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THE IMPORTANCE OF THE VITRIFICATION SYSTEM DURING EMBRYO CRYOSTORAGE: MICROBIOLOGICAL INFECTION RISKS

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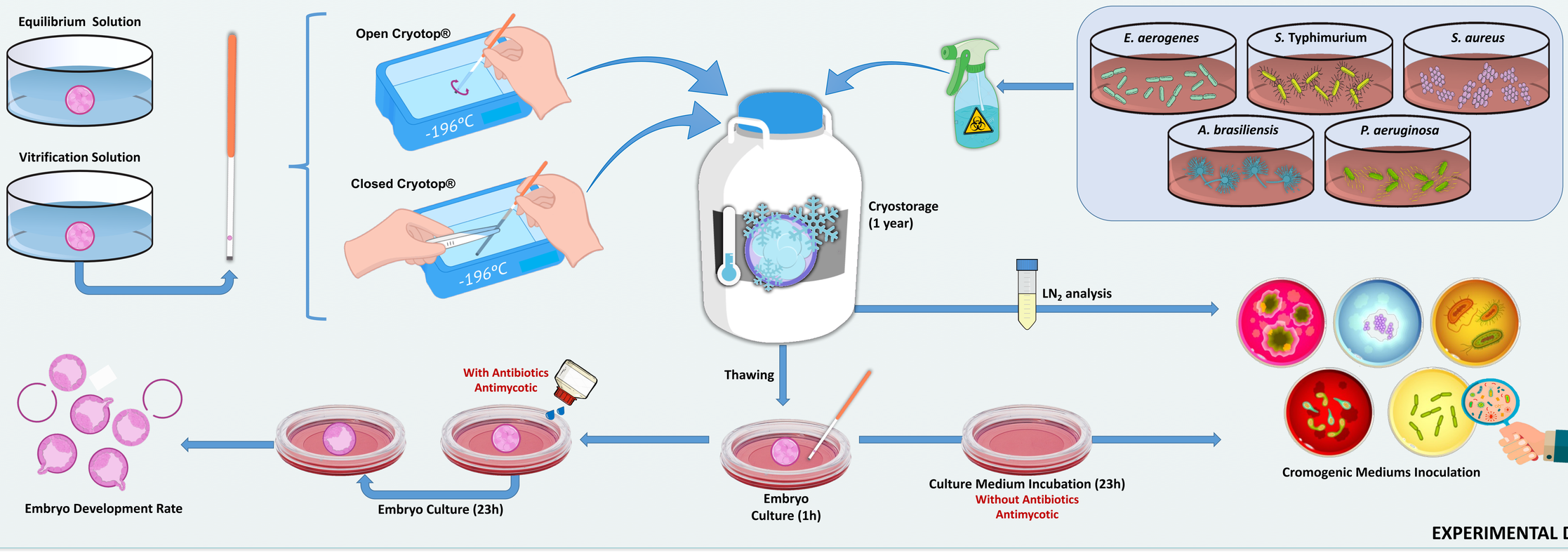
Introduction

Nowadays, infertility affects more than 186 million people worldwide. As consequence, approximately 1.5 millions of cycles that apply assisted reproductive technologies (ARTs) are performed, with a progressive rise in banking cycles in which all embryos are frozen for future ARTs cycles. However, it has been reported that many factors that influence the infection of embryos before and during cryopreservation with pathogens that can survive during cryostorage and transmitted via embryo transfer. During ART procedures, cryostorage is the only situation where large quantities of biologic materials of patients are kept together transmit infective agents from between samples if they are not sealed properly. Therefore, this experiment was carried to examine whether bacteria and fungi were still present on the LN2 after 1 year and to test the cross-contamination from LN2 to embryos contained in open and closed vitrification devices.

Materials and Methods

With this purpose, pools of 10 embryos (n=16) where vitrified using the Cryotop® methodology, with open (n=8) and closed systems (n=8), and stored in the same 11L container. The LN2 was artificially contaminated with several bacteria (*Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* Typhimurium and *Enterobacter aerogenes*) and one fungi (*Aspergillus brasiliensis*), using 10^6 UFC of each bacteria and 10^5 spores. After 1 year, embryos were warmed and cultured for 1h in medium without antibiotics nor antimycotics. Then, embryos were placed in medium containing 1% antibiotic-antimycotic solution to evaluated their development until hatching/hatched blastocyst stage. Remaining medium and LN2 samples (n=6) were inoculated into chromogenic mediums for bacteria detection.



EXPERIMENTAL DESIGN

Results and Conclusions

Our results showed that some microorganisms survive in LN_2 during 1 year. Viable *S. aureus* (100%), *E. aerogenes* (100%) and *A. brasiliensis* (100%) were isolated (Figure 1). In addition, viable *S. aureus* were found in 12.5% of embryos vitrified using the open system (Figure 2). No cross-infection from LN2 was observed in embryos vitrified in closed devices (Figure 2). Nevertheless, no differences in embryo development rates (Figure 3) were obtained between embryos stored in open or closed systems (58.8%), indicating that the use of antibiotics-antimycotics can avoid the microbial activity.

