# A case report of unsuccessful conventional in vitro fertilization in a patient who had previously demonstrated excellent fertilization

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## Abstract

Numerous instances of unsuccessful cIVF inseminations in patients who had previously had fertilizations using the same technique are well known, but this occurrence does not appear to have been documented. The objective of the current case report is to document this occurrence. In the year 2019, a couple approached our clinic to seek treatment for unexplained infertility. At that time, the woman was 38 years old with no prior obstetric history. The man, who was 57 years old, had previously fathered seven children—the youngest of whom is 14 years old—with his first wife. The couple underwent typical IVF investigations. For IUI, cIVF, and ICSI, spermatozoa were prepared using density gradient centrifugation. In October 2019, an IUI treatment cycle was attempted but failed. The couple underwent IVF therapy in January 2020. A total of 15 oocytes were retrieved. Eight oocytes were fertilized by cIVF; 6 oocytes underwent ICSI, of which 4 fertilized. Two grade 3AA expanded blastocysts (n=2) were transferred on day 5. In the fourth month of the pregnancy, the patient miscarried. In August 2022, the couple came back for another IVF treatment. Eight oocytes were retrieved and inseminated by cIVF. The oocytes were denuded at 4hrs post-insemination. Five oocytes were at the metaphase-II stage, but none of them had the second polar body. Two were at the GV stage and 1 was abnormal. Three of 5 oocytes were selected at random for ICSI, of which 2 fertilized. The remaining 2 oocytes were left to proceed with cIVF but did not fertilize. On day 3, the 2 ICSI-fertilized oocytes were transferred at the 16- and 8-cell stages of grades A and AB, respectively, but did not result in pregnancy. This case study has shown that previous successful cIVF fertilization is not a reliable indicator of fertilization in the following cycle of cIVF treatment. It would be prudent to attempt rescue ICSI in a proportion or all oocytes to ensure fertilization if the second polar body had not extruded by 4 to 6 hours post cIVF insemination.

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## Introduction

The fertilization rate for the conventional IVF (cIVF) procedure in our clinic is 77%. However, we have noted up to 97% cIVF fertilizations even when the spermatozoa morphology is suboptimal (AlShalian et al., 2020). The fertilization rate after cIVF is generally assumed to be in the range of 60-70% (Nagy et al., 1993). Instances of decreased or complete fertilization failures following cIVF inseminations have been reported. The reasons for this are an unfavorable hormonal environment brought on by the stimulation protocol utilized, and/or problems in the spermatozoa and/or oocytes (Benadiva et al., 1999). It is now well recognized that attempts to rescue unfertilized oocytes by ICSI when they are about 24hrs old have not been very encouraging (Chen et al., 1995; Sjogren et al., 1995; Tsirigotis et al., 1995; Morton et al., 1997), probably due to oocyte ageing,

One strategy used to avoid fertilization failure in cIVF is to detect the extrusion of the second polar body beginning from 2 to 6hrs post-insemination. Almost 90% of oocytes would have extruded the second polar body by 6hrs post cIVF insemination (Chen and Sathanathan, 1986; Plachot et al., 1986; Nagy et al., 1994; Payne et al.,1997) if penetrated by the spermatozoa. As a result, some fertility clinics

have two shifts of workers so that if the second polar body is not detected 6 hours after cIVF insemination, rescue ICSI can be performed by the second shift of workers to avoid total fertilization failure. In the authors' clinic that has a limited workforce, rescue ICSI was performed in about 50-65% of oocytes that had not extruded the second polar body by 4hrs post cIVF insemination. We do not perform 100% rescue ICSI on such oocytes at 4hrs post-insemination because of the risk of polyploidy.

In the past four decades of cIVF, there must have been numerous instances of failed cIVF inseminations in patients that had previously had fertilizations following the same insemination procedure. The reasons for this are not clear. What is of interest is that such failed cIVF inseminations after previously successful ciVF inseminations have not been reported even though such failures do occur from time to time. A search of the literature resulted in no publications or reports relating to this occurrence. It would be useful for new entrants to the ART profession if this matter was widely communicated so that new workers would be better prepared and could take preventive measures to avoid total fertilization failure in cIVF. The objective of this report is to document this occurrence and to highlight the potential for total cIVF insemination failure in patients that may have had excellent cIVF fertilizations in previous cycles. This documentation is intended to create awareness of this issue so that new entrants to the profession can take appropriate measures to avoid total fertilization failure.

## Subjects and methods

The couple approached our clinic seeking treatment for unexplained infertility in the year 2019, at which time the female was 38 years old with no prior obstetric history. The male was 57 years old and had earlier fathered 7 children, the youngest being 14yrs old, with his first wife. Following standard investigations, the female was treated for IUI. Spermatozoa were prepared by density gradient centrifugation for IUI, cIVF, and ICSI. An IUI was performed in October 2019, and cIVF treatment cycles in 2020 and 2022. The PN check was performed at 17 to 18hrs post cIVF or ICSI insemination.

