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Review

Warfarin-Induced Developmental Toxicity: Insights into Embryogenesis, Teratogenicity, and Molecular Pathways

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Abstract

Warfarin is a coumarin-derived oral anticoagulant widely used for the prevention and treatment of thromboembolic disorders, particularly in patients with mechanical heart valves. The drug exerts its anticoagulant effect by inhibiting vitamin K epoxide reductase, thereby impairing γ-carboxylation of vitamin K–dependent coagulation factors. Despite its clinical efficacy, warfarin therapy is associated with a narrow therapeutic index, substantial interindividual variability in dose response, numerous drug interactions, and significant hemorrhagic risk. Maternal warfarin therapy during pregnancy is strongly associated with fetal warfarin syndrome (FWS), a characteristic pattern of embryopathy resulting from in utero exposure to the drug. This review summarizes current knowledge regarding the physicochemical properties, pharmacological mechanisms, dose variability, toxicity, and developmental effects associated with warfarin exposure. Evidence from human clinical studies and vertebrate animal models is discussed to elucidate conserved developmental and molecular mechanisms underlying warfarin teratogenicity. The review also examines signaling pathways disrupted by warfarin exposure that are involved in bone morphogenesis, vascular homeostasis, and tissue mineralization, contributing to the observed phenotypes. Collectively, this review integrates clinical, molecular, and experimental findings to provide a comprehensive understanding of warfarin-induced developmental toxicity. Current knowledge is insufficient to fully elucidate the complex mechanisms underlying warfarin-induced embryopathy and fetal toxicity. Further investigations are warranted to identify safer anticoagulant regimens during pregnancy and to inform the development of novel therapeutic strategies that minimize fetal risk while maintaining maternal anticoagulation.

Keywords: warfarin; developmental toxicity; embryogenesis; warfarin fetal syndrome; warfarin signaling; teratogenicity

1. Introduction

Warfarin is a synthetic derivative of 4-hydroxycoumarin and belongs to the coumarin class of anticoagulants widely used for the prevention and treatment of thromboembolic disorders. Chemically, warfarin is identified as 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one, with the molecular formula $C_{19}H_{16}O_4$ and a molecular weight of 308.33 g/mol. Warfarin is a synthetic coumarin-derived anticoagulant that appears as a white to off-white crystalline powder. The compound has poor aqueous solubility but dissolves readily in several organic solvents, including ethanol, methanol, and acetone. Due to its limited aqueous solubility, dissolution and oral absorption can vary. To overcome these limitations and enhance pharmaceutical utility, warfarin is frequently formulated as warfarin sodium, which is more water-soluble. Chemically, warfarin behaves as a weak acid due to the presence of a hydroxyl functional group in the coumarin structure, with a pKa of approximately 5.0–5.2. Under physiological conditions, a considerable proportion of the drug

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remains ionized, influencing its absorption, tissue distribution, and protein-binding characteristics. Warfarin is highly bound to plasma albumin, with protein binding exceeding 99%, thereby limiting its volume of distribution and increasing its susceptibility to clinically significant drug–drug interactions via protein displacement [1].

Structurally, it is a δ-hydroxy ketone that exists as two enantiomers with distinct pharmacological activities (Figure 1). Warfarin also demonstrates keto–enol tautomerism, a structural feature that contributes to its chemical reactivity, stability, and pharmacological activity. The molecular structure contains both hydrophobic aromatic moieties and polar functional groups, resulting in moderate lipophilicity, with reported logP values ranging from approximately 2.7 to 3.2. The melting point of warfarin is generally reported between 160°C and 165°C, although slight variations may occur depending on crystal form and purity. In addition, the compound may degrade upon prolonged exposure to light and moisture, necessitating controlled storage conditions to preserve stability and therapeutic efficacy. Collectively, the physicochemical properties of warfarin significantly influence its formulation characteristics, pharmacokinetic profile, and overall clinical performance (Table 1). These physicochemical attributes support effective gastrointestinal absorption after oral administration and influence hepatic metabolism and elimination. The 4-hydroxycoumarin scaffold undergoes tautomerism between the 4-hydroxycoumarin, 2-hydroxychromone, and 2,4-chromandione forms and constitutes the active moiety of several clinically relevant anticoagulants, including bishydroxywarfarin, phenprocoumon, and acenocoumarol [2]. These coumarin derivatives exert their anticoagulant activity by inhibiting the vitamin K cycle, thereby impairing the regeneration of vitamin K hydroquinone, an essential cofactor for γ-glutamyl carboxylation and subsequent activation of vitamin K–dependent coagulation factors [3].

Table 1. Physicochemical Properties and Pharmacological Observations of Warfarin and Its Derivatives.

Name	Mol. Weight (g/mol)	Pharmacological Observations		Source
Warfarin	308.3	Parent compound used as comparator		PubChem [1]
6-hydroxywarfarin	324.3	Significantly earlier minimum mean percent of normal prothrombin complex activity (%PCA), with activity prior to mean similar to regular warfarin (in rabbit model).		Obaseki (1987) [90]
7-hydroxywarfarin	324.3			
8-hydroxywarfarin	324.3			
4′-hydroxywarfarin	324.3	Similar to other hydroxy-analogs with the exception of half the minimum mean %PCA (in rabbit model).		
6-chlorowarfarin	342.77	Decrease in 'real' anticoagulant activity, minimum mean %PCA was higher and occurred around 2/3rds of a day sooner, although activity to that point mirrored that of regular warfarin (in rabbit model).		
6-bromowarfarin	387.22			
6-fluorowarfarin	326.32	IC_{50} was 0.79 times R-warfarin in rat microsomes. Tighter HSA binding than S-	Not metabolized to 6- or 7-hydroxywarfarin by CYP2C9, despite binding with similar high affinities	Zhang (1997) [92], Kerr (1997) [89]

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		warfarin. Increased PT time comparable or longer than S-warfarin.	(measured in human liver microsomes with competitive inhibition of S-warfarin binding).	
7-fluorowarfarin	326.32	IC_{50} was 0.87 times R-warfarin in rat microsomes.		
6,7,8-trifluorowarfarin	362.30			Zhang (1997) [92]
^11C alcohol analog	324.37	IC_{50} was twice that of R-warfarin in rat microsomes. Did not increase PT time above controls at 1600 ug/day.		
4C $COCH_3$ ether substituent	350.4	Increased prothrombin time (PT) time comparable or longer than S-warfarin. IC_{50} was 1.70 times that of R-warfarin.		Kerr (1997) [89]
4C $CO(CH_2)_2CH_3$ ether substituent	378.4	IC_{50} was 1.80 times that of R-warfarin.		

Note: Molecular weights were calculated from the molecular formulas of the respective compounds using standard atomic masses.

The anticoagulant properties of warfarin were first recognized following investigations into fatal hemorrhagic disease in cattle that consumed spoiled sweet clover in the early twentieth century [3]. After its commercial introduction as a rodenticide in 1948, warfarin was subsequently adapted for clinical use in the 1950s and gained widespread medical acceptance following its administration to President Dwight Eisenhower after his 1955 myocardial infarction [4]. Since then, warfarin has remained a cornerstone therapy for the prevention and management of thromboembolic disorders, including deep vein thrombosis (DVT), pulmonary embolism, and cardioembolic stroke [5]. Although several direct oral anticoagulants have emerged as alternatives to warfarin, specific clinical scenarios, particularly anticoagulation in patients with mechanical heart valves, continue to require warfarin due to the limited efficacy or safety of current alternatives [6].

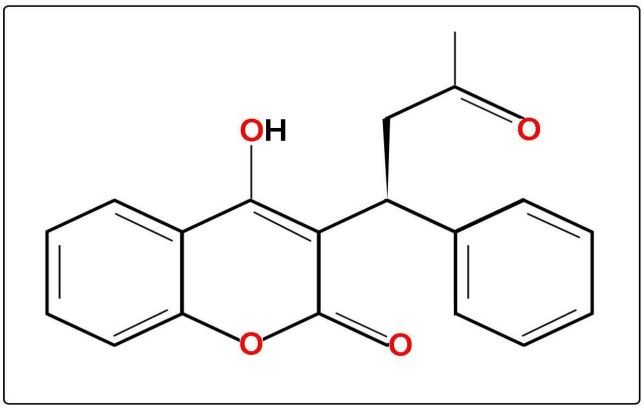

Figure 1. Structure of S-Warfarin. An anticoagulant drug, (S)-warfarin sodium, is an organic sodium salt having 2-oxo-3-[(1S)-3-oxo-1-phenylbutyl]-2*H*-1-benzopyran-4-olate. It is an enantiomer of (R)-warfarin. Reference: PubChem CID 54688261.

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Despite its therapeutic importance, warfarin is a well-established teratogen associated with dose-dependent embryofetal toxicity and a spectrum of developmental abnormalities collectively termed warfarin embryopathy [7]. Warfarin readily crosses the placenta and can enter the fetal circulation, thereby exposing the developing fetus to its pharmacological and teratogenic effects. Consequently, anticoagulation management during pregnancy presents a significant clinical challenge in balancing maternal thromboembolic risk against fetal developmental toxicity. Here, we aim to combine all information on the properties, mechanism of action, and signaling mechanisms underlying warfarin toxicity, as well as its teratogenic effects during development.

The search for original articles was performed using databases, including Scopus, PubMed, Google Scholar, and ScienceDirect, with MeSH terms such as *warfarin, anticoagulant therapy, warfarin embryopathy, fetal warfarin syndrome, warfarin teratogenicity, and effects of warfarin during development.* Web sources such as the National Institutes of Health (NIH), the World Health Organization (WHO), and NCBI-PubChem were also referred to obtain information on warfarin. The AI tools ChatGPT and Gemini were also used to identify any sources missed by the referenced database sources. Peer-reviewed studies published in English, including original research studies, clinical trials, reviews, and case reports, were considered for inclusion. Relevant studies were selected through title, abstract, and full-text screening, and additional references were identified from the bibliographies of selected articles. The retrieved literature was critically evaluated and synthesized to provide a comprehensive overview of warfarin pharmacology, clinical applications, adverse effects, teratogenicity, and alternative anticoagulant therapies.

