Acta Veterinaria-Beograd 2025, 75 (3), 326-338
UDK: 636.2.082.4:[612.646:579.61
636.2.082.454
DOI: 10.2478/acve-2025-0025

Research article

MICROBIAL CONTAMINATION OF IN VITRO-DERIVED CATTLE EMBRYOS AND RESISTANCE GENES

Gokcenur SANIOGLU GOLEN[1*], Kadir AKAR[2], Tahir KARAŞAHIN[3], Göktuğ ŞENTÜRK[3], Selçuk GÖLEN[4], Zeki ARAS[1]

[1]Aksaray University, Faculty of Veterinary Medicine, Department of Microbiology, Aksaray, Türkiye;
[2]Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Microbiology, Van, Türkiye;
[3]Aksaray University, Faculty of Veterinary Medicine, Department of Physiology, Aksaray, Türkiye;
[4]Ministry of Agriculture and Forestry, Aksaray, Türkiye.

(Received 16 April, Accepted 11 August 2025)

The global trend of in vitro embryo systems, particularly the in vitro fertilization (IVF) culture system, is gaining momentum. Despite the strict standards followed in in-vitro embryo procedures, microbiological contamination is occasional, and the relevant literature is scarce. In this study, for the first time, IVF culture dishes with microbial contamination and resistance genes of isolates were evaluated in veterinary medicine. Samples were microscopically taken from IVF tissue cultures suspected of bacterial or fungal contamination and sent to the microbiology laboratory for further examination. The total contamination rate was 11.1% in IVF cultures where cell division did has stopped or turbidity occurred. Identification of contaminant microorganisms showed that infections were mainly caused by *E. coli* 9.5% and *Candida* spp. 1.58%. A set containing multiplex antibiotic primers was used during the IVF protocol to determine antibiotic resistance genes. All *E. coli* isolates were resistant to penicillin used in the Kirby-Bauer, and 16% was resistant to streptomycin. This study is the first systematic evaluation of microbial contamination of bovine IVF culture vessels in veterinary medicine. IVF culture should be evaluated in more detail to learn more about the source of the microorganism and to develop adequate measures to prevent microbial contamination.

Keywords: Cattle, contamination, *E. coli,* embryo, PCR

INTRODUCTION

The global trend of in vitro embryo systems, particularly the in vitro fertilization (IVF) culture system, is gaining momentum. This system, designed to provide a secure and controlled environment for the survival and development of gametes and embryos in vitro, is not immune to occasional microbial intrusions [1].

*Corresponding author: e-mail: gokcenursanioglu@aksaray.edu.tr

Despite the strict standards followed in in-vitro embryo procedures, microbiological contamination occurs occasionally, and the relevant literature is scarce. Therefore, it is crucial to assess the incidence and impact of contamination events in IVF laboratories [2]. Most reports do not contain information on contamination incidents, so the frequency of microbial contamination in their laboratories is not clearly known. Monitoring practices for regulating sterility levels and microbiological control should be followed. Due to the widespread presence of microorganisms in the environment and follicular and seminal fluids, it is reasonable to assume that contamination cases are mainly underestimated [3,4]. Even if microbial contamination appears low, the absolute number of contamination cases can cause severe damage to cultured oocytes or embryos, leading to economic losses. In addition, yeast and bacterial contamination, such as *Candida* spp. and *Enterobacteriaceae*, which are challenging to maintain laboratory sterilization standards and can sometimes cause abortions, can result in time and cost losses [5-7]. Microbial contamination threatens the survival of gametes and embryos, causing degeneration of the culture system, developmental arrest, and cross-infection. Pathogens can be transmitted to the offspring through embryo transfer [8]. There have been previously reported cases of patients infected with hepatitis B virus (HBV) and human immunodeficiency virus (HIV) resulting from IVF treatment in human medicine [9]. Similarly, in veterinary medicine, embryos and oviduct cells derived in vitro from slaughterhouse material from cows experimentally and naturally infected with Bovine Viral Diarrhoea Virus (BVDV) and Bovine Herpes Virus (BHV-1) are contaminated [1,10].

