The Development of the Chick Embryo Heart and Using it as a Model for Atrial Septal Research

Taking 24 hours to generate one egg, hens can produce upwards of 300 eggs per year (Biggs, 2022). From that one egg, a fully developed chick hatches in 21 days (Vergara and Canto-Soler, 2012). As the egg is laid, the chick embryo is already in the blastula stage, and within two to three days, the embryo will complete gastrulation, neurulation, and histogenesis (Vergara and Canto-Soler, 2012). Throughout these 21 days, embryos can be visualized without microscopes, and experimentally manipulated, leading to important embryological discoveries and concepts. Using the chick, Malpighi identified the neural tube, somites, and capillaries; Pander and von Baer discovered the germ layers; and Waddington discovered the avian organizer (Stern, 2005). These crucial discoveries illustrate the importance of chicks as a model organism. Chicks develop and hatch rapidly, allowing for quick experiments; chicken eggs are highly accessible; the embryo's large size makes it simple for experimental manipulation, and provides easy access to vital organs; the embryo's large size makes it amenable to genetic and cellular modifications; and there is a significant similarity to human embryos – both at the genetic and structural level (Vergara and Canto-Soler, 2012). Scientists can manipulate genes, cells, and molecules to track and visualize changes pertaining to development; moreover, since chicks are amniotes, development and changes in development may be directly applied to human development (Vergara and Canto-Soler, 2012). Since chicks have a four chambered heart, like humans, chicks can be used to track heart development (Vergara and Canto-Soler, 2012). Cellular and mechanical processes can be observed, recorded, and manipulated to gain insight into how the human heart develops, ultimately contributing to the scientific community. Identifying the development and movement of the chick heart, and its structures, can be compared to abnormal development and movement, providing researchers with details and information that connects gaps in knowledge pertaining to development. By fully understanding chick development, information can be adjusted to fit human heart development, contributing to inferences regarding human embryological development and disease mechanisms and prevention. Overall, this contributes to the scientific community as a deeper understanding of life and science is attained, leading to advances that improve everyday life. Current research uses chick embryos to understand heart development, and the mechanisms that occur during development. Researchers studying this process utilize information about normal development,

and compare it to abnormal development, to gain more knowledge about cardiac diseases that may occur, such as atrial septal defect, and how this disease may be treated.

In the developing chick embryo, the heart is the first functional organ to be formed, with the circulatory system being the first functional unit formed (Wittig and Münsterberg, 2016). During the first day of incubation, two epithelial, endocardial tubes form on opposite sides of the embryo (Taber and Perucchio, 2000). These tubes are formed by mesodermal cells undergoing an epithelial to mesenchyme transition (Taber, 1998). Promptly, the two tubes undergo cylindrical bending to merge and fuse along the embryo's ventral midline to form a straight anterior to posterior cardiac tube (Taber, 1998). The single cardiac tube consists of one tube with two branches and three distinct layers: an outer myocardium, a middle cardiac jelly, and an inner endocardium layer (Taber, 1998). Following the formation of the cardiac tube, heart contractions begin; however, initially, peristatic waves of contractions causes blood to flow through the heart (Taber and Perucchio, 2000). By day three, blood flow becomes pulsative, with the presence of endocardial cushions acting as values to prevent backflow of blood (Taber and Perucchio, 2000).

The beginning of pulsative blood flow triggers the beginning of heart looping (Männer, 2000). Heart looping consists of four phases: the pre-looping phase, the dextral-looping phase, transformation of the c-shaped heart into the s-shaped heart, and a final phase of late positional changes that leads to the primitive outflow tract (Männer, 2000). The pre-looping phase is marked by the formation of bulges in the cardiac tube, giving rise to the sinus venosus, the primitive atrium, the ventricle, and the conotruncus (Taber, 1998). Once these bulges are established, dextral looping of the heart occurs: the straight heart tube begins to change into a c-shaped heart loop (Männer, 2000). The looping of the heart into a c-shape allows for the pre-formed bulges to move into the correct location to form important future structures (Männer, 2000). Following the formation of the c-shaped heart, activation of the third phase occurs, transforming the c-shaped heart into an s-shaped heart (Männer, 2000). The s-shaped heart begins to take shape of a mature heart, finalizing the movement of structures into the correct location (Männer, 2000). The s-shaped heart consists of a single atrium and ventricle; however, the final phase of heart looping consists of creating the four chambers of the heart, finalizing the movement of important veins and arteries, and any final growth necessary to have a normal, healthy heart (Männer, 2000). Within 72 hours of incubation, looping is complete, the heart is fully developed, and by day four of development there is visible beating of the heart (Taber and

