ROLE OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (ENOS) IN FETAL HEART DEVELOPMENT AND ITS IMPLICATIONS ON SEPTAL DEFECTS

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Abstract.

Endothelial nitric oxide synthase is crucial for the formation of the circulatory system during cardiomyogenesis and for maintaining its homeostasis (eNOS). This enzyme synthesizes nitric oxide and it is expressed in the cells of endothelium, an essential signaling molecule involved in several physiological processes. Throughout the development of the embryonic heart, many key processes that influence cardiac morphogenesis and function include angiogenesis, vasculogenesis, and cardiomyocyte proliferation. The regulation of eNOS-mediated nitric oxide generation is critical for the growth of heart chambers and septation in developing hearts. Conditions such as septal defects of ventricles, atrium, and other congenital heart issues can result from disruption with eNOS function. In order to treat symptoms and avoid consequences from these anomalies, which could harm heart function, surgical operations are frequently required. Understanding how eNOS controls activities throughout the development of the embryonic heart is essential to understanding the etiology of congenital cardiac disorders, especially those involving septal abnormalities. There is possibility of improved outcomes for those with these conditions by focusing on medicines that aim to mitigate the consequences of abnormalities through genes in the eNOS pathway. Therefore, this review focuses on the foundational to current significance of the role of eNOS in the formation of the fetal heart and its impacts on congenital cardiac abnormalities that result from eNOS and its signaling pathways being impaired. (www.actabiomedica.it)

Key words: Fetal heart development, eNOS mutant, Cardiomyocyte proliferation, Paracrine signaling.

Introduction

The NOS3 gene, which is responsible for producing the enzyme can be found on chromosome 7q36 and was initially discovered in specialized cells known as endothelial cells. This gene consists of 21,000 base pairs. Includes 26 coding regions (exons) that are interspersed with 25 coding regions (introns). When activated, eNOS is produced by the NOS3 gene stimulates the

release of nitric oxide (NO) resulting in the dilation of blood vessels, for circulation. It also contributes to the formation of new blood vessels (angiogenesis). However, NO production is unstable because the expression of the NOS3 gene is often suppressed or stimulated during the development and growth of the cardiovascular system by various factors (1,2). The release of NO plays a major role in maintaining a healthy cardiovascular development in fetal heart. It helps control blood pressure by widening blood vessels (vasodilatation), allows proper muscle tone development, and ensures strong and regular heartbeats (cardiac contractility) (3). Issues may arise in the development of a fetal heart due to impairment in eNOS potentially resulting in heart defects at birth and elevated blood pressure, in the lungs. (4). Knowing how eNOS affects the development of a fetus's heart and how it causes congenital heart disease, especially septal heart defects of ventricles and atrium, is crucial for improved outcomes of people who have been diagnosed with CHD. (5). Therefore, in this review, we discuss on understanding the molecular pathways played by eNOS in the formation of the heart during the fetal heart development. We also concentrate on how a disruption in this process can result in birth defects related to the CHD's.

eNOS and its expression during fetal heart development:

To regulate and maintain proper heart development and functioning, a substance called NO is produced by eNOS (6). Blocking the production of NO hampers the development of heart muscle cells. When NO production is restored, it stimulates specific signals that promote the growth of endothelial cells. Both eNOS and another form of NO synthase iNOS, are essential for the early stages of heart muscle cell development. As the heart grows (around E14.5), the production of NO by eNOS significantly decreases. Moreover, NOS inhibitors stop terminally differentiated cardiomyocytes from maturing via the Embryonic Stem cell pathway, where NO-generation is necessary for cardiomyogenesis. This is a crucial aspect of fetal heart development during embryogenesis (7). Further, alpha-myosin heavy chain (a-MHC) expression increases in WT mouse hearts while atrial natriuretic peptide (ANP) expression gradually declines from day 1 to day 7 postnatally. On the other hand, at postnatal day 7, the levels of a-MHC are greatly reduced while the ANP levels remains high in hearts that lack NOS3. These findings suggest that NOS3 disruption impacts the temporal alterations in ANP and a-MHC throughout postnatal development of heart, supporting the essential role of NOS3 in promoting postnatal heart maturation (1). NO produced by eNOS also helps to regulate blood vessel functions. When eNOS function is impaired, it leads to endothelial dysfunction. This dysfunction is associated with several cardiovascular conditions, including atherosclerosis, hypertension, thrombosis, and stroke. In studies involving eNOS-null mice the findings revealed significant abnormalities like ventricular myocardium (heart muscle) and irregularities in arteries of the pharyngeal arch. Furthermore, there were very fewer baroreceptors (pressure-sensing cells) in the aortic arch than normal, and adult eNOS-null survivors exhibited signs of cartilaginous changes, aortopathy (aortic disease), and cardiac hypertrophy in the area of periduct and aortic arch. Disruption of eNOS during development affected neuregulin and Notch1 communication between the ventricles and arteries of the

