A new method to rescue embryos contaminated by bacteria

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Objective: To report a case of successful pregnancy involving embryos that were affected by bacterial contamination.

Design: A case report.

Setting: Academic assisted reproductive center.

Patient(s): A 31-year-old infertile patient with obstructed fallopian tubes facing bacterial contamination in her embryos during in vitro fertilization.

Intervention(s): The zona pellucida (ZP) of the embryos that were contaminated by bacteria was removed by acidic Tyrode’s solution. The ZP-free embryos were then cultured in a time-lapse culture dish with 1 zygote per well until day 5 when a single ZP-free blastocyst was selected for transfer.

Main Outcome Measure(s): The rate of obtaining embryos without recurrence of bacterial contamination and the developmental potential of the embryos.

Result(s): Twenty oocytes were retrieved and were coincubated with sperm in vitro overnight. A total of 9 zygotes with 2 pronuclei and potential of the embryos.

Conclusion(s): Zona pellucida removal is a safe and effective method to rescue embryos contaminated with bacteria. (Fertil Steril Rep® 2022;3:168–71. ©2022 by American Society for Reproductive Medicine.)

Key Words: Bacterial contamination, zona pellucida, in vitro fertilization, pregnancy

INTRODUCTION

Bacterial contamination during embryo culture is a serious problem faced in all in vitro fertilization (IVF) laboratories. According to previous reports, the frequency of microbial contamination is between 0.35% and 0.86% (1–3). Although the frequency of contamination is <1%, it is still a serious problem when it occurs, resulting in no embryos being available for transfer (1, 4, 5). The damage caused by microbial contamination in IVF procedures can be directly translated into financial and psychological burdens for patients (6). With the sheer number of IVF cycles performed worldwide each year, microbial contamination should not be underestimated as a complication in an assisted reproductive technology treatment cycle. Therefore, it is of utmost importance for any IVF laboratory to prevent bacterial contamination. Likewise, the method to rescue contaminated embryos is equally important.

At present, the most commonly used method when bacterial contamination occurs involves washing the embryos thoroughly with a medium containing antibiotics as an attempt to remove microorganisms from their...
surfaces. However, because of the unique porous network structure of the zona pellucida (ZP) that surrounds the oocyte (7, 8), it is often difficult to remove microorganisms from it completely. In most cases, bacterial contamination may happen again even after the embryos have been washed. In this study, the recontamination with bacteria was successfully prevented by the removal of the ZP from embryos affected by bacterial contamination. To our knowledge, this is a new method of rescuing contaminated embryos, eventually leading to a successful pregnancy.

**CASE REPORT**

The key patient in this study is a 31-year-old woman with a 3-year history of secondary infertility and regular periods. Her right fallopian tube was obstructed (distal obstruction), and her left fallopian tube suffered partial blockage. Her baseline follicle-stimulating hormone, luteinizing hormone, estradiol, and antimüllerian hormone levels as well as her husband’s semen analysis were normal. Controlled ovarian stimulation was performed using the long gonadotropin-releasing hormone agonist protocol.

Sperm preparation and insemination procedures were as described previously (9). Briefly, the man washed his hands and penis with soap and disinfected his hands with alcohol before semen collection. The semen sample was collected through masturbation, and the sperms were selected via disinfected his hands with alcohol before semen collection. The semen sample was collected through masturbation, and the sperms were selected via

The semen suspensions used for the insemination of the oocytes, follicular fluid, and the IVF medium were collected and sent to the Department of Medical Microbiology for microbiological examination.

Test results showed that the contaminating microorganism was the *Klebsiella pneumoniae* bacteria. It is, however, noteworthy that the follicular fluid samples were found to contain *Enterococcus faecalis*, whereas no bacteria were detected in the semen samples. Each zygote was then washed several times in the G-IVF medium and then cultured in the G-1 PLUS medium with a ratio of 1 embryo to 1 drop of culture medium. Nonetheless, bacteria were still present in all embryo culture droplets by the afternoon of day 1. Considering that washing the zygotes again may have little effect, the ZP of some zygotes was removed instead after obtaining the patient’s informed consent.