2. Pharmacological Mechanism of Warfarin

The anticoagulant activity of warfarin is mediated through inhibition of the vitamin K cycle, thereby impairing the post-translational activation of vitamin K-dependent proteins (VKDPs). Vitamin K compounds are lipophilic naphthoquinones that occur naturally as phylloquinone (vitamin K_1) and the menaquinone (vitamin K_2) family of isoprenologues. Dietary vitamin K consists predominantly of phylloquinone, which is absorbed in the proximal small intestine after incorporation into mixed micelles containing bile salts and dietary lipids [8]. Intestinal uptake is facilitated by membrane transport proteins, including scavenger receptor class B type 1 (SR-BI), cluster of differentiation 36 (CD36), and Niemann–Pick C1-like 1 (NPC1L1). During absorption, a fraction of vitamin K_1 undergoes side-chain cleavage to menadione, which is subsequently converted by UBIAD1 (UbiA prenyltransferase domain-containing protein 1) into menaquinone-4 within peripheral tissues. In addition to its role in γ-glutamyl carboxylation, menaquinone-4 has been implicated in cellular signaling and gene regulatory pathways[8,9].

Vitamin K serves as an essential cofactor for γ-glutamyl carboxylase (GGCX), an integral membrane enzyme localized primarily in the endoplasmic reticulum and Golgi apparatus [10]. GGCX catalyzes the conversion of glutamate residues to γ-carboxyglutamate residues within VKDPs, enabling calcium-dependent conformational activation. This reaction occurs concomitantly with the oxidation of vitamin K hydroquinone to vitamin K epoxide. Although hepatic activation of coagulation factors represents the principal physiological role of GGCX, VKDPs are also involved in bone mineralization, growth regulation, and intracellular signaling pathways [11].

Maintenance of VKDP activation depends upon efficient recycling of vitamin K through the vitamin K cycle. Vitamin K epoxide is reduced to vitamin K quinone by vitamin K epoxide reductase (VKOR), followed by reduction to vitamin K hydroquinone via mechanisms that have been proposed to involve either VKOR itself or alternative NAD(P)H-dependent reductases [9]. Warfarin exerts its pharmacological effect through competitive inhibition of VKOR, thereby depleting intracellular vitamin K hydroquinone stores and impairing γ-carboxylation of VKDPs. Consequently, synthesis of biologically active coagulation factors II, VII, IX, and X, together with proteins C and S, is reduced.

Warfarin is rapidly absorbed following oral administration, with peak plasma concentrations achieved within 0.3–4 hours[3]. More than 99% of circulating warfarin is bound to plasma proteins, predominantly albumin, leaving only a small pharmacologically active free fraction. Hepatic uptake

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may involve organic anion transporter 2 (OAT2), while breast cancer resistance protein (BCRP) has also been implicated in experimental models [12,13]. Following distribution, warfarin accumulates primarily in hepatic and renal tissues [14].

The drug is administered as a racemic mixture of R- and S-warfarin, with S-warfarin demonstrating substantially greater anticoagulant potency. Hepatic metabolism occurs in a stereo- and regioselective manner via cytochrome P450 enzymes located within the endoplasmic reticulum. S-warfarin is metabolized predominantly by CYP2C9 to 7-hydroxywarfarin and 6-hydroxywarfarin, with smaller quantities of 10- and 4′-hydroxywarfarin also produced. A minor proportion undergoes reduction to inactive alcohol metabolites. R-warfarin undergoes comparatively slower metabolism, generating primarily 6- and 7-hydroxywarfarin, as well as reduced alcohol derivatives [3]. Metabolites are excreted primarily in urine, and elimination of S-warfarin occurs approximately twice as rapidly as that of R-warfarin. Inter-individual variability in warfarin response is strongly influenced by genetic polymorphisms affecting both pharmacokinetic and pharmacodynamic pathways. Variants in *CYP2C9* significantly alter metabolic clearance and dose requirements, while polymorphisms in *VKORC1* contribute substantially to differences in warfarin sensitivity and resistance [15,16]. Additional contributions from *CYP4F2* polymorphisms have also been reported[5].

At the molecular level, warfarin binds reversibly within the VKOR active site through hydrophobic and aromatic stacking interactions. Structural modeling studies have demonstrated a critical T-shaped interaction between warfarin and the tyrosine residue Y139, with residues Y25 and A26 further contributing to ligand stabilization [17]. Mutations affecting these residues significantly diminish binding affinity and confer resistance phenotypes. In particular, alteration of Y139 markedly weakens warfarin–VKOR interactions and has been associated with profound resistance, whereas Y25 mutations have been identified in resistant rodent populations[17,18]. Inhibition of VKOR ultimately disrupts vitamin K recycling and prevents activation of VKDPs, forming the mechanistic basis for both the therapeutic anticoagulant effect of warfarin and the developmental abnormalities associated with warfarin embryopathy.

3. Warfarin Dose Variability, Toxicity, and Clinical Complications

Warfarin therapy requires meticulous dose individualization owing to its narrow therapeutic index, marked interindividual variability, and complex pharmacokinetic and pharmacodynamic profile. Therapeutic dosing is influenced by numerous clinical variables, including age, body mass, hepatic function, dietary vitamin K intake, comorbidities, and concomitant pharmacotherapy. Warfarin is administered as a racemic mixture comprising the pharmacologically distinct S- and R-enantiomers, which exhibit elimination half-lives of approximately 32 h and 43 h, respectively[3]. Owing to considerable variability in anticoagulant response, therapeutic monitoring is achieved through serial assessment of the prothrombin time, a blood test that measures how long it takes blood to clot through the extrinsic and common coagulation pathways and is standardized as the international normalized ratio (INR), which remains the cornerstone of dose titration and anticoagulation management.

Genetic determinants contribute substantially to variability in warfarin sensitivity and maintenance dose requirements. Polymorphisms in *CYP2C9*, the principal enzyme responsible for oxidative metabolism of S-warfarin, reduce metabolic clearance and prolong systemic exposure to the active enantiomer. In particular, the *CYP2C9**3 allele has consistently been associated with lower maintenance dose requirements, delayed attainment of stable anticoagulation, and an increased risk of over-anticoagulation during treatment initiation[15]. Variants in *VKORC1*, which encodes vitamin K epoxide reductase complex subunit 1, exert additional pharmacodynamic effects by altering sensitivity to VKOR inhibition, thereby representing a major determinant of dose variability across populations [16]. Polymorphisms in *CYP4F2*, which participate in vitamin K oxidation, have also been implicated in modulating dose requirements, although their contribution appears to be comparatively modest [5]. Consequently, incorporation of pharmacogenetic data into dosing

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algorithms has emerged as an important strategy for optimizing anticoagulation therapy and minimizing adverse outcomes.

Hemorrhage remains the most clinically significant adverse effect associated with warfarin therapy and represents the principal limitation to its long-term use[19,20]. Bleeding complications may range from minor mucocutaneous manifestations to severe or fatal hemorrhage involving the gastrointestinal tract, intracranial compartment, or other major organ systems[21]. The risk of hemorrhage is influenced by excessive anticoagulation, advanced age, interacting medications, hepatic dysfunction, and comorbid disease states[3]. In addition to bleeding complications, warfarin therapy has been associated with systemic atheroembolism and cholesterol microembolization syndromes.

A broad spectrum of non-hemorrhagic adverse effects has also been described. Immunologic and hypersensitivity manifestations include allergic reactions and vasculitic phenomena, while hepatobiliary complications encompass hepatitis and elevations in hepatic transaminases[22]. Gastrointestinal adverse effects may include nausea, vomiting, diarrhea, abdominal pain, bloating, flatulence, and dysgeusia. Dermatologic complications, such as rash, dermatitis, pruritus, and alopecia, have also been reported. Less frequently, respiratory complications, including tracheal and tracheobronchial calcification, may occur, together with generalized constitutional symptoms such as chills [22]. Among the most severe non-hemorrhagic complications is warfarin-induced skin necrosis, a rare but potentially life-threatening condition associated with substantial morbidity and mortality[23,24]. This complication most commonly develops in the initial days of therapy and is believed to arise from a transient hypercoagulable state secondary to the rapid depletion of the endogenous anticoagulant proteins C and S relative to procoagulant factors. Clinically, affected patients initially develop painful erythematous lesions that may rapidly progress to purpura, hemorrhagic bullae, and full-thickness cutaneous necrosis, particularly within adipose-rich anatomical regions such as the breasts, thighs, buttocks, and abdomen. Early recognition and prompt discontinuation of warfarin are critical to limiting tissue destruction and improving clinical outcomes.

4. Phenotypic Spectrum of Fetal Warfarin Syndrome in Humans

Warfarin exposure during pregnancy is associated with significant embryotoxic and fetotoxic risk. The severity of fetal complications demonstrates a dose-dependent relationship, with higher maternal doses correlating with increased incidence of spontaneous abortion, stillbirth, and warfarin embryopathy[19]. Maternal doses exceeding 5 mg/day were associated with substantially greater risk of adverse fetal outcomes. The teratogenic effects of warfarin are primarily attributed to disruption of vitamin K-dependent γ-carboxylation pathways essential for normal skeletal and connective tissue development during embryogenesis.

Warfarin remains one of the few anticoagulants considered effective for preventing thromboembolism in pregnant patients with mechanical heart valves, despite its well-established teratogenic potential[19,25]. Maternal warfarin dosage may influence the likelihood of adverse fetal outcomes. Pregnant patients receiving warfarin doses greater than 5 mg daily have demonstrated increased rates of fetal complications, including spontaneous abortion, compared with patients maintained on doses of 5 mg daily or less. Most documented cases of warfarin embryopathy involve maternal doses between 5 and 7.5 mg daily, although many do not specify a dosage. Importantly, the most common abnormalities—including nasal hypoplasia, saddle nose deformity, epiphyseal stippling, and distal phalangeal hypoplasia—have been reported following both low- and high-dose warfarin exposure. Fetal exposure to warfarin during pregnancy can result in a spectrum of congenital abnormalities collectively referred to as warfarin embryopathy (WE) or fetal warfarin syndrome (FWS)[26–28] (Table 2). The resulting phenotypes are highly variable and depend on the timing, duration, and dosage of exposure during gestation. The risk of classic warfarin embryopathy is greatest when exposure occurs during the first trimester, particularly between the sixth and ninth weeks of gestation, when organogenesis and cartilage development are highly active[26,29,30]. However,

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adverse fetal outcomes may also occur following second- and third-trimester exposure, resulting in severe neurological injury, growth restriction, and fetal loss, indicating that teratogenicity is not restricted to early gestation.

