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Microbiological contamination in IVF laboratories: A narrative review

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ABSTRACT

In vitro fertilization (IVF) is one of the most used assisted reproductive technology (ART) techniques today. However, the success of IVF procedures heavily relies on maintaining a sterile environment in laboratories. This narrative review examines the effects of microbiological contamination in IVF laboratories, exploring its sources, impacts on IVF outcomes, and preventive measures. We conducted a complete literature search using databases such as PubMed and Google Scholar, focusing on studies published within the last fifteen years. Our findings highlight that microbiological contamination can significantly impair embryo quality, reduce implantation and pregnancy rates, and increase the risk of miscarriage and infection. The review also discusses current best practices for contamination prevention and identifies areas for future research. This work emphasizes the critical importance of stringent sterility protocols in IVF laboratories and calls for continued vigilance and innovation in maintaining optimal conditions for assisted reproduction.

KEYWORDS: *In vitro* fertilization; Assisted reproductive technologies; Microbial contamination; Embryonic arrest; Infertility

1. Introduction

Assisted reproductive technology (ART) comprises fertility treatments where oocytes or embryos are manipulated outside a woman's body to enhance the chances of achieving successful pregnancies and delivering healthy offspring[1]. The methods that involve manipulating oocytes outside the body are called ART. The most common technique in ART is *in vitro* fertilization (IVF). "*In vitro*" means that the process occurs outside a living organism. Oocytes mature spontaneously in the ovary, and embryos develop into pregnancy inside the uterus. Still, fertilization of the oocytes

occurs in a petri dish[2]. The quality of gametes (sperm and oocytes), the laboratory setting, the conditions of the culture, and the knowledge of the clinical team are all important variables in the success of IVF procedures[3]. Microbiological contamination in IVF laboratories refers to the unintended introduction of microorganisms, such as bacteria, fungi, and viruses, into the sterile culture environment where gametes and embryos are handled and cultured[4]. Human gametes and their associated fluids contain numerous microorganisms. Biological fluids like semen, ovarian follicular fluid, fallopian tube washings, peritoneal fluid, and endometrial aspirates are all possible sources for microbial infection to enter the IVF environment, while contaminating embryos and the person carrying them[5–7].

Microorganisms have the potential to contaminate embryos created using ART at different phases of the process. Viral infections like the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are given special attention, and antiretroviral therapy procedures are specifically designed to reduce the risk of transmission of these frequently fatal viruses[8]. These microbes may originate from various places, such as the air in the lab, surfaces, instruments, culture media, and even the lab workers themselves. Contaminants can cause the culture environment to change, changing things like the pH and oxygen levels, or they

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can directly affect the quality of gametes and embryos by causing cytotoxic effects or inflammatory reactions[9]. These alterations may impair an embryo's capacity to develop, lower implantation rates, raise the chance of miscarriage, and, in some cases, cause infections in the mother or fetus[10].

Although there are many protocols and guidelines for best practices in IVF laboratories to minimize the risk of introducing unwanted agents, there are no standardized protocols for detecting and monitoring other potential sources of bacterial and fungal contamination, such as biological fluids and environmental air[11]. Furthermore, while current guidelines require IVF clinics and laboratories to document all procedures, their annual reports rarely provide data on the prevalence of microorganisms. This lack of information makes it challenging to accurately determine the frequency of microbial contamination in ART laboratories[4].

Therefore, this review examines the sources of microbiological contamination in IVF laboratories, including environmental factors, contaminated culture media, and improper handling practices. It will also attempt to comprehend the effect of such contaminants on embryo quality, rates of implantation, and total success rates of IVF. An analysis of available literature on mechanisms through which microorganisms act on reproductive cells and embryos has further underlined the need for effective measures in contamination control by strict adherence to aseptic practices.

2. Methods

The search methodology for this narrative review involved conducting a comprehensive literature search using databases such as PubMed, Google Scholar, Scopus, and Embase, focusing on studies published within the last 15 years to ensure relevance. The search terms included keywords like "in vitro fertilization (IVF)," "assisted reproductive technologies (ART)," "microbiological contamination," and "embryo culture contamination." Both Medical Subject Headings (MeSH) and free-text terms were used to capture a broad range of studies. The review included articles written in English, focusing on microbiological contamination in IVF laboratories, sources of contamination, and the impact on IVF outcomes such as embryo quality, implantation, and pregnancy rates. Studies unrelated to IVF or not addressing contamination risks were excluded.

