OBSTETRICS

A review of the mechanisms and evidence for typical and atypical twinning

Helen C. McNamara, MB, BS; Stefan C. Kane, MB, BS; Jeffrey M. Craig, PhD; Roger V. Short, ScD; Mark P. Umstad, MD

The mechanisms responsible for twinning and disorders of twin gestations have been the subject of considerable interest by physicians and scientists, and cases of atypical twinning have called for a reexamination of the fundamental theories invoked to explain twin gestations. This article presents a review of the literature focusing on twinning and atypical twinning with an emphasis on the phenomena of chimeric twins, phenotypically discordant monozygotic twins, mirror-image twins, polar body twins, complete hydatidiform mole with a coexistent twin, vanishing twins, fetus papyraceus, fetus in fetu, superfetation, and superfecundation. The traditional models attributing monozygotic twinning to a fission event, and more recent models describing monozygotic twinning as a fusion event, are critically reviewed. Ethical restrictions on scientific experimentation with human embryos and the rarity of cases of atypical twinning have limited opportunities to elucidate the exact mechanisms by which these phenomena occur. Refinements in the modeling of early embryonic development in twin pregnancies may have significant clinical implications. The article includes a series of figures to illustrate the phenomena described.

Key words: assisted reproductive technologies, chimeric twins, complete hydatidiform mole with coexistent twin, dizygotic, fetus in fetu, fetus papyraceus, mirror-image twins, monozygotic, twins, phenotypically discordant monozygotic twins, polar body twins, superfecundation, superfetation, vanishing twin

Widespread use of assisted reproductive technologies (ART) involving the manipulation of the natural mechanisms of fertilization and implantation has been accompanied by an increasing number of reports of atypical twinning. Cases of atypical twinning have inspired a reexamination of the fundamental theories of twinning. Traditional models of twinning pertaining to a fission event have been contested. New models involving fusion of fetal membranes have been proposed.

This article presents a review of the literature regarding the typical and atypical twinning, highlighting the phenomena of chimeric twins, phenotypically discordant monozygotic twins, mirror-image twins, polar body twins, complete hydatidiform mole with coexistent twin, vanishing twins, fetus papyraceus, fetus in fetu, superfetation, and superfecundation. A detailed discussion of monoamniotic twins, conjoined twins, and twin-reversed arterial perfusion sequence is beyond the scope of this review and has been presented elsewhere.[1,2]

Traditional models of twinning

Traditionally it has been thought that dizygotic twins result from fertilization of 2 distinct ova by 2 separate spermatozoa, whereas monozygotic twins are the product of a single ovum and sperm that subsequently divide to form 2 embryos.[1]

Widely accepted models of monozygotic twinning are based on the unproven hypothesis of postzygotic division of the conceptus (Figure 1). In this model, the number of fetuses, chorions, and amnions are determined by the timing of the embryo splitting (Table 1).

Proposed triggers for splitting include postzygotic gene mutations, abnormalities in cell surface proteins, and abnormalities in the formation of the zona pellucida.[3] The incidence of monozygotic twins is increased 2- to 5-fold in ART pregnancies,[3] which might be predisposed to splitting because of handling, media, and microinjection or because of the intrinsic abnormalities associated with infertility.[4-6]

It has conventionally been asserted that monochorionicity confirms monozygosity, opposite-sex twins confirm dizygosity, and same-sex dichorionic twins remain of uncertain zygosity until postnatal evaluation occurs.[7]

New models of twinning

In 2013 Herranz[8] argued that the hitherto-unchallenged hypothesis of

From the Department of Maternal-Fetal Medicine (Dr McNamara, Dr Kane and Dr Umstad), Royal Womens Hospital, Department of Obstetrics and Gynaecology (Dr Kane and Dr Umstad), and Faculty of Medicine, Dentistry and Health Sciences (Dr Short), University of Melbourne, and Murdoch Childrens Research Institute (Dr Craig), Royal Children's Hospital, Melbourne, Victoria, Australia.

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Corresponding author: Mark P. Umstad, MD. mark.umstad@thewomens.org.au

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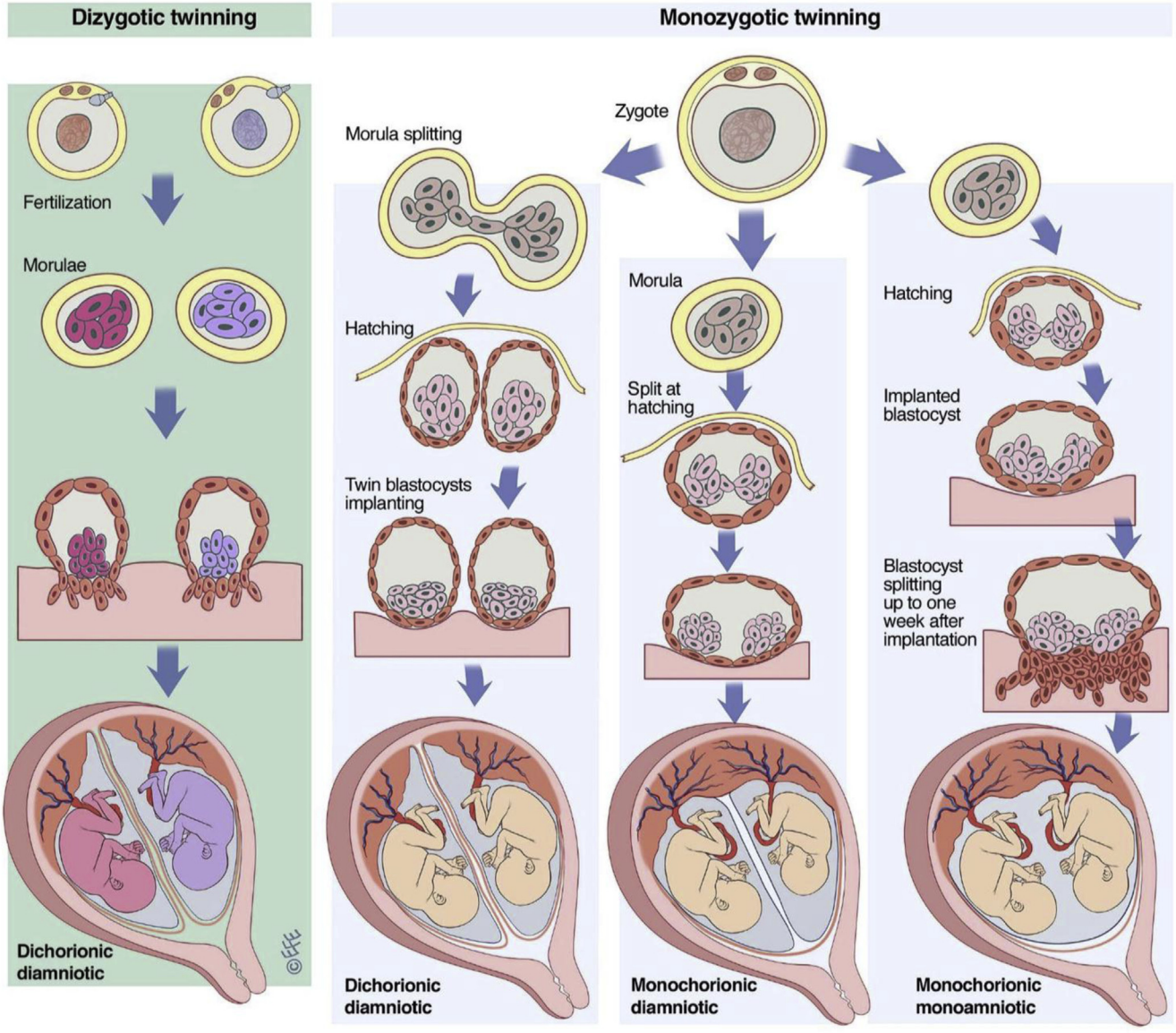
FIGURE 1
The traditional model of twinning



Dizygotic twins are the product of 2 distinct fertilization events, resulting in dichorionic diamniotic twins with each conceptus developing to become a genetically distinct individual. Monozygotic twins result from postzygotic splitting of the product of a single fertilization event. Splitting on days 1—3 (up to the morula stage) results in dichorionic diamniotic twins, on days 3—8 (during which blastocyst hatching occurs) in monochorionic diamniotic twins, on days 8—13 in monochorionic monoamniotic twins, and if no split has occurred by day 13, in conjoined twins (not shown). In this diagram, 2 of the 3 oocyte-derived polar bodies are shown at the zygote stage.

