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Microstructural observations of villous and membrane histology in monochorionic triplet placenta after *in vitro* fertilization

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Summary. The frequency of triplet gestation is low in humans, estimated at 1:6400 deliveries. Monochorionic gestations represent a subpopulation of approximately 10% of these triplet pregnancies. Hypertensive complications are known to occur with greater frequency in the context of multiple gestation. In this report we describe microscopic placental changes associated with pre-eclampsia and proteinuria in the setting of an uncommon monochorionic-triamniotic triplet pregnancy achieved via in vitro fertilization. Histologic features observed in this case include placental stromal fibrosis and increased syncytial nodularity (Tenney-Parker change). In this triplet delivery resulting from two consecutive fissions of a single embryo, chorion and amnion configuration are also characterized with a review of the literature discussing the potential relationship between in vitro culture conditions and monozygotic multiple gestation.

Key words: IVF, Multiple gestation, Hypertension, Prematurity, Syncytial nodularity

Introduction

The histology of membrane and villous structures associated with monochorionic triplet pregnancy has been poorly characterized because the entity is not frequently encountered in clinical practice (Schieve et al., 1999). Indeed, triplet gestations occur with an estimated frequency of only 1:6400 pregnancies, and among triplet gestations those with a unified chorion represent an even smaller subset (10-15%). It has long been recognized that disorders of blood pressure regulation develop more commonly in multiple gestations (Suzuki et al., 2003), and previous investigators have commented on histologic findings observed in the context of preeclampsia (Risteli et al., 1984; Li et al., 2000). Whether the membrane microarchitecture evident in higher-order multiple gestation is a factor contributing to development of intrapartum complications, or represents the end result of a compensatory or adaptive process is not known. This is the first report to describe clinical and histopathologic findings in the unusual setting of monochorionic triplet pregnancy complicated by hypertension.

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Materials and methods

We evaluated a 21 year-old nulligravida and her partner for infertility of two years duration. Both husband and wife were in good general health and the female's past gynecological history was unremarkable. Physical exam revealed no abnormality; BMI was 20kg/m^2 and her blood type was O$^+$. Cervical cytology and laboratory tests results were all normal, and saline hysterography identified no intrauterine filling defects. The partner was healthy and took no regular medications. He had established no previous pregnancies and had been diagnosed with azoospermia four years earlier. Urological evaluation was normal and the karyotype was 46,XY, and obstructive azoospermia (rete testes obliteration) had been diagnosed after exploratory scrotal surgery. For personal reasons, the couple declined intrauterine insemination using anonymous donor sperm, and elected in vitro fertilization utilizing surgically retrieved spermatozoa with intracytoplasmic sperm injection.

After pituitary downregulation with intranasal nafarelin acetate, controlled ovarian hyperstimulation followed a combined FSH+hMG protocol. Subcutaneous hCG was administered on stimulation day 11 (Sills et al., 2001), when serum estradiol was 2110 pg/ml. Twenty-two oocytes were retrieved via ultrasound-guided transvaginal needle aspiration, undertaken in parallel with spermatozoa collection via testicular biopsy. Ten

oocytes advanced to the 2pn stage after intracytoplasmic sperm injection. Two embryos were transferred on day three after assisted hatching by acid Tyrode's method; one blastocyst was cryopreserved at -196 ºC. Two weeks later, serum hCG was 423mIU/ml. Transvaginal sonogram at eight weeks' gestation showed a single 28 mm intrauterine gestational sac (chorion) with three distinct fetal poles, each with cardiac activity. Amnion configuration could not be precisely determined. The diagnosis was revised to monochorionic-triamniotic triplet pregnancy at follow-up ultrasound one week later. Daily folic acid dose was increased to 1mg/d, and antenatal care was co-managed with a perinatologist.

The obstetrical course was uneventful through the 19th gestational week, when cervical funneling was observed on transvaginal sonogram. Prompt hospital admission was advised and a McDonald cerclage was placed. Her post-operative course was uneventful and the patient was discharged home the next day.