In the human medicine literature, microbial contamination in IVF cycles is reported to be approximately 0.1% to 1% [11]. There is no systematic study in veterinary medicine on the frequency of microbial contamination in bovine embryo IVF cultures. Despite the use of gentamicin in the embryo collection procedure, sterile embryo culture conditions, and the use of penicillin and streptomycin-containing media, it is observed that the number of infections increases from time to time. In this study, for the first time, 63 IVF cultures (culture dishes) with microbial contamination, turbidity, or cell division arrest in Aksaray University Embryo Transfer Education Application Research Centre were evaluated in Aksaray University Faculty of Veterinary Medicine, Department of Microbiology to determine microbial contamination and resistance genes of embryos.

MATERIAL AND METHOD

Collection of embryos

The ovaries obtained from the slaughterhouse were brought to the laboratory within 3 hours at 30 °C in 0.9% saline containing 100 mg/I gentamicin. In the laboratory, oocytes were aspirated from 2-8 mm diameter follicles on the ovarian surface with the help of an 18-gauge needle attached to a 5 ml syringe. The collected oocytes were

placed in Phosphate-buffered saline (PBS) with 5% calf serum and evaluated in the same solution, and the oocytes classified as A and B were taken into the maturation process [12]. Ethical approval for the study was obtained from the local ethics committee (Number: 24/7/40).

A total of 63 embryos were obtained and analyzed in this study. The sample size was determined based on a power analysis using G Power software, with a confidence level of 95% ($\alpha = 0.05$) and a power of 80% ($1–\beta = 0.80$). The analysis indicated that a minimum of 60 embryos was necessary to detect statistically significant differences in embryo quality outcomes with medium effect size (Cohen's $d = 0.5$). This number was chosen to ensure robustness and reproducibility of the statistical results.

After maturation, oocytes were fertilized with bull spermatozoa frozen in 0.25ml aliquots containing $17.5x10^5$ spermatozoa. After this process, they will be kept in a suitable medium for 7-8 days until the blastocyst stage. The embryos obtained were examined under a stereomicroscope. The developmental quality of the embryos was evaluated according to the criteria of the International Embryo Transfer Association [13].

The assessment of embryo quality was based on morphological integrity. Code I (excellent or good) corresponded to a very low level of disorganization between cells, a viable embryonic cell proportion of more than 85%, and a round, intact zona pellucida. Code II (fair) was characterized by moderate irregularity between cells and a viable embryonic cell proportion of approximately 50–85%. Code III (poor) was defined by pronounced irregularities in the form of the embryo and a viable cell proportion of approximately 25–50%. Code IV (degenerated/dead) was assigned to embryos with less than 25% viable cells, or oocytes and dead cells that had not completed division. Based on these criteria, Code I and Code II embryos are considered of transferable quality [13].

Microbiologic examination

An embryo culture dish was transported by cold chain to the Department of Microbiology, Faculty of Veterinary Medicine, Aksaray University, for microbiological analysis when it appeared muddy, the cell division stages stopped, and microbial contamination was suspected.

Samples were microscopically taken from IVF tissue cultures suspected of bacterial or fungal contamination and sent to the microbiology laboratory for further examination. These samples were cultured according to standard procedures. Samples were inoculated on blood agar (Oxoid, Basingstoke, UK) plates and MacConkey agar (Oxoid, Basingstoke, UK) plates for detecting *Enterobacteriaceae* and Sabouraud Dextrose Agar (SDA)(Oxoid, Basingstoke, UK) agar for detecting yeast or fungal infections. Blood agar and MacConkey plates were incubated aerobically at 37 °C, and isolatates were observed within 24 hours. With the help of eosin methylene blue (EMB) (Oxoid, Basingstoke, UK) agar, isolates showing metallic blue-green color

were identified, antibiotic resistance was determined by culture, and resistance genes were determined by multiplex PCR. SDA agar was incubated at room temperature for five days, and the presence of *Candida* spp. was determined by microscopic and biochemical tests [14].