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postzygotic splitting lacked scientific proof. He argued that factors that initiate cleavage have not been specified, that coexistence of separate embryos within a single zona pellucida is unlikely, that postzygotic splitting becomes more unlikely with the passage of time, and that splitting has never been observed in vitro.

Herranz[8] offered an alternative theory of twinning based on the following 2 principles: (1) monozygotic twinning occurs at the first cleavage division of the zygote and (2) subsequent chorionicity and amnionicity is determined by the degree of fusion of embryonic membranes within the zona pellucida (Figure 2).

Denker[9] opposed Herranz's argument, emphasizing that a lack of evidence may stem from ethical limitations on scientific experimentation with human embryos. He highlighted that data regarding twinning mechanisms in animals, differences in the nature of the zona pellucida in vivo and in vitro, and

TABLE 1
Chorionicity and amnionicity by time of zygote splitting

Zygosity	Twins	Time of split	Chorions	Amnions	Fetal mass
Dizygotic	DC DA	No split	2	2	2
Monozygotic	DC DA	Days 1–3	2	2	2
Monozygotic	MC DA	Days 3–8	1	2	2
Monozygotic	MC MA	Days 8–13	1	1	2
Monozygotic	Conjoined	After day 13	1	1	1

DA, diamniotic; *DC*, dichorionic; *MA*, monoamniotic; *MC*, monochorionic.

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the developmental potential of cell lineages after fertilization were not discussed. Denker[9] concluded that both the traditional fission model and Herranz's fusion model were unsubstantiated.

Further examination of twinning processes in typical and atypical twins might confirm which, if either, of these models is more accurate.

Atypical twinning

A review of the evidence for atypical twinning provides insights into the mechanisms of twinning and challenges aspects of traditional models of twinning.

Chimeric twins

A chimera is a single organism containing 2 populations of genetically distinct cells originating from 2 different zygotes.[1] Chimerism in humans was initially observed in studies of the ABO blood group.[10] Blood group chimerism was demonstrated via genetic testing in 1976[11] and is now considered common[10,12] and persistent.[13]

Chimerism has since been described in twins with monochorionic dizygotic (MCDZ) placentation. Early reports of MCDZ twins were discounted because of the absence of formal placental histopathology[14] or confirmatory genetic testing.[15]

In 2003 Souter et al[15] reported the first confirmed case of sex-discordant MCDZ twins born to a 48 year old woman following in vitro fertilization (IVF). Cytogenetic analyses demonstrated chimerism in peripheral blood leukocytes. A further 20 cases of MCDZ twins with confined hematological and/or tissue chimerism have been reported (Appendix 1).[7,12,16-29]

Concerns have been raised that chimeric twins might exhibit reproductive dysfunction analogous to that of the bovine freemartin.[30] Early follow-up studies described normal genitalia, gonads and endocrinological function in gender-discordant chimeric twins to a maximum of 18 months of age. However, in 2013 Choi et al[26] reported a case of MCDZ twins complicated by death in utero of the female twin and severe gonadal failure in the male cotwin. The authors concluded that close observation of chimeric infants is necessary to ensure that gonadal failure/dysfunction is identified and appropriately managed.

The mechanisms underlying human twin chimerism and monochorionic dizygotic twin pregnancies remain incompletely defined.[18] Theories proposed are outlined in Table 2 and depicted in Figure 3.[7,14,17,19,25,27,31-35] Nevertheless, it is clear that the dogma of monochorionicity being synonymous with monozygosity is no longer appropriate.

Assumptions regarding the antenatal diagnosis of zygosity on the basis of sonographic features[24] may be unreliable, with important implications for antenatal risk stratification,[16] screening, and diagnosis. Failure to diagnose MCDZ twins might have long-term consequences.[26] Individuals with blood or tissue chimerism might be at increased risk in the context of transfusion or transplantation,[22] and modeling of epigenetic and genetic factors of disease in monochorionic twins may lead to erroneous conclusions.[36]

Phenotypically discordant monozygotic twins

Phenotypic discordance in monozygotic twins commonly occurs as a consequence of epigenetic, mitochondrial, and genetic discordance.[1,37,38] Epigenetics has been implicated as a mediator of stochastic and twin-specific environmental factors.[39-41] Genetic differences within monozygotic pairs must arise de novo soon after zygotic cleavage if they are found in multiple somatic tissues and later in development if they are mosaic.[42] Such differences can be single base pair mutations[42,43] or copy number variation[44,45] or involve whole chromosomes.[46]

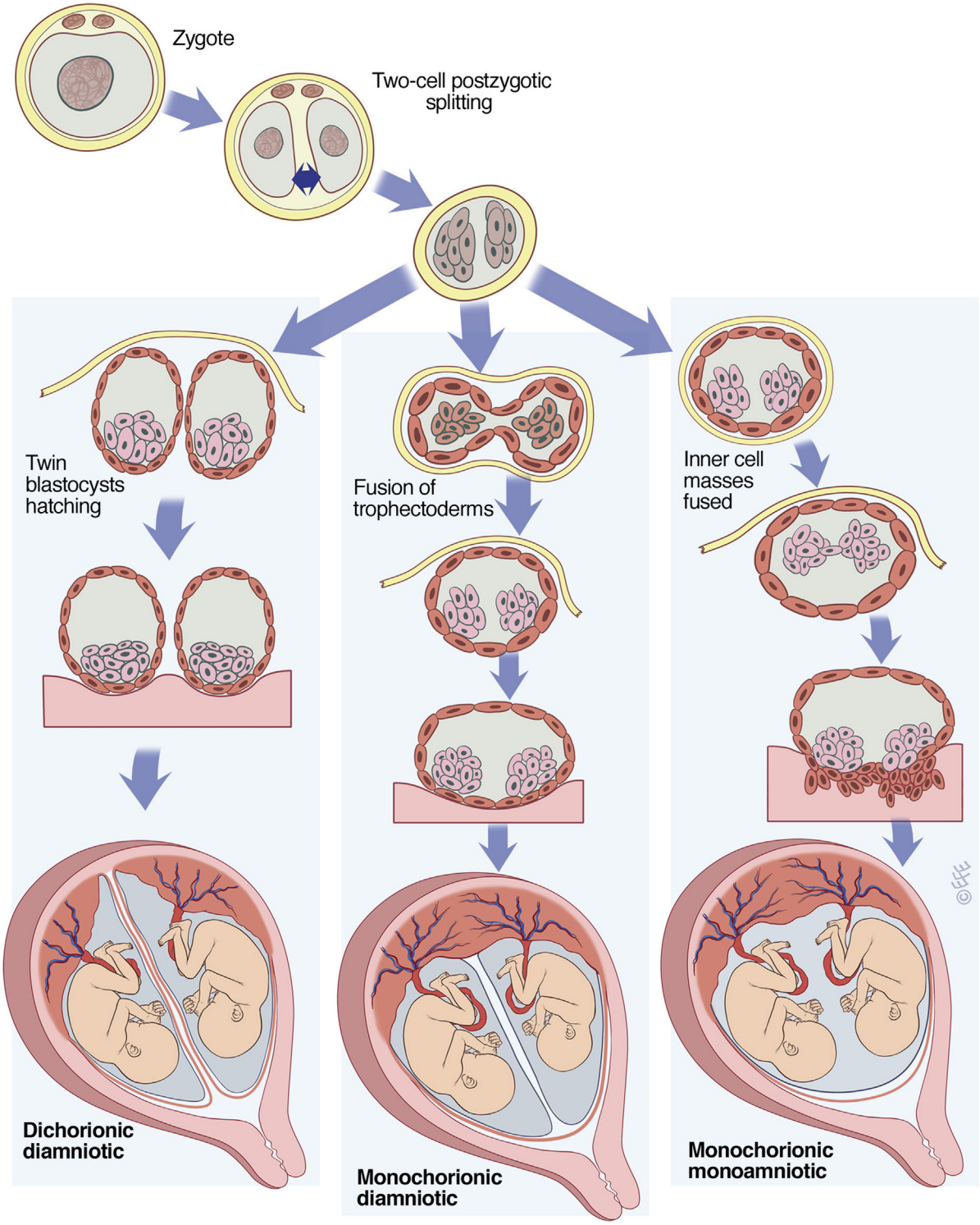
On a genome-scale, the frequency of epigenetic differences within monozygotic pairs is likely to be high.[39,40] Less is known about the frequency of genetic discordance in monozygotic twins, although it is likely to be low.[47,48] However, more genetic variation might occur outside coding regions.[49] Little is known about the frequency of mitochondrial discordance in twins.[50]

Mirror-image twins

Mirror-image twinning has been described in monozygotic twins with phenotypic features that are asymmetrical. As many as 25% of monozygotic twins may have mirror-image features.[51] Mirror asymmetries observed include direction of occipital hair whorl, dental patterns, unilateral eye and ear defects, cleft lip and palate, bony abnormalities, and tumor patterns (Appendix 2).[51-62] Mirror-image central nervous system abnormalities, including optic glioma, colpocephaly, and arachnoid cysts,[63-66] and cases of mirror-image organ laterality in heterotaxy syndromes[67,68] have been described.

