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**Running title**

ICSI quadruplets and successful delivery

**Successful pregnancy and delivery of two sets of monozygotic twins after ICSI and embryo transfer: case report and literature review**

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## **Capsule**

The positive outcome and delivery of two pairs of monozygotic twins after ICSI and embryo transfer of two blastocysts was achieved with intensive survey and prophylactic therapy throughout pregnancy.

## **Abstract**

**Objective:** To report a unique case of successful pregnancy and delivery of two pairs of monozygotic twins (quadruplets) after intracytoplasmic sperm injection (ISCI) and transfer of two blastocysts.

**Design:** Case report.

**Setting:** University medical center.

**Patient:** A 30 year old nulligravida, who had first ISCI, assisted hatching and fresh embryo transfer because of a male factor of infertility (severe olygoasthenozoospermia).

**Interventions:** Prophylactic cervical cerclage sec. McDonald in 21<sup>st</sup> week of pregnancy, hospitalization and intensive care of pregnancy, ending with delivery by planned cesarean section (CS) in 34<sup>th</sup> week of pregnancy.

**Main Outcome Measure:** Successful pregnancy and delivery of two male and two female twins in 34<sup>th</sup> week of pregnancy.

**Results:** Two blastocysts were divided and 4 embryos developed. After extensive counseling the couple decided to keep all embryos. In 21<sup>st</sup> week prophylactic cerclage was preformed. During hospitalization the ultrasound examination was performed every 2 weeks, and from 30<sup>th</sup> week on a cardiotocogram was recorded. At 33<sup>rd</sup> week her blood pressure increased and she received antihypertensive therapy. At 34<sup>th</sup> week planned CS was performed and four newborns (two male monochorionic monoamniotic twins, and two female monochorionic biamniotic twins) weighted between 1300 – 2170 grams were born.

**Conclusion:** Even without embryoreduction the intensive care throughout pregnancy including prophylactic cerclage, bed rest, prophylactic anticoagulant and antihypertensive therapy results in delivery of four healthy newborns.

**Key Words:** ICSI, embryo transfer, simultaneous division, monozygotic twins, quadruplets, pregnancy care, delivery.

## **Introduction**

Monozygotic twins (MZT) arise from division of a fertilized ovum at various early stages of embryogenesis. Division of the cells less than 72 hours after fertilization will result in formation of diamniotic dichorionic MZT. Furthermore, division between 4<sup>th</sup> and 8<sup>th</sup> day will result in diamniotic monochorionic MZT. Splitting after 8<sup>th</sup> day will result in monoamniotic monochorionic MZT (1-3).

The incidence of MZT in general population is less than 0.4% of all births (4). Several factors could be associated with increased frequency of MZT. The use of medication for induction of ovulation (clomiphene citrate and gonadotrophines) raises the incidence of MZT three times (1.2% vs. 0.4% in general population) (5). Some authors found the incidence of MZT 5% after IVF and blastocyst transfer (6,7). Others have described significantly higher rate only after ICSI and blastocyst transfer (8.9%) (8). Increased frequency of MZT after ICSI may be a result of changes in the zona pellucida at during ICSI procedure, extended culture and assisted hatching procedure (8-12).

Simultaneous division of two transferred embryos is extremely rare (less than 0.16% of all pregnancies), and only a few cases are reported in the literature (13-16).

Quadruplet pregnancy is a pregnancy with extreme risks, and in all previously reported cases the selective embryo reduction was performed. In our country, the selective reduction is not allowed by the law, and our pregnant woman agreed to keep all four embryos. Extensive survey, prophylactic cerclage, anticoagulant and antihypertensive therapy and bed rest throughout pregnancy result in successful pregnancy outcome.

Therefore, we present a unique case of the successful pregnancy and delivery of four newborns at 34<sup>th</sup> week of pregnancy after simultaneous division of two transferred embryos.



## Case report

A 30-year old Caucasian nulligravida was referred to our centre in August 2008 for her first ICSI and embryo transfer procedure. Her physical examination, body mass index, hormonal status, and cervical swabs were unremarkable. The reason for ICSI procedure was partner's severe oligoasthenozoospermia ( $< 10^6/ml$ ).

