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Current Epidemiology of HOXB2 Mutation in Aborted Fetuses of Infected Mothers

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ABSTRACT

The intricate and significant relationship between maternal infections, HOXB2 (Homeobox B2) genes mutation and fetal development is underscored by the association of congenital TORCH infections with various complications such as encephalitis, microcephaly, hydrocephaly, hepatitis, lymphadenopathy, and intrauterine death. TORCH infections pose substantial risks to both mother and fetus, resulting in adverse outcomes like congenital infections, intrauterine growth restriction, and stillbirth. Pathogens can breach the placental barrier via transplacental transmission, ascending infections during childbirth or blood-borne transmission. HOXB2, a pivotal gene in embryonic development and placental formation, may influence susceptibility to TORCH infections. In particular, mutations affecting HOXB2 could disrupt placental integrity, nutrient exchange and immune response, potentially heightening the risk of vertical transmission to the fetus and exacerbating adverse outcomes. Understanding transmission routes is essential for comprehending the association between maternal infections and HOXB2 mutation. Proper infection control measures during pregnancy, labor, and delivery are indispensable for minimizing risk. Further research is imperative to elucidate the molecular mechanisms underlying HOXB2 mutation in aborted fetuses of infected mothers and its implications for maternal and fetal health. By unravelling these complexities, more effective strategies can be devised for preventing and managing maternal infections and their impact on fetal development.

Keywords: *Aborted Fetuses, Diagnosis, Fetal Development, HOXB2 Gene Mutation, Infected Mothers, Pregnancy Loss, TORCH Infection.*

Introduction

Prabhu Das et al. (2015) articulated that "Pregnancy comprises a unique immunological condition, safeguarding the fetus from maternal rejection and facilitating optimal fetal development while providing protection against microorganisms." [1]. The American Society of Reproductive Medicine (ASRM) defined that, recurrent pregnancy loss (RPL) is characterized by two or more consecutive pregnancy losses. Jacob L et al. noted that pregnancy loss, involving miscarriage, abortion, or fetal loss, is a prevalent adverse outcome in contemporary pregnancies [2].

In 2006, Rai and Regan stated that, “RPL or recurrent miscarriage is characterized as three or more pregnancy loss (consecutive) prior to 20 weeks from the last menstrual period. Moreover, the estimated prevalence of spontaneous miscarriage is approximately 15% among clinically diagnosed pregnancies” [3]. Suzumori and Sugiura-Ogasawara estimated that, “around 50% of early missed abortions, the fetus exhibit chromosomal aberrations” [4]. In 2023, Cuenca noted varied responses to pregnancy loss among women, influenced by factors like age, race, culture, or religious beliefs. Health providers, accustomed to the common occurrence, can enhance women's mental well-being by effectively managing the diagnostic process and treatment [5].

Kolte reported that “miscarriage classified into two groups based on different factors associated with miscarriage i.e., embryonic loss and fetal loss” Table 1 [6]. Kolte et al. categorized spontaneous abortion as "early pregnancy loss" before 10 weeks and "late abortion" between 10 and 22 weeks [6]. Losses in at least two gestations were indicated as recurrent miscarriage by Christiansen et al. [7]. The American College of Obstetricians and Gynecologists & Society for Maternal-Fetal Medicine in 2018, after 22 weeks of gestation, pregnancy loss is referred to as "fetal loss" or "stillbirth." ESHRE Capri Workshop Group defined induced abortion as a controlled procedure due to reasons like unplanned gestation [8, 9]. Fetal reduction, according to Evans et al. is the voluntary termination of one or more fetuses in a multiple gestation [10].

Genetic and Molecular Factors in Recurrent Pregnancy Loss.

Recurrent pregnancy loss, often linked to intrauterine fetal damage, arises from maternal or paternal chromosomal abnormalities. Structural abnormalities, like translocations and deletions, elevate the risk of miscarriage due to improper genetic information segregation [5]. Genetic factors contribute to 3-5% of recurrent miscarriages, with chromosomal, hormonal and anatomical defects accounting for additional cases [11].