It has been suggested that higher-order cerebral functions including dominant handedness,[69] eye dominance,[52] and cerebral lateralization for language and mental rotation tasks[70] also exhibit mirror asymmetries. However, Derom et al[69] demonstrated that although left-handedness may be more

FIGURE 2
An alternative model of monozygotic twinning



In this model, splitting occurs at the postzygotic 2 cell stage, with each cell forming a distinct individual. If twin blastocysts hatch from the zona pellucida together, dichorionic diamniotic twins will result. If the 2 trophectoderms fuse before hatching and the inner cells masses are separated within the shared trophectoderm, monochorionic diamniotic twins will result. If the inner cell masses are fused and separated later, monochorionic monoamniotic twins will result.

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TABLE 2
Theories proposed to explain chimeric and MCDZ twinning

	Hypothesis	Evidence
1	Placental anastomoses allowing early transfer of genetic material	• In cases of chimeric twins affected by TTTS, recipient twins are significantly more chimeric than donor twins.[7,25] • Tissue chimerism might be due to migration and subsequent ectopic differentiation of chimeric hematopoietic stem cells.[27] • Chimerism has been demonstrated to persist after selective laser photocoagulation of placental anastomoses.[25,31]
2	Fusion of elements of 2 genetically distinct zygotes	• Chorions might fuse in early pregnancy with subsequent degeneration of intervening tissue.[14] • Trophoblasts might fuse preimplantation. Fusion of preimplantation embryos has been achieved in vitro.[34] • MCDZ twinning is more common in ART pregnancies. Handling with disruption of the zona pellucida, and multiple embryo transfer with spatial proximity of embryos, might predispose to fusion.[17,19]
3	Fertilization of a binovular follicle	• Binovular follicles have been observed in women undergoing ovulation induction with gonadotropins.[33] • Fertilization of a binovular follicle has been achieved in vitro, but progression to a viable pregnancy has not been observed.[150-152]

ART, assisted reproductive technologies; *binovular follicles*, follicles in which 2 oocytes exist within a single zona pellucida; *MCDZ*, monochorionic dizygotoic; *TTTS*, twin-to-twin transfusion syndrome.

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common in twins, there is no evidence to suggest that discordant handedness represents mirror imaging. These conclusions were supported by large-scale data from Australia and The Netherlands.[71,72]

According to traditional models of twinning, mirror-imaging results from late zygotic splitting at days 9-12, just prior to the formation of conjoined twins (Table 1).[1,60] Cases of heterotaxy syndrome have been likened to cases of conjoined twins in whom the close proximity of the body axes gives rise to organ laterality of one twin affecting that of the other.[73] Nevertheless, little evidence exists to support these hypotheses.[67,69,74]

Polar body twins

A polar body is a small, cellular by-product of the meiotic division of an oocyte. Apoptosis usually occurs within 17-24 hours of formation, and the resulting fragments remain within the zona pellucida.[75]

It has been hypothesized that fertilization of an ovum and its first or second polar body by 2 distinct sperm may result in polar body twinning (Figure 4). In 1981 Bieber et al[76] described a monochorionic twin pregnancy with a normal male (XY karyotype) and an acardiac female (triploid XXX karyotype). Cytogenetic studies suggested a diploid contribution from the mother in the acardiac twin. Human leukocyte antigen (HLA) typing suggested dispermic fertilization. Thus, it was proposed that independent fertilizations of a haploid ovum and its diploid first polar body had occurred. The authors hypothesized that the proximity of the ovum and its first polar body allowed the development of distinct inner cell masses within a common trophoblast. A fusion mechanism for twinning was deemed unlikely.[76]

In contrast, Fisk et al[77] performed cytogenetic analyses on the tissues of 9 twin pregnancies affected by twin-reversed arterial perfusion sequence. All twin pairs were monochorionic and discordant for the acardiac anomaly. Deoxyribonucleic acid (DNA) fingerprinting revealed monozygosity. The calculated likelihood of fertilization of an ovum and its first or second polar body in any twin pair and in all twin pairs was less than 3.6% and 0.0003%, respectively. The authors disputed the existence of polar body twinning, ascribing previously reported cases to chimerism, and instead suggested embryonic fusion as an alternative but poorly understood possibility.[77]

Complete hydatidiform mole with coexistent twin

A multiple pregnancy with a complete hydatidiform mole (CHM) and a coexisting live fetus (CLF) is characterized by the presence of a fetus with normal karyotype, anatomy, and placentation alongside a molar component with no identifiable fetal parts, a placenta with diploid paternal chromosomes, and the characteristic sonographic and histological features of a CHM (Figure 5). CHM-CLF is rare, with a reported incidence of 1 in 22,000 to 1 in 100,000 pregnancies.[78,79]

Three conditions require differentiation from CHM-CLF. First, a singleton pregnancy with a partial hydatidiform mole may occur in which the fetus has triploidy resulting from dispermic fertilization of a haploid normal oocyte. Second, a twin pregnancy may occur with a normal twin in one sac and a partial mole in the other sac. Third, mesenchymal dysplasia may occur and is associated with an enlarged cystic

placenta, fetal growth restriction, and, occasionally, fetal death.[80]

Historically, most CHM-CLF pregnancies have been terminated. More recently it has become apparent that the prognosis is not as poor as previously thought. The live birth rate varies from 21% to 40%.[81,82] Complications include vaginal bleeding, hyperemesis gravidarum, thyrotoxicosis, early-onset severe preeclampsia, and fetal death. Outcomes in higher-order multiple pregnancies remain poor.[83,84]