A short-protocol IVF-ICSI cycle was initiated with GnRH analogue - triptorelin acetate 0.1 mg/day from the 1<sup>st</sup> day of the cycle until the administration of chorionic gonadotropin (Decapeptyl, Ferring Pharmaceuticals Inc, Tarrytown, USA) to achieve suppression of the pituitary gland. Subsequently, follicular stimulation was achieved with 14 ampoules of menotropins: 75 IU/d (Menopur, Ferring Pharmaceuticals Inc, Tarrytown, USA). Follicular growth and maturation as well as endometrial thickness were assessed by transvaginal ultrasound. On 9<sup>th</sup> day of the stimulation 5000 IU of chorionic gonadotropin (Choragon, Ferring Pharmaceuticals Ltd, Tarrytown, USA) was administered, followed by follicular puncture 36 hours after the choriogonadotropin alfa administration. Four oocytes were obtained, and all of them were microinjected in our laboratory. Two embryos were cryopreserved for future procedures whereas remaining two embryos at the stage of blastocyst were transferred. Additional treatment with micronized progesterone, 600 mg per day (Utrogestan, Ferring Pharmaceuticals Ltd, Tarrytown USA), and two single doses of 5000 IU of chorionic gonadotropin (Choragon, Ferring Pharmaceuticals Ltd, Tarrytown, USA) were started 12 hours after ET. The micronized progesterone therapy was suspended at 16<sup>th</sup> week of pregnancy.

Ultrasonographic assessment on the 38<sup>th</sup> day after procedure showed two intrauterine gestational sacs, with one monochorionic monoamniotic twin gestation, and one monochorionic biamniotic twin gestation (Figure 1). Cardiac activity was present in all embryos. The couple was informed about the increased maternal and fetal risks in this

condition, and they were also counseled about the possibility of performing selective embryo reduction in one of the neighboring countries. The couple decided to continue the pregnancy without reduction.

The initial laboratory tests (complete blood count, renal and liver function tests, C reactive protein, coagulation tests, urinalysis, urine culture, and oral glucose tolerance test) were unremarkable, except for anemia. Iron supplement therapy and anticoagulant therapy with low molecular weight heparin, enoxaparine sodium 0.6 ml s.c. per day (Clexane, Sanofi-Aventis, Paris, France) was started at the time of hospitalization.

At 21<sup>st</sup> week of pregnancy the cervical cerclage sec. McDonald was performed because the cervix rapidly started to shorten. The laboratory tests and ultrasound assessment of fetal biometric measures (biparietal diameter, abdominal circumference, femur length, placental site, amniotic fluid index as well as biophysical profile (BPP) (since 28<sup>th</sup> week) and Doppler parameters (resistance index in umbilical and medial cerebral artery) were performed every 2 weeks. Cardiotocogram was recorded every day since 28<sup>th</sup> week of pregnancy. At 25<sup>th</sup> week the cervical swabs were taken, and were normal except fungal infection. The fungal infection was treated with one course of antifungal vaginal tablets. The control cervical swabs three weeks later were normal.

At 32<sup>nd</sup> week of pregnancy her blood pressure exceeded 150/90 and methyldopa: 250 mg tid (Aldomet, Merck Sharp & Dohme, Whitehouse Station, USA) was started. The kidney function was normal without proteinuria. The pregnant woman had no preexisting history of hypertension, and with antihypertensive therapy her blood pressure was within normal limits. The sporadic uterine contraction due to the increased intrauterine pressure started at 33<sup>rd</sup> week, and tocolytic therapy with beta mimetic agent (fenoterol): 15 ml/h i.v. infusion (Partusisten, Boehringer Ingelheim GmbH, Ingelheim, Germany), and corticosteroid

prophylaxis with dexamethason: 12 mg i.m. per day for three days (Dexamethason, TEVA, Petach Tikva, Israel) was administered.

At 34<sup>th</sup> week a planned cesarean section was performed due to the intensive uterine contractions. The male pair of twins was delivered first, two neonates weighting 1970g and 2170g, with Apgar scores were 9/10 and 10/10 and umbilical venous pH values 7.23 and 7.24. The first pair of twins was monochorionic, monoamniotic. After that the second pair of female twins was born, two neonates weighting 1300g and 2010g, with Apgar scores 9/9 and 10/10 and umbilical venous pH values 7.22 and 7.22, respectively. The second pair of twins was monochorionic, diamniotic and the first female twin was in breech presentation. During the cesarean section the uterine contractility was excellent and the bleeding was minimal. The histopathological analysis of placentas confirmed the chorionicity and amnionicity of two sets of twins. Seven days after delivery the patient's blood pressure was normal, and all newborns were healthy.

