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REVIEW

Microbial contamination in assisted reproductive technology: source, prevalence, and cost

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Abstract

Even the strictest laboratories and clinics are prone to the occurrence of microbial contamination. In the case of in vitro fertilization (IVF) research and practice facilities, the number of possible sources is particularly vast. In addition to ambient air, personnel, and non-sterilized materials, follicular fluid and semen from patients are a very common gateway for a diverse range of bacteria and fungi into embryo cultures. Even so, reports of contamination cases are rare, what leads many clinics to see the issue as a negligible risk. Microbiological contamination may result in the demise of the patient's embryos, leading to additional costs to both the patient and the clinics. Regardless of financial loss, emotional costs, and stress levels during IVF are highly distressing. Other worrisome consequences include DNA fragmentation, poor-quality embryos, early pregnancy loss or preterm birth, and possible long-term damages that need further investigation. In this review, we aimed to shed a light on the issue that we consider largely underestimated and to be the underlying cause of poor IVF outcomes in many cases. We also discuss the composition of the microbiome and how its interaction with the reproductive tract of IVF-seeking patients might influence their outcomes. In conclusion, we urge clinics to more rigorously identify, register, and report contamination occurrences, and highlight the role of the study of the microbiome to improve overall results and safety of assisted reproduction.

Keywords Microbial contamination · Assisted reproductive technology · Microbiota · IVF · Follicular fluid · Semen

Introduction

Embryos generated by assisted reproductive technologies (ARTs) are susceptible to contamination by microorganisms at various stages of the process. Special attention is given to viral pathogens such as HIV, HBV, and HCV, with ART protocols directly aimed to minimize the risk of transmission of these often-fatal viruses [1, 2]. However, sperm, oocytes, and embryos are also affected by contaminants of bacterial and fungal origins with worrisome results [3–7]. Contamination may arise from ambient air in the workplace, reproductive tract microbiota of donors, or the inadvertent introduction of microorganisms during in vitro procedures [4, 8–12].

Human gametes and their accompanying fluids are known to carry a number of microorganisms. As such, biological fluids like semen, ovarian follicular fluid, Fallopian tube washings, peritoneal fluid, and endometrial aspirates are possible gateways to microbiological infection into the IVF system, risking contamination of embryos and its carrier [3, 8, 10, 11]. When transferred, embryos can carry microorganisms to the microenvironment of the uterus, altering the local microbiota and compromising implantation and survival during pregnancy [4, 8, 10]. Usually, some attention is given to the occurrence of microorganisms in our bodies only when they are associated with an active infection; however, the human body is in constant interaction with its personal ecological community of microorganisms, called microbiota, which have direct influence in many aspects of human physiology, including reproductive health. Research in this field is relatively incipient but recent studies have shown how this interaction might influence IVF results [13–16].

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As previously mentioned, endogenous sources are far from being the only way of microorganisms inside ART laboratories, as many reagents, devices, equipment, personnel, and even the environmental air represent a potential risk of contamination [4, 9, 12]. This scenario justifies the necessity of applying strict aseptic techniques in each procedure and manipulation step throughout the process. As affirmed in the latest consensus on IVF laboratory environment [17], exposure time of gametes to the outside, not controlled, environment should be kept at minimum. Nevertheless, even the strictest laboratories are prone to the occurrence of contamination cases [8].

While there are numerous protocols and guidelines for good IVF laboratory practice that aim to reduce the possibility of introducing an adventitious agent into the embryology laboratory, there are no standard protocols available to detect and monitor other sources of bacterial and fungal contamination, such as biological fluids and the environmental air [8, 17]. Moreover, although current guidelines demand that all IVF clinics and laboratories keep records of all procedures, their annual report rarely includes information about the prevalence of microorganisms, making the precise estimation of the frequency of microbial contaminations in ART laboratories quite difficult. The limited number of publications and case reports dealing with this subject suggests that contamination events are being largely underestimated [8].

Therefore, in this review, we summarize the most recent information regarding the influence of microorganisms, from both endogenous and exogenous sources, on IVF cycle outcomes. We also discuss about the incidence of confirmed contamination cases and what could be done to minimize the impact of such events to clinics and patients alike.