With persistent blood pressures at 140/80, the patient was readmitted to hospital for mild preeclampsia at 28 weeks' gestation. To promote fetal lung maturity, betamethasone (12 mg x 2 doses) was given via intramuscular injection. Although no focal neurological signs were present, ALT and AST were elevated (97 and 121 u/l, respectively). Liver function tests normalized soon after admission. Platelet count was 157,000 and protein (325 mg) was present in 24 h urine collection. Multifetal biophysical profiles were performed every 48h with reassuring results. At $30^{5/7}$ weeks gestation, serum uric acid was 7.8 mg/dl and platelets fell to 115,000. Concurrently, the patient began to experience visual scotomata. Given these findings consistent with preeclampsia, the patient underwent a cesarean delivery resulting in the births of three viable female infants (1475, 1021, and 1021 g). Apgar scores were and 8/9, 9/9, and 8/9, respectively. At delivery, umbilical artery blood gas values for triplet A were: pH=7.307, pCO_2=47.8, pO_2=28.1, HCO_2=23.2, base excess=-2.2, and for triplet C: pH=7.308, pCO_2=52.3, pO2=18.7, HCO_3=25.4, base excess=-0.1 (the umbilical cord from triplet B demonstrated velamentous insertion and could not be sampled).

Results

A single three-umbilical cord placenta (weight=693 g) was delivered intact; each umbilical cord was trivascular and morphologically normal. The placenta measured 19x17x3 cm *en toto*, and was devoid of any gross parenchymal lesion. No abnormality of the placental surface vascularity was noted. Membrane examination confirmed monochorionic-triamniotic triplet gestation (Fig. 1). Umbilical cord lengths for triplet A, B, and C were 30, 21, and 25 cm, respectively. Cord diameters for triplet A, B, and C were 1.2, 1.1, and 1.1 cm, respectively.

Placental insertion sites for triplet A and B (velamentous) were 3 and 5 cm, respectively, from the nearest margin. The umbilical cord insertion site for triplet C was indeterminate because it was separated from the placenta. Microscopic examination of placental parenchyma showed well-vascularised villi with no evidence of ischemia or hemorrhage. Focal acceleration of villous maturation was noted, as were areas of diminished villous diameter in tandem with Tenney-Parker change (Fig. 2). Occasional areas of fibrinoid necrosis and lipid-containing macrophages localised to spiral arteries (acute atherosis) were present with hyperplastic arteriosclerosis, consistent with the clinical history of preeclampsia.

Discussion

Because monochorionic triplet pregnancy is exceedingly rare, typical villous and membrane findings have proven difficult to characterize. Indeed, since triplet pregnancy accounts for some 1:6400 of all deliveries, the single chorion renders this triplet pregnancy particularly striking as monochorionic triplets comprise only 10-15% of the overall triplet population. In our report, we describe microscopic placental features in the context of preeclampsia and monochorionic triplet delivery after IVF.

Preeclampsia is a gestational emergency, with serious implications both for mother and newborn (Suzuki et al., 2003). The disorder is usually defined as hypertension in pregnancy accompanied by proteinuria after 20 weeks gestation. Placentas from preeclamptic women are smaller and tend to have lower average weights than placentas from normal pregnancies (Fox, 1967). While histologic examination of placentas obtained from preeclamptic women show no reliable or predictable features, most investigators concur that infarcts are more prevalent among placentas from preeclamptic patients than from uncomplicated gestations. It may be that the extent of infarction is related to the severity of the prevailing hypertensive condition, yet even this is not universally seen in preeclampsia. Indeed, some degree of placental infarction is relatively common and its significance in preeclampsia relies more on quantitative assessment. Impaired choriodecidual vascularity and associated uteroplacental insufficiency are the most characteristic placental findings present in preeclampsia. Cytotrophoblastic proliferation and thickening of the trophoblastic membrane are often observed in the setting of preeclampsia (Risteli et al., 1984; Naeye, 1989; Li et al., 2000). In our case, placental villi were relatively small and punctuated by scattered syncytial nodules, the so-called Tenney-Parker change (Tenney and Parker, 1940; Las Heras et al., 1980, 1985; Naeye, 1989). Additionally, villous arterial luminal constriction or obliteration is often identified in the placentas of preeclamptic women (Van Der Veen et al., 1982; Las Heras et al., 1985). While the etiology of preeclampsia remains unknown, the higher observed frequency of preeclampsia in multiple gestations has been proposed to