Hogge et al. described that chromosome abnormalities cause 50 to 80 percent of pregnancy losses, depending on maternal and gestational age [12]. Miller and Therman noted aneuploidy and polyploidy as common occurrences, contributing to 47.9% and 9.8% of spontaneous abortuses, respectively [13]. Turner syndrome, a prevalent sex chromosomal abnormality, constitutes 20-25% of cytogenetically abnormal abortuses. Triploidy (16%), tetraploidy (8%), and structural chromosomal abnormalities (3%) were reported as factors in chromosomally abnormal abortions [14]. Balanced translocations, explained by Bhasin et al., account for the majority of karyotypic abnormalities and may lead to pregnancy loss due to meiotic segregation [15]. Macklon suggested that undetectable single gene defects may contribute to multiple miscarriages [16]. Genes related to immunity, angiogenesis, and apoptosis differ in expression during pregnancy failure [17, 18, 19]. Analyzing these genes in normal pregnancies could help identify high-risk cases for recurrent pregnancy loss (RPL) and guide management.

The intricate biological processes essential for maintaining a healthy pregnancy involve differential gene expression. Key genes like HOXB2, MTHFR, and CYP family genes are dysregulated in pregnancy loss. Factor V Leiden mutation and natural killer cells also play roles in these complex mechanisms [17]. Daftary & Taylor, emphasized that second-trimester pregnancy loss can result from either very preterm delivery or fetal demise. The significant role of homeobox genes, both known and undiscovered, during gestation is highlighted [20]. Studies by Bhatlekar et al. explained that an altered HOX gene expression network disrupts tissue homeostasis during tumor development [21]. Gehring et al. and Seifert et al. denoted that HOX genes encode transcription factors involved in the regulation of differentiation, cell growth, and organogenesis. Mutations in this gene may lead to disruptions in normal developmental processes, potentially contributing to pregnancy loss [22, 23].

In the study by Chaithra et al. molecular analysis using PCR-based subtractive hybridization identified a limited number of genes in chorionic villi from patients with recurrent pregnancy loss (RPL). However, this method may not capture all differentially expressed genes, suggesting the involvement of more genes in establishing and maintaining pregnancy [11]. Further research is required to confirm the clinical relevance of these genes and understand their physiological significance in controlling subsequent pregnancies.

Human infertility and recurrent pregnancy loss due to implantation defects remain poorly understood [24]. Capecchi suggested certain genes regulate cell proliferation during development, but their specific molecular effectors remain unknown [25]. Du and Taylor emphasized HOX genes' critical role in establishing positional identity and proper

development of the female reproductive tract. Abnormalities in reproductive tract development can result from mutations or altered expression of HOX genes. Environmental factors like diethylstilbestrol (DES) can induce Müllerian defects by affecting HOX gene expression. Additionally, HOX genes are essential for regulating adult endometrial development. Changes in HOX gene expression can result in uterine developmental abnormalities and impaired adult endometrial development, contributing to female infertility or pregnancy loss [26].

Table 1: Classification of Pregnancy Loss

CLASSIFICATION		DEFINITION	REFERENCES
Miscarriage or Spontaneous Abortion	Spontaneous Abortion, Early Pregnancy Loss	Pregnancy Occurring Before 10 Weeks	[6]
	Late Abortion, Fetal Miscarriage or Late Miscarriage	Occurs between 10 and 22 weeks of gestation	[6]
	Recurrent Miscarriage	Spontaneous loss in two separate pregnancies, whether consecutive or not, or in three or more non-consecutive pregnancies.	[27]
	Fetal Loss or Stillbirth	Pregnancy loss occurring after 22 weeks of gestation.	[8]
Voluntary termination of pregnancy	Abortion or Induced Abortion	Controlled procedure to terminate a pregnancy, performed for reasons such as unplanned gestation, fetal malformation, and maternal health complications.	[9].
	Fetal Reduction	Specific type of induced abortion in a multiple gestation, where one or more fetuses are voluntarily selected for termination.	[10]