Warfarin embryopathy demonstrates considerable phenotypic variability and can affect the craniofacial, musculoskeletal, cardiopulmonary, and central nervous systems. The most characteristic feature is nasal hypoplasia, typically presenting with a depressed nasal bridge, a short upturned nose, and underdevelopment of the nasal cartilage (Figure 2A) [19,26,29–42]. Additional nasal abnormalities commonly reported alongside nasal hypoplasia include a flattened or absent nasal bridge, deep ala nasi grooves, anteverted nostrils, nasal stenosis, absent nasal bones, choanal atresia, and respiratory complications secondary to laryngomalacia or tracheomalacia[29,31,35,43]. Other craniofacial findings include micrognathia, cleft lip or palate, frontal bossing, hypertelorism, and facial asymmetry[31,32,43]. Some infants also present with choanal stenosis or airway abnormalities, including laryngomalacia and tracheomalacia, which may contribute to neonatal respiratory distress (Table 2).

Table 2. Craniofacial Phenotypes Reported Following Prenatal Warfarin Exposure in Humans.

Phenotype	Key Findings	Sources	Representative Exposure Range	Timeline
		Hypoplasia		
Nasal hypoplasia:	Incomplete development of these regions, leading to affected morphology	Stevenson (1980) [29], Gupta (2010) [43], Howe (1997) [30], Harrod (1981) [32], Birbal (2023) [34], Tongsong (1999) [35], Hou (2004) [36], Ferreira (2018) [38], Dilli (2011) [41], Vitale (1999) [19], Pauli (1987) [27], Basu (2016) [42], Vilhena (2015) [40], Chan (2003) [31], Raghav (2007) [37].	5 mg/day [29,34,36,38,43] 5 mg/day alternating with 10 mg/day [41] 11 mg/day, reduced to 6 mg/day [30] 12.5 mg/day [32] 10 mg/day [35].	Throughout pregnancy [29,35,36,41], Until 9 gestational weeks then from 12 gestational weeks onwards [43], Until 4 gestational weeks then from 12 gestational weeks onward [34], Until 6 gestational weeks then from 12 gestational weeks onward [38], Reduced dose mid gestation discontinued at 2 weeks before delivery [30], From the 6th through 24th week of gestation [32].
Maxillary hypoplasia:		Howe (1997) [30]	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Midfacial hypoplasia:		Barr (1976) [45], Pati (1994) [46].	7.5 mg/day [45].	Discontinued one week prior to delivery [45].
Mandibular hypoplasia:		Chan (2003) [31], Stevenson (1980) [29].	5 mg/day [29].	Throughout pregnancy [29].
Microstomia	Reduced size of the oral opening, causing difficulty when opening the mouth	Chan (2003) [31].		
Absent nasal bones	Lack of nasal bones in the facial structure	Ferreira (2018) [38], Mehndiratta (2010) [39], Basu (2016) [42].	5 mg/day [38] 3 mg/day [39].	Until 6 gestational weeks, then from 12 gestational weeks onward [38], throughout pregnancy [39].
Saddle nose	Collapse or deformity of the nasal bridge, creating a saddle-like appearance	Stevenson (1980) [29], Chan (2003) [31], Harrod (1981) [32], Tongsong (1999) [35], Hou (2004) [36], Mehndiratta (2010) [39], Vitale (1999) [19], Basu (2016) [42], Dilli (2011) [41], Songmen (2017) [44], Vilhena	5 mg/day [29] 5-6 mg/day [44] 10 mg/day [35] 3 mg/day [39] 5 mg/day alternating with 10 mg/day [41].	Throughout pregnancy [29,35,36,39,41], Until 35 weeks of gestation [44].

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		[29,35,36,39,41(2015) [40], Wainwright (2010) [47].		
Short or small nose	Reduced length or profile of the nose	Vilhena (2015) [40], Wainwright (2010) [47], Howe (1997) [30], Hou (2004) [36], Pauli (1987) [27], Dilli (2011) [41].	11 mg/day, reduced to 6 mg/day [30] 5 mg/day [36].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30], Throughout pregnancy [36].
Nasal stenosis	Weakening or narrowing of the nasal passages	Stevenson (1980) [29], Gupta (2010) [43], Chan (2003) [31], Songmen (2017) [44].	5 mg/day [29,43] 5-6 mg/day [44].	Throughout pregnancy [29], Until 35 weeks of gestation [44], Until 9 gestational weeks then from 12 gestational weeks onwards [43].
Deep ala nasi groove	Pronounced groove between the ala and the tip of the nose	Stevenson (1980) [29], Hou (2004) [36], Pauli (1987) [27], Basu (2016) [42], Dilli (2011) [41], Songmen (2017) [44].	5 mg/day [29,36] 5-6 mg/day [44].	Throughout pregnancy [29, 36,41], Until 35 weeks of gestation [44].
Anteverted nostrils	Front- and/or upward-facing nostrils	Stevenson (1980) [29], Basu (2016) [42].	5 mg/day [29].	Throughout pregnancy [29].
Laryngomalacia with tracheomalacia	Weakness and lacking rigidity of the tracheal cartilage and the tissue covering the vocal cords	Hou (2004) [36], Basu (2016) [42].	5 mg/day [36].	Throughout pregnancy [36].
Choanal atresia	Blockage of the posterior nasal passage by soft tissue or bone formation	Chan (2003) [31], Songmen (2017) [44].	5-6 mg/day [44].	Until 35 weeks of gestation [44].
Flattening of the face	Depression of facial structures creating a diminished side profile	Chan (2003) [31], Tongsong (1999) [35].	10 mg/day [35].	Throughout pregnancy [35].
Hypertelorism	Increased intercanthal (between the eyes) distance	Barr (1976) [45], Dilli (2011) [41], Songmen (2017) [44].	7.5 mg/day [45] 5 mg/day alternating with 10 mg/day [41] 5-6 mg/day [44].	Throughout pregnancy [41] Until 35 weeks of gestation [44] Discontinued one week prior to delivery [45].
Auricular fold hypoplasia	'Cupped ear' presentation where folds inside the pinna have failed to fully develop	Chan (2003) [31].		
Pinnae hypoplasia	Incomplete development of the external ear cartilage	Chan (2003) [31].		
Flattening of the occipital area		Chan (2003) [31].		
Macrocephaly		Tongsong (1999) [35], Stevenson (1980) [29].	10 mg/day [35] 5 mg/day [29].	Throughout pregnancy [29,35].

Note: Gestational exposure periods are reported as described in the original studies. Craniofacial abnormalities are most commonly associated with warfarin exposure during weeks 6–12 of gestation, although abnormalities have also been reported following exposure throughout pregnancy.

Another hallmark of warfarin embryopathy is epiphyseal stippling, also known as chondrodysplasia punctata, which results from abnormal calcification in developing cartilage. Epiphyseal stippling may affect multiple skeletal regions, including the ankles, hips, tarsals, patellae, phalanges, talus, and most frequently within the humerus, vertebral column, femur, and calcaneus (Figure 2B-C) [26,27,30–32,34–36,38,39,41–44]. Infants with nasal hypoplasia frequently demonstrate concomitant epiphyseal stippling, reflecting the high prevalence of these two defining phenotypes within fetal warfarin syndrome[29–32,35,36,38]. Although stippling may diminish with age, affected individuals

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frequently exhibit persistent orthopedic abnormalities, including limb shortening, scoliosis, joint deformities, and abnormal gait.

Skeletal abnormalities beyond epiphyseal stippling are also frequently observed. Distal phalangeal hypoplasia, limb shortening, and generalized growth restriction have been repeatedly documented in affected infants. Although these findings occur less consistently than nasal hypoplasia or stippled epiphyses, they remain important diagnostic indicators of prenatal warfarin exposure. Hypoplastic distal phalanges, brachydactyly, shortened extremities, and nail abnormalities have also been described in infants with FWS. Additional musculoskeletal findings include vertebral anomalies, pectus deformities, hip dysplasia, and delayed skeletal maturation. In severe cases, generalized growth restriction and low birth weight may accompany these structural abnormalities, suggesting broader disruption of fetal growth and connective tissue development (Table 3) [19,26,27,29,30,32,35,36,39,45,46].

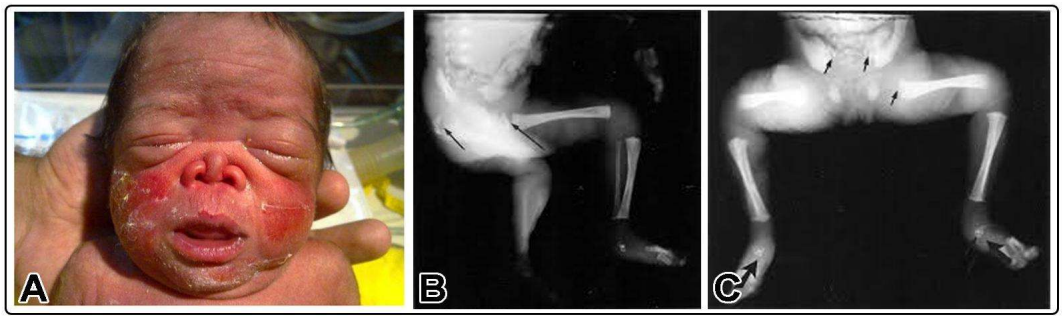

Figure 2. Photographic (A) and radiographic (B, C) representation of Warfarin Embryopathy. (A) Preterm newborn at 33 weeks of pregnancy delivered a male fetus with nasal hypoplasia characterized by a depressed nasal bridge and flat upturned nose (taken from Gupta et al., 2010) [43]. **(B-C)** Aborted fetus following termination of pregnancy at 23 2/7 weeks' gestation, showing bilateral calcific stippling of the proximal femoral epiphyses (B), the iliac wings, and the tarsal bones (C) (taken from Chan et al., 2003) [31]. .

Table 3. Skeletal and Musculoskeletal Abnormalities Reported Following Prenatal Warfarin Exposure in Humans.