Methods/research criteria

Bibliographic search was made using relevant keywords in three international search engines/databases (PubMed, Scopus, and Google Scholar). Search terms included a combination of either "assisted reproduction technology" or "in vitro fertilization" with "microorganisms" or "microbiological contamination"; any combinations were then paired with terms "outcome," "prevalence," "incidence," and "costs." Only human studies were included, and recent review articles were prioritized to avoid the inclusion of redundant information. Most cited articles are written in the English language, but works in Portuguese, Spanish, French, Russian, and Chinese were also included.

Microorganisms in the reproductive tract

The human body hosts a community of microorganisms that is even larger than the number of human cells [18]. Collectively, the communities of microorganisms that inhabit each organism are called "microbiota", while the interactions of those communities with the human physiology, including their products and catalog of genes, are called "microbiome" [16]. Recently, there has been great interest in the study of these interactions and, as more is learned, evidence strongly suggest that most (if not all) organs have their physiologic functions greatly influenced by its local microbiota [13]. Although not a long time ago many components of the reproductive tract of both men and women were believed to be sterile, it is now known that they also host microbiota of their own [19, 20]. It has been recently reported that the female reproductive tract microbiota accounts for approximately 9% of the total bacterial load in humans [15]. The use of modern molecular techniques such as next-generation sequencing (NGS) of DNA and the development of the Human Microbiome Project have been paramount to the precise study of microorganisms and how they interact with the human physiology at the different body sites, even allowing for the construction of a catalog of the microbial diversity inhabiting the female reproductive tract [15].

Influence of the microbiome on female fertility

It is well established that clinically symptomatic infection associated with inflammation affects reproductive function [13, 16]. However, it has been reported that subtle changes in the human microbiota, often not clinically detected, might play an important role in reproductive health, influencing various disease states in other body sites [16]. In addition, recent studies suggest that infertile women harbor a differential reproductive tract microbiota compared to healthy and fertile women [15].

The correlation between the isolation of some bacterial groups (e.g. *Chlamydia*, *Gonococcus*, *Enterococcus spp.*) from the female reproductive tract with poor pregnancy outcomes and increased associated risks such as higher miscarriage rates has been reported [15, 21]. Similarly, evidence shows a link between the absence of these bacterial groups and the presence of *Lactobacillus* and better results [15]. Recently, the vaginal microbiome has been categorized into five groups considering its predominant species. Four of them were classified as "Lactobacillus-dominated" (LD) with one of the following *Lactobacillus spp*. being the predominant representative (*Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *Lactobacillus jenseii*), while the remaining group is classified as "non-Lactobacillus dominated" (NLD), with a more diverse number of genera being present, including *Gardnerella*, *Pretovella*, *Corynebacterium*, *Atopobium*, *Megasphaera*, and *Sneathia*. Interestingly,

healthy women with high rates of pregnancy success were consistently associated with the LD groups [15, 16].

The uterine microbiome has also been investigated. It demonstrated an even stronger association between the abundance of *Lactobacillus* and reproductive outcomes. The percentage of Lactobacilli was clearly associated with reproductive outcomes; implantation, pregnancy, and live-birth rates were all higher in women belonging to LD groups than those in the NLD [15]. A similar pattern was observed in studies that analyzed the endometrial fluid composition. The results indicate that the majority of patients going through ART had an NLD microbiome, suggesting that the LD endometrium might favor implantation [15, 22, 23].

In a series of studies, Pelzer et al. [10, 20, 24] demonstrated that the human follicular fluid is also colonized by a number of microbial species that are not necessarily associated with an active infection. They observed that microorganisms isolated from follicular fluid could persist for more than 28 weeks without any exogenous nutrient. Most of bacterial species identified within fluid samples were anaerobes and generally not targeted by penicillin, streptomycin, or gentamicin, the antimicrobials traditionally included in IVF culture media. Ultimately, the presence of certain bacterial species was linked to adverse IVF outcomes.

