Candida contamination of embryos in the ART laboratory: a case report and literature review

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ABSTRACT

Objective: To report a case of yeast contamination with adhesion of hyphae to the zona pellucida. **Design:** A case report and brief literature review. **Setting:** Nextclinic Fertility Center, Merano (Italy). **Patient(s):** A 32-year infertile patient facing microbial contamination of her embryos after thawing. **Results:** Due to the heavy contamination of the embryo by yeast, embryo transfer (ET) was not carried out, resulting in cancellation of the cycle. **Conclusions:** A very rare case involving embryos contaminated by Candida is here described. Strategies and practical measures to prevent the risk of cross-contamination in the storage tanks of cryobanks are illustrated. Zona pellucida removal may be a possible method to rescue contaminated embryos for ET.

KEYWORDS

In vitro fertilization, yeast contamination, cryopreservation, zona pellucida.

Introduction

Despite strict quality control protocols and aseptic techniques, microbial contamination of assisted reproductive technology (ART) cultures is a known possibility. Only a few studies report the incidence of this phenomenon, which can affect from 0.29% [1] to 0.68% [2] of embryonic cultures. There are even fewer data available on the incidence of yeast contamination, which can be up to 0.18% [3].

The main sources of contamination are follicular fluid and semen, therefore intracytoplasmic sperm injection (ICSI) is an established method to minimize the risk of microbial contamination [2]. Most reported contaminants are caused by bacteria strains, with *E. Coli* [1] accounting for 83% of cases.

We report a case of contamination caused by *Candida spp*, which resulted in cancellation of the planned embryo transfer (ET).

Case report

A 32-year-old patient with primary infertility was referred to our clinic in February 2022. She had been trying to conceive for 7 years. Her baseline follicle-stimulating hormone, luteinizing hormone, estradiol and anti-Müllerian hormone levels were normal. Her husband's semen analysis showed moderate asthenozoospermia (sperm concentration 42 x 10^6/mil; motility 28%; normal morphology 33%, leukocytes 340000) (Table 1). She had already undergone a cycle of ovarian stimulation in another IVF clinic, where a sperm culture was not performed before the fresh cycle. A low-dose gonadotropin antagonist protocol was used to obtain a controlled ovarian stimulation.

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Table 1 Patient's baseline data.

MATERNAL	VALUES	PATERNAL	VALUES
Baseline FSH (U/L)	6,6	Sperm concentration	42 x 10^6/ mil
Baseline LH (U/L)	7,5	Motility	28
Baseline E2 (U/L)	5,6	Normal morphology	33%
AMH (ng/mL)	4,47	--	--

FSH = follicle-stimulating hormone; **LH** = luteinizing hormone; **E2** = estradiol; **AMH** = anti-Müllerian hormone. Levels of FSH, LH, E2 on day 3 during the patient's menstruation.

After oocyte retrieval, 18 oocytes were collected, 16 of which were mature. Eight oocytes were cryopreserved for later use and 8 were fresh fertilized using the ICSI technique, which resulted in four embryo transfers (ETs) at the blastocyst stage, but only one biochemical pregnancy was achieved.

From subsequent ICSI fertilization of the cryopreserved oocytes, four blastocysts were obtained that were still available for ET. The embryos were cryopreserved using an open system (Cryotop, Kitazato).

The couple decided to perform ET in our clinic due to per-

sonal preference. Transport of the four embryos was organized and documentation and protocols regarding the embryos were collected and evaluated in order to ensure compliance with national and our internal quality regulations [4].

To achieve a personalized ET, we performed an Endometrio test (Igenomix, Italy) which demonstrated a low endometrial microbiome. Vaginal lactobacillus (Avidif CV) was prescribed during preparation for ET. No pathogens were detected and the endometrium appeared to be in the receptive stage.

