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CASE REPORT

A case of conjoined twins after a transfer of a multinuclear embryo

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Key Clinical Message

A pregnancy with conjoined twins was observed after transfer of a multinuclear embryo. As nuclear mechanisms have a role in cellular differentiation, association between multinucleation and fetal malformations is possible. Follow-up studies on children born after transfer of embryos with bi/multinuclear blastomeres are needed.

Keywords

Conjoined twins, embryo, IVF/ICSI, multinucleation, nuclear envelope.

Introduction

Assisted reproduction treatments (ART) increase the rate of monozygotic (MZ) multiples. The natural prevalence of MZ is 0.4–0.45% of live births but ART with single-embryo transfer increases to rate to 1.0–2.3% [1–3]. This increase seems to associate especially with ICSI and transfer of blastocysts. The mechanisms behind the increase remain unknown, although ovarian stimulation, hardening and manipulation of zona pellucida, prolonged culture and suboptimal culture conditions are proposed as putative explanations [1, 4]. A rare complication of monozygotic twinning is conjoined twins (CT) with prevalence of 1.47 per 100,000 births. The prevalence varies, however, markedly among different countries with highest prevalence in Finland [5].

The true prevalence of CT after ART is not known. As ART increases monozygotic twin rate, it is logical to assume that the risk of CT is increased likewise. A recent review reported that 14.8% of the 75 CT pregnancies diagnosed during the first trimester had begun with ART [6]. However, the cases originating from ART are more likely to be detected early and thus it remains unknown if ART is a risk factor for CT.

In this case report, we present a case of conjoined twins after a transfer of a frozen-thawed embryo with multinuclear blastomeres (MNB). This is the first case of conjoined twins after ART in Finland since the beginning of the National data collection on infertility treatments (M.Gissler, personal communication). This demonstrates rarity of CT after ART as data on 32,180 pregnancies is collected during 1992–2011 (www.thl.fi).

Case Report

A thirty-three year-old healthy, nonsmoking nullipara was referred to our infertility unit because of primary male infertility. Her husband was azoospermic secondary to stem cell transplantation for hematologic malignancy. Normal sperm was frozen prior to the transplantation. The menstrual period was 22–26 days and the ovarian reserve tests were within the normal limits (FSH 7.2 U/L, AMH 1.0 g/L, AFC 18). The clinical and ultrasound findings were normal. GnRH analog was used for scheduling the treatment but for controlled ovarian stimulation, GnRH antagonist protocol was used (Gonal-F® Merck Serono, Modugno, Bari, Italy 175 IU 9 days and Cetrotide® 0.25 mg (Merck Serono) for 6 days). When three leading follicles were ≥17 mm, human chorionic gonadotrophin (10,000 IU) was administered. Ten normal oocytes were aspired. Of these, nine mature oocytes were fertilized with

intracytoplasmic injection of frozen-thawed sperm. Embryos were cultured in Vitrolife sequential medium (G5 Series) in 7% CO_2 and 8% O_2 and 85% N_2 in single-embryo droplets.

Eight oocytes showed normal fertilization with two pronuclei and two polar bodies. All fertilized oocytes cleaved to 2-cell stage in 25 h after the fertilization but carried multiple small nuclei in all blastomeres (Fig. 1A). All embryos cleaved further and at a 4-cell stage they contained mostly mononuclear, even-sized blastomeres and <25% fragmentation (Fig. 1B). An embryo with one nucleus in each of the four blastomeres was transferred unsuccessfully. Seven 4-cell embryos with one bi- or multinuclear and three mononuclear blastomeres were frozen by slow method according to manufacturers (Vitrolife) protocol and stored in liquid nitrogen (−196°C). Later, three embryos were thawed and cultured overnight. An 8-cell embryo with three multinuclear, even-sized blastomeres was transferred successfully 2 days after ovulation. An intrauterine pregnancy with single heartbeat was confirmed with ultrasound at 7 weeks gestation. At 12 weeks gestational age, monozygotic, conjoined twins with two heads, four limbs and a common heart was detected (Fig. 2). Termination of pregnancy was recommended on the basis of poor prognosis of thoracopagus twins. Medically induced abortion was performed without complications.

The patient has given a written consent for publication of this report. Institutional ethical approval was not required as this is a case report.

Discussion

To our knowledge, this is the first report of conjoined twins after a transfer of embryo with multinuclear blastomeres.

