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Investigating The Rare Occurrence of Male-Female Conjoined Twinning: Incomplete Embryonic Division with Divergent Sexual Differentiation, Symmetrical Conjoined Twins Opposite Phenotypic Sex

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Abstract:

Conjoined twinning is a very rare congenital disorder that results from partial separation during the development of the embryos in cases of monozygotic twins. Male-female symmetrical conjoined twins with an opposite phenotype in relation to their biological sex are an extremely rare developmental abnormality due to the complications involved, from an embryological, genetic, hormonal, clinical, and ethical standpoint. In this review, we discuss the embryological causes of conjoined twinning, sexual differentiation processes, and the potential causes of discordant phenotypical sex development through chromosomal mosaicism, epigenetics, asymmetry of hormone distribution, or receptors. Furthermore, Disorders of Sex Development (DSD), prenatal diagnosis and molecular analyses, psychosocial impacts, surgery, and ethical issues related to sexual discordance among conjoined twins are evaluated. Our current scientific knowledge is limited since such cases are extremely rare.

Keywords: Conjoined Twins, Monozygotic Twinning, Sexual Differentiation, Disorders of Sex Development, Divergent Phenotypic Sex, Embryonic Division, Developmental Genetics, Gonadal Differentiation, Prenatal Diagnosis, Embryology

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1. INTRODUCTION

The human embryo development is a very intricate biological process that consists of cell differentiation, tissue organization, and genetic regulation during early fetal development stages[1]. Any irregularities in embryonic cell divisions or developmental signal transduction can lead to congenital disorders concerning morphological abnormalities, organogenesis defects, or sexual determination issues. Among those congenital disorders, conjoined twinning constitutes a very rare and complex developmental abnormality observed in medical practice. Not only does it

pose numerous anatomical and surgical complications but also it raises several embryological questions on individuality and developmental plasticity[2].

Thanks to progress in the field of embryology, developmental genetics, endocrinology, and prenatal imaging, scientists gained a significant insight into congenital disorders and DSD. But the actual existence of male and female conjoined twins whose phenotype contradicts monzygosity is an extremely rare and little-known developmental abnormality[3]. This type of disorder poses certain doubts concerning the monochromaticity of genetic material, hormone levels, epigenetics, and the divergence of the developmental pathway.

1.1 Background and Context

Conjoined twinning is one of the least common congenital developmental disorders in human embryology that arises from the failure of monozygotic twins to separate completely at the embryonic stage. Conjoined twins are typically genetically identical and are thus normally similar in their genetic and physical sexual traits. Nonetheless, unusual developmental conditions that result in sexual differentiation have brought into question current knowledge on embryology, gonad development, and sexual phenotypes[4]. The rare possibility of having male and female symmetrical conjoined twins with contrasting phenotypical sex is considered a very unusual developmental event that could be associated with genetic mosaicism, epigenetics, hormonal differences, and sexual differentiation disorders[5].

1.2 Introduction to the Problem

Conjoined twins present a range of physiological complications that include different types of body fusions like thoracopagus, pygopagus, parapagus, craniopagus, and rachipagus arrangements[5]. While monozygotic twins derive from a solitary egg cell fertilization, there could be rare instances of developmental deviations that result in unique physical features, such as sex reversal. These deviations pose critical issues regarding embryonic structure, hormonal control, morphological flexibility, and DSD[6].

1.3 Objectives of the Review

The objectives of this review are:

- To study the embryological factors involved in the formation of conjoined twinning and partial embryo separation.
- To discuss the biological processes involved in sex determination and DSD.
- To understand the theoretical concepts related to differential sex phenotype in conjoined twins.
- To explore clinical aspects, imaging techniques, psychological issues, surgery, and ethical considerations in symmetrical conjoined twinning.
- To find out research gaps and future directions in embryology and sex differentiation studies.

