Invited Review

Cardio-respiratory development in bird embryos: new insights from a venerable animal model

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ABSTRACT - The avian embryo is a time-honored animal model for understanding vertebrate development. A key area of extensive study using bird embryos centers on developmental phenotypic plasticity of the cardio-respiratory system and how its normal development can be affected by abiotic factors such as temperature and oxygen availability. Through the investigation of the plasticity of development, we gain a better understanding of both the regulation of the developmental process and the embryo's capacity for self-repair. Additionally, experiments with abiotic and biotic stressors during development have helped delineate not just critical windows for avian cardio-respiratory development, but the general characteristics (e.g., timing and dose-dependence) of critical windows in all developing vertebrates. Avian embryos are useful in exploring fetal programming, in which early developmental experiences have implications (usually negative) later in life. The ability to experimentally manipulate the avian embryo without the interference of maternal behavior or physiology makes it particularly useful in future studies of fetal programming. The bird embryo is also a key participant in studies of transgenerational epigenetics, whether by egg provisioning or effects on the germline that are transmitted to the F1 generation (or beyond). Finally, the avian embryo is heavily exploited in toxicology, in which both toxicological testing of potential consumer products as well as the consequences of exposure to anthropogenic pollutants are routinely carried out in the avian embryo. The avian embryo thus proves useful on numerous experimental fronts as an animal model that is concurrently both of adequate complexity and sufficient simplicity for probing vertebrate cardio-respiratory development.

Key Words: bird embryos, cardiovascular, epigenetics, fetal programing, respiratory plasticity, toxicants

Introduction

The avian embryo has a been a model for exploring animal biology for more than two millennia, encompassing ancient Egyptians, Aristotle, Leonardo Da Vinci, and William Harvey (Stern, 2004). Legions of modern-day biologists have used the bird embryo to provide insights into numerous facets of biology, including development, evolution, physiology, morphology, and taxonomy. In fact, analysis of the PubMed data base maintained by the US National Institutes of Health (www.pubmed.org) reveals that the number of papers on the embryo of chickens alone (not including other birds) is second only to mice among the grouping of mouse, chicken, rat, cow, and fish (Figure 1). Given that nearly 60,000 papers in PubMed contain the word pairing of "chick" and "embryo", it is obviously beyond the scope of this review to consider this enormous literature on avian development. For an entry into this literature, the reader is referred to both classic and modern reviews of avian embryonic morphology (Goodrich, 1930; Hamburger and Hamilton, 1951; Romanoff, 1960; Bellairs and Osmond, 2014), physiology (Romanoff, 1960; Burggren and Keller, 1997; Burggren and Crossley, 2002; Elfwing et al., 2011; Mueller et al., 2015a), and biochemistry and cellular/molecular biology (Romanoff, 1967; Stern, 2004; Gabrielli and Accili, 2010; Streit et al., 2013; Kain et al., 2014; Hirst and Marcelle, 2015; Schneider, 2015). Rather, the focus of this paper is to examine specifically how avian embryos have contributed to our growing understanding of cardio-respiratory development, not just in birds but also in vertebrates generally. The PubMed database lists more than 5000 papers that contain the search terms "chick" plus "embryo" plus "heart", and more than 1250 papers containing the search terms "chick" plus "embryo" plus "lung". These cited statistics do not even include the considerable research on the embryonic cardio-respiratory system of bird species other than chickens that
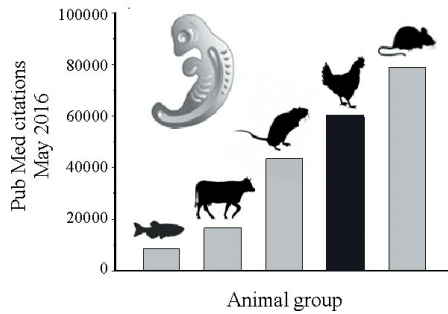
Figure 1 - Representation of papers in the PubMed data base that reference embryos of five major examples of vertebrate animals, determined May 2016.

are commonly used in experiments (e.g., zebra finch, duck, emu, etc.). Thus, our strategy for limiting this review to reasonable length is to first provide a simple overview of the basic cardio-respiratory ontogeny of the avian embryo. More correctly, we point the reader to some key papers that will provide an introduction to this topic. This brief overview will then serve as a back drop for more in-depth consideration of several relatively new areas of cardio-respiratory research on avian embryos that are providing — or have the potential to provide — major new insights into developing vertebrates. These topics are the ontogeny of organ systems interactions, developmental phenotypic plasticity, critical developmental windows, fetal programming, and epigenetic inheritance and toxicology.

Ontogeny of cardio-respiratory morphology, physiology, biochemistry, and celluar/molecular biology

As introduced above, the avian embryo has been extensively studied to provide insight into the ontogeny of the respiratory and cardiovascular systems and their regulation in vertebrates. *What are the unusual features of the avian embryo that have made this such a popular animal model for cardio-respiratory studies?* The answer to this question is the same for many areas of avian embryo investigation, and hinges on three key characteristics. First, the circulation of the avian embryonic circulation is readily accessible, even in the early formative periods, allowing both sampling of blood and injection of agonists/antagonist. Second, the embryo in the egg is not subjected to direct maternal influences (as is, for example, the mammalian embryo or fetus in the uterus). This allows the experimenter to modulate the embryonic environment during growth (e.g., inducing hypoxia at controlled levels). When these two attributes are combined with the relatively large size of the embryos of some avian species, it becomes apparent

that avian embryos have allowed invasive experimental manipulations (Burggren et al., 2004; Kowalski et al., 2013; Shell et al., 2016). Such invasive procedures have primarily focused on the embryo of the chicken but also the embryos of other birds such as the emu (*Dromaius novaehollandiae*) and ostrich (*Struthio camelus*). Importantly, such experimental procedures simply are difficult if not impossible in the early developmental stages of mice or rats or other popular animal models.

Experimental approaches to study the avian embryonic cardio-respiratory system have involved the topical application of drugs (agonists or antagonists) directly onto the heart and central vessels of the very early embryo (Borkowf and Kolesari, 1986; Rosenbruch et al., 1993; Branum et al., 2013). As the embryo and its circulation grow (and in particular as the vessels of the chorioallantoic membrane become accessible), drug injection directly into the blood vessels of the chorioallantoic membrane can be through a "keyhole" in the shell, and in some cases through an indwelling catheter, e.g., Altimiras and Crossley (2000), Burggren and Crossley (2002), Crossley et al. (2003a), Crossley et al. (2003b), Crossley and Altimiras (2000), Lindgren et al. (2011), Mueller et al. (2013a), Mueller et al. (2014a), and Tintu et al. (2009).

These approaches are often combined with exposure of the developing embryo to hypoxia or hypercapnia as stressors on the cardiovascular, respiratory, and acid-base balance systems to tease apart elements of cardio-respiratory physiological control (Adair et al., 1987; Strick et al., 1991; Burton and Palmer, 1992; Rouwet et al., 2002; Crossley et al., 2003a; Villamor et al., 2004; Fisher and Burggren, 2007; Copeland and Dzialowski, 2009; Lindgren and Altimiras, 2009; Acosta and Hernandez, 2012; Zhang and Burggren, 2012; Mueller et al., 2013b; Andrewartha et al., 2014; Mueller et al., 2014b; Burggren et al., 2015b; Jonker et al., 2015; Itani et al., 2016).

An additional approach to understanding embryonic hemodynamics and the ontogeny of their control involves the mechanical rather than pharmaceutical modification of cardiovascular function. For example, arterial outflow tract restriction has proven useful in understanding the hemodynamics of blood flow through the heart, and how they may influence cardiac development (Burggren and Keller, 1997; Broekhuizen et al., 1999; Lucitti et al., 2005; Lucitti et al., 2006; Stekelenburg-de Vos et al., 2007).

The discussion above has only scratched the surface in describing the "established" approaches applied towards "conventional" lines of inquiry on the ontogeny of the avian cardio-respiratory physiology and its control. In the limited remaining space in this review, let us now consider some of

the more recent, less conventional uses of the avian embryo model in cardio-respiratory biology.

Ontogeny of interactions of system components and organ systems

Effective gas exchange in vertebrates — mature or immature — depends upon adequate ventilation of gas exchange organs and their perfusion with appropriate levels of blood pumped by the heart. Thus, a pivotal point in the organogenesis, growth, and maturation of the avian embryo (or, indeed, the mammalian fetus) is the regulatory control over the heart, vessels, and lungs geared towards the onset of air breathing. Such control systems involve motor neurons releasing neurotransmitters, endocrine organs releasing circulating hormones, and cellular receptors on effector organs that are stimulated by these neurotransmitters and hormones. At first glance, it might seem logical that all necessary elements of a physiological regulatory system would develop concurrently, such that the embryonic animal is largely or fully functional upon hatching/parturition. *Do all the components of a regulatory system develop functionality concurrently or do they develop in a sequence?* In fact, numerous studies have revealed a temporal mismatch of the onset of functionality of the various regulatory components. For example, the neural and hormonal control of the heart in perinatal birds involves a complex interplay between vagal tone, sympathetic tone, locally acting hormones (e.g., nitric oxide) and circulating hormones such as catecholamines and angiotensin II (Altimiras and Crossley, 2000; Crossley and Altimiras, 2000; Burggren and Crossley, 2002; Crossley et al., 2003a; Crossley et al., 2003b; Chiba et al., 2004; Mueller et al., 2013a; Iversen et al., 2014; Mueller et al., 2014a). Both muscarinic and adrenergic receptors are present in the first few days of normal embryonic development in the chicken, potentially enabling both sympathetic and parasympathetic neural control. Yet, early signs of sympathetic innervation appear several days *after* the presence of receptors capable of being stimulated by them. Full vagal innervation does not appear until a few days before hatching (Burggren and Crossley, 2002). Thus, most of normal embryonic development of the chicken is characterized by adrenergic cardiac control, with little or no involvement of vagal innervation (Crossley and Altimiras, 2000; Burggren and Crossley, 2002). Similar temporal displacement of the onset of numerous components of the renin-angiotensin system and its control of the cardiovascular system is evident from analysis of the literature of ANG II and its effects in chicken embryos (Brand et al., 2007; Crossley et al., 2010; Mueller et al., 2013a; Mueller et al., 2014a).

A schematic of how normal regulatory control system development can be viewed during normal development is shown in Figure 2. In Panel A, all elements of a regulatory system mature simultaneously, allowing full physiological regulation by a comprehensive control system to appear concurrently at the end of embryonic development. In Panel B, the development of motor neurons affecting a
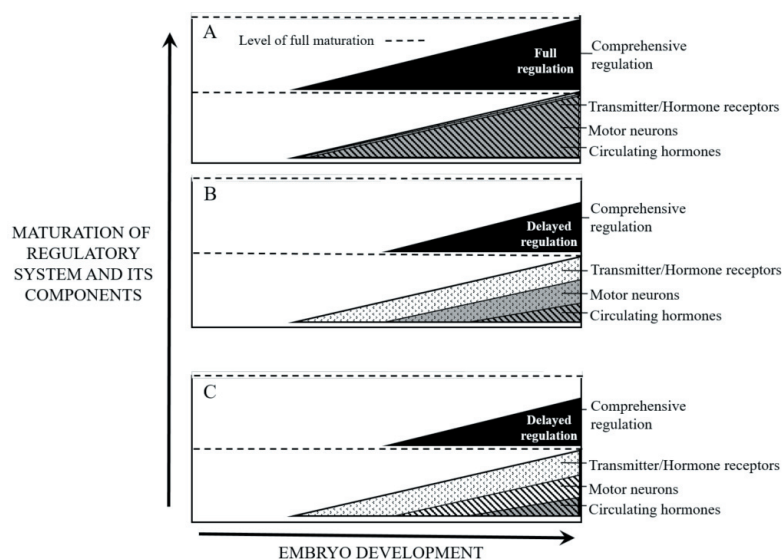

Figure 2 - A schematic illustration of different scenarios of maturation of a physiological regulatory system and its constituent components during normal embryonic development. A) All components appear and mature at a similar rate, and full regulation occurs at the end of embryonic development. B) and C) the normal onset of one of the regulatory components is delayed relative to other components, resulting in a delayed onset and maturation of the physiological regulatory system.

cardiovascular response lags behind formation of receptors on cells of the heart and vasculature, and the appearance of circulating hormones lags even further. The net effect is that the comprehensive regulatory system is not fully mature at the end of embryonic development. Panel C presents the reverse of Panel B, with the development of circulating hormones lagging the appearance of motor neurons. However, the effect in C is the same as in B — the lack of a fully developed comprehensive regulatory system.