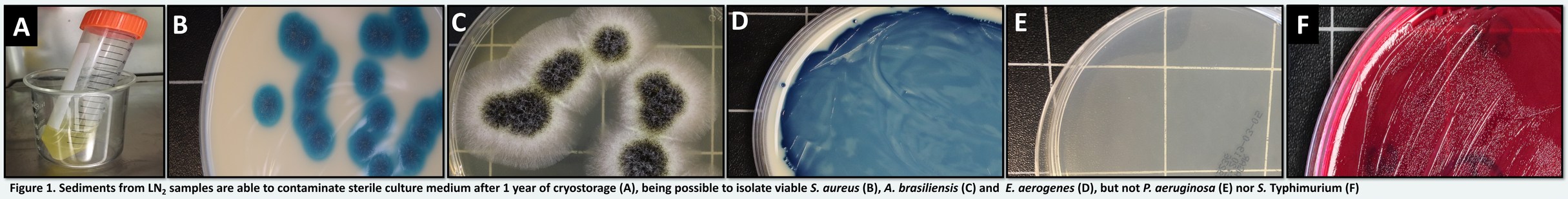


Figure 1. Sediments from LN_2 samples are able to contaminate sterile culture medium after 1 year of cryostorage (A), being possible to isolate viable *S. aureus* (B), *A. brasiliensis* (C) and *E. aerogenes* (D), but not *P. aeruginosa* (E) nor *S.* Typhimurium (F)

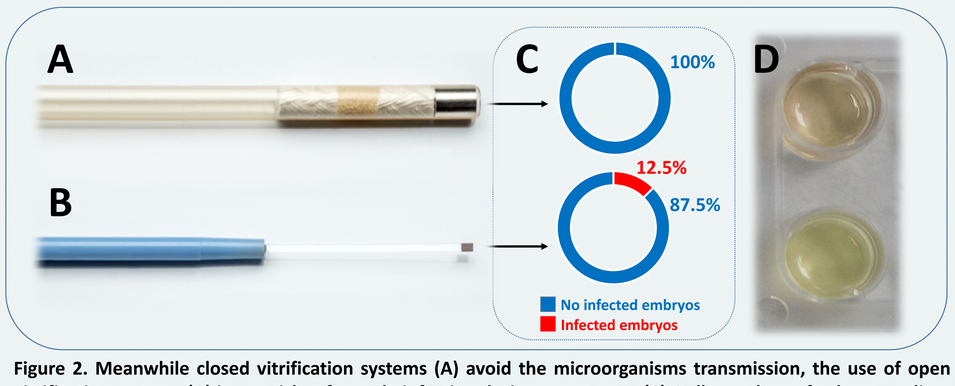


Figure 2. Meanwhile closed vitrification systems (A) avoid the microorganisms transmission, the use of open vitrification systems (A) incurs risks of sample infection during cryostorage (C). Yellow colour of culture medium with phenol red indicates that some cultured embryos vitrified in open systems contain viable bacteria (D)

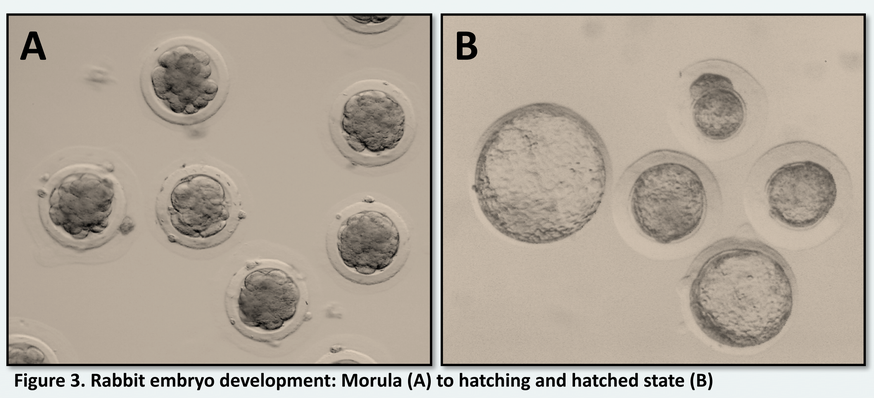


Figure 3. Rabbit embryo development: Morula (A) to hatching and hatched state (B)

In conclusion, pathogens can long-term survive in LN2 and can spread to open vitrified samples. Then, the appearance of resistant strains could bring on infections to recipients via embryo transfer. ***Research supported by Ministry of Economy and Competitiveness, Spain (AGL2017-85162-C2-1-R)*.**