1.4 Importance of the Topic

Male-female conjoined twins with sexual divergence have significant implications for the fields of embryology, reproductive medicine, genetics, pediatric endocrinology, and bioethics. Insights into these birth defects can enhance scientific understanding about the organization of the embryo, gonadal development, developmental flexibility, and prenatal diagnosis. Additionally, research into such birth defects aids in progress toward better care for neonates with rare congenital conditions[7].

2. EMBRYOLOGICAL BASIS OF CONJOINED TWINNING

Embryogenesis is a process that requires synchronized cell proliferation, differentiation, and organization along the embryonic axis at an early stage of pregnancy[8]. Errors in embryologic separation or embryonic development signals can cause various birth defects including conjoined twins, where the failure to separate results in the joining of the twins. Knowledge about the embryology of conjoined twins helps explain variations in anatomy, as well as abnormal sexual phenotypes[9].

2.1 Monozygotic Twinning and Incomplete Embryonic Division

Conjoined twinning is thought to be caused by the failure to separate completely the embryonic discs in cases of monozygotic twinning that occur within the period ranging from the 13th to 15th day after conception[10]. In contrast to the normal development of monozygotic twins, there is a possibility that the failure to separate might cause the permanent fusion of the twins.

There are two predominant embryologic explanations for the formation of conjoined twins:

- **Fission Theory**

The fission theory states that the partial division of the embryonic disc causes the existence of persistent physical fusion between the twins[11]. The fission theory asserts that the separation of the embryonic axis is never complete at an early stage of fetal development, hence causing the sharing of certain body parts between the twins. The fission theory is the most credible mechanism explaining symmetrical conjoining of twins[12].

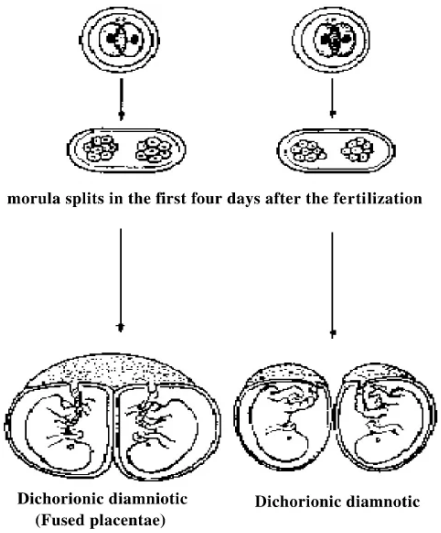

Figure 1: Early Embryonic Division and Monozygotic Twin Formation

Fusion Theory

According to the fusion theory, it is possible for two individual embryos, initially separated, to re-fuse through stem cell communication during the development of the embryo[13]. The fusion theory argues that neighboring embryonic tissues can fuse at particular sites of development, creating shared body parts and vascular connections. While not as popular as the fission theory, some rare cases of conjoined twinning can be explained through fusion[14].

The extent and area of fusion are determined by the time and direction of separation between the two embryos as well as the stage of development[15]. Differences in these embryologic factors result in various forms of conjoined twinning, asymmetrical developments, shared organs, and congenital conditions related to the cardiovascular, gastrointestinal, musculoskeletal, and nervous systems.

2.2 Classification of Symmetrical Conjoined Twins

Conjoined twins with symmetrical anatomy are differentiated based on the specific regions of their physical connection. It is essential for determining the embryologic arrangement, prenatal identification, surgical treatment, and clinical outcome[16]. Various types of fusions might include organs, blood vessels, nerves, and bones, based on the areas of the embryo that are affected.

Table 1: Classification and Anatomical Sites of Symmetrical Conjoined Twins

Type of Conjoined Twins	Site of Fusion
Thoracopagus	Thoracic region
Omphalopagus	Abdominal region
Craniopagus	Cranial region
Pygopagus	Sacral region
Parapagus	Lateral trunk fusion
Rachipagus	Vertebral region

Thoracopagus is the most prevalent type of conjoined twinning, which includes fusion of the chest cavity with sharing of cardiac tissue. Omphalopagus is characterized by fusion of the abdominal wall with sharing of parts of the liver and gastrointestinal organs. The craniopagus type of conjoined twinning includes fusion of the skull vault and is accompanied by neural and vascular connections. Pygopagus conjoined twinning involves fusion in the area of the sacrum, while parapagus conjoined twinning includes lateral fusion of the body from the trunk down to the pelvic area. Rachipagus refers to the fusion of the spine and is the rarest type of symmetric conjoined twinning.