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pharyngeal arch. This study suggests that eNOS mutations and gene variation may contribute to congenital cardiac abnormalities in humans and could also play a role in hypertension (8).

Table: 1 represents a summary of studies investigating eNOS and its role in heart development.

GENES INVOLVED	TYPE OF STUDY	ANIMAL MODEL	CELL SIGNALING PATHWAYS STUDIED	FINDINGS OF THE STUDY	REFERENCE NO.
Gata4, tgf-β, bmp2, vegf, bfgf, Erythropoietin	In vivo	Mice	eNOS expression during heart development	Impact on CHDs in eNOS-/-/- mice, valvular malformations, impaired heart function, embryonic apoptosis, and valve formation abnormalities.	(1)
caspase-3, s-nitrosylation, superoxide dismutase	In vivo	Mice	eNOS role in cell growth and protection against apoptosis, NO generation triggered by shear stress	Suppression of caspase-3 activity resulted in the control of cell proliferation, defense against apoptosis, and decrease in endothelial cell death.	(9)
cardiac-specific genes	In vitro	Embryonic stem cells	NOS inhibitors on cardiomyocyte maturation, NO donor treatment	Inhibition of cardiomyocyte maturation by NOS inhibitors, promotion of differentiation into cardiomyocytes by NO donor or	(7)

				NOS, induction of cardiac-specific gene expression.	
-	In vitro	Mouse embryonic stem cells	NOS role in cardiomyocyte differentiation	Promotion of cardiomyocyte differentiation by NO donor or NOS treatment.	(10)
-	In vitro	Mouse E14.5 ventricular tissues and induced pluripotent stem cells	Transcriptome profiles, glucose metabolism genes	Consistent transcriptome profiles, glucose metabolism genes were overexpressed in eNOS-deficient cells.	(11)
gata4	In vivo	Mice	eNOS modulation by Gata4	Modulation and increase in eNOS expression by Gata4.	(12)
-	In vivo	eNOS-null mice	CHDs, valvular malformations, embryonic apoptosis, impaired heart function	, valvular malformations, improper heart functioning, elevated embryonic apoptosis, and valve formation abnormalities in eNOS-null mice.	(13-14)
-	In vivo	Individuals suffering from	eNOS protein expression	valve disease(bicuspid) of the aorta	(15)

		bicuspid aortic valve disease		had lower expression of the eNOS than those with tricuspid aortic valves.	
caspase-3	In vivo	Mice	eNOS role in protecting early cardiac progenitors	Protection of early cardiac progenitors against apoptosis through S-nitrosylation and caspase-3 inactivation.	(16)
superoxide dismutase	In vivo	Mice	eNOS role in reducing endothelial cell death	Reduction of endothelial cell death by the inhibition of caspase-3 activation.	(17)
vegf	In vivo	Mice	eNOS role in cardiomyocyte proliferation and VEGF expression	Requirement of eNOS for cardiomyocyte proliferation, VEGF expression, and capillary network formation.	(18)
gata4, wt1, vegf, bfgf, erythropoietin	In vivo	Mice	eNOS role in coronary artery development	Control of coronary artery development, downregulation of key transcription	(19)

				factors in eNOS-null mice, restoration of normal vasculature by eNOS overexpression.	
snail+, tgf-β, bmp2	In vivo	Mice	eNOS-null mice and endocardial cushions	Reduced amounts of genes involved in valve creation at the molecular level and impaired endocardial-to-mesenchymal transition (EndMT)	(20)

eNOS-Mediated Regulation of Endocardial-to-Mesenchymal Transition (EndMT)

