The embryology of Siamese twins – what we know about their mechanisms of development today?

Review article

LEOPOLD ŚLIWA, BARBARA MACURA

Uniwersytet Jagielloński Collegium Medicum
Wydział Nauk o Zdrowiu
Zakład Biologii Rozwoju Człowieka
Kierownik: dr hab. Leopold Śliwa

Address for correspondence:
Leopold Śliwa
Zakład Biologii Rozwoju Człowieka
ul. Kopernika 7, 31- 034 Kraków
tel./fax 12 422 99 49, leosliwa@cm-uj.krakow.pl

Statistic

Word count	3216
Tables	0
Figures	0
References	14

Received: 26.02.2015
Accepted: 29.04.2015
Published: 30.09.2015

Summary
Etiology of the Siamese (conjoined) twins formation is currently unclear. An article discusses the universally well-known, although contradictory, theories of Siamese twins development. This phenomenon is demonstrated in the context of normal process of embryo development during critical period for this malformation (the third week of development). Probable factors responsible for the conjoined twins formation are discussed. What is more, the other types of abnormal connections between fetuses are described, e.g. parasitic twins, fetuses in fetu or acardiacs. In addition, main medical and ethical problems, as well as the gaps in embryological knowledge connected with conjoined twins are briefly described.
Key words: Siamese (conjoined) twins; embryology; etiology

INTRODUCTION

The anatomical structure and physiological functioning of the female reproductive system predisposes to conception of one embryo which, following implantation, develops into a fetus and is born naturally as a normal neonate at term. At times, however, various disorders may disrupt this series of processes. A particular example of such a disorder is multiple pregnancies, commonly and wrongly called twin pregnancies since there can be more than two fetuses. Ovulation disturbances can lead to the release of several oocytes which, when connected with sperm cells, form independent zygotes, and subsequently, polyzygotic fetuses. Embryonic regulation is a specific property of an embryo's early development. It can lead to the development of genetically identical (monozygotic) twins. This phenomenon is not treated as a pathology, particularly because the further embryonic development, labor and postnatal development are frequently normal. Sometimes, however, when an embryo is damaged at an early stage of development and further attempts of its repair by means of biological regulation are not entirely normal, considerable developmental anomalies can occur, including the formation of fetuses connected with one another in various ways, called conjoined or Siamese twins. Such a phenomenon is always a considerable pathology and causes various problems, particularly of medical nature, concerning the chances for survival of such children and their comfort of life. In order to facilitate their subsequent functioning, they can be separated surgically. Such a procedure depends on the degree to which organs are shared, which translates into the prognosis for separated individuals. This, in turn, evokes ethical, moral and social problems associated with these chil-

dren's future fate. A question arises: can the life of one child be sacrificed to save another or increase their comfort of life? Because of various religious and moral convictions of parents in different regions of the world as well as their cultures and outlook, the answers to this question vary. As for this developmental pathology, the causes and factors that contribute to its occurrence remain unclear [1,2].

The common name, i.e. "Siamese twins," comes from the most famous conjoined boys born in 1811 in Siam (today's Thailand). Following their emigration to the United States, Eng and Chang Bunker became famous thanks to their performances in an international circus group. They were probably conjoined with the lower part of their chests and had the common liver. The connection with an extensible band of tissue of their xiphoid processes enabled them not only to stand next to one another, but also to move. Currently, they would be eligible for a separation surgery. The brothers had a relatively normal and prosperous life. They were married to two sisters and fathered a total of 21 children. The death of one brother quickly led to the death of the other. They lived together for 63 years [3,4].

The global occurrence of conjoined twins is not known for certain, but merely estimated at 1.47 per 100,000 births. It is one of the rarest congenital developmental defects in children. Moreover, it is suggested that the occurrence can vary in various regions of the world or even in different ethnic groups. The differences in data concerning the occurrence of conjoined twins in the world can also be associated with the differences in the sizes of investigated populations, the inclusion or exclusion of stillborn conjoined twins and the readiness to terminate pregnancy if such a pathology is identified. The percentage of stillborn conjoined twins is approximately 40–60%. Also, the value is exceptionally high in spontaneously miscarried pregnancies. Moreover, Siamese twins present more congenital defects that are not directly associated with the shared organs [3].