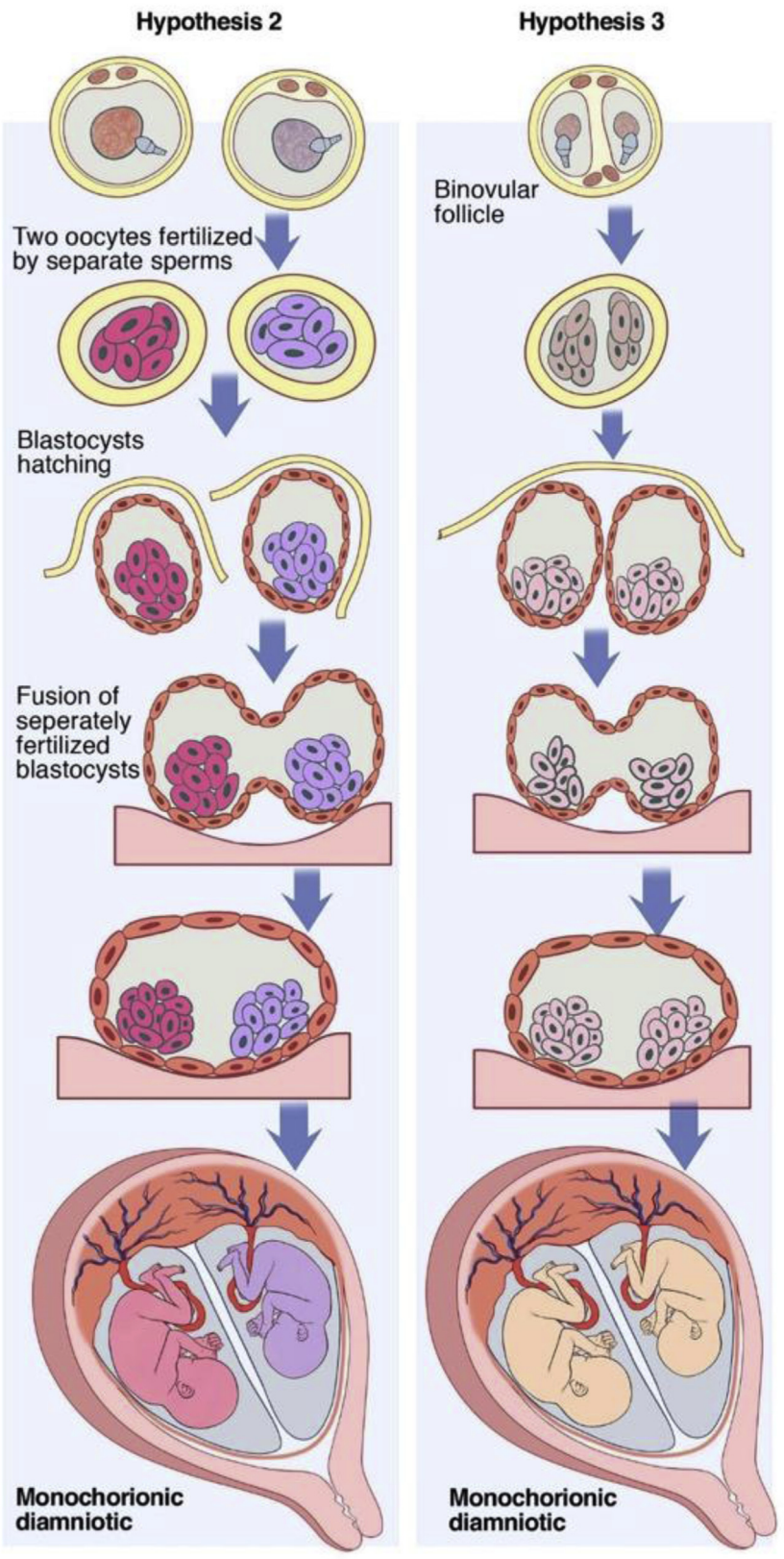
Conflicting evidence exists regarding rates of persistent gestational trophoblastic disease in CHM-CLF compared with CHM alone. Sebire et al[82] showed a rate of 19% for CHM-CLF compared with 16% for CHM alone. Massardier et al[81] showed a rate of 50% for CHM-CLF compared with 14% for CHM alone. The risk of gestational trophoblastic disease is independent of gestation and whether the pregnancy is terminated or is allowed to continue.[85]

Although fetal loss is the most likely outcome for CHM-CLF, continuing the pregnancy is possible as long as maternal complications are manageable and the pregnancy is closely monitored.

Vanishing twins

Vanishing twin syndrome (VTS) refers to multiple pregnancies affected by the

FIGURE 3
Models of chimeric twinning



Hypothesis 1 (not shown) accords with the traditional model of monochorionic diamniotic twinning (Figure 1) in which placental anastomoses may result in intertwin transfer of blood cells with subsequent blood cell chimerism. Such blood cells might subsequently infiltrate saliva. Hypothesis 2 follows the traditional model of dizygotic twinning up to the hatching stage. If 2 hatched blastocysts are in close proximity, as with the use of assisted reproductive technologies, trophectoderm fusion may occur. Hypothesis 3 involves fertilization of a binovular follicle in which 2 oocytes exist within a single zona pellucida. In each hypothesis, fusion might also occur after implantation. In rare cases, cells from the inner cell mass may be transferred between twins, resulting in some degree of somatic chimerism (not shown).

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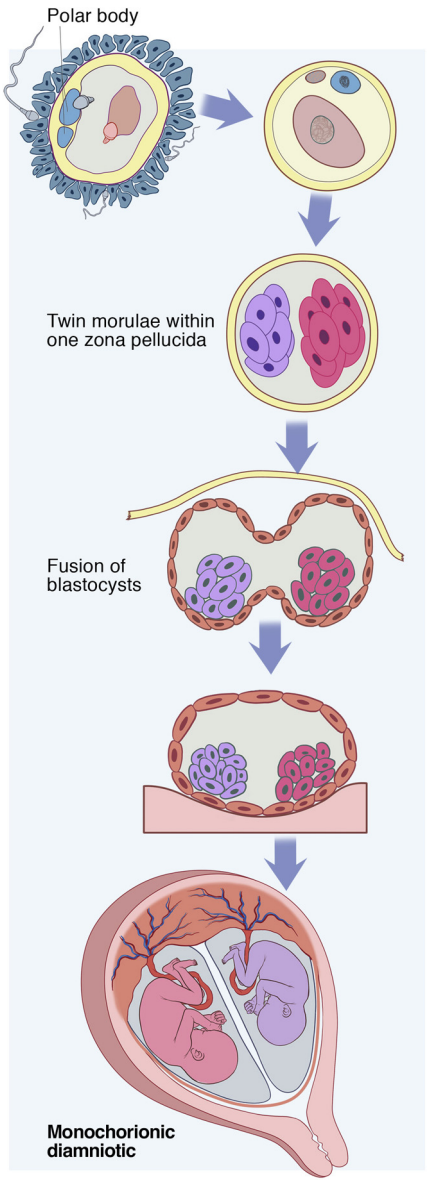
FIGURE 4
Polar body twinning



If an oocyte and one of its polar bodies are each fertilized by a different sperm (illustrated in *red* and *blue*), 2 zygotes within a single zona pellucida may result. If these 2 products of fertilization fuse at the blastocyst stage, monochorionic diamniotic twins (shown) or monochorionic monoamniotic twins (not shown) may result. If the first polar body, from the first meiotic division, is fertilized, the twin will be triploid. If one of the second polar bodies is fertilized, the twin will be diploid.

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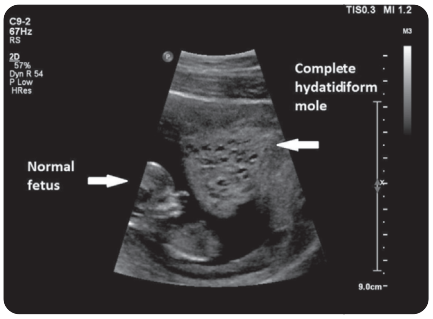
FIGURE 5
Complete hydatidiform mole with coexistent twin



A transabdominal ultrasound scan of a dichorionic diamniotic twin pregnancy at 12 weeks' gestation demonstrating a normal fetus (*left panel*) and a complete hydatidform mole (*right panel*).

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spontaneous loss of an embryo or fetus in the first trimester. In 1976 it was observed that twin pregnancies diagnosed on ultrasound prior to 15 weeks' gestation frequently gave rise to the delivery of singleton infants.[86] After the introduction of routine first-trimester ultrasound, VTS was increasingly observed.[87-90]

VTS is thought to be underreported in spontaneous twin pregnancies.[91] Fetal reduction may occur prior to recognition of pregnancy, with up to 80% occurring prior to 9 weeks' gestation (Figure 6).[92] Most cases are asymptomatic but can be accompanied by vaginal bleeding.[93] Alternatively, VTS is well characterized in ART pregnancies, with reported rates ranging from 10.4% to 18.8%.[35,92,94]

Conflicting evidence exists regarding potential adverse effects of a vanishing twin on the remaining pregnancy. Pinborg et al[94,95] described the outcomes of 642 survivors of VTS. When compared with singletons, survivors were found to be at an increased risk of small for gestational age, low and very low birthweight, and preterm birth. The degree of risk was inversely proportional to the timing of fetal loss.[94,95] Further studies have demonstrated inconsistent results (Appendix 3).[35,96-99]

Concerns have been raised regarding the impact of VTS on neurodevelopmental outcomes. Cases of focal cortical sclerosis, microcephaly, and multicystic encephalomalacia have been reported.[100] Initially it was suggested that VTS might increase the risk of cerebral palsy for survivors.[101] Subsequent studies revealed no statistically significant increase in cerebral palsy or adverse neurological sequelae.[94,102] Longer-term follow-up has suggested a possible increased risk of cerebral impairment at 1 year of age, but again, findings did not reach statistical significance.[100] Furthermore, survivors of VTS are deemed less psychologically vulnerable by their parents to a maximum age of 11 years.[103]