Similarly, Ricci et al. [25] showed that genital tract pathogens could be associated to IVF failure. A study with 285 infertile couples revealed that 46.3% of them had an ongoing asymptomatic genital tract infection prior to IVF treatment. Microbiological analysis was conducted on a total of 855 samples (of which 285 were semen specimens, 285 vaginal swabs, and 285 endocervical swabs) and found 195 clinical strains belonging to 25 different microbial species. *Enterococcus faecalis* represented the most common finding with a prevalence of 24.1% (47/195). Other frequently identified microbial species included *Streptococcus agalactiae* (15.9%), *Escherichia coli* (15.4%), *Mycoplasma hominis* (10.8%), *Candida spp*. (8.2%), and *Ureaplasma urealyticum* (5.1%). Co-presence of two different pathogens was detected in 14 semen specimens and in 16 vaginal or endocervical swabs, while the simultaneous presence of three pathogens was observed in three samples. The same study also investigated if specific genital tract pathogens could be associated to IVF failure, looking for a correlation between microbiological testing results and IVF outcomes [25]. Success rates were slightly higher in non-infected than in infected couples under ART treatment. Microbiological data indicated that specific pathogens (*E. faecalis*, *U. urealyticum*, *M. hominis*, *G. vaginalis*, *E. coli*) were more prevalent in unsuccessful (IVF−) than successful (IVF+) couples; however, they did not find significant differences when each pathogen was tentatively associated with IVF outcome. Analysis was performed by examining couples positive for groups of genital tract pathogens after sequential exclusion of the pathogens that seemed not to affect IVF outcome. The group constituted of *E. faecalis*, *U. urealyticum*, *M. hominis*, *Gardnerella vaginalis*, and *Trichomonas vaginalis* was more prevalent in IVF− than IVF+ couples, but the differences were not significant. Elimination of *T. vaginalis* showed that prevalence of the microbial group was significantly higher in IVF− (36.3%) compared to IVF+ (16.7%) couples. Excluding *G. vaginalis*, the smallest infectious group significantly associated with IVF failure included *E. faecalis* and/or *U. urealyticum* and/or *M. Hominis*. Analysis of the IVF+ couples showed that 30/35 (85.7%) were negative to microbiological testing, whereas out of the couples infected with *E. faecalis* and/or *U. urealyticum* and/or *M. hominis*, only5/67 (7.5%) obtained a successful IVF. Among the IVF couples positive for this microbial group, *E. faecalis* and *U. urealyticum* were found in approximately 90% of cases, whereas *M. hominis* was detected in all the couples with a poor IVF outcome [25].

Pelzer et al. [10] and Ricci et al. [25] showed that determined microorganisms in the female genital tract negatively affected embryo transfer (ET) and pregnancy rates during ART, leading to early pregnancy loss or preterm birth, an increasing global problem. Their presence might be either pre-existing or acquired during IVF procedures, highlighting the importance of a more in-depth study about this correlation.

The co-culture of some of the bacterial species colonizing human follicular fluid in vivo may cause DNA fragmentation in mouse oocytes following 12 h of in vitro incubation, suggesting that it is one of the mechanisms affecting oocytes and/or embryos quality, leading to poor IVF outcomes [10]. Another hypothesis is that the presence of high concentrations of endotoxins (components of gram negative Bacteria) induces a reaction of Th1 inflammatory cells. Th1 cells may predispose a hostile endometrial environment [13].

As studies advance, it is becoming clear that the many bacterial species in the vagina greatly influence reproductive health and pregnancy outcomes [26]. Although the composition of each woman's microbiome is unique and highly variable, a current hypothesis suggests that it can be altered by exogenous factors, such as controlled ovarian hyperstimulation required for IVF, with direct impact on reproductive health outcomes [13, 26]. Much research is still needed, however, to assess the impact of external factors and how they could be controlled [26]. Taken together, recent studies confirm the importance of investigating the female microbiota prior to an IVF cycle looking for microorganisms associated with poor outcomes using cultures, as well as using more advanced technology to characterize the complete microbiome associated with the best pregnancy outcomes [14].