The concept of concurrent vs. sequential development of regulatory systems can be extended beyond regulatory controls of a single organ system, such as the cardiovascular system, to include multiple organ systems. Embryonic development is characterized not just by the progressive growth of organ systems in isolation, but also by the progressive maturation of the interactions between developing systems. For example, in most vertebrates, the kidney influences cardiovascular function through the renin-angiotensin system, but the influence is not just one way. Hormones secreted from heart tissue such as atrial natrietic peptide (ANP) also influence kidney function. Yet, very poorly understood is the *ontogeny of the regulatory interactions* of these two systems, as opposed to the ontogeny of each system by itself (Burggren et al., 2014). Making matters more complex, the heart begins to function much earlier in embryonic development than do the various stages (pro-, meso-, and metanephros) of the embryonic kidneys. Thus, the heart potentially develops control over the developing kidneys before the kidneys can exert control over the already well-developed cardiovascular system (Burggren et al., 2014). This hypothesis remains to be fully tested but, if true, indicates another example where there is failure of temporal symmorphosis in the development of the avian embryo.

Noteworthy is that the discussion above considers "normal" timing of developmental processes. Yet, the timing of onset or components of a developing regulatory system can also be somewhat plastic as a consequence of environmental stressors. This developmental plasticity in the timing of appearance of the components of a regulatory system (or indeed, of any developmental process) affected by an altered environment (broadly defined) will now be discussed in the general framework of developmental phenotypic plasticity.

Developmental phenotypic plasticity and critical windows of the cardiorespiratory system

The study of avian embryos is showing us how the embryonic environment modulates the development of these systems — that is, to what extent avian embryos are plastic in their growth and maturation. To fully appreciate this, we now consider the intertwined concepts of developmental phenotypic plasticity and critical windows.

Developmental phenotypic plasticity and critical windows

Phenotypic plasticity is usually interpreted as the interactions of an organism's *G*enotype with its *E*nvironment, so-called Gene × Environment or G × E interactions (Burggren and Reyna, 2011; Hutchings, 2011; Grishkevich and Yanai, 2013; Crews et al., 2014; Danchin and Pocheville, 2014; Keller, 2014; Wada and Sewall, 2014). By extension, *developmental* phenotypic plasticity — of morphology, physiology, or both — represents the modification of phenotype of a developing organism, which may or may not be fully carried in to the mature, adult form (Spicer and Burggren, 2003; West-Eberhard, 2003; Reeves and Gozal, 2005; Bjorklund et al., 2007; Spicer and Rundle, 2007; Burggren and Reyna, 2011; Domyan and Sun, 2011; Rymer and Pillay, 2012). Put differently, such plasticity results from interactions of an organism with its environment during the course of its development, or the less explored "G × E × D".

How environment affects the cardio-respiratory phenotype of the developing bird egg has been explored using environmental factors (stressors) such as temperature, oxygen, carbon dioxide, and various toxicants, as will be explored below (see section "Fetal programming of the avian cardio-respiratory system"). Key interrelated questions that have emerged include *When in development are the most susceptible or vulnerable periods for environmental stressors?* and *To what extent can the developing organ systems mitigate the impact of these stressors?* Answering these questions requires considering the concept of critical windows in development.

The "critical window" in development (sometimes described as "susceptible period" in psychology and other fields) is that period of time during development when an organism's phenotype is most vulnerable for the effects of an environmental stressor or toxicant (Burggren, 1998; Nijland et al., 2008; Loepke, 2010; Burggren and Reyna, 2011; Burggren and Mueller, 2015). A tragic but illustrative example was the use of the drug thalidomide as an anti-nauseant to treat morning sickness in pregnant women. Unknown before it began being prescribed to pregnant women, thalidomide is also a powerful teratogen that disrupts human limb development beginning with week three and continuing through week five, then diminishing afterwards. Thus, the period of morning sickness, for

which thalidomide was originally prescribed, unfortunately coincided with the critical window for limb development in the human fetus. The prescription of this drug for morning sickness consequently resulted in the birth of thousands of children in Europe and Canada with deformed or absent limbs before the correlation was discovered and thalidomide hurriedly pulled from the market (Franks et al., 2004; Knobloch and Rüther, 2008). This tragedy not only illustrates the concept of the critical window, but also the specificity of critical windows; that is, there are *specific* critical windows during development for *specific* organ systems. Thus, while limb development as mentioned above is in the human fetus most susceptible to teratogens in weeks 3-5, as evident from the thalidomide experience, the human fetal teeth, palate, and urogenital systems, for example are most sensitive from weeks 7-9.

A key question derives from the metaphor of the "window" - *What defines the "edges" of the windows, are they sharp or fuzzy, and can they be moved by environmental factors*? Typically, critical windows are described as distinct periods, that is, windows with sharp edges, e.g., "Day X through Day Y" of development (Figure 2A). Sometimes, in a slightly more nuanced approach, they are presented as "primary effects on Days X to X_n with lesser secondary effects beyond Days X_{n+1}". In either approach, however, there is a prevailing two-dimensional view of the critical window, with time and effect as the only two variables. Recently, we have advocated a more realistic "3D" approach that takes into account not only time and effect, but also dose of the environmental stressor (Burggren and Mueller, 2015; Mueller et al., 2016). In this approach, the injection of stressor dose adds a third dimension that can potentially alter our characterization of the critical window for a stressor (Figure 3). For example, while high doses of

a stressor produce easily recognizable phenotypic changes, lower doses may produce modified phenotypes that are less likely to be noticed but are still biologically significant. Thus, the perception and reality of the extent of the critical window in development depends, in fact, on the dose-response relationships between stressor and phenotypic characteristics.

The following question arises: "*What is the role of bird embryos in studies of developmental phenotypic plasticity and critical windows cardio-respiratory systems?*" Bird embryos have great promise as animal models in this area of experimentation for several reasons. First, as alluded to above, the bird egg physically is a closed, independent system, and as such the environment surrounding the embryo is much more tractable to experimental manipulation. Second, substances can be injected directly into the egg and the circulation of the embryo without mitigation by maternal influences, as would happen in placental mammals. Third, the distinct morphological stages of bird development have been highly characterized (Hamburger and Hamilton, 1951; Bellairs and Osmond, 2014), which allows subtle but significant phenotypic modifications (at least of morphology) to be readily detected. Fourth, the incubation period of some of the commonly used avian models (chicken, quail) is in the range of 20-25 days, which allows for the completion of critical windows experiments within a reasonable time frame (contrast this with the gestation period of ~114 days in swine and ~152 days in sheep, two commonly used large mammal models). Fifth, the cardio-respiratory morphology and physiology of the avian embryo is relatively well characterized (Xu and Mortola, 1989; Crossley and Altimiras, 2000; Burggren and Crossley, 2002; Crossley et al., 2003a; Crossley et al., 2003b; Maina, 2003, Maina, 2006; Ferner and Mortola, 2009;
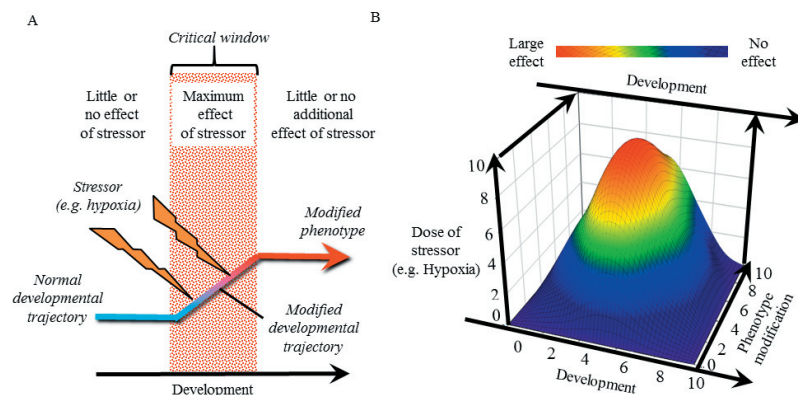

Figure 3 - Depictions of critical windows. A) Conventional linear view of critical windows, where a stressor results in a modified developmental trajectory. B) A more complex three-dimensional view of critical windows, where the degree and timing of the modification of the phenotype is dependent upon the stressor dose. After Burggren and Mueller (2015).

Mortola, 2009; Maina, 2012; Lewallen and Burggren, 2015; Mueller et al., 2015a). Finally, and in some ways among the most important of reasons, bird eggs are typically very inexpensive both to purchase and to maintain, compared with rodent models, for example. This ready availability provides for high throughput experiments with large n values. This feature is crucial, as large numbers of observations are often a requisite to achieve the level of granularity for defining critical windows (Burggren and Reyna, 2011). As we consider a more refined 3D approach of delineating critical windows in a dose-response context, access to a tractable, well understood model that has economic advantages (inexpensive and easily available) will likely accelerate the use of the avian embryo as a model for studying cardio-respiratory ontogeny.

Hypoxia, hypercapnia, and avian critical windows

Because cardiovascular and respiratory systems have specific critical periods, specific probes for these systems are often required to define and explore their critical windows. The respiratory gases oxygen and carbon dioxide have been primary tools for delineating critical windows for development of the cardiovascular and respiratory system of developing birds.

Hypoxia. Oxygen level is effective in modifying phenotype because the avian embryo directly depends upon diffusion across the bird egg shell for respiratory gas exchange – see Mortola (2009), Mueller et al. (2015a), and Rahn et al. (1987) for reviews of the extensive literature on this subject. Thus, changes in environmental oxygen levels, which can be readily induced at controlled levels in developing embryos by precisely regulating incubator gases, will be reflected in internal oxygen levels within the egg and embryo. Numerous studies have used environmental hypoxia (typically chronic exposure to 12-15% O_2) to reveal phenotypic developmental plasticity in both the cardiovascular and respiratory systems of the developing chicken embryo. The results reveal a complex and somewhat variable pattern, depending upon oxygen exposure used, period in incubation of exposure, and even the specific study using similar experimental protocols. Organs directly associated with the uptake and delivery of oxygen may be affected. Thus, the chorioallantoic membrane, the gas exchange organ of the embryo through most of the incubation period, shows proliferation in both size and vascularization as a result of incubation in chronic hypoxia (Adair et al., 1987; Stock and Metcalfe, 1987; Corona and Warburton, 2000; Chan and Burggren, 2005; Acosta and Hernandez, 2012; Druyan and Levi, 2012),

although hypoxic incubation has also been reported to decrease the size of the chorioallantoic membrane (CAM) (Burton and Palmer, 1992). The increase in the mass and size of the CAM allow the embryo to survive the low oxygen conditions, but the increased growth in this structure means the relocation of nutrients from the embryo with direct consequences for growth (Nowak-Sliwinska et al., 2010).

Both pulmonary surface area and structure are also modified to lesser or greater extents by chronic hypoxia incubation (Xu and Mortola, 1989; Azzam and Mortola, 2007; Zhang and Burggren, 2012; Lewallen and Burggren, 2015). Hypoxia also has varying degrees of effect on the morphology and performance of the heart and systemic vasculature, ranging from no effect to profound disturbance (Asson-Batres et al., 1989; Richards et al., 1991; Burton and Palmer, 1992; Altimiras and Phu, 2000; le Noble et al., 2000; Dzialowski et al., 2002; Rouwet et al., 2002; Villamor et al., 2002; Ruijtenbeek et al., 2003; Villamor et al., 2004; Chan and Burggren, 2005; Zhang and Burggren, 2012). Corresponding with these cardio-respiratory phenotypic modifications are changes in overall metabolic rate, typically measured as oxygen consumption. Thus, for example, the embryos of the domestic chicken show a lower rate of basal oxygen consumption when incubated in 15% hypoxia during the middle period of incubation (days 6-12), but not during early (days 1-6) or late (days 12-18) incubation (Dzialowski et al., 2002). More recent studies have suggested that chronic hypoxic incubation throughout avian development leads to a lowered oxygen consumption without the creation of an oxygen debt (Mortola et al., 2012).