The endothelium-to-mesenchymal transition (EndMT), a comparable dedifferentiation process, has also been observed in endothelial cells (ECs) in recent years (21). During cardiogenesis and vasculogenesis, subsets of ECs were shown to exhibit EndMT during the initial observations of the condition in developing embryos. Cardiac cushions are finally produced by ECs that undergo EndMT in the endocardium and infiltrate the cardiac cushion. Defective heart valve development and embryonic mortality are the outcomes of EndMT disruption at this developmental period (22,23,24). Recent research has outlined the pivotal function that EndoMT plays in the development and course of cardiac fibrosis. After myocardial infarction, endocardial cells can produce myofibroblasts by EndoMT. These distinct modifications align with molecular shifts in the polarity and structure of the endothelial cells. The study analysis also involves in obtaining markers related to cells such, as VE cadherin, eNOS and CD31 along with factors for mesenchymal cells like α SMA, FSP 1, transgelin and SM22a or calponin. The expression of pSMAD2 and/or pSMAD3 by ECs in damaged hearts demonstrated that the embryonic endoMT system is regulated by TGFβ signaling activation (25). The formation of the outflow tract and valves of atrium and ventricle is dependent on EndMT. Any disturbance in the OFT's development can cause Congenital heart disorders (CHD), such as conotruncal defects (CTD) (26). Using salvianolic acid (SAL) the potential inhibitory effects on homocysteine (Hcy)-induced Endothelial-to-Mesenchymal Transition (EndMT) was evaluated. This finding revealed that α-SMA and KLF4

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expression increased as VE-cadherin marker decreased among cells treated with Hcy, suggesting an occurrence of EndMT. However, these impacts were overturned by prior exposure to SAL through upregulating eNOS/NO signaling pathway and downregulating KLF4 expression. Also, knocking down KLF4 in endothelial cells raised eNOS/NO signaling pathways. This may imply that targeting the KLF4/eNOS pathway may be a potential therapeutic option for CHDs and other cardiac related malformations (27).

