CASE REPORT

Birth of a healthy child after preimplantation genetic screening of embryos from sperm of a man with non-mosaic Down syndrome

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Abstract

Purpose The purpose of this study is to present a case of healthy infant born after intracytoplasmic sperm injection-in vitro fertilization (ICSI-IVF) with preimplantation genetic screening (PGS) using sperm from a man with non-mosaic trisomy 21 and a literature review.

Materials and methods A 26-year-old euploid female and 29-year-old male with non-mosaic trisomy 21 and male factor undergoing ICSI-IVF treatment for primary infertility with embryo biopsy for PGS with comprehensive chromosomal screening (CCS) presented to the Infertility Clinic at Highland Hospital, the Alameda County Medical Center, California, with 6-year history of primary infertility. The outcome measure is a live birth of a healthy child and ploidy status of biopsied blastocysts.

Results Egg retrieval yielded 33 oocytes, 29 of which underwent ICSI with ejaculated sperm. Twenty-eight 2PN zygotes were cultured, and 13 blastocysts underwent trophectoderm biopsy and vitrification 5 or 6 days after retrieval. CCS analysis revealed that 12 out of 13 (92 %) of blastocysts were euploid and one was a complex abnormal mosaic. Transfer of two grade I hatching blastocysts resulted

in a singleton pregnancy with normal prenatal genetic screening and delivery of a healthy male infant at 41 weeks via primary cesarean section for non-reassuring fetal status.

Conclusion This is the first report of a live birth of a healthy child after ICSI-IVF with PGS using ejaculated sperm from a man with non-mosaic trisomy 21 and male factor infertility.

Keywords Trisomy 21 infertility · Down syndrome · Male factor infertility · Preimplantation genetic screening · Comprehensive chromosomal screening · Repetitive trophectoderm embryo biopsy

Introduction

Trisomy is the most common chromosomal abnormality in humans. Trisomy 21, commonly known as Down syndrome, is the most common nonlethal trisomy in humans usually arising de novo within the embryo in association with advanced maternal age. Detection of fetuses with trisomy 21 is the primary goal of prenatal genetic screening. In developed countries, the life expectancy of Down syndrome patients has reached 56–60 years [1, 2]. In addition to cognitive impairment, Down syndrome is associated with congenital heart defects and early onset of Alzheimer's disease. Down syndrome is the only autosomal trisomy compatible with survival beyond puberty and reproduction [3]. Patients with Down syndrome engage in sexual relations, may conceive spontaneously, and, on occasion, seek treatment for infertility. Their pursuit of children raises several complex but intriguing issues.

Limited information is available on fertility in people with Down syndrome. Females are generally fertile albeit subfertility in some has been linked to a reduced number of ovarian follicles with an increased rate of atresia [4–6]. They are, however, at a high risk of having children with trisomy 21,

Capsule Healthy life birth after ICSI with PGS in non-mosaic Down syndrome male.

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presumably from vertical transmission rather than de novo origin. In one series, ten of 28 children (35.7 %) born to 26 women with non-mosaic trisomy 21 inherited the abnormality from their mothers [3, 5, 7]. Men with trisomy 21 are usually infertile with impaired sperm production being compounded by sexual ignorance [5, 8]. They are frequently hypogonadal and azoospermic, but the pathophysiologic basis of their male factor remains obscure [5, 9].

To date, four live births have been reported from men with Down syndrome [8, 10–12]. However, in marked contrast to the experience in women, all four children born to men with trisomy 21 have been normal. Three conceptions were spontaneous [8, 10, 11] while one case required intracytoplasmic sperm injection (ICSI) of round-headed sperm obtained by testicular aspiration from a globozoospermic man mosaic for trisomy 21 [12]. Of the four reported live births, one was in a man with mosaicism [12] and three in non-mosaic men [8, 10, 11]. In men who are mosaic for trisomy 21, i.e., having two cell lines (46, XY and 47, XY+21), it is hard to determine which cell line predominates within seminiferous tubules and primarily impacts spermatogenesis. The percentage of disomic sperm with an extra chromosome 21 in either mosaic or non-mosaic men with Down syndrome has not been determined. Therefore, the risk of paternal transmission of trisomy 21 to offspring remains unknown.