Given that the organs exchanging and supplying oxygen and nutrients are often affected by hypoxic incubation, it is not surprising that the structures beyond just these organs and organ systems (e.g., brain, eye, liver, stomach, beak, toes, and other structures) are also potentially affected. Yet again there are conflicts in the literature regarding the magnitude and even presence of the effects (Chan and Burggren, 2005; Azzam and Mortola, 2007; Zhang and Burggren, 2012). *What might be the source of this variation in the morphological and physiological responses, and even the apparent timing of the critical windows, of avian embryos in response to hypoxic incubation?* Certainly, there are specific physiologies and morphologies attached to specific strains of chickens, with often profound differences in magnitude and developmental timing of the response (Sato et al., 2006; Everaert et al., 2008b; Druyan, 2010; Ho et al., 2011; Crossley and Altimiras, 2012; Burggren et al., 2015b; Buzala et al., 2015; Huth and Archer, 2015). All too often, the specifics of the strain of chicken are not

provided in studies, leaving open the fact that strain-specific differences are contributing to the apparent variability between published studies. Other examples of possible sources of variation in the physiological literature – e.g., prandial state, photoperiod, and parental history – have recently been reviewed (Burggren, 2014).

Hypercapnia. Hypercapnia has been widely used as a stressor to probe the onset of acid-base balance; discussion of this extensive literature is beyond the scope of this review, and the reader is referred to the following studies for an entry into the literature (Bruggeman et al., 2007; Everaert et al., 2008a; Everaert et al., 2011; Tazawa et al., 2012; Burggren et al., 2012; Mueller et al., 2013b; Andrewartha et al., 2014; Mueller et al., 2014b; Burggren et al., 2015b; Mueller et al., 2015a). Like hypoxia, hypercapnia has differential levels in mortality/hatchability at different points in development (Taylor and Kreutziger, 1966; Taylor and Kreutziger, 1969; Everaert et al., 2007). As just a few examples, hypercapnic tolerance is high during D9-D12 of incubation in chicken embryos (Taylor and Kreutziger, 1969). Elevated CO_2 during the last half of development affects time to hatching but not a number of other key parameters at either hatching or day seven after hatching, including body mass, relative growth, or hatching success, suggesting a "remarkable tolerance" to high CO_2 levels during the second half of development (Everaert et al., 2007). However, hypercapnic effects specifically on the cardio-respiratory system have not been specifically examined.

Extrapolation of data on hypoxic and hypercapnic exposure to an actual determination of cardio-respiratory critical windows is problematic. In part, this arises because low levels of CO_2 are beneficial to incubation, while there is no parallel advantage to mild hypoxia, and more severe hypoxia has long been understood to be detrimental (Grabowski and Paar, 1958; Mueller et al., 2015a; Riddle, 1924). Moreover, a wide range of different exposure protocols and O_2 and CO_2 exposure levels have been used. Not unexpectedly, then, there is variation in reported periods of respiratory gas susceptibility in development of the chicken embryo. In fact, even when experiments using similar protocols are repeated by the same experimental laboratory, both qualitative and quantitative differences may emerge. However, despite the absence of clear patterns of change in developmental plasticity, an overarching factor in the interpretation of these data on hypoxia and hypercapnia is that there is no reason *a priori* to expect that the lungs or heart share identical critical windows for development. Indeed, analysis of the literature on hypoxia effects and avian critical windows suggests an overall pattern in which the greatest vulnerability to phenotypic

modification appears in the middle of avian development (Figure 3). While there is the above-mentioned considerable variation around this theme of hypoxia susceptibility, early in avian incubation the developing structures are not sufficiently developed to be heavily perturbed by hypoxia, while towards the end of incubation the embryo is developing the ability to physiologically mitigate hypoxia through adjustments in cardiac output, blood oxygen transport and, late in development, through the onset of pulmonary respiration as internal pipping starts. Thus, the middle period of incubation — a period of rapid cell growth — appears to be the most likely period in which phenotypic plasticity of the cardiorespiratory system arises. In (partial) contrast, induction of phenotypic plasticity (or death) by hypercapnia appears to be greatest in early development, with a growing tolerance to elevated CO_2 during the second half of the incubation period suggested by several long-standing studies (Riddle, 1924; Barott, 1937; Sadler et al., 1954; Taylor and Kreutziger, 1966).

Noteworthy is that the perspective of greatest hypoxia sensitivity in the mid-incubation period (or the reduced hypercapnic sensitivity) depicted in Figure 4 is heavily driven by observations of *morphological* perturbations to normal development. Our understanding of how cardio-respiratory *physiology* is altered by hypoxia, hypercapnia, or other stressors is far more fragmentary (see "Fetal programming" in the next section).

Studying heterokairy with avian embryo models

Avian embryos may also prove to be highly useful animal models for studying the malleability or plasticity
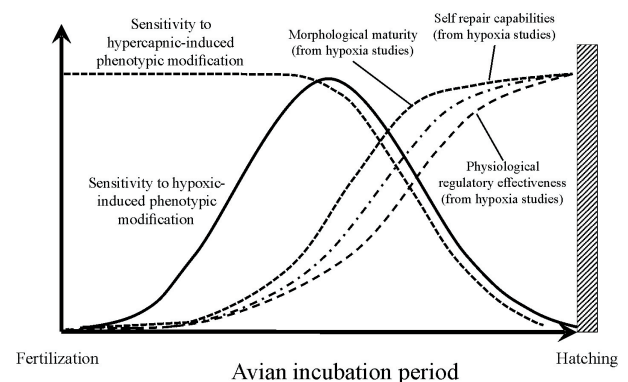

Figure 4 - Relationship between susceptibility to hypoxia and hypercapnia induced phenotypic modification, morphological and physiological maturity, and self-repair capabilities during the avian incubation period. Note that the timing of susceptibility varies for hypercanpia and hypoxia.

of the *order of appearance* of developmental landmarks. Such malleability is incorporated in the concept of heterokairy, the change in developmental sequence of the onset of physiological regulatory mechanisms or supporting morphological structures within an individual or population (Spicer and Burggren, 2003; Spicer and Rundle, 2007; Spicer et al., 2011). This contrasts with heterochrony, which is a change in the development sequence between species over evolutionary time (Spicer and Burggren, 2003; Blom and Lilja, 2005; Spicer et al., 2011; Keyte and Smith, 2014; Mueller et al., 2015b). The relatively few experimental demonstrations of heterokairy have centered on either invertebrates or lower vertebrates. Thus, for example, exposure to predator cues alters the timing of onset of several key developmental events in embryos of the gastropods *Radix balthica* and *Radix auricularia* (Rundle et al., 2011). More recently, rearing oxygen level has been used as a stressor on larval air breathing fishes, where hypoxia accelerated the onset of air breathing in larval *Betta splendens*, but delayed the onset of air breathing in larval *Trichopodus trichopterus* (Mendez-Sanchez and Burggren, 2014). Additionally, development of rivulid annual killifish, as well as their diapause state, can reflect elements of heterokairy (Varela-Lasheras and Van Dooren, 2014). Collectively, these data on fishes show not only developmental phenotypic plasticity and heterokairy, specifically, but also show that a complex behavior that can be moved back and forth in the normal developmental timetable must be supported by equally complex morphology and physiology (Warkentin, 2011; Mendez-Sanchez and Burggren, 2014).

Despite their promise as experimental models, avian embryos have rarely been examined in the context of heterokairy. In one of the few such studies, hypoxia during incubation was used to investigate the ontogeny of pulmonary surfactant lipids in the lungs of chicken embryos (Blacker et al., 2004). Hypoxic exposure during key critical windows around day 16 of incubation accelerated the production of surfactants in developmental time in chicken embryos, likely through the mechanism of increased corticosterone production. Why the paucity of studies on the plasticity of developmental timing in individuals or populations of bird embryos? Perhaps this results from the perception that avian development — especially development of the chicken — is viewed as extraordinarily invariant and predictable (Bellairs and Osmond, 2014; Hamburger and Hamilton, 1951), even accounting for differences between species ascribed to heterochrony (Blom and Lilja, 2005). On the other hand, studies of heterokairy pivot around inherent *variability* in development. Yet, the very fact that

avian development has been characterized in such detail is advantageous. Thus, subtle changes in developmental timing (i.e., heterochrony), which has been described for intraspecific comparisons of avian embryo development (Blom and Lilja, 2005), may actually be more likely to be teased apart when individuals or populations of avian embryos are perturbed by environmental modifications.

Fetal programming of the avian cardio-respiratory system

What is fetal programming?

Fetal programming is the shaping of the adult animal by events that occur early in its development. First described by Barker in more than 25 years ago (Barker et al., 1989; Barker, 1990), fetal programming has proven to be a robust conceptual framework for understanding developmental phenotypic plasticity, particularly in the context of disease — for an entry in to the voluminous literature, see Alexander et al. (2015), Chiossi et al. (2011), Fernandez-Twinn et al. (2015), Mueller et al. (2015b), Roberts et al. (2015), Sedaghat et al. (2015), Stangenberg et al. (2015), and Thornburg and Louey (2005). Essential, fetal programming describes the phenomena by which early fetal experiences in the uterus actual "program" physiologically and morphologically modified phenotypes subsequent to birth. Importantly, such phenotypes may change their expression during development to adulthood, and may actually not present themselves until later in adult life. A classic example of this is fetal programming caused by fetal intrauterine growth restriction (IUGR), which can be caused by maternal malnourishment, by placental blood flow insufficiencies, or other toxicological factors such as alcohol or tobacco exposure, e.g., Cohen et al. (2016). The resulting very low birth weight (VLBW) infants are undersized at first, but at varying rates show "catch up" growth due to a "thrifty phenotype" that is highly efficient at assimilating nutrients into their body tissues for accelerated tissue growth (Bergmann et al., 2008; Luo et al., 2010). However, the accompanying so-called "metabolic syndrome" results in adult onset problems including diabetes, obesity, and cardiovascular and renal problems, to name but a few (Gonzalez-Bulnes et al., 2016).

Fetal programming — Beyond mammals to birds

Although the term "fetus" implies mammals in popular usage, the concept of fetal programming should, in theory, extend beyond mammals within the vertebrates to the

embryos of birds and the fetuses of live bearing reptiles, and the embryos and larvae of fish and amphibians. Yet, there is little evidence of this; not because studies have failed to show the consequences of early developmental experiences on adult phenotype, but rather because few studies outside mammals have been attempted. The following questions thus arise:

Is fetal programming of cardio-respiratory biology uniquely mammalian, or is it more broadly a vertebrate phenomenon? and

If fetal programming occurs specifically in birds, are there unique aspects to bird development that can aid our understanding of the fetal programming phenomenon in all animals?

Some long-term experiments that go beyond just embryonic plasticity evident through embryonic development (see above) have also been carried out in birds. "Catch up growth" occurs in a variety of birds that experience some form of malnutrition that occurs *after* hatching, with achievement of normal body mass following a recovery period from the original perturbation (Palo et al., 1995; Criscuolo et al., 2008; Honarmand et al., 2010; Hector and Nakagawa, 2012; Burness et al., 2013; Chin et al., 2013). Catch up growth related to *embryonic* malnutrition (i.e., lowered provisioning of the egg manifested in smaller egg size) or other perturbation has also been demonstrated in late stage chicken embryos (Azzam and Mortola, 2007). Despite such catch-up growth, earlier experiments have shown that albumin removal from the egg produces smaller individuals at hatching (Hill, 1993; Finkler et al., 1998) and smaller eggs (with less yolk and albumin) within a species result in smaller hatchlings (Williams, 1994; Dzialowski and Sotherland, 2004). However, catch up growth evident in juveniles or adults specifically following abnormal embryonic experiences has not been observed in adult birds to our knowledge. Indeed, in one recent study, a *lack*

of catch up growth was evident. Albumen removal from chicken eggs resulted in reduced body weight of the hens 55 weeks after hatching (Willems et al., 2015a), and egg laying performance of adult hens is also altered (Willems et al., 2013). Similar results occurred in a separate study by this research group, but feed intake was increased in juveniles undernourished as embryos, apparently without accompanying body mass increases up to normal levels (Willems et al., 2015b). Interestingly, albumen removal also results in adult hens with modified DNA methylation and long-term alterations in the hepatic transcriptome (Willems et al., 2016). The extraction of a percentage of the egg contents reduces embryonic growth and results in a low hatching weight, but apparently without any vascular consequences (Ruijtenbeek et al., 2003).