Perucchio, 2000). Normal occurrence of these processes lead to a health heart; however, when heart development or looping falters, cardiac diseases occur, specifically atrial septal defect.

Atrial septal defect is a congenital defect, a defect present at birth, that causes a hole in the wall of the heart that separates the atria of the heart (Geva et al. 2014). Atrial septal defect is the third most common congenital defect, causing the heart difficulty to pump blood around the body (Geva et al. 2014). Currently, the cause of atrial septal defect is unknown; therefore, researchers have been experimentally manipulating chick heart development to obtain details of the disorder. Xu and colleagues used chick hearts to identify gene and transcription factor mutations that are associated with atrial septal defect. NKX2.5 and GATA4 are genes transcribed very early in chick heart development (Durocher, 1997). These genes, working in unison, produce transcription factors that bind to specific cardiac cell DNA promoters, causing cardiac cell formation and cardiomyocyte differentiation (Durocher, 1997). Normally, NKX2.5 and GATA4 causes heart tube formation and growth, along with proper heart looping; Xu and colleagues identified that mutations in these genes causes the alteration of transcription factor formation, ultimately modifying the formation and differentiation of cardiac cells (Xu, et al. 2017). Mutations of the genes that prevent transcription factor formation, ultimately leading to the arresting of cardiac looping and inhibition of heart growth (Xu, et al. 2017). The heart tube cannot properly loop into the c-shape, and eventual s-shape, causing improper joining of the atrial septum (Xu et al. 2017). Although these genes cause detrimental mutations, Xu and colleagues indicate that these mutations are an uncommon cause of atrial septal disorder in humans, and that other mutations are more likely to contribute to the disorder.

Another more likely cause of atrial septal defect is the mutation of the TLL1 gene, mammalian tolloid-like 1 gene (Sieron, et al. 2019). BMP1 was the first discovered tolloid in mammals (Sieron, et al. 2019). TLL1 genes cause cleavage of chordin, resulting in BMP2 and BMP4 to interact with receptors to trigger epithelial to mesenchymal transition of cells into endocardial panel cells – the endocardium of the heart tube (Sieron, et al. 2019). Sieron and colleagues, introduced noggin, a signaling molecule, into a chick embryo's heart. The researchers found that noggin inhibits BMP2 and BMP4, resulting in reduced expression of genes that cause cardiogenesis, specifically NXK2.5 and GATA4 (Sieron, et al. 2019). Normally, BMP2 forms a positive feedback loop with SMAD1, NXK2.5, and GATA4, causing the differentiation and proliferation of cardiac cells to form the secondary, s-shaped heart tube

(Sieron, et al. 2019). However, mutations in TLL1, ultimately causing mutations in BMP2 and BMP4 expression, causes a change in the formation of endocardial cushion cells; changes in cushion cells causes partial atrial septal formation in the s-shaped heart and semilunar valve defects (Sieron, et al. 2019). Ultimately, Sieron et al. illustrated those deficiencies in BMP2 and BMP4 molecules, or presence of noggin inhibitors, contribute to the formation of atrial septal defect in chick hearts (Sieron, et al. 2019).