ETIOPATHOGENESIS OF CONJOINED TWINS

The etiopathogenesis of this developmental defect has not been unequivocally explained. Embryologists and doctors associate it with the mechanism of monozygotic twin formation. Monozygotic twins are genetically identical individuals that develop from the same fertilized oocyte. The division into two embryos takes place at the time of gastrulation. If the division occurs early, twins have separate fetal membranes, amnions and chorions. They constitute 25% of all monozygotic twins. When the embryo divides later, biamniotic twins form, each of which has their own individual amnion but they share the same chorion (65% of monozygotic twins). In the situation when the division concerns the embryonic disc and occurs after the primordium of the amnion forms, both fetuses develop in the common amnion and chorion. This type is referred to as monoamniotic monochorionic twins. If the embryonic disc ruptures after two weeks of development, the division of cells is often incomplete thus resulting in the formation of conjoined twins. The possibility of the development of embryos damaged in such a way is associated with considerable abilities of an embryo to repair the damage thanks to processes called embryonic regulation. The chances for the formation of conjoined twins disappear in humans after the fourth week of pregnancy when the elements of the central nervous system develop and an embryo folds. The presence of conjoined twins in the uterus can be detected in the 12th week of pregnancy in a proper US examination [3–8].

When attempting to explain the mechanism in which conjoined twins are formed, it is necessary to look into the mechanisms and disorders of embryological development of monozygotic twins. Moreover, the elements of classical embryology that are relevant to the developmental anomalies described herein, are worth reminding. At the beginning of the third week from fertilization, a human embryo assumes the form of a flat disc which consists of three germ layers. The amnion constitutes its upper border, and the yolk sac cavity is its lower border. The cephalic end of the disc contains the oropharyngeal membrane, and the caudal end contains the cloacal membrane. The primordia of the heart and diaphragm are located cephalically from the oropharyngeal membrane. In the middle of the disc, in the longitudinal line, there is the notochord. Above it, on the dorsal surface of the disc, there forms a longitudinal cavity called the neural groove. It is the primordium of the axial elements of the central nervous system. The folds that surround the neural groove close up and create the neural tube, open at both ends. The final stage of neurulation is the closure of the anterior (26th day) and posterior (28th day) neural tube opening. The cephalic end of the neural tube develops into the brain, and the remaining part forms the spinal cord. In the fourth week of development, the cephalic and caudal ends of an embryo begin to fold; they "roll up" under the ventral part of the embryonic disc. This causes, among others, the movement of the primordia of the heart and diaphragm to the embryo's ventral side. At the same time, its lateral sides are folded. Finally, an embryo becomes surrounded by the amniotic cavity filled with amniotic fluid. In about the 20th day of development, a pedicle forms on the embryo's ventral side, which will later transform into the umbilical cord that connects it with the placenta and ensures gas and metabolic substance exchange between the mother and the fetus [5,7,8].

Currently, the widely accepted and prevailing theory that might elucidate the formation and development of conjoined fetuses is the cleavage theory. It assumes that during gastrulation, when an embryo still has a form of an elliptical disc, the disc spontaneously cleaves incompletely along the cephalocaudal axis. Due to embryonic regulation processes, Siamese twins are

formed. They are characterized by a homologous connection, which means that both individuals share the same organs, e.g. head to head or back to back. The fragmentation of the embryonic disc and the separation of tissue elements can be caused by environmental factors, cellular adhesion disorders or enhanced genetically-determined apoptosis, i.e. death of cells. The formation of free spaces in dense tissues of germ layers decides about separate differentiation of non-combined embryo tissues [3,9,10].