Phenotype	Key Findings	Sources	Representative Exposure Range	Timeline
Pectus carinatum deformity	Anteriorly protruding sternum and ribs	Stevenson (1980) [29], Hou (2004) [36].	5 mg/day [29,36].	Throughout pregnancy [29,36].
Pectus excavatum	Posterior displacement of the sternum leading to a sunken-chest appearance	Vilhena (2015) [40].		
Depressed muscle tone	Low passive muscle resistance	Stevenson (1980) [29], [D] Howe (1997) [30], Sherman (1976) [33].	5 mg/day [29] 11 mg/day, reduced to 6 mg/day [30].	Throughout pregnancy [29], Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
		Chondrodysplasia punctata (epiphyseal stippling)		
Unspecified	Appearance of 'dots' of calcification in the cartilaginous growth plates of bones and other cartilage tissue	Gupta (2010) [43], Birbal (2023) [34], Wainwright (2010) [47].	5 mg/day [34,43].	Until 4 gestational weeks then from 12 gestational weeks onward [34], Until 9 gestational weeks then from 12 gestational weeks onwards [43].
Vertebral epiphyses		Stevenson (1980) [29], Howe (1997) [30], Harrod (1981) [32], Tongsong	5 mg/day [29]	Throughout pregnancy [29,35,36,41], Reduced dose mid gestation discontinued at 2 weeks before delivery [30], From the 6th

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	(1999) [35], Hou (2004) [36], Pauli (1987) [27], Basu (2016) [42], Dilli (2011) [41].	5 mg/day alternating with 10 mg/day [41] 11 mg/day, reduced to 6 mg/day [30] 12.5 mg/day [32].	through 24th week of gestation [32].
Talus / ankle epiphyses	Howe (1997) [30], Basu (2016) [42], Dilli (2011) [41].	11 mg/day, reduced to 6 mg/day [30] 5 mg/day alternating with 10 mg/day [41].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30], Throughout pregnancy [41].
Humerus	Howe (1997) [30], Basu (2016) [42].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Carpal bones	Howe (1997) [30].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Elbow epiphyses	Howe (1997) [30].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Distal tibia	Howe (1997) [30].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Femur epiphyses	Chan (2003) [31], Tongsong (1999) [35], Ferreira (2018) [38], Mehndiratta (2010) [39], Basu (2016) [42].	10 mg/day [35] 5 mg/day [38] 3 mg/day [39].	Throughout pregnancy [35,39], Until 6 gestational weeks then from 12 gestational weeks onward [38].
Patellar epiphyses	Basu (2016) [42].		
Hips and Pelvic bones	Basu (2016) [42], Chan (2003) [31], Howe (1997) [30].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Tarsal epiphyses	Chan (2003) [31], Harrod (1981) [32], Basu (2016) [42].	12.5 mg/day [32].	From the 6th through 24th week of gestation [32].
Calcaneal epiphyses	Harrod (1981) [32], Hou (2004) [36], Mehndiratta (2010) [39].	12.5 mg/day [32] 5 mg/day [36] 3 mg/day [39].	From the 6th through 24th week of gestation [32], Throughout pregnancy [36,39].
Phalangeal epiphyses	Harrod (1981) [32], Raghav (2007) [37], Basu (2016) [42].	12.5 mg/day [32].	From the 6th through 24th week of gestation [32].

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Epiphyseal and paraspinal punctate calcification	Punctate calcification within cartilage and adjacent tissue	Birbal (2023) [34].	5 mg/day [34].	Until 4 gestational weeks then from 12 gestational weeks onward [34].
Distal phalangeal hypoplasia	Underdevelopment in the distal finger and toe regions	Howe (1997) [30], Harrod (1981) [32], Hou (2004) [36], Pauli (1987) [27], Hall (1980) [26], Barr (1976) [45].	12.5 mg/day [32] 11 mg/day, reduced to 6 mg/day [30] 7.5 mg/day [45] 5 mg/day [36].	From the 6th through 24th week of gestation [32], Reduced dose mid gestation discontinued at 2 weeks before delivery [30], Discontinued one week prior to delivery [45], Throughout pregnancy [36].
Cervical vertebra hypoplasia	Underdevelopment of the cervical vertebrae	Howe (1997) [30].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Kyphosis	Hunch-back posture caused by excess outwards rounding of the spine	Howe (1997) [30], Raghav (2007) [37].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Short neck		Hou (2004) [36], Dilli (2011) [41].	5 mg/day [36] 5 mg/day alternating with 10 mg/day [41].	Throughout pregnancy [36,41].

Note: Epiphyseal stippling (chondrodysplasia punctata) represents the most characteristic skeletal manifestation of fetal warfarin syndrome and may involve multiple skeletal regions. Dosages and gestational exposure periods are reported as described in the original studies.

Warfarin exposure also causes cardiovascular (Table 4) and ophthalmologic (Table 5) abnormalities during development. Although less consistently observed than skeletal and craniofacial findings, congenital heart defects including septal defects and outflow tract abnormalities have occasionally been reported following prenatal warfarin exposure[37,42]. Ocular phenotypes such as microphthalmia, optic nerve abnormalities, cataracts, and visual impairment have also been reported[26,37]. Further, in a few cases, hearing disability and genitourinary abnormalities have been documented[27,47].

Table 4. Cardiorespiratory Phenotypes Associated with Prenatal Warfarin Exposure.

Phenotype	Key Findings	Sources	Representative Exposure Range	Timeline
Atrial Septal Defect (ASD)	Abnormal opening in the interatrial septum permitting left-to-right shunting of blood between the atria.	Hou (2004) [36], Shil (2020) [90].	5 mg/day [36] 6 mg/day followed by 5 mg/day [90].	Throughout pregnancy [36], Until 10 weeks of gestation then from 14 weeks onward [90].
Ventricular Septal Defect (VSD)	Abnormal opening in the interventricular septum permitting left-to-right shunting of blood between the ventricles.	Chan (2003) [31], Starling (2012) [50], Vitale (1999) [19], Shil (2020) [90].	5-6 mg/day [50] 6 mg/day followed by 5 mg/day [90].	Until 17 weeks then from 23 weeks onward [50], Until 10 weeks of gestation then from 14 weeks onward [90].
Patent Ductus Arteriosus (PDA)	Persistence of the fetal ductus arteriosus after birth, resulting in abnormal blood flow between the aorta and pulmonary artery.	Hou (2004) [36].	5 mg/day [36].	Throughout pregnancy [36].
Aortic Arch Coarctation	Congenital narrowing of a segment of the aorta, typically involving the aortic arch or descending aorta.	Chan (2003) [31], Starling (2012) [50].	5-6 mg/day [50].	Until 17 weeks then from 23 weeks onward [50].

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Preductal / postductal hypotension	Differential blood pressure and/or oxygen saturation between preductal and postductal circulations, suggestive of congenital cardiovascular pathology.	Starling (2012) [50].	5-6 mg/day [50].	Until 17 weeks then from 23 weeks onward [50].
Anemia	Reduced hemoglobin concentration or red blood cell mass.	Starling (2012) [50], Hou (2004) [36].	5-6 mg/day [50] 5 mg/day [36].	Until 17 weeks then from 23 weeks onward [50], Throughout pregnancy [36].
Respiratory distress	Respiratory compromise characterized by labored breathing, tachypnea, or inadequate oxygenation.	Stevenson (1980) [29], Gupta (2010) [43], Howe (1997) [30], Ferreira (2018) [38], Dilli (2011) [41].	5 mg/day [29,38,43] 5 mg/day alternating with 10 mg/day [41] 11 mg/day, reduced to 6 mg/day [30].	Throughout pregnancy [29,41], Until 9 gestational weeks then from 12 gestational weeks onwards [43], Until 6 gestational weeks then from 12 gestational weeks onward [38], Reduced dose mid gestation discontinued at 2 weeks before delivery [30].

Table 5. Ophthalmologic Phenotypes Associated with Prenatal Warfarin Exposure.

Phenotype	Key Findings	Sources	Representative Exposure Range	Timeline
Optic atrophy	Degeneration of the optic nerve resulting in visual impairment or vision loss.	Stevenson (1980) [29], Hall (1980) [26].	5 mg/day [29].	Throughout pregnancy [29].
Corneal opacity	Loss of corneal transparency that may impair the transmission of light to the retina.	Harrod (1981) [32].	12.5 mg/day [32].	From the 6th through 24th week of gestation [32].
Shallow anterior chamber	Reduced depth of the anterior chamber between the cornea and iris.	Dilli (2011) [41].	5 mg/day alternating with 10 mg/day [41].	Throughout pregnancy [41].
Cataract	Opacification of the crystalline lens resulting in impaired vision.	Harrod (1981) [32].	12.5 mg/day [32].	From the 6th through 24th week of gestation [32].
Blindness	Partial or complete loss of vision.	Sherman (1976) [33], Hall (1980) [26].	Not reported	Not reported

Neurological abnormalities associated with prenatal warfarin exposure are often the most clinically significant outcomes compared to the classic skeletal and craniofacial manifestations of warfarin embryopathy because of their potential to cause lifelong disability[26,47,48]. CNS anomalies are more likely to develop following exposure during the second and third trimesters of pregnancy that result from fetal hemorrhage, subsequent scarring, and secondary impairment of normal brain development (Table 6) [26,49]. Reported CNS findings include intracranial hemorrhage, cerebral cysts, ischemic lesions, hydrocephalus, calcified brain regions, and ventriculomegaly[26,30,31,42,47–51]. Hydrocephalus, agenesis of the corpus callosum, cerebral hypoplasia, cortical atrophy, seizures, and intellectual disability have also been documented in multiple reports[31–33,35,37,46–49]. Collectively, these findings demonstrate that fetal warfarin exposure results in a broad and variable constellation of developmental abnormalities. While craniofacial and skeletal defects are the most recognizable manifestations of warfarin embryopathy, CNS involvement contributes substantially to long-term morbidity and developmental impairments, including speech, motor coordination, and learning. The diversity and severity of these phenotypes emphasize the importance of careful anticoagulant management during pregnancy and continued investigation into safer therapeutic alternatives for patients requiring long-term anticoagulation.

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Table 6. Neurodevelopmental and Central Nervous System Phenotypes Associated with Prenatal Warfarin Exposure.