Institutional review board approved the case report, and the woman gave informed written consent.

## **Discussion**

As a part of our investigation we performed a MEDLINE search of the literature from 1966 to February 2009 using the key words: IVF, ICSI, embryo transfer, simultaneous division, monozygotic, twins, triplets, quadruplets, and delivery. We found only four similar cases of simultaneous division of two transferred embryos. Unger et al. referred a case of quintuplet pregnancy (triamniotic monochorial and diamniotic monochorial) after the transfer of two blastocysts (13). However, in this case a selective embryo reduction of one of the triplets was performed in the 11<sup>th</sup> week of pregnancy due to the increased nuchal translucency (8 mm) and increased risk of complications. The remaining two triplets died after the procedure. The outcome of the remaining twins was unknown (13).

Wehbe et al. reported a case of quadruple gestation (two sets of MNZ twins) after ICSI with assisted hatching and transfer of four embryos on day 3 (14). At 12<sup>th</sup> week a chorionic villous sampling (CVS) was performed on the first two twins and the karyograms were normal. The selective reduction of the remaining two embryos was performed. The remaining two male twins were successfully vaginally delivered at 36<sup>th</sup> week of pregnancy (14).

The second case of quintuplet gestation (triamniotic monochorial and diamniotic monochorial) after the transfer of two blastocysts was published by Zikopoulos et al. (15).

After counselling, the patient underwent embryo reduction of the monochorionic triamniotic pregnancy, continuing her gestation carrying the monochorionic biamniotic pregnancy. The outcome of the remaining twins was unknown (15).

The last report of two sets of MZG twins after ICSI was by Carrillo-Vadillo et al. (16). After counseling, the couple decided to proceed to a nonselective reduction of a set of monozygotic twins. The remaining set of twins died at 13<sup>th</sup> week of pregnancy (16).

In contrast to the previously reported cases, our case is the first without selective embryo reduction. The strategy to survey this extremely high-risk pregnancy included following:

elective cerclage in the 21<sup>st</sup> week of pregnancy to prevent cervical insufficiency due to the increased intrauterine pressure. The anticoagulant therapy with low molecular weight heparin was started at admission to hospital, in the early second trimester, in order to facilitate the invasion of trophoblast. It was continued until dismissal from hospital to prevent the thrombosis due to intensive bed rest. At 33<sup>rd</sup> week of pregnancy pregnant woman developed hypertension and antihypertensive therapy was started. Interestingly, the uterine contractions were low and sporadic until the end of 33<sup>rd</sup> week.

The intensive sonographic assessment of pregnancy with biophysical profiles and Doppler parameters in addition of cardiotocogram was a basic survey of the fetuses. The ultrasound examinations were assessed every 2 weeks. At the first set of twins there were no discrepancies neither in estimated fetal weight or amniotic fluid volume. At the second set of twins the significant growth discordance more than 20 % was present as well as polyhydraminos at the first twin and oligohydraminos at the second. The urine bladder was visible at second twin (Quintero Staging System for Twin-Twin Transfusion Syndrome – stage 1) (17). The umbilical and middle cerebral artery velocimetry and BPP was unremarkable at all fetuses.

The cardiotocogram in duration of 30 minutes was recorded every day since 28<sup>th</sup> week. She received one course of antifungal vaginal capsules, and iron supplementation throughout pregnancy. At 34<sup>th</sup> week we decided to perform a planned cesarean section due to the quadruplet pregnancy and increased frequency of uterine contractions. During the cesarean section the uterine contractibility was excellent and the bleeding was minimal.

In the current report, we report an IVF/ICSI pregnant woman with two normal intrauterine gestational sacs, both of which appear to have split between days 35 and 42 of gestation. Blastocyst transfer, ICSI procedure, and assisted hatching procedure are known to increase the risk for development of this rare phenomenon (8-12).

Accordingly, our report emphasizes the importance of transferring a single blastocyst in IVF/ICSI procedure. The increased possibility of MZT when a blastocyst is transferred should be included into the counseling with couples who enter the assisted reproductive program.

In conclusion, even with a conservative policy of selective embryo reduction in early pregnancy, this extremely risk pregnancy successfully ended with delivery of four healthy newborns owing to the intensive survey and therapy during pregnancy.

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## Figure 1

Sonographic scan of 2 pairs of monozygotic twins in 9<sup>th</sup> week of pregnancy

