Comp Med 2019 May 1;69(3):184-203. doi: 10.30802/AALAS-CM-18-000061.

**Embryonic Chicken ( *Gallus gallus domesticus*) as a Model of Cardiac Biology and Development**

José G Vilches-Moure [1]

Abstract

Cardiovascular disease remains one of the top contributors to morbidity and mortality in the United States. Increasing evidence suggests that many processes, pathways, and programs observed during development and organogenesis are recapitulated in adults in the face of disease. Therefore, a heightened understanding of cardiac development and organogenesis will help increase our understanding of developmental defects and cardiovascular diseases in adults. Chicks have long served as a model system in which to study developmental problems. Detailed descriptions of morphogenesis, low cost, accessibility, ease of manipulation, and the optimization of genetic engineering techniques have made chicks a robust model for studying development and make it a powerful platform for cardiovascular research. This review summarizes the cardiac developmental milestones of embryonic chickens, practical considerations when working with chicken embryos, and techniques available for use in chicks (including tissue chimeras, genetic manipulations, and live imaging). In addition, this article highlights examples that accentuate the utility of the embryonic chicken as model system in which to study cardiac development, particularly epicardial development, and that underscore the importance of how studying development informs our understanding of disease.

Scand J Immunol. 2007 Aug-Sep;66(2-3):113-21.

**Avian model for B-cell immunology--new genomes and phylotranscriptomics.**

Kohonen P[1], Nera KP, Lassila O.

Extract: Avian B-cell development – another perspective

Removal of the chicken bursa of Fabricius first demonstrated the existence of bursa-derived cells or B cells as the source of antibody responses. Avian species use gut- associated lymphoid tissues (GALT) as a site for primary B-cell development instead of foetal liver and bone marrow. They also differ from human and mouse in that they have a very simple immunoglobulin (Ig) locus. In order to produce Ig diversity they make use of homologous recombination (called gene conversion) to transfer genetic material from unexpressed V pseudogenes in the vicinity to the rearranged active Ig locus. A number of mammalian species such as rabbits and sheep also carry out part of their B-cell development in GALT tissues. Rabbit and swine also use gene conversion to generate diversity whereas sheeprely mainly on somatic hypermutation. However, the chicken bursa of Fabricius, located in the distal cloaca, is probably the most highly specialized primary B-cell organ. Despite the differences outlined here the B-cell development in chicken and mammals is a very similar process. It can be argued that similarities are even greater at the molecular level and at the level of regulatory networks.

Eur Cell Mater. 2013 Sep 11;26:91-106; discussion 106.

**A new take on an old story: chick limb organ culture for skeletal niche development and regenerative medicine evaluation.**

Smith EL[1], Kanczler JM, Oreffo RO.

Abstract

Scientific research and progress, particularly in the drug discovery and regenerative medicine fields, is typically dependent on suitable animal models to develop new and improved clinical therapies for injuries and diseases. In vivo model systems are frequently utilized, but these models are expensive, highly complex and pose a number of ethical considerations leading to the development and use of a number of alternative ex vivo model systems. The ex vivo embryonic chick long bone and limb bud models have been utilized in the scientific research field as a model to understand skeletal development for over eighty years. The rapid development of avian skeletal tissues, coupled with the ease of experimental manipulation, availability of genome sequence and the presence of multiple cell and tissue types has seen such model systems gain significant research interest in the last few years in the tissue engineering field. The models have been explored both as systems for understanding the developmental bone niche and as potential testing tools for tissue engineering strategies for bone repair and regeneration. This review details the evolution of the chick limb organ culture system and presents recent innovative developments and emerging techniques and technologies applied to these models that are aiding our understanding of

skeletal developmental and regenerative medicine research and application.

PLoS Genet. 2020 Nov 11;16(11):e1009164. doi: 10.1371/journal.pgen.1009164

**The PAX-FOXO1s trigger fast trans-differentiation of chick embryonic neural cells into alveolar rhabdomyosarcoma with tissue invasive properties limited by S phase entry inhibition**

Gloria Gonzalez Curto [1] , et al.