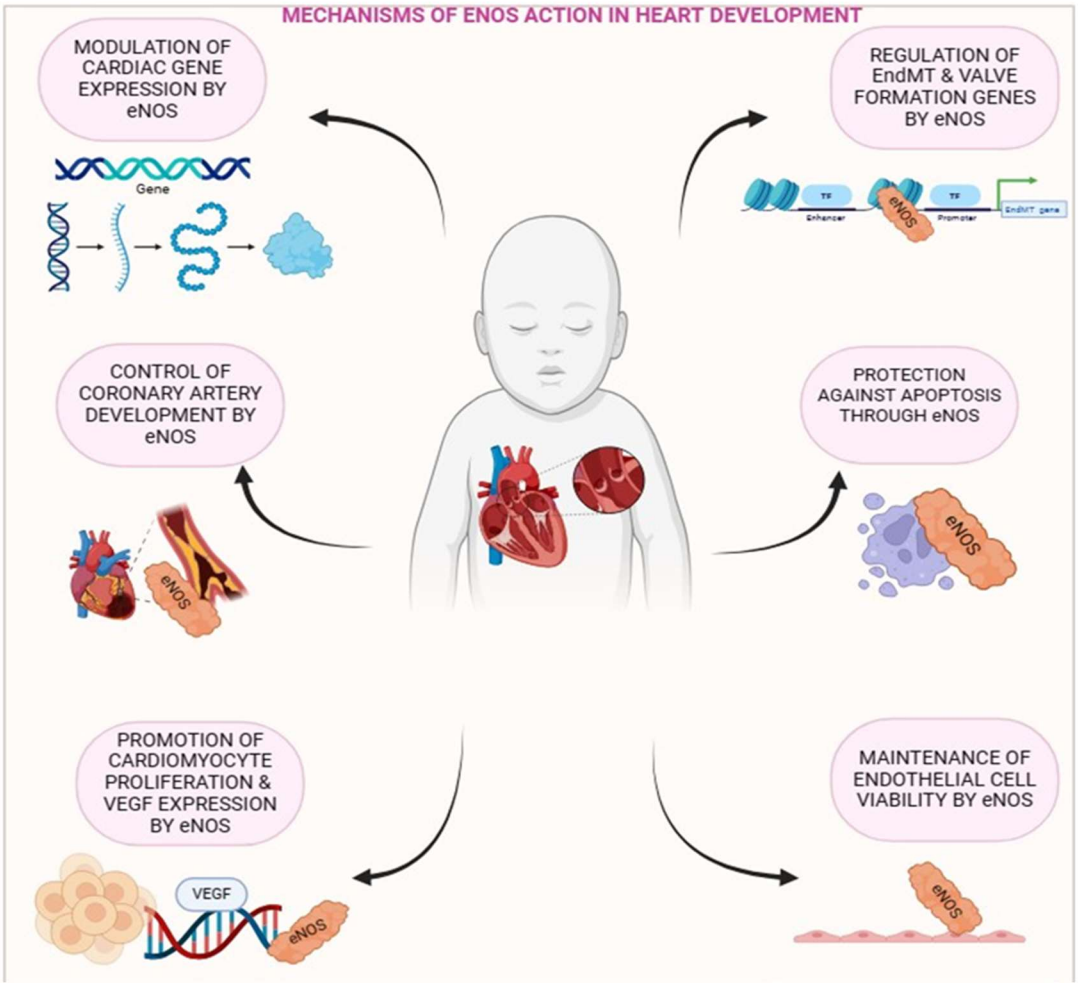

Figure 1: The diagrammatic illustration represents the multifaceted position of endothelial nitric oxide synthase (eNOS) in fetal heart development throughout embryogenesis. The central node represents the overarching theme of eNOS's involvement, with branches depicting its expression in the developing heart., The mechanisms of eNOS action including gene modulation and protection against apoptosis, and the overall impact of eNOS and nitric oxide (NO) on heart morphogenesis is represented here.

Hemodynamic Stress and Its Influence on eNOS function in fetal heart

Endothelium-derived hyperpolarizing factor (EDHF), prostacyclin (PGI2), peptides of natriuretic, NO, and other redundant processes are among the vasodilators that together account for the aterial

placenta and uterine low vascular resistance during typical pregnancy. Many researchers believe that NO has a significant, if not crucial, role in vascular adaptation related to pregnancy. (28) During pregnancy, there is a noticeable increase in the levels of second messenger cyclic guanosine monophosphate (cGMP) and NO. (29)(30) in the feto and uteroplacenta in particular (31)(32)(33). Additionally, long-term NO production inhibition results in intrauterine fetal growth restriction (IUGR), which is linked to reductions in fetoplacental blood flow, and small for gestational age (SGA) weights.(34)(35)(36). The fact that the uteroplacental and fetoplacental endothelium release more NO during pregnancy provides strong evidence in favor of this hypothesis.(30)(37). Furthermore, the most extensive growth rate of fetus of ovine develops between 70 and 90 percent in 110 to 130 days with total 147 days of gestation. Throughout the third trimester, the ovine placenta's production of NO and the fetoplacental artery endothelium's expression of the endothelial NO synthase (eNOS) protein both steadily rise (38)(39). Research employing animal models has indicated that extended suppression of NO synthesis during gestation results in fetal growth restriction, perhaps due to a reduction in uterine and fetoplacental supply of blood (40)(41). All of these results indicate an association between increased blood flow to the placenta and uterus, which supports normal fetal development and locally produced endothelium-derived NO. Pretreating with the NOS inhibitor L-NAME or stripping the endothelium can both greatly diminish or completely abolish the acute flow-induced dilatation of isolated arteries. The inhibitor substrate selectivity of the eNOS enzyme is demonstrated by the ability of L-arginine, a NOS substrate, to reverse NOS inhibition of flow-mediated vasodilation (42). Posttranslational changes in endothelial cells of ovine placenta and fetal artery promote eNOS levels, NO generation, and phosphorylation of eNOS in the placental circulation, which is the main emphasis of this study (43).

eNOS-Driven Angiogenesis During Fetal heart development:

NO is a pleiotropic diffusible gas that has several important functions, such as blood pressure management, heart and bone formation, neo-angiogenesis stimulation, and maintenance of vascular tone. NOS3 is an enzyme constitutively expressed in endothelial cells (ECs) and is responsible for the production of nitric oxide (NO), a well-known regulator of angiogenesis (44). Studies on fetal growth impairment and increased neonatal mortality during placental insufficiency suggest that decreased vascularization in the placenta causes these negative outcomes. Studies on pregnant mice lacking the Akt1 gene also found that significant reductions in Akt1 and eNOS phosphorylation emphasize the role of NO signaling in preserving vascular health during pregnancy (45). The increased expression of FGF2 and VEGF with high vascular density, and blood flow to the placenta, during a normal sheep pregnancy are correlated with an increase in placental NO generation, indicating the potential role of eNOS derived NO in placental angiogenesis (46, 47). Rats with preeclampsia-like symptoms and smaller litter sizes are the effects of pharmacological suppression of NOS by L-NG-nitroarginine methyl ester, according to preliminary research. The development of proteinuria and growth restriction in the fetuses of eNOS-null mice confirmed this. Pregnant mice lacking eNOS exhibit defective uteroplacental

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remodeling and dysregulated vascular adaptations, leading to lower placental and uterine blood flow as well as significantly reduced placental vascularization (48,49). Several investigations have proven the importance of activation of the PI3K/Akt1, MAPK and eNOS/NO pathways for VEGF and FGF2 stimulated angiogenesis in a vast amount of endothelial cells. The importance of controlling tube formation, cell proliferation and migration in placental endothelial cells contribute to in vitro angiogenesis in response to VEGF and FGF2 stimulation has been identified. These results emphasize how intricately signaling pathways interact to coordinate angiogenic processes, especially when it comes to placental vascular development (50,51,52,53)

