Use of the Lambda Sign in Fetal Reduction of Dizygotic Triplets after Intracytoplasmic Sperm Injection

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With the increased use of artificial reproductive technologies, there are ever greater numbers of multifetal pregnancies. The increased incidences of monozygotic twins and triplet gestations can be attributed to several factors. It is important to differentiate the chorionicity in the management of multifetal pregnancies because monochorionic gestations lead to an increased risk of prematurity, twin-to-twin transfusion, morbidity, and mortality. In a dizygotic triplet pregnancy, increased risks of obstetric complications result from the monozygotic twins, such as twin-to-twin transfusion, as well as from the presence of triplets. Fetal reduction can lower these risks. During the first trimester, a "lambda sign" on ultrasound can differentiate between monozygotic twins and a separate fetus. In this paper, we present selective fetal reduction of 1 of the monozygotic twins in order to reduce the risks resulting from either monozygotic twins or from triplets. By sacrificing 1 monozygotic twin, we believe the quality of life of the remaining babies in this case were improved. *(Chang Gung Med J 2002;25:469-73)*

Key words: **dizygotic triplet, monozygotic twin, fetal reduction.**

Multifetal pregnancies are increasing due to the use of artificial reproductive technologies, and are associated with serious complications. Most fetuses are non-identical. The incidence of monozygotic twinning (MZT) is rare and estimated to be 0.42% of all births,[1] but it is more frequent with in-vitro fertilization (IVF) programs.[2] The true incidence of MZT is difficult to estimate because of multiple embryo transfer and early embryonic demise. Increased incidence of MZT has been attributed to several factors, including the in vitro culture system,[2] ovulation induction,[3] zona hardening and assisted hatching,[4] other zona manipulations such as intracytoplasmic sperm injection (ICSI),[5] advanced female age,[5] and blastocyst transfer.[6]

Monochorionic diamniotic twins are formed in the latter part of the first week of blastocyst implantation. They share a single placental disc, and possible vascular anastomosis may occur. Twin-to-twin syndrome (TTTS) complicates 15%-30% of diamniotic monochorionic twin pregnancies[7] and accounts for 15%-17% of perinatal mortality.[8] TTTS may pose a hazard to either fetus. Early diagnosis of diamniotic monochorionic twins can be achieved by the twin-peak or lambda sign on ultrasound (US),[9-10] or by observing the thickness of the separating membranes.[11]

A dizygotic triplet pregnancy after IVF was first reported in 1993.[12] Triplet pregnancy also poses risks such as preterm labor and perinatal mortality to

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both mother and fetuses, and thus, is a candidate for fetal reduction. We present a dizygotic triplet pregnancy after IVF with a lambda sign on ultrasound. One of the monozygotic twins was sacrificed in the first trimester in order to minimize the risk of TTTS during pregnancy.

CASE REPORT

A 30-year-old woman (gravida 0, para 0) with a 4-year history of infertility due to oligoasthezospermia (count, 1.4¡ 10^3/ml; motility, 71.4%; normal morphology, 78.6%; antisperm antibody, negative) underwent ICSI. She denied any history of familial twinning. This patient received the so-called "long protocol" for ovulation induction. Briefly, pituitary suppression was achieved using GnRH agonist (leuprolide acetate, Lupron; Abbott Laboratories, Chicago, IL, USA). Once down-regulation was confirmed, follicular development was stimulated with gonadotropins (Gonal-F; Serono Laboratories, Switzerland). When the size of at least 2 to 3 follicles was greater than 18 mm in diameter, 10,000 IU of hCG (Serono) was administered. Thirty-four hours later, oocytes were retrieved transvaginally under ultrasound guidance. Oocytes were washed in human tubal fluid (HTF-HEPES, Sigma Chemical, City??, MA, USA), supplemented with penicillin (10,000 IU/ml) and streptomycin (10,000 mg/ml), and then placed in a culture dish containing 3 ml of a chemically defined commercial IVF medium (Medi-Cult, Copenhagen, Denmark). Retrieved oocytes were fertilized by conventional IVF with 50,000 spermatozoa/ml added to the culture media 3-5 hours after retrieval. Fertilization occurred 18-20 h after insemination (day 1). Oocytes with 2 pronuclei and 2 polar bodies were maintained for another 24 h in the same culture conditions. On day 2, the embryos were classified based on the number of blastomeres and percentage of cytoplasmic fragments. On day 3, two well-developed embryos were transferred into the endometrial cavity. Assisted hatching was not performed in this IVF program. Micronized progesterone at 400 mg/d was given from the day of oocyte retrieval.