Abstract

The chromosome translocations generating PAX3-FOXO1 and PAX7-FOXO1 chimeric proteins are the primary hallmarks of the paediatric fusion-positive alveolar subtype of Rhabdomyosarcoma (FP-RMS). Despite the ability of these transcription factors to remodel chromatin landscapes and promote the expression of tumour driver genes, they only inefficiently promote malignant transformation in vivo. The reason for this is unclear. To address this, we developed an in ovo model to follow the response of spinal cord progenitors to PAX-FOXO1s. Our data demonstrate that PAX-FOXO1s, but not wild-type PAX3 or PAX7, trigger the trans-differentiation of neural cells into FP-RMS-like cells with myogenic characteristics. In parallel, PAX-FOXO1s remodel the neural pseudo-stratified epithelium into a cohesive mesenchyme capable of tissue invasion. Surprisingly, expression of PAX-FOXO1s, similar to wild-type PAX3/7, reduce the levels of CDK-CYCLIN activity and increase the fraction of cells in G1. Introduction of CYCLIN D1 or MYCN overcomes this PAX-FOXO1-mediated cell cycle inhibition and promotes tumour growth. Together, our findings reveal a mechanism that can explain the apparent limited oncogenicity of PAX-FOXO1 fusion transcription factors. They are also consistent with certain clinical reports indicative of a neural origin of FP-RMS.

Int J Dev Biol. 2018;62(1-2-3):97-107. doi: 10.1387/ijdb.170321ja.

**Craniofacial development: discoveries made in the chicken embryo.**

Abramyan J[1], Richman JM.

Abstract

The aim of this review is to highlight some of the key contributions to our understanding of craniofacial research from work carried out with the chicken and other avian embryos. From the very first observations of neural crest cell migration to the fusion of the primary palate, the chicken has proven indispensable in facilitating craniofacial research. In this review we will look back to the premolecular studies where "cut and paste" grafting experiments mapped the fate of cranial neural crest cells, the role of different tissue layers in patterning the face, and more recently the contribution of neural crest cells to jaw size and identity. In the late 80's the focus shifted to the molecular underpinnings of facial development and, in addition to grafting experiments, various chemicals and growth factors were being applied to the face. The chicken is above all else an experimental model, inviting hands-on manipulations. We describe the elegant discoveries made by directly controlling signaling either in the brain, in the pharyngeal arches or in the face itself. We cover how sonic hedgehog (Shh) signals to the face and how various growth factors regulate facial prominence identity, growth and fusion. We also review abnormal craniofacial development and how several type of spontaneous chicken mutants shed new light on diseases affecting the primary cilium in humans. Finally, we bring out the very important role that the bird beak has played in understanding amniote evolution. The chicken, duck and quail have been and will continue to be used as experimental models to explore the evolution of jaw diversity and the morphological constraints of the vertebrate face.

Biochem Cell Biol. 2018 Apr;96(2):98-106. doi: 10.1139/bcb-2017-0205.

**The avian embryo as a model for fetal alcohol spectrum disorder.**

Flentke GR[1,1], Smith SM[1,1].

Abstract

Prenatal alcohol exposure (PAE) remains a leading preventable cause of structural birth defects and permanent neurodevelopmental disability. The chicken (Gallus gallus domesticus) is a powerful embryological research model, and was possibly the first in which the teratogenicity of alcohol was demonstrated. Pharmacologically relevant exposure to alcohol in the range of 20-70 mmol/L (20-80 mg/egg) disrupt the growth of chicken embryos, morphogenesis, and behavior, and the resulting phenotypes strongly parallel those of mammalian models. The avian embryo's direct accessibility has enabled novel insights into the teratogenic mechanisms of alcohol. These include the contribution of IGF1 signaling to growth

suppression, the altered flow dynamics that reshape valvuloseptal morphogenesis and mediate its cardiac teratogenicity, and the suppression of Wnt and Shh signals thereby disrupting the migration, expansion, and survival of the neural crest, and underlie its characteristic craniofacial deficits. The genetic diversity within commercial avian strains has enabled the identification of unique loci, such as ribosome biogenesis, that modify vulnerability to alcohol. This venerable research model is equally relevant for the future, as the application of technological advances including CRISPR, optogenetics, and biophotonics to the embryo's ready accessibility creates a unique model in which investigators can manipulate and monitor the embryo in real-time to investigate the effect of alcohol on cell fate.