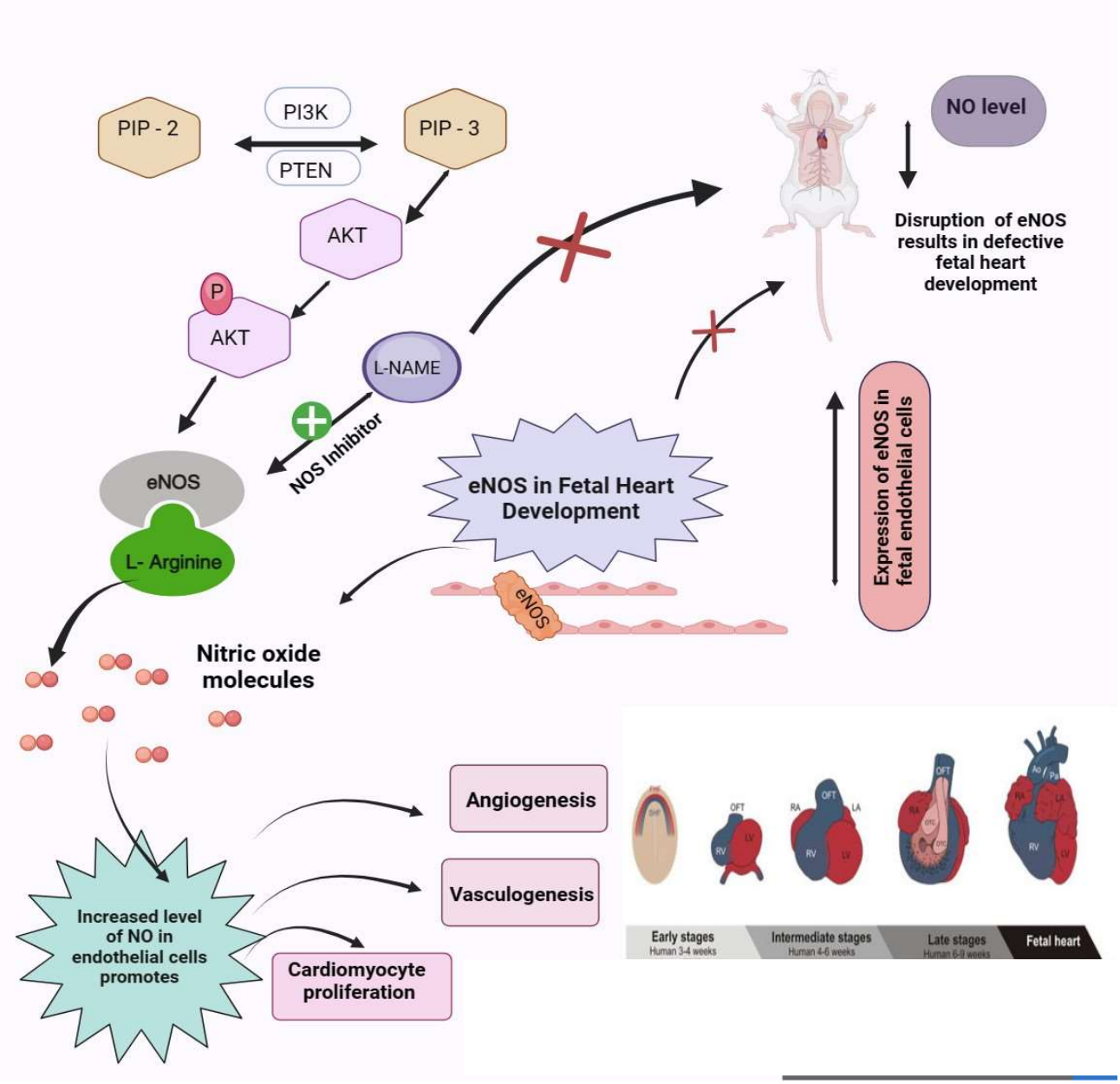

Figure 2: The diagram depicts the mechanisms through which nitric oxide (NO) is produced in the context of placental cardiomyogenesis leading to angiogenesis and vasculogenesis and cardiomyocyte proliferation in fetal heart during pregnancy. According to the NO Production Pathway, endothelial nitric oxide synthase (eNOS) and L-arginine together produce NO, which promotes angiogenesis and controls vascular tone. On the other hand, the Inhibition of NO Pathway shows how eNOS and the nitric oxide inhibitor L-NAME interact in order to limit the generation of NO, which causes congenital heart abnormalities which was well studied in mice models. Any disruption in the PI3K/AKT, PTEN and eNOS can lead to abnormal septation and reduced vascularization of the fetal heart thereby leading to congenital heart abnormalities.

Role of non-coding RNAs in eNOS Regulation:

Based on nucleotide length, non-coding RNAs are classified as lncRNAs and sncRNAs. The bulk sncRNAs are composed of miRs, which have an average of 22 nucleotides, whereas lncRNAs, which include circRNAs and linear lncRNAs, contain more than 200 nucleotides. The pre-miRNAs are transported into the cytoplasm by the nucleocytoplasmic protein exportin-5 (XPO5). These pre-miRNA gets integrated into the complex RNA induced silencing complex (RISC) interacts with argonate and develops into miRNA (53,54,55). A well-conserved miR that modulates the TGF-β and WNT signaling pathways is crucial in the formation of the heart muscle. The miR-335-5p and miR-335-3p, a significant regulators of heart morphogenesis were identified using silico method. Overexpression of miR-335-5p and miR-335-3p resulted in increased levels of TNNT2 and CNX43 suggesting the significance of TGF-β and WNT signaling as a critical indicator of dysregulated cardiac development (56). The role of certain miRNAs in endothelial cells (ECs) is important for vascular signaling and function. Several studies show that the two main miRNA-regulating enzymes, Drosha and Dicer, are necessary for endothelial cells to perform their angiogenic roles (57). The association between miR-92a and eNOS was demonstrated in a study. In animal models of arterial damage, nitric oxide (NO) largely prevents the establishment of neointimal hyperplasia by preventing the smooth vascular muscle proliferation (58). A rise in NO bioavailability and an inhibitory effect on SMC proliferation are, thus, the functional outcomes of the miR-92a inhibition. Represented miR-222 and miR-221, these microRNAs exhibit yet another negative connection with eNOS activity. With their anti-angiogenic properties, these microRNAs are extensively expressed in ECs (59). Interestingly, overexpression of miR-221 and miR-222 decreases eNOS levels indirectly. The receptor for stem cell factor (SCF), c-kit, is targeted by miR-221 and miR-222 where C-kit is essential for endothelial cell motility (60).

Paracrine Signaling by eNOS between endothelial Cells and cardiomyocytes.