Pregnancy Loss and TORCH Infections

Singh et al. reported that 15.6 million abortions in India in 2015, with a global concern highlighted by World Health Organization (WHO, 2016) stating that the majority of stillbirths occur in developing countries [28, 29]. WHO estimated 2.5 million child deaths within the first month of life in 2018 [30]. Singh et al. revealed an abortion rate of 47.0 per 1000 women aged 15 to 49 years, raising global concern [28, 29]. WHO estimated 2.5 million child deaths within the first month of life in 2018 [30]. Addressing multifaceted mechanisms, the research links TORCH infections to pregnancy loss, involving direct pathogen impact, placental engagement and inflammation-induced premature delivery, with both DNA and RNA viruses crossing the maternal-fetal interface. Various risk factors for miscarriage, including ethnic origin, psychological parameters, BMI, stress, anti-inflammatory drug use and smoking, were identified, emphasizing the role of endocrine disorders, genetic anomalies, anatomical abnormalities, immunological factors, infections, and environmental influences in recurrent abortions.

The influence of TORCH infections on the expression and regulation of the HOXB2 gene contributes to genetic mutations within the HOXB2 gene. The potential influence of TORCH infections on the genetic makeup of the HOXB2 gene, providing insights into the intricate relationship between these infections and the molecular processes associated with HOXB2 gene mutations.

Materials and Methods

The research focuses on examining the intricate interplay between genetic factors and molecular aspects. A systematic exploration of diverse databases including PubMed, Google Scholar, Scopus and Scopus was conducted to gather comprehensive information. The review was guided by specific keywords, including "HOXB2 gene mutation," "pregnancy loss," "aborted fetuses," "infected mothers," "TORCH infection," "fetal development" and "diagnosis." From an initial pool of 679,000 articles, a stringent screening process was applied, resulting in the identification of 180 relevant articles. Subsequently, 25 articles emerged as the most pertinent for constructing the review article, titled "HOXB2 mutation in aborted fetuses of infected mothers." The selected articles elucidate the complex connections among genes linked to pregnancy loss, genetic factors, and molecular aspects. They illuminate the role of the HOXB2 gene and its mutations in infected mothers, as well as the impact of TORCH infection. The insights gained from this synthesis provide valuable perspectives for future research and clinical understanding.

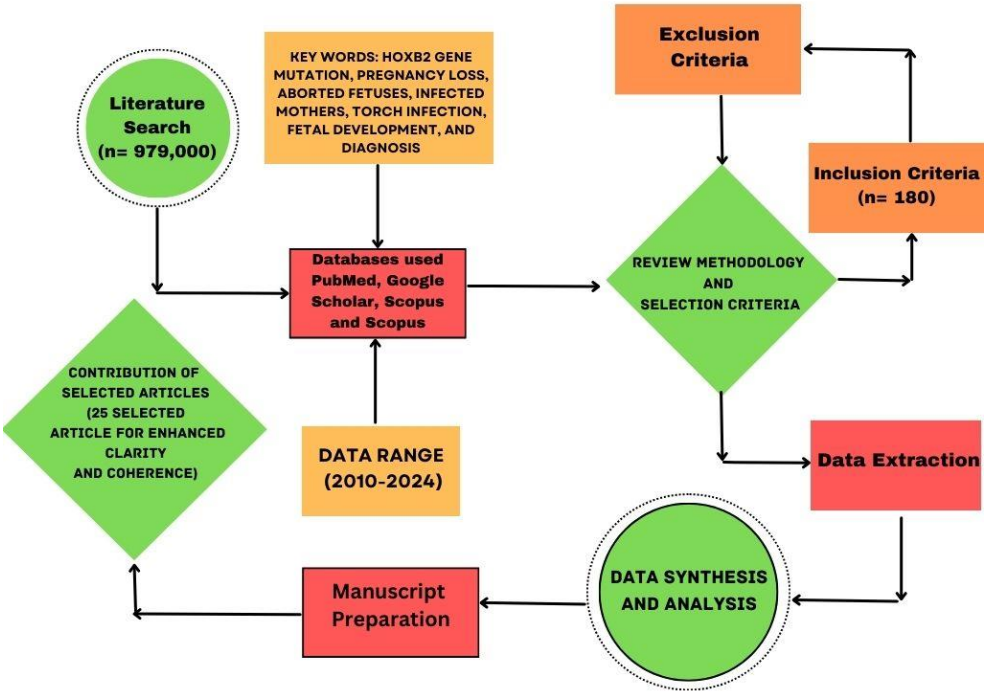

Fig.1: Methodology for Investigating HOXB2 Mutation and Its Implications in Aborted Fetuses of Infected Mothers.