Prog Retin Eye Res. 2017 Nov;61:72-97. doi: 10.1016/j.preteyeres.2017.06.004.

**The chick eye in vision research: An excellent model for the study of ocular disease.**

Wisely CE[1], Sayed JA[1], Tamez H[1], Zelinka C[2], Abdel-Rahman MH[1], Fischer AJ[3], Cebulla CM[4].

Abstract

The domestic chicken, Gallus gallus, serves as an excellent model for the study of a wide range of ocular diseases and conditions. The purpose of this manuscript is to outline some anatomic, physiologic, and genetic features of this organism as a robust animal model for vision research, particularly for modeling human retinal disease. Advantages include a sequenced genome, a large eye, relative ease of handling
and maintenance, and ready availability. Relevant similarities and differences to humans are highlighted for ocular structures as well as for general physiologic processes. Current research applications for various ocular diseases and conditions, including ocular imaging with spectral domain optical coherence tomography, are discussed. Several genetic and non-genetic ocular disease models are outlined, including for pathologic myopia, keratoconus, glaucoma, retinal detachment, retinal degeneration, ocular albinism, and ocular tumors. Finally, the use of stem cell technology to study the repair of damaged tissues in the chick eye is discussed. Overall, the chick model provides opportunities for high-throughput translational studies to more effectively prevent or treat blinding ocular diseases.

Z Kinderchir 1990 Dec;45 Suppl 1:20-2. doi: 10.1055/s-2008-1042628.

**Spina bifida: a chick embryo experimental model**

B Lopez de Torre [1] , J A Tovar, P Aldazabal, S Uriarte, A Rey, I Ruiz, M San Vicente

Abstract

Neural Tube Defects (NTD) can be induced in the chick embryo with relative ease in order to provide an experimental tool for investigation of such disabling malformations. Domestic hen (Gallus gallus) eggs were incubated at 37.5 degrees C and 80% humidity for 24 h. At that moment, 5 ml of albumen were aspirated by sterile puncture of the shell, and the incubation was resumed. The embryos were recovered and studied at the 8th, 10th and 14th days. Almost half (45%) of the 602 treated embryos survived and 73 of them (12%) had various malformations. Thirty-six (6%) suffered NTD of which 30 were open myelomeningocele, 2 meningocele and 4 encephalocele. The anatomy of the defects was astonishingly similar to that of the human malformation. Whether these experimental NTD are induced by mechanical or nutritional modifications of the internal environment of the egg is unknown, but the similarity of the lesion with those in humans make them suitable for further investigation of these issues. We believe that this relatively simple and inexpensive model is a suitable tool for research on spina bifida.

Neurosci Biobehav Rev. 2015 Mar;50:86-102. doi: 10.1016/j.neubiorev.2014.07.006.

**Memory-related brain lateralisation in birds and humans.**

Moorman S[1], Nicol AU[2].

Abstract

Visual imprinting in chicks and song learning in songbirds are prominent model systems for the study of the neural mechanisms of memory. In both systems, neural lateralisation has been found to be involved in memory formation. Although many processes in the human brain are lateralised--spatial memory and musical processing involves mostly right hemisphere dominance, whilst language is mostly left hemisphere dominant--it is unclear what the function of lateralisation is. It might enhance brain capacity, make processing more efficient, or prevent occurrence of conflicting signals. In both avian paradigms we find memory-related lateralisation. We will discuss avian lateralisation

findings and propose that birds provide a strong model for studying neural mechanisms of memory-related lateralisation.