3. Sources of microbiological contamination in IVF labs

In ART, microbiological contamination can cause serious risks in IVF labs. Various sources of contamination can quickly compromise the gametes' or embryos' quality and culture conditions and are subsequently associated with various risks, such as low embryo

development, lack of fertilization, or infection of patients[12]. IVF laboratory air quality plays a critical role in microbial contamination. Airborne microorganisms, including bacteria, fungi, and viruses, may gain access to the laboratory from different sources such as heating, ventilation, and air conditioning (HVAC) systems, personnel, and materials brought into the laboratory[13]. Particulate matter is a combination of the minute solid particles and liquid drops where the size ranges within 1 to 100 microns in the air. Microorganisms like viruses, spores, and bacteria are also within <1 and 8 microns and present all over, in every non-living surface and in air suspension, so they also pose a possible source of contamination in an embryology lab. Volatile organic compounds (VOCs) are defined as any organic solid or liquid compound containing carbon that can evaporate at room temperature. They react with indoor ozone to create submicronic particles and some harmful by-products that contain toxic properties, which are potentially mutagenic[14]. Microbial contamination in IVF labs is frequently caused by human biological fluids, including semen, ovarian follicular fluid, peritoneal fluid, fallopian tube washings, and endometrial aspirates. During the collection or processing of these fluids, bacteria, fungus, and other microorganisms may be introduced into the lab setting[15]. *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus* species are among the bacteria that are frequently found in semen samples. If aseptic procedures are not followed, these germs can quickly contaminate culture media and lab equipment, whereas follicular fluid taken during oocyte retrieval could contain microorganisms that risk the laboratory's sterility, putting other samples at the chance of contamination and possibly impacting the IVF process overall[16]. In IVF labs, several microbial species are commonly found, posing risks to both sample integrity and the success of fertility treatments. The most prevalent microbe identified is *Enterococcus faecalis*. Other frequently detected microbes include *Streptococcus agalactiae*, *Escherichia coli*, *Mycoplasma hominis*, *Candida* species, and *Ureaplasma urealyticum*. These isolates could be isolated from semen samples, follicular fluid samples or even from vaginal or endocervical swabs[4].

4. Effects of microbiological contamination on IVF outcomes

The presence of bacteria, fungi, viruses, or any other laboratory environment microorganism may adversely affect gametes, embryos, and culture media, thus bringing a variety of related complications that may be detrimental to the outcomes of IVF[12].

Bacterial contamination during embryo culture is probably the most serious issue occurring in all IVF laboratories. Although its frequency is <1%, it is still a serious problem when it occurs and results in the availability of no embryos for transfer. The extent to

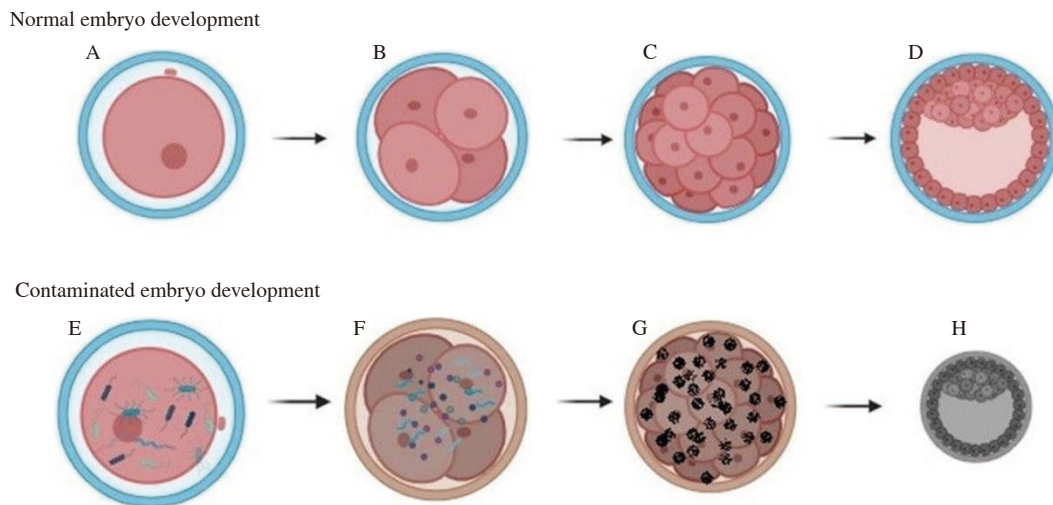


Figure 1. Normal embryo development (A-D) and contaminated embryo development (E-H) to distinguish the two rows clearly. A: Fertilisation; B: Cleavage; C: Blastulation; D: Blastocyst; E: Contaminated oocyte fertilisation; F: Contaminated cleavage stage embryo; G: Delayed blastulation; H: Embryonic arrest.