A urinary pregnancy test was positive 17 days after transfer. Transvaginal US (28 days after retrieval) showed 3 gestational sacs in the uterus. All of the embryos had a heartbeat. Two embryos shared the same amniotic cavity. At 10 gestational weeks, transabdominal US examination still showed 3 gestational sacs. Two closer sacs shared a thin membrane between them and a thicker separation (2 mm) from the third one. A lambda sign could be observed in this pregnancy (Fig. 1). However, the woman requested to reduce 1 fetus because of economic considerations. After explaining the risks of triplets and monozygotic twins and the risks of bleeding, infection, preterm labor and co-twin loss after injection of potassium chloride (KCl) during

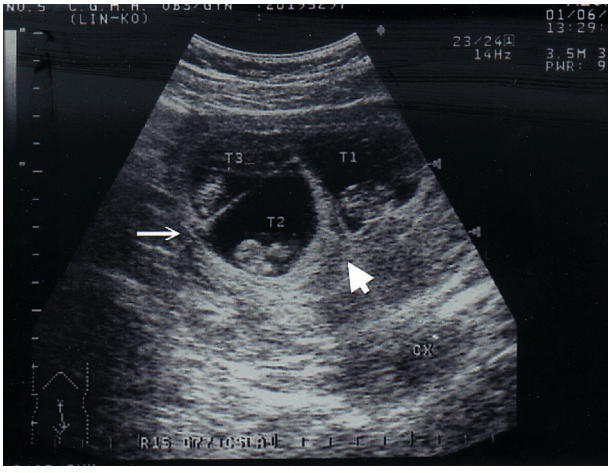

Fig. 1 Three gestational sacs with T sign (small arrow) and lambda sign (big arrow) as seen at 10 weeks of gestation.

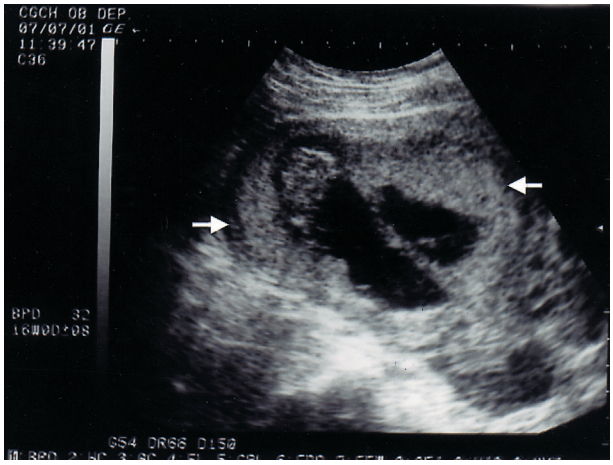

Fig. 2 Two separate placentae (arrows) noted at 15 weeks of gestation (5 weeks after fetal reduction)

the procedure, the patient agreed, and fetal reduction was performed. One of the monozygotic twins was sacrificed by intrathoracic puncture and injection of KCl. In order to reduce the risk of infusion of KCL to the co-twin, KCL was injected 0.1 ml at a time, and another 0.1 ml was injected if a heartbeat was still present after a 10-s observation. After injection of 0.2 ml of KCl, cessation of the heartbeat of the target embryo was observed. Embryo death was confirmed after a 1-min observation. Each surviving embryo now had its own amniotic cavity. Ultrasound at 15-weeks' gestation revealed that both embryos had heartbeats and separate placentae (Fig. 2). The pregnancy was uneventful beyond 20 weeks of gestation, and ultrasound showed that both female fetuses were well developed without organic defects or discordance.

DISCUSSION

The mechanism of monozygotic twining and the pattern of the separating membrane depend upon the time which the zygotes split.[5] In this case, the embryo was transferred on day 3 after oocyte retrieval, when only a single chorion had developed, and so the twins shared the same placenta. In the first trimester, dizygotic embryos developing from 2 zygotes will demonstrate a twin peak or lambda sign between the 2 sacs at the placental sites on ultrasound.[9-10] First reported in 1992,[9] the lambda sign can predict chorionicity with a sensitivity and speci ficity of over 90% at 10-14 weeks of gestation.[13] The thickness of the separating membrane is also helpful in differentiating monozygotic and dizygotic twins: in the latter, the membrane thickness will exceed 2 mm,[11] as observed in our triplet. The present case showed 3 gestational sacs, 2 placental sites, and a lambda sign.