An alternative theory that explains the formation of Siamese twins is the spherical theory. It assumes that embryonic discs, which form after the complete cleavage of an embryo at an early stage of development, can have the common yolk sac. The cells that form it can "swim" or "float" on its surface and fuse again, usually endodermally, thus creating ventrally united twins. Dorsally united twins, in turn, are a result of ectodermal fusion due to the approximation of embryonic discs that share the common amniotic cavity at an early stage [9,11]

The exact site of disc connection and their location in relation to each other leads to the formation of a given type of Siamese twins. The fusion of embryos forces their adaptation to new anatomic conditions thus resulting in changes in organ position and/or aplasia of certain tissues. Anatomic defects at the fusion of critical organs and other developmental defects, not necessarily associated with the site of fusion, are a reason for exceptionally high mortality among Siamese twins. In multiple pregnancies, with three or more embryos, fusions between fetuses are very rare. Interestingly, conjoined embryos have been observed not only in viviparous species (mammals), but also in oviparous animals, such as birds or reptiles [3,10,11].

It seems that mechanisms of embryo cleavage and Siamese twin development are based on mechanical factors whereas genetic, biochemical or environmental factors might be less important. Chemical or physical factors that can cause the development of conjoined twins are not fully known. It is believed that vitamin A, hyperthermia or hypoxia can affect developing embryos. Moreover, it has also been tested whether the orientation of the ovum affects the development of the embryo axis in the critical moment of body symmetry axis formation. Furthermore, other unknown teratogenic substances, which act during a strictly specified period of embryonic development, probably around the third week of development, i.e. in the critical teratogenic period of human development, can be associated with this developmental anomaly [3,10].

NOMENCLATURE ASSOCIATED WITH CONJOINED FETUSES

Classical Siamese twins are two fused individuals of a similar degree of development. Generally, conjoined twins are divided into those united ventrally (approximately 69.3% of cases) and those united dorsally (5.4%). The remaining types include connections of non-specific nature (21.4%) and so-called parasitic twins (3.9%), i.e. those characterized by considerable morphological disproportions [3].

The nomenclature associated with the pathologies observed in the case of conjoined fetuses is based on the morphology of the connection. Ventrally united twins can be divided into cephalically united represented by *thoracopagus* (42%), *cephalopagus* (5.5%) and *omphalopagus* (5.5%) types. An example of caudal union is *ischiopagus* type (1.8%), and the lateral union – *parapagus* type (14.5%). Ventrally joined twins initially share the common yolk sac that takes part in the development of the intestine, liver and pancreas. They usually share the common peritoneal cavity and umbilical cord. Their hearts can be either common or separate depending on the level of commonness.

The *thoracopagus* type develops by the connection at the cardiac primordium. The connection involves the chest and the upper part of the abdominal cavity. In this case, numerous common organs prevent separation. The comfort of life of conjoined individuals in very low. The *cephalopagus* type develops by the initial connection at the oropharyngeal membrane in the anterior aspect of the embryonic disc. Subsequently, it extends from the crown of the head to the umbilicus. In this case, the trunks of both fetuses share the head and chest. The prognosis concerning their survival and development is very unfavorable. The *omphalopagus* type develops by the connection of the primordia of the diaphragm. The connection involves the chest and the upper part of the abdominal cavity. The *ischiopagus* type is associated with the common cloacal membrane and developing posterior intestine and rectum. It also involves the muscle and skeletal system of the posterior parts of the body. Such a connection can also be found in the lower part of the abdominal cavity and in the region of the urogenital tract. The connection does not usually involve critical organs and neonates can be separated effectively. The *parapagus* type develops by the connection of the primordia of the anus, frequently retaining two notochords, and ultimately lower segments of the spine. The connection involves the pelvis and torso to various degrees; there is one or two heads and two faces.

Dorsally united twins are divided into the following types: *craniopagus* (3.4%), *rachipagus (*1.0%) and *pygopagus* (1.0%). These twins have a common part of the axial nervous system originating from the neural tube. The spine and its muscles are common, but there are two abdominal cavities. Umbilical cords are usually separate.