## Results and Discussion

At that time, IUI was performed. The semen specimen was oligozoospermic with a total spermatozoa concentration of 8 million per ml, of which 60% were motile. Although the specimen was not suitable for IUI, nevertheless, the treatment was performed using washed spermatozoa at 2 million per ml, of which 90% were motile. The IUI treatment cycle failed.

Subsequent to the failed IUI, the couple were recruited for IVF treatment. The semen characteristics obtained during this treatment cycle had improved significantly (see Table 1). This was in late January 2020. Fifteen oocytes (n=15) were retrieved following ovarian stimulation. Eight oocytes (n=8) were subjected to conventional cIVF while the remaining 7 oocytes were denuded for ICSI. ICSI was performed on 6 metaphase-II oocytes. One oocyte was atretic.

The following day, all 8 oocytes inseminated by cIVF had fertilized and all of them were 2PNs. Four (n=4) of the 6 oocytes inseminated by ICSI were fertilized (4x2PNs). One showed a faint 2 PN but was later proven to be not fertilized, while the other oocyte was not fertilized. On day 5, 2 (n=2) expanded grade 3AA blastocysts were transferred, one from cIVF and the other from ICSI inseminations. The patient became pregnant (singleton) but aborted in the fourth month.

The couple returned for a second IVF treatment in August 2022. The semen quality was lower (Table 1) than it was in the previous cycle, but the post-wash spermatozoa specimen appeared sufficient and suitable for cIVF insemination. Eight oocytes (n=8) were retrieved from the female. Considering the excellent cIVF insemination obtained in the previous cycle, all 8 oocytes were subjected to cIVF insemination.

The inseminated oocytes were denuded at 4hrs post-insemination. Subsequent to denuding, the stripped oocytes were examined for the presence of the second polar body. At this point was that 2 oocytes were at the GV stage. 1 was atretic, and 5 oocytes were at the metaphase-II stage with a single polar body. The second polar body was not visible in all 5 oocytes. Three of the oocytes (n=3) were selected at random for

**Table 1: Semen and spermatozoa characteristics pre- and post-density gradient wash**

| Description | Semen and spermatozoa characteristics | | | |
|---|---|---|---|---|
| | Pre wash | | Post wash | |
| | First cycle | Second cycle | First cycle | Second cycle |
| **Volume (ml)** | 1.5 | 1.2 | 1.0 | 1.0 |
| **Viscosity** | Normal | Normal | Washed | Washed |
| **Liquefaction Time (mins)** | 20 | 10 | N/A | N/A |
| **pH** | 8.5 | 8.5 | N/A | N/A |
| **WBC/Pus cells** | 6-8 | 5-6 | 0 | 0 |
| **Agglutionation** | 0 | 0 | 0 | 0 |
| **Total spermatozoa count** | 59 | 19 | N/A | N/A |
| **Spermmatozoa count/ml** | 39 | 16 | 27 | 15 |
| **Total motility (%)** | 74 | 69 | 89 | 93 |
| **Progressive motility** | 49 | 31 | N/A | N/A |
| **Weak motility** | 25 | 38 | N/A | N/A |
| **Non-motile** | 26 | 31 | N/A | N/A |
| **Morphology** | 2 | 2 | 3 | 3 |

ICSI while the remaining two were left to proceed with cIVF. Of the 3 oocytes inseminated by ICSI, 2 had intact healthy oolemma that offered resistance to injection by the ICSI pipet. The 3rd oocyte had an oolemma that offered no resistance to injection by the ICSI pipet. The latter was cultured separately.

The next morning, it was observed that the 2 cIVF inseminated oocytes were not fertilized (2 x 0PN), the 2 ICSI-inseminated oocytes with intact and healthy oolemma had fertilized (2x 2PNs), while the oocyte with an oolemma that offered no resistance to the ICSI injection needle had lysed. The latter oocyte was probably undergoing apoptosis due to senescence. The apoptotic oocyte probably succumbed as a consequence of the trauma of injection. On day 3, the 2 fertilized oocytes were transferred at the 16-cell and 8-cell stages, which were graded as grades A and AB respectively. This treatment cycle failed to elicit a pregnancy.

## Conclusion

In cIVF treatment cycles, it is imperative to check for extrusion of the second polar body at about 4 to 6hrs post-insemination to determine whether spermatozoa penetration of the oocyte has occurred. If the second polar body has not extruded at 6 hours following cIVF insemination, rescue ICSI must be carried out. This case study has demonstrated that past cIVF fertilization is not a clear predictor for fertilization in a subsequent cIVF treatment cycle.

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