Phenotype	Key Findings	Sources	Representative Exposure Range	Timeline
Microcephaly	Abnormally reduced head circumference resulting from impaired brain growth.	Sherman (1976) [33], Pati (1994) [46].	Not reported	Not reported
Sluggish pupil / poor pupillary response	Delayed or diminished pupillary constriction in response to light.	Harrod (1981) [32].	12.5 mg/day [32].	From the 6th through 24th week of gestation [32].
Poor suck	Impaired neonatal sucking reflex affecting feeding ability.	Stevenson (1980) [29], Matar (2016) [49].	5 mg/day [29].	Throughout pregnancy [29].
Absent or decreased deep-tendon reflexes	Reduced or absent neuromuscular reflex responses elicited by tendon percussion.	Stevenson (1980) [29], Howe (1997) [30].	5 mg/day [29] 11 mg/day, reduced to 6 mg/day [30].	Throughout pregnancy [29], Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Spinal cord thinning	Reduced spinal cord diameter or volume.	Raghav (2007) [37].	Not reported	Not reported
Spinal cord compression	Application of pressure to the spinal cord	Raghav (2007) [37].	Not reported	Not reported
Spina bifida	Congenital neural tube defect resulting from incomplete closure of the vertebral arches.	Vitale (1999) [19].	Not reported	Not reported
Ventriculomegaly	Abnormal enlargement of the cerebral ventricles.	Tongsong (1999) [35], Lee (2003) [48], Pati (1994) [46].	10 mg/day [35] 7.5 mg/day, then 7 mg/day warfarin [48].	Throughout pregnancy [35], Until 5 weeks of gestation then from 14 weeks onward [48].
Frontal cystic lesions	Cystic lesions involving the frontal cerebral region.	Starling (2012) [50].	5-6 mg/day [50].	Until 17 weeks then from 23 weeks onward [50].
Arachnoid cyst	Fluid-filled sac located between the arachnoid mater and central nervous tissue	Lee (2003) [48].	7.5 mg/day, then 7 mg/day warfarin [48].	Until 5 weeks of gestation then from 14 weeks onward [48].
Dandy-Walker malformation	Congenital malformation involving hypoplasia of the cerebellar vermis and cystic dilation of the fourth ventricle.	Chan (2003) [31], Hall (1980) [26], Basu (2016) [42].	Not reported	Not reported
Porencephalic cyst	Cystic cavity within the cerebral hemisphere, typically resulting from focal brain injury.	Matar (2016) [49].	Not reported	Not reported
Hemorrhagic posterior fossa cyst	Hemorrhagic cystic lesion located within the posterior cranial fossa.	Matar (2016) [49].	Not reported	Not reported
Spinal cord lesion	Spinal cord tissue alteration or damage	Howe (1997) [30].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Intracranial hemorrhage	Bleeding within the cranial cavity.	Lee (2003) [48], Matar (2016) [49], Shan (2023) [51], Wainwright (2010) [47].	7.5 mg/day, then 7 mg/day warfarin [48] 3.75 mg/day [51].	Until 5 weeks of gestation then from 14 weeks onward [48], Until 5 gestational weeks then from 16 gestational weeks onward [51].
Intracranial mass	Space-occupying intracranial lesion identified by neuroimaging.	Lee (2003) [48].	7.5 mg/day, then 7 mg/day warfarin [48].	Until 5 weeks of gestation then from 14 weeks onward [48].

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Ischemic changes in basal ganglia	Ischemic injury involving the basal ganglia.	Howe (1997) [30].	11 mg/day, reduced to 6 mg/day [30].	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Subdural hematoma	Accumulation of blood between the brain and the dura mater	Lee (2003) [48], Matar (2016) [49].	7.5 mg/day, then 7 mg/day warfarin [48].	Until 5 weeks of gestation then from 14 weeks onward [48].
Dystrophic calcification associated with white matter necrosis	Pathological calcium deposition associated with cerebral white matter necrosis.	Wainwright (2010) [47].	Not reported	Not reported
Calcified basal ganglia	Abnormal calcium deposition within the basal ganglia.	Starling (2012) [50].	5-6 mg/day [50]	Until 17 weeks then from 23 weeks onward [50].
Quadriplegia	Paralysis affecting all four limbs and the trunk.	Howe (1997) [30].	11 mg/day, reduced to 6 mg/day [30]	Reduced dose mid gestation discontinued at 2 weeks before delivery [30].
Hydrocephalus	Abnormal accumulation of cerebrospinal fluid within the cerebral ventricular system.	Chan (2003) [31], Matar (2016) [49], Wainwright (2010) [47].	Not reported	Not reported
Language Delay	Delayed acquisition of age-appropriate language abilities.	Harrod (1981) [32].	Not reported	Not reported
Intellectual disability, unspecified	Impairment of intellectual functioning and adaptive behavior.	Sherman (1976) [33], Raghav (2007) [37].	Not reported	Not reported
Seizures	Episodes of abnormal, excessive neuronal activity resulting in convulsions or altered neurological function.	Hall (1980) [26], Matar (2016) [49].	Not reported	Not reported

Prenatal warfarin exposure has also been associated with a range of additional abnormalities beyond the classic manifestations of fetal warfarin syndrome, including impaired fetal growth, hearing deficits, developmental delay, nail hypoplasia, and coagulation disturbances (Table 7). These findings suggest that warfarin embryopathy may affect multiple organ systems and developmental processes.

Table 7. Additional Developmental and Systemic Abnormalities Reported Following Prenatal Warfarin Exposure in Humans.

Phenotype	Description	Sources	Dosages	
Polyhydramnios	Excess accumulation of amniotic fluid during pregnancy.	Songmen (2017) [44].	5-6 mg/day [44]	Until 35 weeks of gestation [44].
Low birth weight	Birth weight below the expected range for gestational age.	Stevenson (1980) [29], Hou (2004) [36].	5 mg/day [29,36]	Throughout pregnancy [29,36].
Fetal growth restriction (FGR)	Impaired fetal growth resulting in size below that expected for gestational age.	Stevenson (1980) [29], Tongsong (1999) [35], Hou (2004) [36], Mehndiratta (2010) [39], Vitale (1999) [19], Pati (1994) [46].	5 mg/day [29,36] 10 mg/day [35] 3 mg/day [39]	Throughout pregnancy [29,35,36,39].
Fetal maceration	Postmortem degenerative changes characterized by skin discoloration, peeling, and tissue softening following prolonged intrauterine fetal demise.	Chan (2003) [31], Barr (1976) [45].	7.5 mg/day [45]	Discontinued one week prior to delivery [45].

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Widely spaced nipples	Increased distance between the nipples relative to thoracic width.	Harrod (1981) [32].	12.5 mg/day [32]	From the 6th through 24th week of gestation [32].
Mild hearing loss	Partial impairment of auditory function.	Harrod (1981) [32], Pauli (1987) [27].	Not reported	Not reported
Deafness	Severe or complete loss of hearing.	Raghav (2007) [37], Hall (1980) [26].	Not reported	Not reported
Hypoplastic nails	Underdevelopment of the fingernails and/or toenails.	Hou (2004) [36], Basu (2016) [42].	5 mg/day [36]	Throughout pregnancy [36].
Delayed developmental milestones	Delayed attainment of expected developmental milestones during infancy or childhood.	Raghav (2007) [37], Hall (1980) [26], Basu (2016) [42].	Not reported	Not reported
Prolonged prothrombin time (PT)	Prolongation of blood clotting time as measured by the prothrombin time assay.	Pauli (1987) [27].	Not reported	Not reported

FWS is a complex teratogenic disorder with substantial phenotypic variability. Although typical phenotypes presented include nasal hypoplasia and epiphyseal stippling, warfarin exposure has been associated with a broader range of craniofacial, skeletal, neurological, cardiovascular, and respiratory abnormalities. Understanding the pathogenic basis of warfarin-induced embryopathy may aid in improving prenatal risk assessment and the development of safer anticoagulant therapies.

5. Insights from Animal Models of Fetal Warfarin Syndrome

Vertebrate animal models provide valuable systems for studying the molecular and phenotypic mechanisms underlying fetal warfarin syndrome (FWS) due to rapid embryonic development, high fecundity, and conserved genetic pathways between zebrafish and humans. Several signaling pathways involved in coagulation and vitamin K metabolism, which are required for skeletal and vascular development, are evolutionarily conserved between vertebrate model organisms and humans, making them useful for developmental toxicology studies [52,53].

5.1. Zebrafish

Experimental exposure of zebrafish (*Danio rerio*) embryos to warfarin has demonstrated dose-dependent, developmental-stage-specific effects during embryogenesis and organ development. Zebrafish embryos exposed to warfarin concentrations ranging from 62.5 to 1500 μM showed a relationship between increasing warfarin concentration and the severity of teratogenic and lethal outcomes[54,55]. At the highest concentration, 1500 μM warfarin exhibited 92% mortality rate within 2–3 days post-fertilization (dpf) in embryos, and developmental defects were detectable within 1 dpf, indicating accelerated toxicity. The embryos exposed to a higher concentration of warfarin showed severe developmental abnormalities such as absent cardiac activity and coagulation prior to death. Survived embryos demonstrated delayed early development at 1 dpf followed by severe morphological abnormalities during later stages. Morphological phenotypes include defects of the head, eyes, sacculi and otoliths, notochord, tail, and tail tip, as well as scoliosis, yolk deformities, and generalized growth impairment. Notochord abnormalities were dose-dependent, appearing as isolated lesions in lower-dose groups but progressing to extensive structural disintegration at higher concentrations[54,55]. At lower concentrations (62.5–250 μM), teratogenic abnormalities generally became apparent at approximately 3 dpf, whereas embryos exposed to 500–1000 μM exhibited abnormalities as early as 2 dpf. Toxic effects of warfarin at concentrations of 5, 25, and 125 mg/L during embryonic development (1 hour to 2.5 dpf) and endotrophic development (2.5–5 dpf) were recorded to manifest reduced survival rate, reduced body size, and persistent growth impairment with hemorrhagic events[56]. Warfarin exposure also induced substantial cardiovascular and skeletal abnormalities. Cardiac edema occurred more frequently during embryonic-stage exposure, whereas

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swim bladder reduction was observed at the highest concentration (125 mg/L) regardless of developmental stage. Embryos exposed to warfarin frequently exhibit cardiac edema, reduced heart rate, altered cardiac looping, and circulatory defects. Such observations may help explain the congenital cardiovascular abnormalities occasionally reported in human fetal warfarin syndrome. Warfarin exposure at 20 μM from 4 to 96 hours post-fertilization (hpf) in zebrafish embryos has significantly reduced cardiac cone area and heart tube size, indicating impaired early cardiac development[57]. Although heart tube formation still occurred, affected embryos exhibited incomplete and defective cardiac looping, reduced chamber size, and impaired rhythmic contraction. Structural cardiac abnormalities included a markedly smaller ventricle, atrial dilation, and defective endocardial cushion formation. Minor looping abnormalities were also observed, suggesting disruption of normal cardiac patterning and morphogenesis. This study identified a broad critical exposure window extending from 4 to 72 hpf, indicating that warfarin-induced cardiotoxicity affects multiple stages of zebrafish cardiac development.