Atrial septal defects may also arise form teratogens (Harris, et al. 2018). Trichloroethylene, abbreviated TCE, a contaminant of drinking water, is a cardiac teratogen in chicks (Harris, et al. 2018). TCE is directly linked to inhibiting HNF4a mRNA production; HNF4a produces transcription factors that help cardiac development and valve formation (Harris, et al. 2018). TCE inhibits HNF4a transcription, causing the fusion of the s-shaped heart walls to fail (Harris, et al. 2018). In vivo, TCE inhibits formation of molecules that enable epithelial to mesenchymal transition of cardiac cells to form the septal wall and valve tissues (Harris, et al. 2018). By preventing this transition, a hole in the heart of chicken embryos manifests itself, causing a difficulty of pumping blood around the embryo (Harris, et al. 2018).

Finally, researchers determined to find a mechanism that aims to prevent atrial septal formation. A transcriptional subunit, specifically a transcriptional cascade, comprised of TGIF1, EST1, and SOX8 work in tandem for cardiac neural crest cells and heart development to occur; Gandhi and colleagues illustrate that this subunit may be used to reprogram neural crest cells into functional cardiac cells (Gandhi, et al. 2020). Loss of genes, such as PAX3 or EDN1 result in cardiac abnormalities; however, TGIF1, EST1, and SOX8, a cardiac-crest specific subcircuit, can be recruited to program cardiac neural cells, and other important cells, to migrate to the heart for development and looping (Gandhi, et al. 2020). Gandhi and colleagues altered transcription of specific genes and triggered the expression of the subunit; the researchers determined that SOX8 influences TGIF1, which then influences ETS1; with all 3 genes active, ETS1 produces a protein, NACC2, that interact with neural-crest cells, causing an alteration in neural crest cells to develop a cardiac crest-like identity (Gandhi, et al. 2020). These cells exhibit migratory behaviours like normal cardiac cells, moving into specific locations determined by NACC2 (Gandhi, et al. 2020). Altered and unaltered chick hearts were labeled with GFP to visualize neural crest cells and their movement. In unaltered chicks, neural crest cells were located towards the ventral midline of the chick embryo; however, in altered chicks GFP was in the

heart, with majority of GFP located in the atrial wall (Gandhi, et al. 2020). Therefore, reprograming cells, specifically neural crest cells in chick embryos, can be used as a mechanism to prevent formation of atrial septal defects (Gandhi, et al. 2020).

Currently, atrial septal defect can be fixed through open-heart surgery (Jiminez, et al. 2022). This method, however, causes difficult recovery for patients (Jiminez, et al. 2022). Additionally, after surgery, patients must keep up with regular checkups, monitoring for possible complications, such as: arrythmias, heart valve issues, pulmonary hypertension, and heart failure (Jiminez, et al. 2022). Therefore, researchers are developing experiments to find therapies and mechanisms that can provide patients with less invasive methods of treatment. Currently, one researcher is working on a less-invasive surgical method that would not introduce any foreign materials into the heart, like a catheter. Researcher Hattam hypothesised, used chick models, to develop a new mechanism with minimal limitations. Hattam, *in utero,* created an artificial atrial septum near a pre-existing septum (Hattam, 2018). Doing so caused the developing myocytes to respond to the artificial septum, causing myocyte hyperplasia (Hattam, 2018). The increased number of myocytes caused the release of a "pressure-flow" signal, triggering the increased myocyte number to close the artificial septum, in addition to the pre-existing septum (Hattam, 2018). Although successful with chick models, Hattam stated that his next steps would be to experiment on a more complex model, such as a sheep, that is much more like the human heart (Hattam, 2018). If successful, this mechanism would be applicable to all but the rarest atrial septal defects, allowing a wide range of individuals with atrial septal defect to obtain treatment (Hattam, 2018).

Using chicks as a cardiac model organism to understand normal development allows researchers to understand human heart development. Normal and abnormal development can be compared, contrasting similarities and differences that contribute to specific a deeper understanding of development and embryology. Diseases, like atrial septal defect, can be studied, determining molecules and factors that contribute to the defect. Finding treatments in chick models can be applied to humans to provide novel therapies, treating heart defects and diseases. Most importantly, using chicks as a model organism contributes to the scientific community, but also contributes globally, as details about development and life are becoming easier to access allowing more people to become educated. As well, chicks as a model organism allows researchers to share data that can be used to greatly improve the health of the global population.

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