We present a case of a normal male infant born to a normal woman as a result of transfer of euploid embryos after ICSI using ejaculated sperm from her husband who has non-mosaic trisomy 21 demonstrated by fluorescent in situ hybridization (FISH). Embryo selection for transfer was based on results of preimplantation genetic screening (PGS) following trophectoderm biopsy of 13 blastocysts.

Materials and methods

A 26-year-old woman and her 29-year-old husband, both of Middle Eastern origin, presented to the Infertility Clinic at Highland Hospital, the Alameda County Medical Center, California, with 6-year history of primary infertility. The man carried the diagnosis of non-mosaic trisomy 21 made by FISH, while his wife had no cognitive impairment or known chromosomal abnormalities. Both partners were otherwise healthy and had strong extended family support for starting a family. The infertility workup of the female partner included a normal HSG and saline infusion sonohysterogram, cycle day 3 FSH, and normal antral follicle count of 34.

Semen analysis had volume 0.4 mL, concentration 14.2 million/mL, 50 % motility (TMC=2.84 million).

In view of male factor and the unknown risk of paternal transmission of extra chromosome 21 to offspring, in vitro fertilization with ICSI and PGS was recommended and

accepted. The treatment was completed through the income-based sliding scale program at the Alta Bates IVF.

Results

The intended mother underwent controlled ovarian stimulation with GnRH antagonist protocol after pretreatment with monophasic OCP. She was triggered on stimulation day 12 with peak estradiol level of 3414 mIU/mL. GnRH agonist trigger was given 35 h before transvaginal ultrasound-guided retrieval. Thirty-three oocytes were retrieved and 29 mature metaphase II eggs underwent ICSI using ejaculated semen with the above parameters.

The following morning, 28 injected eggs exhibited two pronuclei and were cultured to blastocyst stage. Twenty grade I and II blastocysts underwent trophectoderm biopsy on day 5 or 6 after retrieval. After the biopsy, trophectoderm samples were placed in microtubes for shipping; the blastocysts were collapsed and vitrified in CryoTips (Irvine Scientific, Santa Ana, CA). However, the single-nucleotide polymorphism microarray platform used by Natera (San Carlos, CA) was nondiagnostic for chromosome 21 because their algorithm requires that both gamete providers are euploid.

Therefore, the 18 (euploid/no call on chromosome 21) blastocysts were thawed and 13 re-biopsied (the rest did not re-expand sufficiently to allow re-biopsy and refreeze) with specimens sent to Genesis Genetics (Plymouth, MI), which uses a comparative genomic hybridization (CGH)-based platform for CCS and does not require parental euploidy. After re-biopsy, the blastocysts were re-vitrified in CryoTips.

The CCS finding was that 12 out of 13 (92 %) blastocysts had normal chromosomal complement and one was abnormal with mosaicism and aneuploidy of multiple chromosomes (Table 1). No embryo had isolated trisomy 21.

In a frozen embryo transfer cycle, two euploid hatching grade I (46,XY) blastocysts were warmed and transferred after endometrial preparation with estradiol and progesterone per standard protocol, resulting in a singleton intrauterine pregnancy.

First trimester prenatal genetic screening results included normal nuchal translucency of 0.15 mm and a negative triple screen for Down syndrome (estimated risk of 1:3100) and trisomy 18 (estimated risk of 1:20,000). Maternal serum alpha fetoprotein was also normal. Anatomic fetal ultrasound survey at 23 weeks revealed normal fetal anatomy.