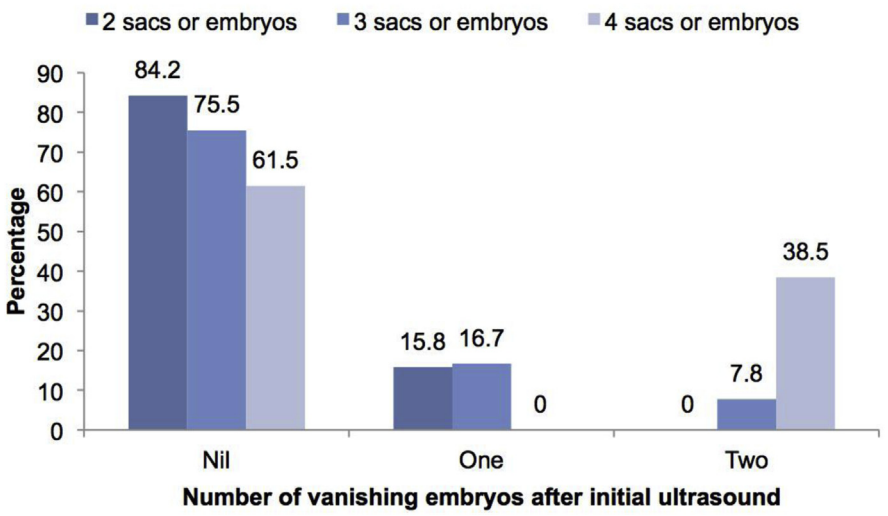
VTS may have an impact on prenatal screening and diagnosis. Studies evaluating serum markers in pregnancies affected by VTS have reported inconsistent results, perhaps because of differences in mean gestational age at sampling and the interval between sampling and fetal loss. Recent evidence suggests the presence of a vanishing twin is associated with a 21% increase in pregnancy-associated plasma protein A ($P = .0026$), a 10% increase in alpha-fetoprotein ($P < .0001$), and a 13% increase in dimeric inhibin A ($P = .0470$).[104]

VTS might also affect the interpretation of noninvasive prenatal testing (NIPT) using cell-free fetal DNA. Cases of misdiagnosis of fetal sex using NIPT may be due to VTS with subsequent persistence of sex chromosome sequences from the vanishing twin.[105,106] In 2015 a large-scale evaluation of results of NIPT in 30,795 consecutive cases identified 130 cases with additional fetal haplotypes, 76 of which could be clinically correlated. VTS was evident in 42.1% of these 76 cases. Fetal haplotypes remained detectable via NIPT for up to 8 weeks after the fetal loss.[107] It has been concluded that early ultrasound monitoring and careful pretest and posttest counseling regarding NIPT are essential.

Fetus papyraceus

Fetus papyraceus refers to a fetus in a multiple pregnancy that dies in utero and then appears as a compressed, mummified mass at delivery (Figure 7).[108] Fetal death is thought to occur between 12 and

FIGURE 6
Fetuses continuing at 11 weeks' gestation



Number of fetuses continuing at 11 weeks' gestation following the initial diagnosis of 2, 3, or 4 gestational sacs or embryos early in the first trimester.

Adapted from Rodríguez-González et al.[92]

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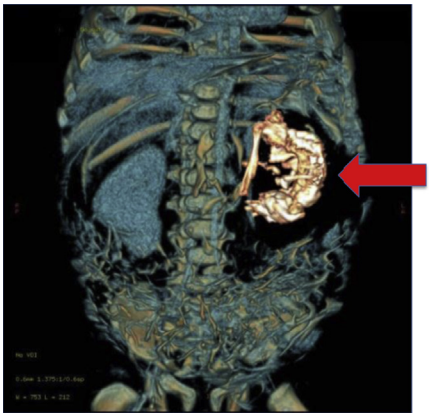
FIGURE 8
Computed tomography scan of the bony outline of a fetus in fetu



A 64 slice computed tomography scan of the bony outline of a fetus in fetu presenting as an abdominal mass in a 2 month old child is shown.

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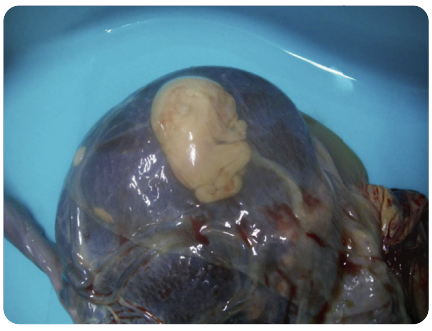
20 weeks' gestation. The incidence is 1 in 200 twin pregnancies and 1 in 12,000 pregnancies overall.[109]

In most cases, fetus papyraceus is of no consequence to the surviving pregnancy. However, associations with aplasia cutis congenital[110-114] intestinal atresia, gastroschisis, and cardiac and pulmonary anomalies have been described.[109,110]

FIGURE 7
Fetus papyraceus



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Fetus papyraceus has been reported in both monochorionic[109,111,112] and dichorionic[109,113] twin pregnancies and higher-order multiple pregnancies.[114] There are no associations with age or parity, but there is a trend toward increased frequency with monochorionicity and velamentous cord insertion.[109]

Many of the abnormalities associated with fetus papyraceus can be attributed to thromboembolic events following the death of a monochorionic twin.[108] However, shared vascular anastomoses alone are not a complete explanation because the condition is seen in dichorionic twins.[113] In dichorionic twins, fetal death might be due to placental ischemia leading to fetus papyraceus and consequences for the cotwin.[108]

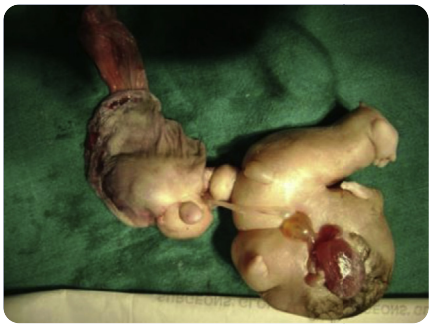
Fetus in fetu/parasitic twins

Fetus in fetu refers to 1 or more partially formed fetuses situated entirely within the body of another normally formed fetus (Figure 8). First described in approximately 1800,[115,116] this event has been estimated to occur once in every 500,000 births.[117] Fetus in fetu remains rare despite the advent of ART, with fewer than 200 cases documented.

Historically, fetus in fetu was considered to represent a well-formed mature teratoma. However, in contrast to the disorganized tissues derived from uncontrolled pluripotent cell replication in teratomata, fetus in fetu is characterized by the presence of vertebrae with appropriately organized limbs and organs (Figure 9).[118] Serology and molecular genetic testing have indicated that fetus in fetu represents a monozygotic, monochorionic diamniotic twin gestation.[119,120] Persistent anastomoses of the vitelline circulation lead to the absorption of one twin inside the other during the ventral folding of the trilaminar embryonic disc.[121]

Nevertheless, an association between teratomata and fetus in fetu has been observed.[122] Both are commonly located in the retroperitoneum[123] and are histopathologically similar.[124] Cases of teratoma and fetus in fetu occurring in the same individual have been reported.[125,126] Fetus in fetu has been described as part of a parasitic continuum including conjoined twins, acardiac twins, and teratomata.[127]

FIGURE 9
Pathological specimen of fetus in fetu



Pathological specimen of fetus in fetu demonstrates 2 miniature fetuses joined by a cord-like structure.

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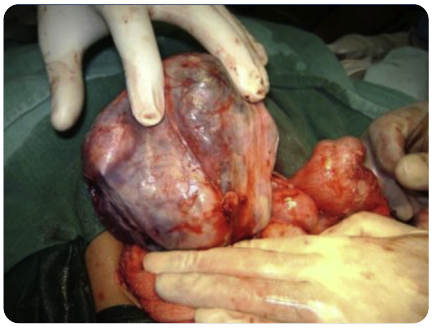
Fetuses in fetu have been identified in the mediastinum, scrotum, mouth, and skull.[123,128,129] Usually there is 1 fetal mass, but cases of up to 11 fetuses in fetu have been reported.[130] The diagnosis is commonly made following the incidental identification of an abdominal mass in a neonate or infant.[131] Advances in fetal sonography have led to increased prenatal diagnosis.[120,132,133] Unlike teratomata, fetuses in fetu do not demonstrate malignant potential but may cause significant mass effect, necessitating surgical removal (Figure 10).[126,134]

FIGURE 10
The fetus in fetu mass enveloped in a sac at the time of surgery



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McNamara. Typical and atypical twinning. Am J Obstet Gynecol 2016.