Chronic hypoxia in chicken embryos causes cardiac enlargement and beta adrenoceptor desensitization in juvenile birds (Lindgren and Altimiras, 2009). Incubation of chicken eggs at high altitude causes reduced arterial PO_2 and hemoglobin saturation, elevated hematocrit, lower systemic arterial blood pressure, and gender-dependent alterations in the gain of the arterial baroreflex (Herrera et al., 2013). Hypoxia incubation in bobwhite quail results in modifications to both the structure and function of the cardiovascular system (Flores Santin, 2016) (Figure 5). *In ovo* administration of glutamate inhibitors reduces intestinal size in young chickens (Li et al., 2014). Embryonic hypoxic incubation in chicken embryos leads to adult birds with dilated hearts (Tintu et al., 2009). Embryonic incubation also results in altered vascular structure and function in late chicken embryos, and the authors suggest that these effects *may* carry into the adult (Rouwet et al., 2002).

To conclude, the limited data on embryonic programming of adult modified phenotype in birds appears to strongly resemble the fetal programming of laboratory mammals as well as those human populations. This suggests that fetal programming is not exclusive to placental vertebrates and likely extends to other animal groups. The use of the avian model, and its highly tractable nature in experiments, introduces new and different insights into the comparative developmental physiology of vertebrates and more specifically to the phenomenon of fetal programming.

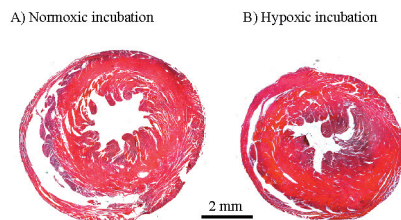A) Normoxic incubation   B) Hypoxic incubation

2 mm

Figure 5 - Left ventricular anterior wall thickness in 12-month-old adult quail (*Colinus virginianus*) chronically incubated in either A) normoxic or B) hypoxic conditions. Note the hypertrophy of the adults exposed to hypoxia *in ovo*. Note the thicker walls of the hypoxia-exposed birds (Flores Santin, 2016).

Avian epigenetic phenomena and mechanisms

Interest in epigenetic phenomena and mechanisms has burst onto the forefront of experimental biology. Increasing numbers of studies are examining intragenerational effects,

including the epigenetics of disease and fetal programming (see above) as well as transgenerational epigenetic inheritance, either or both forms of epigenetic phenotype modification potentially induced by a wide variety of stressors. Interestingly, the vast majority of the focus of epigenetic studies has been on the underlying mechanisms (e.g., DNA methylation, histone modification, microRNA) for intragenerational epigenetic effects (Burggren, 2016). A comprehensive review of this fascinating field of epigenetics is well beyond the scope of this article, so the reader is referred to reviews that emerge from medicine and physiology (Ho and Burggren, 2010; Zhang and Ho, 2011; Holland and Rakyan, 2013; Burggren, 2014; Burggren and Crews, 2014; Hala et al., 2014; Burggren, 2015), evolutionary biology (Kuzawa and Thayer, 2011; Zhang and Ho, 2011; Duncan et al., 2014; Varriale, 2014; Mendizabal et al., 2014; Burggren, 2016), ecology, and environment (Crews and McLachlan, 2006; Baccarelli and Bollati, 2009; Crews and Gore, 2012; Kim et al., 2012; Collotta et al., 2013; Kilvitis et al., 2014), etc.

Curiously, although avian embryos have been used extensively in many types of developmental biology studies, they have been far less exploited in either intra- or transgenerational epigenetic studies. Certainly, DNA methylation and histone modification patterns have been investigated as a function of development in avian embryos and hatchlings (Ellis et al., 2012; Li et al., 2015), and in some cases these molecular findings have been related to the respiratory transition from allantoic to pulmonary gas exchange (Gryzinska et al., 2014). Perhaps the biggest contributions to date from studies of avian embryos attempts to answer the question *What is the role of "maternal provisioning" in epigenetic inheritance?* While the terminology and semantics of "epigenetics" are still quite messy and continue to evolve (Burggren and Crews, 2014; Felsenfeld, 2014; Deans and Maggert, 2015), many embracing a broad definition of epigenetics regard maternal effects and the associated maternal provisioning as a form of transgenerational epigenetics. Bird eggs are, of course, pivotal in the investigation of maternally-derived yolk factors and their effect on the F_1 generation. A variety of hormones, nutrients, antibodies, and other substances that the mother puts into the egg yolk during oogenesis ultimately affect the development and eventual phenotype of the offspring (Hasselquist and Nilsson, 2009; Ho et al., 2011; Reed and Clark, 2011; Adkins-Regan et al., 2013; Ho, 2014). The bird egg and embryo should prove to be an ideal model for more detailed study of maternal effects through provisioning. Not only can the nature of the egg be changed by modifying parental diet (Karadas et al., 2005;

Koutsos et al., 2007; Labaque et al., 2013), but also by direct injection into the yolk prior to or during incubation (Tissier et al., 2014; Herrington et al., 2015).

Studies on transgenerational epigenetics have focused far more on the inheritance of molecular phenotypes and far less on system-level physiology (Burggren, 2016). The bird embryo can also help us answer the question *Can modified phenotypes of cardiovascular and respiratory processes be epigenetically inherited?* Because of the relative precision with which stressors like hypoxia or osmotic stress can be delivered to the avian embryo, it is likely to be much easier to look for causation for subsequent phenotypic plasticity in the F_1 generation than in mammals, for example, where maternal influences can mitigate or at least modify the stressors as externally applied.

Avian embryos in toxicology and pollution research

It has long been appreciated that the development of the bird embryo, especially its cardiovascular and respiratory system, can be affected by anthropogenic toxicants as well as abiotic factors such as hypoxia and temperature (Mueller et al., 2015a). *Do developing bird embryos respond to toxicants in unique ways that reflect their development within the bird egg, or do their responses more generally reflect the responses of other vertebrates?* The answer of relevance to other vertebrates is indirectly answered by the existence of an extensive literature using the bird embryo for toxicology testing, e.g., Kishore et al. (2008), Kormos et al. (2009), Kue et al. (2015), Sharma et al. (2001), and Varnagy et al. (2001). However, beyond screening of cosmetics and putative drugs for human use, the bird embryo is increasingly being used to understand the effects of environmental pollutants on animals, including men, as we will now explore.

Inorganic toxicants as developmental disruptors

Anthropogenic environmental toxicants can be divided into inorganic substances (e.g., heavy metals, metalloids, non-metals, and radioactive isotopes. Some metals (e.g., arsenic, cadmium, cobalt, copper, iron, mercury, and zinc) may be extremely toxic at low levels, while in trace amounts some comprise essential nutrients required for biochemical and physiological functions. Some inorganic substances including metals not only have general effects in bird embryos such as reduced hatchability (Oliveira et al., 2015), but also specifically affect cardiovascular system through altered angiogenesis (Bertossi et al., 2004;

Gheorghescu et al., 2015; Mroczek-Sosnowska et al., 2015), disruption to hematology (Newkirk et al., 2014), or *in vitro* changes in cardiomyoctes (Canga et al., 1993; Natarajan et al., 2006). Inorganic pesticides, organometals, and halogenated polycyclic aromatic hydrocarbons (HPAH) are some of the chemicals identified as endocrine disruptors (ED) in part because of their high lipophilicity, their ability to irreversibly bind to macromolecules (i.e., DNA), and their ability to reversibly react at specific sites of the receptors and enzymes (Yu et al., 2011). The exploration of ED in early development has extensively used the avian embryonic model ― for an entry into the literature see Malir et al. (2013) and Roig et al. (2014). As one example, however, compounds that interfere with gonadal estrogen (responsible for differentiation of female phenotype in birds) such as the ERα agonists propyl-pyrazole-triol (PPT) and diarylproprionitrile (DPN) and the ERα antagonist methyl piperidino pyrazole (MPP) disrupted the normal development of the female reproductive tract in chicken embryos, resulting in embryonic retention of the right Müllerian duct (which normally regresses) and the malformation of both ducts (Mattsson et al., 2011).

Organic toxicants as developmental disruptors

Organic toxicants, including complex pesticides, organometallic compounds, aliphatic compounds (alkanes and alkenes), and aromatic compounds (e.g., polycyclic aromatic hydrocarbons, PAH) represent a significant portion of contaminant mixtures found in ground- and surface waters, coastal areas, soil, and the global atmosphere. One of the main sources of aliphatic and aromatic compounds are petroleum hydrocarbons (alkanes, alkenes, and aromatic hydrocarbons). Such compounds (e.g., DDT, PCBs, TCDD, PAH) resist degradative processes and thus persistence in the environment for extremely long periods of time. Sources of PAH include the incomplete combustion of organic material due to both anthropogenic and natural sources ― high temperature combustion of fuels, emissions from crude and refined oil, forest and prairie fires, volcanic eruptions, and direct biosynthesis by microbes and plants (Näf et al., 1992). Some of these PAH are readily metabolized by organisms. For example, benzoperylene (a PAH) has a half-life of 14 months on soil, but it is quickly absorbed and metabolized in animal tissues, including those of the embryos of chickens and eider ducks (Albers, 1978; Näf et al., 1992; Albers, 2006; Fournier et al., 2010).

Polycyclic aromatic hydrocarbons are both mutagenic and carcinogenic – see Yu et al. (2011). They enter tissues through inhalation, ingestion, and dermal contact, and their rate of absorption increases when they are present in mixtures. The liver is generally the most important site where toxicants undergo biotransformation (e.g., Westman et al. (2013)), but generally most if not all organs are affected to some extent by PAH, with their toxicity dependent upon dose, concentration, time, route of exposure, further distribution, sensitivity, and ability of the organ to metabolize these compounds (Canga et al., 1993).

Bird embryos, once again, have been heavily exploited to probe the effects on the cardiovascular system of PAH, either individually or generally as a component of petroleum products. For example, vascularization decreases in the 72 h chicken embryo in responses to diesel exhaust particles (DEP), and between 30-80% of exposed embryos were poorly developed (Simsek et al., 2012).

Accelerated attention is now being devoted to the effects of PAH on animals, driven in part by the devastating Deep Horizon oil spill in the Gulf of Mexico (Burggren et al., 2015a). In this context, the bird embryo is proving to be useful not only as a tractable animal model for studying PAH effects, but more specifically to see what the effects might be on nesting birds and their eggs exposed either to oil transferred to the eggs or as a result of direct exposure to PAH in oil vapors in the air. Particularly useful in this regard is the ability to grow bird embryos in either the opened egg (Romanoff, 1931) or in *in vitro* culture (Dunn, 1991; Ho et al., 2011; Jourdeuil et al., 2015). Such unparalleled access to the early developmental stages has
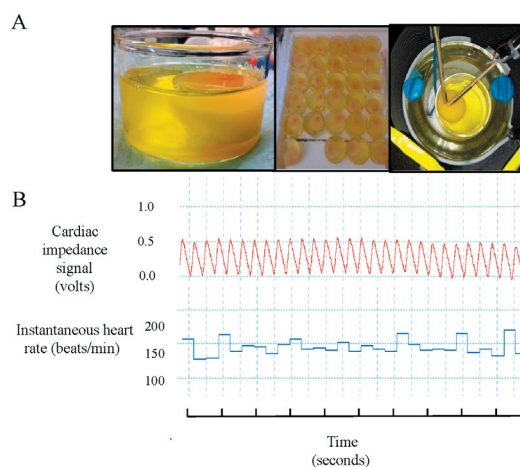

Figure 6 - Shell-less *in vitro* culture of chicken embryos. A) An individual embryo on yolk surrounded by albumin in a culture dish (left), mass production of embryos for experimentation (center), and using recording electrodes to determine physiological parameters such as heart rate (right). B) The cardiac impedance signal produced by heart contraction and relaxation and the derived instantaneous heart rate in a 72-h chicken embryo at 37.5 °C in shell-less culture.

enabled study of organogenesis, angiogenesis, hematology, and the physiological function of the cardiovascular system in bird embryos just a few days old (Burggren et al., 2004; Datar and Bhonde, 2005; Khorrami et al., 2008; Branum et al., 2013). Figure 6 illustrates the process of growing embryos in shell-less culture as well as physiological information that can be derived from them. Experiments are currently underway to determine the specific cardio-toxic effects of PAH released from the Deep Water Horizon oil spill on early cardiac function in birds. In this respect, bird embryos will be illustrative in terms of the critical windows of sensitivity of the cardiovascular system to PAH in developing vertebrates. However, not only do nesting birds inadvertently foul their eggs with oil they have come in contact with, but the incubating embryos are exposed to PAH vapors emanating from the landed oil on the shoreline (Burggren et al., 2015a).