Some rare types include asymmetrical organization, parasitic structures, or neurological, skeletal, and visceral congenital anomalies. These cases indicate that more than one mechanism can cause conjoined twinning along with its complications[17].

3. SEXUAL DIFFERENTIATION AND DISORDERS OF SEX DEVELOPMENT

The phenomenon of sexual differentiation is a complicated process requiring the interaction of genetics, hormones, gonads, and environment in the embryo[18]. Sexual development starts with the determination of chromosomal sex during fertilization and continues with gonadal development, hormone action, internal development of reproductive organs, and development of external genitalia. Disruption of any of the highly regulated processes above might lead to abnormal sexual development or DSDs. In some exceptional developmental cases like conjoined twins, developmental differences in the embryonic stage could theoretically cause different phenotypes of sexual differentiation from a common embryonic source[19].

3.1 Biological Mechanisms of Sexual Differentiation

Sexual differentiation in humans is a stepwise process that includes sexual differentiation of chromosomes, gonads, hormones, and external genitalia. At the onset of embryonic development, the bipotential gonads can potentially differentiate into either testes or ovaries based on the genetic signal cascade pathway. SRY, a gene present in the Y chromosome, triggers the development of testes, which results in androgen secretion and male sexual differentiation. Sertoli cells secrete Anti-Müllerian Hormone (AMH) to inhibit the development of Müllerian ducts, whereas testosterone stimulates Wolffian duct development. In the absence of androgen action, the female pathway of sexual differentiation usually prevails.

$$\mathrm{XY} \xrightarrow{\mathrm{SRY}} \mathrm{Testis} \rightarrow \mathrm{Testosterone} + \mathrm{AMH} \rightarrow \mathrm{Male\ Phenotype}$$

In addition to genetics, sex differentiation is also determined by hormones, receptors, epigenetics, transcription factors, and timing. If there are any changes with respect to androgen levels, androgen receptors, and gene expression, it will change the manner by which sex differentiation takes place and result in the formation of abnormal sexual features and divergence[20].

3.2 Disorders of Sex Development (DSD)

DSD are considered to be congenital problems with an abnormal development of chromosomes, gonads, or the external genitalia phenotype. There may be a discrepancy between chromosomes, gonads, hormone levels, and the appearance of external genitals. Such disorders are clinically significant because they involve multiple aspects of development and reproduction.

Common DSD conditions include:

- Androgen Insensitivity Syndrome
- Gonadal dysgenesis
- Congenital adrenal hyperplasia
- Ovotesticular DSD
- Chromosomal mosaicism

Disturbances in androgen production, receptor activity, gonad development, or chromosome structure can lead to diverse sex differentiation even if there is a genetically identical profile[21]. In

certain cases of DSD, problems such as ambiguous genitalia, unusual reproductive structures, infertility, hormone imbalances, and psychological stress related to gender identity or sexuality could arise. Progress has been made in molecular genetics and endocrinology toward identifying factors in sex development processes linked to DSD, though many factors are yet unknown.

3.3 Divergent Sexual Differentiation in Conjoined Twins

Conjoined twins that possess different phenotypes of opposite sexes are extremely rare since monozygotic twins share almost identical genes and should therefore possess the same type of chromosomes and phenotypes[22]. Nonetheless, there exist rare cases of developmental deformities associated with asymmetry of the embryo, hormone differences, or other genetic factors that can cause sexual differentiation in different ways[23].

