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Congenital Tuberculosis After In Vitro Fertilization: First Twin Cases In Spain And Review Of The Literature

Eduardo Lagarejos[1], Mª Isolina Campos-Herrero[1], Michele Hernández[2] and Elena Colino[3]

[1]Servicio de Microbiología. Hospital Universitario de Gran Canaria Dr. Negrín. Las Palmas De Gran Canaria. Spain

[2]Unidad de Enfermedades Infecciosas. Complejo Hospitalario Universitario Materno-Insular. Las Palmas de Gran Canaria. Spain

[3]Unidad de Enfermedades Infecciosas. Servicio de Pediatría. Complejo Hospitalario Universitario Materno-Insular. Las Palmas de Gran Canaria. Spain

Corresponding author:
Mª Isolina Campos-Herrero,
Servicio de Microbiología. Hospital Universitario de Gran Canaria Dr. Negrín. Las Palmas De Gran Canaria. Spain
Email: mcamnavl@gobiernodecanarias.org.

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1. Abstract

Genital tuberculosis is a major cause of infertility in women living in endemic areas. Since In-vitro fertilization (IVF) has been introduced as a treatment for infertility, congenital tuberculosis cases have been published. We report the first premature twins born with CTB after IVF in Spain and perform a literature review.

2. Introduction

The global burden of tuberculosis remains enormous, especially in developing countries. Genital tuberculosis (GTB) is a form of extrapulmonary tuberculosis (TB) secondary to hematogenous spread from foci in the lungs that, due to diverse clinical manifestations and the lack of specific clinical symptoms and signs, can be easily misdiagnosed. GTB accounts for 7-19% of female infertility in endemic areas [1] and accordingly, thus, congenital tuberculosis (CTB) has been considered to be rare with only approximately 400 cases reported in the literature [2]. In vitro fertilization (IVF) and embryo transfer (ET) represents a useful treatment for infertility and since this technology has been introduced, CTB cases following IVF have been reported. We herein report two cases that, to the best of our knowledge, are the first cases of premature twins born with CTB after IVF-ET in Spain and perform a literature review to describe reported cases. Since the first case was published in 2008 [3] another 22 cases have been reported so ours would be the 23 and 24th.

3. Cases Report

3.1. Mother's History

In 2019 a 30-year-old Spanish woman who was living on the island of Lanzarote (Canary Islands) successfully underwent an IVF-ET. She had no significant past medical history apart from her in fertility. Since the first trimester of pregnancy, she has had intermittent vaginal bleeding. At 24 weeks of gestational age began with fever, cough, and night sweats hence she was treated with empirical antibiotic therapy with no improvement. Due to unresolved fever and the menace of premature delivery, she was transferred to the reference hospital located on the island of Gran Canaria. Chest radiograph and pulmonary CT scan were normal; respiratory virus detection and blood and urine cultures were negative. Despite several courses of antibiotic therapy, the patient remained with the same symptoms, and she besides complained of headache. At 28 weeks gestation, chorioamnionitis was diagnosed, a cesarean section was performed, and female twins were born. After delivery, as the fever persisted an endometrial biopsy was made showing caseificant epithelioid granulomas, extensive areas of necrosis, and acid-fast bacilli (AFB) on Ziehl-Neelsen staining. A positive result for *M. tuberculosis* complex (MTB) nucleic acid amplification technique (NAAT) was obtained and subsequently grew drug-susceptible MTB. The mycobacterial culture was also positive from urine samples and negative from several sputa. Once a diagnosis of GTB was achieved, their babies were promptly evaluated for CTB. The mother was treated with a 2-month course of isoniazid, rifampicin, and pyrazinamide followed by 10 months of isoniazid and rifampicin. She recovered totally.