Temperature as a developmental disruptor

Temperature is sometimes excluded in lists of pollutants *per se*, but this abiotic factor results from local anthropogenic effects (e.g., power plants, urban environments) but also as a chronic pollutant with broad reach; e.g., climate change in the form of global warming extending over decades. The potential future effects of global warming on developing birds is beyond the scope of this article, but here we introduce some of the known effects of changes in incubation temperature on bird egg development.

Bird embryos chronically incubated under hypothermia (35.5 ºC) not unexpectedly show significant retardation of embryonic development (Tazawa, 1973; Boehm et al., 1987; Lourens et al., 2007; Reyna and Burggren, 2012). Additionally, acute hypothermia increases the P_{O_2} and pH and decreases the P_{CO_2} of both arterialized blood and mixed venous blood and creates cardiac hypotrophy in chicken embryos (Boehm et al., 1987; Tazawa, 1973; Tazawa and Mochizuki, 1978). On the other hand, hyperthermia during incubation decreases hatching success in the chicken and the bobwhite quail (*Colinus virginianus*) (Stoleson and Beissinger, 1999; Reyna, 2010; Reyna and Burggren, 2012) and alters hemodynamics beyond the expected rate function increases with elevated temperature (Lee and Lee, 2010). Prenatal incubation temperature affects many aspects of post-natal performance. For example, the early postnatal development of neuronal hypothalamic thermal sensitivity in birds, with birds incubated during the last week at 38.5 ºC exhibiting elevated neuronal hypothalamic

cold sensitivity at the 10th day post-hatching, likely due to an increased proportion of cold-sensitive neurons and a reduced proportion of warm-sensitive neurons (Tzschentke and Basta, 2002). Periodic cooling studies had shown a decrease in embryo mass and yolk reserves in the zebra finch (*Taeniopygia guttata*), and it also delayed development and increased metabolic costs (Olson et al., 2006). Interestingly, temperature disruption also had disproportionately larger effects on soft tissues compared to long bones and skull in zebra finch (Olson et al., 2008).

Even pre-incubation storage temperatures at the early blastocyst stage of bird embryos can affect viability (Reyna and Burggren, 2012; Branum et al., 2016). While many species are continuously incubated by a parent (e.g., chicken), other species may leave the eggs exposed to the environment either upon first laying or later during incubation while parents are off foraging (e.g., ratites, quail, etc.). Thus, temperature changes (especially habitat warming) may have detrimental effects on embryos in these birds.

The ease with which the temperature of the bird embryo is manipulated has also led to their use as models in many different lines of experimental inquiry. For example, bird embryos have been instrumental in our understanding of the evolution of endothermy (Walter and Seebacher, 2009; Tzschentke and Rumpf, 2011; Sirsat et al., 2016). Hypothermic bird embryos have also led to an understanding of how cardiac function is altered during hypothermic surgery in humans (Sarre et al., 2006; Shao et al., 2007). Finally, temperature manipulation has provided insights into strain-based physiological differences in the poultry industry (Collin et al., 2007).

Hypoxia as a developmental disruptor

Finally, hypoxia as a natural environmental stressor for birds and especially their embryos can result from natural conditions such as inundation of the nest by water, poorly ventilated burrows, and high altitude habitats (Mueller et al., 2015a). The potentially disruptive effects of hypoxia on cardiovascular and respiratory development have already been discussed in earlier sections of this essay and will not be elaborated upon here. Noteworthy, however, is that for many animals, the developmentally disruptive effects of hypoxia are multiplied by the simultaneous presence of other factors such as environmental temperature and toxicants. Indeed, the suite of hypoxia, elevated temperature, and toxicants are often present concurrently in polluted environments; consider, for example, the so-called "dead zone" of the Gulf of Mexico (Stow et al., 2005).

Conclusions

Developmental biology, comparative physiology, evolutionary biology, and numerous other areas of contemporary biological research continue to benefit from lessons learned using the venerable bird egg and its embryo within. Indeed, as we identify similarities between all vertebrates from molecular to organismal levels, the use of the bird embryo continues to expand, including animal modeling for areas as diverse as nonclinical safety studies of pharmaceuticals and vaccines (Saw et al., 2008; Bjornstad et al., 2015) to toxicology (Kopf and Walker, 2009; Smith et al., 2012) to prototyping surgical and measurement apparatus (Filas et al., 2011; Ford and Mertz, 2013; Lee and Ha, 2013). Moreover, the structure of the bird embryo within its egg has allowed focus on specific organs, such as the chorioallantoic membrane (Branum et al., 2013; Nowak-Sliwinska et al., 2014; Yuan et al., 2014), or on large specific and circumscribed energy and nutrient pools, such as albumin and yolk (Williams, 1994; Dzialowski et al., 2009; Nangsuay et al., 2015). Finally, a compelling aspect of the bird embryo is its size, particularly when considering the embryos of the ratites (ostriches, emus, rheas), which allows surgical and other interventions not possible in many other common embryonic models (Burggren et al., 2000; Burggren et al., 2004; Dzialowski and Greyner, 2008; Khorrami et al., 2008; Shell et al., 2016), including *in vitro* cultivation (Ho et al., 2011; Brand et al., 2014).

Like the ancient Egyptians, Aristotle, and William Harvey, we continue to peer into the bird egg, transfixed by what we see within. In doing so, we gain insight into developmental processes, structures, and outcomes in all vertebrates.

Acknowledgments

References

Acosta, E. and Hernandez, A. 2012. Vascular density, hypoxia inducible factor, vascular endothelial growth factor, and its receptor expression in the chorioallantois of relatively normoxic and hypoxic chicken embryos, at 6 and 7 days of incubation, and corresponding weight values. Poultry Science 91:2637-2644.

Adair, T. H.; Guyton, A. C.; Montani, J. P.; Lindsay, H. L. and Stanek, K. A. 1987. Whole body structural vascular adaptation to prolonged hypoxia in chick embryos. American Journal of Physiology 252:H1228-1234.

Adkins-Regan, E.; Banerjee, S. B.; Correa, S. M. and Schweitzer, C. 2013. Maternal effects in quail and zebra finches: Behavior and hormones. General and Comparative Endocrinology 190:34-41.

Albers, P. 2006. Birds and polycyclic aromatic hydrocarbons. Avian and Poultry Biology Reviews 17:125-140.

Albers, P. H. 1978. The effects of petroleum of different stages of incubation in bird eggs. Bulletin of Environmental Contaminants and Toxicology 19:624-630.

Alexander, B. T.; Dasinger, J. H. and Intapad, S. 2015. Fetal programming and cardiovascular pathology. Comprehensive Physiology 5:997-1025.

Altimiras, J. and Crossley, D. A. 2000. Control of blood pressure mediated by baroreflex changes of heart rate in the chicken embryo (*Gallus gallus*). American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 278:R980-R986.

Altimiras, J. and Phu, L. 2000. Lack of physiological plasticity in the early chicken embryo exposed to acute hypoxia. Journal of Experimental Zoology 286:450-456.

Andrewartha, S.; Tazawa, H. and Burggren, W. W. 2014. Acute regulation of hematocrit and acid-base balance in chicken embryos in response to severe intrinsic hypercapnic hypoxia. Respiratory Physiology & Neurobiology 195:1-10.

Asson-Batres, M. A.; Stock, M. K.; Hare, J. F. and Metcalfe, J. 1989. O2 effect on composition of chick embryonic heart and brain. Respiration Physiology 77:101-109.

Azzam, M. A. and Mortola, J. P. 2007. Organ growth in chicken embryos during hypoxia: implications on organ "sparing" and "catch-up growth". Respiratory Physiology and Neurobiology 159:155-162.

Baccarelli, A. and Bollati, V. 2009. Epigenetics and environmental chemicals. Current Opinion in Pediatrics 21:243-251.

Barker, D. J. 1990. The fetal and infant origins of adult disease. British Medical Journal - Clinical Research 301:1111.

Barker, D. J.; Osmond, C.; Golding, J.; Kuh, D. and Wadsworth, M. E. 1989. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. British Medical Journal - Clinical Research 298:564-567.

Barott, H. G. 1937. Effect of temperature, humidity, and other factors on hatch of hens' eggs and on energy metabolism of chick embryos. USDA Technical Bulletin 533:1-45.

Bellairs, R. and Osmond, M. 2014. Atlas of chick development. 3rd ed. Elsevier, Amsterdam, The Netherlands.

Bergmann, R. L.; Bergmann, K. E. and Dudenhausen, J. W. 2008. Undernutrition and growth restriction in pregnancy. Nestle Nutrition workshop series. Paediatric Programme 61:103-121.

Bertossi, M.; Girolamo, F.; Errede, M.; Virgintino, D.; Elia, G.; Ambrosi, L. and Roncali, L. 2004. Effects of methylmercury on the microvasculature of the developing brain. Neurotoxicology 25:849-857.

Bjorklund, D. F.; Ellis, B. J. and Rosenberg, J. S. 2007. Evolved probabilistic cognitive mechanisms: an evolutionary approach to gene x environment x development interactions. Advances in Child Development and Behavior 35:1-36.

Bjornstad, S.; Austdal, L. P.; Roald, B.; Glover, J. C. and Paulsen, R. E. 2015. Cracking the egg: Potential of the developing chicken as a model system for nonclinical safety studies of pharmaceuticals. The Journal of Pharmacology and Experimental Therapeutics 355:386-396.

Blacker, H. A.; Orgeig, S. and Daniels, C. B. 2004. Hypoxic control of the development of the surfactant system in the chicken: evidence for physiological heterokairy. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 287:R403-410.

Blom, J. and Lilja, C. 2005. A comparative study of embryonic development of some bird species with different patterns of postnatal growth. Zoology 108:81-95.

Boehm, C.; Johnson, T. R.; Caston, J. D. and Przybylski, R. J. 1987. Cardiac hypertrophy in chick embryos induced by hypothermia. The American Journal of Physiology 252:C97-104.

Borkowf, A. and Kolesari, G. L. 1986. Uptake and blood pressure studies in the chick embryo following treatment with a teratogenic dose of dopamine. Teratology 34:97-102.

Brand, M.; Lamande, N.; Larger, E.; Corvol, P. and Gasc, J. M. 2007. Angiotensinogen impairs angiogenesis in the chick chorioallantoic membrane. Journal of Molecular Medicine 85:451-460.

Brand, Z.; Cloete, S. W.; Malecki, I. A. and Brown, C. R. 2014. Embryonic development in the ostrich (*Struthio camelus*) during the first 7 days of artificial incubation. British Poultry Science 55:68-75.

Branum, S. R.; Tazawa, H. and Burggren, W. W. 2016. Phenotypic developmental plasticity induced by preincubation egg storage in chicken embryos (*Gallus gallus domesticu*s). Physiological Reports 4:e12712.

Branum, S. R.; Yamada-Fisher, M. and Burggren, W. 2013. Reduced heart rate and cardiac output differentially affect angiogenesis, growth, and development in early chicken embryos (*Gallus domesticus)*. Physiological and Biochemical Zoology 86:370-382.

Broekhuizen, M. L.; Hogers, B.; DeRuiter, M. C.; Poelmann, R. E.; Gittenberger-de Groot, A. C. and Wladimiroff, J. W. 1999. Altered hemodynamics in chick embryos after extraembryonic venous obstruction. Ultrasound in Obstetrics & Gynecology 13:437-445.