Possible factors include:

- Chromosomal mosaicism
- Epigenetic divergence
- Hormonal asymmetry
- Differential androgen receptor sensitivity
- Localized gonadal dysgenesis
- Embryonic axis irregularity

These anomalies can lead to gonadogenesis, hormonal control, and external genitalia development despite having a common embryological derivation. Chromosomal mosaicism can lead to different cells being produced in the embryonic tissue, whereas epigenetics can affect the expression of the gene that regulates sexual development. Alternatively, differential exposure to hormones or hormone receptors can lead to different responses to androgens in fraternal twins[24]. Though there is a lack of clinical studies due to the rarity of the condition, these theoretical concepts demonstrate the complexity in the development of conjoined twins and sex differentiation.

4. CLINICAL AND DIAGNOSTIC PERSPECTIVES

Conjoined twinning must be thoroughly investigated using clinical evaluation, prenatal assessment, anatomical studies, and molecular testing to determine possible abnormalities and complications that may result from the condition[25]. The early diagnosis of conjoined twinning and DSD is especially crucial in exceptional instances where the condition presents an unusual type of sexual differentiation since this will allow for better prenatal counseling, maternal management, surgical planning, and neonatal treatment. Modern diagnostic techniques like fetal imaging and embryology have greatly increased our understanding of the mechanisms behind conjoined twinning and DSD.

4.1 Prenatal Diagnosis and Imaging

The pre-natal diagnosis procedure is very vital in detecting conjoined twinning along with associated congenital problems during pregnancy[26]. The early detection enables physicians to conduct tests on anatomical fusion, identify whether there are shared body systems, the

likelihood of survival of the twins in the womb, and also make necessary preparations in advance for surgical operations after birth. There have been many advancements in the field of prenatal diagnosis owing to the use of imaging techniques.

The major methods used in prenatal diagnosis include ultrasonography, Doppler imaging, fetal MRI, three-dimensional reconstruction imaging and genetic testing. Among all the above-mentioned procedures, ultrasonography is the major method used in detecting conjoined twinning, and Doppler imaging is used to detect blood flow patterns in the twins.

Figure 2: Prenatal Ultrasonographic Imaging of Conjoined Twins

In advanced imaging during prenatal care, there is early detection of organs being shared, cardiovascular union, neural tube fusion, bone defects, and other anomalies that arise from these processes. When there is rare occurrence of different sexual development, imaging can help determine the condition of gonads and reproduction systems. The importance of early prenatal diagnosis cannot be underestimated in clinical decisions and neonatal management.

4.2 Molecular and Genetic Investigation

Recent advancements in molecular technology have provided insight into embryonic development and developmental problems that are related to conjoined twinning and abnormal sexual development[27]. Molecular studies have proven to be very crucial in studying rare cases of developmental problems that result in phenotypic variations as there may be chromosomal, epigenetic, and hormone-related causes of abnormality during embryonic development.

Molecular approaches like whole-genome sequencing, transcriptomic studies, and epigenetic studies enable researchers to analyze the gene expression and regulation processes during embryonic development, gonadal development, and hormone regulation. Hormone receptor studies and single-cell sequencing techniques have proven useful in analyzing problems with androgen sensitivity and endocrine signaling during embryonic development.

The above-mentioned molecular approaches could be beneficial in studying biological processes leading to abnormal phenotypic sex, gonadal development, embryonic asymmetry, and developmental plasticity in rare cases of conjoined twins.

5. ETHICAL, PSYCHOSOCIAL, SURGICAL, AND THEORETICAL PERSPECTIVES ON OPPOSITE PHENOTYPIC SEX IN CONJOINED TWINS

Conjoined twins with different phenotypic sexes represent an extremely complicated developmental disorder with issues related to ethics, psychology, surgery, and biology[28]. These disorders pose many questions about identity, gender assignment, reproduction, and quality of life. The combination of conjoined twinning and atypical sex determination adds another layer of complexity in treating such cases.