which microbial contamination causes damage in IVF procedures can be directly related to the financial and psychological burdens on patients[17].

Contamination can have a direct impact on embryo development and viability, as shown in Figure 1. Microorganisms or their byproducts can disrupt essential developmental processes, resulting in compromised embryo growth, lower rates of blastocyst formation, and diminished overall embryo quality[18].

Research has demonstrated a connection between microbiological contamination and decreased rates of successful implantation and pregnancy. The presence of specific bacteria or their by-products in the culture environment can hinder the embryo's capacity to implant effectively in the uterus, thereby lowering the likelihood of achieving pregnancy[19].

5. Mechanism of microbial impact on reproductive cells

Microbial contamination has been linked to a higher risk of miscarriage in IVF pregnancies. Moreover, such contamination can cause infections in either the mother or the developing fetus, potentially leading to severe complications[20]. At present, the technique most widely applied in the face of bacterial contamination involves washing embryos with an antibiotic-containing medium to attempt to remove microorganisms from the surface of embryos[21]. Microbiological contamination can have a significant, negative impact on various aspects of the IVF process and its outcomes[22]. Certain microorganisms produce toxins or metabolites that can directly harm gametes and embryos. These cytotoxic effects can result in cell death or disrupt cellular functions essential for normal embryo development[6]. Microbial growth in culture media disrupts pH levels, oxygen concentrations, and other critical parameters of the culture environment. These alterations can create suboptimal

conditions for embryo development, even if the microorganisms do not contact the embryos directly[23]. The presence of microorganisms or their components in reproductive cells can provoke inflammation. This inflammation can disrupt normal cellular processes with the end result of potentially impairing embryo development and implantation[24]. The uterus is highly responsive to even slight changes during pregnancy, and bacterial infections can lead to pregnancy loss. While less frequent now due to vaccines, *Brucella abortus* remains a pathogen associated with causing abortions. Other pathogens, such as *Listeria monocytogenes*, *Salmonella dublin*, and *Campylobacter* species, operate similarly, but the time between infection and pregnancy loss can extend up to 3 months[25]. Bacteria seem able to modify the environment surrounding sperm, oocytes, and embryos, thereby affecting the essential processes necessary for sustaining pregnancy. The conditions in which an oocyte matures are crucial for determining its quality and its potential to be successfully fertilized[26]. Various bacteria, including *Escherichia coli*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*, have been shown to impact fertility in both men and women. These pathogens may cause infections that lead to inflammatory responses, damage to reproductive tissues, and alteration in the microbiome. For instance, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are associated with pelvic inflammatory disease, which can lead to fallopian tube damage, increasing the risk of infertility and ectopic pregnancy in women. Similarly, *Ureaplasma* and *Mycoplasma* infections can alter sperm quality, reduce sperm motility, and impair fertilization capabilities in men. These microbial infections not only affect natural conception but also pose a challenge for ART, where microbial contamination can negatively influence IVF outcomes, embryo quality, and pregnancy rates, as shown in Figure 2. Proper diagnosis and treatment of these infections are crucial for improving reproductive health and IVF success rates[27].

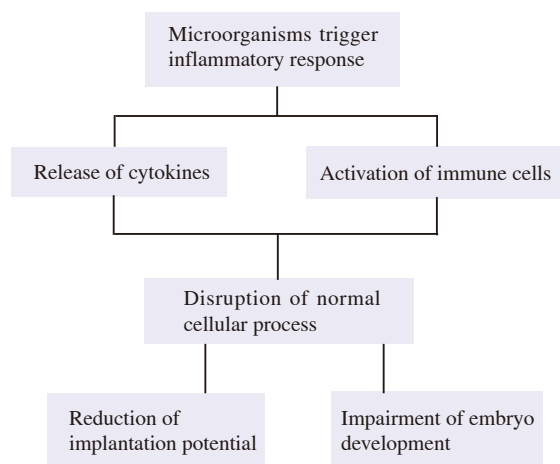


Figure 2. The flowchart illustrates how a microorganism can trigger an inflammatory response and its subsequent effects on cellular processes, particularly regarding reproduction.

