Received: 30 August 2023 | Revised: 10 January 2024 | Accepted: 19 January 2024

DOI: 10.1002/ccr3.8483

CASE REPORT

Clinical Case Reports Open Access WILEY

Zona pellucida removal resulted in a successful live birth: Report on a case with recurrent implantation failure due to embryonic bacteria contamination

Son Truong Dang[1] | **Huy Phuong Tran**[2] | **Tuong Nguyen Ho**[3] | **Loc Thai Ly**[2] | **Tuyet Thi-Diem Hoang**[2] | **Trang Nguyen-Khanh Huynh**[4] | **Anh Tuan Do**[1] | **Thuan Duc Nguyen**[1] | **Phuong Thi Dao**[1]

[1]HP Fertility, Hai Phong International Hospital of Obstetrics and Pediatrics, Vietnam

[2]Infertility Department, Hung Vuong Hospital, Ho Chi Minh City, Vietnam

[3]Taipei Fertility Center, Taipei, Taiwan

[4]Department of Obstetrics and Gynaecology, Pham Ngoc Thach University of Medicine, Ho Chi Minh City, Vietnam

Correspondence
Huy Phuong Tran, Infertility Department, Hung Vuong Hospital, 128 Hong Bang Street, Ward 12, District 5, Ho Chi Minh City 700000, Vietnam.
Email: tranphuonghuy1994@gmail.com

Key clinical message

In in vitro fertilization (IVF), laser offers several advantages. In this study, we employed laser to eliminate the zona pellucida of a contaminated embryo. This approach helps to rescue embryo with bacterial contamination, and improve embryo-endometrium interaction.

Abstract

To present a case report on the removal of a contaminated zona pellucida from an embryo of patient with a history of recurrent implantation failure (RIF), which was followed by a successful live birth. We present the case of a 34-year-old patient with a history of 3 years of infertility who underwent in vitro fertilization. During the culture process, the embryos became contaminated, leading to three failed implantations. Despite the aneuploidy of the embryo and the implementation of a washing technique, the contamination persisted. In the final attempt, the contaminated zona pellucida was successfully removed using laser, followed by embryo transfer, resulting in a live birth. We provided detailed clinical information, including patient demographics, infertility history, ovarian response, evidence of bacterial contamination, embryo development, treatment protocols, and outcomes. Laser excision of the zona pellucida is a safe and effective method for addressing bacterial infection in embryos.

KEYWORDS

contaminated embryo, embryo transfer, IVF, recurrent implantation failure, zona pellucida removal

1 | INTRODUCTION

The success of in vitro fertilization (IVF) relies on a series of crucial laboratory procedures, including the culture and development of embryos in a controlled environment. However, despite strict quality control, there is a risk of bacterial contamination during the embryo culture process.[1] Such contamination poses a threat to the IVF outcomes. Bacterial contamination in the IVF laboratory can arise from various sources, including air, water, equipment, and personnel.[2] It can also occur at different stages of the IVF process, such as during oocyte retrieval, sperm preparation, handling of culture dishes, or manipulation of embryos. Bacteria introduced into the culture media can proliferate rapidly, producing harmful byproducts and triggering inflammatory responses that may negatively impact embryo development. Additionally, certain bacteria may directly adhere to the embryo's zona pellucida, compromising its integrity and reducing the chances of successful implantation.

Commonly, the washing technique is employed to rescue embryos that have been contaminated. It involves the gentle removal of external contaminants from the embryo's surface. The washing process begins by transferring the contaminated embryo to a clean petri dish containing a culture media supplemented with antibiotics or antifungal agents. Gentle swirling motions are then used to perform the washing. After that, the embryo is transferred to a fresh, uncontaminated dish with a clean culture medium. However, a study has demonstrated that washing alone is not effective in eliminating bacteria, as some still remain in the zona pellucida.[3] In a recent case report by Li R et al.,[4] a new method was proposed to rescue contaminated embryos, involving the removal of the contaminated zona pellucida using Tyrode acid. It has been observed that the porous structure of the zona pellucida makes it difficult to completely eliminate microorganisms.[5] Additionally, the zona pellicida itself can prevent embryo development in later stages.[6] Therefore, removing zona pellucida is a promissing method to solve the contamination problems.

