

## Dichorionic twins and monochorionic triplets after the transfer of two blastocysts

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### Abstract

**Purpose** To describe a unique case of MZ dichorionic twins and MZ monochorionic triplets in a quintuplet gestation after intracytoplasmatic sperm injection (ICSI) and blastocyst transfer.

**Methods** Case report. A 24-year-old woman underwent ICSI and received two blastocysts transferred. A quintuplet gestation was established .Transvaginal ultrasonography was performed sequentially during early pregnancy.

**Results** Three intrauterine gestational sacs were revealed at about 5th week. At the 7th week, five gestational sacs presenting heart beats were detected and a quintuplet pregnancy consisting of two monozygotic (MZ) dichorionic twins and three MZ monochorionic triplets was determined. At the 10th week, a single gestational sac with heart beats was detected. The prenatal course was uneventful. A healthy baby was born at 36th week.

**Conclusion** Few other reports have described the occurrence of a quintuplet gestation after the transfer of two blastocysts generated by ICSI. Our case is unique in that the two blastocysts underwent two different splitting processes, which occurred possibly at a similar time giving rise to MZ dichorionic twins and MZ monochorionic triplets.

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**Capsule** A case is reported on the birth of MZ dichorionic twins and monochorionic triplets after ICSI and embryo transfer.

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### Introduction

Extended embryo culture to the blastocyst stage represents an effective means of embryo selection, aiming to reduce the number of embryos to be transferred and the rate of multiple pregnancies [1]. However, it seems that the extended period of time that embryos spend in culture to reach the blastocyst stage may be responsible for an observed increase in monozygotic (MZ) twinning after ART [2–4]. In addition, there are suggestions of an association between the breach of the zona pellucid (ZP) for ICSI [5–9] and an increased rate of MZ multiple gestations.

Monozygotic twins arise from the splitting of an embryo from the pronuclear to the blastocyst stage. Depending on the stage of embryonic development that the embryo splits, monozygotic multiplets may have individual chorions and amnions or a single chorion and separate multiple amnions [10]. We report, to our knowledge, the first case of a quintuplet pregnancy consisting of two MZ dichorionic twins and three MZ monochorionic triplets achieved after the transfer of two blastocysts.

### Case report

A 24-year-old woman underwent her first ICSI attempt due to andrological subfertility, in February 2009

The patient was stimulated with 225 IU of recombinant FSH (Gonal F, Serono) and 75 IU of HMG (Menopur, Ferring) in a down-regulated cycle using GnRH agonist

(Nafarelin, Pfizer) according to the long protocol, starting on midluteal phase of the previous cycle. Twelve cumulus-oocyte complexes were collected 36 hrs after an injection of 10.000 IU of hCG (Choragon, Ferring). Eleven MII oocytes were injected, resulting in 11 two-pronuclei zygotes. After three days of culture in Early Cleavage medium (Irvine), embryos were placed in MultiBlast Medium (Irvine) and two blastocysts, grade G1 and G2 [11] were transferred (Fig. 1) and two others were cryopreserved. The luteal phase was supported with 800 mg of micronized progesterone daily. Fourteen days after transfer the patient had a positive  $\beta$ -hCG exam (121 ng/ml). The clinical pregnancy was defined as the presence of three intrauterine gestational sacs, at about five weeks gestational age. Two to three weeks later, the ultrasonographic exam was repeated and