6. Preventive measures

The primary purpose of embryo culture is to provide an optimal environment that supports the health and developmental potential of embryos while reducing the risk of any unintended stress caused by the procedure. Since the inception of IVF in the 1970s, numerous advancements and improvements have been made to the culture system to increase the number of high-quality embryos suitable for transfer[28]. Minimizing contamination risks in IVF labs requires a comprehensive approach, beginning with the lab's design and infrastructure. Advanced air filtration systems, like high-efficiency particulate air (HEPA) filters and positive-pressure ventilation, are essential to maintain a sterile airflow and prevent the entry of airborne contaminants[29]. Strict environmental controls, such as regulating temperature, humidity, and air quality, are crucial for minimizing the risk of microbial contamination and growth. Moreover, routine microbiological monitoring of air, surfaces, and equipment using validated techniques like swabbing and air sampling helps detect contamination early, allowing for timely corrective action[30]. Contaminated embryos are typically treated by repeatedly washing them. Currently, the culture media used in ART embryology labs contain antibiotics, and most bacterial contaminants can be eliminated using techniques such as gradient centrifugation and swim-up methods[31]. A key factor in preventing microbial contamination in IVF labs is the sterilization of equipment, culture media, and consumables. Essential methods include autoclaving, UV sterilization, and aseptic techniques when preparing culture media. Using high-purity reagents and materials is crucial, with single-use items favoured to prevent cross-contamination. Additionally, rigorous adherence to validated cleaning protocols for surfaces, incubators, and workstations is vital to ensure no residues remain

that could promote microbial growth[32].

The review exhibits few limitations in its coverage of microbiological contamination in IVF laboratories. While bacterial contamination is extensively discussed, there is insufficient attention given to viral and fungal contaminants, equally important threats to IVF success. The mechanistic aspects of how different microorganisms interfere with embryo development are presented superficially, lacking detailed molecular and cellular pathways through which these organisms compromise embryonic growth and viability.

7. Conclusions

The microbiological contamination of IVF laboratories is a serious menace to the success of assisted reproductive technologies. Contamination from various sources, as reviewed in this paper, includes biological fluids, air, and equipment surfaces that can adversely affect gametes, embryos, and the overall culture environment, with possible outcomes of poor embryo development, lower implantation rates, and increased risks for infection and miscarriage. However, despite all these preventative measures—air filtration systems, monitoring of the environment, plans of sterilization, and antibiotics—there is a big deficiency in the standards of IVF laboratories regarding the issue of contaminant detection and elimination. Therefore, strict asepsis practice, routine monitoring, and introducing new methods of contamination control are absolutely necessary to improve IVF results. Future studies need to be done in the direction of refinement of these strategies, enhancing detection techniques, and uniform preventive protocols to assure quality embryos for successful results in IVF procedures.

Conflict of interest statement

The authors declare no competing interests.

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Authors' contributions

Sudheer Singh contributed to the conception or design of the work, drafting of the work, approval of the final version of the manuscript, and agreed on all aspects of the work. Nancy Nair contributed to the literature review, revision of the manuscript for important intellectual

content, approval of the final version of the manuscript, and agreed to all aspects of the work. Akash More contributed to the literature review, revision of the manuscript for important intellectual content, approval of the final version of the manuscript, and agreed to all aspects of the work. Neeraj Vishwakarma contributed to the review and revision of the manuscript for important intellectual content, offering a lot of feedback and revision to make it rich in intellectual content. Avanti Kalbande was quite instrumental in the review, as he handled the statistical analysis by organizing, and interpreting data through advanced methods. Her work was ensuring the accuracy and clarity of the results to make the review credible and impactful.