Through desmosomes, adherens junctions, and gap junctions, cardiomyocytes are physically connected to one another within the myocardium (61). They also exchange signals through paracrine pathways and direct physical contact with other cardiac cell types. In order to communicate endocrinely with peripheral tissues and paracrinely with other cardiac cells, cardiomyocytes generate and secrete proteins and peptides. These Cardiokines, also known as

cardiomyokines ranges between 30 and 60 distinct proteins or peptides secreted by the heart (62) However, as suggested by analytical method advancement, this number could rise significantly. Cardiokines are essential for maintaining growth of heart normally as well as acting as mediators for stress response. They include growth factors, endocrine hormones, cytokines, extracellular matrix proteins, and peptides. The natriuretic peptides A (ANP) and B (BNP), which functions primarily as cardioprotective factors for Cardiomyocytes and involves in the production of NO and endothelin-1 (63). Vascular endothelial growth factor (VEGF) plays a significant role as it attaches and activates the VEGF receptor 2 (VEGFR2, also known as KDR and FLK1) in endothelial cells, which further controls activation and proliferation of cardiomyocytes (64). It is very important to consider that VEGF further regulates the phosphorylation of AKT which involves in the activation of eNOS thereby increasing the NO production in endothelial cells. These NO molecules upregulated by paracrine signaling will lead to vasculogenesis, angiogenesis and increased cardiomyocyte proliferation. In order to improve circulation, the availability of oxygen and nutrients in areas that are deficient, such as growing or infarcted hearts the VEGFR2 signaling triggers angiogenesis where the formation of new blood vessels from the pre-existing ones occur. The other members of the VEGF family, VEGF-B (65), VEGF-C (66), and placental growth factor (PlGF), as well as fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), and angiopoietin-1 also plays a major role in angiogenesis. Additionally, cardiomyocytes generate and release multiple members of the TGF-β superfamily, which have an adverse effect on the heart as well as cardioprotective effects and also increase the expression of eNOS through Smad pathway. Follistatin like-1 is one of these that has been studied the most and has been found to impact both Cardiomyocytes and Endothelial cells. They protect endothelial dysfunction by increasing eNOS activity (67). Furthermore, studies have shed light on the processes by which eNOS activity affects cardiac progenitor cells (CPCs) fate in co-culture settings. Also, recent studies suggest that nitric oxide (NO) significantly affects CPC development in a set of tests using a co-culture model of Sca-1+ CPCs and newborn mouse cardiac myocytes (NNCMs) in low glucose DMEM. Notable alterations in CPC behavior were seen upon administration of NO, either endogenously released by paracrine signaling from parenchymal cells or exogenously supplied through sources such as DETA-NO. In particular, these investigations showed that NO, which is mostly produced by eNOS activity, improved CPC's capacity to differentiate into cardiomyocytes (68).

Table 2 summarizes the primary findings on eNOS and paracrine signaling in the development of the cardiovascular system. With regard to cardiac physiology, it provides a thorough understanding of target genes, nitric oxide levels, study findings, experimental settings, and pathways explored.

Experimental Setup and Models Used	Pathway Involved	Nitric Oxide Expression	Research Findings	Cardiomyocyte Differentiation/Proliferation	Target Genes
Co-culture of Sca-1+ CPCs (WT) with NNCMs (NOS3-tg) (68)	Wnt/β-catenin pathway	High	Increased proportion of α-sr-actinin+ CPCs in co-culture (*P < 0.05) - L-NAME and ODQ inhibit NO-mediated differentiation.	Yes	Wnt4, Axin2, Snai2
Injection of GFP-expressing Sca1+ CPCs in infarcted LV areas (NOS3-tg mice, WT) (68)	Wnt/β-catenin pathway	High	Nearly five-fold increase in doubly positive CPCs in hearts from NOS3-tg mice compared to WT post-AMI.	Yes	No target genes
Inhibition of canonical Wnt/β-catenin pathway in Sca-1+ CPCs using NO (68)	Wnt/β-catenin pathway	High	Decreased β-catenin levels and down-regulation of Axin2, Wnt4, and Snai2 transcripts in NO-treated CPCs - Reversed by	Yes	β-catenin, Axin2, Wnt4, Snai2