The pregnancy was uncomplicated until 41 weeks when oligohydramnios was diagnosed, and the patient's labor was induced. Delivery was accomplished by emergency primary low transverse Cesarean section for non-reassuring fetal status and cephalopelvic disproportion with second stage arrest. A 3538 g (7 lb 13 oz) male infant with Apgar scores of 8 and 9 at 1 and 5 min, respectively, was delivered. The child had no

Table 1 Testing results from Genesis Genetics

Sample number	Genesis-24 chromosome results	Interpretation
3	46, XY	Euploid
4	46, XX	Euploid
5	46, XX	Euploid
7	Complex abnormal	Mosaic for multiple aneuploid chromosomes
10	46, XX	Euploid
11	46, XY	Euploid
13	46, XY	Euploid
15	46, XX	Euploid
19	46, XX	Euploid
22	46, XY	Euploid
24	46, XY	Euploid
26	46, XY	Euploid
27	46, XX	Euploid

congenital anomalies associated with Down syndrome and has developed normally to the current age of 5 months (at the moment of this article preparation).

Discussion

We report the first successful application of preimplantation genetic screening (PGS) by means of trophectoderm biopsy of blastocyst stage embryos and comprehensive chromosomal microarray analysis employed in order to avoid vertical transmission of trisomy 21 from an infertile man with non-mosaic Down syndrome. In this case, PGS revealed that all but one of the 13 biopsied blastocysts were euploid with equal distribution of 46, XX and 46, XY embryos (Table 1). The one aneuploid embryo exhibited multiple lethal abnormalities as well as mosaicism which are unlikely to have arisen through fertilization by a spermatozoan disomic for just chromosome 21.

While the finding that 12 out of 13 (92.1 %) of the biopsied blastocysts had normal chromosomal complements suggests that most sperm produced by men with trisomy 21 carry a normal haploid genotype, this conclusion must be tempered by the fact that we do not know the genetic makeup of the eight embryos which were normally fertilized through ICSI but failed to develop to blastocyst stage and the seven rewarmed blastocysts that did not undergo a second biopsy. It is possible that, if transferred at the cleavage stage, some of these embryos could have initiated a pregnancy with a fetus affected by trisomy 21. After all, trisomy 21 is one of the most common autosomal abnormalities detected in first trimester spontaneous abortion samples [13, 14]. In fact, the couple was counseled by a genetic counselor that they are at increased risk of having embryos with a chromosomal abnormality, most likely trisomy 21, and PGS was recommended.

Nonetheless, putting the results of PGS in the current case together with the four previously reported normal children indicates that 16 out of 17 (94.1 %) embryos with known ploidy status obtained from sperm of men with trisomy 21 have been normal. This result stands in stark contrast to the 35.7 % transmission rate of trisomy 21 by women with the same condition [3]. It appears that the aneuploidy surveillance and elimination mechanisms during spermatogenesis are much more efficient than in oogenesis (reviewed in [15]).

The finding of lower than theoretically expected number of disomic sperm in trisomy 21 is consistent with the much wider experience in men with Klinefelter's syndrome (47, XXY), who are infertile. In two studies, the majority of biopsied embryos (54–61 %) from men with Klinefelter's syndrome were euploid by fluorescence in situ hybridization with the remainder showing abnormalities in both sex chromosomes and autosomes [16, 17]. In 47, XXY men, the frequency of disomic (24, XY) sperm is 3–4 % compared to 0.2 % in fertile 46, XY men and 1 % in infertile 46, XY men [18]. In the mouse model of Klinefelter's syndrome, the frequency of disomic spermatozoa is also much lower than the theoretical expectation of 50 % [19].

Trophectoderm biopsy at the blastocyst stage with comprehensive chromosomal screening (CCS) by microarray has largely replaced cleavage stage embryo biopsy with FISH [20, 21]. Whereas the latter technique usually led to a transfer of fresh embryos on day 5 or 6 after retrieval, the current approach usually involves vitrification of blastocysts after biopsy and subsequent transfer of warmed euploid embryos [20]. In experienced hands, embryo survival after trophectoderm biopsy and vitrification approaches 100 % and can result in high embryo implantation rates even when the procedure is repeated as in our case [21, 22].