The *craniopagus* type develops by the initial connection in the region of the anterior neural opening which ultimately includes the cranial vault. The *rachipagus* type develops by the connection of the central part of the neural tube that includes the spine and its muscles. The *pygopagus* anomaly results from the

connection of the caudal segments of the neural tube in the region of the posterior neural opening, and ultimately includes the sacrum and coccyx with pelvic muscles [3,12].

It seems that all of the above mentioned defects have common etiology. It must be emphasized that the anatomy of connections between Siamese twins is changeable, even within one twin type [4]. Most conjoined twins are female fetuses; however, this relationship strongly depends on the connection site. For instance the *thoracopagus* type is usually observed among female fetuses whereas the *parapagus* type and the parasitic type is more common is males [3].

Conjoined twins that can result from amorphous and irregular division of embryonic cells are characterized by a different type of pathological disorders. Developmental disorders that arise this way include: parasitic twins, fetus in fetu type or acardiacs. It is suspected that these anomalies can also result from the damage or death of one of Siamese twins, and its localization corresponds to the site of the primary connection.

A parasitic twin is not properly developed or consists of only a part of the fetus and its existence is determined by the connection with a normal fetus that functions as a host. Such a twin usually consists of additional extremities; it frequently has no heart or brain. This anomaly is observed 20 times more rarely than symmetrically conjoined twins. It seems that a parasitic element develops as a result of the death of one of conjoined twins, and its tissues remain vascularized by the host twin's vessels [3,9,13].

As for fetus in fetu, a fetus-like tissue is located inside a morphologically normal fetus. This tissue frequently protrudes from the fetal oral cavity in the form of so-called *epignathus.* As with parasitic twins, such an element does not usually have the heart or brain and its histological and anatomical differentiation is extremely abnormal. Such pathological fetuses are characterized by being enclosed in a sac which is partially or completely covered with skin. Sometimes, certain anatomical parts, such as bones or muscles, are distinguishable and the entire body is attached to the host by a thin pedicle that contains few vessels [9,13].

Acardia is a situation in which an abnormal fetus without a heart is completely separated from the host but remains connected with the host's circulation by its own umbilical vessels, without direct contact with the placental circulation. Such a fetus is supplied with blood that flows through the dominant embryo first; the blood contains very low levels of oxygen and nutrients. The parasitic host is surrounded with the amniotic membrane (his own or common with the host). As with the fetus in fetu, it is covered with skin and contains differentiated but teratogenically altered anatomical elements [13].

Another type of embryonic development disorders is the formation of teratomas (multi-tissue neoplasm). There are numerous theories to explain their genesis. It is postulated that they are formed as a result of blastomere proliferation, fertilization of a polar body, parthenogenesis, degeneration of a fertilized oocyte or transformation of stem cells (due to mutation) into neoplastic cells of benign or malignant nature. Teratomas can also be found in various normally shaped organs or in pathological conditions described above [13].

These definitions, however, do not fully reflect the actual state. For instance, there are cases of parasitic twins, conditions of acardia and fetus in fetu with some elements of a teratoma, or a teratoma with a fetus in fetu. Moreover, one host may include a teratoma as well as fetus in fetu. The question arises whether the *epignathus* pathology is a type of a parasitic twin, fetus in fetu or teratoma. Furthermore, there are cases of Siamese twins conjoined in such an atypical way that it is difficult to understand the mechanism of their formation. The only organ that is essential for fetal survival is the heart, and even the heart itself can be replaced with the host's heart that supplies oxygen and nutrients to the malformed tissue [12,13].

Perhaps the aforementioned division of anomalies is artificial and should be replaced with a new one based on disorders in embryonic development that have caused it rather than on morphological features. Since human embryonic development is not fully explored and factors that might disrupt it are not entirely known, such a division would be very difficult to develop. Perhaps studies in the field of experimental teratology conducted on appropriate animal models could provide answers. However, the interpretation of results and their extrapolation to human development is frequently difficult and ambiguous.