3.2. Twin 1

The infant weighed 1235 g and the Apgar scores at 1 and 5 minutes were 8 and 10, respectively. Immediately after delivery due to respiratory distress, she was admitted to the Neonatal Intensive Care Unit (ICU) and nursed in an incubator with continuous positive airway pressure (CPAP) for three days. She was started with broad-spectrum antibiotics until blood cultures were negative. Her condition improved and remained stable until 14 days of age when her respiratory state worsened, and biphasic CPAP was required. On the day of life 18, after her mother's diagnosis of GTB, she was evaluated for CTB. A positive result was obtained for TST, interferon-gamma release assay (IGRA, Quantiferon-TB Gold) and AFB staining, and MTB NAAT from the gastric aspirate. Abdominal ultrasound examination

did not show features of a primary complex of the liver. Treatment with isoniazid, rifampicin, and pyrazinamide was started. Subsequently, the culture of gastric aspirates revealed drug susceptible MTB. Urine and CSF mycobacterial cultures were negative. After two weeks of antituberculous therapy, she suffered a clinical worsening that required ventilatory support again. At this moment, blood analysis revealed elevated procalcitonin and anemia so oral prednisolone was added, and blood transfusion was required. On the day of life 53, respiratory support was stopped. At 2 months of age, while in the hospital, the girl developed an enlarged left submandibular lymphadenopathy that drained spontaneously. An excision biopsy of the cervical lymph node showed necrotizing granulomatous inflammation and AFB on Ziehl- Neelsen staining. After 4 months of antimycobacterial therapy, further cervical lymphadenopathies were surgically removed again with the same histological findings as previously. Mycobacterial cultures were negative in both excision biopsies. The baby completed a 2-month course of isoniazid, rifampicin, and pyrazinamide followed by a 10-month course of isoniazid and rifampicin and she fully recovered.

3.3. Twin 2

Her birth weight was 1095 g and the Apgar scores at 1 and 5 minutes were 2 and 6, respectively. Resuscitation was needed at birth, and she was transferred to the neonatal ICU with the requirement of ventilatory support (biphasic CPAP). Intravenous antibiotic therapy was instaurated and stopped several days later due to negative blood cultures. At 18 days of life, she was evaluated for CTB. An abdominal ultrasound showed liver lesions highly suggestive of granulomas. TST, IGRA, AFB staining, and MTB NAAT from gastric aspirate were positive. Therapy with isoniazid, rifampicin, and pyrazinamide was started. A culture of gastric aspirate and urine yielded drug susceptible MTB and was negative for CSF. After two weeks of antituberculous therapy due to general worsening, ventilatory support with biphasic CPAP was reinitiated. A paradoxical effect to antituberculous therapy was suspected and intravenous prednisolone was added with a good response. On the day of life 48, respiratory support was removed, and she was finally discharged from the hospital at 3 months of age. After the initial 2 months of therapy, pyrazinamide was stopped, and she completed a 12-month therapy. In the following four years, the girl had recurrent episodes of fever treated with corticosteroids with a probable diagnosis of PFAPA (Periodic Fever, Adenitis, Pharyngitis, and Aphthous stomatitis). Hepatic involvement was solved during the follow-up.

When CTB evaluation began, both infants were separated from the rest of the babies hospitalized. All workers from ICU and Neonatology Units were screened for TB, with no transmission of tuberculosis detected.

4. Review of Literature

Available characteristics of reported cases in the literature [3-16] and our two cases are summarized in Tables 1 and 2. Nineteen women and 24 babies (5 pairs of twins) were involved. The majority (71%, 12/17) of the women with available data were natives of countries with a high- level incidence of tuberculosis. None of them, even those (9 women) with a history suggesting previous TB, were evaluated for GTB before IVF. Of 15 women with data about symptoms, 5 were fully asymptomatic, and 10 had symptoms: 4 during pregnancy (3 in the first and 1 in the second trimester), 4 after delivery, and in two cases data about onset of symptoms were not available. The most frequent symptom was fever (70%) and others were cough, vaginal bleeding, and headache.

