—Case Report—

Conjoined Twin in Triplet Pregnancy Occurring after ICSI, Cryopreservation, and Assisted Hatching

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Abstract: *Conjoined twins result from an abnormal process in the development of monozygotic twins. Monozygotic twins are closely associated with assisted reproductive technology, especially assisted hatching. The prognosis for conjoined twins is very poor. This risk underscores the importance of fetal screening by ultrasonography in the early stages of pregnancy, since the conjoined twin can be detected by ultrasonography. Here we reported a case of conjoined twin in a triplet pregnancy after a transfer of cryopreserved embryos with assisted hatching that were obtained by ICSI.*

Key words: *Monozygotic twin, Conjoined twin, Assisted hatching, ICSI*

Conjoined twins result from an abnormal process in the development of monozygotic twins. The incidence of conjoined twins ranges from 1 in 50,000 to 1 in 100,000 pregnancies [1]. *In vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have increased the frequency of multiple pregnancies [2]. The incidence of monozygotic twins is generally constant at one in 250 pregnancies [3]. There were two reports of conjoined twins which occurred after assisted reproductive technology. One case occurred after a combination of IVF and assisted hatching [4]. The other occurred after a combination of ICSI and cryopreservation of embryos [5].

In our patient, we performed ICSI and cryopreserved the embryos obtained. Subsequently, we thawed 2 embryos in a natural cycle. After performing assisted hatching, we transferred the embryos. This resulted in a pregnancy, which was determined to be a triplet pregnancy by transvaginal ultrasonography performed at week 10 weeks' gestation. And a thoraco-omphalopagus as a conjoined twin was diagnosed in two fetuses.

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A 30-year old gravida 0, para 0 woman underwent her first ICSI with testicular sperm extraction (TESE) in a case involving obstructive azoospermia in the husband. Since spermatozoa could not be obtained from the epididymis, TESE was performed, followed by cryopreservation of testicular spermatozoa. From the first day of the treatment cycle, 900 μg of GnRHa (Suprecur, Hoechst) was administered daily (short protocol). Stimulation was administered in the treatment of menstrual cycles with pure FSH and hMG, with a 10,000 IU dose of hCG administered when the maximum diameter of follicles reached 20 mm. The oocytes were collected 35 hours thereafter. Five oocytes, all grade 1, according to Veeck's classification [6], were retrieved. ICSI with motile thawed spermatozoa was performed for all oocytes. All oocytes were successfully fertilized. Three of the 5 oocytes were transferred to the uterus during the treatment cycle. The oocytes, all were grade 1, were transferred at the 7 to 8 cell stage three days after oocyte retrieval. Two oocytes out of 5 were cryopreserved at the 4 cell stage by the slow-freeze method. Finally, none of the transferred oocytes resulted in pregnancy in the first

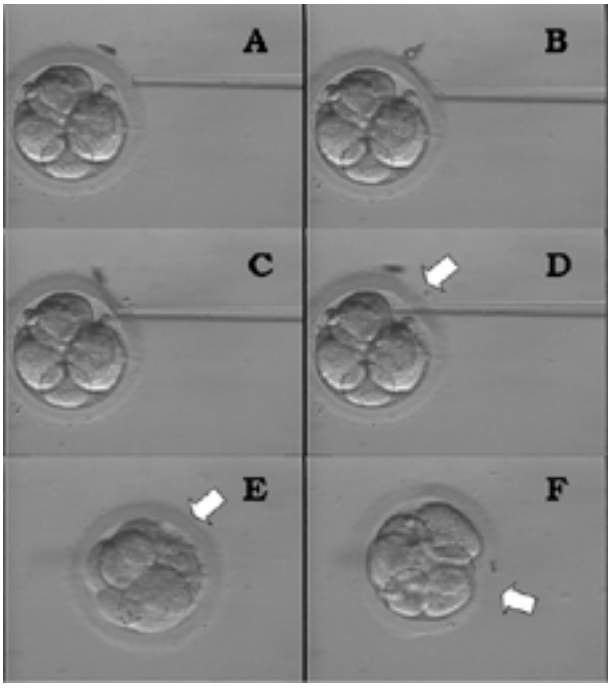

Fig. 1. Embryos in which assisted hatching was done and transferred. Two embryos were performed assisted hatching with acid Tyrode's solution (A–D). A small hole was created by assisted hatching (arrow in D). It took 1 to 2 minutes. Two embryos were transferred into the uterus (E, F). The diameter of the hole made by assisted hatching was about 20 μm (arrows in E, F).

