



## Embryologic Development and Monozygotic Twinning

**J.G. Hall, E. Lopez-Rangel**

*Department of Pediatrics, University of British Columbia and Children's Hospital, Vancouver, Canada*

### INTRODUCTION

In 1875, Sir Francis Galton was the first to propose that by comparing the concordance of a specific trait or disorder in monozygotic (MZ) twins (assumed to be genetically identical) and dizygotic (DZ) twins (which were assumed to be as similar or as different as any other pair of sibs), it would be possible to distinguish between environmental and heritable effects. DZ twins are derived from the fertilization of two eggs by two sperm. MZ twins are the result of the fertilization of one egg by one sperm that then divides to form two embryos.

Genetic research has made amazing progress since 1875. Advances in molecular DNA techniques and developmental genetics have made it possible to differentiate unequivocally between MZ and DZ twins [1, 14]. It is in this new light that this manuscript will review the recent knowledge about twins and the twinning process.

### DZ twins

The incidence of DZ twins differs between population [11, 18]. A DZ twin birth in North America is estimated to occur 9–11 times in every 1000 live births or 1 in 100 births; i.e. 1 in every 50 people is a DZ twin.

The DZ twinning rate is closely related to maternal age, parity, height, weight and gonadotropin levels. An increased DZ twinning rate is seen with increasing maternal age and peaks around 35–39 years; higher parity is also associated with a higher DZ twinning rate [19]. Tall heavy women are more likely to give birth to DZ twins than short thin women. A higher incidence of DZ twins has also been reported with the use of new reproductive techniques.

## MZ twins

The incidence of MZ twins has been thought to be constant around the world at 3–4 in every 1,000 live births (1 in every 330 births). The rate of MZ twinning appears to be unaffected by maternal age, parity, height or weight [2]. A recent study [5] reported that women who were given periconceptual folic acid supplementation had a higher incidence of recognizable miscarriages and multiple births (both MZ and DZ twins).

Suggestions for the etiology of MZ twins include lack of oxygen prior to implantation causing developmental arrest and splitting [23], rupture of the zona pellucida [7] and “developmental” separation during embryogenesis [3]. Familial MZ twinning also occurs [9, 20] and is suggested to be due to a single dominantly inherited gene [22] possibly producing a zone pellucida prone to rupture.

## Congenital anomalies in twins

Twins are known to have an excess of congenital anomalies [21, 15, 6]. Defects may be due to shared circulation (e.g., acardia), due to fetal crowding or constraint (e.g., contractures) or, in the case of MZ twins, congenital anomalies are thought to be due to defects which are part of the twinning process (e.g., conjoined twins). Twin conceptions have a high frequency of “vanishing” twins. Ultrasound studies have shown that as many as 70% of twin pregnancies convert to singletons or are lost in early pregnancy [16].

## Placentation in twins

Placentation in twins differs between DZ and MZ twins. By definition, DZ twins are expected to have two placentas, two chorions and two amnions, that is, to be diamniotic dichorionic. However, placentas in DZ conceptions may fuse and look like one. Fused-placenta DZ twins have been shown to have vascular connections in as many as 8% of cases [24]. A careful pathological and histological examination of the placenta and other membranes will help in establishing the zygosity of a twin pregnancy.

MZ twins may have any number of different types of placentation according to the number of chorionic membranes and amniotic sacs. MZ twins may have separate or contiguous placentas and may be monochorionic monoamniotic (extremely rare at birth), monochorionic diamniotic or dichorionic diamniotic (with separate or contiguous placentas). In the case of contiguous placentas, MZ twins usually share vascular connections and circulation [17]. Twins thought to be MZ because of a monochorionic placenta may need to be more carefully studied if discordance is present.

## Vanishing twin

Ultrasound studies have shown that at least 70% of twin pregnancies diagnosed by ultrasound before the 10th week miscarry or convert to a singleton in early pregnancy. The disappearance of a cotwin has been termed the “vanishing twin” [16]. Estimates of van-

ishing twin rates vary depending on the time (i.e., how early) and the number of ultrasound scans [12, 13]. Some of the mechanisms that have been suggested for the vanishing twin include vascular compromise, life-threatening malformations, or spontaneous mutations incompatible with life [16].

## Sex ratio

The sex ratio in MZ twins is lower than in DZ twins or singletons, and even lower among conjoined twins. Female mammalian embryos have been found to be somewhat behind male twins in early embryonic development. Many studies have shown different patterns of X inactivation in female MZ twins discordant for X-linked traits and a number of normal female MZ twins are also discordant for X inactivation [4, 8]. The relative delay in the development of female twin conceptions and the presence of X inactivation may play a key role in the excess of female MZ twins.

## Application of new genetic techniques to the study of twins

Advances in the field of molecular genetics have allowed for the unequivocal differentiation between MZ and DZ twins. DNA techniques have also made it possible to determine genetic abnormalities such as submicroscopic deletions and duplications, and to distinguish non traditional genetic mechanisms such as mosaicism, mitochondrial inheritance and uniparental disomy.

## Mz twinning hypothesis

There are many reports of MZ twins discordant for genetic disorders. We suggest that the development of discordance due to genetic abnormalities, which may be the result of any of the newly recognized mechanisms of genetic disease as well as more traditional mutation, chromosome anomalies and mosaicism, may be the underlying cause for some cases of MZ twinning.

This hypothesis proposes that at some point after fertilization (8 to approximately 360 cells of inner cell mass), a genetic change arises and is passed along to the daughter cells. This change renders a small group of cells distinct enough to be recognized as "alien". This alien change then leads to separation (loss of cell-to-cell connection) of a group of cells leading to two distinct cell masses. The alien recognition must occur at a time in human tissue development when tissue differentiation has not yet occurred and thus allows an entirely separate and additional embryo to form. One embryo will be expected to have mostly normal original cells and the other will have mostly changed cells. This means that the twin embryos may be mosaic for the differences. This change may not necessarily be a DNA difference or mutation in the classical sense and it may not necessarily produce a recognizable disease or disorder, but it is expected to lead to some type of discordance between cells.

It is important to remember that 70% of monozygotic twins who come to live birth

share circulation in utero so that a discordance is not likely to be demonstrated in studies done on blood cells. Skin is a much better tissue to look for discordance.

Keeping this hypothesis in mind allows for the possibility that some phenotypically different twins may be related to discordant monozygotic twinning. This hypothesis may also provide information regarding:

1. the "mutation" rate (or change in expression by various mechanisms), involved in the mechanism of alien recognition and MZ twinning in early embryonic development;
2. whether the excess of congenital anomalies in MZ twins is related to mutations or changes in expression of genetic material;
3. the number of cells required for self recognition in early development;
4. the number of cells and stage in development at which mosaicism may develop and be tolerated without a separate cell mass developing;
5. the number of harmful "mutations" or changes in control which arise spontaneously;
6. the relative frequency and timing of various types of MZ twinning events and
7. the number of cells present when various types of MZ twins arise.

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**Correspondence:** Judith G. Hall, M.D., Department of Pediatrics, University of British Columbia and Children's Hospital, 4480 Oak Street Room 2D15, Vancouver, V6H 4V4 Canada.