Superfetation

Superfetation refers to fertilization and implantation of a second conception during pregnancy (Figure 11). Early cases of suspected superfetation were reported in the context of growth discordance. In 1989 Bhat et al[135] reported a case of dichorionic diamniotic twins delivered at 36 weeks' gestation with discordant birthweights and the subsequent death of the second twin. Given the significantly different Dubowitz scores,[136] superfetation was presumed.

In 2003 Singhal et al[137] reported a case of twins born to a mother with uterus pseudodidelphys. At the time of presentation, discordant estimated gestational age was determined. In the absence of Doppler parameters suggesting intrauterine growth restriction, superfetation was presumed. The demise of the second twin was confirmed at 32 weeks' gestation, whereas the first twin was live born at 35 weeks' gestation.[137]

The plausibility of the phenomenon of superfetation has been questioned.[138] Whereas ovulation has been reported to occur in the first trimester of pregnancy,[139] the up-regulation of the hypothalamic-pituitary-ovarian axis by luteal and then placental progesterone typically suppresses ovulation. Progesterone-induced changes in cervical mucus may limit successful fertilization. The presence of a gestational sac in the uterus may limit a successful implantation. Therefore, it has been argued that spontaneous superfetation is unlikely.[140]

Three alternative explanations for described cases of superfetation have been proposed. First, pregnancies complicated by growth discordance may give rise to the appearance of twins of differing gestational ages. Growth discordance because of placental insufficiency, infection, congenital anomalies, or twin-to-twin transfusion syndrome is not uncommon in twins.[141] Second, interval delivery might contribute to the subsequent appearance of twins with different gestational ages. Third, in cases in which there is a misdiagnosis of a singleton pregnancy, multiple pregnancy on further imaging might be attributed to superfetation rather than to sonographic diagnostic error.[140]

In contrast, with the advent of ART, it has been recognized that natural barriers to superfetation can be overcome. Cases of superfetation resulting from ART with or without additional spontaneous conception have been reported (Appendix 4).[142,143]

In 2005 Harrison et al[141] attempted to confirm superfetation in a triplet pregnancy by estimating the gestational age postnatally using neurosonography and ophthalmic evaluation. The authors raised the possibility that superfetation can be determined conclusively.[141] Further research is necessary to definitively prove or disprove this phenomenon.

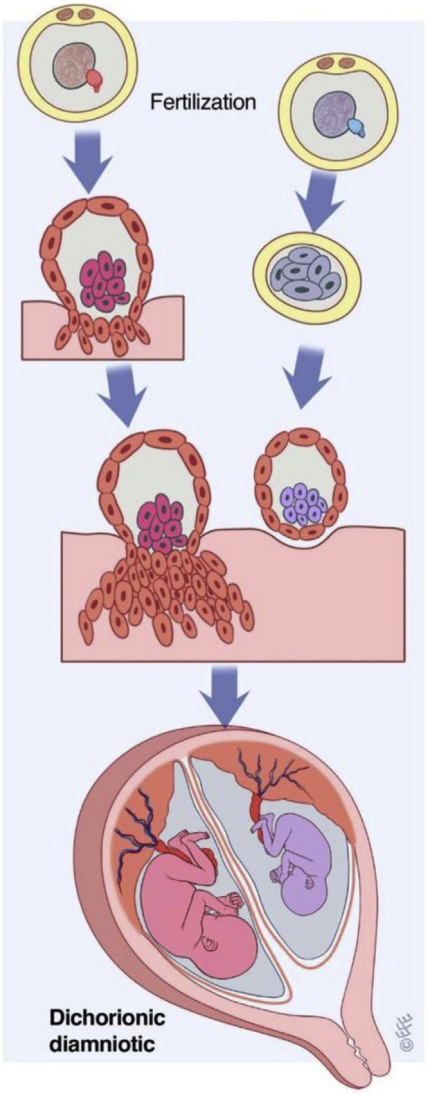
Superfecundation

Superfecundation refers to the fertilization of 2 oocytes via separate instances of coital or artificial insemination during the polyovulatory period (Figure 11). Heteropaternal superfecundation occurs after coitus with multiple partners. Monopaternal superfecundation occurs after coitus with one partner on multiple occasions. The latter is probably more common and not frequently detected.[142]

In 1978 Terasaki et al[144] described a case of suspected heteropaternal superfecundation on the basis of HLA typing. In 1982 heteropaternal superfecundation was again suspected in a pair of twins born with different skin colors.[145] Additional cases have been described in the context of paternity disputes (Appendix 5).[146]

More recently, superfecundation has been associated with ART. In 2001 Amsalem et al[142] reported spontaneous monopaternal superfecundation in a 25 year old woman undergoing IVF for secondary infertility. After an

FIGURE 11
Superfetation and superfecundation



Superfetation may result when 2 embryos are produced at different time points, resulting in asynchronous development in utero. At birth, this may result in apparent growth discordance of dichorionic diamniotic twins. Superfecundation may result when the fertilization of 2 oocytes via separate instances of coital or artificial insemination occur during a single polyovulatory period. Because superfecundation is in many ways similar to superfetation, both are illustrated by the same figure. Fertilization in superfecundation may be monopaternal or heteropaternal and both are illustrated by *red* and *blue sperm*.

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uncomplicated transfer of 2 embryos, 5 embryos were detected on ultrasound. Following multifetal pregnancy reduction, male twins were born at term. Subsequent cytogenetic testing confirmed monopaternity and suggested that cleavage/duplication was unlikely. In 2005 Peigné et al[143] reported a case wherein monopaternal superfecundation was observed following multiple intrauterine inseminations and IVF cycles. Again, a selective fetal reduction resulted in the delivery of live-born twins.[143]

Evaluation of large parentage databases has led to a reported overall frequency of heteropaternal superfecundation of 2.4%.[147] It has been proposed that 1 in 400 twin pairs born to married white women in the United States are the result of heteropaternal superfecundation. Monopaternal superfecundation is thought to be more common, with an estimated prevalence of 1:12 dizygotic twins born to mothers in the United Kingdom.[148] The rate of superfecundation depends on community rates of coital frequency, polyovulation, and subsequent DNA detection. The reported incidence may be increasing because of increased paternity testing.[146]

Given that superfecundation may occur with ART, women should be advised to consider avoiding intercourse after embryo transfer to reduce the risk of subsequent higher-order multiple pregnancy and/or ectopic pregnancy.[142]

Conclusion

Twinning is a complex and multifactorial phenomenon and elements of the twinning process remain incompletely understood. A conventional model of monozygotic twinning is based on fission events in the developing embryo. This model lacks definitive evidence and is challenged by cases of atypical twinning.

An alternative model proposes that embryonic fusion events underlie monozygotic twinning. However, supporting evidence is similarly limited. Elucidating the precise mechanisms by which twinning occurs will have significant implications for managing complications unique to multiple gestations; utilizing cell-free DNA for aneuploidy screening in multiple pregnancies; interpreting twin data to determine the relative contributions of genetic, epigenetic, and environmental factors to various phenotypic outcomes; and reducing the incidence of spontaneous and assisted multiple pregnancies. An examination of the anomalies of the placenta and umbilical cord in twin gestations has been recently published and provides an excellent review.[149] Further research, including series of extended cytogenetic analyses, is needed to refine our understanding of early embryonic development in twin pregnancies. ■

