Live birth of monochorionic triamniotic triplets after in vitro fertilization and blastocyst transfer: case report and review of the literature

Poród trojaków z ciąży jednokosmówkowej jednojajowej trójowodniowej po zapłodnieniu pozaustrojowym i transferze blastocysty

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Streszczenie

Ciąże trojacze monozygotyczne są bardzo rzadkie również po zapłodnieniu in vitro (IVF). Prezentujemy opis przypadku żywych narodzin trojaczków z ciąży trojaczej trójowodniowej, po transferze blastocysty.

Opis przypadku: *29-letnia pacjentka została zakwalifikowana do drugiej próby zapłodnienia pozaustrojowego z mikroiniekcją plemnika do komórki jajowej (IVF-ICSI). Wywiad medyczny obejmował cztery lata leczenia w tym stymulację owulacji, trzy cykle inseminacji domacicznej, histeroskopię oraz nieudane zapłodnienie pozaustrojowe (IVF-ICSI). Podczas drugiej próby zapłodnienia pozaustrojowego owulacja stymulowana była tzw. protokołem długim z agonistą GnRH. Pobrano jedenaście prawidłowych komórek jajowych (MII). Wykonano mikroiniekcje plemnika do 11 oocytów MII uzyskując cztery ekspandujące blastocysty. Na prośbę pacjentki dwie blastocysty klasy 4AA zostały przeniesione do jamy macicy. W 7 tygodniu ciąży zdiagnozowano ciążę trojaczą monozygotyczną trójowodniową.*

Ze względu na rozpoczęty poród w 33 ciąży wykonano cięcie cesarskie. Urodzono trzy dziewczynki ważące odpowiednio: 2060g (Apgar 7), 1860g, (Apgar 6) i 2000g

(Apgar 6). Po 13 dniach hospitalizacji zostały wypisane do domu.

Wniosek: *Przyczyny ciąż monozygotycznych zarówno w cyklach spontanicznych jak po metodach rozrodu wspomaganego nie są w pełni znane. Wydaje się, że występują one częściej po zapłodnieniu in vitro. Jednakże, związek między poszczególnymi etapami zapłodnienia pozaustrojowego a jednojajowymi ciążami mnogimi nadal pozostaje kontrowersyjny.*

Słowa kluczowe: **ciąża trojacza monozygotyczna** / **zapłodnienie pozaustrojowe** / / **rozród wspomagany** / **blastocysta** / **ciąża wielopłodowa** /

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Otrzymano: **12.03.2012**
Zaakceptowano do druku: **30.09.2013**

Abstract

Monozygotic triplet pregnancies are very rare, even after in vitro fertilization (IVF). We present a case of a live birth of triplets from a monochorionic triamniotic pregnancy after blastocyst transfer.

A 29-year-old woman underwent intracytoplasmic sperm injection (IVF-ICSI). Her medical history included a 4-year infertility treatment with ovarian stimulation, three cycles of intrauterine insemination, hysteroscopy and an unsuccessful attempt at IVF. During the second IVF attempt, the patient underwent ovulation simulation according to the long GnRH agonist protocol. Eleven metaphase II (MII) oocytes were injected with spermatozoa, resulting in four expanded blastocysts. In the end, two blastocysts (4AA) were transferred into the uterine cavity. Ultrasound examination performed at 7 weeks of gestation showed an ongoing triamniotic triplet pregnancy. Regular uterine contractions began at 33 weeks of gestation and a cesarean section was performed, resulting in a birth of three healthy girls, weighing 2060g (Apgar 7), 1860g (Apgar 6), 2000g (Apgar 6). After 13 days of hospitalization the infants and the mother were discharged home.