DNA extraction

E. coli isolates were subcultured overnight in Luria-Bertani (LB) broth (Merck, Darmstadt, Germany) at 37 °C with constant shaking. Genomic DNA was then extracted manually using the Genomic DNA Purification Kit (Fermentas, Thermo Scientific, Bremen, Germany) following the manufacturer's protocol, with the steps detailed below to ensure reproducibility:

First, 1.5 mL of overnight bacterial culture was transferred into a microcentrifuge tube and centrifuged at 10,000 × g for 5 minutes to pellet the cells. The supernatant was discarded, and the pellet was resuspended in 180 µL of lysis solution containing lysozyme. The suspension was incubated at 37 °C for 30 minutes to allow enzymatic degradation of the bacterial cell wall.

Following lysis, 25 µL of proteinase K and 200 µL of binding buffer (containing chaotropic salts) were added to the tube, and the mixture was incubated at 56 °C for 30 minutes to degrade proteins and facilitate DNA binding. Then, 200 µL of absolute ethanol was added, and the sample was mixed thoroughly by vortexing.

The entire mixture was transferred to a spin column provided in the kit and centrifuged at 10,000 × g for 1 minute. The flow-through was discarded, and the column was washed sequentially with 500 µL of wash buffer I and 500 µL of wash buffer II, each followed by centrifugation at 10,000 × g for 1 minute. After the final wash, the column was centrifuged again for 2 minutes to remove residual ethanol.

Finally, the DNA was eluted by adding 100 µL of elution buffer (pre-warmed to 65 °C) directly onto the column membrane and incubating for 5 minutes at room temperature. The column was then centrifuged at 10,000 × g for 1 minute to collect the purified genomic DNA, which was stored at −20 °C until use.

Antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion method was used Mueller-Hinton agar (Oxoid, Basingstoke, UK) and gentamicin (30 µg/disc), penicillin (10 µg/disc), and streptomycin (10 µg/disc) (Oxoid, Basingstoke, UK) discs. After the inoculated plates were incubated under aerobic conditions at 37 °C for 18 to 24 hours, the susceptibility of *E. coli* isolates to each antimicrobial agent was measured, and the results were interpreted according to the criteria provided by CLSI [15].

Primers and PCR assay

A set containing multiplex antibiotic primers was used during the IVF protocol to determine antibiotic resistance genes (Table 1).

Table 1. Multiplex primer sequences of the antibiotic resistance genes

Antimicrobial agent	Resistance gene	Sequence (5' – 3')	Size (bp)	References
Gentamycin	aac(3)-IV	F) CTTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT	286	[16,17]
Streptomycin	aadA1	(F) TATCCAGCTAAGCGCGAACT (R) ATTTGCCGACTACCTTGGTC	447	[17]
Beta-lactam	blaOXA	(F) GCAGCGCCAGTGCATCAAC (R) CCGCATCAAATGCCATAAGTG	198	[16]
Beta-lactam	blaSHV	(F) TCGCCTGTGTATTATCTCCC (R) CGCAGATAAATCACCACAATG	768	[16]

PCR reactions were performed in a total volume of 25 µL, including 1.5mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 µm of each dNTP (Fermentas, Thermo, Bremen, Germany), 1 µm primers, 1 IU of Taq DNA polymerase ((Fermentas, Thermo, Bremen, Germany) and 5 µl (40–260 ng/µl) of DNA. Amplification reactions were carried out using a DNA thermo-cycler as follows: Three min at 95 °C, 35 cycles each consisting of 1 min at 94 °C, 90 s at ~55 °C and 1 min at 72 °C, followed by a final extension step of 10 min at 72 °CTo ensure the accuracy of the PCR results, positive controls consisted of bacterial DNA samples that had been previously confirmed to carry the relevant resistance genes, while negative controls were prepared by replacing the template DNA with PCR-grade distilled water. The amplification products were visualized by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and observed under UV illumination. A 100 bp DNA ladder (Fermentas, Thermo Scientific, Bremen, Germany) was used as a molecular size marker to estimate the length of the amplified fragments [16,17].

Statistics Analysis

All data analyses were performed with SPSS software version 20.0 (IBM). Categorical data were described as frequencies and percentages and analyzed using the Chi-square test. Categorical data, such as the relationship between microbial contamination and embryo cell division status, were compared using the Chi-square test. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

Embryo cell division

The division rates of bovine oocytes on day 2, oocyte maturation rates on day 7, and the status of fertilized oocytes developing to the morula-blastocyst stage were monitored by stereomicroscope. Culture dishes where cell division did not continue, and turbidity was observed were considered infected (Fig. 1).