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APPENDIX 1

Cases of monochorionic dizygotic twins

Studies		Maternal	ART status	Antenatal	Gestation delivery	Placental histology	Twin 1	Twin 2	Blood	Tissue	Genitalia	Follow-up
Nylander et al,[14] 1970, western Nigeria	1	35 y old Multi	Spontaneous	—	—	MCDA anastomoses present	Male 2490 g	Female 1760 g	—	—	Examination (N)	—
Viëtor et al,[12] 2000, The Netherlands	2	29 y old G1P0	—	Spontaneous reduction triplet	34.5	—	Male	Female	Yes	No (skin fibroblast)	Examination (N) Ultrasound (N)	—
Quintero et al,[16] 2003, United States	3	28 y old G1P0	—	TTTS	—	MCDA Anastomoses ×3 lasered	FDIU Male	FDIU Female	Yes	—	Examination (N)	NA
Souter et al,[15] 2003, United States	4	48 y old G1P0	IVF with donor eggs; 3 ET	—	37	MCDA Anastomoses present	Male 2114 g	Female 2183 g	Yes	No (skin fibroblast)	Examination (N) Ultrasound (N)	5 mo
Williams et al,[17] 2004, United States	5	38 y old 0111	Ovarian stimulation, IVF, ICSI	Severe preeclampsia	28 Emergency CD	MCDA Twin 2 velamentous insertion of the cord	Male 2114 g	Female 654 g	Yes	No (ovarian biopsy)	Twin 2 clitoromegaly; ultrasound: ovaries not seen	7 wks
Ginsberg et al,[18] 2005, United States	6	35 y old	Ovarian stimulation (clomiphene)	Preterm labor	22	MCDA	Male	Female	Yes	—	Limited autopsy: normal internal and external genitalia	Expired d 14
Miura and Niikawa,[19] 2005, Japan	7	—	IVF, ET	—	—	MC	Male	Female	Yes	No	Examination (N)	—
	8	—	FSH ovarian stimulation, IUI	—	—	MC	Male	Female	—	—	Examination (N)	—
	9	—	TESE, ICSI	Spontaneous fetal reduction	—	MC	Male	Female	Yes	No	—	—
	10	—	IVF, ET	—	—	—	Male	Female	—	—	—	—
	11	—	IVF, ET	—	—	—	Male	Female	Yes	—	—	—
Yoon et al,[20] 2005, United States	12	34 y old G1P0	IVF, ICSI, 3 ET	—	33.5	MCDA	Male 2660 g XXY	Male 2221 g	Yes	—	Examination (N)	—
Aoki et al,[21] 2006, Japan	13	27 y old G1P0	Ovarian stimulation (clomiphene)	—	34 Elective CD	MCDA Anastomoses present	Male 2002 g	Male 2132 g	Yes	No (hair root cells)	—	3 mo
Walker et al,[22] 2007, Australia	14	30 y old G2P1	IVF, 2 ET	—	36 Elective CD	MCDA Not sent for histology	Male 2395 g	Male 1960 g	Yes	No (buccal cells)	Examination (N) Ultrasound (N)	15 mo

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(continued)

APPENDIX 1

Cases of monochorionic dizygotic twins *(continued)*

Studies		Maternal	ART status	Antenatal	Gestation delivery	Placental histology	Twin 1	Twin 2	Blood	Tissue	Genitalia	Follow-up
Ekelund et al,[23] 2008, Denmark	15	38 y old Multi	ICSI, assisted hatching, 3 ET	TTTS	32 Elective CD	MCDA	Male 2265 g	Female 1550 g	Yes	No (buccal cells)	Examination (N) Ultrasound (N; 1 identifiable ovary)	6 mo
Hackmon et al,[24] 2009, United States	16	32 y old G3P2	Spontaneous	—	37 NVD	MCDA	—	—	Yes	No (buccal cells)	Examination (N) Ultrasound (N)	18 mo
Assaf et al,[25] 2010, United States	17	28 y old G1P0	ICSI, 2 ET	TTTS	37+4 Elective CD	MCDA Ablation of vascular communications	Female 3487 g	Male 2892 g	Yes	No (buccal cells)	Examination (N)	9 mo
Hawcutt et al,[32] 2011, United Kingdom	18		IVF, 2ET	Preterm labor	25+1 Emergency CD	—	Male 710 g	Female 740 g	Yes	No (Buccal cells)	Examination (N) Ultrasound (N)	10 mo
Umstad et al,[7] 2012, Australia	19	36 y old	Spontaneous	TTTS	36 Elective CD	MCDA	Male 2490 g	Male 2310 g	Yes	No (Buccal cells)	Examination (N)	14 mo
Choi et al,[26] 2013, South Korea	20	31 y old 0020	IVF, assisted hatching, 3 ET	—	33.8 CD	MCDA	Male 2160 g	FDIU Female 2335 g	Yes	No (Skin fibroblast)	Twin 1: undescended left testis	Twin 1: 39 mo
Fumoto et al,[27] 2014, Japan	21	32 y old	IVF, 2 ET	—	35 Elective CD	—	Female 1880 g	Male 2548 g	Yes	Yes (Buccal cells)	Examination (N) Ultrasound (N)	7 mo
Lee et al,[28] 2014, Korea	22	—	IVF-ET	—	—	—	Female	Male	Yes	No	Hydrocele in male twin	23 mo
Lee et al,[29] 2014, Korea	23	34 y old G1P0	IVF, 2ET	—	38+0 NVD	MCDA	Female 2490 g	Male 2340 g	Yes	No (skin fibroblast)	Examination (N) Ultrasound (N)	23 mo

ART, assisted reproductive technologies; *CD*, cesarean delivery; *ET*, embryo transfer; *FDIU*, fetal death in utero; *FSH*, follicle stimulating hormone; *ICSI*, intracytoplasmic sperm injection; *IUI*, intrauterine insemination; *IVF*, in vitro fertilization; *MC*, monochorionic; *MCDA*, monochorionic diamniotic; *Multi*, multiparous; *N*, normal; *NA*, not applicable; *NVD*, normal vaginal delivery; *TTTS*, twin-to-twin transfusion syndrome; *US*, ultrasound.

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APPENDIX 2

Cases of mirror-image twins

Studies		Maternal	ART status	Antenatal	Delivery	Twin 1	Twin 2	Zygosity	Twin 1	Twin 2	Evaluation
Rife,[52] 1933, United States	1										Dominant handedness, direction of hair whorl, eye dominance
Letts and Shokeir,[153] 1975, Canada	2	—	—	No family history, uncomplicated	36 NVD	Female 1979 g	Female 1097 g	Nixon's discriminant, probability 0.9695 MZ; chromosome studies	—	—	Coxa vara
Springer and Searleman,[51] 1978, United States	3	—	—	—	—	Female	Female	HLA antigens were similar; considered MZ	52 y	52 y	Breast ductal carcinoma in situ
Novak, 1981,[56] United States	4	39 y old G10	—	Spontaneous preterm labor	35 CD	Female 2210 g	Female 1940 g	Placenta examined = MCDA	PN	PN	Laryngotracheosophageal cleft, displacement of heart, unilateral pulmonary hypoplasia
Nigro et al,[64] 1991, United States	5	—	Clomid	Spontaneous preterm labor	33+4 CD	Male 1616 g	Male 1758 g	Identical	7 y, 9 y	7 y, 9 y	Colpocephaly (enlarged occipital horns of the lateral ventricles), dominant handedness
Williams and Kapila,[154] 1993, United Kingdom	6	—	—	—	—	Male	Male	—	5 y	5 y	Posterior urethral valves with unilateral hydronephrosis and bladder diverticulum
Morison et al,[62] 1994, United States	7	—	—	—	—	Male	Male	—	60 y	62 y	Lung adenocarcinoma
Sperber et al,[53] 1994, Canada	8	G2P1	—	Nonconsanguineous, no family history	—	Female	Female	Peripheral blood DNA analysis = MZ	4 y	4 y	Dental fusion (mandibular lateral incisor and canine fusion)
Karaca et al,[57] 1995, Turkey	9	30 y old G5P4	—	Consanguineous, uncomplicated	NVD	—	—	Monozygotic	6 mo	6 mo	Cleft lip and palate
Satoh et al,[58] 1995, Japan	10	—	—	Nonconsanguineous, uncomplicated	NVD, term	Female	Female	ABO blood typing HLA typing, DNA typing = MZ	PN, 5 y	PN, 5 y	First and second branchial arch syndrome, spinal scoliosis, unilateral cleft lip/palate
Sommer et al,[70] 1999, The Netherlands	11	—	—	Uncomplicated	No trauma	Female 3300 g	Female 3500 g	Genotyping = MZ	—	—	Cerebral lateralization for language mental rotation
Okamoto et al,[155] 2001, Japan	12	—	—	—	38	2360 g Male	2500 g Male	Monozygotic	20 months	20 months	Myopic anisometropia

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(continued)