In the first 3 days, the embryos were cultured in the G-1 PLUS medium (Vitrolife, 10128). Next, the embryos were moved into a G-2 PLUS medium (Vitrolife, 10132) for blastocyst culture.

![Image](https://via.placeholder.com/150)

The embryo culture medium used in this study contains gentamicin as the antibiotic unless otherwise stated.

On the first day after fertilization, cloudy particles were observed in the fertilization dishes, and a large number of sperms were noticed to be immotile (Fig. 1A). Thus, we suspected that bacteria contaminated the fertilization dishes. The semen suspensions used for the insemination of the oocytes, follicular fluid, and the IVF medium were collected and sent to the Department of Medical Microbiology for microbiological examination.

**TABLE 1**

| Baseline information of the patient. | Maternal | Values | Paternal | Values | Reference ranges for semen analysisb |
|-------------------------------------|----------|--------|----------|--------|-------------------------------------|
| Baseline FSH (U/L)a                  | 1.86     | Sperm concentration | 62 × 10⁶/mL | 47%   | ≥ 15 × 10⁶/mL                        |
| Baseline LH (U/L)a                   | 5.66     | Motility          | 47%         | ≥ 40% |
| Baseline E₂ (pg/mL)a                 | 132      | Normal morphology | 10%        | ≥ 4%  |
| AMH (ng/mL)                          | 5.04     | Sperr DNA fragmentation index | 14%       | <25%  |
| Total Gn (IU)                        | 1,775    |                    |            |       |
| Oocyte number                        | 20       |                    |            |       |
| 2PN                                 | 9        |                    |            |       |
| 1PN                                 | 3        |                    |            |       |
| Multiple PN                         | 2        |                    |            |       |
| MII                                 | 3        |                    |            |       |
| MI                                  | 1        |                    |            |       |
| Gv                                  | 2        |                    |            |       |

Note: 1PN = 1 pronucleus; 2PN = 2 pronuclei; MII = metaphase II stage; MI = metaphase I stage; GV = germinal vesicle stage; AMH = antimüllerian hormone; DNA = deoxyribonucleic acid; E₂ = estradiol; FSH = follicle-stimulating hormone; LH = luteinizing hormone; Gn = Gonadotropin.

a Levels of FSH, LH, and E₂ on day 2 during the patient’s menstruation.

b According to the World Health Organization laboratory manual for the examination and processing of human semen, 5th edition.

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times using the G-1 PLUS medium and then cultured in 35 mm culture dishes containing G-1 PLUS medium, 1 zygote per drop (washing treatment group). The embryos in the washing treatment group were incubated in an incubator at 37°C, with saturated humidity containing 5% CO2, 5% O2, and 89% N2. In the washing treatment group, all embryos experienced recontamination on day 2 and were dead by day 3. As for the ZP-free group, 2 of 7 zygotes experienced recontamination on day 2 and were dead by day 3 because of disrupted development. The remaining 5 embryos remained uncontaminated and were transferred to the G-2 PLUS medium on day 3. Two cultured embryos developed into blastocysts by day 5; hence, 1 blastocyst was transferred on day 5 of the fresh cycle (Fig. 1B) and the other was cryopreserved (Fig. 1C). No antibiotic was prescribed for the patient after embryo transfer. Luteal support was administered twice a day using vaginal capsules at 400 mg each time. Luteal support was continued until 12 weeks into pregnancy.

A single intrauterine gestation was confirmed by vaginal ultrasound examination 4 weeks after embryo transfer. At the time of writing this article, this patient was in the 30th week of pregnancy, and no intrauterine infection had occurred throughout this period of time.