5.1 Ethical Complexities

Conjoined twins raise substantial ethical concerns regarding:

- Personhood
- Bodily autonomy
- Surgical separation
- Consent
- Quality of life
- Survival prioritization

Ethical issues tend to become highly complicated when surgery will endanger the life of one of the twins or when there is an incongruity between the biological and psychological sexual characteristics that might pose challenges for their identity in the future. Issues surrounding parental authorization, reproductive freedom, and post-natal quality of life also pose ethical concerns. Issues become more important if there is sharing of organs or complications during surgery.

5.2 Psychosocial Implications

Conjoined twins and those born with DSD may encounter many psychological, social, and emotional issues during their formative years and adult life. Issues with identity, body image, social stigma, gender identification, and difficulties forming interpersonal relationships can impact the overall psychological wellbeing of the patient. For the few that suffer from different phenotypic sex, psychosocial adaptation can be even harder as there is bound to be confusion regarding identity development, social acceptance, and gender identity.

Psychological care as well as social work is, thus, important for the wellbeing, social adaptation, and quality of life of the patients[29]. Long-term psychological interventions may also be useful in helping such individuals adapt emotionally and socially.

5.3 Surgical Considerations

The surgical separation of symmetrical conjoined twins is among the most technically challenging operations within pediatric surgery due to the anatomical fusion, which can lead to shared organs, blood vessels, nervous tissue, as well as bones. The result of separation surgery

will depend on the degree of organ sharing, the circulation system interaction, neural involvement, and the prognosis for both twins.

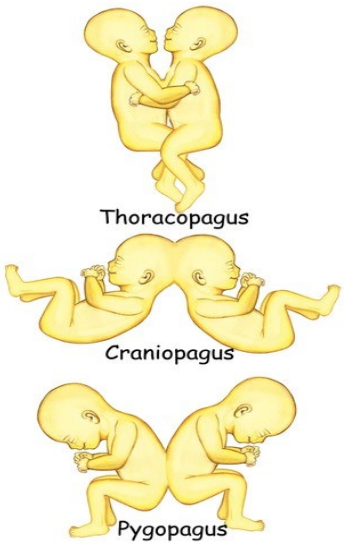

Figure 3: Common Anatomical Types of Conjoined Twins Based on Site of Fusion

The use of advanced imaging technologies for prenatal imaging and anatomical evaluation, including fetal magnetic resonance imaging (MRI), ultrasonography, Doppler ultrasound imaging, and three-dimensional reconstruction imaging, is important in planning surgery and assessing risks. Rehabilitation following surgery, ongoing medical observation, and reconstructive surgery might be required to enhance chances of survival and functional outcome. Moreover, the health care environment and skills of surgeons can be critical determinants of successful surgery.

5.4 Theoretical Perspectives on Opposite Phenotypic Sex in Conjoined Twins

Conjoined twins with contrasting phenotypic sex occur due to an extremely rare phenomenon in development which contradicts conventional notions regarding the same-sex chromosomal makeup of monozygotic twins[30]. Despite monozygotic twins being formed through a single fertilized egg and thus having identical sex chromosomes, rare occurrences such as chromosomal mosaics, epigenetics differences, hormone discrepancies, and differential hormone receptor sensitivity could account for variations in sexual development of the embryos.

Table 2: Theoretical Mechanisms Contributing to Divergent Phenotypic Sex in Conjoined

Proposed Mechanism	**Potential Developmental Effect**
Chromosomal mosaicism	Divergent gonadal differentiation
Epigenetic variation	Altered sexual gene expression
Hormonal asymmetry	Opposite genital differentiation
Receptor sensitivity variation	Different androgen responsiveness

Embryonic axis irregularity	Asymmetrical body development

Mosaicism in chromosome constitution, epigenetic differences, hormonal imbalance, and receptor differences could be some of the factors that lead to differing sexual phenotypes in spite of the common origin in embryogenesis between conjoined twins[31]. Nevertheless, scientific knowledge regarding the occurrence of opposite sexual phenotypes in conjoined twinning is still inadequate owing to its rare occurrence[32].