Influence of the microbiome on male fertility

The male reproductive tract has also been shown to possess an active microbiome and might also host infections; this is

supported by the presence of bacteria in seminal fluid samples that might persist even after applying washing techniques [11, 19, 27]. A recent study revealed a high prevalence of gram-positive cocci in semen samples, such as *Staphylococcus spp.* (80%) and *Viridans streptococci* (50%). In this case, microorganisms were not observed in semen samples after a micro swim-up procedure for sperm preparation in intracytoplasmic sperm injection (ICSI) cycles [28]. A study by Qing et al. [29] used a novel testing method based on an RNA-detection technique to identify the presence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* on urine samples of men diagnosed with infertility and correlate it with semen parameters. Their results showed a relative high prevalence of bacterial species *U. urealyticum* and *M. genitalium* that could be associated with impaired fertility trough damage caused to sperm DNA [29]. Elevated bacteriospermia in semen has been previously correlated to DNA fragmentation and poorer seminal parameters overall, which in turn are connected to some types of male infertility [11]. Also of interest, a study showed that IVF cycles are more prone to yeast contamination in comparison with ICSI, suggesting a great influence of paternal source in infection cases, which may be eliminated during ICSI preparation [7].

However, current available data on the prevalence of microorganisms and their influence on male fertility are not conclusive. In an also very recent and comprehensive review, Jue and Ramasamy [30] concluded that while positive semen culture for some of the most common microorganism was correlated with decrease in some aspects of semen quality among fertile men, there was no significant difference between the incidence observed among fertile men and men seeking ART. This suggests that there may not be an indication for routine semen culture prior to IVF or cryopreservation [30]. It is important to note that infertility is a very complex condition that involves multiple factors. Further studies are needed in order to better understand this relationship and change the approach from a focus on pathology to physiology; we may find that the knowledge of the precise microbiome and the ability to manipulate it may dramatically improve reproductive outcomes both in vitro and in vivo [16].

Antibiotics prophylaxis

Administration of antibiotics is a common way of dealing with or preventing unwanted microbiological infections. Considering the invasive nature of some gynecologic procedures carried out during ART, the use of antibiotics has been proposed to decrease the possibility of seeding the upper genital tract with microorganisms from the skin, vagina, or endocervix [31]. However, despite being routinely offered by many clinics, recent review studies concluded that antibiotic prophylaxis is generally not recommended for the majority of patients undergoing ART-related procedures [32–34]. The exception being those affected by risk factors such as history of pelvic inflammatory disease (PID), endometriosis, or multiple prior pelvic surgeries [33]. For healthy patients, antibiotic prophylaxis at the time of ET did not influence clinical pregnancy rates [32]. In addition, it seems that the use of broad-spectrum antibiotics may decrease the number of Lactobacillus species, which have been associated to successful implantation outcomes [35]. Similarly, while antibiotic treatment is efficient and significantly increased sperm concentration in men with active infections [36], prophylaxis for healthy men is also not recommended since the prolonged use of antimicrobials is one of the causes of male infertility [37].

IVF and embryo culture protocols include the prophylactic use of antibiotics, such as penicillin, streptomycin, or gentamicin as media supplements to reduce infection rates. However, embryos cultured in antibiotic-free media had a higher rate of cleavage, and the number of embryos that reached the blastocyst stage was also significantly higher as compared with those embryos cultured in medium containing penicillin and streptomycin, irrespective of the concentration [38]. While gentamicin, an aminoglycoside and inhibitor of protein synthesis, is currently presented as the safest option, its addition to culture media has not improved embryo development. Further studies are needed to establish the optimal use of antibiotics for increased efficiency during IFV cultures and, meanwhile, the best recommendation is to minimize the risk of contamination from external sources [39–41].

Microorganisms from external sources

It is not rare for even the strictest laboratories to suffer with the occurrence of contamination cases. It is important to note that, to some extent, the presence of microorganisms in IVF culture system seems to be the rule rather than the exception. As previously discussed, it is possible that follicular fluid and/or semen bring microorganisms to all cultures [42, 43]. Sometimes, washing procedures may dilute microbial colonies and prevent them from being visible in the form of flocculation or, most likely, culture conditions are not favorable for large colony formation. This, however, does not mean microorganisms are not present. Especially when the possibility of contamination through external sources is considered.