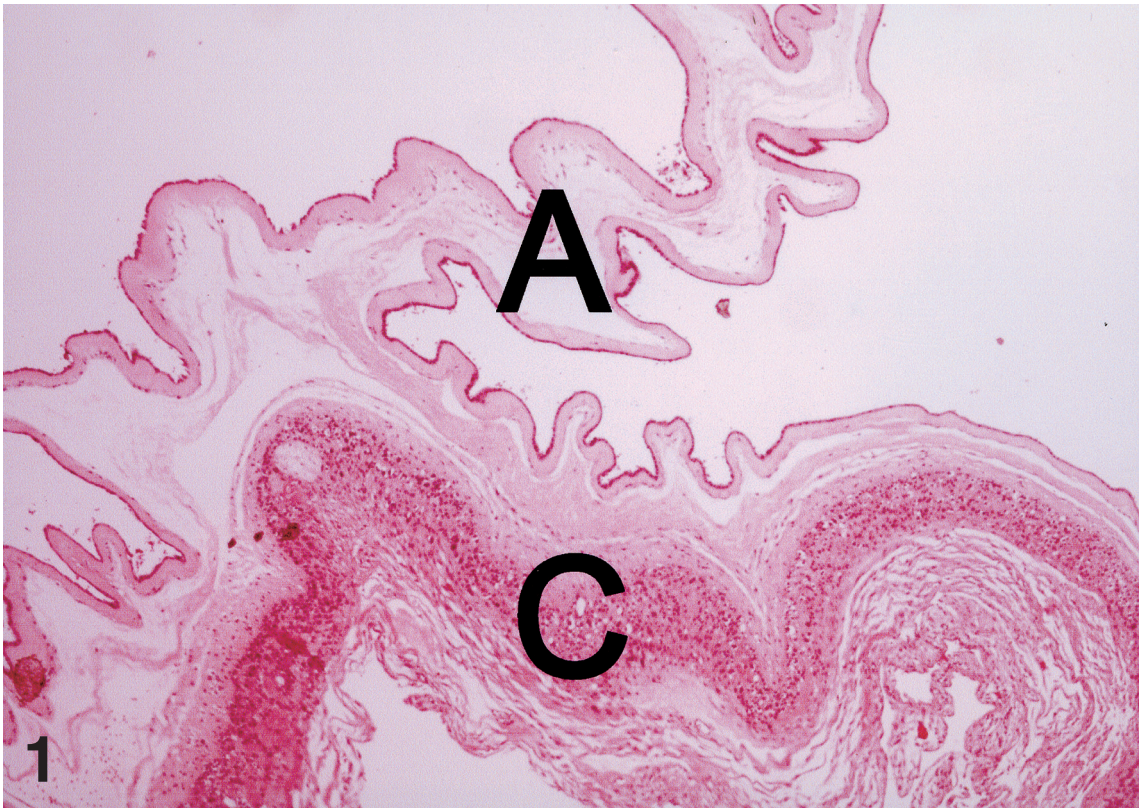

Fig. 1. Amnion (A) with chorion (C) confined to basal elements in monochorionic-triamniotic conception resulting from a two-embryo transfer following in vitro fertilization and intracytoplasmic sperm injection (original magnification 40X, hematoxylin and eosin).