In assisted reproductive technology (ART), the use of laser offers several advantages, primarily due to their precision. For example, an application of laser is assisted hatching (AH), involving the creation of a small opening in the zona pellucida.[7] It enhances the embryo's ability to hatch from its protective shell and successfully implant into the uterine lining. While the technique is well-established in the field of ART, our specific approach, as detailed in this report, aims to address contamination concerns. We describe an alternative method using lasers to remove the contaminated zona pellucida from an embryo. The significance lies in adapting this established technique to a unique context, deviating from its routine application. We present the clinical details, procedural methodology, and subsequent outcomes of this unique case, thereby shedding light on the promising prospects for assisted reproductive technologies.

2 | CASE PRESENTATION

The key patient, born in 1987, presented with a body mass index (BMI) of 20.34. She had a history of nulliparity (PARA: 1021) and had been actively trying to conceive for 3 years. After unsuccessful attempts at intrauterine insemination (IUI) in 2018, she was recommended for IVF. Table 1 provides an overview of the patient's baseline characteristics. Preimplantation genetic testing for aneuploidy (PGT-A) was also carried out to investigate the ploidy status of embryos.

The IVF treatment followed a standardized protocol. Controlled ovarian stimulation was achieved through a GnRH-antagonist protocol, with daily administration of 150 IU of Gonal-F® and 150 IU of Pergoveris® (Merck Serono S.p.A., Italy). Cetrotide® (Merck KGaA, Germany) was introduced on the seventh day of stimulation. The patient received an intravenous administration of 10,000 units of hCG IVF-C (LG Chem, Korea).

Oocyte retrieval was performed on September 23, 2021, followed by embryo vitrification on September 28, 2021. The embryos were cultured in a Geri™ Time-lapse Incubator at 37°C with a controlled atmosphere consisting of 6% CO_2, 5% O_2, and 89% N_2. On the day of oocyte retrieval, her husband collected semen through masturbation. The sperm analysis yielded a favorable result. The swim-up method was applied to prepare the sperm sample for fertilization. The sample was incubated in G-IVF

TABLE 1 Baseline characteristics of the key patient.

Characteristics	Value
Male age	38
Female age	34
BMI	20.34
PARA	1-0-2-1
Reason for IVF	IUI failed 3 cycles
Infertility duration	3 years
Baseline FSH (mIU/mL)	4.88
Baseline LH (mIU/mL)	3.64
AMH (ng/mL)	5.0
Prolactin (mIU/mL)	15.42

Abbreviations: AMH, Anti-mullerian hormone; BMI, Body mass index; FSH, Follicle stimulating hormone; IVF, In vitro fertilization; LH, Luteinizing hormone; PARA, parity.

TABLE 2 Semen quality at the day of ovum pickup.

Characteristics	Value
Volume	2.0 mL
Sperm concentration	32×10^6/mL
Progressive motile	33%
Non-progressive motile	4%
Normal morphology	2%

PLUS media (Vitrolife, 10,136) for 30 min to collect motile spermatozoa. Detailed information on semen quality is described in Table 2.

For oocyte retrieval, we used Modified HTF Medium with Gentamicin (FUJIFILM Irvine Scientific, 90,126). Following retrieval, the oocyte-cumulus complexes (OCCs) were cultured using G-IVF PLUS media (Vitrolife, 10,136). A total of 23 oocytes were retrieved, comprising 18 mature (MII) oocytes, 3 immature (MI) oocytes, and 2 germinal vesicle (GV) stage oocytes. Out of these, 18 oocytes underwent successful fertilization, resulting in the formation of two pronuclei (2PN) embryos.

Embryo development proceeded using Geri medium (Genea Biomedx, ONE-50), resulting in 13 blastocysts on the fifth day. Trophectoderm cells from eight good-quality blastocysts were selected for aneuploidy screening using next-generation sequencing (NGS) technology. The screening identified five euploid embryos, indicating a favorable prognosis for successful implantation. Detailed information regarding the IVF with PGT-A cycle is provided in Tables 3 and 4. All embryos were preserved using the Vitrification Kit 101 (Cryotech, Japan).