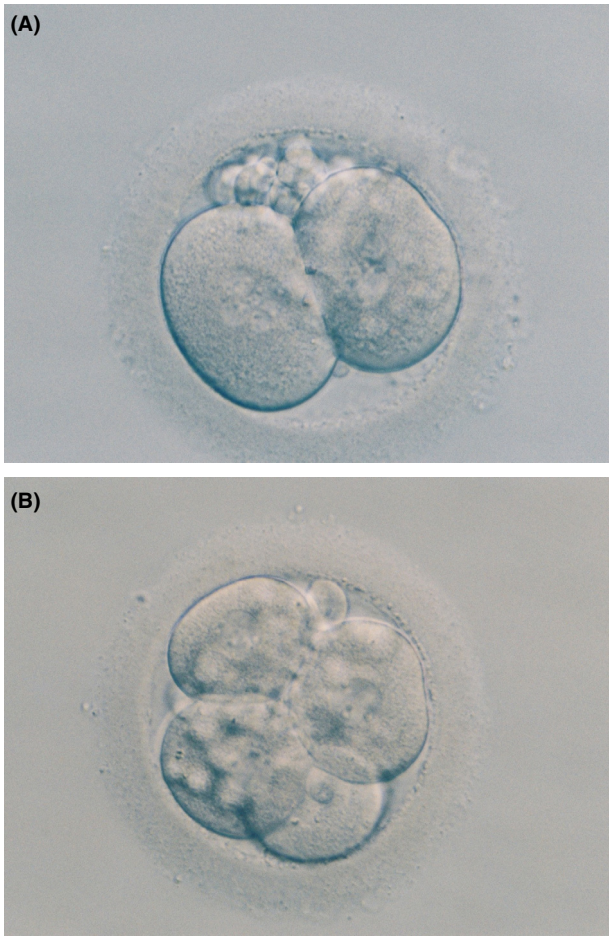

Figure 1. Nuclear status of an MNB embryo on day one and two: (A) Good quality 2-cell embryo with <25% fragmentation 25 h after fertilization. Both, even-sized blastomeres have several clearly visible nuclei. (B) The same embryo on day two, 44 h after fertilization. Each blastomere have only one, even-sized nucleus.

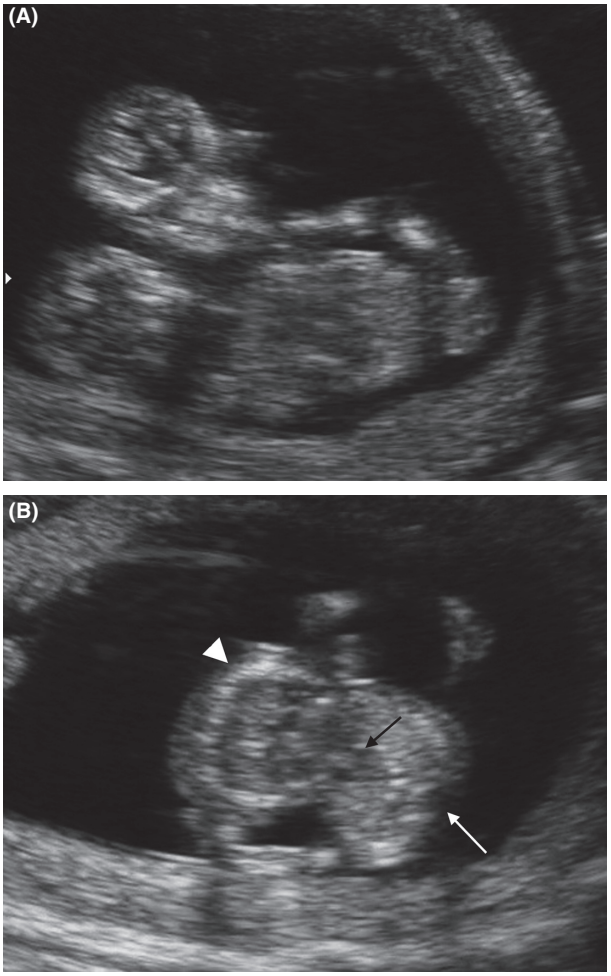

Figure 2. (A) Conjoined twins with two heads opposing each other in a fixed manner. (B) A transverse image of fused thoraxes showing a common heart (black arrow – common heart; white arrow head – rib of the left fetus; white arrow – spine of the fetus on right).

Table 1. A summary of the reported cases of conjoined twins after assisted reproduction treatments.