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References

- [1] Graham ME, Jelin A, Hoon AH Jr, Wilms Floet AM, Levey E, Graham EM. Assisted reproductive technology: Short- and long-term outcomes. *Dev Med Child Neurol* 2023; 65(1): 38-49. doi: 10.1111/dmcn.15332.
- [2] Morato ALC, Verruma CG, Furtado CLM, dos Reis RM. *In vitro* maturation of oocytes: What is already known? *Biol Reprod* 2024; ioae147. doi: 10.1093/biolre/ioae147.
- [3] Cairo Consensus Group. 'There is only one thing that is truly important in an IVF laboratory: Everything' Cairo Consensus Guidelines on IVF Culture Conditions. *Reprod Biomed Online* 2020; 40(1): 33-60. doi: 10.1016/j.rbmo.2019.10.003.
- [4] Borges ED, Berteli TS, Reis TF, Silva AS, Vireque AA. Microbial contamination in assisted reproductive technology: Source, prevalence, and cost. *J Assist Reprod Genet* 2020; 37(1): 53-61. doi: 10.1007/s10815-019-01640-5.
- [5] Pelzer ES, Allan JA. The isolation and identification of microorganisms in the reproductive environment: The potential impact on the IVF culture system and on IVF outcomes. *J Clin Embryol* 2012; 15(3): 44-53.
- [6] Pelzer ES, Allan JA, Waterhouse MA, Ross T, Beagley KW, Knox CL. Microorganisms within human follicular fluid: Effects on IVF. *PLoS One* 2013; 8(3): e59062. doi: 10.1371/journal.pone.0059062.
- [7] Domes T, Lo KC, Grober ED, Mullen JBM, Mazzulli T, Jarvi K. The incidence and effect of bacteriospermia and elevated seminal leukocytes on semen parameters. *Fertil Steril* 2012; 97(5): 1050-1055. doi: 10.1016/j.fertnstert.2012.01.124.
- [8] Alqawasmeh OAM, Jiang XT, Cong L, Wu W, Leung MBW, Chung JPW, et al. Vertical transmission of microbiomes into embryo culture media and its association with assisted reproductive outcomes. *Reprod BioMed Online* 2024; 49(2). doi: 10.1016/j.rbmo.2024.103977.
- [9] Wale PL, Gardner DK. The effects of chemical and physical factors on mammalian embryo culture and their importance for the practice of assisted human reproduction. *Hum Reprod Update* 2016; 22(1): 2-22. doi: 10.1093/humupd/dmv034.
- [10] Cimadomo D, Fabozzi G, Vaiarelli A, Ubaldi N, Ubaldi FM, Rienzi L. Impact of maternal age on oocyte and embryo competence. *Front Endocrinol (Lausanne)* 2018; 9: 327. doi:10.3389/fendo.2018.00327.
- [11] Mortimer D, Cohen J, Mortimer ST, Fawzy M, McCulloh DH, Morbeck DE, et al. Cairo consensus on the IVF laboratory environment and air quality: Report of an expert meeting. *Reprod Biomed Online* 2018; 36(6): 658-674. doi: 10.1016/j.rbmo.2018.02.005.
- [12] Du F, Luo H, Li R, Zhu Y, Zhao J, Yuan P, et al. Microbe contamination during embryo cultures in an *in vitro* fertilization-embryo transfer system. *Ann Obstet Gynecol* 2021; 4(1): 1015.
- [13] Sciorio R, Rapalini E, Esteves SC. Air quality in the clinical embryology laboratory: A mini-review. *Ther Adv Reprod Health* 2021; 15: 1-11. doi: 10.1177/2633494121990684.
- [14] Esteves SC, Varghese AC, Worriolow KC. *Clean room technology in ART clinics: A practical guide*. Boca Raton: CRC Press; 2016.
- [15] Schenk M, Huppertz B, Obermayer-Pietsch B, Kastelic D, Hörmann-Kröpfl M, Weiss G. Biobanking of different body fluids within the frame of IVF—A standard operating procedure to improve reproductive biology research. *J Assist Reprod Genet* 2017; 34(2): 283-290. doi: 10.1007/s10815-016-0847-5.
- [16] Eini F, Kutenaei MA, Zareei F, Dastjerdi ZS, Shirzeyli MH, Salehi E. Effect of bacterial infection on sperm quality and DNA fragmentation in subfertile men with leukocytospermia. *BMC Mol Cell Biol* 2021; 22(1): 42. doi: 10.1186/s12860-021-00380-8.
- [17] Li R, Du F, Ou S, Ouyang N, Wang W. A new method to rescue embryos contaminated by bacteria. *F S Rep* 2022; 3(2): 168-171. doi: 10.1016/j.xfre.2022.05.002.
- [18] Vajta G, Rienzi L, Cobo A, Yovich J. Embryo culture: Can we perform better than nature? *Reprod BioMed Online* 2010; 20(4): 453-469. doi: 10.1016/j.rbmo.2009.12.018.
- [19] Pelzer ES, Allan JA, Theodoropoulos C, Ross T, Beagley KW, Knox CL. Hormone-dependent bacterial growth, persistence and biofilm formation – A pilot study investigating human follicular fluid collected during IVF cycles. *PLoS One* 2012; 7(12): e49965. doi: 10.1371/journal.pone.0049965.
- [20] Franasia JM, Scott RT. Endometrial microbiome. *Curr Opin Obstet Gynecol* 2017; 29(3): 146. doi: 10.1097/GCO.0000000000000357.
- [21] Litscher ES, Wassarman PM. Zona pellucida proteins, fibrils, and matrix. *Annu Rev Biochem* 2020; 89: 695-715. doi: 10.1146/annurev-biochem-011520-105310.
- [22] Cottell E, Harrison RF, McCaffrey M, Walsh T, Mallon E, Barry-Kinsella C. Are seminal fluid microorganisms of significance or merely contaminants? *Fertil Steril* 2000; 74(3): 465-470. doi: 10.1016/s0015-0282(00)00709-3.
- [23] Swain JE. Optimizing the culture environment in the IVF laboratory:

- Impact of pH and buffer capacity on gamete and embryo quality. *Reprod Biomed Online* 2010; **21**(1): 6-16. doi: 10.1016/j.rbmo.2010.03.012.
- [24]Defreyne J, Van Schuylenbergh J, Motmans J, Tilleman KL, T'Sjoen GGR. Parental desire and fertility preservation in assigned female at birth transgender people living in Belgium. *Fertil Steril* 2020; **113**(1): 149-157. e2. doi: 10.1016/j.fertnstert.2019.09.002.
- [25]Owens CE, Daniels KM, Ealy AD, Knowlton KF, Cockrum RR. Graduate student literature review: Potential mechanisms of interaction between bacteria and the reproductive tract of dairy cattle. *J Dairy Sci* 2020; **103**(11): 10951-10960. doi: 10.3168/jds.2019-18050.
- [26]Usman SF, Shuaibu IR, Durojaiye K, Medugu N, Iregbu KC. The presence of microorganisms in follicular fluid and its effect on the outcome of *in vitro* fertilization-embryo transfer (IVF-ET) treatment cycles. *PLoS One* 2021; **16**(2): e0246644. doi: 10.1371/journal.pone.0246644.
- [27]Feng T, Liu Y. Microorganisms in the reproductive system and probiotic's regulatory effects on reproductive health. *Comput Struct Biotechnol J* 2022; **20**: 1541-1553. doi: 10.1016/j.csbj.2022.03.017.
- [28]Sciorio R, Rinaudo P. Culture conditions in the IVF laboratory: State of the ART and possible new directions. *J Assisted Reprod Genet* 2023; **40**: 1-17. doi: 10.1007/s10815-023-02934-5.
- [29]Graham ME, Jelin A, Hoon AH, Wilms Floet AM, Levey E, Graham EM. Assisted reproductive technology: Short- and long-term outcomes. *Dev Med Child Neurol* 2023; **65**(1): 38-49. doi: 10.1111/dmcn.15332.
- [30]Dubey S, Rohra H, Taneja A. Assessing effectiveness of air purifiers (HEPA) for controlling indoor particulate pollution. *Heliyon* 2021; **7**(9): e07976. doi: 10.1016/j.heliyon.2021.e07976.
- [31]Zheng T, Li Q, Chen N, Du P, Ye H. Analysis of the clinical outcomes of microbial contamination caused by environmental contamination of the embryology laboratory during IVF-ET treatment cycles. *BMC Pregnancy Childbirth* 2023; **23**: 190. doi: 10.1186/s12884-023-05516-6.
- [32]ESHRE Guideline Group on Good Practice in IVF Labs, De los Santos MJ, Apter S, Coticchio G, Debrock S, Lundin K, et al. Revised guidelines for good practice in IVF laboratories (2015). *Hum Reprod* 2016; **31**(4): 685-686. doi: 10.1093/humrep/dew016.