In May 2022, during thawing of the first embryo the embryologist immediately noticed the presence of a mass, presumably hyphae, attached firmly to it. The mass appeared tightly adhered to the outer surface of the zona pellucida and covered a significant part of the blastocyst itself (Fig. 1A). The embryologist attempted to detach the mass by repeatedly rinsing the blastocyst with a pipette with a diameter of 300 micrometers, but without success.

In order to ensure a transfer for the couple and avoid cancelling the cycle, all the remaining blastocysts were thawed (Fig. 1B) and similar findings were observed in all except for the last one which had no evident contamination with hyphae. The embryos were classified according to the Gardner classification [5] which showed a good embryo grading for all four blastocysts: Bl4BB, Bl3BB, early Bl, Bl3BB.

No additional information regarding embryo cultures before freezing was available (single/double embryo culture, evidence of contamination during the embryo culture, presence of hyphae before loading blastocysts on the cryotops, etc.), and therefore, after consultation with the couple, we decided not to perform ET.

The embryo culture media were sampled and sent to the microbiology laboratory for further investigation.

Following the sampling, all blastocysts were vitrified using a closed device (Vitrisafe).

The workstations were thoroughly cleaned with a surface disinfectant (Oosafe). After 15 minutes microbiological samples were taken from the work surfaces to ensure that contamination had not spread.

Candida contamination was confirmed by the microbiology laboratory for the embryo culture media of three blastocysts, while the fourth was found to be contaminated by *E. Coli*. The microbiological samples from the work surfaces were negative, confirming the effectiveness of the laboratory's sanitization procedures. The relevant surveillance institute (Centro Nazionale Trapianti - CNT) was immediately notified of the serious adverse event, as was the original laboratory that had performed the insemination, so that they could also take the necessary precautions.

Discussion

Although ART procedures have to be carried out in accordance with strict standards, microbiological contamination can occasionally still occur, with serious consequences for the patients. Literature regarding this occurrence is rare and refers mainly to aspecific contamination of cultures by bacteria, yeast or viruses.

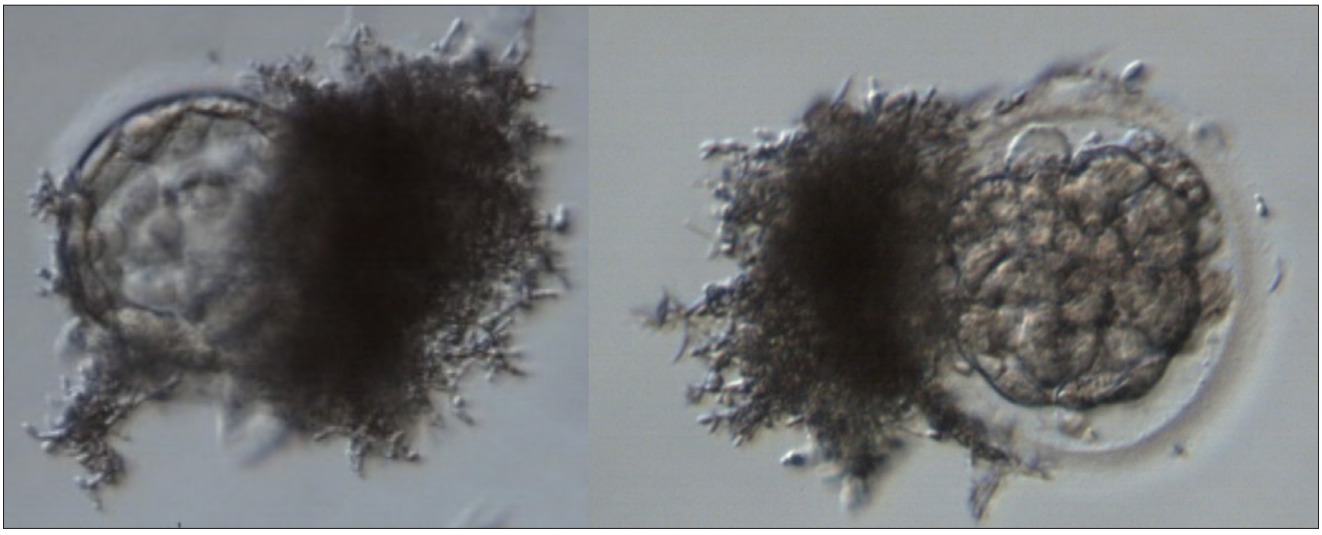
Over a period of 8 years, Kastrop and his study group analyzed 13977 ART cycles, finding a contamination rate of 0.68%; contamination was due to *E. Coli* in 59% of cases and to *Candida* species in 25% [2].