APPENDIX 2

Cases of mirror-image twins *(continued)*

Studies		Maternal	ART status	Antenatal	Delivery	Twin 1	Twin 2	Zygosity	Twin 1	Twin 2	Evaluation
Morini et al, 2002,[156] Italy	13	33 y old	—	Prenatal diagnosis hydronephrosis	NVD	Male	Male	Placenta examined = MCDA, ABO typing	PN	PN	Posterior urethral valves, bilateral vesicoureteric reflux, hydroureter, nonfunctioning kidney, cyst
Dirani et al,[54] 2006, Australia	14	—	—	No family history	3 wks preterm	1500 g	2000 g	Standard genotyping = MZ	55 y	55 y	Congenital esotropia
Hwang et al,[68] 2006, Taiwan	15	26 y old	—	No family history, uncomplicated	38 CD	2580 g Male	2934 g Male	DNA testing = MZ	PN	PN	Asplenia syndrome
Helland and Hester,[65] 2007, Norway	16	24 y old	—	Uncomplicated	40 NVD	2900 g Female	3370 g Female	Placenta examined = MC	12 y	12 y	Arachnoid cysts in cerebellopontine angle
Goto et al,[61] 2008, Japan	17	—	—	—	—	Male	Male	Monozygotic	10 y	11 y	Solitary bone cysts of the humerus, handedness, hair whorl direction
Thacker et al,[67] 2009, United States	18	37 y old G2P3	—	US cardiac defect	34 EI CD	Female 1720 g	Female 2000 g	Monozygotic	PN	PN	Heterotaxy syndrome
Zhou et al,[66] 2011, China	19	24 y old	—	Uncomplicated	35 CD	1700 g Male	2300 g Male	Placenta examined = MC	14 mo	14 mo	Arachnoid cysts in temporal lobe
Riess et al,[59] 2012, Germany	20	G2P1	—	US cardiac defect; Karyotype NAD	32+6 CD	1660 g Male	1945 g Male	DNA testing (peripheral blood leukocytes) = MZ	PN	PN	Kabuki syndrome, mirror cleft lip and palate
Wang et al,[60] 2012 United States	21	—	—	—	—	Male	Male	Genetic testing = MZ with > 99% CI	11 mo	17 mo	Trigger thumb
Hu et al,[55] 2014 China	22	—	—	—	—	Female	Female	DNA testing = MZ	10 y	10 y	External ear deformity: microtia

ART, assisted reproductive technologies; *CD*, cesarean delivery; *DNA*, deoxyribonucleic acid; *HLA*, human leukocyte antigen; *MCDA*, monochorionic diamniotic; *MZ*, monozygotic; *NVD*, normal vaginal delivery; *PN*, postnatal; *US*, ultrasound.

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APPENDIX 3

Vanishing twins: outcomes of survivors of a vanishing twin vs primary singletons

Studies		Years	ART status	Case, n	Control, n	Mean GA (delivery)	LBW < 2500 g	VLBW < 1500 g	SGA	CP	Adverse neurology
Dickey et al,[96] 2002, United States	1	1976–2000	Infertility treatment	140	4683		15.7 vs 4.5[a] $P = .32$				
Chasen et al,[97] 2006, United States	2	2003–2005	IVF	55	168	39.4 vs 39.1 $P = .44$	14.5 vs 9.6[a] $P = .32$				
La Sala et al,[35] 2006, Italy	3	1992–2004	IVF with or without ICSI	84	602	38.2 vs 38.3 $P > .05$	OR 1.2 (0.6–2.8)	OR 2.7 (0.4–18.9)			
Shebl et al,[98] 2008, Austria	4	1999–2005	IVF with or without ICSI	46	92	39.0 vs. 39.3 $P = .69$	26.1% vs 12.0% $P = .036$[a]	4.3% vs 1.1% $P = .22$	32.6% vs 16.3% $P = .029$[a]		
Almog et al,[99] 2010, Israel	5	1999–2007	IVF with or without ICSI	57	171	35.1 vs. 38.2 $P = .001$[b]	33.3% vs 11.7% $P = .0001$[b]	3.5% vs 0.6% NS	14.0% vs 17.5% NS		
Pinborg et al, 2005,[94] and 2007,[95] Denmark	6	1995–2001	IVF with or without ICSI	642	5237		OR 1.7 (1.2–2.2)	OR 2.1 (1.3–3.6)	OR 1.50 (1.03–2.20)[b]	OR 1.9 (0.7–5.2)	4.7% vs 4.1 % $P = .5$
Anand et al,[100] 2007, United Kingdom	7	1999–2001	Mixed	22	103						RR 6.1 (1.5–8.3) $P = .03$[b]

ART, assisted reproductive technologies; *CP*, cerebral palsy; *GA*, gestational age; *ICSI*, intracytoplasmic sperm injection; *IVF*, in vitro fertilization; *LBW*, low birthweight; *NS*, not statistically significant; *OR*, odds ratio; *RR*, relative risk; *SGA*, small for gestational age; *VLBW*, very low birthweight.

[a] Birthweight less than 10th percentile; [b] Statistically significant.

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APPENDIX 4

Cases of superfetation

		Maternal	ART status	Antenatal	Progress	Delivery	Twin 1	Twin 2	Evaluation
Bhat et al,[135] 1989, India	1	Gravida 5	—	Admitted 36/40	—	Spontaneous NVD at 36/40	Female 2100 g Dubowitz 36/40	Male 1500 g Dubowitz 30/40	Presumed superfetation on the basis of different estimated gestational age (Dubowitz score) without evidence of IUGR
Kobayashi et al[157] 1996, Japan	2	33 y old G1P0	Spontaneous	Presented with abdominal pain; E/O left anterior broad ligament Serial hCG monitoring	Second pregnancy identified with increase in hCG; estimated 4/40	—	—	—	Presumed superfetation
Tuppen et al,[158] 1999, United Kingdom	3	38 y old G3P2	Spontaneous	Vaginal bleeding 7/40	US 7/40: 4 wks growth discordance	Spontaneous NVD	Female 3288 g Dubowitz 39/40	Male 2324 g Dubowitz 35/40	Presumed superfetation on the basis of different estimated gestational age (Dubowitz score)
Singhal et al,[137] 2002, India	4	20 y old G1P0	—	US 28/40: 5 wks growth discordance; no IUGR; bicornuate uterus	US 32/40: FDIU twin 2	Emergency CD Confirmed pseudodidelphys uterus	Female 2200 g	Female 660 g (macerated stillbirth)	Presumed superfetation on the basis of different estimated gestational age (Dubowitz score) without evidence of IUGR

ART, assisted reproductive technologies; *CD*, cesarean delivery; *E/O*, excision of; *FDIU*, fetal death in utero; *hCG*, human chorionic gonadotropin; *IUGR*, intrauterine growth restriction; *NVD*, normal vaginal delivery; *US*, ultrasound.

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APPENDIX 5

Cases of superfecundation

		Maternal	ART status	Antenatal	Progress	Delivery	Twin 1	Twin 2	Evaluation	Context
Archer,[159] 1810	1								One white twin One mulatto twin	Racially different in appearance
Terasaki et al,[144] 1978	2								HLA typing	Paternity suit
Harris et al,[145] 1982	3								One white twin One black twin	Racially different in appearance
Wenk et al,[147] 1991, United States	4								HLA typing	Paternity suit
Verma et al,[160] 1992	5								Chromosome heteromorphic markers	Paternity suit
Lu et al,[161] 1994	6								HLA typing Conventional marker typing	Paternity suit
	7								HLA typing Conventional marker typing	Paternity suit
Girela et al,[146] 1997, Spain	8						Female 2100 g	Female 1750 g	PCR typing of loci HLA Restricted fragment length polymorphism	Paternity suit
Amsalem et al, 2001,[142] Israel	9	25 y old	IVF with ICSI; 2 ET (7 cell/8 cell stage)	US Day 27: 5 embryos	Selective fetal reduction × 3	37 Elective CD	Male 2390 g	Male 2170 g	DNA analysis of 3 embryos (amniotic fluid), live-born twins (cord blood), parents; genetically distinct	Antenatal care; IVF
Peigné et al, 2011,[143] France	10	31 y old G0	3 × IUI; 2 × IVF; 2 ET second cycle	US 4/40 5 embryos	Selective fetal reduction × 3	31 Elective CD	Female 780 g	Male 1240 g	DNA analysis of 3 embryos, live-born twins, parents; genetically distinct	Antenatal care; IVF

ART, assisted reproductive technologies; *CD*, cesarean delivery; *DNA*, deoxyribonucleic acid; *ET*, embryo transfer; *HLA*, human leukocyte antigen; *ICSI*, intracytoplasmic sperm injection; *IUI*, intrauterine injection; *IVF*, in vitro fertilization; *NVD*, normal vaginal delivery; *US*, ultrasound.

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