Cartilage development was also disrupted by warfarin treatment. Embryos exposed to the highest concentration (125 mg/L) exhibited reduced cartilage growth plate size in the ethmoid, Meckel's, and ceratohyal cartilages, with embryonic-stage exposure again producing more severe defects. These defects are thought to arise from impaired neural crest cell differentiation and abnormal extracellular matrix mineralization. Given that craniofacial anomalies such as nasal hypoplasia represent hallmark features of fetal warfarin syndrome in humans, zebrafish provide an experimentally accessible model for investigating the developmental origins of these abnormalities. Skeletal analyses demonstrated impaired mineralization of several dermal and endochondral skeletal structures, including the cleithrum, parasphenoid, basioccipital, and ceratobranchial bones. Exposure during endotrophic development primarily delayed mineralization of the parasphenoid, whereas embryonic-stage exposure resulted in a near-complete absence of mineralization in the cleithrum and parasphenoid at 5 and 7 dpf. Severe anti-mineralogenic effects were therefore most pronounced following early developmental exposure. Mineralization of vertebral centra was also markedly reduced, particularly in the caudal and caudal fin vertebrae. Additional histological abnormalities included hepatocellular shrinkage and thinning of retinal layers, especially following embryonic-stage exposure. Significant reductions in the ganglion cell layer, inner plexiform layer, and inner nuclear layer were observed at 7 dpf. Collectively, these findings demonstrated that warfarin exposure increased mortality, impaired growth, induced hemorrhage, shortened lifespan, disrupted skeletal mineralization, and altered cartilage development, with embryos exposed during early embryogenesis showing the most severe phenotypes (Table 8) [56].

These findings further support the utility of zebrafish as a model for investigating the developmental and molecular mechanisms underlying fetal warfarin syndrome. Together, zebrafish studies consistently demonstrate that warfarin exposure disrupts vascular integrity, skeletal mineralization, cartilage formation, and cardiac morphogenesis in a dose- and developmental-stage–dependent manner, closely paralleling many of the abnormalities observed in human fetal warfarin syndrome.

Table 8. Developmental Abnormalities Observed in Zebrafish (*Danio rerio*) Following Warfarin Exposure.

Phenotype	Malformation Description	Reference
Craniofacial		
Craniofacial hypomineralization	Impaired mineralization of the dermal and facial cartilaginous structures, reducing hard tissue content in the cleithrum, basioccipital, parasphenoid, and ceratobrachial 5 bone.	Granadeiro (2019) [56].

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Craniofacial cartilage reduction	Decreased cartilage content in growth plates of jaw and nasal bones with greatest impact in embryonic development.	Granadeiro (2019) [56].
Ceratohyal hypoplasia	Decreased length of throat-structural ceratohyal cartilage at 7 days post-fertilization.	Granadeiro (2019) [56].
	Circulation/Musculoskeletal	
Delayed skeletal mineralization, generalized	Delayed skeletal ossification and mineral deposition during development.	Granadeiro (2019) [56].
Decreased vertebral mineralization	Reduced vertebral mineralization accompanied by decreased birefringence, indicative of impaired bone formation.	Granadeiro (2019) [56].
Tail malformation	Abnormal morphology of the tail.	Fernández (2014) [78].
Scoliosis	Sideways curvature of the spine observed as early as 1 day post-fertilization.	Strecker (2013) [55].
Cardiac edema	Accumulation of fluid within tissues secondary to impaired cardiac function.	Granadeiro (2019) [56].
Decrease in cardiac cone area	Impaired cross-sectional area of fusing myocardial precursors during development, which occurred around 20 hours post-fertilization	Liu (2025) [57].
Cardiac hypoplasia	Underdevelopment of the heart characterized by reduced cardiac size and incomplete formation of cardiac structures, indicative of impaired cardiogenesis.	Fernández (2014) [78].
Pathological heart calcification	Calcified regions present adjacent to the aortic bulb	Fernández (2014) [78].
Cardiac looping defects	Defect in heart formation during the twisting of the cardiac tube to establish basic structure	Liu (2025) [57].
Impaired ventricle/atrium morphogenesis	Decreased size of the ventricle and atrium of the heart	Liu (2025) [57].
Absence of heartbeat (cardiac arrest)	Present at 3 days post-fertilization	Strecker (2013) [55].

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Impaired circulation	Reduced or abnormal blood flow through the embryonic vasculature, indicative of disrupted cardiovascular function and compromised circulation during development.	Fernández (2014) [78].
	CNS/Neuro	
Incomplete forebrain formation	Incomplete development of the forebrain observed following embryonic warfarin exposure, indicating disruption of normal central nervous system development.	Fernández (2014) [78].
Reduced ganglion cell / inner plexiform / inner nuclear layer ratios	Reduced retinal ganglion cell, inner plexiform layer, and inner nuclear layer thickness ratios, indicating impaired retinal development.	Granadeiro (2019) [56].
Brain hemorrhage	Intracranial hemorrhage observed predominantly in the highest exposure group.	Fernández (2014) [78].
	Other	
Growth retardation	Reduced body size and growth compared with controls at 16 days post-fertilization following embryonic exposure, though endotrophic exposure caused growth reduction in all dose groups.	Granadeiro (2019) [56], Strecker (2013) [55].
Hemorrhage, generalized	Bleeding throughout the body, observed in the highest-dosage group.	Granadeiro (2019) [56].
Swim bladder reduction	Reduction in size of the gas-filled swim bladder in the highest-dosage group.	Granadeiro (2019) [56].
Hepatocellular shrinkage	Reduction in size of liver mesenchymal cells.	Granadeiro (2019) [56].
Yolk deformity	Altered composition and/or size of yolk, presence of edema in yolk.	Strecker (2013) [55].
Underdeveloped somites	Underdevelopment of mesoderm blocks which differentiate into skeletal and connective tissue.	Fernández (2014) [78].

5.2. Chicken

The chick embryo is particularly useful for teratogenicity studies because embryonic development occurs externally, allowing direct manipulation and observation of skeletal and vascular development during defined developmental stages. The effects of dietary warfarin exposure in Leghorn and broiler chicks fed diets containing 25, 50, or 75 mg warfarin/kg body weight were reported[58]. Hemorrhagic lesions increased in severity with increasing warfarin dosage. Large subcutaneous hemorrhages were particularly evident in the wings of both breeds receiving the highest warfarin concentration. Intramuscular hemorrhages were also observed in several muscle

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groups, including the major and minor pectoralis, biceps femoris, and sartorius muscles, with occasional hemorrhage extending into the intraperitoneal region. Higher warfarin concentrations were additionally associated with increased mortality rates. Both Leghorn and broiler chicks fed warfarin-containing diets demonstrated significant reductions in feed consumption and body weight gain throughout most observation periods compared with controls (Table 9). Prothrombin times were also significantly prolonged in warfarin-treated birds, confirming the anticoagulant effects of warfarin exposure [58].

Table 9. Phenotypic Effects of Postnatal Warfarin Exposure in Chickens.

Phenotype Category/ Effect	Description	Reference
Circulation/Musculoskeletal: Intramuscular hemorrhage	Dose-dependent hemorrhage is observed in the major and minor pectoralis, biceps femoris, and sartorius muscles, as well as the intraperitoneal region.	Veltmann (1981) [58].
Growth: Decreased body weight	Reduced feed consumption resulting in decreased body weight gain.	Veltmann (1981) [58].
Hematological: Prolonged prothrombin time	Increased prothrombin time, with peak effects generally observed one week after exposure; in broiler chicks receiving 100 ppm warfarin, peak prolongation occurred after three weeks.	Veltmann (1981) [58].

5.3. Rats

Rodent models have provided important insight into the developmental and skeletal effects of prenatal warfarin exposure and have helped clarify the mechanisms underlying fetal warfarin syndrome[59]. The effects of warfarin exposure in pregnant Sprague–Dawley rats were studied by administering 100 mg/kg warfarin orally each day in combination with intramuscular injections of 10 mg/kg vitamin K1. When treatment occurred during gestational days 1–12, no major maternal or fetal abnormalities were observed apart from fetal growth impairment. However, treatment during gestational days 9–20 produced severe fetal hemorrhage, increased fetal resorption, and a significant reduction in litter size at day 21 of gestation. Approximately 36% of hemorrhagic lesions were externally visible in live fetuses, with many presenting as subcutaneous hemorrhages. More severe hemorrhages involved the brain, face, eyes, and occasionally the limbs. Intracranial hemorrhage, primarily intraventricular, was observed in 13% of exposed fetuses. Hemorrhage localized within the walls of the cerebral hemispheres resulted in focal areas of brain destruction, while hemorrhage affecting the eyes and ears caused tissue distortion and degeneration. Notably, classic skeletal features of human fetal warfarin syndrome, such as nasal hypoplasia and epiphyseal stippling, were not observed. The authors suggested that this difference may reflect species-specific developmental timing, as much of the skeletal ossification in rats occurs postnatally [59]. In a subsequent study, postnatal warfarin exposure in Sprague–Dawley rats was investigated by administering 100 mg/kg warfarin daily and simultaneously administering intramuscular injections of 10 mg/kg vitamin K1, beginning the day after birth and continuing for up to 12 weeks[60]. Treated rats demonstrated substantial growth impairment, including significant reductions in body weight, tail length, nasal length, and overall body length. Craniofacial abnormalities included shorter and broader snouts, smaller ear pinnae, and maxillonasal hypoplasia resulting from impaired skull growth. Significant reductions were observed in the dimensions of the skull, frontal bone, maxilla, premaxilla, nasal bone, and limb bones. Histological analysis revealed extensive calcification within the septal cartilage, particularly in the inferior half of the septum, with calcification extending throughout the full height of the anterior septum. These calcium deposits persisted for up to 15 months following treatment. Although classical stippling was absent, ectopic calcification formed “bridges” across

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growth plates, accompanied by disorganization and reduced cellularity within growth plate columns. These findings suggested that warfarin exposure disrupts normal cartilage maturation and endochondral ossification[60].