A decade later, Li-Ling et al. retrospectively reviewed 4118 ART cycles and found 12 cases of microbial contamination, extrapolating an incidence of nearly 0.29%. The contamination was mainly caused by *E. Coli*, while no other microorganisms such as yeast species were isolated [1].

When we sought literature specifically concerning yeast contamination in ART cultures, the available data were found to be even scarcer. To our knowledge, there are only two scientific publications on this topic: in 1996 Ben-Chetrit et al. found yeast colonization in 5 dishes out of 729 cycles [6]; in 2007, Klein *et al*., overviewing 11816 cycles, reported an incidence of 0.18% [3].

In all of the mentioned publications, microbial contamination was suspected after microscopic evaluation of the culture

Figure 2 *Candida* contamination: adhesion of hyphae to the zona pellucida. **A**: First thawed embryo. **B**: second thawed embryo.



dish revealed a turbid liquid which was then sampled and sent for confirmatory microbiology assessment. In our case, hyphae of *Candida* were directly visible and firmly attached to the zona pellucida of the thawed blastocysts. In all three visibly contaminated embryos, the contamination was confirmed by microbiological analysis.

Reviewing the limited existing literature, it becomes clear that microbial contamination is much more frequent when the fertilization technique involved is IVF. The ICSI technique is proposed as a strategy to minimize contamination. Considering that seminal fluid is considered to be the main contaminant [(1)], the fact that ICSI involves isolation of a single spermatozoon from the solution may explain the low risk of contamination associated with this procedure [2].

Our clinical case is therefore a rare event in itself, given the minimal risk of *Candida* contamination in ART laboratories; however, the fact that the contamination was found in embryos derived from fertilization by ICSI performed on thawed oocytes makes it even more unusual. This aspect underlines the importance of ensuring that operators observe all the necessary measures to prevent contamination of cultures, such as the use of disposable personal protection, a controlled air system in the laboratory room, and regular cleaning of the laboratory equipment, forceps, tongs, canisters, canes, and so on.

In an important review on cryostorage management of reproductive cells and tissue in ART, published in 2023, the authors remark that temporary storage tanks, dewar flasks and devices used for transporting straws or devices between long-term storage tanks and the IVF laboratory bench should be cleaned after use by rinsing with a cleaning solution and then left to dry upside down in order to prevent contamination [7].

There is still no unanimous consensus regarding the transfer of embryos from contaminated culture. In 1996, a positive effect deriving from the co-culture of embryos with *Candida* was even hypothesized thanks to a reduced concentration of oxidative free radicals [6]. On the other hand, a later report suggested that earlier contamination, at day one, has a detrimental effect on embryo development [3]. The authors of this report still proceeded with ET after adequate counseling of patients regarding the decreased likelihood of clinical success. They reported no statistically significant differences in clinical outcomes between the control cycles and the yeast cycles. However, they noticed that all live births resulted from cycles in which contamination was noted on day 3 or 4. No live birth occurred when contamination was noted on day one, due to poorer embryo quality [3].

In our specific case, we decided, together with the couple, not to proceed with ET of the contaminated blastocysts due to the patient's favorable ovarian reserve and the favorable prognosis for the couple in a subsequent cycle. Moreover, the contamination we detected was not limited to the culture medium, but more extensive, being firmly attached to the blastocyst itself, even though the quality of the embryo did not appear to be compromised. We were concerned about the possibility that the embryo might undergo degeneration under the faster and thriving development of the hyphae, and about the danger of introducing a considerable amount of yeast into the uterus, with the risk of causing infections. We notified the original center of the contamination because we deemed it implausible that such substantial contamination could have occurred within a brief time frame, meaning that the contamination likely transpired at the source center.