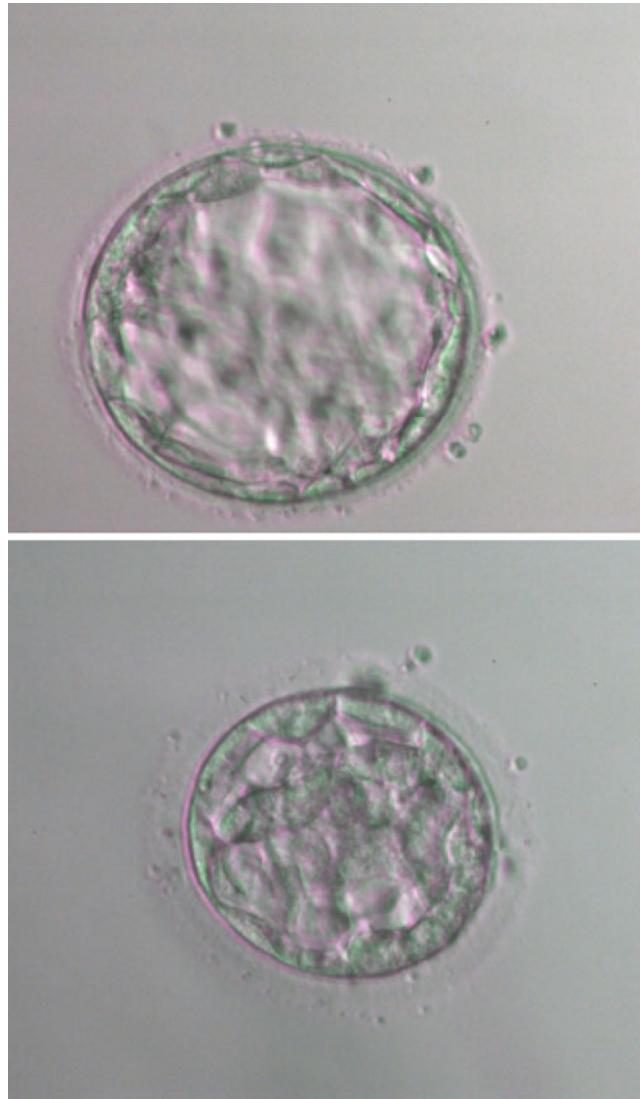
five gestational sacs presenting heart beats were detected. A quintuplet pregnancy, consisting of two MZ (biamniotic) dichorionic twins and three MZ (triadniotic) monochorionic triplets was then determined (Fig. 2). At the 9–10th week of gestation a new transvaginal sonography revealed one gestational sac with a single beating heart. The prenatal course was uneventful. A normal healthy baby girl was born at the 36th week of gestation by spontaneous vaginal delivery (weight 1.800 g, apgar 8/9).

## Discussion

This case report describes the occurrence of a quintuplet gestation after the transfer of two blastocysts generated by ICSI. One blastocyst split into MZ dichorionic twins and the other split into three MZ embryos with a single chorion.

Monozygotic twinning is a rare phenomenon among humans. Its incidence among natural conception is about 0.4% of births [12, 13]. There seems to be an increased rate of MZ twinning following ART, especially after embryo culture till blastocyst [14]. Culture to the blastocyst stage was envisaged as a means of embryo selection, to reduce multiple gestations. However, higher –order multiple pregnancies have been reported following the transfer of two blastocysts [9, 15, 16]. The etiology of the quintuplet gestation here described is difficult to know. Culture conditions may affect embryonic metabolism, favoring the splitting of the inner cell mass (ICM) before or during hatching [17]. This was our first experience, in which the number of gestational sacs and fetal heart beats exceeds the number of blastocysts transferred. Two other similar cases were found in literature. One described a quintuplet pregnancy consisting of monochorionic diamniotic and monochorionic triadniotic triplets [15]. The other report described a gestation consisting of MZ triplets (monochorionic, triadniotic) and monozygotic twins (monochorionic diamniotic) [9]. The striking fact of the present case is that the quintuplets, two MZ dichorionic and three MZ monochorionic were possibly generated by splitting processes that happened in each embryo at a similar time. Our case differs from the other reports [9, 15] in that the patient was significantly younger. This agrees with results from a review on MZ twinning after IVF [4] showing a higher incidence of this condition among young women undergoing ART. Also, in none of the previous reports and in our case, assisted hatching or pre-implantation genetic diagnosis (PGD) were performed.

The monochorionic triplets may have resulted from multiple herniations of the ICM, due to several ICSI openings in the ZP [8]. However, we have no records of difficulties during ICSI of that cycle and a single opening in the ZP should have been made. Other studies have also



**Fig. 1** Day-5 blastocysts prior to transfer

**Fig. 2** Ultrasonographic image of the quintuplets at 7 to 8-weeks of gestation



found no relationship between the incidence of MZ twinning and micromanipulation techniques [3, 18–20].

In conclusion, we believe that multiplet pregnancies after the transfer of one or two blastocysts are a rare, unwanted outcome of ART that should not discourage blastocyst transfers [21]. However, the possibility of MZ twinning after blastocyst transfers should be disclosed to the couples and single embryo transfer should be considered. Despite the existence of guidelines, legislation in Brazil does not limit the number of embryos to be transferred, however the present case prompt us to question the transfer of more than one blastocyst to young patients (<30 yrs). Also, predictive factors that may promote MZ twinning should be identified from the available literature to avoid its recurrence and unwanted results.

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