Table1: Data of mothers with GTB

ND: no data; EP: extremely preterm; VP: very preterm; LAD: lymphade-

Case (year of publication) citation	Age/ Country of origin	Prenatal TB history	Symptoms (onset)	EGA in weeks/ cesarean section	TB diagnosis	Disseminated TB
1 (2008)3	40/ Turkey	None	Fever, headache, myalgia (after delivery)	28/ Yes	Microbiological	Yes
2 (2009)4	40/ Kurdistan	None	ND	27/ Yes	Microbiological	Yes
3 (2009)5	29/ Bosnia	Granulomas in the endometrium.	None	28/ Yes	IGRA, radiological findings	No
4 (2009)6 5 (2009)6	34/Turkey	None	Fever, abdominal pain (after delivery)	30/ Yes	Microbiological	Yes
6 (2013)7 7 (2013)7	38/ India	Supraclavicular cold abscess, chronic ascites 7 years before	Persistent fever (ND)	31/ Yes	Microbiological	Yes
8 (2013)7	30/ India	None	None	32/ Yes	Microbiological	Yes
9 (2013)7 10 (2013)7	33/ India	CT calcified nodules and hilar lymph nodes	Vaginal bleeding (after delivery)	35/ Yes	Microbiological	Yes
11 (2014)8	34/ Nigeria	Salpingitis with necrosis, psoas abscess, paraspynal abscess	Fever, back pain (after delivery)	27/ Yes	Microbiological	Yes
12 (2014)9	35/ China	CT fibronodular lesions, calcifications in bilateral upper lung	Fever (first trimester)	34/ Yes	TST	ND

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13 (2015)10	32/ Ireland	3-years history of intermittent vaginal discharge, bilateral salpingitis	None	Full term/ Yes	Microbiological	ND
14 (2016)11	ND/ Turkey	Granulomatous Salpingitis	None	36/ Yes	Unknown	No
15 (2017)12	37/ SE Asia	None	Intermittent vaginal bleeding, seizures (first trimester)	24/ Yes	Microbiological	Yes
16 (2017)13	ND	None	Cough (second trimester)	Full term/ Yes	Microbiological	Yes
17 (2018)14	ND/ China	Salpingitis, spontaneous abortion	Fever, headache (ND)	30/ Yes	Radiological findings	Yes
18 (2018)14	ND/ China	Leucorrhea	ND	31/ Yes	Microbiological	Yes
19 (2019)15 20 (2019)15	ND/ Italia	None	None	27/ Yes	Microbiological	ND
21 (2020) 16	ND	ND	ND	ND	Microbiological	ND
22 (2020) 16	ND/ Moroco	ND	ND	ND	Microbiological	ND
23 (PR) 24(PR)	30/ Spain	None	Intermittent vaginal bleeding, cough, fever (first trimester)	28/ Yes	Microbiological	Yes

