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Case Report OMICS International

Quadruple Pregnancy after Post-advanced Assisted Technique Transfer of Blastocysts

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Abstract

Report the case of a quadruple pregnancy (monochorionic diamniotic and dichorionic diamniotic) after the transfer of two blastocysts generated by intracytoplasmic sperm injection (ICSI). A 29-year-old woman patient with transfer of two blastocysts after long cultivation of 6 embryos generated by ICSI and vitrified in d+3, in a couple with primary infertility diagnosis due to male factor. This revealed quadruple pregnancy (monochorionic diamniotic and dichorionic diamniotic) of 56 days of evolution by transvaginal ultrasound. The couple decided to have selective embryo reduction of the monochorionic diamniotic gestation after receiving information about the risks arising from it. After that embryo reduction the uncomplicated pregnancy continued until 36 weeks of gestation, achieving reproductive success with the birth of two babies alive and healthy.

Keywords: Quadruple pregnancy; Dichorionic diamniotic pregnancy; Monochorionic diamniotic pregnancy; Embryo reduction; Elective single embryo transfer

Abbreviations ASEBIR: Association for the Study of Reproductive Biology; B-hcG: Chorionic Gonadotropin Hormone; ER: Embryonic Reduction; eSET: Elective Single Embryo Transfer; FSH: Follicle Stimulating Hormone; ICSI: Intracytoplasmic Sperm Microinjection; IGR: Intrauterine Growth Retardation; IVF: *In vitro* Fecuntation; GnRH: Gonadotropin Releasing Hormone; MZT: Monozygotic Twin; SUZI: Subzonal Insemination; TEC: Transfer of Devitrified Embryos

Introduction

The monozygotic twin (MZT) pregnancy is a biological process that occurs by accidental partition of an embryo in a typical fertilization originated from an oocyte and a sperm. Depending on the time that this division occurs, placentation will be dichorionic diamniotic if it occurs between 48 and 72 hours post-fertilization or monochorionic diamniotic if done 3-8 days after fertilization.

In recent years the rate of multiple pregnancies associated with assisted reproductive technologies (IVF/ICSI) has decreased due largely to the current trend towards elective single embryo transfer (eSET). However, the risk of gemellarity due to embryo division in IVF/ICSI pregnancies is higher than in spontaneous gestations, presenting a ratio in the general population of 0.42%, 0.72% for pregnancies from conventional IVF and 0.86% after an intracytoplasmic sperm microinjection (ICSI) [1].

We describe a case of quadruple gestation (monochorionic diamniotic gestation and dichorionic diamniotic gestation) after the transfer of two blastocysts generated after long culture of early devitrified embryos.

Case Report

A 29-year-old woman came along with her partner to the Assisted Reproduction Unit of our hospital for primary infertility of 4 years of evolution. The couple had previously done an IVF-ICSI cycle with 2 embryo transfers of 2 embryos each, cell stage (D+3) without getting pregnant. After performing the basic infertility study, the definitive diagnosis was primary moderate male factor infertility, since the male semen presented a moderate teratozoospermia (percentage of normal sperm forms of 3%, according to strict Kruger criteria). The rest of seminal parameters were normal and no associated female factor was detected, so it was decided to perform advanced assisted reproductive technique (IVF/ICSI). The long protocol starts with GnRH agonists insert in oral contraceptives with leuprolide acetate, 0.1 cc/24 hours (Procrin; AbbviePharmaceutical, Madrid, Spain). When pituitary slowing and ovarian rest was observed ovarian stimulation began with recombinant FSH, 150 U/24 hours (Gonal; Serono Laboratories, Madrid, Spain) for 10 days. Ovulation induction with HCG is performed, 250 mg subcutaneous (Ovitrelle; Serono Laboratories, Madrid, Spain) 36 hours before the ovarian puncture.

Twenty sevenoocyte clusters were recovered. Of these, 12 were inseminated by conventional IVF with 50,000 sperm/cluster. The rest were decumulated and we decided to microinject by ICSI another 12 metaphase II oocytes (MII) with conjugal sperm. Due to the high ovarian response obtained after ovarian stimulation and the risk of ovarian hyperstimulation syndrome it was decided to defer the transfer and proceed to the vitrification of 10 embryos, 8 from ICSI and 2 generated by IVF, in D+3 (MedicultVitrification Cooling, Origio Laboratories, Denmark). After performing a transfer of devitrified embryos (TEC) in D+3 that failed and because the couple had a history of three previous transfers of 6 embryos in cell stage of good quality without success, it was decided to devitrify 6 early embryos (D +3) generated by ICSI (MedicultVitrification Warming, Origio Laboratories, Denmark) and keep them in long cultivation to D+5 to perform transfer in blastocyst stage (Vitrolife Plus G1 andG2 Media, Vitrolife Laboratories, Sweden).