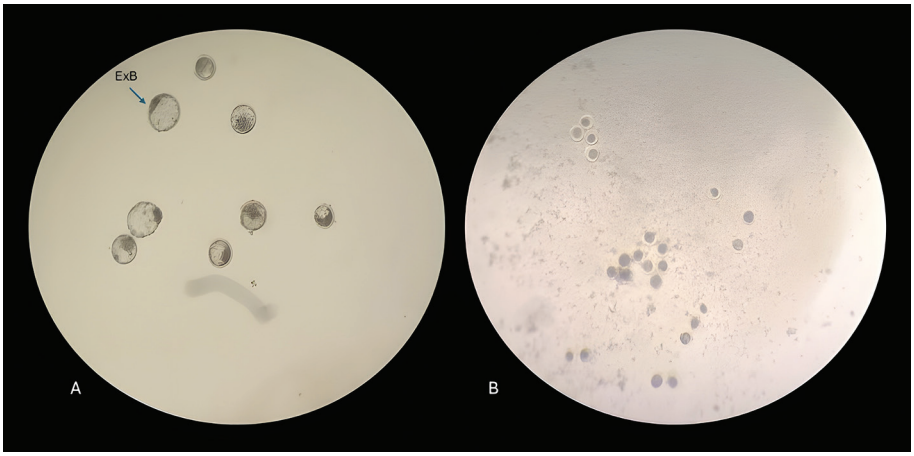

Figure 1. Day 7 maturation rates by stereomicroscope **(A)** oocytes in which cell division continues ExB (Expanded Blastocyst); **(B)** oocytes in which cell division has stopped.

Antimicrobial susceptibility testing

The total contamination rate was 7/63 (11.1%) in IVF cultures where cell division did not continue or turbidity occurred. Identification of contaminant microorganisms showed that infections were mainly caused by *E. coli* 6/63 (9.5%) and *Candida* spp. 1/63 (1.58%). All *E. coli* isolates were resistant to penicillin used in the culture medium (Kirby-Bauer), and 1 (16%) was resistant to streptomycin. All isolates were susceptible to gentamicin. All IVF cultures where *E. coli* was isolated were turbid, and division did not continue. However, although turbidity was observed in the culture dish in which *Candida* spp. was isolated, it was determined that embryo division continued.

Detection of resistance genes by PCR

The presence of the blaOXA (beta-lactam) resistance gene was detected in all isolates, the blaSHV (beta-lactam) resistance gene was detected in 50%, and the aadA1 (streptomycin) resistance gene was detected in 16%. No aac(3)-IV (gentamicin) resistance gene was identified in any isolate (Fig. 2).