Following endometrium preparation, the patient was recommended for frozen embryo transfer (FET). The embryologists thawed one embryo for transfer using the Warming Solution Set 205 (Cryotech, Japan). Unfortunately, the patient experienced three unexplained implantation failures. During the culture process, we observed cloudy particles in the petri dishes (Figure S1A) and even at the blastocyst stage (Figure S1B). Those particles proliferated afterward, raising concerns about bacterial contamination. To investigate this, we collected IVF medium and sent it to the Department of Medical Microbiology for examination. The later result confirmed the contamination of Rhizobium radiobacter bacteremia, an infrequent cause of human infection. It's important to highlight that the contamination occurred during the initial stages of embryo culture. Consequently, bacteria became cryopreserved along with the embryos. For the second and third FET attempts, we implemented a washing step for the embryos in culture medium before transfer. However, the patient continued to experience unsuccessful outcomes. In the final attempt, we employed the Octax NaviLase® Laser from Vitrolife to remove the zona pellucida. Following this intervention, the patient achieved pregnancy, and ultimately, a live birth was accomplished. The baby was born healthy, weighing 2608 g at 37.5 weeks' gestation. Comprehensive details regarding the four FET cycles of the patient are presented in Table 5.

TABLE 3 Result of IVF cycle.

Characteristics	Value
Oocyte number	23
MII	18
MI	3
GV	2
Fertilized oocyte	18
Day-3 embryo	18
Grade A	8
Grade B	5
Grade C	5
Day-5 embryo	13
Grade A	7
Grade B	2
Grade C	4

Abbreviations: GV, Germinal vesicle; IVF, In vitro fertilization; MI, Immature oocyte; MII, Mature oocyte.

TABLE 4 Result of preimplantation genetic testing for aneuploidies (PGT-A).

Characteristics	Value
No. embryo biopsied	8
Euploidy	5
Aneuploidy	1
Mosaic	2

3 | DISCUSSION

During the proliferation process, bacteria created endotoxin, which is harmful to embryonic development.[8] Washing is a commonly used method to remove microorganisms from the embryo surface. However, it may not be the most effective approach, as demonstrated by the patient in our study who experienced failed pregnancies despite careful washing of the embryos during the second and third transfers. This could be attributed to the high bacterial load in the culture medium and the qualitative nature of the washing procedure, leading to recontamination.[4]

The zona pellucida is a critical structure that surrounds the oocyte and later, the developing embryo in humans. The ultrastructure of zona pellucida has been clearly described in a related study.[9] It can act as a protective barrier

TABLE 5 Detailed information of 4 frozen embryo transfer cycles.

Number	Number of embryo transfered	Ploidy status	Endometrium thickness	Intervention	Outcome
1st FET	1	Euploidy	9.5 mm	None	beta hCG = 0.1
2nd FET	1	Euploidy	10 mm	Wash 4 times in culture medium	beta hCG = 13.9
3rd FET	1	Euploidy	10.5 mm	Wash 4 times in culture medium	beta hCG = 0.1
4th FET	1	Euploidy	12 mm	Zona pellucida removal	Successful Live birth

Abbreviations: Beta hCG, Beta human chorionic gonadotropin; FET, Frozen embryo transfer.

to prevent multiple sperm from fertilizing the same oocyte. It also protects the developing embryo during its journey through the fallopian tubes towards the uterus. However, in our study, the zona pellucida cannot protect embryo from bacteria. This can be explained due to the porous structure of the zona pellucida, which makes it challenging to completely eliminate microorganisms.[5] Indeed, Familiari et al.[10] performed a scanning electron microscopy (SEM) to prove the spongy structure of zona pellucida of mature oocytes. Additionally, during the cleavage and blastocyst stages of the embryo, the outer surface of the zona pellucida also showed a spongy texture with wide and flattened fenestrations. Therefore, we suggest that zona pellucida removal is an optimal method to stop bacterial contamination. This approach can be done successfully using both chemical (Tyrode acid) and physical method (Laser). However, the effectiveness of Tyrode acid can vary depending on the thickness the zona pellucida. Additionally, there is a slight risk of exposing the embryo to Tyrode acid, which could potentially affect its viability or development. In our study, by utilizing a laser to remove the contaminated zona pellucida from the embryos, the recontamination of bacteria was prevented, leading to a successful live birth for the patient. Moreover, the removal of the barrier between the embryo and the uterine environment could potentially enhance implantation and increase the chances of a successful pregnancy.[11]

