

Embryo Culture Challenge: Microbial Contamination

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Dear Editor,

The success rate of embryo production critically depends on several factors. One of the pivotal factors is a technique used for the whole procedure of *in vitro* fertilization (IVF) and *in vitro* embryo culture (IVC) (1, 2). Besides the technique selected for IVF / *in vitro* embryo production (IVEP), there is an important consideration called IVF / IVEP culture dishes disinfection (3). For many years, microbial contamination of embryo culture dishes has caused several deficiencies in the success rate of assisted reproductive technique (ART) laboratory. Despite precise culture conditions, strict trafficking regulations, rigorous discipline of aseptic technique and applying culture medium supplemented with antibiotics including Penicillin and Streptomycin, a dramatic increase in the number of infections was recorded routinely worldwide (4, 5).

There is a great demand to overcome the abovementioned issue in human fertility & sterility clinics and laboratory procedures. Results of intensive studies regarding the identification of the main sources of such contamination revealed that *Escherichia coli* (*E. coli*) and *Candida* species have caused more than 59 % and 26 % of these contaminations, respectively (6-8). Among *E. coli* species, 74 % showed a great resistant to both *Penicillin* and *Streptomycin* in the culture medium. On the other hand, 24 % of *E. coli* appeared to be resistant to both antibiotics. "Infections in IVF culture dishes are mainly caused by bacterial strains insensitive to the antibiotics used or due to yeast colonization by *Candida* species, which frequently reside in the vagina" (6, 9).

IVF culture dishes catch *E. coli* / *Candida* either via ejaculation which is the paternal source of infection obtained from semen samples, and / or maternal sources. The maternal source of contamination is either ovum pick up

procedure (OPU) or vaginal environment (10). In a report, identification of the contaminating micro-organisms revealed that some of the materials utilized in the culture procedure such as "mineral oil", which is used to cover the fertilization / culture drops, were infected with *Aspergillus terreus*. Therefore, not only, a great variation has been observed in case of the origin of culture dishes infections, but also there would be a great number of bacterial / fungal species transferred by the infecting sources to the culture dishes (6). However, as mentioned before, many of the infectious bacterial or fungal colonies are insensitive to antibiotics (6). Therefore, it would be an obligation to develop an efficient and simple prevention method to reduce the high rate of culture dishes contaminations. Animal biotechnologist and other scientists who are involved in IVEP aspired to reduce the mentioned deficiency caused by these microorganisms.

Disinfection procedure of IVF / IVC culture dishes involved two main obstacles, in which there were complications; selection of an appropriate and efficient antibiotic as well as wide variation of bacterial / fungal sensitivity to the selected antibiotic. Diverse techniques have been suggested to overcome the current problem, such as different combinations of antibiotics added to culture mediums and / or antibiotic administration to oocyte and sperm donors. Getting to work all these directions, a great number of bacterial / fungal contaminations are yet being reported. On the other hand, the current knowledge has a huge doubt about side effects of antibiotics administered to patients with subfertility and / or added to embryo culture medium, on oocyte / embryo competence and survival (6, 9). In recent years some laboratories such as University of Tehran IVF / IVC laboratory has employed and proven the efficiency of applying a specific regiment of Ultra Violet lamp (UV)

Implication for health policy / practice / research / medical education:

Embryo production laboratory and animal biotechnologist may use the presented information

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along with non-toxic disinfectant gaseous phase “Ozone (O₃)” (physicochemical disinfection method (PDM)). The results showed that besides administrating and supplementing culture mediums with common antibiotics for disinfection of IVF / IVC culture medium, the new horizon in physicochemical disinfection procedure could also be promising in this context; through the applications of PDM. Results revealed that, when this regiment was applied, no infection was observed through 2367 IVF treatment cycles. The same combination of UV and O₃ recently has been applied in an incubator (Simple Embryo Culture Chamber (SECC)) designed by Dadashpour Davachi

et al. (unpublished data) to protect all kinds of cell and embryo culture dishes against infecting microorganism. Results showed that using SECC compared to other commercial incubators led to a dramatic decrease in culture dishes contamination (Table 1). In SECC only before putting the culture dishes in the chamber the UV lamp was on. It would be demonstrated that many kinds of the infecting microorganism are introduced to culture dishes via external sources such as mineral oil, tissue culture medium and other reagents. Applying different UV and O₃ regiment in IVF / IVC laboratory would be promising to reduce antibiotic consumption in IVEP.

Table 1. Differences in the Number of Infected IVC Dishes Between two Types of Incubators

Type of Incubator	No. Culture Dishes	Infected IVC Dishes, Mean ± SEM	Infected IVC Dishes, Mean ± SEM
Commercial Incubator	100	72 ± 1.0	28 ± 0.0
SECC Equipped with UV and O ₃	100	15 ± 0.5	85 ± 1.0

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Authors' Contribution

The authors have participated equally in manuscript preparation.

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