Conclusion: *The causes of monozygotic multiple gestations in spontaneous and ART pregnancies are poorly understood. They seem to occur more often after IVF. Any definitive relationship between particular stages of the IVF procedure and monozygotic multiple pregnancies remains controversial.*

Key words: **monozygotic triplet pregnancy** / **in vitro fertilization** / **artificial reproductive techniques** / **blastocyst transfer** / **multiple pregnancy** /

Introduction

Monozygotic triplet gestations are very rare, even after in vitro fertilization (IVF). They account for 3.5-4% of all triplets and occur in 0.001-0.004% of all pregnancies [1]. The prevalence of spontaneous triplet pregnancy is reported to be 1 in 6500-7000 deliveries.

Monozygotic twinning following in vitro fertilization was first reported in 1984 and since that time numerous studies have demonstrated an increased occurrence of monozygotic twins associated with Artificial Reproductive Technologies (ART) [2-8]. Recently, the considerable attention has been put to the issue of reducing the rate of multiple pregnancies [9,10]. However the incidence of monozygotic multiple pregnancies has not been reduced because they can still occur after single embryo transfer (SET) [11-13].

According to the Fertility and Sterility Special Interest Group, Polish Gynecological Society, in 2010 in Poland there were 2962 deliveries as a result of ART and 9 (3%) of them were polyzygotic triplet (personal communication). Some factors have been proposed to explain the etiology of monozygotic pregnancies, including hereditary factors, vitro fertilization, zona pellucida manipulation, ovarian stimulation, blastocyst transfer and maternal age [1,5,7,14-17]. In our paper we present the case report of live birth of triplets from monochorionic triamniotic gestation, following transfer of two blastocysts in 29-year old women who underwent intracytoplasmic sperm injection (ICSI).

Case report

A 29-year-old woman underwent her second IVF-ICSI attempt at Gameta Fertility Center in August 2010. Her medical history included a 4-year infertility treatment at another center, with ovarian stimulation and three cycles of intrauterine insemination. At our center the patient underwent hysteroscopy and an unsuccessful attempt at IVF. The sperm parameters of the partner were normal according to the WHO 2010 criteria. During the second attempt at IVF she was stimulated according to long GnRH agonist protocol with a daily dose of Triptorelin equal to 0.1 mg (Decapeptyl, Ferring Pharmaceuticals), starting from the 21st day of the cycle, and 150 IU of recombinant FSH (Puregon, Organon), administered daily for a period of 11 days. The estradiol level at the end of stimulation was 3500 pg/ml. After an injection of 250 μg of recombinant hCG (Ovitrelle, Merck Serono), 15 cumulus-oocyte complexes (COCs) were retrieved. Eleven metaphase II (MII) oocytes were injected with spermatozoa resulting in 8 two-pronuclear zygotes. After 5 days of culture in sequential media (G1, G2, Vitrolife), 4 expanded blastocysts were obtained. Three blastocysts were grade 4AA and 1 was 4 CC according to Gardner and Schoolcraft scoring system. The patient was advised to have one top quality blastocyst transferred. However, due to previous IVF failure, she requested to have two blastocysts transferred. In the end, two blastocysts (4AA) were transferred into the uterine cavity under ultrasonographic guidance (Image 1). Two surplus blastocysts were vitrified (Cryotop, Kitazato). The luteal phase was supported with 600 mg of vaginal progesterone daily (Luteina, Adamed). Ultrasound performed at 7 weeks of gestation showed an ongoing triamniotic triplet pregnancy with three fetal heart rates. The second ultrasound at 10 weeks of gestation confirmed the first ultrasound test. The examination was repeated at 14 weeks of gestation and the patient was sent to an obstetric center for further tests. Due to a high likelihood of premature labor (before 32 weeks), the patient was referred to the Department of Perinatology, Silesian Medical University at 29 weeks of gestation. Regular uterine contractions began at 33 weeks of gestation. After antenatal corticosteroid therapy, a caesarian section was preformed, resulting in a birth of three healthy girls weighing 2060g (Apgar 7), 1860g (Apgar 6), and 2000g (Apgar 6). All three of the neonates experienced some respiratory problems and were in need of nasal continuous airway

pressure (NCPAP). After 13 days of hospitalization the infants were discharged in good overall condition. The cesarean section was not associated with any complications for the mother.