6. DISCUSSION

The existing literature shows that conjoined twinning with divergent phenotypic gender is a very rare and complicated developmental abnormality. Embryology, genetics, hormones, psychosocial, and ethical considerations are some of the important factors related to conjoined twinning with divergent phenotypic gender. The recent studies show that the development of phenotypic divergence in conjoined twins may be influenced by several biological factors such as embryonic development, hormonal factors, and epigenetics.

Table 3: Summary of Selected Literature on Twin Studies and Rare Conjoined Twinning Disorders

Author(s) & Year	Study Focus	Major Findings	Relevance to Present Study
Segal (2018)[33]	Twin research and developmental discordance	Discussed growth discordance and developmental variations in twins	Supports developmental variability in monozygotic twins
Segal (2021)[34]	Twins and triplets raised apart	Examined genetic and environmental influences in twin development	Relevant to developmental plasticity and phenotypic divergence
Segal (2023)[35]	Twin-related developmental abnormalities	Reported technological advances and rare monozygotic twin conditions	Supports discussion on rare congenital and conjoined twin anomalies
Segal (2025)[36]	Twin neonatal outcomes and rare twin cases	Highlighted unusual conjoined twin and developmental conditions	Relevant to rarity and clinical significance of conjoined twinning
Shrestha et al. (2020)[37]	Dicephalic parapagus twins with congenital anomalies	Reported discordant developmental abnormalities in conjoined twins	Supports embryological irregularities and phenotypic divergence in conjoined twins

6.1 Interpretation and Analysis of Findings

As can be seen from the above findings, the failure of embryonic cell division along with developmental abnormalities such as chromosome mosaic, hormone imbalance, epigenetics, and alterations in receptor sensitization might play a role in developing sexual differences between symmetrical conjoined twins[38]. According to the data at hand, the occurrence of embryonic asymmetry, coupled with abnormalities in gonad development, may influence the phenotype even in the case of monozygotic individuals.

6.2 Implications and Significance

The scientific study of the occurrence of opposite sexual phenotypes in conjoined twins is crucial for the development of various disciplines including embryology, reproduction, genetics, pediatrics, and neonatology[39]. An increase in knowledge regarding such cases can help in making advancements in the fields of prenatal diagnosis, molecular embryology, counseling, ethics, and neonatology.

6.3 Research Gaps and Limitations

Our current scientific knowledge about divergent sex development in conjoined twins is constrained by the rare occurrence of such cases and a lack of comprehensive research into their molecular biology, genetics, and development over time. The available literature is largely speculative and focuses on individual case reports without substantial information on the role of embryonic gene expression, epigenetics, hormones, and psychological impact[40].

6.4 Future Research Directions

Further studies need to be carried out in the areas of embryonic transcriptomics, epigenetics, prenatal genome testing, molecular imaging, hormonal pathways, and psychosocial assessment in order to gain deeper insights into the biological underpinnings of the distinct sexual differentiation process of conjoined twins. More collaboration among experts from different fields like embryology, genetics, endocrinology, pediatric surgery, psychology, and bioethics is required for further scientific advances in this extremely rare condition.

7. CONCLUSION

Conjoined twinning can be considered among the least understood and most intricate developmental disorders in the field of human embryology. The theoretical possibility of opposite-sex, symmetrical conjoined twins raises even greater challenges regarding our current understanding of the biological processes of embryonic development, gonadal differentiation, and developmental plasticity. The available data indicate that mechanisms such as chromosomal mosaicism, hormonal imbalance, epigenetics, and abnormal development through cell-surface receptor interaction might play a role in the different phenotypic sexual differentiation of these individuals, even though they come from the same embryo. Despite recent progress in developmental genetics, prenatal ultrasound imaging, and genetic diagnostics, there still exists a lack of scientific knowledge about these rare developmental disorders due to their extreme rarity.

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