Bruggeman, V.; Witters, A.; De Smit, L.; Debonne, M.; Everaert, N.; Kamers, B.; Onagbesan, O. M.; Degraeve, P. and Decuypere, E. 2007. Acid-base balance in chicken embryos (*Gallus domesticus*) incubated under high CO2 concentrations during the first 10 days of incubation. Respiratory Physiology & Neurobiology 159:147-154.

Burggren, W. W. 1998. Studying physiological development: past, present and future. Biological Bulletin of the National Taiwan Normal University 33:71-84.

Burggren, W. W. 2014. Epigenetics as a source of variation in comparative animal physiology - or - Lamarck is lookin' pretty good these days. Journal of Experimental Biology 217:682-689.

Burggren, W. W. 2015. Dynamics of epigenetic phenomena: Intergenerational and intragenerational phenotype 'washout'. Journal of Experimental Biology 218:80-87.

Burggren, W. W. 2016. Epigenetic Inheritance and its role in evolutionary biology: Re-evaluation and new perspectives. Biology (Basel) 5:24.

Burggren, W. W.; Andrewartha, S. J. and Tazawa, H. 2012. Interactions of acid-base balance and hematocrit regulation during environmental respiratory gas challenges in developing chicken embryos (*Gallus gallus*). Respiratory Physiology & Neurobiology 183:135-148.

Burggren, W. W.; Christoffels, V. M.; Crossley, D. A., 2nd; Enok, S.; Farrell, A. P.; Hedrick, M. S.; Hicks, J. W.; Jensen, B.; Moorman, A. F.; Mueller, C. A.; Skovgaard, N.; Taylor, E. W. and Wang, T. 2014. Comparative cardiovascular physiology: future trends, opportunities and challenges. Acta Physiologica 210:257-276.

Burggren, W. W. and Crews, D. 2014. Epigenetics in comparative biology: why we should pay attention. Integrative and Comparative Biology 54:7-20.

Burggren, W. W. and Crossley, D. A. 2nd. 2002. Comparative cardiovascular development: improving the conceptual framework. Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology 132:661-674.

Burggren, W. W.; Dubansky, B.; Roberts, A. and Alloy, M. 2015a. Deepwater horizon oil spill as a case study for interdisciplinary cooperation within developmental biology, environmental sciences and physiology. World Journal of Engineering and Technology 3:7-23.

Burggren, W. W. and Keller, B. B. 1997. Development of cardiovascular systems. Cambridge University Press, Cambridge.

Burggren, W. W.; Khorrami, S.; Pinder, A. and Sun, T. 2004. Body, eye, and chorioallantoic vessel growth are not dependent on cardiac output level in day 3-4 chicken embryos. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 287:R1399-1406.

Burggren, W. W. and Mueller, C. A. 2015. Developmental critical windows and sensitive periods as three-dimensional constructs in time and space. Physiological and Biochemical Zoology 88:91-102.

Burggren, W. W.; Mueller, C. A. and Tazawa, H. 2015b. Hypercapnic thresholds for embryonic acid-base metabolic compensation and hematological regulation during CO2 challenges in layer and broiler chicken strains. Respiratroy Physiology and Neurobiology 215:1-12.

Burggren, W. W. and Reyna, K. S. 2011. Developmental trajectories, critical windows and phenotypic alteration during cardio-respiratory development. Respiratory Physiology & Neurobiology 178:13-21.

Burggren, W. W.; Warburton, S. J. and Slivkoff, M. D. 2000. Interruption of cardiac output does not affect short-term growth and metabolic rate in day 3 and 4 chick embryos. The Journal of Experimental Biology 203:3831-3838.

Burness, G.; Huard, J. R.; Malcolm, E. and Tattersall, G. J. 2013. Post-hatch heat warms adult beaks: irreversible physiological plasticity in Japanese quail. Proceedings of the Royal Society - Biological Science 280:20131436.

Burton, G. J. and Palmer, M. E. 1992. Development of the chick chorioallantoic capillary plexus under normoxic and normobaric hypoxic and hyperoxic conditions: a morphometric study. The Journal of Experimental Zoology 262:291-298.

Buzala, M.; Janicki, B. and Czarnecki, R. 2015. Consequences of different growth rates in broiler breeder and layer hens on embryogenesis, metabolism and metabolic rate: A review. Poultry Science 94:728-733.

Canga, L.; Paroli, L.; Blanck, T. J.; Silver, R. B. and Rifkind, A. B. 1993. 2,3,7,8-tetrachlorodibenzo-p-dioxin increases cardiac myocyte intracellular calcium and progressively impairs ventricular contractile responses to isoproterenol and to calcium in chick embryo hearts. Molecular Pharmacology 44:1142-1151.

Chan, T. and Burggren, W. 2005. Hypoxic incubation creates differential morphological effects during specific developmental critical windows in the embryo of the chicken (*Gallus gallus*). Respiratory Physiology & Neurobiology 145:251-263.

Chiba, Y.; Fukuoka, S.; Niiya, A.; Akiyama, R. and Tazawa, H. 2004. Development of cholinergic chronotropic control in chick (*Gallus gallus domesticus*) embryos. Comparative Biochemistry and Physiology A 137:65-73.

Chin, E. H.; Storm-Suke, A. L.; Kelly, R. J. and Burness, G. 2013. Catch-up growth in Japanese quail (*Coturnix Japonica*): relationships with food intake, metabolic rate and sex. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology 183:821-831.

Chiossi, G.; Costantine, M. M.; Tamayo, E.; Orise, P.; Hankins, G. D.; Saade, G. R. and Longo, M. 2011. Effect of age and gender on the progression of adult vascular dysfunction in a mouse model of fetal programming lacking endothelial nitric oxide synthase. American Journal of Physiology. Heart and Circulatory Physiology 301:H297-305.

Cohen, E.; Wong, F. Y.; Horne, R. S. and Yiallourou, S. R. 2016. Intrauterine growth restriction: impact on cardiovascular development and function throughout infancy. Pediatric Research 79:821-830.

Collin, A.; Berri, C.; Tesseraud, S.; Rodon, F. E.; Skiba-Cassy, S.; Crochet, S.; Duclos, M. J.; Rideau, N.; Tona, K.; Buyse, J.; Bruggeman, V.; Decuypere, E.; Picard, M. and Yahav, S. 2007. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poultry Science 86:795-800.

Collotta, M.; Bertazzi, P. A. and Bollati, V. 2013. Epigenetics and pesticides. Toxicology 307:35-41.

Copeland, J. and Dzialowski, E. M. 2009. Effects of hypoxic and hyperoxic incubation on the reactivity of the chicken embryo (*Gallus gallus*) ductus arteriosi in response to catecholamines and oxygen. Experimental Physiology 94:152-161.

Corona, T. B. and Warburton, S. J. 2000. Regional hypoxia elicits regional changes in chorioallantoic membrane vascular density in alligator but not chicken embryos. Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology 125:57-61.

Crews, D.; Gillette, R.; Miller-Crews, I.; Gore, A. C. and Skinner, M. K. 2014. Nature, nurture and epigenetics. Molecular and Cellular Endocrinology 398:42-52.

Crews, D. and Gore, A. C. 2012. Epigenetic synthesis: a need for a new paradigm for evolution in a contaminated world. F1000 Biology Reports 4:18.

Crews, D. and McLachlan, J. A. 2006. Epigenetics, evolution, endocrine disruption, health, and disease. Endocrinology 147:S4-10.

Criscuolo, F.; Monaghan, P.; Nasir, L. and Metcalfe, N. B. 2008. Early nutrition and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. Proceedings of the Royal Society - Biological Sciences 275:1565-1570.

Crossley, D. A., 2nd and Altimiras, J. 2012. Effect of selection for commercially productive traits on the plasticity of cardiovascular regulation in chicken breeds during embryonic development. Poultry Science 91:2628-2636.

Crossley, D. A., 2nd; Bagatto, B. P.; Dzialowski, E. M. and Burggren, W. W. 2003a. Maturation of cardiovascular control mechanisms in the embryonic emu (*Dromiceius novaehollandiae*). The Journal of Experimental Biology 206:2703-2710.

Crossley, D. A., 2nd; Burggren, W. W. and Altimiras, J. 2003b. Cardiovascular regulation during hypoxia in embryos of the domestic chicken Gallus gallus. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 284:R219-226.

Crossley, D. A., 2nd; Jonker, S. S.; Hicks, J. W. and Thornburg, K. L. 2010. Maturation of the angiotensin II cardiovascular response in the embryonic White Leghorn chicken (*Gallus gallus*). Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology 180:1057-1065.

Crossley, D. A. II. and Altimiras, J. 2000. Ontogeny of cholinergic and adrenergic cardiovascular regulation in the domestic chicken (*Gallus gallus*). American Journal of Physiology - Regulatory, Integrative & Comparative Physiology 279:R1091-R1098.

Danchin, E. and Pocheville, A. 2014. Inheritance is where physiology meets evolution. The Journal of Physiology 592:2307-2317.

Datar, S. and Bhonde, R. R. 2005. Shell-less chick embryo culture as an alternative in vitro model to investigate glucose-induced malformations in mammalian embryos. The Review of Diabetic Studies 2:221-227.

Deans, C. and Maggert, K. A. 2015. What do you mean, "epigenetic"? Genetics 199:887-896.

Domyan, E. T. and Sun, X. 2011. Patterning and plasticity in development of the respiratory lineage. Developmental Dynamics 240:477-485.

Druyan, S. 2010. The effects of genetic line (broilers vs. layers) on embryo development. Poultry Science 89:1457-1467.

Druyan, S. and Levi, E. 2012. Reduced O2 concentration during CAM development--its effect on angiogenesis and gene expression in the broiler embryo CAM. Gene Expression Patterns 12:236-244.

Duncan, E. J.; Gluckman, P. D. and Dearden, P. K. 2014. Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype? Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution 322:208-220.

Dunn, B. E. 1991. Methods for shell-less and semi-shell-less culture of avian and reptilian embryos. p.409-418. In: Egg incubation: Its effects on embryonic development in birds and reptiles. Deeming, C. D. and Ferguson, M. W. J., eds. Cambridge University Press, Cambridge, UK.

Dzialowski, E. M. and Greyner, H. 2008. Maturation of the contractile response of the Emu ductus arteriosus. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology 178:401-412.

Dzialowski, E. M.; Reed, W. L. and Sotherland, P. R. 2009. Effects of egg size on Double-crested Cormorant (Phalacrocorax auritus) egg composition and hatchling phenotype. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 152:262-267.

Dzialowski, E. M. and Sotherland, P. R. 2004. Maternal effects of egg size on emu Dromaius novaehollandiae egg composition and hatchling phenotype. The Journal of Experimental Biology 207:597-606.

Dzialowski, E. M.; von Plettenberg, D.; Elmonoufy, N. A. and Burggren, W. W. 2002. Chronic hypoxia alters the physiological and morphological trajectories of developing chicken embryos. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 131:713-724.

Elfwing, M.; Lundengard, K. and Altimiras, J. 2011. Fetal development of baroreflex sensitivity: the chicken embryo as a case model. Respiratory Physiology & Neurobiology 178:75-83.

Ellis, H. L.; Shioda, K.; Rosenthal, N. F.; Coser, K. R. and Shioda, T. 2012. Masculine epigenetic sex marks of the CYP19A1/aromatase promoter in genetically male chicken embryonic gonads are resistant to estrogen-induced phenotypic sex conversion. Biology of Reproduction 87:23; 1-12.

Everaert, N.; De Smit, L.; Debonne, M.; Witters, A.; Kamers, B.; Decuypere, E. and Bruggeman, V. 2008a. Changes in acid-base balance and related physiological responses as a result of external hypercapnia during the second half of incubation in the chicken embryo. Poultry Science 87:362-367.

Everaert, N.; Kamers, B.; Witters, A.; De Smit, L.; Debonne, M.; Decuypere, E. and Bruggeman, V. 2007. Effect of four percent carbon dioxide during the second half of incubation on embryonic development, hatching parameters, and posthatch growth. Poultry Science 86:1372-1379.