Higher-order pregnancies are often associated with a high incidence of maternal and/or fetal morbidity and mortality, especially with monozygotic twins or triplets. An investigation concerning adverse outcomes of triplet pregnancies demonstrated that the incidences of preterm labor and neonatal death were higher in triplet pregnancies. Meanwhile, birth weights of the newborn triplets were lower than those of twins, which necessitate longer hospital stays. Neonatal outcome was improved in pregnancies after selective embryo reduction.[14]

Monozygotic twins are often complicated with TTTS, and increased morbidity is primarily associated with monozygotic twins who have vascular anastomoses associated with diamniotic-monochorionic placentation, and with monoamniotic twins.[15] TTTS is proposed to result from transfusion of blood via placental vascular anastomoses between the 2 fetuses, causing anemia, hypoxia, and growth retardation in the donor and polycythemia with circulatory overload in the recipient. A vicious cycle was proposed to explain the development of TTTS.[5] It was postulated that the placenta of the donor twin causes increased peripheral resistance in the placental circulation that promotes the shunting of blood to the recipient. The donor suffers from hypovolemia, while the recipient compensates for its overloaded circulation. Consequently, hypervolemia and the subsequent polyuria and hyperosmolality lead to heart failure and polyhydramnios. Once severe TTTS with polyhydramnios develops in the second trimester, there is a high risk of perinatal death or brain damage. The death of 1 fetus, usually the donor, is associated with subsequent death or hypoxic or ischemic sequelae in the other one.

There is evidence that monozygotic twins are associated with congenital or chromosome anomalies.[10] In addition, the risk of structural or chromosomal anomalies is higher in monozygotic twins than in dizygotic twins or singletons. However, the chromosomal or structural status cannot be recognized at the time of fetal reduction. Nonetheless, sacrificing 1 of the monozygotic twins may provide the remaining fetus with the opportunity for survival.

Timing of fetal reduction is also a concern. The advantages of performing fetal reduction in the second trimester are that fetuses with chromosomal or structure anomalies can be selectively sacrificed.[17] However, outcomes of second-trimester feticide are controversial.[17-18] In addition, the lambda sign disappears in the second trimester, which makes differentiation of chorionicity difficult, and TTTS may still have the opportunity of harming the fetuses if the singleton is inadvertently sacrificed.

Developed from the same zygote, monozygotic twins share the same placenta and chromosomes, which increases the risks of TTTS as well as of structural and chromosomal anomalies. Intrathoracic instillation of a small amount of KCL during fetal reduction still raises the risk of co-twin loss, because

there is the possibility of transfusion of KCL to the co-twin. The concern is that, if the singleton is sacrificed, the risks of TTTS and structural and chromosomal anomalies become higher, which leads to no normal, healthy fetuses remaining. Conversely, to sacrifice 1 of the monozygotic twins, despite the risk of co-twin loss, will reduce the risk, and the chance of preserving at least 1 normal, healthy fetus is increased. In dizygotic triplet pregnancies, the mother is at higher risks of obstetric complications due to the triplets, and the fetuses are at risk of TTTS. Although embryo reduction decreases the number of babies surviving to go home, we believe sacrificing 1 of the monozygotic twins in this case improved the quality of life of the remaining babies.

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Lambda Sign在精蟲注射後之雙合子三胞胎減胎之應用

洪上國　趙安祥　王馨世　宋永魁

隨著人工協助生殖技術的使用，多胞胎的機率愈來愈多。而同卵雙胞胎及三胞胎的發生受許多因素影響。在處理多胞胎之時，絨毛膜屬性便很重要，因爲在單一絨毛膜的多胞胎妊娠其早產、雙胞胎輸血症候群、罹病率及死亡率等風險會隨之增加。在雙卵三胞胎，主要的產科風險來自於單一絨毛雙胞胎，如雙胞胎輸血症候群，及三胞胎妊娠本身。減胎可以降低這個風險。在妊娠前三個月中，利用lambda sign來區別是否爲同卵雙胞胎。在此篇文章中，我們藉此來減胎，以改善其餘胎兒的品質。(長庚醫誌 2002;25:469-73)

關鍵字：雙合子三胞胎，單羊膜雙胞胎，減胎。

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