly and annual examinations performed over a period of 12 to 13 years. Their volumes, however, were reduced due to evaporation. No bacterial growth was noted in the agar plates or nutrient broth tubes for all CMs tested. The stained smears too did not detect the presence of microorganisms.



Fig 1: Cellcure® embryo culture, gamete handling and flushing media. Picture taken immediately after production.

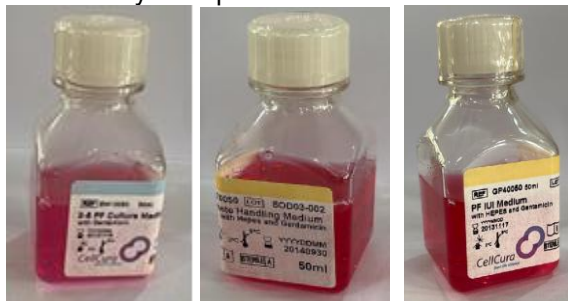


Fig 2: Sealed sterile Cellcure® embryo culture, gamete handling and IUI media manufactured in 2013 and 2014 showing no microbial growth after storage at room temperature for 12-13 years. Photo was taken in 2026



Fig 3: The nutrient broths inoculated with Cellcure® embryo culture medium were clear, with no visible turbidity. Similar observations were noted for remaining gamete and IUI media.

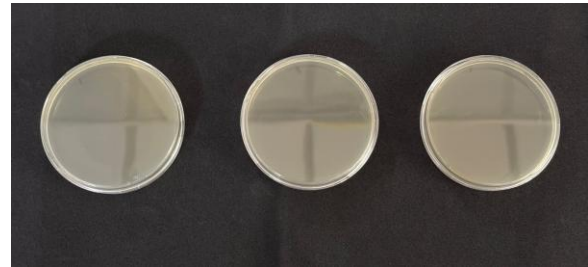


Fig 4: The nutrient agar plates inoculated with the Cellcure Cellcure® embryo culture medium were clear, with no growth of microbial colonies. Similar observations were noted for remaining media.

The results of the repeat study performed on all the same media bottles opened previously three weeks earlier showed no microbial growth identical to the previous results on previously sealed and unopened bottles.



Fig 5: The nutrient broths inoculated with Cellcure® embryo culture medium were clear, with no visible turbidity. Similar observations were noted for remaining gamete and IUI media.

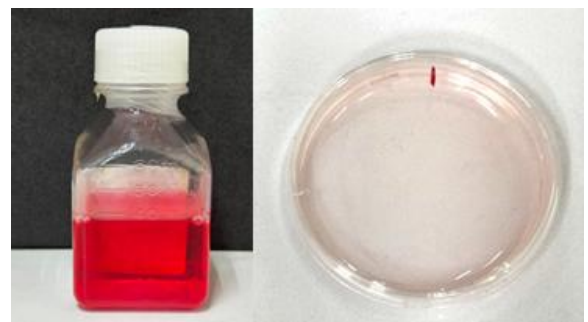


Fig 6a,b: The nutrient agar plates inoculated with Cellcure® embryo culture medium from a bottle that was previously opened three weeks earlier were clear, with no visible turbidity.

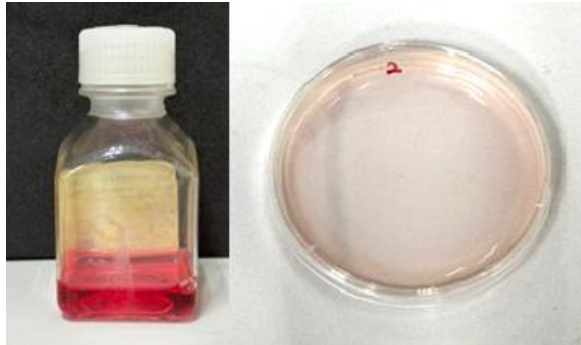


Fig 7a,b: The nutrient agar plates inoculated with Cellcura® gamete handling medium from a bottle that was previously opened three weeks earlier were clear, with no visible turbidity.

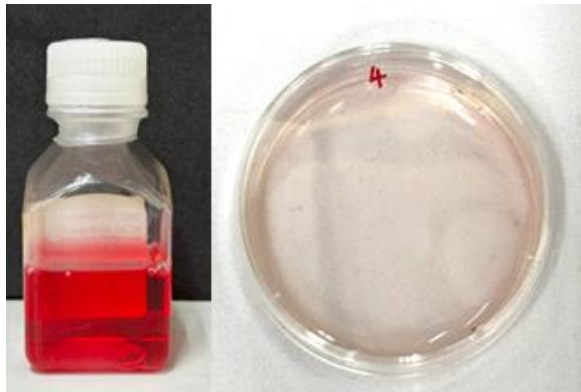


Fig 8a,b: The nutrient agar plates inoculated with Cellcura® IUI medium from a bottle that was previously opened three weeks earlier were clear, with no visible turbidity.

Discussion

The results of this study indicated that microbial growth will not occur in CMs if manufactured under stringent conditions compliant with industrial norms even after years of storage in conditions that normally would have encouraged growth of microbes in fluids produced under less stringent manufacturing practices. This assumption is further strengthened when the same bottles of media when tested three weeks later even though their seals were removed and its cap opened, but remained sterile with no microbial growth. This is a testimony to the excellent media manufacturing process adhered to by embryo culture and handling media manufacturers.

A widely recognized indicator of quality in the culture media (CM) manufacturing industry is

certification by the International Organization for Standardization (ISO, 2020). ISO is an independent, non-governmental international organization responsible for developing voluntary standards that are applicable across a broad range of industries, including CM manufacturing.

Within the pharmaceutical industry, ISO standards establish a structured framework for quality management, risk assessment, environmental management, and laboratory practices, thereby supporting the production of safe, effective, and internationally compliant products.

In contrast to regulatory requirements such as Good Manufacturing Practices (GMP), which are legally mandated, ISO standards are voluntary in nature but are extensively implemented to strengthen organizational credibility, improve operational efficiency, and promote continuous quality improvement (ISO, 2020). ISO 11133:2014 is an international standard for microbiological culture media used in food and water safety. While ISO 11133 is not specific to human embryo culture, it is relevant because the embryo culture media is a medical device and therefore its manufacture is a regulated process of quality management systems specifically under ISO 13485 for medical devices.

Therefore, manufacturers of various CMs for therapeutic IVF must comply with the quality standards of manufacture. Manufacturers of CMs go to great lengths to ensure and comply with stringent manufacturing practices as evidenced by the present findings. This, primarily, is to manufacture CMs that met the stringent mandatory requirements instituted by relevant authorities to provide the IVF laboratory with quality sterile CMs devoid of microbial contamination.

Conclusion

This study has confirmed the efficacy and capability of the manufacturers of human embryo culture, handling cryopreservation media in producing culture medium very high quality completely devoid of not only the vegetative forms of microorganisms and even the microbial spores. This is a testimony to the excellent production standards practiced by

manufacturers of IVF culture media. This is probably the first report of its kind.

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Author contributions

NBA: Designed the experiments, analysed the outcome of the study and in part drafted the manuscript – 60%

NMANAR and MAO: contributed equally to the interpretation of the results and assisted in drafting the manuscript – 20% each.

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