Everaert, N.; Willemsen, H.; De Smit, L.; Witters, A.; De Baerdemaeker, J.; Decuypere, E. and Bruggeman, V. 2008b. Comparison of a modern broiler and layer strain during embryonic development and the hatching process. British Poultry Science 49:574-582.

Everaert, N.; Willemsen, H.; Kamers, B.; Decuypere, E. and Bruggeman, V. 2011. Regulatory capacities of a broiler and layer strain exposed to high CO2 levels during the second half of incubation. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 158:215-220.

Felsenfeld, G. 2014. The evolution of epigenetics. Perspectives in Biology and Medicine 57:132-148.

Fernandez-Twinn, D. S.; Constancia, M. and Ozanne, S. E. 2015. Intergenerational epigenetic inheritance in models of developmental programming of adult disease. Seminars in Cell & Developmental Biology 43:85-95.

Ferner, K. and Mortola, J. P. 2009. Ventilatory response to hypoxia in chicken hatchlings: a developmental window of sensitivity to embryonic hypoxia. Respiratory Physiology & Neurobiology 165:49-53.

Filas, B. A.; Varner, V. D.; Voronov, D. A. and Taber, L. A. 2011. Tracking morphogenetic tissue deformations in the early chick embryo. Journal of Visualized Experiments e3129.

Finkler, M. S.; Van Orman, J. B. and Sotherland, P. R. 1998. Experimental manipulation of egg quality in chickens: influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology 168:17-24.

Fisher, S. A. and Burggren, W. W. 2007. Role of hypoxia in the evolution and development of the cardiovascular system. Antioxidant and Redox Signalling 9:1339-1352.

Flores Santin, J. 2016. Cardiovascular fetal programming in quail (*Colinus virginianus*), an avian comparative model. Ph.D Dissertation. Department of Biological Sciences. University of North Texas, Denton, Texas, USA.

Ford, T. N. and Mertz, J. 2013. Video-rate imaging of microcirculation with single-exposure oblique back-illumination microscopy. Journal of Biomedical Optics 18:066007.

Fournier, A.; Feidt, C.; Dziurla, M. A.; Grandclaudon, C. and Jondreville, C. 2010. Transfer kinetics to egg yolk and modeling residue recovered in yolk of readily metabolized molecules: polycyclic aromatic hydrocarbons orally administered to laying hens. Chemosphere 78:1004-1010.

Franks, M. E.; Macpherson, G. R. and Figg, W. D. 2004. Thalidomide. Lancet 363:1802-1811.

Gabrielli, M. G. and Accili, D. 2010. The chick chorioallantoic membrane: a model of molecular, structural, and functional adaptation to transepithelial ion transport and barrier function during embryonic development. Journal of Biomedicine & Biotechnology 2010:940741.

Gheorghescu, A. K.; Tywoniuk, B.; Duess, J.; Buchete, N. V. and Thompson, J. 2015. Exposure of chick embryos to cadmium changes the extra-embryonic vascular branching pattern and alters expression of VEGF-A and VEGF-R2. Toxicology and Applied Pharmacology 289:79-88.

Gonzalez-Bulnes, A.; Astiz, S.; Ovilo, C.; Garcia-Contreras, C. and Vazquez-Gomez, M. 2016. Nature and nurture in the early-life origins of metabolic syndrome. Current Pharmaceutical Biotechnology 17:573-586.

Goodrich, E. S. 1930. Studies on the structure and developmnt of vertebrates. Macmillan, London.

Grabowski, C. T. and Paar, J. A. 1958. The teratogenic effects of graded doses of hypoxia on the chick embryo. American Journal of Anatomy 103:313-348.

Grishkevich, V. and Yanai, I. 2013. The genomic determinants of genotype x environment interactions in gene expression. Trends in Genetics TIG 29:479-487.

Gryzinska, M.; Andraszek, K. and Jezewska, W. G. 2014. Estimation of global content of 5-methylcytosine in DNA during allantoic and pulmonary respiration in the chicken embryo. Folia Biologica 62:97-101.

Hala, D.; Huggett, D. B. and Burggren, W.W. 2014. Environmental stressors and the epigenome. Drug Discovery Today: Technologies 12:e3-e8.

Hamburger, V. and Hamilton, H. L. 1951. A series of normal stages in the development of the chick embryo. Journal of Morphology 88:49-92.

Hasselquist, D. and Nilsson, J. A. 2009. Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity.

Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 364:51-60.

Hector, K. L. and Nakagawa, S. 2012. Quantitative analysis of compensatory and catch-up growth in diverse taxa. Journal of Animal Ecology 81:583-593.

Herrera, E. A.; Salinas, C. E.; Blanco, C. E.; Villena, M. and Giussani, D. A. 2013. High altitude hypoxia and blood pressure dysregulation in adult chickens. Journal of Developmental Origins of Health and Disease 4:69-76.

Herrington, J.; Vallin, C. and Lickliter, R. 2015. Increased yolk progesterone interferes with prenatal auditory learning and elevates emotional reactivity in bobwhite quail (*Colinus virginianus*) chicks. Developmental Psychobiology 57:255-262.

Hill, W. L. 1993. Importance of prenatal nutrition to the development of a precocial chick. Developmental Psychobiology 26:237-249.

Hirst, C. E. and Marcelle, C. 2015. The avian embryo as a model system for skeletal myogenesis. Results and Problems in Cell Differentiation 56:99-122.

Ho, D. H. 2014. Transgenerational epigenetics: the role of maternal effects in cardiovascular development. Integrative and Comparative Biology 54:43-51.

Ho, D. H. and Burggren, W. W. 2010. Epigenetics and transgenerational transfer: a physiological perspective. The Journal of Experimental Biology 213:3-16.

Ho, D. H.; Reed, W. L. and Burggren, W. W. 2011. Egg yolk environment differentially influences physiological and morphological development of broiler and layer chicken embryos. The Journal of Experimental Biology 214:619-628.

Holland, M. L. and Rakyan, V. K. 2013. Transgenerational inheritance of non-genetically determined phenotypes. Biochemical Society Transactions 41:769-776.

Honarmand, M.; Goymann, W. and Naguib, M. 2010. Stressful dieting: nutritional conditions but not compensatory growth elevate corticosterone levels in zebra finch nestlings and fledglings. Plos One 5:e12930

Hutchings, J. A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. Heredity 106:421-437.

Huth, J. C. and Archer, G. S. 2015. Effects of LED lighting during incubation on layer and broiler hatchability, chick quality, stress susceptibility and post-hatch growth. Poultry Science 94:3052-3058.

Itani, N.; Skeffington, K. L.; Beck, C.; Niu, Y. and Giussani, D. A. 2016. Melatonin rescues cardiovascular dysfunction during hypoxic development in the chick embryo. Journal of Pineal Research 60:16-26.

Iversen, N. K.; Wang, T.; Baatrup, E. and Crossley, D. A., 2nd. 2014. The role of nitric oxide in the cardiovascular response to chronic and acute hypoxia in White Leghorn chicken (*Gallus domesticus*). Acta Physiologica 211:346-357.

Jonker, S. S.; Giraud, G. D.; Espinoza, H. M.; Davis, E. N. and Crossley, D. A., 2nd. 2015. Effects of chronic hypoxia on cardiac function measured by pressure-volume catheter in fetal chickens. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 308:R680-689.

Jourdeuil, K. A.; Hammer, C. L. and Franz-Odendaal, T. A. 2015. A comparative analysis of chick culturing methods on skeletogenesis. Anatomical Record 298:810-819.

Kain, K. H.; Miller, J. W.; Jones-Paris, C. R.; Thomason, R. T.; Lewis, J. D.; Bader, D. M.; Barnett, J. V. and Zijlstra, A. 2014. The chick embryo as an expanding experimental model for cancer and cardiovascular research. Developmental Dynamics 243:216-228.

Karadas, F.; Surai, P. F.; Sparks, N. H. and Grammenidis, E. 2005. Effects of maternal dietary supplementation with three sources of carotenoids on the retinyl esters of egg yolk and developing

quail liver. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 140:430-435.

Keller, M. C. 2014. Gene x environment interaction studies have not properly controlled for potential confounders: the problem and the (simple) solution. Biological Psychiatry 75:18-24.

Keyte, A. L. and Smith, K. K. 2014. Heterochrony and developmental timing mechanisms: changing ontogenies in evolution. Seminars in Cell & Developmental Biology 34:99-107.

Khorrami, S.; Tazawa, H. and Burggren, W. 2008. 'Blood-doping' effects on hematocrit regulation and oxygen consumption in late-stage chicken embryos (*Gallus gallus*). The Journal of Experimental Biology 211:883-889.

Kilvitis, H. J.; Alvarez, M.; Foust, C. M.; Schrey, A. W.; Robertson, M. and Richards, C. L. 2014. Ecological epigenetics. Advances in Experimental Medicine and Biology 781:191-210.

Kim, M.; Bae, M.; Na, H. and Yang, M. 2012. Environmental toxicants-induced epigenetic alterations and their reversers. Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology Reviews 30:323-367.

Kishore, A. S.; Surekha, P. A.; Sekhar, P. V.; Srinivas, A. and Murthy, P. B. 2008. Hen egg chorioallantoic membrane bioassay: an in vitro alternative to draize eye irritation test for pesticide screening. International Journal of Toxicology 27:449-453.

Knobloch, J. and Rüther, U. 2008. Shedding light on an old mystery: thalidomide suppresses survival pathways to induce limb defects. Cell Cycle 7:1121-1127.

Kopf, P. G. and Walker, M. K. 2009. Overview of developmental heart defects by dioxins, PCBs, and pesticides. Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology Reviews 27:276-285.

Kormos, E.; Tavaszi, J.; Budai, P.; Pongracz, A. and Lehel, J. 2009. Eye irritation study of some pesticides on chorioallantoic membrane of the egg. Communications in Agricultural and Applied Biological Sciences 74:125-128.

Koutsos, E. A.; Garcia Lopez, J. C. and Klasing, K. C. 2007. Maternal and dietary carotenoids interactively affect cutaneous basophil responses in growing chickens (*Gallus gallus domesticus*). Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology 147:87-92.

Kowalski, W. J.; Dur, O.; Wang, Y.; Patrick, M. J.; Tinney, J. P.; Keller, B. B. and Pekkan, K. 2013. Critical transitions in early embryonic aortic arch patterning and hemodynamics. Plos One 8:e60271.

Kue, C. S.; Tan, K. Y.; Lam, M. L. and Lee, H. B. 2015. Chick embryo chorioallantoic membrane (CAM): an alternative predictive model in acute toxicological studies for anti-cancer drugs. Experimental Animals 64:129-138.

Kuzawa, C. W. and Thayer, Z. M. 2011. Timescales of human adaptation: the role of epigenetic processes. Epigenomics 3:221-234.

Labaque, M. C.; Martella, M. B.; Maestri, D. M. and Navarro, J. L. 2013. The influence of diet composition on egg and chick traits in captive Greater Rhea females. British Poultry Science 54:374-380.

le Noble, F. A.; Ruijtenbeek, K.; Gommers, S.; de Mey, J. G. and Blanco, C. E. 2000. Contractile and relaxing reactivity in carotid and femoral arteries of chicken embryos. American Journal of Physiology. Heart and Circulatory Physiology 278:H1261-1268.

Lee, J. Y. and Lee, S. J. 2010. Thermal effect on heart rate and hemodynamics in vitelline arteries of stage 18 chicken embryos. Journal of Biomechanics 43:3217-3221.

Lee, S. J. and Ha, H. J. 2013. In vivo measurement of blood flow in a micro-scale stenosis model generated by laser photothermal blood coagulation. IET Systems Biology 7:50-55.

Lewallen, M. A. and Burggren, W. W. 2015. Chronic hypoxia and hyperoxia modifies morphology and VEGF concentration of the lungs of the developing chicken (Gallus gallus variant domesticus). Respiratory Physiology and Neurobiology 219:85-94.

Li, C.; Guo, S.; Zhang, M.; Gao, J. and Guo, Y. 2015. DNA methylation and histone modification patterns during the late embryonic and early postnatal development of chickens. Poultry Science 94:706-721.

Li, X. G.; Sui, W. G.; Yan, H .C.; Jiang, Q. Y. and Wang, X. Q. 2014. The in ovo administration of L-trans pyrrolidine-2,4-dicarboxylic acid regulates small intestinal growth in chicks. Animal 8:1677-1683.