Molecular Basis of HOXB2 Mutation

Homeobox genes (HOX), identified by a conserved 180-bp DNA sequence, function as transcription factors, regulating target genes [31]. Hogge et al. emphasized the crucial role of the HOX gene family, comprising 39 members grouped into A, B, C, and D based on chromosomal location, in controlling the body plan and specifying segment identity in the embryo [12]. Davenne et al. and Barrow and Capecchi underscored the significance of HOXB2 in embryonic development, particularly in hindbrain development and specifying skeletal structures in the second brachial arch [32, 33]. Acampora et al. added insights into HOXB2's genomic location on chromosome 17 [34].

Bhatlekar et al. emphasized the disruption of tissue balance in tumor development due to altered HOX gene expression [21]. Gehring et al. and Seifert et al. highlighted HOX genes' role as transcription factors governing differentiation, cell growth, and organogenesis (Figure 1) [22, 23]. Skinner et al. underscored Hox genes' critical function in controlling body plan and segment identity along the craniocaudal axis in embryonic tissues [35].

Human embryo and HOXB2

Seifert et al. emphasized the pivotal roles of Hox genes in both embryonic development and adult body repair mechanisms, influencing cell fate and driving stem cell differentiations in vitro and in vivo [23]. Barber and Rastegar highlighted the core principles of Hox gene functions in differentiation, offering pathways for stem cell manipulation in regenerative medicine. These genes are expected to significantly impact stem cell differentiation, both in vivo and in vitro, directly or inferred from tissue development effects. Additionally, epigenetic regulation of the Hox code may maintain stem cell characteristics in specific niches. Specifically, HOXB2 is essential for coordinating central nervous system and skeletal development, crucial for proper axial patterning and organogenesis by regulating downstream target genes in embryonic development [36].

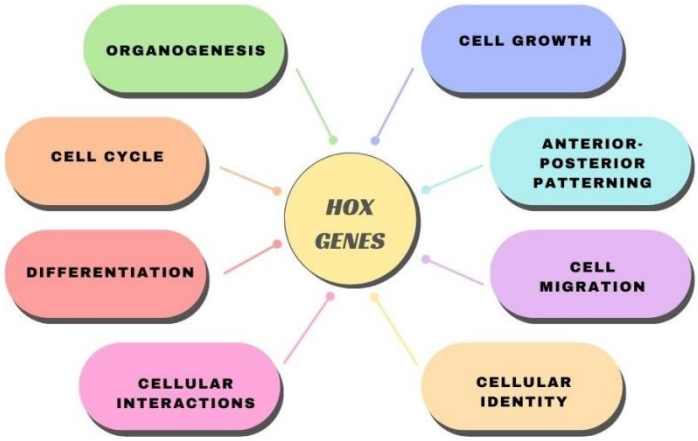

Figure 2: Functions of HOX Genes in Organisms and Cells

HOXB2 genes are crucial in various stem cells and differentiation processes. They maintain ESC pluripotency by repressing specific genes, while guiding adult hematopoiesis transition in HSCs [37]. Although Hox genes are generally absent, their induction in NCCs under specific conditions influences differentiation [38]. Diverse expressions of Hox genes in MSCs from different sources affect their differentiation potential [39]. Altered Hox gene expressions in CSCs are linked to tumor progression and therapy resistance [40]. iPS cells, like ESCs, regulate Hox genes during quiescence and differentiation, indicating the versatile functions of Hox genes in stem cell biology [41].