ND: no data; PR: present report

Table 2: Data of children with CTB

Case (year of publication) citation	Infant's sex	Prematurity	Clinical manifestations of baby	Age at diagnosis /Diagnosis after mother	Diagnosis	HM/SM /LAD	TB therapy (months)	Outcome
1 (2008)3	F	Yes / VP	Fever	20 days / No	Microbiological	HM, LAD	H, R, Z, E (2) / H, R (10)	Recovered
2 (2009)4	F	Yes / EP	Respiratory distress, fever, hypotonia	3 months / Yes	Microbiological	HM,SM \LAD	H, R, Z, E (3) / H, R (9)	Recovered
3 (2009)5	M	Yes / VP	Severe respiratory distress	6 weeks / No	Microbiological	LAD	H, R, Z, E (2) / H, R (10)	Recovered
4 (2009)6	M	Yes / VP	Pneumonia	23 days (twin 1) / Yes	Microbiological	No	H, R, Z, AN (2) / H, R (U)	Recovered
5 (2009)6	F	Yes / VP	Mild cough and poor feeding	6 weeks (twin 2) / Yes	Microbiological	No	H, R, Z, S (2) / H, R (U)	Recovered
6 (2013)7	M	Yes / VP	Late-onset sepsis, respiratory distress	19 days (twin 1) / Yes	Microbiological (post-mortem)	No	R, AN, LZD, CPR (U)	Died (33 days)
7 (2013)7	F	Yes / VP	Bradycardia, mild respiratory distress	33 days (twin 2) / Yes	Clinical, TST	No	AN (3 weeks) H, R, Z (2) / H, R (10)	Recovered
8 (2013)7	M	Yes / VP	Lethargy, mild respiratory distress, Late-onset sepsis	24 days / No	Microbiological	No	H, R, Z, E (2) / H, R (10)	Recovered
9 (2013)7	F	No	Fever, respiratory distress	29 days (twin1) / Yes	Microbiological	No	H, R, Z, AN (3) / H, R (12)	Recovered
10 (2013)7	M	No	Fever, respiratory distress	29 days (twin 2) / Yes	Clinical	No	H, R, Z, AN (3) / H, R (12)	Recovered
11 (2014)8	F	Yes / EP	Bradycardia, pneumonia	28 days / Yes	Microbiological	No	H, R, Z, AN (U)	Recovered

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12 (2014)9	M	No	Fever, non-productive cough, respiratory distress	41 days / No	Microbiologcal	HSM, LAD	H, R, Z, S (2) / H, R (6)	Recovered
13 (2015)10	F	No	Cough, fever and failure to thrive	4 months / No	Microbiological	HSM, LAD	H, R, Z, E (U)	Recovered
14 (2016)11	F	No	Cough, fever, malnutrition, respiratory distress	3 months / No	Microbiological	HM	H, R, Z, E (2) / H, R (10)	Recovered
15 (2017)12	M	Yes / EP	Severe respiratory distress	ND / Yes	Clinical	No	H, R, Z, E (2) / H, R (4)	Recovered
16 (2017)13	F	No	None	8 weeks / Yes	Microbiological	No	H, R, Z (2) / H, R (4)	Recovered
17 (2018)14	M	Yes / VP	Apnea, bradycardia, edema	41 days / Yes	Microbiological	No	H, R (2 weeks)	Died (56days)
18 (2018)14	F	Yes / VP	Respiratory distress, fever	28 days / No	Microbiological	No	H, R, Z (1)	Died (56 days)
19 (2019)15	F	Yes / EP	Respiratory distress, luminal obstruction caused by tuberculous granuloma	4 weeks (twin 1) / No	Microbiological	LAD at 4 months	H, R, Z, E (2) / H, R, E (13)	Recovered
20 (2019)15	F	Yes / EP	Respiratory distress and sepsis	Post-mortem (twin 2) / No	Post-mortem	No	No	Died (60 days)
21 (2020)16	ND	Yes	ND	ND	ND	ND	Yes (U)	Recovered
22 (2020)16	ND	Yes	ND	ND	ND	ND	Yes (U)	Recovered
23 (PR)	F	Yes / VP	Respiratory distress	18 days (twin 1) / Yes	Microbiological	LAD at 2 months	H, R, Z (2) / H, R (10)	Recovered
24 (PR)	F	Yes / VP	Respiratory distress	18 days (twin 2) / Yes	Microbiological	HM	H, R, Z (2) / H, R (10)	Recovered

nopathy; HM: hepatomegaly; SM: splenomegaly; HSM: hepatosplenomegaly; H: Isoniazid; R: Rifampicin; Z: Pyrazinamide; E: Ethambutol; S: Streptomycin; AN: Amikacin; CPR: Ciprofloxacin; LZD: Linezolid; U: unknown