Fig. 2. A normal fetus and conjoined twin at 10 weeks' gestation. a: normal fetus with 35.8 mm crown-rump length. b: a thoraco-omphalopagus (conjoined twin).

Fig. 3. A thoraco-omphalopagus. Two heads (h) are seen. Two fetuses were joined between the thorax and abdomen of each fetus (joined region indicated by the marks (▽△).

treatment cycle.

Two months later, the 2 frozen embryos were thawed and transferred to the uterus, with hormone replacement therapy administered concurrently. Cryopreserved at the 4 cell stage, the 2 thawed embryos developed to the 6 to 7 cell stage after 24 hours of incubation. We undertook assisted hatching for the 2 embryos by using zona drilling with acidic Tyrode's solution), after inspecting the thickness of the zona pellucida (≥15 μm) under the microscope (Fig. 1) [7]. Urine hCG tests were positive at fourteen days after the transfer. At 20 days after the transfer, the first sonographic examination indicated two gestational sacs exhibiting lambda signs. Repeated sonography clearly showed one sac with a normal fetus, with a crown-rump length (CRL) of 35.8 mm at 10 weeks' gestation (Fig. 2). In the other sac, two embryos were observed as a complete duplication of conjoined twins on transvaginal ultrasound examination by the sonographer and obstetrician (Figs. 2 and 3). This double-embryo had two heads, two thoraxes, single heart and two abdomens with CRLs of 36.2 mm and 34.5 mm and nuchal translucency of 5.2 mm and 3.1 mm at the neck of each fetus respectively. And they were joined at their thoracic and abdominal wall (Figs. 4A, 4B). There was a heartbeat. A diagnosis of thoraco-omphalopagus was made for the embryos. At 12 weeks' gestation, the conjoined twin fetus no longer exhibited a heartbeat. Thereafter, the obstetrical course was not eventful. The outcome of the pregnancy was the birth of a normal healthy child at 38 week's gestation by spontaneous delivery (weight of 3336 g, male). Evidence of

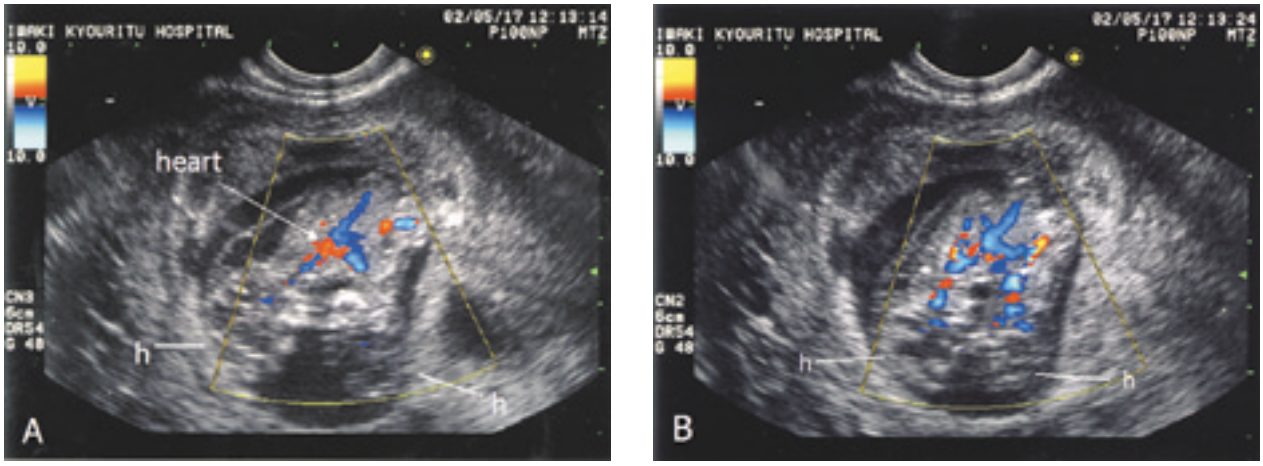

Fig. 4. The color Doppler flow mapping of the conjoined twins. A: The shared heart (arrow) was diagnosed by color Doppler flow mapping. "h" indicates the head of each fetus. B: The color Doppler flow mapping at another time. Major circulatory systems of fetuses were seen.

conjoined twins which resulted in intra-uterine fetal death was not clear at delivery.