The main external sources of microorganisms are the personnel, environmental air, and contaminated materials. Bacteria and fungi are both known to thrive in all sorts of environments and can be easily carried into the laboratory from external areas by people and instruments [44]. Mycoplasma, a genus of extra small bacteria, is especially difficult to detect and control. The use of materials from animal origins, such fetal bovine serum, can be a source of mycoplasma species *M. arginini and A. laidlawii*,

while personnel, especially by the use of mouth-pipetting techniques, are the main sources of *M. orale*, *M. fermentans*, and *M. hominis*. The last three species represent more than half of all mycoplasma infections in cell cultures in general [45].

The human skin is also a possible source of contamination. It is colonized with *Staphylococcus epidermidis* and *Corynebacterium spp.* that "snows down" constantly along skin cells at a rate of 30,000 to 40,000 cells per minute. The shed skin cells make up for most of the dust particles that are swept up during routine clean ups in the laboratory [46]. Because many microorganisms can attach themselves to these particles, it is of utmost importance to reduce the number of particles; this can be achieved with the use of high efficiency filtration systems [47].

The laboratory air quality is a factor that plays a significant role in IVF outcome [4, 9, 47]. Improvements in the environmental conditions and air quality have been associated with overall positive effects on clinical outcomes [48]. The impact of volatile organic compounds (VOC) is well documented and have been pivotal to necessity of implementing air filtration system improvement measures, including replacing the air filtration system. Equally important are the actions to minimize the presence of microorganisms and subsequent contamination of cultures [47, 48]. Given the importance of the air quality for an IVF laboratory, regulatory guidelines have been described by the European Union and Brazil to ensure specific requirements for air quality control [49, 50]. These state that the laboratory should be kept clean and free of microbial contamination, the air in incubators must be purified, and incoming gases should be filtered to minimize bacteria and fungi contamination. To reach the required quality, all the products used throughout the process should be chosen carefully aiming not to expose embryos to sterilizing agents or its residues, among many other recommendations [51].

Recently, a Brazilian fertility clinic totally fulfilled the Brazilian directive on air quality standards and, in result, improved many aspects of its service (e.g., increased live birth rates, decreased miscarriage rates) [9]. This demonstrates that implementation of strict air quality control is advantageous and allows the optimization of outcomes [9, 47]. Despite the seemingly win-win scenario, there are those who are critical of such strict guidelines [52]. Therefore, it is important to consider all variables that influence laboratory environment in order to improve the decision-making process that may, in turn, result in the improvement of clinical outcomes. In this sense, more controlled studies on air quality as well as more precise guidelines on how to implement air quality and monitoring practices are needed [46, 47].

Impact of contamination cases on IVF

An important factor that should be considered to aid the decision-making process in ART clinics is the assessment of the real incidence of contamination events and its impact. Even though most current guidelines demand that all IVF clinics and laboratories keep records of every procedure in detail, most reports do not include information about contamination events, thus not allowing the precise estimation of the frequency of microbial contaminations in ART laboratories. The lack of standardization between guidelines and protocols regarding sterility requirement levels and especially monitoring practices for microbiological control adds to the problem. Considering the prevalence of microorganisms in the environment as well as in both follicular and seminal fluids, it is logical to assume that contamination cases are being largely underestimated [8, 53, 54].

Recently, bacterial contamination was detected in the ET catheter (mainly by *E. coli*, *Staphylococcus spp.*, and *Streptococcus* spp.) and was associated with a reduction in the clinical pregnancy rate [12]. Additionally, yeast-colonized embryo dishes and detrimental effects in IVF have been evidenced in different reports [5, 7, 55]. According to data described by Klein et al. [7], the confirmed incidence of yeast contamination was significantly higher in IVF cycles as compared with in ICSI cycles and was strikingly consistent with the reports by Kastrop et al. [8]. An incidence of 0.18% in all ART cycles, compared with 0.17% (24/13,977), and 0.28% in IVF-only cycles, compared with 0.22% (24/11,051) was observed by Missmer and Kastrop studies, respectively.