All but one of the women were diagnosed after delivery, mostly by microbiological methods (15/19). The disseminated disease was present in 12/14 women, 4 with meningeal and one with vertebral involvement. Regarding the babies with CTB, most of them (79%, 19/24) were born prematurely (less than 38 weeks of gestational age), 9 were very preterm (28 to 32 weeks), and 5 were extremely preterm (less than 28 weeks); 59% (13/22) were diagnosed after their mothers at a median age of 29 (range 18-120) days. Symptoms were predominantly respiratory distress and sepsis-like. Lymphadenopathy (LAD) was seen in 7, hepatosplenomegaly (HSM) in 3, and hepatomegaly (HM) in 3. The microbiological diagnosis (Table 3) was achieved in 20/24 (83.3%) babies, in 16 of them (80%) by a rapid method (AFB staining in 75% (12/16) and NAAT in all 11 cases with data about this technique) and only by culture in 4 patients. CTB was diagnosed by histological examination and/or a high clinical-epidemiological suspicion in 4 patients. Four (18.2%) babies died at the age of 33,56,56 and 60 days respectively. The remaining 20 babies were treated with different antituberculous therapies, most of them with isoniazid, rifampicin, ethambutol, and pyrazinamide for 12 months, and all fully recovered with no reported sequelae.

Table 3: Microbiological diagnosis of children with CTB

Case (year of publication) citation	Positive samples	AFB	NAAT	Culture
1 (2008)3	Gastric aspirate, feces	ND	ND	Positive
2 (2009)4	Gastric and tracheal aspirate, feces	Positive	Positive	Positive
3 (2009)5	ETA, lymph node biopsy	Positive	Positive	Positive
4 (2009)6	ETA, gastric aspirate	Positive	ND	Positive
5 (2009)6	ETA	ND	Positive	Positive
6 (2013)7	Lung and pleural autopsy	Positive	ND	Positive
7 (2013)7	None			
8 (2013)7	ETA, gastric aspirate, blood	Positive	ND	Positive
9 (2013)7	Gastric aspirate	ND	ND	Positive
10 (2013)7	None			
11 (2014)8	ETA	Positive	ND	Positive
12 (2014)9	ETA	Negative	ND	Positive

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13 (2015)10	Lymph node biopsy, brochial wasings	Positive	ND	Positive
14 (2016)11	Gastric aspirate	Negative	Positive	Positive
15 (2017)12	None			
16 (2017)13	Gastric aspirate	Negative	ND	Positive
17 (2018)14	ETA	ND	Positive	ND
18 (2018)14	Aspirate	Positive	Positive	ND
19 (2019)15	Gastric aspirate	Negative	Positive	Positive
20 (2019)15	None			
21 (2020) 16	Yes (U)	Positive	Positive	Positive
22 (2020) 16	Yes (U)	Positive	Positive	Positive
23 (PR)	Gastric aspirate	Positive	Positive	Positive
24 (PR)	Gastric aspirate, urine	Positive	Positive	Positive

AFB: Acid-fast bacili; NAAT: Nucleic-Acid Amplification test; ETA: endotracheal aspirate; ND: No data; U: unknown

5. Discussion

GTB is a major cause of infertility in women living in endemic areas. Its incidence varies widely with the highest incidence in India and South Africa [17] and it is increasing in Western countries due to migration of people from endemic areas. Moreover, 76% of women with GTB are infertile in these areas [3]. With the increasing application of IVF technology as treatment of infertility, TB has gradually increased posing a serious threat to the health of pregnant women and fetuses. On one hand, during pregnancy due to endocrine and immune disorders, latent TB infection (LTBI) can be and reactivated and disseminated. Besides, it has been reported that acute miliary TB is significantly higher in IVF pregnant women than in naturally pregnant ones [18]. On the other hand, vertical transmission is more common in women with miliary and genital patterns of TB than among mothers with pulmonary TB. The consequence for the fetus is a higher risk of miscarriage and preterm delivery, intrauterine growth retardation and stillbirth, and CTB. In this literature review, most of the involved women were migrants from high TB-burden areas. The cases herein reported are very unusual because the woman was native to Spain, a country with a low TB incidence in 2019 (9.34/100,000 inhabitants) and even lower in the Canary Islands (5.04/100,000 inhabitants) [19]. We only found two previously reported cases in Spain [16], one of the women involved was from Morocco and there was no information about the other one. None of the women in this series had been evaluated for TB before IVF-ET, not even those with a history suggesting previous TB. At IVF-ET time, most women were asymptomatic. During pregnancy, in those women who became symptomatic, the onset of symptoms occurred in the first or second trimester with fever as the main symptom, so TB was difficult to suspect. Besides, as pregnancy symptoms overlap those of TB, diagnosis is usually delayed as happened in this series in which almost all women were diagnosed after delivery. Most of them presented a disseminated disease, mainly with meningeal involvement, as previously reported [20]. Fortunately, despite the severity of the disease, all women completed antituberculous treatment and were cured.