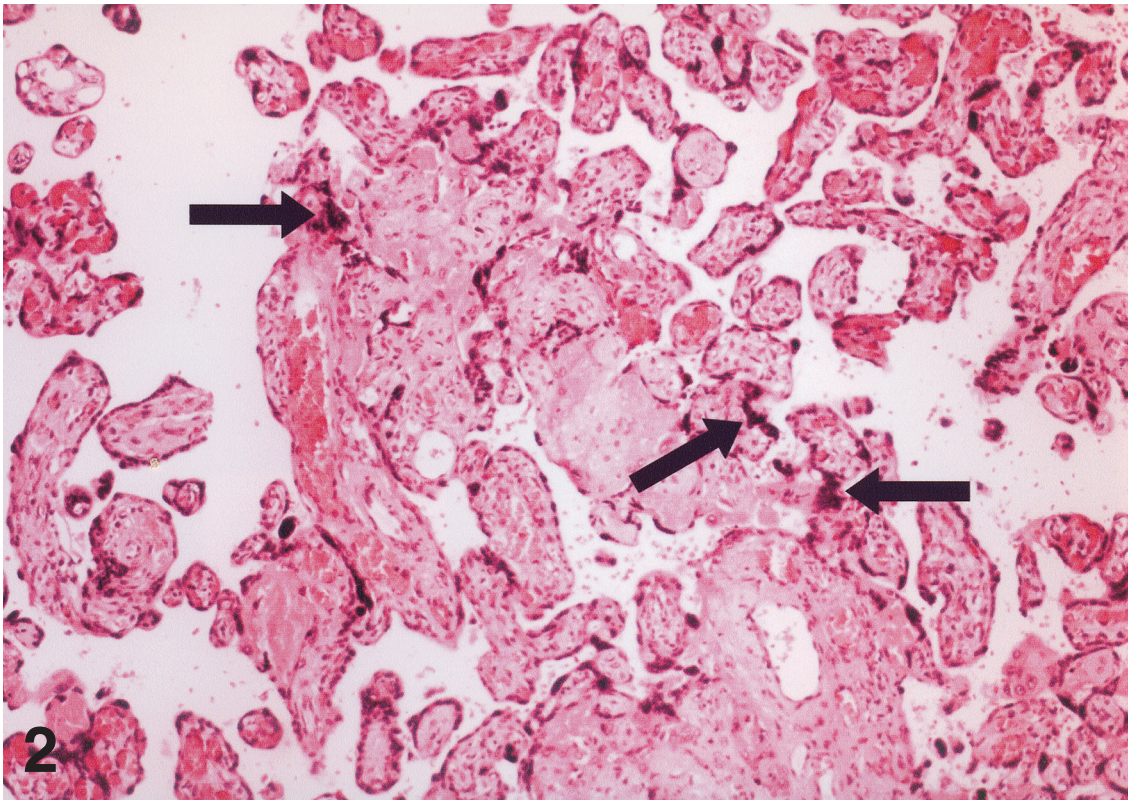

Fig. 2. Cytotrophoblast proliferation with multiple syncytial nodes (arrows) identified in placental tissue obtained from monochorionic-triamniotic triplet gestation, delivered at $30^{5/7}$ weeks gestation due to preeclampsia (original magnification 40X, hematoxylin and eosin).

result from maternal exposure to a superabundance of chorionic villi at the endometrial-placental interface (Beer, 1978).

It must be acknowledged that microarchitectural changes observed in this case may have developed without hypertension or proteinuria, existing as a placental adaptation necessary to support three fetuses. Similarly, whether or not the advanced reproductive technologies influence monozygotic multiple gestation remains controversial and has been reviewed elsewhere (Sills et al., 2000). It has been hypothesized that artificial openings of the zona pellucida could afford the expanding blastocyst an opportunity to extrude in such a way that a "figure of eight" distortion and embryo splitting occurs. While zygosity refers to the number of source zygotes comprising the gestational set (with profound life-long implications for a sibling cohort), characterization of chorion and amnion for any multiple gestation is more important in obstetrical management. The number of placentas correlates with embryo number for multizygotic gestations, but monochorionic multiplets without mosaicism (Souter et al., 2003) are considered monozygotic.

The problem of higher-order multiple births resulting from a large number of transferred embryos has received comment by epidemiologists (Schieve et al., 1999), and some authors have suggested that reductions in multiple gestation can best be accomplished by transfer of only one embryo after IVF (De Neubourg et al., 2002). However, this monozygotic/monochorionic triplet pregnancy occurred in the context of a single blastocyst implantation after transfer of two embryos, a clinical practice generally regarded as conservative. Although preeclampsia requiring cesarean delivery was encountered in this case, whether fertility therapy or in vitro embryo culture conditions were causative or merely associative interventions is unknown. Further observational studies are needed to characterize the physiology and natural history of monozygotic/monochorionic multiple pregnancy, particularly those accompanied by preeclampsia.

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