In one of the very few studies regarding the number of contamination events, Kastrop et al. [8] reported that an 8-year observation on their own laboratory showed an incidence of 0.86% of microbial contamination among all IVF procedures (0.68%, if ICSI procedures, that did not present any case of contamination, are included). The incidence ranged from 0.40% to as high as 1.30% during the observation period. These numbers are in accordance with previous studies that reported incidences of 0.35% [42] and 0.69% [5]. In 2004, a study in Brazilian IVF clinics and laboratories reported a prevalence of 4.8% [6] of bacteria and fungi contamination despite following sanitary steps and supplementing culture media with antibiotics.

In a similar retrospective study of the association between yeast-contaminated media with IVF outcomes, Klein et al. [7] analyzed all oocyte retrievals performed between the years of 1998 and 2006 in order to confirm possible yeast contamination of embryo culture that were suspected based on microscopic evaluation any time between the fertilization check and ET. Out of the 11,816 oocyte retrievals performed during the 8-year period, 51 (0.43%) were suspected to have yeast contamination after microscopic examination of spent media. Previously acquired microbiological data were available for

26 of the in vitro production cycles in which these oocytes were used and the presence of yeast contamination was confirmed in 21 of them, which represented a 0.18% overall incidence of confirmed yeast contamination. Of the 21 yeast-contaminated cycles, 20 underwent day 3 ET, and one underwent a day 5 ET. In seven of the 21 cycles, yeast contamination was noted at the fertilization check on day 1. In the remainder of the cycles, contamination was noted on day 3 (n .13) or day 4 (n .1, in the case having a day 5 transfer). In the absence of a program policy regarding whether to transfer contaminated embryos, the decision to transfer was made on a "case-by-case" basis after discussion between the physician and patient [7].

The American Society for Reproductive Medicine (ASRM) and European Society for Human Reproduction and Embryology (ESHRE) provide guidelines for good IVF laboratory practice, including ambient air monitoring [8, 17]. However, there are no guidelines or standard protocols to detect and monitor other sources of microbial contamination, such as biological fluids. In order to reduce or eliminate the potential of introducing an adventitious agent into the embryology laboratory, critical steps that pose the highest biosecurity risk, such as biological fluids handling and air quality assessment, have to be correctly monitored. Different approaches have been proposed to reduce the microbial contamination introduced by biological fluids into the human embryo culture. The micro swim-up (MSU) applied in ICSI cycles may contribute to prevent infection problems that could arise from the normal microbiota of the semen, perhaps implicating a paternal source that may be eliminated during ICSI. However, its efficacy in terms of fertilization, embryo development, and pregnancy remain to be investigated. The transfer of zona-free frozen blastocysts that were previously contaminated during IVF culture has also been suggested as a successful approach to prevent effects of contamination on pregnancy rates, but there is not enough data to support the safety of this technique [56].

Although ET and pregnancy are the usual parameters considered when IVF outcome is discussed, only a longitudinal study following IVF-born individuals would be able to assess all possible effects of bacterial and fungal contamination during embryo production [57]. Epigenetics studies give us some hints of possible consequences. According to Bierne et al. [58], bacterial pathogens can be considered as potential epimutagens, reshaping the epigenome with long-lasting effects on host cells. It is known that epigenetics effects on spermatozoa might lead to oligozoospermia, one of the most common causes of male infertility [59]. The consequences, should the target of epigenetic changes caused by contamination be the embryo, could be even more critical [57].

Costs

Although the occurrence of microbial contamination seems to be low, taking into account the total number of annual procedures, the absolute number of contamination cases raises an alarm, especially considering that it may cause serious damage to cultured oocytes or embryos, resulting in cancelation or delaying of a fresh ET. This scenario represents a big waste of money and resources that ultimately affects the accessibility of ART procedures.