Discussion

Conjoined twins are defined as monochorionic monozygotic twins joined at a part of their bodies. Within the general population, monochorionic monozygotic twins occur at the rate of 1 in 250 pregnancies (approximately 0.4%) [3]. Several reports state that the overall rate of monozygotic twins in pregnancies involving assisted reproductive technology was 0.84 to 8.9% of all pregnancy [8–15]. Not only assisted reproductive technology, but also ovulation induction induced monozygotic twins [14]. Monozygotic twins are typically associated with assisted hatching or a thin zona pellucida [9]. Monochorionic monozygotic twins are frequently observed in older women [16]. Sixty-five percent of monozygotic twins occurr in women aged ≥35 years old [17]. This may be related to the finding that the zona pellucida is often thin in older women [18]. The timing of the embryo splitting is hypothetically classified as follows, according to the stage at which a fertilized ovum divides: division within 72 hours of fertilization will result in diamniotic dichorionic twins; division after 72 hours but within 8 days after fertilization will result in diamniotic monochorionic twins; and division after 8–12 days after fertilization (after formation of the amnion) will result in monoamniotic monochorionic twins. Conjoined twins may result from division more than 10 days after fertilization (after formation of the blastocyst). Conjoined twins may arise from incomplete separation of an embryonic disk that forms from 6 to 15 days after fertilization, when hatching occurs.

Conjoined twins are classified as follows; conjoined twins in which heads and limbs maintain their identity are referred to as duplicata completa, whereas conjoined twins featuring more extensive fusion are referred to as duplicata incompleta. The most common type of conjoined twins is thoraco-omphalopagus (28%), followed by thoracopagus (18%), omphalopagus (10%), incomplete duplication (10%), and craniopagus (6%). Of all conjoined fetuses, 39% result in stillbirths, and 34% die on or before the day after birth [19]. Seventy percent of monochorionic twins are boys, but 70% to 95% of conjoined twins are girls [20]. Another type of conjoined twin is the heteropagus in which an unequal, asymmetrical conjoined twin is fused to an almost normal fetus. An heteropagus is the name for a parasite or conjoined parasitic twin and is very rare (4.5% of conjoined twins [21]), but the prognosis for this type is the best among all conjoined twins. The case of conjoined twins reported herein were thoraco-omphalopagus. In our patient, the two fetuses were joined at their thoracic and abdominal wall and shared a single heart. This appears to point to an abnormality in the early stages of hatching. The cause of intra-uterine fetal death in the conjoined twins reported here was not clear. It might be thought that the conjoined twin had a chromosomal abnormality and disorders of the blood-circulation systems were due to sharing a single heart,

since there is the possibility that a fetus with nuchal translucency of > 5 mm has a chromosomal abnormality.

As previously stated, assisted reproductive technology involves a risk of conjoined twins. One published report involves a case of triplets, including conjoined twins, conceived by assisted hatching after IVF [4]. The conjoined twins in the patient reported in this case were diagnosed as thoraco-omphalopagus at 12 weeks' gestation by transvaginal ultrasonography. Selective termination was performed after obtaining informed consent from the patient. Another report involves a case of triplets, including conjoined twins, conceived by transfer of cryopreserved embryos obtained from fertilized ova obtained by ICSI [5]. The fetuses were diagnosed as thoraco-omphalopagus twins sharing a heart at 8 weeks' gestation by transvaginal ultrasonography. The patient accepted selective termination, which was performed at 12 weeks' gestation. In our patient, fertilized ova obtained by ICSI were cryopreserved and thawed. Subsequently, assisted hatching was performed, and 2 embryos were transferred, resulting in triplets, including thoraco-omphalopagus twins.

In conclusion, assisted reproductive technology involves a risk not only of monochorionic monozygotic twins, but of conjoined twins. This risk underscores the importance of fetal screening by ultrasonography in the early stages of pregnancy.

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