Cantile et al. highlighted the crucial roles of specific Hox genes like Hoxa4, a7 and Hoxd4 in adipogenic differentiation, linking their dysregulation to obesity-related diseases. In chondro-osteogenesis, Deprez et al. 2013 noted that the contribution of Hox genes, such as Hoxa2, Hoxd9 and Hoxd13, to bone and joint formation, influencing chondrogenesis and osteogenic

differentiation [42]. Potdar and Prasannan, emphasized the significance of Hox genes in cardiovascular lineages, influencing differentiation toward cardiomyocytes, endothelial cells and smooth muscle cells [43]. Additionally, Briscoe and Wilkinson, discussed the role of Hox genes in neurogenesis, impacting the development and differentiation of neural stem cells and shaping neural tissue formation. These Hox gene functions, as highlighted by various authors, provide valuable insights for potential therapeutic applications in regenerative medicine and various disease treatments [44].

Mechanisms of Mutation in the HOXB2 Gene

HOXB2 gene mutations can result from various mechanisms, including point mutations, insertions, deletions, or chromosomal rearrangements. These changes may disrupt normal HOXB2 function, leading to abnormal gene expression and potential developmental issues. In hematopoietic cells, research identified a crucial 5'-flanking sequence with a promoter fragment necessary for tissue-specific HOXB2 gene expression. Within this sequence, a GATA-binding site was found to be vital for promoter activity, confirmed through point mutation experiments. Gel shift analysis showed the formation of a specific complex involving the GATA-1 protein in erythroleukemic lines, indicating GATA-1's regulatory role upstream of the HOXB2 gene in erythroid cells (Vieille-Grosjean I & Huber P, 1995) [45].

During hindbrain development, Hoxb2 expression in rhombomere r4 relies on specific Hox response elements interacting with related proteins like Hoxa1 and Hoxb1, forming heterodimeric complexes with PBC homeodomain proteins (Pbx genes). Interestingly, while Hoxb1 and Hoxb2 enhancers in r4 share similar activity, they differ in the organization of Pbx/Hox sites, hinting at additional regulatory components [46]. HOXB2 is pivotal in coordinating embryonic development along the anterior-posterior axis by engaging in cross-regulatory interactions within the Hox gene cluster, ensuring precise spatial and temporal expression patterns. Furthermore, HOXB2's activity is subject to modulation by signalling molecules like retinoic acid, crucial for specifying neuronal identity during neural development [47].

HOX genes, like HOXB2, are crucial for determining cell identity and fate during embryonic development," states Steens & Klein. They govern complex molecular pathways influenced by diverse stem cell-related signalling cascades essential for adult stem cell functions. HOXB2 likely contributes to signalling pathways guiding cellular differentiation and tissue morphogenesis in various organs [48]. Its expression patterns across different developmental contexts suggest roles in organogenesis, including the nervous and skeletal systems, and other

embryonic mesoderm and ectoderm-derived structures. As a transcription factor, HOXB2 regulates downstream genes critical for development, including transcription factors, signalling molecules, and structural proteins. Moreover, HOXB2 is a target of retinoic acid, a crucial differentiation inducer maintaining mammary gland homeostasis. Upon activation, HOXB2 encodes a transcription factor inhibiting growth in breast cancer cells [49].

In various human breast cancer subtypes, HOXB2 has a distinct role, notably in the aggressive triple-negative subtype (TNBC), where its expression diminishes. This decline is linked to heightened metastatic potential, often associated with epithelial-to-mesenchymal transition (EMT) in TNBC cells. HOXB2 also restrains TNBC aggressiveness by influencing the organization of the extracellular matrix (ECM) through interactions with MATN3 and ECM2 promoter regions. Overexpression of HOXB2 effectively inhibits TNBC progression and metastasis in mouse xenograft models. Additionally, HOXB-AS1, a long non-coding RNA, in collaboration with the epigenetic regulator SMYD3, controls HOXB2 expression. Transcriptional regulation by HOXB2 is further influenced by interactions with cofactors and chromatin modifiers, refining gene expression during development [45].

Steens J & Klein D emphasized the pivotal role of the HOXB2 gene in embryonic development. They highlight its significance in orchestrating various cellular processes through interactions with other genes, signalling molecules and chromatin modifiers. Mutations in HOXB2 have the potential to disrupt this complex signalling pathway, leading to developmental abnormalities. Hence, comprehending the underlying molecular mechanisms of HOXB2 function is critical [48].