			ODQ co-treatment.		
NO improvement in CPC differentiation via Wnt/β-catenin down-regulation (68)	Wnt/β-catenin pathway	High	NO inhibits intracellular β-catenin activity and Wnt morphogen production in CPCs - NO enhances CPC differentiation through Wnt/β-catenin signaling pathway.	Yes	β-catenin, Wnt morphogens
Notch pathway mutants in ventricular myocardium development (8)	Notch signaling	High	Abnormalities in ventricular myocardium development similar to eNOS null mutants at E10.5.	Yes	Notch1, Nrg1
Notch1 and Jag1 expression patterns in eNOS null and control embryos at E14.5 (8)	Notch signaling	High	Notch1ICD in cells of endothelium of ductus arteriosus in eNOS null mice at E14.5 showed	No	Notch1, Jag1

			decreased Notch1 levels. Also, Downregulated Jag1 in eNOS mutants were observed.		
Notch1ICD and Jag1 distribution at E10.5 and E11.5 in eNOS null embryos (8)	Notch signaling	High	Less Notch1ICD in forming trabeculae at E10.5 of eNOS null mutants. eNOS null embryos showed reduction in Notch1ICD of endocardial ventricles. Increased Nrg1 expression in ventricles of eNOS nulls.	Yes	Notch1, Nrg1

eNOS Genetic variants linked to congenital heart defects

Septal defects have been linked to genetic variation in endothelial nitric oxide synthase (eNOS), especially in CHD's and PAH. Studies has demonstrated a strong correlation between the vulnerability to certain conditions and particular polymorphisms associated with the eNOS gene. For example, a higher risk of PAH in individuals with congestive heart failure (CHD) is associated with the eNOS rs1799983 T allele, particularly in GT/TT genotypes, potentially because of decreased nitric oxide (NO) production (69). Similarly, there is a substantial correlation between the eNOS G894T polymorphism and the risk of CHD, with a greater allele frequency seen in CHD

patients, especially in genotypes TT and GT (70). Additionally, the eNOS 894 GT and TT genotypes in children are linked to an increased risk of congestive heart failure (CHD), particularly in relation to the mother 894TT genotype and tobacco use during pregnancy (71). The polymorphism of the eNOS-786 gene however, has no apparent impact on the probability of developing coronary artery disease (CAD) prematurely (72). Furthermore, there is a significant increase in the risk of CHD associated with the eNOS rs7830 polymorphism, especially in cases with peri-membranous ventricular septal defects (73). It's important to consider that in individuals with atrial septal defect (ASD), eNOS G894T and 4b/4a polymorphisms do not contribute to the development of PAH (74). In contrast, there appears to be a link between the eNOS 894T genotype and pulmonary arterial hypertension, since the eNOS 894T allele is considerably greater in people with PAH associated to CHD (75). Therefore, these findings suggest that NO production regulated by eNOS is much more important in septum formation and maintenance in fetal heart, where gene variant in eNOS can lead to abnormal septum formation during cardiogenesis.

Conclusion:

In conclusion the crucial function of eNOS within the intricate mechanism of embryonic heart development and the implications this has for septal defects is discussed on this review. eNOS regulates the cardiac development and valve formation via an organized molecular pathway and signaling networks. Also, this sheds light on the numerous functions that eNOS plays, from its participation in vasculogenesis and angiogenesis to its important role in preserving hemodynamic balance and regulating vascular tone for the duration of the development of fetal heart during cardiogenesis. The mechanisms behind molecular signaling of eNOS-mediated cardiac development and septum formation has extraordinary potential for the improvement of particular therapeutic techniques for CHD's including ASD, VSD and conotruncal defects. Potential therapeutic goals that can mitigate the outcomes of septal defects and different cardiac anomalies can be achieved by targeting eNOS paracrine signaling pathways thereby increasing the upregulation of NO molecules in endothelial cells. Furthermore, this highlights how essential it is to carry out studies on eNOS signaling cascades and validate their relevance therapeutically in treating CHD. More research and innovation in this field also are made viable way of a combination of contemporary generation along with genetic studies, animal models, and medical observations. By using nitric oxide (NO) molecule by targeting eNOS signaling pathways and eNOS modulation, novel approaches can be made to provide personalized therapy strategies which may advance the clinical outcomes and improve the quality of life for individuals affected with CHD's.

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