Author	Age	Parity	Treatment	Transfer day (D)	Embryos Transferred	Embryo description	Dg at gw	Gest sacs (fetuses)	CT type	Outcome of Conjoined twins (Normal fetuses)
Poret et al. [7]	30	0	ICSI	D2	1	Even sized <10% fragm.	9	1 (2)	Thoracopagus	Termination
Mercan et al., [8]	32	0	ICSI	D3	3	8-cell good quality	10	1 (2)	Thoracopagus	Termination
Hirata et al., [9]	34	1	ICSI	D5	2	4BB + early bla	8	2 (3)	Thoracopagus	Miscarriage 10 gw, (Live birth 39 gw)
Sugawara et al., [10]	30	0	ICSI, FET, AHA	D3	3	6 and 7 cell	10	2 (3)	Thoracopagus	Miscarriage (Live birth 38 gw)
Shimizu et al., [11]	36	0	IVF	D3 and D5	2 and 1	4 cell and extended bla	10	1 (1)	Cephalophagus	Termination
Boulot et al. [12]	27	0	IVF	NA	2	NA	10	2 (3)	Craniophagus	Selective termination (Live birth)
Skupski et al. [13]	35	G3P2	IVF, AHA	D3	4	NA	12	2 (3)	Thoracoomphalopagus	Selective termination (Ongoing pregnancy)
Hill [14]	32	NA	ART	NA	NA	NA	9	2 (3)	Ischiopagus	Selective termination (Miscarriage)
Goldberg et al. [15]	28	0	ICSI	NA	NA	NA	8	2 (3)	Thoracoomphalopagus	Selective termination (Ongoing 26 gw)
Ericson and Källén [16]	NA	NA	ICSI	NA	NA	NA	NA	NA	NA	NA
MacKenzie et al. [17]	30	NA	IVF	NA	NA	NA	9	1 (2)	Ischiopagus	Miscarriage
Fujimori et al., [18]	30	0	ICSI	NA	2	NA	28	NA	Omphalopagus (heteropagus)	CS 30 gw neonatal death
Maymon et al., [19]	33	2	ICSI	NA	4	NA	9	3 (4)	Thoraco omphalopagus	Selective terminations of CT and one normal fetus (CS 38 gw singleton)
Charles et al., [20]	NA	NA	IVF	NA	2	NA	10	3 (3)	Minimally conjoined omphalopagus	Selective termination (21 gw delivery)
Allegra et al., [21]	34	0	ICSI, AHA	D3	3	NA	11.3	3 (4)	Thoraco-omphalopagus	Selective termination 12 gw (CS twin 38 gw)
Varma et al., [22]	33	G3P0	IVF FET	Bla/NA	NA	NA	9	1 (2)	Thoracopagus	Miscarriage

CT, conjoined twins; NA, data not available; gw, gestational weeks; Bla, blastocyst, AHA, assisted hatching; CS, caesarian section; fragm., fragmentation.

In literature, sixteen cases [7–22] of conjoined twins after ART have been reported to date (Table 1). Description of embryos transferred is included in only five case reports: thoracopagus twins are reported after a transfer of a 4-cell embryo with even-sized blastomeres and <10% fragmentation on day 2 [7], a transfer of three good quality 8-cell embryos on day 3 [8], a transfer of two blastocysts (early and 4BB) on day 5 [9] and 6- and 7-cell embryos on day 3

[10]. Cephalopagus CT was reported after a sequential transfer of 4-cell embryo on day 3 and an extended blastocyst on day 5 [11]. Information on the nuclei in blastomeres is not included in these reports.

Embryos with multinuclear blastomeres (MNB) are commonly seen in IVF/ICSI treatments. In several reports, around 15–35% of embryos in up to nearly 80% of IVF/ICSI cycles have seen to be bi/multinucleated [23–25]. After IVF and ICSI, 5–10% of the 2-cell embryos have been reported to carry solely bi/multinuclear blastomeres [26]. In embryos with even-sized blastomeres, the multinucleation rate is significantly lower than in embryos with uneven cleavage (2.1% vs. 45.5%) [27]. In general, the presence of MNB in human embryos is considered abnormal, but when there are no other embryos available, embryos with <50% MNB can be transferred and frozen [26]. Multinucleation occurring at the first mitotic division and affecting both blastomeres is considered pathological. These embryos are not usually transferred, although a birth of a healthy child after a transfer of an embryo with two multinuclear blastomeres at the 2-cell stage has been reported [28]. Even if chromosomal abnormality has been detected in 71.4% of embryos containing only MNB [29], multinucleation is not a definite sign of aneuploidy. The majority of 2-cell embryos carrying multiple nuclei in both blastomeres cleave further and 30.4% of those contain only mononuclear diploid blastomeres at 3- and 8-cell stages [26]. Thus, in some cases, multinucleation is a temporary and reversible phenomenon.