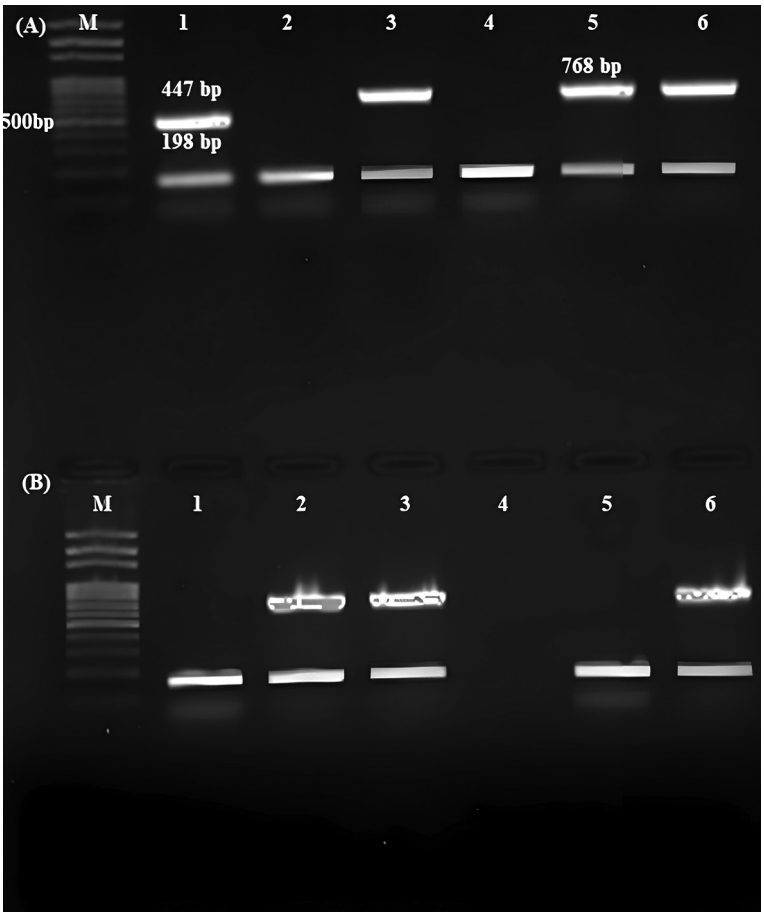

Figure 2. Agarose gel image of the PCR products. **(A) M:** Marker, **1:** aadA1, blaOXA; **2:** blaOXA; **3:** blaSHV blaOXA; **4:** blaOXA; **5-6:** blaSHV blaOXA; **(B) M:** Marker, **1:** blaOXA Positive control; **2,3,6:** blaSHV blaOXA positive control; **4:** Negative Control.

Statistical Comparison of Contamination and Embryo Development

A Chi-square test was used to evaluate the association between microbial contamination and embryo development status. Among the 63 IVF cultures, contamination was significantly associated with the absence of cell division (Chi-square = 12.47, p = 0.0004). All cultures in which *E. coli* was isolated (n = 6) showed both turbidity and arrest in cell division, whereas embryo development continued in the single culture contaminated with *Candida spp.*. This difference was statistically significant, indicating that *E. coli* contamination had a stronger negative impact on embryo viability compared to *Candida spp.*

DISCUSSION

In vitro embryo technologies, particularly in vitro fertilization (IVF) culture systems, are increasingly adopted worldwide to provide a stable and controlled environment for the development and viability of gametes and embryos. Despite adherence to strict aseptic procedures, these systems remain vulnerable to occasional bacterial, fungal, or viral contamination [1,2,26]. Monitoring microbial contamination in IVF cultures is essential not only for identifying contamination patterns but also for taking preventive measures. Therefore, routine microbiological screening should be conducted in all IVF cultures suspected of contamination [1,2].

Studies in human medicine have shown that most infections in IVF culture are caused by bacterial strains insensitive to the antibiotics used or by yeast colonization by *Candida* spp., which are present in the vaginal flora and can sometimes cause abortions [2,3]. Kastrop et al. [3] found a contamination rate of 0.68% in an 8-year retrospective study and reported that 59% of the contamination was caused by *E. coli* and 25% by *Candida* species. A retrospective analysis of the IVF laboratory procedure by Lin et al. [18] revealed 12 occurrences of microbial contamination or an incidence of about 0.29%. No additional bacteria were detected, and *E. coli* was the source of all contaminations. In a 10-year study by Du et al. [19], the microbial contamination rate was determined as 0.23%. It was reported that contaminations were caused by *E. coli* (60.4%) and *Enterococcus faecalis* (*E. faecalis)* (58.3%). In veterinary medicine, no study has monitored microbial contamination in bovine embryo IVF laboratories. This study showed that *E. coli* (9.5%) and *Candida* spp. (1.58%) caused contamination. Like other human embryo studies, most contaminations were caused by *E. coli* and *Candida* spp.

Kastrop et al. [3] reported that *E. coli* caused 59% of contamination in human embryo laboratories, and 91.4% of bacterial strains were resistant to penicillin and streptomycin used in the standard procedure. He suggested that contamination decreased in cultures to which gentamicin was added, even though almost no contamination occurred. Bielansky et al. [20] experimentally performed in vitro fertilization with sperm infected with *Mycoplasma* (*M.*) *bovis* or *Mycoplasma* (*M.*) *bovigenitalium* and showed that these pathogens could be transmitted through the IVF system and thus infect embryos. They also showed that supplementation of the media used for in vitro culture with standard antibiotics and the IETS-recommended washing procedure of embryos were not effective in clearing IVF embryos from *M. bovis* and *M. bovigenitalium*. This is the first study in veterinary medicine dealing with the evaluation of IVF culture microbial contamination in IVF laboratories by antibiotic resistance. The contaminants isolated in our study were *E. coli* strains with increased resistance to penicillin and streptomycin, which were used in the procedure of our IVF laboratory. In line with the findings obtained in this study, the efficacy of penicillin and streptomycin supplementation to the culture medium should be questioned increasingly. In addition, it is thought that this study may be a guide for IVF culture media supplemented with other antibiotics or chemical agents in future studies.