Since Italian law prohibits the destruction of embryos, the blastocysts had to be cryopreserved again. Initially they were stored in an open device (Cryotop, Kitazato). In this regard, it should be noted that there is a risk of both cross-infection and cross-contamination of embryos and gametes in liquid nitrogen tanks [8].

Our clinic has always worked with a closed carrier device to guarantee a safe procedure [9].

As this device is hermetically sealed, there is no contact with the liquid nitrogen during cooling, storage and warming. This device offers the highest protection during the entire vitrification process compared with open or semi-closed cooling devices [10].

Despite this, the embryos that turned out to be contaminated only at the time of thawing had been stored in the cryobiology room of our clinic in an open device in liquid nitrogen containers. This arrangement might result in contamination of the liquid nitrogen itself. One of the main biosafety measures to prevent contamination is segregation of samples. It is therefore recommended that samples from infected or suspected donors be quarantined, tested, and if contamination is found, stored in separate containers [11]. In this case, however, it was not possible to use the quarantine tank as *Candida* infection is not among the contaminants regularly tested in the transport of gametes and embryos between fertility clinics.

Since the majority of commercial liquid nitrogen is not sterile, long-term storage tanks also require periodic cleaning. An interesting strategy, proposed by the working group of Parmegiani *et al*., involves the use of UV radiation to sterilize the liquid nitrogen and the tanks [12]. This procedure requires that all cryopreserved samples be transferred in a witnessed way to a similar cryostorage vessel, after which the long-term tank is sterilized by UV radiation.

The damage caused by microbial contamination in ART procedures has a direct impact on laboratory and fertility clinics' costs [13]. The greatest damage, however, is suffered by couples trying to achieve a pregnancy: ART treatments place an extremely high emotional and financial burden on patients. The destiny of oocytes and embryos is a sensitive issue, especially if they have to be stored indefinitely and can no longer be used. For this reason, although we initially wondered whether an alternative solution might be found to salvage the ET, given that the transfer of embryos contaminated by *Candida* is generally considered possible [3,6], in our case the contamination was not limited to the culture medium, and therefore ET was not deemed feasible.

From a later evaluation of our clinical case and after a careful review of the international literature, we found two publications regarding two interesting approaches to this problem.

In 2016, Shu *et al*. reported live births after removing the zona pellucida from contaminated blastocysts. The two cases they analyzed concerned the transfer of blastocysts contaminated by bacteria [14]. After thawing, the authors performed zona pellucida removal with laser pulse at the fifth day of embryo

development, in order to avoid microbial growth.

In 2022 Ruiqi et al. performed a similar procedure but with different timing: they removed the zona pellucida using acidic Tyrode's solution on zygotes, and obtained one ongoing pregnancy. Again, in this case, the contamination was caused by bacteria, specifically *Klebsiella pneumoniae* [15].

With hindsight, we can state that laser removal of the zona pellucida might perhaps have been used to perform a rescue ET for our couple. However, we have not found any specific reports of zona pellucida removal in cases of *Candida* infection with adhesion to the blastocyst. Moreover, considering the impracticality of conducting an extended culture period, given that we only discovered the contamination at the blastocyst stage, we would not have been able to definitively exclude residual contamination.

Conclusions

In conclusion, this report describes a very rare case of *Candida* contamination of embryos derived from fertilization by ICSI performed on thawed oocytes. Two aspects can be highlighted: first, in order to minimize the risk of contamination in IVF laboratories the application of aseptic techniques is of fundamental importance. Second, zona pellucida removal might be a feasible method to rescue *Candida* contaminated embryos, but more studies are needed to safely implement the technique.

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***Conflict of Interest**: The authors declare that they have no conflict of interest*