Conjoined twins are usually delivered by a cesarean section following the previous diagnosis. Developmental defects in Siamese twins usually involve the following systems: cardiovascular, musculoskeletal, nervous and urogenital. Numerous Siamese twins die in the neonatal period, including individuals that die during a separation surgery. Their survival mainly depends on the type of connection and shared organs as well as the time and precision of surgery and non-surgical treatment. The postoperative mortality of conjoined twins is often caused by postoperative stress, presence of accompanying defects, lack of material to cover body defects or infection. If critical organs are shared, separation is impossible. A scheduled surgery is more likely to be effective than a "life-saving" procedure. This is mainly associated with the fact that before a scheduled procedure, all examinations are conducted to find out the exact course of the connection between twins, and the risk of finding unexpected anatomical structures during the surgery is lower [1,3,4,14].

The difficulties in the separation of Siamese twins are presented in the clinical analysis of children treated in a center in Krakow, Poland, in 1977–2006. Of 19 pairs of conjoined twins, 10 were separated surgically

and 9 died. As a result of the surgery, one child in 8 pairs of twins survived, in one pair both twins died, and in one pair (separated abroad) both children survived [14].

CONCLUSION

The review of the contemporary literature on the formation of conjoined twins demonstrates how little is known about the pathogenesis of this phenomenon. This is associated with the lack of exact knowledge about embryological processes and teratogenic factors that underlie this anomaly. The lack of sufficient knowledge about embryonic development and underlying mechanisms should prompt thorough molecular and genetic studies in this field of medicine. Perhaps then, it would be possible to elucidate the causes and the mechanisms of Siamese twin formation along with many other developmental defects. At present, however, when the emphasis is put on practical and interventional problem-solving, it is more difficult to obtain funds for basic research, which does not always yield simple and readily applicable effects.

References:

1. **Jaczyńska R, Niemiec KT, Przybyłkowska M i wsp**. Bliźnięta niecałkowicie rozdzielone - problemy diagnostyczne i terapeutyczne. *Ginekol Pol* 2005;76:602-611.
2. **Kobylarz K.** Aspekty psychologiczno-etyczne związane z leczeniem zrośląków. *Bioet Zesz Pediatr* 2005;2:96-107.
3. **Mutchinick OM, Luna-Muňoz L, Amar E et al.** Conjoined twins: a worldwide collaborative epidemiological study of the International Clearinghouse for Birth Defects Surveillance and Research. *Am J Med Genet Part C Semin Med Genet* 2011;157:274-287.
4. **Spitz L, Kiely EM.** Conjoined twins. *JAMA* 2003; 289:1307-1310.
5. **Bartel H**. Embriologia. Warszawa: Wydawnictwo Lekarskie PZWL;2010.
6. **Grzesiak M, Podciechowski L, Kaczmarek P et al.** Ultrasonograficzna diagnostyka bliźniąt niecałkowicie rozdzielonych w I trymestrze ciąży. *Ultrason Ginekol Położ* 2006;2:216-219.
7. **Jura C, Klag J**. Podstawy embriologii zwierząt i człowieka. (tom 1) Warszawa: Wydawnictwo naukowe PWN; 2005.
8. **Moore KL, Persaud TVN, Torchia MG.** Embriologia i wady wrodzone. Od zapłodnienia do urodzenia. Wrocław: Elsevier Urban & Partner; 2013.
9. **Cunningham FG, Leveno KJ, Bloom SL et al.** Williams Obstetrics. 24th edition, 2014.
10. **Kaufman MH.** The embryology of conjoined twins. *Childs Nerv Syst* 2004;20:508-525.
11. **Spencer R.** Theoretical and analytical embryology of conjoined twins: Part I: Embryogenesis. *Clin Anat* 2000; 13:36-53.
12. **Spencer R.** Theoretical and analytical embryology of conjoined twins: Part II: Adjustments to Union. *Clin Anat* 2000;13:97-120.
13. **Spencer R**. Parasitic conjoined twins: external, internal (fetuses in fetu and teratomas), and detached (acardias). *Clin Anat* 2001;14:428-444.
14. **Kózka M, Ociepka M.** Analiza kliniczna bliźniąt syjamskich leczonych w ośrodku krakowskim w latach 1977-2006. *Prz Chir Dziec* 2009;4:185-190.