Semin Cell Dev Biol. 2011 Dec;22(9):985-92. doi: 10.1016/j.semcdb.2011.09.019.

**Early arterial differentiation and patterning in the avian embryo model.**

Garriock RJ[1], Mikawa T.

Abstract

Of the many models to study vascular biology the avian embryo remains an informative and powerful model system that has provided important insights into endothelial cell recruitment, assembly and remodeling during development of the circulatory system. This review highlights several discoveries in the avian system that show how arterial patterning is regulated using the model of dorsal aortae development along the embryo midline during gastrulation and neurulation. These discoveries were made possible through spatially and temporally controlled gain-of-function experiments that provided direct evidence that BMP signaling plays a pivotal role in vascular recruitment, patterning and remodeling and that Notch-signaling recruits vascular precursor cells to the dorsal aortae. Importantly, BMP ligands are broadly expressed throughout embryos but BMP signaling activation region is spatially defined by precisely regulated expression of BMP antagonists. These discoveries provide insight into how signaling, both positive and negative, regulate vascular patterning. This review also illustrates similarities of early arterial patterning along the embryonic midline in amniotes both avian and mammalians including human, evolutionarily specialized from non-amniotes such as fish and frog.

Nature 2016 Mar 3;531(7592):105-9. doi: 10.1038/nature16951.

**Deriving human ENS lineages for cell therapy and drug discovery in Hirschsprung disease**

Faranak Fattahi [1 2 3], Julius A Steinbeck [1 2], Sonja Kriks [1 2], Jason Tchieu [1 2], Bastian Zimmer [1 2], Sarah Kishinevsky [1 2 3], Nadja Zeltner [1 2], Yvonne Mica [1 2], Wael El-Nachef [4], Huiyong Zhao [4], Elisa de Stanchina [4], Michael D Gershon [5], Tracy C Grikscheit [6], Shuibing Chen [7], Lorenz Studer [1 2]

Abstract

The enteric nervous system (ENS) is the largest component of the autonomic nervous system, with neuron numbers surpassing those present in the spinal cord. The ENS has been called the 'second brain' given its autonomy, remarkable neurotransmitter diversity and complex cytoarchitecture. Defects in ENS development are responsible for many human disorders including Hirschsprung disease (HSCR). HSCR is caused by the developmental failure of ENS progenitors to migrate into the gastrointestinal tract, particularly the distal colon. Human ENS development remains poorly understood owing to the lack of an easily accessible model system. Here we demonstrate the efficient derivation and isolation of ENS progenitors from human pluripotent stem (PS) cells, and their further differentiation into functional enteric neurons. ENS precursors derived in vitro are capable of targeted migration in the developing chick embryo and extensive colonization of the adult mouse colon. The in vivo engraftment and migration of human PS-cell-derived ENS precursors rescue disease-related mortality in HSCR mice (Ednrb(s-l/s-l)), although the mechanism of action remains unclear. Finally, EDNRB-null mutant ENS precursors enable modelling of HSCR-related migration defects, and the identification of pepstatin A as a candidate therapeutic target. Our study establishes the first, to our knowledge, human PS-cell-based platform for the study of human ENS development, and presents cell- and drug-based strategies for the treatment of HSCR.

Neuroscience 2005;134(4):1285-300. doi: 10.1016/j.neuroscience.2005.05.020

**The chick embryo appears as a natural model for research in beta-amyloid precursor protein processing**

J A Carrodeguas [1], A Rodolosse, M V Garza, A Sanz-Clemente, R Pérez-Pé, A M Lacosta, L Domínguez, I Monleón, R Sánchez-Díaz, V Sorribas, M Sarasa