To verify the effectiveness of this method, some discarded embryos were infected with *Escherichia coli*. A total of 14 triploid embryos were cultured with a medium containing *E. coli* the next day. These embryos were then randomly divided into 2 groups of 7 embryos each. Each group was cultured for 72 hours, and the bacterial growth in the embryo culture droplet was observed every day. All 7 embryos in the washing treatment group suffered from *E. coli* contamination again on the next day after treatment. In contrast, the ZP-free group exhibited no bacterial growth after 3 days of culture, and the embryo morphology was maintained without blastomere lysis and separation.

**DISCUSSION**

In this study, the recontamination of embryos by bacteria was prevented through the removal of the ZP of the contaminated embryos, which in turn enabled the patient to achieve pregnancy. To our knowledge, this is a new method of rescuing contaminated embryos, which ultimately leads to a successful pregnancy. In 1 other report, Shu et al. (10) removed the ZP of contaminated frozen blastocysts, which led to successful clinical pregnancies after the transfer of ZP-free blastocysts that were previously contaminated during IVF culture. However, the removal of the ZP in that study was not done on the day the contamination was discovered but right before embryo transfer.

In our case, the microorganism that had contaminated the embryo culture medium was *K. pneumoniae*. However, *E. faecalis* was found in the follicular fluid samples instead, and no bacteria were detected in the semen samples. Some studies suggest that the work environment and improper operation may be the possible causes of contamination, besides the reproductive tract microbiota of donors (1, 11). Therefore, the microbes may have originated from ambient air in the workplace or the inadvertent introduction during in vitro procedures performed in this case.

Bacterial endotoxin, which is produced in the process of proliferation, has a serious impact on embryonic development (3). Apart from causing fragmentation and blebbing to embryos, it also reduces the pregnancy rate (4, 5). Therefore, washing bacteria-contaminated embryos with a medium containing antibiotics is a method of rescuing the embryos by attempting to remove microorganisms from the embryo surface. Studies have shown that the growth of bacteria can be inhibited by adding exogenous penicillin (31.5 IU/mL) and streptomycin (10 μg/mL) or gentamicin (0.15 g/L) into the washing medium (12, 13). However, this might not be the most effective method for decontaminating embryos. Our previous study showed that only 7 of 42 cases of contamination had embryos available for transfer after treatment with this method (14). There are two possible explanations for this outcome: there was a high bacterial load in the culture medium or inaccuracy in the applied antibiotic susceptibility test. Because bacterial susceptibility testing usually requires several days before results can be obtained, the antibiotic choice to reverse contaminations in embryos can therefore only be selected subjectively.
The human ZP is primarily composed of 4 glycoproteins (ZP1, ZP2, ZP3, and ZP4). The outer surface of the ZP is comprised of a delicate network of thin, interconnected filaments in a regular alternating pattern of pores and compact meshes. (7, 15). The pores appear larger at the outer surface of the ZP matrix than at the inner surface, giving it a “spongy” appearance (16). This also means that microorganisms can easily adhere to the surface of the ZP. Henceforth, it can be difficult to remove the microorganisms found on the surface of the ZP by washing. In this case, we opted to remove the ZP itself from the contaminated embryos after the washing method failed to remove the bacteria. Interestingly, no recontamination was observed in 5 of the 7 embryos with the ZP removed; however, in the washing treatment group, all embryos suffered recontamination. Moreover, time-lapse observation showed that when recontamination occurred, bacteria began to grow around the embryo, indicating that the ZP was the carrier of the bacteria, making it difficult to remove the bacteria by washing alone (Video 1).

It is worth noting that the ZP plays vital roles during oogenesis, fertilization, and preimplantation development (7). The ZP is of critical importance to maintain the spatial structure of the embryo before compaction occurs, which limits the separation of blastomeres from each other. Therefore, a culture dish with independent wells is recommended for the ZP-free embryos to restrict blastomere movement and limit external disturbances.

In conclusion, this study utilized a novel method to safely and successfully assist a patient in getting pregnant with an embryo that had previously experienced bacterial contamination. This method could effectively rescue embryos that were contaminated by bacteria in a timely manner, thus enabling embryo transfer during the fresh cycle and preventing possible damage during the freezing and/or thawing process.

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