Table 2: Mechanisms, Functions and Roles of HOXB2 Gene

Mechanism of Mutation in HOXB2 Gene	Description	Reference
Transcriptional Regulation	HOXB2 gene expression in hematopoietic cells is regulated by a crucial 5'-flanking sequence containing a GATA-binding site, confirmed by GATA-1 protein's involvement in erythroid cells via gel shift analysis.	[45]
	Hoxb2 expression in hindbrain development is regulated by interactions between Hox response elements, Hoxa1, Hoxb1 and PBC homeodomain proteins, with distinct organization of Pbx/Hox sites in enhancers of r4.	[46]

Developmental Functions	Hox gene cluster cross-regulation coordinates anterior-posterior embryonic development, modulated by retinoic acid for neural identity specification.	[47]
	HOXB2 regulates embryonic cell identity and fate, influencing stem cell signalling cascades crucial for adult function and participates in pathways governing cellular differentiation and morphogenesis.	[48]
Role in Breast Cancer	HOXB2 involvement spans breast cancer subtypes, notably triple-negative, correlating its down regulation with heightened metastasis, while also regulating ECM and inhibiting TNBC progression.	[49]
	HOXB2 interacts with HOXB-AS1 and SMYD3, controlling its expression and modulating transcriptional regulation during development through interactions with cofactors and chromatin modifiers.	[50]
Importance in Embryonic Development	HOXB2 orchestrates diverse cellular processes, interacting with genes, signalling molecules and chromatin modifiers, with mutations leading to developmental abnormalities.	[45]
	HOXB2 coordinates embryonic development by ensuring precise spatial and temporal expression patterns.	[48]

Maternal Infections and HOXB2 Mutation

Maternal Infections on Fetal Development

Maternal infections play a significant role in influencing fetal development, with potential implications for HOXB2 mutation. The impact of these infections on fetal development involves intricate processes and pathways that warrant exploration Figure 2. Tosto et al. highlighted the critical role of placental histopathological examination in understanding immunohistopathological mechanisms involved in unfavourable perinatal outcomes. While SARS-CoV-2 infection can induce specific changes in placental tissue, the correlation between maternal infection, placental lesions and obstetric outcomes is not absolute [51]. Kwok J et al. discovered that maternal infections during pregnancy, particularly in the third trimester, were associated with decreased verbal IQ at age 4 and reduced verbal and performance IQ, as well as total IQ, at age 8. These findings suggest that maternal infections during specific gestational periods may have latent effects on cognitive development [52].

Tosto et al. and Neu et al. underscored the risks posed to both mother and fetus with TORCH infections representing Toxoplasmosis, Rubella, Cytomegalovirus (CMV), Herpes simplex virus (HSV) and others, can lead to diverse fetal abnormalities. Early pregnancy infections tend to result in worse outcomes, highlighting the importance of prompt medical intervention [51, 53]. Megli & Coyne, noted that TORCH infections can lead to pregnancy complications such as premature birth, intrauterine growth restriction, and miscarriage. Although the majority of miscarriages are not directly attributed to TORCH infections, these pathogens significantly contribute to adverse perinatal outcomes [54].

Scott & Lappin, emphasized the crucial role of the HOXB2 gene in embryonic development, particularly in forming various essential pregnancy-related tissues and organs. HOXB2's regulatory functions extend to processes like organogenesis, tissue patterning and cell differentiation [55]. As emphasized by Tosto et al. the significance of HOXB2 becomes evident in relation to TORCH infections. TORCH infections pose risks to both pregnant individuals and their developing fetuses, with adverse outcomes including congenital infections, intrauterine growth restriction, hearing impairment, low birth weight, and stillbirth. These pathogens can be transmitted to the fetus through the placenta during pregnancy, delivery or breastfeeding. HOXB2's involvement in placental development suggests a potential link between HOXB2 dysregulation and susceptibility to TORCH infections. Mutations affecting HOXB2 function may disrupt placental integrity, impacting nutrient exchange and fetal growth. Furthermore, altered HOXB2 expression or function could compromise the immune response to TORCH pathogens, potentially increasing the risk of vertical transmission to the fetus [51].