Additional evidence of warfarin-induced skeletal dysplasia was reported after administering daily warfarin doses of 0.05 mg/kg or 0.1 mg/kg to pregnant Wistar albino rats during gestational days 0–15, corresponding to the organogenesis period [61]. Fetuses exposed to warfarin exhibited incomplete ossification of the skull, enlarged fontanelles, incomplete development of the sacral and coccygeal vertebrae, abnormal metatarsal ossification, hind limb defects, and wavy ribs. At the higher dose, calcification was markedly impaired, and bone development was incomplete. Histopathological examination demonstrated hemorrhage and moderate degeneration within intervertebral tissues, while higher-dose exposure produced pronounced vertebral necrosis and degeneration[61]. Prenatal warfarin exposure in Sprague–Dawley rats treated daily from gestational days 8–22 was reported [62]. At 150 μg/kg, dams exhibited no external bleeding, and fetuses showed no gross abnormalities. However, higher doses of 185 and 200 μg/kg were lethal to the dams. At 175 μg/kg, maternal survival was reduced to approximately 57%, and fetuses demonstrated reductions in mandibular length, mandibular depth, and maxillary length, although these differences were not statistically significant after correction for fetal body weight. Histological analyses revealed widened hypertrophic zones in growth plates, disruption of the normal columnar arrangement of hypertrophic chondrocytes, and irregular cellular organization within cartilage. One dam exhibiting a markedly elevated prothrombin time produced fetuses with more severe skeletal abnormalities, including absence of ossification centers in the proximal and distal phalanges of both forelimbs and hind limbs, widened growth plates, and calcified hypertrophic zones. These findings further supported the relationship between excessive anticoagulation and impaired skeletal development [62]. The effects of therapeutic and toxic postnatal warfarin exposure in Sprague–Dawley rats were reported[63]. Rats received either a toxic dose consisting of 100 mg/kg warfarin combined with 10 mg/kg phylloquinone or a therapeutic dose of 0.07 mg/kg warfarin daily after birth. Neither treatment group demonstrated hemorrhage at the end of the study period. However, rats exposed to the toxic dose exhibited statistically significant reductions in skull and radius length. Animals receiving the therapeutic dose also demonstrated significantly reduced skull length, although radius length was not significantly affected (Table 10). These findings indicated that even therapeutic warfarin exposure may adversely affect craniofacial growth and skeletal development [63].

Table 10. Developmental and Skeletal Abnormalities Associated with Warfarin Exposure in Rats.

Phenotype	Malformation Description	Reference
	Craniofacial	
Maxillonasal Hypoplasia	Nasal bone length was reduced by 11–13% compared with controls.	Howe (1992) [60]
Reduced skull length	Cranial shortening, with the anterior skull region more severely affected; total skull length reduced by 15.7 ± 2.3% relative to controls.	Howe (1992) [60], Chetot (2020) [63]
Shortened and broadened snout	Altered snout morphology characterized by reduced length and increased width.	Howe (1992) [60]
Reduced pinna size	Decreased size of the external ear (pinna).	Howe (1992) [60]

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Decreased mandibular length	Reduction in mandibular length; differences were not significant after adjustment for fetal body weight.	Feteih (1990) [62]
Decreased maxillary length	Reduced maxillary and premaxillary dimensions; maxilla and premaxilla decreased by approximately 6–12% and 5–7%, respectively.	Feteih (1990) [62], Howe (1992) [60]
Septal cartilage calcification	Premature or abnormal calcification of the nasal septal cartilage.	Howe (1992) [60]
Craniofacial/Skeletal		
Incomplete ossification of the skull	Delayed or incomplete cranial bone ossification.	Abdulsamad (2023) [61]
Enlarged fontanelles	Large persistent fontanelles resulting from delayed cranial ossification.	Abdulsamad (2023) [61]
Circulatory/Hemorrhagic		
Subcutaneous hemorrhage	Hemorrhage within subcutaneous tissues; represented a substantial proportion of observed bleeding events.	Howe (1990) [59]
Brain hemorrhage	Predominantly intraventricular hemorrhage, observed in approximately 13% of fetuses.	Howe (1990) [59]
Facial hemorrhage	Hemorrhage affecting facial soft tissues.	Howe (1990) [59]
Ocular hemorrhage	Hemorrhage involving periocular or ocular tissues.	Howe (1990) [59]
Limb hemorrhage	Hemorrhage affecting limb soft tissues.	Howe (1990) [59]
Musculoskeletal/Skeletal		
Reduced radius length	Radius length was 11.3 ± 0.6% reduced. Postnatal exposure.	Chetot (2020) [63]
Reduced tail length	12-17% reduction. Postnatal exposure.	Howe (1992) [60]
Reduced forelimb bone length	Forelimb bones shortened by approximately 4–5%.	Howe (1992) [60]
Wavy ribs	Abnormal rib curvature and morphology.	Abdulsamad (2023) [61]
Generalized incomplete bone	Extensive reduction in skeletal mineralization and calcification.	Abdulsamad

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development		(2023) [61]
Vertebral necrosis and degeneration	Marked vertebral degeneration and necrosis observed in the high-dose group.	Abdulsamad (2023) [61]
	Growth/Development	
Growth restriction	Postnatal exposure. Generalized reduction in fetal growth and development.	Howe (1992) [60]
Reduced body weight	Body weight is reduced by approximately 7–13% compared with controls.	Howe (1992) [60]
Reduction in cell count or disorganization of growth plate column cells	Reduced numbers and disorganization of growth plate columnar chondrocytes.	Howe (1992) [60]
Disorganized hypertrophic zones	Hypertrophic cartilage zones were widened, calcified, and disorganized, occupying a greater proportion of total cartilage length than in controls. Mean width was 18.3% of total cartilage length compared to control (14.2%).	Feteih (1990) [62]

6. Gene Signaling Networks in Warfarin-Induced Teratogenesis

Warfarin teratogenicity is primarily attributed to the disruption of vitamin K–dependent biochemical pathways required for normal embryonic development. Experimental studies in zebrafish, rodent, and avian models, as well as in cell culture systems, have identified alterations in signaling pathways regulating cartilage maturation, osteogenesis, vascular integrity, neuronal survival, and extracellular matrix organization following prenatal warfarin exposure. Importantly, several neurological manifestations associated with fetal warfarin syndrome have been reported prior to significant fetal coagulation factor expression, suggesting that mechanisms beyond anticoagulation-mediated hemorrhage contribute to developmental toxicity[64]. These observations support the hypothesis that warfarin may directly influence gene expression programs and signaling pathways critical for embryonic tissue differentiation and organogenesis. Understanding the molecular signaling networks affected by warfarin exposure is therefore essential for clarifying the pathogenesis of fetal warfarin syndrome and identifying potential targets for safer anticoagulation strategies during pregnancy.

6. 1γ-. Glutamyl Carboxylase (GGCX), Thrombin, and Protease-Activated Receptor-1 (PAR-1) Regulate Vitamin K–Dependent Protein Activation

Warfarin inhibits vitamin K epoxide reductase complex subunit 1 (VKORC1), preventing the regeneration of the reduced form of vitamin K required for γ-glutamyl carboxylase (GGCX) activity. γ-Glutamyl carboxylase (GGCX) catalyzes the vitamin K–dependent γ-carboxylation of several proteins involved in coagulation and embryonic development. Known GGCX substrate proteins include coagulation-associated proteins such as prothrombin, protein C, protein S, protein Z, and coagulation factors VII, IX, and X, as well as developmental proteins including growth arrest-specific protein 6 (Gas6), matrix Gla protein (MGP), proline-rich γ-carboxylated proteins 1 and 2,

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nephrocalcin A–D, and the transmembrane proteins TMG3 and TMG4 [65]. Warfarin reduces vitamin K-mediated GGCX activity and subsequent γ-carboxylation of vitamin K–dependent proteins [66,67]. Experimental studies have demonstrated that impaired γ-carboxylation disrupts the activation of osteocalcin and matrix Gla protein (MGP), resulting in abnormal cartilage mineralization and skeletal dysplasia [68]. Mutations in *GGCX* produce phenotypes resembling fetal warfarin syndrome, including chondrodysplasia punctata and ectopic calcification [69]. While impaired γ-carboxylation is considered the central mechanism underlying fetal warfarin syndrome, increasing evidence suggests that warfarin exposure also disrupts broader developmental signaling networks involved in embryogenesis.

Warfarin-induced reduction of coagulation factors decreases thrombin generation and downstream PAR-1 signaling. PAR-1 regulates vascular stability, angiogenesis, and neural development during embryogenesis [70]. Experimental evidence suggests that impaired thrombin-PAR-1 signaling contributes to vascular fragility and fetal hemorrhage, particularly intracranial hemorrhage associated with CNS injury in fetal warfarin syndrome [71]. Although bleeding abnormalities are commonly observed in humans with γ-carboxylase mutations, developmental defects associated with non-coagulation γ-carboxylated proteins are comparatively rare. Experimental studies in mice have demonstrated the critical developmental importance of GGCX signaling. Homozygous *Ggcx*$^{-/-}$ mice exhibit severe embryonic lethality, with approximately half of embryos dying between embryonic days 9.5 and 18.5, while surviving embryos succumb to intra-abdominal hemorrhage shortly after birth [65]. Interestingly, these embryos did not demonstrate ectopic calcification despite the absence of γ-carboxylation activity. Similar embryonic lethality has been observed in mice lacking protease-activated receptor-1 (*Par-1*), a thrombin receptor involved in vascular and developmental signaling. However, adult *Par-1*$^{-/-}$ mice exhibit normal hemostasis, suggesting that mid-embryonic lethality in these models may result from impaired developmental signaling rather than hemorrhage alone. This finding supports the hypothesis that thrombin-mediated signaling pathways contribute to embryonic survival independently of their role in coagulation. In contrast, knockout models targeting several individual vitamin K–dependent proteins, including osteocalcin, Gas6, MGP, protein C, protein Z, and coagulation factors VII and IX, do not demonstrate embryonic lethality. Nevertheless, mutations in the *MGP* gene in humans cause Keutel syndrome, a disorder characterized by abnormal cartilage calcification, brachytelephalangia, and midfacial hypoplasia, phenotypes that overlap with the skeletal manifestations observed in fetal warfarin syndrome [65]. These findings suggest that disruption of multiple γ-carboxylated developmental pathways collectively contributes to the teratogenic effects of warfarin exposure.