Multinucleation of blastomeres may result from insufficient culture medium or cooling of the oocyte during manipulation leading to alterations of the cytoskeleton [30]. Also, accelerated response to ovarian stimulation and intrafollicular hypoxia disturbing the oocyte′s spindle organization are proposed as causative factors [23, 31, 32]. Different mechanisms causing multinuclear blastomeres have been suggested: karyokinesis without cytokinesis, defective migration of chromosomes at mitotic anaphase or partial fragmentation of nuclei [26, 27]. As stated above, some of the multinuclear 2-cell embryos develop further and carry a diploid chromosome constitution. This may indicate that in these cases multinucleation results from fragmentation of the nucleus or from poorly coordinated reconstitution of the nuclear envelope at the end of mitotic division.

During mitosis, the nuclear envelope (NE) dissolves at prophase and is rebuilt at late telophase. The nuclear envelope is composed of the outer nuclear membrane, which is continuous with the endoplasmic reticulum, the inner nuclear membrane (INM) and nuclear pore complexes (NPC) [33]. In multicellular organisms, the nuclear lamina lies beneath the INM and maintains the nuclear shape and structure. The nuclear lamina is composed of type A/C and B lamin filaments and it stays in contact with the cytoskeleton through NPC and linker of nucleoskeleton and cytoskeleton (LINC) complexes [34]. The reformation of the NE involves the reassembly and organization of NE components as well as lamins around chromosomes [33, 35]. It is possible that in some cases MNB may arise from disturbed reassembly of NE components and lamins at the end of mitosis. In mice, paternal exposure to cyclophosfamide causes micronuclei formation during the first mitotic division. These micronuclei have incomplete peri-nuclear and peri-nucleolar lamin B1 membrane formation [36]. In animal, B type lamins are present in all embryonic stages [37] and interestingly B-type lamin is reported to be essential for organogenesis in mouse [38]. Such an association renders logical, as changes in NE composition affect heterochromatin positioning and play a role in gene expression and cellular differentiation during development [39].

Increased prevalence of congenital malformations is seen both in ART pregnancies and in MZ twins [40, 41]. Monozygotic (MZ) twins are the result of a single-embryo splitting during early embryo development to form two separate embryos. MZ twins arise probably by multiple mechanisms: (1) insult to an early embryo leading to development of two blastocysts; (2) disruption of communication between inner blastomeres of a morula; (3) entrapment and mechanical dissection of the inner cell mass during hatching; (4) blastocyst collapse and adhesion of IC blastomeres to another point; and (5) dissection of IC by apoptosis of some cells [1]. Conjoined twins are proposed to arise from incomplete fission of inner cell mass or embryonic disc or alternatively, a fusion of two dizygotic or monozygotic embryos [13, 19]. Monozygotic twinning is suggested to represent a form of blastogenesis defects occurring before organogenesis [42, 43]. Blastogenesis defects affect usually the formation of the midline and they include defects of fusion, lateralization, segmentation, morphogenetic movement, and symmetry [43]. There is a close relationship between nuclear mechanics and cellular organization, function and movement [44] needed for normal organogenesis. Studies are needed to explore if impaired assembly and function of NE associate with disturbed organization, communication, and movement of the embryonic cells. Such an association could increase the risk of developmental diversity like monozygotic twinning and congenital anomalies.

We need data from single-embryo transfers to see if embryo morphology correlates with monozygotic twinning and the health of children. Such studies are scarce. An old study did not show significant difference between the mean percentage of MNB embryos leading to singleton and MZ twin pregnancies, neither there was difference in mean cell number or fragmentation volume of

the transferred embryos [45]. However, in the study the mean number of transferred embryos was 3.2 (range 1–6) and the percentage of MNB and other markers of embryo quality were calculated from all transferred embryos. Only studies with single-embryo transfer can answer the question what is the significance of embryo morphology on pregnancy outcome and children′s health.

Taken together, a question arises whether the formation of multiple nuclei in the blastomeres and the embryo′s incomplete division during development share a common origin in the presented case. It is plausible, that several mechanisms cause multinuclear blastomeres, and the significance of the phenomenon varies accordingly. Therefore, studies on the mechanisms behind multinucleation of cleavage stage embryos are warranted, as well as follow up of children born after transfer of embryos with bi/multinuclear blastomeres.

Conflict of Interest

None declared.

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