Transmission Routes of Infections to the Fetus

The transmission routes of infections to the fetus are crucial aspects to consider in comprehending the association between maternal infections and HOXB2 mutation [56]. Magklara et al. and Dadwal & Bhatt, emphasized the placenta as the primary route for infections to reach the developing fetus. Transplacental transmission, particularly significant for vertically transmitted infections like TORCH pathogens, occurs when pathogens breach the placental barrier during pregnancy. This can lead to adverse outcomes such as congenital infections, intrauterine growth restriction, and stillbirth. Understanding transplacental transmission dynamics is essential for unravelling the interplay between maternal infections and fetal development [57, 58].

In addition, infections can ascend from the maternal reproductive tract during childbirth, described by Klein [59]. This ascending route involves pathogens moving from the lower genital tract upward into the uterus and amniotic cavity. Rupture of membranes during labor or invasive procedures like amniocentesis can expose fetus to pathogens. Proper infection control measures during labor and delivery are crucial to minimize the risk of ascending infections. Additionally, blood-borne transmission, discussed by Aeeza Malik, poses a risk as pathogens circulate in the maternal bloodstream and cross the placenta to reach the fetus. These transmission routes underscore the importance of early detection and management of maternal infections. Moreover, the impact of HOXB2 mutations in aborted fetuses of infected mothers should be considered, potentially exacerbating adverse outcomes associated with maternal infections [60].

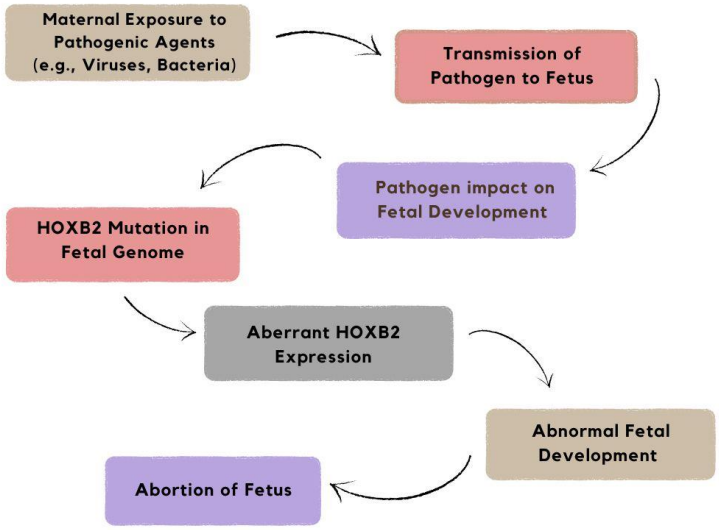

Figure 3: Scheme of HOXB2 Mutation in Aborted Fetuses of Infected Mothers.

Conclusion

There is an intricate relationship between maternal infections, HOXB2 mutation and their impact on fetal development. Maternal infections, particularly TORCH infections, pose substantial risks to both mother and fetus, leading to adverse outcomes like congenital infections and intrauterine growth restriction. Pathogens can breach the placental barrier through transplacental transmission, ascending infections during childbirth or blood-borne transmission. HOXB2, critical in embryonic development and placental formation, may influence susceptibility to TORCH infections. Mutations in HOXB2 could disrupt placental integrity, nutrient exchange, and immune response, potentially increasing vertical transmission risk and adverse outcomes. The impact of maternal infections on fetal brain development involves complex interactions, potentially mediated by inflammatory cytokines.

Understanding transmission routes is crucial for comprehending maternal infection-HOXB2 mutation associations. Proper infection control during pregnancy, labor and delivery is vital. Further research is needed to elucidate molecular mechanisms underlying HOXB2 mutation in aborted fetuses of infected mothers and its implications for maternal and fetal health. By unravelling these complexities, more effective strategies can be developed so as to prevent and manage maternal infections and their impact on fetal development.

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Author's Contributions

Abdul Adil V K – Drafted the manuscript

Paneer Selvam K – Critically reviewed the manuscript

K V Nisha – Contributed to reference collections

Dinesh Roy D – Helped in drafting the manuscript and critically reviewed the manuscript

Conflict of Interest

The authors declare that there is no conflict of interest.

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