Research conducted in human IVF labs has shown that poor-quality growing embryos result from obvious bacterial contamination of culture dishes; nevertheless, yeast contamination does not lower embryo quality. Ben-Chetrit et al. [21] explained that *Candida* spp. do not have a harmful effect on human embryos because they are generally present in the human flora. On the other hand, Klein et al. [22] suggested that early contamination with yeast had a detrimental effect on embryo development; in case of late contamination, there was no difference with the control group. In another study, it was determined that the stages progressed normally in the embryo in which turbidity was detected in IVF culture, and *Candida* spp. adhesion to the zona pellucida was observed in microscopy [2]. In this study, this situation was determined for the first time in bovine embryos, and it was observed that yeast contamination did not prevent divisions. However, it caused turbidity in the culture. The main reason for this situation may be due to the natural presence of *Candida* spp. in the bovine vaginal flora.

In this study, the presence of antimicrobial resistance genes in *E. coli* isolates obtained from IVF cultures represents an important finding, particularly given the clinical relevance of these genes. The universal detection of the blaOXA gene, a beta-lactamase gene associated with resistance to penicillins, is consistent with previous reports indicating the widespread presence of beta-lactam resistance among environmental and clinical *E. coli* strains [3,23]. The blaSHV gene, found in 50% of isolates, has also been reported in extended-spectrum beta-lactamase (ESBL)-producing *E. coli*, especially in strains isolated from reproductive tract infections and hospital settings [3]. This is significant in the context of IVF, where such resistance may compromise sterility and embryo viability.

Detection of the aadA1 gene in 16% of isolates, conferring resistance to streptomycin, also aligns with earlier studies indicating its role in horizontal gene transfer among *Enterobacteriaceae* [24,25]. Interestingly, no aac(3)-IV gene (associated with gentamicin resistance) was detected, supporting the phenotypic findings that all isolates were susceptible to gentamicin, an encouraging result for its continued use in embryo culture media.

Although *Candida* spp. was isolated from only one culture and embryo division continued despite visible turbidity, the presence of fungal contaminants in IVF environments has been reported in literature as a rare but critical risk factor [2]. However, molecular resistance profiling was not conducted for the *Candida* isolate due to its limited occurrence. Further studies could focus on expanding the sample size to better understand antifungal resistance trends in such settings.

The findings presented here, particularly the detection of resistance genes in IVF-related microbial contamination, highlight the need for routine screening and the careful selection of antibiotics in culture systems. Comparisons with similar studies in bovine and human IVF settings further underscore the importance of microbiological

monitoring to prevent reduced embryo development and compromised reproductive outcomes.

It has been demonstrated that hygienic control at different stages of embryo production can be performed on maturation and culture fluids. Such control has been reported to be very important in bacterial and viral colonization. Although contaminations in the IVF system can often be detected, it is thought that latent contamination can lead to even more severe consequences. Therefore, effective infection prevention is the basis for ensuring IVF laboratories' safe and stable operation. To control infectious factors and inhibit pathogenic microorganisms growth, asepsis should be strictly applied in IVF laboratories, and contaminations should be routinely monitored [5].

This study is the first systematic evaluation of microbial contamination of bovine IVF culture vessels in veterinary medicine. It can be concluded that the incidence of contamination in our IVF program is significant. Very few studies and case reports have been published in bovine IVF laboratories, so a complete comprehensive data comparison could not be made. In addition, these findings may be due to the rigorous and thorough examination of our IVF laboratory cultures to detect contamination. Given the many IVF cycles performed worldwide, microbial contamination should not be underestimated as an IVF treatment cycle complication. IVF culture should be evaluated in more detail to learn more about the source of the microorganism and to develop adequate measures to prevent microbial contamination.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

We wish to acknowledge the entire staff of both the Embryo Transfer Training Application Research Center and the Department of Microbiology at Aksaray University for their immeasurable support throughout this work.