6.2. Receptor Tyrosine Kinases (RTKs) and Eyk/Axl Signaling

Growth-arrest-specific protein 6 (Gas6) is a vitamin K–dependent protein (VKDP) that shares significant structural homology with protein S, a coagulation-associated protein affected by warfarin exposure. Gas6 functions as a ligand for the receptor tyrosine kinase (RTK) family members Tyro3, Axl, and MerTK, which regulate cellular proliferation, differentiation, survival, migration, and signal transduction during embryonic development [72,73]. Warfarin inhibits γ-carboxylation of GAS6, thereby reducing receptor activation and impairing signaling involved in endothelial survival, vascular stabilization, and neuronal development [74]. Experimental studies linked disrupted GAS6–AXL signaling to vascular instability and defective tissue remodeling. Because RTK signaling plays a central role in tissue morphogenesis and organogenesis, disruption of Gas6-mediated pathways has been proposed as a contributing mechanism in warfarin teratogenesis. Experimental studies using chicken embryo models demonstrated that warfarin exposure induces a dose-dependent reduction in tyrosine phosphorylation via pathways involving c-Eyk, the avian homolog of the Tyro3 receptor tyrosine kinase, which is widely expressed during embryogenesis [75]. Additional signaling molecules affected by warfarin exposure included pp125FAK (focal adhesion kinase), pp60$^{c\text{-}src}$, and paxillin, all of which are critical regulators of cytoskeletal organization, signal transduction, and cellular adhesion during embryonic development. pp125FAK regulates cytoskeletal assembly and

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phosphorylates paxillin, a protein involved in actin-membrane interactions during organogenesis. pp60$^{c\text{-}src}$ functions in intracellular signaling pathways and is highly concentrated in developing neural tissue, whereas paxillin contributes to cell adhesion and morphogenesis [75]. Vitamin K administration increased phosphorylation of c-Eyk, pp125FAK, and paxillin and upregulated Src kinase activity in chicken embryos [76]. Warfarin exposure inhibited these vitamin K–mediated signaling responses, suggesting that disruption of RTK-associated phosphorylation pathways may contribute to impaired embryonic development during prenatal warfarin exposure. These findings support the hypothesis that warfarin teratogenicity extends beyond coagulation abnormalities to include disruption of developmental signaling networks that regulate cellular proliferation, cytoskeletal organization, and tissue morphogenesis.

6.3. Pregnane X Receptor (PXR) Pathway

Vitamin K functions as a specific ligand for the pregnane X receptor (PXR), a nuclear receptor that is a major regulator of xenobiotic metabolism and bile acid homeostasis [77]. In addition to its metabolic functions, PXR signaling plays important roles in skeletal development by regulating osteoblast differentiation and extracellular matrix (ECM)-associated genes, including *tsukushi* (*tsku*), *matrilin-2*, and *CD14*[78]. Altered PXR activity following warfarin exposure has been associated with impaired skeletal development and reduced ossification. Experimental studies have demonstrated that *PXR*-knockout mice develop osteopenia, underscoring the importance of PXR signaling in bone homeostasis and mineralization. In zebrafish models, warfarin exposure increased expression of the PXR-regulated genes *tsukushi* and *cyp3a65* [78]. The skeletal abnormalities observed in warfarin-exposed zebrafish have been proposed to result partly from tsukushi-mediated antagonism of bone morphogenetic protein (BMP) signaling, a pathway essential for skeletal patterning and osteogenesis. Disruption of BMP signaling may therefore contribute to impaired mineralization and skeletal dysplasia associated with prenatal warfarin exposure. The proposed interaction between Tsukushi and BMP signaling may also explain the observed *in vivo* upregulation of osteocalcin following warfarin exposure, which contrasts with findings reported in C2C12 cell culture models. These findings suggest that PXR-mediated signaling pathways may contribute to the skeletal manifestations of warfarin teratogenicity by modulating extracellular matrix organization and BMP-dependent developmental processes.

6.4. Sulfatide Metabolism and CNS Sequelae

Warfarin exposure has been shown to reduce the biosynthesis of sulfatides, essential sphingolipid components of myelin in the central nervous system [79]. Sulfatides play a critical role in myelin stability, neuronal signaling, and normal neural development. Disruption of sulfatide synthesis has therefore been proposed as a potential mechanism contributing to the neurological manifestations and developmental delays associated with fetal warfarin syndrome. Importantly, central nervous system abnormalities observed following prenatal warfarin exposure cannot be fully explained by hemorrhagic injury secondary to impaired coagulation. CNS abnormalities in a fetus exposed to warfarin between gestational weeks 8 and 12, a developmental period preceding significant fetal expression of coagulation factors [64]. These findings suggest that warfarin may directly interfere with neurodevelopmental pathways, independent of its anticoagulant effects, potentially by disrupting myelin-associated lipid metabolism and other developmental signaling mechanisms.

6.5. Ras Family Signaling Pathways

GTPases of the Ras protein family are critical regulators of embryonic development and participate in cellular processes including cell division, nuclear assembly, vesicle transport, cytoskeletal organization, and differentiation [80]. Ras signaling activity is tightly controlled by Ras-GTPase-activating proteins (Ras-GAPs), which accelerate the hydrolysis of active Ras-GTP to inactive

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Ras-GDP, thereby regulating downstream developmental signaling pathways [81]. One Ras-associated regulatory protein implicated in warfarin teratogenesis is SH3-domain binding protein (G3BP), which plays an essential role in embryogenesis and neurodevelopment. Experimental studies identified four redundant G3BP isoforms in embryonic stem cells, all of which were upregulated following warfarin exposure [80]. Warfarin-mediated disruption of GAS6/TAM receptor signaling alters downstream Ras-MAPK pathway activation, which is involved in cell proliferation, differentiation, and skeletal morphogenesis[82]. These findings suggest that warfarin may alter Ras-mediated signaling networks involved in cellular differentiation and neural development, potentially contributing to the neurodevelopmental abnormalities observed in fetal warfarin syndrome.

6.6. Wnt Signaling Pathway

Low-dose warfarin exposure has been shown to induce cleft palate formation in a subset of zebrafish embryos, producing phenotypes similar to the craniofacial abnormalities reported in human fetal warfarin syndrome. Experimental studies demonstrated that inhibition of Wnt signaling exacerbated the cleft palate phenotype, whereas treatment with Wnt agonists, including BIO, WAY-262611, and CHIR-99021, significantly rescued palatal development in affected embryos. Warfarin exposure was additionally associated with reduced expression of the canonical Wnt downstream transcription factors *tcf7* and *lef1*, indicating suppression of Wnt/β-catenin signaling activity[83]. This reduction was accompanied by decreased cellular proliferation and impaired viability of developing palatal structures. These findings suggest that disruption of canonical Wnt signaling contributes to warfarin-induced craniofacial malformations and may play an important role in the pathogenesis of cleft palate associated with prenatal warfarin exposure.

Transglutaminase 2 (TG2) has been identified as a critical mediator of warfarin-induced vascular calcification by activating canonical β-catenin signaling in vascular smooth muscle cells (VSMCs). Experimental studies in rat A10 VSMCs demonstrated that warfarin exposure promotes vascular calcification by activating the TG2/β-catenin signaling axis, thereby inducing osteogenic transformation of VSMCs. Inhibition of either TG2 activity or canonical β-catenin signaling significantly reduced warfarin-induced calcification, thereby identifying the TG2/β-catenin pathway as a potential therapeutic target for preventing warfarin-associated vascular calcification[84,85]. Disrupted Wnt signaling has been associated with delayed ossification, abnormal cartilage mineralization, and craniofacial defects characteristic of fetal warfarin syndrome.

7. Warfarin Derivatives and Alternative Anticoagulants

Several coumarin-derived anticoagulants structurally and pharmacologically related to warfarin are used clinically, including phenprocoumon and acenocoumarol. These agents act by inhibiting vitamin K epoxide reductase, thereby impairing γ-carboxylation of vitamin K–dependent coagulation factors, via a mechanism similar to that of warfarin. Phenprocoumon is sometimes preferred in patients with poor warfarin metabolism because of its prolonged elimination half-life. The S- and R-enantiomers of phenprocoumon have half-lives of approximately 172 and 156 hours, respectively, with the S-enantiomer accounting for most of the anticoagulant activity[16]. Structurally, phenprocoumon differs from warfarin by lacking the ketone group attached to the asymmetric carbon center[3]. Acenocoumarol, another coumarin derivative, has a substantially shorter half-life, with elimination half-lives of approximately 2 hours for the S-enantiomer and 8 hours for the R-enantiomer[16]. In contrast to warfarin, acenocoumarol contains a nitro group on the aromatic ring adjacent to the asymmetric carbon[3]. Despite pharmacokinetic differences, both phenprocoumon and acenocoumarol exert anticoagulant effects by inhibiting vitamin K–dependent pathways and may therefore share a teratogenic potential similar to that of warfarin. Indandione anticoagulants are another class of vitamin K antagonists with mechanisms of action comparable to those of warfarin. These compounds inhibit the synthesis of vitamin K–dependent clotting factors by interfering with vitamin K recycling pathways [86]. Although less commonly used today, indandione derivatives have historically been employed as oral anticoagulants and demonstrate similar anticoagulant and

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developmental toxicity profiles. Heparin and low-molecular-weight heparins (LMWHs) are widely used alternatives to vitamin K antagonists during pregnancy because they do not readily cross the placenta. Unlike warfarin (molecular weight 308.3 Da), which readily crosses the placenta, heparin is a large polysaccharide with an average molecular weight of 12–15 kDa and does not traverse the placental barrier, thereby minimizing fetal exposure. Comparatively, heparin exerts its anticoagulant effect by potentiating antithrombin III activity, thereby inhibiting thrombin and factor Xa[87,88]. Due to their limited placental transfer, heparin-based anticoagulants are generally considered safer for fetal development and are frequently recommended for anticoagulation management during pregnancy.

8. Conclusions

This review highlights the multifaceted nature of warfarin pharmacology, toxicity, and teratogenicity, emphasizing its continued clinical importance alongside its significant developmental risks during pregnancy. Although warfarin remains an effective and widely used anticoagulant, prenatal exposure can result in fetal warfarin syndrome (FWS), a complex disorder characterized by craniofacial, skeletal, neurological, cardiovascular, and growth abnormalities. Clinical and experimental evidence demonstrates that the severity of these phenotypes depends on the timing, duration, and dosage of exposure. Importantly, findings from zebrafish, chicken, and rodent models demonstrate that warfarin-induced developmental toxicity extends beyond hemorrhagic complications and involves disruption of multiple vitamin K–dependent molecular signaling pathways regulating cartilage formation, skeletal mineralization, vascular stability, cardiac morphogenesis, and neurodevelopment. Pathways involving GGCX, GAS6/TAM receptors, thrombin–PAR-1, PXR, Ras, and Wnt signaling appear to play critical roles in the pathogenesis of FWS. Collectively, these studies provide important mechanistic insight into the developmental effects of warfarin and support the need for careful anticoagulation management during pregnancy. Continued integration of developmental biology, pharmacogenomics, and experimental modeling will be essential for improving understanding of warfarin teratogenesis and for developing safer anticoagulant strategies with reduced fetal toxicity.

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