In this case, the use of laser for zona pellucida removal allowed for precise and controlled manipulation. It can also be used in a wide range of cases, including embryos with thick or tough zona pellucida. It is worth noting that the successful outcome obtained in this patient after repeated implantation failures, with a healthy live birth, suggests the potential benefit of this technique. However, further studies with larger sample sizes and controlled settings are needed to validate these findings. Additionally, it is important to consider the risks and limitations associated with zona pellucida removal. The procedure requires skilled personnel and specialized equipment to ensure proper execution and minimize the risk of damage to the embryo. There is a possibility of damage to the embryo during the removal process, potentially impacting its viability. Long-term follow-up studies are necessary to evaluate the potential effects of zona pellucida removal on fetal development and health.

4 | CONCLUSION

In conclusion, the removal of the zona pellucida can mitigate bacterial contamination and improve embryo-endometrium interaction. While these findings are promising, additional research is needed to establish the efficacy and safety associated with zona pellucida removal. As this technique continues to evolve, this study aims to contribute to the existing literature and stimulate further investigation in this field.

AUTHOR CONTRIBUTIONS

Son Truong Dang: Conceptualization; methodology. **Huy Phuong Tran:** Writing – original draft; writing – review and editing. **Tuong Nguyen Ho:** Investigation. **Loc Thai Ly:** Resources; validation; writing – original draft; writing – review and editing. **Tuyet Thi-Diem Hoang:** Validation; writing – original draft; writing – review and editing. **Trang Nguyen-Khanh Huynh:** Supervision; writing – original draft; writing – review and editing. **Anh Tuan Do:** Investigation; resources; visualization. **Thuan Duc Nguyen:** Resources; validation. **Phuong Thi Dao:** Resources; supervision; validation.

FUNDING INFORMATION

None.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study. No identifiable information of the patient was disclosed.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

ORCID

Huy Phuong Tran https://orcid.org/0000-0001-8737-5208

REFERENCES

1. Peter MM. Kastrop and others, microbial contamination of embryo cultures in an ART laboratory: sources and management. *Hum Reprod*. 2007;22(8):2243-2248. doi:10.1093/humrep/dem165
2. 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;37:53-61. doi:10.1007/s10815-019-01640-5
3. Shu Y, Prokai D, Berga S, Taylor R, Johnston-MacAnanny E. Transfer of IVF-contaminated blastocysts with removal of the zona pellucida resulted in live births. *J Assist Reprod Genet*. 2016;33:1385-1388. doi:10.1007/s10815-016-0776-3
4. 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
5. Pellerin JL, Alsaleh A, Mermillod P, et al. Attachment of Coxiella burnetii to the zona pellucida of in vitro produced goat embryos. *Theriogenology*. 2018;106:259-264.
6. Yumoto K, Shimura T, Mio Y. Removing the zona pellucida can decrease cytoplasmic fragmentations in human embryos: a pilot study using 3PN embryos and time-lapse cinematography. *J Assist Reprod Genet*. 2020;37:1349-1354.
7. Li D, Yang DL, An J, et al. Effect of assisted hatching on pregnancy outcomes: a systematic review and meta-analysis of randomized controlled trials. *Sci Rep*. 2016;6(1):31228.
8. Dumoulin JC, Menheere PP, Evers JL, et al. The effects of endotoxins on gametes and preimplantation embryos cultured in vitro. *Hum Reprod*. 1991;6(5):730-734.
9. Wassarman PM. Zona pellucida glycoproteins. *J Biol Chem*. 2008;283(36):24285-24289.
10. Familiari G, Heyn R, Relucenti M, Sathananthan H. Structural changes of the zona pellucida during fertilization and embryo development. *Front Biosci*. 2008;13(17):6730-6751.
11. Jelinkova L, Pavelkova J, Strehler E, Paulus W, Zivny J, Sterzik K. Improved implantation rate after chemical removal of the zona pellucida. *Fertil Steril*. 2003;79(6):1299-1303.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Dang ST, Tran HP, Ho TN, et al. Zona pellucida removal resulted in a successful live birth: Report on a case with recurrent implantation failure due to embryonic bacteria contamination. *Clin Case Rep*. 2024;12:e8483. doi:10.1002/ccr3.8483