However, the complexity of genetic analysis using microarrays and the lack of a uniform platform in genetic

laboratories can lead to a misunderstanding about the technical limitations of these powerful techniques. In this case, despite direct communication between the clinician and a genetic counselor at Natera, it was not recognized that the laboratory's analysis of single-nucleotide polymorphisms compares the embryonic samples to parental controls and, therefore, requires euploidy of both genetic parents for a valid result. Genesis Genetics employs comparative genomic hybridization assessing the embryonic DNA against a population-based, rather than parental, normal control so parental aneuploidy does not present an issue. Thus, in unusual cases, such as this one, as well as cases of known single-gene mutations and translocations, clinicians must communicate directly with geneticists in the laboratory in order to avoid this potential pitfall.

Cognitive impairment may preclude effective family planning in both women and men with Down syndrome whose reproductive needs are very different. Women with trisomy 21 are liable to conceive spontaneously and thus in need of contraception to minimize the risk of vertical transmission. Long-term reversible contraceptives are the method of choice for women with cognitive impairment. When desirous of children, they would clearly be candidates for preimplantation genetic screening (PGS) to define the ploidy status of embryos available for transfer. Men with Down syndrome, on the other hand, may seek treatment for infertility because of male factor. In both women and men, the social prerequisites for fertility treatments have not been defined but are clearly central to clinical management.

Over the past several decades, people with Down syndrome and other cognitive disabilities have become increasingly integrated into the educational system and community life rather than confined to institutions as used to be the case in the past. They often live with families or in a group home holding basic jobs and interacting with others [23]. These adults need to be educated about their bodies, sexual development, contraception, and fertility options [24]. Since the optimal reproductive management is exceedingly complex for both men and women with Down syndrome, explaining these options to individuals with cognitive impairment is a major challenge. However, focusing on the desired outcome of having or not having children rather than technical aspects of the procedures should render informed consent attainable. Similar concerns arise with Fragile X syndrome, which is the most common nonchromosomal cause of mental retardation and which is inherited like an X-linked disorder affecting only males.

Adults with Down syndrome are generally considered competent to make their own medical choices which would include reproductive decisions [23]. Moreover, they have access to reproductive services under the Americans with Disabilities Act. It is likely that in the past, a high percentage of women with trisomy 21 had unintended, and in some cases

probably nonconsensual, pregnancies. Since women with Down syndrome have a high chance of transmitting this condition to offspring while the added cost of raising such children exceeds their earning capacity, their decisions would have a major impact on their families and society at large. The additional cost of raising a child with Down syndrome was estimated at \$500,000 in 1998 dollars which corresponds to \$709,617 in 2013 dollars [25]. This incremental cost is more than 28 times higher than the current cost estimate of IVF with PGS (\$25,000) which makes this option highly cost-effective. Since most women with Down syndrome in the USA are covered under Medicare or Medicaid programs, coverage for IVF with PGS would reduce the long-term cost of these programs.

However, it cannot be assumed that the reproductive decisions of people with Down syndrome would necessarily be the same as in the general population. A recent survey of 284 persons with Down syndrome above the age of 12 years found that 99 % of them are happy and satisfied with their lives, which is no doubt higher than the percentage in their unaffected siblings [26]. Therefore, when offered a free choice of embryos through PGS, people with trisomy 21 might select an embryo which shares their own genetic makeup thus propagating this condition, a decision which raises ethical concerns.

In the case of males with Down syndrome, the risk of vertical transmission appears to be low but the presence of male factor may require fertility treatment to achieve a pregnancy. In the present case, the wife's desire for a child in the absence of any cognitive impairment on her part clearly trumps any other considerations. Much has been written about the multiple challenges of raising a child with cognitive disability [27], but the research on being raised by a parent with Down syndrome is more limited [28]. What are the general prerequisites to providing fertility treatments, regardless of whether they involve high-tech approaches such as PGS or simple inseminations, for people with Down syndrome and other cognitive disabilities? At a minimum, one must take into account the couple's ability to parent, the potential impact on offspring of having a cognitively impaired parent, as well as the availability of family, medical, and community support systems. In the absence of general guidelines, each case needs to be examined on its own merits with input from physicians, psychologists, social workers, and educators. Advanced reproductive technologies, when appropriate, can provide a margin of safety for these challenging reproductive dilemmas.

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