Data availability

All data generated or analyzed during this study are included in this manuscript.

Authors' contributions

GSG, KA, TK, SG, and ZA contributed to the study's design and conception, while GSG, KA, and TK were involved in data curation and visualization. All authors participated in writing and revising the manuscript, which they then read and approved as the final version.

Conflict of Interests

The authors declare no actual, potential, or perceived conflicts of interest for this article.

Ethics approval and consent to participate

This study was approved by the Aksaray University Animal Experiments Local Ethics Committee , Türkiye (Number: 24/7/40).

ORCID iDs

Gokcenur Sanioglu Golen https://orcid.org/0000-0001-9950-330X
Kadir Akar https://orcid.org/0000-0003-0894-7357
Tahir Karaşahin https://orcid.org/0000-0003-2358-0389
Göktuğ Şentürk https://orcid.org/0000-0002-2093-1510
Selçuk Gölen https://orcid.org/0009-0002-0668-4696
Zeki Aras https://orcid.org/0000-0003-4564-2077

REFERENCES

1. Li D, Gao Y: Microbial contamination of culture media. In: Qual Manag Ass Reprod Lab. Singapore: Springer Nature Singapore; 2024:79-85.
2. Poli E, Obkircher M, Comploj G, Netzbandt P, Sorrentino U, Zuccarello D, Faggionato C: *Candida* contamination of embryos in the ART laboratory: a case report and literature review. Rıver J 2024, 1:47.
3. Kastrop PM, de Graaf-Miltenburg LA, Gutknecht DR, Weima SM: Microbial contamination of embryo cultures in an ART laboratory: sources and management. Hum Reprod 2007, 22:2243-2248.
4. Haahr T, Jensen JS, Humaidan P: Vaginal microbiota and IVF outcomes: poor diagnosis results in flawed conclusions. Reprod Biomed Online 2019, 39:178.
5. Borges ED, Berteli TS, Reis TFD, Silva ASD, Vireque AA: Microbial contamination in assisted reproductive technology: source, prevalence, and cost. J Reprod Genet 2020, 37:53-61.
6. Fiorentino MA, Acuña Y, Sosa E, Cantón GJ, Erreguerena I, Malena R, García JA: Infectious sporadic bovine abortions: retrospective analysis. Trop Anim Health Prod 2024, 56:63.
7. Pal M, Tariku F, Rebuma T, Seth D, Patel KB: Growing role of fungi in the reproductive disorders of animals. J Adv Microb Res 2024, 5:37-42.
8. Malhotra K: Troubleshooting in an IVF laboratory. Fertil Reprod 2023, 5:279.
9. Yi W, Li M, Sun F, Lu H, Zeng Z, Bi X, Xie Y: Impact of in vitro fertilization-embryo transfer on mother-to-infant transmission in women with chronic HBV infection. Liver Int 2022, 42:2167-2174.
10. Gard J: Reproductive technologies and pathogen transmission. Reprod Technol Anim 2020, 263-73.
11. Zhu GJ, Wei YL, Hu J, Liu Q: Microorganism contamination in in vitro fertilization-embryo transfer system and their sources. Zhonghua Fu Chan Ke Za Zhi 2004, 39:382-384.