Treatment for endometrial preparation with estradiol was initiated by transdermal patches, 150 µg/72 hours (Evopad;Janssen-CiLag Laboratories, Madrid, Spain). Once the endometrium acquireda trilaminar appearance of 9 mm it was supplemented with progesterone 200 µg/8 hours vaginally (Progeffik; LaboratoriosEffik, Madrid, Spain) for 5 days prior to the embryo transfer. Two expanded quality B blasts were transferred by Labottec catheter. The quality was assigned in response to the morphological characteristics of trophectoderm and inner cell mass, following the rules and recommendations established by ASEBIR (Association for the Study of Reproductive Biology). Pregnancy was confirmed from the result of serum B-hCG of 2737 IU/L at 14 days of transfer. At week 8 of gestation a transvaginal ultrasound was performed that revealed two gestational sacs with two embryos each (compatible with dichorionic diamniotic twin pregnancy and another monochorionic diamniotic twin pregnancy). The four embryos had positive cardiac activity by echo doppler. After informing of the foetal and maternal risks of multiple pregnancy, the couple decided to have an embryonic reduction (ER). The ER was performed on the monochorionicdiamniotic pregnancy in week 9 by intracardiac injection of potassium chloride, guided by transabdominal ultrasound. Monitoring of pregnancy continued by the Foetal Medicine Unit until 36 weeks of gestation, progressing without complications; Caesarean section was performed for the dichorionic diamniotic twin pregnancy with the first twin in cephalic presentation and the second twin crosswise with the result of two alive and healthy newborns. Both were females with Apgar test for both at 5 minutes of 9 out of 10 and weighing 2450 gr and 2590 gr, respectively.

Discussion

The mechanisms of formation of the monozygotic twins are not fully established although many studies have addressed the division of the inner cell mass in the early stages as a possible cause. This duplication occurs in most cases during the first two weeks after fertilization, resulting in various forms of monozygotic twin pregnancy described [2]. Among the predisposing factors are advanced maternal age (>35 years), morphology of the zona pellucida, time of *in vitro* culture and type of assisted reproduction technology used [3].

For decades the increased incidence of MZT in IVF pregnancies has been well known [4], and even more so if techniques are used that involve manipulation of the zona pellucida such as assisted hatching, subzonal insemination (SUZI) or ICSI [5-7]. These procedures would cause an artificial opening that could interfere with the natural process of embryo hatching prior to implantation. Although there are many studies showing the association, many others do not observe significant differences between ICSI cycles with assisted hatching and IVF cycles without any manipulation [1], thus reflecting the lack of unanimity on the influence of these factors on the phenomenon of twinhood [8].

Another factor to consider is the increased risk of MZT after performing transfer at blastocyst stage compared to cells stage (D+2/D +3) [9,10], contrasting with the main objective of performing long cultivation: the selection of a single blastocyst for singleton pregnancies. Several hypotheses have been proposed to explain this relationship, such as the composition of the sequential media used, especially when they are rich in glucose concentrations [11], abnormal cellular remodelling and abnormal apoptosis of the inner cell mass [12], overgrowth of the inner cell mass [13] and even the coculture to favour the elimination of free radicals during the long cultivation [14]. Other authors relate this association with the presence of low concentrations of calcium during the blastocyst formation and hatching [15]. By contrast, other studies found no significant differences according to the embryonic stage of the embryo at the time of transfer [16,17].

On the other hand the increase in obstetric complications resulting from a twin pregnancy is well known. They include the risk of abortion and premature birth, growth discordance between twins, intrauterine growth retardation (IGR) and transfusion syndromes, especially in the case of monochorionic gestations [4]. Hence, the importance of determining chorionicity in early pregnancy. This routine practice is of even greater interest in cases of multiple pregnancies in which foetal reduction may be an acceptable option. This can be carried out with a "selective" purpose, reducing the genetically affected foetus or with morphological alterations, or "non-selective", using the reduction to benefit the current pregnancy [18].

In our particular case, two of the risk factors of MZT observed and described in the literature are long culture and the ICSI technique used to generate embryos despite there being no manipulation of the zona pellucida. In addition, it is worth noting the evolution of twin pregnancy after ER, without incidents associated with this type of pregnancy and reproductive success was achieved with the birth of two alive and healthy neonates. Despite the success of the case, current scientific societies have included single blastocyst transfer as an effective strategy to reduce the incidence of twin pregnancies and its complications while maintaining the pregnancy rate [19]. It should also be borne in mind that the transfer in D+5 was performed more closely, from the chronological standpoint, to the implantation window [20].

In short, we can say that one of the main objectives of professionals who are dedicated to assisted reproduction should be to avoid multiple pregnancies, thus minimizing the risks associated with twin pregnancies. We can attain this objective by performing elective transfers of a single embryo, especially when it is transferred in blastocyst stage [21,22], provided that there is an optimal sequential culture system that ensures a high percentage of blastocysts, and cryopreservation program with maximum survival rates for this embryonic stage.

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