Discussion

When the division of the embryonic cell mass occurs earlier than within 72h after fertilization, diamniotic dichoronic monozygotic twins will evolve. The division of the embryo which occurs after the inner cell mass has been formed, between 4th and 8th, will result in diamniotic monochorionic twins. Splitting after day 8 will lead to monoamniotic monochorionic twins. The process probably begins with the protrusion of some trophectoderm cells through a small opening in the zona pellucida (ZP). Some cells of the inner cell mass may then break off *in utero* to form monozygous twins [5].

Monozygotic multiple pregnancies have an increased risk of adverse outcome when compared to dizygotic multiple gestations. The elevated risk arises from congenital malformations, cord entanglement, and complications directly connected with monochorionic placentation such as twin to twin transfusion syndrome for example [18, 19]. Monochorionic gestation may be more common after IVF treatment [1, 4, 19]. Schieve et al., calculated the odds ratio of monozygotic twinning in assisted reproduction pregnancies to be 3.2–3.8 [20]. However, the mechanism and the role of ART in this process remains poorly understood. There are several theories why IVF could increase the percentage of monochorionic gestations, including ovarian stimulation, zona pellucida manipulation, embryo denudation and blastocyst culture [4, 17, 21-23]. However none of these theories have been confirmed.

Ovarian stimulation could affect the structure of zona pellucida to such an extent that it could induce not only polyzygotic twins but also increase the probability of monochorionic twins and triplets [15, 16, 22]. An increased frequency of 1.2% for monozygotic twinning after treatment with ovulation induction agents (clomiphene citrate and gonadotropins) was observed by Derom et al., and proved to be significantly higher than the expected frequency after spontaneous ovulation. The authors considered the induction of ovulation to be the first identified biological mechanism influencing the monozygotic twinning rate [17].

Interventions in the zona pellucida, such as assisted hatching, were also considered to augment embryo twinning rate. Some authors noted a significantly increased rate of monozygotic twins after mechanically-assisted hatching in conventional IVF, which reached the rate of 1.2% per embryo transfer [20, 22]. In contrast, Sills et al., showed that in IVF patients the frequency of monozygotic twinning was not statistically different between zona manipulated and zona intact subgroups. They believed that greater overall frequency of monozygotic twinning for IVF patients may be the consequence of the higher number of embryos transferred in IVF, rather than discrete zona manipulations [22].

It is also suspected that prolonged embryo culture to the blastocyst stage might increase the incidence of monochorionic multiple pregnancies. Da Costa et al., reported a significantly higher incidence of monozygotic twinning in pregnancies after blastocyst transfer [24, 25]. In a 7-year retrospective study of a large IVF program based on minimal ovarian stimulation and over 47000 single embryo transfers, monozygotic twinning occurred in 1.01% of 14956 clinical pregnancies. Blastocyst culture was associated with a significantly increased monozygotic twinning risk, whereas embryo freezing, type of stimulation protocol, intracytoplasmic sperm injection (ICSI) or assisted hatching (AH) did not contribute to its incidence. In contrast, a recent study based on over 2000 fresh IVF/ICSI cycles with SET (single embryo transfer) indicated a potential relation between the day of embryo transfer (day 3 or 5) and the incidence of monozygotic twinning. The analysis showed that blastocyst transfer does not increase the probability of monozygotic twins [26].

Conclusion

The causes of monozygotic multiple gestations in spontaneous and ART pregnancies are poorly understood. They seem to occur more often after IVF. Any definitive relationship between particular stages particular Assisted Reproductive Technology (ART) procedures and monozygotic multiple pregnancies remains controversial.

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Źródło finansowania:

Praca nie była finansowana przez żadną instytucję naukowo-badawczą, stowarzyszenie ani inny podmiot, autorzy nie otrzymali żadnego grantu.

Konflikt interesów:

Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

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