12. Karaşahin T, Arıkan S: The effect of oleic and linoleic acids on in vitro bovine embryonic development and embryo quality. Turk J Vet Anim Sci 2015, 39:154-159.
13. Bó GA, Mapletoft RJ: Evaluation and classification of bovine embryos. Anim Reprod 2018, 10:344-348.
14. Costa AR, Silva F, Henriques M, Azeredo J, Oliveira R, Faustino A: *Candida* clinical species identification: molecular and biochemical methods. Ann Microbiol 2010, 60:105-112.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 16th informational supplement. CLSI document M100-S16. Wayne, PA: CLSI; 2006.
16. Van TTH, Chin J, Chapman T, Tran LT, Coloe PJ: Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. Int J Food Microbiol 2008, 124:217-223.
17. Momtaz H, Rahimi E, Moshkelani S: Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. Vet Med 2012, 57:193-197.
18. Lin LL, Guu HF, Yi YC, Kung HF, Chang JC, Chen YF, Chen MJ: Contamination of ART culture media – the role of semen and strategies for prevention. Taiwan J Obstet Gynecol 2021, 60:523-525.
19. Du F, Luo H, Li R, Zhu Y, Zhao J, Yuan P, Wang W: Microbe contamination during embryo cultures in an in vitro fertilization-embryo transfer system. Ann Obstet Gynecol 2021, 4:1015.
20. Bielanski A, Devenish J, Phipps-Todd B: Effect of Mycoplasma bovis and Mycoplasma bovigenitalium in semen on fertilization and association with in vitro produced morula and blastocyst stage embryos. Theriogenology 2000, 53:1213-1223.
21. Ben-Chetrit A, Shen O, Haran E, Brooks B, Geva-Eldar T, Margalioth EJ: Transfer of embryos from yeast-colonized dishes. Fertil Steril 1996, 66:335-337.
22. Klein JU, Missmer SA, Jackson KV, Orasanu B, Fox JH, Racowsky C: In vitro fertilization outcomes after transfer of embryos contaminated with yeast. Fertil Steril 2009, 91:294-297.
23. Elsharkawy SA, Latif H, Purnawarman T, Rahayu P: Analysis of beta-lactam antibiotic resistance genes in *Escherichia coli* isolated from dairy cattle manure in Bogor, Indonesia. Ger J Vet Res 2024, 4:16-26.
24. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, Marusza W: Molecular mechanisms of drug resistance in Staphylococcus aureus. Int J Mol Sci 2022, 23:80-88.
25. Behera M, Parmanand Roshan M, Rajput S, Gautam D, Vats A, De S: Novel aadA5 and dfrA17 variants of class 1 integron in multidrug-resistant *Escherichia coli* causing bovine mastitis. Appl Microbiol Biotechnol 2023, 107:433-446.
26. Šmigoc J, Pavšič Vrtač K, Jakovac Strajn B, Stvarnik M, Mrkun J: Preventive supplementation of vitamin E and selenium as a factor in improving the success rate of embryo transfer in cattle. Acta Vet-Beograd 2023, 73(1):87-101.

MIKROBNA KONTAMINACIJA EMBRIONA GOVEDA DOBIJENIH IN VITRO METODOM I GENI OTPORNOSTI

Gokcenur SANIOGLU GOLEN, Kadir AKAR, Tahir KARAŞAHIN, Göktuğ ŞENTÜRK, Selçuk GÖLEN, Zeki ARAS

Globalni trend sistema in vitro embriona, posebno sistema kulture in vitro oplodnje (IVF), dobija na značaju. Uprkos strogim standardima koji se primenjuju u postupcima in vitro embriona, mikrobiološka kontaminacija je povremeno prisutna, a relevantna literatura je oskudna. U ovoj studiji, po prvi put, posude za IVF kultivaciju sa prisutnom mikrobnom kontaminacijom, kao i geni otpornosti izolata su procenjeni u veterinarskoj medicini. Uzorci su mikroskopski uzeti iz kultura tkiva IVF-a za koje se sumnja na bakterijsku ili gljivičnu kontaminaciju i poslati u mikrobiološku laboratoriju na dalja ispitivanja. Ukupna stopa kontaminacije bila je 11,1% u kulturama IVF-a gde se deoba ćelija nije nastavila ili je došlo do zamućenja. Identifikacija kontaminirajućih mikroorganizama pokazala je da su infekcije uglavnom uzrokovane sa *E. coli* 9,5% i *Candida* spp. 1,58%. Set koji sadrži antibiotske prajmere korišćen je tokom IVF protokola za određivanje gena otpornosti na antibiotike. Svi izolati *E. coli* bili su otporni na penicilin koji se koristi u Kirbi-Bauer studiji, a 16% je bilo otporno na streptomicin. Ova studija je prva sistematska evaluacija mikrobne kontaminacije posuda za kontaminaciju goveđih IVF kultura u veterinarskoj medicini. Kulturu IVF treba detaljnije proceniti kako bi se saznalo više o izvoru mikroorganizma i razvile adekvatne mere za sprečavanje mikrobne kontaminacije.