Abstract

This study reveals that the chick embryo has active the machinery for the production and degradation of the amyloid beta peptide characteristic of Alzheimer's disease. We cloned the principal beta-amyloid precursor protein isoforms in the chick embryo and observed that they are highly homologous to the human sequences and identical at the C-terminal sequence, including the amyloid beta domain. Mammals such as rat or mouse, more commonly used as animal models of human diseases, have a distinct amyloid beta

sequence. The distribution of beta-amyloid precursor protein isoforms in the chick embryo revealed that, as in humans, their expression is ubiquitous and the prototype beta-amyloid precursor protein-695 predominated in the nervous system. We also found that the chick embryo expresses the genes for the main proteolytic proteases implicated in the production of amyloid beta, including BACE-1, BACE-2, presenilin-1, presenilin-2 and nicastrin, as well as the amyloid beta-degrading enzyme neprilysin, or ADAM-17, a protease implicated in the non-amyloidogenic processing of beta-amyloid precursor protein. We have also found that between amyloid beta40 and amyloid beta42, this latter seems to be the major amyloid beta peptide produced during chick embryogenesis. The chick embryo appears as a suitable natural model to study cell biology and developmental function of beta-amyloid precursor protein and a potential assay system for drugs that regulate beta-amyloid precursor protein processing.

J Neurosci Res 2007 Sep;85(12):2726-40. doi: 10.1002/jnr.21174.

**Excitotoxic motoneuron disease in chick embryo evolves with autophagic neurodegeneration and deregulation of neuromuscular innervation**

Jordi Calderó [1], Olga Tarabal, Anna Casanovas, Dolors Ciutat, Celia Casas, Jerònia Lladó, Josep E Esquerda

Abstract

In the chick embryo, in ovo application of NMDA from embryonic day (E) 5 to E9 results in selective damage to spinal cord motoneurons (MNs) that undergo a long-lasting degenerative process without immediate cell death. This contrasts with a single application of NMDA on E8, or later, which induces massive necrosis of the whole spinal cord. Chronic MN degeneration after NMDA implies transient incompetence to develop programmed cell death, altered protein processing within secretory pathways, and late activation of autophagy. Chronic NMDA treatment also results in an enlargement of thapsigargin-sensitive Ca(2+) stores. In particular MN pools, such as sartorius-innervating MNs, the neuropeptide CGRP is accumulated in somas, peripheral axons and neuromuscular junctions after chronic NMDA treatment, but not in embryos paralyzed by chronic administration of curare. Intramuscular axonal branching is also altered severely after NMDA: it usually increases, but in some cases a marked reduction can also be observed. Moreover, innervated muscle postsynaptic sites increase by NMDA, but to a lesser extent than by curare. Because some of these results show interesting homologies with MN pathology in human sporadic ALS, the model presented here provides a valuable tool for advancing in the understanding of some cellular and molecular processes particularly involved in this disease.

J Physiol 2018 Aug;596(15):2991-3006. doi: 10.1113/JP274111.

**The highs and lows of programmed cardiovascular disease by developmental hypoxia: studies in the chicken embryo**

N Itani [1] [2], C E Salinas [3], M Villena [1], K L Skeffington [1], C Beck [1], E Villamor [4], C E Blanco [5], D A Giussani [1] [2]

Abstract

It is now established that adverse conditions during pregnancy can trigger a fetal origin of cardiovascular dysfunction and/or increase the risk of heart disease in later life. Suboptimal environmental conditions during early life that may promote the development of cardiovascular dysfunction in the offspring include alterations in fetal oxygenation and nutrition as well as fetal exposure to stress hormones, such as glucocorticoids. There has been growing interest in identifying the partial contributions of each of these stressors to programming of cardiovascular dysfunction. However, in humans and in many animal models this is difficult, as the challenges cannot be disentangled. By using the chicken embryo as an animal model, science has been able to circumvent a number of problems. In contrast to mammals, in the chicken embryo the effects on the developing cardiovascular system of changes in oxygenation, nutrition or stress hormones can be isolated and determined directly, independent of changes in the maternal or placental physiology. In this review, we summarise studies that have exploited the chicken embryo model to determine the effects on prenatal growth, cardiovascular development and pituitary-adrenal function of isolated chronic developmental hypoxia.