The damage caused by microbial contamination in ART procedures can be translated directly into costs to the laboratory and clinic that might end up affecting people seeking the service. The cost of microbial contamination can be estimated from the prevalence of these contaminations (about 0.7%), the number of IVF cycles per year, and the cost of the IVF procedure. Approximately 284,385 IVF cycles were carried out in the USA in 2017 [60]. Considering these numbers, it is estimated that nearly 1990 cycles resulted in microbiological contamination without ET in most cases. This means that the estimated cost for these contaminations per patient would be about $10,000 and the overall cost of about $19.9 million. In addition, these estimates do not include the costs to women that did not achieve pregnancy due to potentially non-detectable contamination of embryo cultures which might have had reduced the pregnancy rates. Taking these into account, the overall effect is impressive. In 2017, over 75,000 cycles of IVF were carried out in 119 licensed fertility clinics across the UK and the cost of IVF is usually around £5000 (US$6500) per cycle of treatment. Using the same parameters to estimate the cost for microbial contamination in the USA, an overall cost in UK is about US$3.4 million. In Brazil, on average, each IVF cycle costs R$ 15,000 (US$4000). Considering 36,370 IVF cycles reported in 2017 and the estimated rates of 4.8% of microbial contamination, the total cost of microbiological contamination of IVF treatments per year reaches nearly R$ 26.1 million (US$7 million), a non-negligible cost for IVF clinics and patients. Information about the impact of microbial contamination of IVF worldwide is scarce. Of greater concern, IVF treatment has the potential to be an emotionally and financially exhausting experience to patients. Oocytes and embryos have an extremely high emotional value, and it is hard to explain to patients undergoing ART procedures that their cycle is being canceled by a microbial contamination of embryo culture dish [61, 62].

Thus, we consider that the development of a worldwide database with information about the microbiological monitoring of every IVF cycle and annual reports of contamination cases, including the number of delayed and canceled cycles, could be useful to better understand and manage the impact of microbial contamination on ART as well as to assist clinics and patients to make better decisions during the process.

Research limitations

This review is not without limitations. Our work covered a relatively new topic; therefore, previous literature was scarce. This means that, in order to cover all relevant topics, new studies and some from not very well-known publications were included. In addition, the discussion on some of the topics was based on the research made by very few groups, which ended having multiple citations throughout the text. It is important to note, however, that all included references were indexed in trusted databases.

Conclusions

Currently, there is no detailed survey on the incidence of contamination events in ART clinics and laboratories. Annual reports tend not to include this information, and studies compiling this type data are rare. Although the presence of certain groups of bacteria has been correlated to determined IVF outcomes, precise information regarding the possible influence of microorganisms in the culture media during in vitro production of human embryos or in patient microbiome at the time of the procedure is also lacking. Therefore, continued efforts should be made towards the understanding of the relationship between microorganisms and exposed gametes in the ART field in order to identify possible threats and find a way to stop them without interfering with embryo quality and subsequent development.

Despite being very useful and informative, data derived from culture-based approaches to detect and identify microorganisms should be interpreted carefully since the proportion of identified species using such technique is very limited when compared to more advanced technology. The use of more refined techniques, such as 16S rRNA sequencing, in order to achieve a complete prospective microbiological survey of the tissues and fluids involved in IVF is encouraged. Furthermore, longitudinal studies should be carried to identify possible adverse effects that embryos conceived in vitro in contact with microorganism infections carried to adult life. Meanwhile, new approaches to improve or modify the preparation of gametes should also be discussed. These should be developed with a focus on improving the safety of ART by reducing the subtle and overt-contamination impacts in laboratories and embryo cultures. Although the risks might seem negligible, contamination events might have a big impact for a clinic on the long run, especially when the patient physical and emotional well-beings are considered.

Finally, more studies on the human microbiome and its interactions with the reproductive tracts of both men and women seem pivotal to improve the management of microorganisms during ART practices. It will undoubtedly guide the next discussions over the theme and assist with the decision of whether or not to implement routine screening of microbial contamination of biological fluid samples in IVF treatments. Further comprehension about the influence of the microbiome might lead to better results in ART overall, especially for patients with infertility caused by unknown factors.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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