In 1994 criteria for diagnosis of CTB were revised [21]. According to these criteria, the infant must have a proven tuberculous lesion and at least one of the following: 1) lesions occurring in the first week of life 2) a primary hepatic complex or caseating hepatic granuloma, 3) documented tuberculous infection of the placenta or maternal genital tract and 4) exclusion of the possibility of postnatal transmission by investigation of contacts. Our cases met the revised criteria: both twins had proved tuberculosis (positive AFB staining and NAAT and MTB grown from several specimens) in early life, one of them with primary hepatic complex, and their mother was diagnosed with GTB by histological and microbiological diagnosis from endometrium biopsy. Furthermore, postnatal transmission was precluded because the babies were immediately admitted to ICU after birth and were still there when they were diagnosed. CTB is hard to recognize in infants as symptoms are nonspecific and can be easily mistaken with common neonatal illnesses such as bacterial sepsis or pneumonia as occurred in all cases described here. All babies were initially treated with the usual antibiotherapy and most of them were evaluated for CTB only after their mother's diagnosis. Infected infants are usually born prematurely as we have described here. In the literature, the most common presentation is poor feeding, irritability, failure to thrive, failure to gain weight, cough, respiratory distress, HSM, splenomegaly (SM), LAD, and abdominal distension [22]. In this series most of the newborns presented with respiratory distress and LAD and less frequently with HSM y HM. The reported mortality rate is very high nearly 50% [22] often due to delayed diagnosis followed by delayed treatment. However, in this review, despite the median age at diagnosis was 29 days, the mortality rate was much lower. Sequelae have not been found in the cases here analyzed. In one of the cases reported here (twin 2) the baby subsequently had recurrent episodes of a probable PFAPA, a pathology of unknown etiology that has not been associated in the literature with previous TB [23].

Early diagnosis of CTB is imperative to treat promptly the infant hence it is important to maintain a high index of clinical suspicion in babies born from parents native to endemic areas for TB. Nowadays, there are microbiological techniques widely available, such as AFB staining and NAAT, that offer a rapid TB diagnosis. In this series, TB was diagnosed in a few days using these methods in the majority of women and babies. Current mycobacterial culture (the gold standard of TB diagnosis) utilizing automated liquid media is faster than in the past and permits drug susceptibility testing which is mandatory, especially in patients from areas with a high level of drug resistance.

To summarize, as IVF-ET represents a useful way of conception it can be expected that, in the future, more women native from countries with a high endemicity of TB will present for IVF-ET. Even in countries with a low incidence such as Spain, although very unusual, CTB is possible as it is shown in this report. Consequences of CTB in the neonate are severe including long-term hospitalization with many complications and possible death of the baby. In this context, to prevent CTB, LTBI

screening before undergoing IVF-ET of women from areas with high TB burden, and further examinations in those infected to exclude active TB should be done. In low-incidence settings would be necessary to keep in mind a high degree of clinical suspicion when symptoms or a previous history suggesting TB are present. In both diseases, GTB and CTB, an early diagnosis is essential and nowadays is feasible with available microbiological methods that permit to achieve a rapid diagnosis and therefore to start promptly anti-TB therapy to improve the prognosis.

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