Lindgren, I. and Altimiras, J. 2009. Chronic prenatal hypoxia sensitizes beta-adrenoceptors in the embryonic heart but causes postnatal desensitization. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 297:R258-264.

Lindgren, I.; Crossley, D., 2nd; Villamor, E. and Altimiras, J. 2011. Hypotension in the chronically hypoxic chicken embryo is related to the beta-adrenergic response of chorioallantoic and femoral arteries and not to bradycardia. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 301:R1161-1168.

Loepke, A. W. 2010. Developmental neurotoxicity of sedatives and anesthetics: a concern for neonatal and pediatric critical care medicine? Pediatric Critical Care Medicine 11:217-226.

Lourens, A.; van den Brand, H.; Heetkamp, M. J.; Meijerhof, R. and Kemp, B. 2007. Effects of eggshell temperature and oxygen concentration on embryo growth and metabolism during incubation. Poultry Science 86:2194-2199.

Lucitti, J. L.; Tobita, K. and Keller, B. B. 2005. Arterial hemodynamics and mechanical properties after circulatory intervention in the chick embryo. The Journal of Experimental Biology 208:1877-1885.

Lucitti, J. L.; Visconti, R.; Novak, J. and Keller, B. B. 2006. Increased arterial load alters aortic structural and functional properties during embryogenesis. American Journal of Physiology. Heart and Circulatory Physiology 291:H1919-1926.

Luo, Z. C.; Xiao, L. and Nuyt, A. M. 2010. Mechanisms of developmental programming of the metabolic syndrome and related disorders. World Journal of Diabetes 1:89-98.

Maina, J. N. 2003. Developmental dynamics of the bronchial (airway) and air sac systems of the avian respiratory system from day 3 to day 26 of life: a scanning electron microscopic study of the domestic fowl, Gallus gallus variant domesticus. Anatomy and Embryology 207:119-134.

Maina, J. N. 2006. Development, structure, and function of a novel respiratory organ, the lung-air sac system of birds: to go where no other vertebrate has gone. Biological Reviews of the Cambridge Philosophical Society 81:545-579.

Maina, J. N. 2012. Comparative molecular developmental aspects of the mammalian- and the avian lungs, and the insectan tracheal system by branching morphogenesis: recent advances and future directions. Frontiers in Zoology 9:16.

Malir, F.; Ostry, V.; Pfohl-Leszkowicz, A. and Novotna, E. 2013. Ochratoxin A: developmental and reproductive toxicity-an overview. Birth defects research. Part B, Developmental and Reproductive Toxicology 98:493-502.

Mattsson, A.; Olsson, J. A. and Brunstrom, B. 2011. Activation of estrogen receptor alpha disrupts differentiation of the reproductive organs in chicken embryos. General and Comparative Endocrinology 172:251-259.

Mendez-Sanchez, J. F. and Burggren, W. W. 2014. Environmental modulation of the onset of air breathing and survival of the Siamese fighting fish Betta splendens and the three spot gourami Trichopodus trichopterus. Journal of Fish Biology 84:794-807.

Mendizabal, I.; Keller, T. E.; Zeng, J. and Yi, S. V. 2014. Epigenetics and evolution. Integrative and Comparative Biololgy 54:31-42.

Mortola, J. P. 2009. Gas exchange in avian embryos and hatchlings. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 153:359-377.

Mortola, J. P.; Marinescu, D. C.; Pierre, A. and Artman, L. 2012. Metabolic and heart rate responses to hypoxia in early chicken embryos in the transition from diffusive to convective gas transport. Respiratory Physiology & Neurobiology 181:109-117.

Mroczek-Sosnowska, N.; Sawosz, E.; Vadalasetty, K. P.; Lukasiewicz, M.; Niemiec, J.; Wierzbicki, M.; Kutwin, M.; Jaworski, S. and Chwalibog, A. 2015. Nanoparticles of copper stimulate angiogenesis at systemic and molecular level. International Journal of Molecular Sciences 16:4838-4849.

Mueller, C. A.; Burggren, W. W. and Crossley, D. A., II. 2013a. ANG II and baroreflex control of heart rate in embryonic chickens (Gallus gallus domesticus). American Journal of Physiology Regulatory, Integrative and Comparative Physiology 305:R855-863.

Mueller, C. A.; Burggren, W. W. and Tazawa, H. 2015a. The physiology of the avian embryo. p.739-766. In: Sturkie's avian physiology. 6th ed. Whittow, G. C., ed. Elsevier, New York.

Mueller, C. A.; Crossley, D. A., II and Burggren, W. W. 2014a. The actions of the renin-angiotensin system on cardiovascular and osmoregulatory function in embryonic chickens (Gallus gallus domesticus). Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology 178:37-45.

Mueller, C. A.; Eme, J.; Burggren, W. W; Roghair, R. D. and Rundle, S. D. 2015b. Challenges and opportunities in developmental integrative physiology. Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology 184:113-124.

Mueller, C. A.; Tazawa, H. and Burggren, W. W. 2013b. Dynamics of metabolic compensation and hematological changes in chicken (Gallus gallus) embryos exposed to hypercapnia with varying oxygen. Respiratory Physiology & Neurobiology 185:272-280.

Mueller, C. A.; Tazawa, H. and Burggren, W. W. 2014b. Dynamics of acid-base metabolic compensation and hematological regulation interactions in response to CO2 challenges in embryos of the chicken (Gallus gallus). Journal of Comparative Physiology B 184:641-649.

Mueller, C. A.; Willis, C. and Burggren, W. W. 2016. Salt sensitivity of the morphometry of Artemia franciscana during development: A demonstration of 3-D critical windows. Journal of Experimental Biology 219:571-581.

Näf, C.; Broman, D. and Brunström, B. 1992. Distribution and metabolism of polycyclic aromatic hydrocarbons (PAHs) injected into eggs of chicken (Gallus domesticus) and common eider duck (Somateria mollissima). Environmental Toxicology and Chemistry 11:1653-1660.

Nangsuay, A.; Meijerhof, R.; van den Anker, I.; Heetkamp, M. J.; Kemp, B. and van den Brand, H. 2015. Development and nutrient metabolism of embryos from two modern broiler strains. Poultry Science 94:2546-2554.

Natarajan, A.; Molnar, P.; Sieverdes, K.; Jamshidi, A. and Hickman, J. J. 2006. Microelectrode array recordings of cardiac action potentials as a high throughput method to evaluate pesticide toxicity. Toxicology In Vitro 20:375-381.

Newkirk, C. E.; Gagnon, Z. E. and Pavel Sizemore, I. E. 2014. Comparative study of hematological responses to platinum group metals, antimony and silver nanoparticles in animal models. Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering 49:269-280.

Nijland, M. J.; Ford, S. P. and Nathanielsz, P. W. 2008. Prenatal origins of adult disease. Current Opinion in Obstetrics & Gynecology 20:132-138.

Nowak-Sliwinska, P.; Segura, T. and Iruela-Arispe, M. L. 2014. The chicken chorioallantoic membrane model in biology, medicine and bioengineering. Angiogenesis 17:779-804.

Nowak-Sliwinska, P.; van Beijnum, J. R.; van Berkel, M.; van den Bergh, H. and Griffioen, A.W. 2010. Vascular regrowth following photodynamic therapy in the chicken embryo chorioallantoic membrane. Angiogenesis 13:281-292.

Oliveira, T. F.; Bertechini, A. G.; Bricka, R. M.; Kim, E. J.; Gerard, P. D. and Peebles, E. D. 2015. Effects of in ovo injection of organic zinc, manganese, and copper on the hatchability and bone parameters of broiler hatchlings. Poultry Science 94:2488-2494.

Olson, C. R.; Vleck, C. M. and Adams, D. C. 2008. Decoupling morphological development from growthin periodically cooled zebra finch embryos. Journal of Morphology 269:875-883.

Olson, C. R.; Vleck, C. M. and Vleck, D. 2006. Periodic cooling of bird eggs reduces embryonic growth efficiency. Physiological and Biochemical Zoology 79:927-936.

Palo, P. E.; Sell, J. L.; Piquer, F. J.; Soto-Salanova, M. F. and Vilaseca, L. 1995. Effect of early nutrient restriction on broiler chickens. 1. Performance and development of the gastrointestinal tract. Poultry Science 74:88-101.

Rahn, H.; Paganelli, C. V. and Ar, A. 1987. Pores and gas exchange of avian eggs: a review. Journal of Experimental Zoology (Suppl. 1): 165-172.

Reed, W. L. and Clark, M. E. 2011. Beyond maternal effects in birds: responses of the embryo to the environment. Integrative and Comparative Biology 51:73-80.

Reeves, S. R. and Gozal, D. 2005. Developmental plasticity of respiratory control following intermittent hypoxia. Respiratory Physiology & Neurobiology 149:301-311.

Reyna, K. S. 2010. Thermal stress during pre-incubation induces subsequent developmental plasticity in northern bobwhites. Biological Sciences. University of North Texas, Denton, Texas, USA.

Reyna, K. S. and Burggren, W. W. 2012. Upper lethal temperatures of Northern Bobwhite embryos and the thermal properties of their eggs. Poultry Science 91:41-46.

Richards, M. P.; Stock, M. K. and Metcalfe, J. 1991. Effects of brief hypoxia and hyperoxia on tissue trace element levels in the developing chick embryo. Magnesium and Trace Elements 10:305-320.

Riddle, O. 1924. On the necessary gaseous environment of the bird embryo. Ecology Letters 5:348-362.

Roberts, V. H.; Frias, A. E. and Grove, K. L. 2015. Impact of maternal obesity on fetal programming of cardiovascular disease. Physiology (Bethesda, Md.) 30:224-231.

Roig, B.; Cadiere, A.; Bressieux, S.; Biau, S.; Faure, S. and de Santa Barbara, P. 2014. Environmental concentration of nonylphenol alters the development of urogenital and visceral organs in avian model. Environment International 62:78-85.

Romanoff, A. 1967. Biochemistry of the embryo: A quantitative analysis of prenatal development. Interscience Publishers, New York. p.133-328.

Romanoff, A. L. 1931. Cultivation of the chick embryo in an opened egg. The Anatomical Record 48:185-189.

Romanoff, A. L. 1960. The avian embryo: Structural and functional development. Macmillan, New York.

Rosenbruch, M.; Kniepen, J. and Weishaupt, C. 1993. The early chick embryo as a model to evaluate cardiovascular effects of adrenaline and nicotine. Toxicology In Vitro 7:541-545.

Rouwet, E. V.; Tintu, A. N.; Schellings, M. W.; van Bilsen, M.; Lutgens, E.; Hofstra, L.; Slaaf, D. W.; Ramsay, G. and Le Noble, F. A. 2002. Hypoxia induces aortic hypertrophic growth, left ventricular dysfunction, and sympathetic hyperinnervation of peripheral arteries in the chick embryo. Circulation 105:2791-2796.

Ruijtenbeek, K.; Kessels, C.; Janssen, B.; Bitsch, N.; Fazzi, G.; Janssen, G.; De Mey, J. and Blanco, C. 2003. Chronic moderate hypoxia during in ovo development alters arterial reactivity in chickens. Pflügers Archiv - European Journal of Physiology 447:158-167.

Rundle, S. D.; Smirthwaite, J. J.; Colbert, M. W. and Spicer, J. I. 2011. Predator cues alter the timing of developmental events in gastropod embryos. Biology Letters 7:285-287.

Rymer, T. L. and Pillay, N. 2012. The development of exploratory behaviour in the african striped mouse rhabdomys reflects a gene x environment compromise. Behavior Genetics 42:845-856.

Sadler, W. W.; Wilgus, H. S. and Buss, E. G. 1954. Incubation factors affecting hatchability of poultry eggs. 2. Some effects of carbon dioxide upon morphogenesis. Poultry Science 33:1108-1115.

Sarre, A.; Maury, P.; Kucera, P.; Kappenberger, L. and Raddatz, E. 2006. Arrhythmogenesis in the developing heart during anoxia-reoxygenation and hypothermia-rewarming: an in vitro model. Journal of Cardiovascular Electrophysiology 17:1350-1359.

Sato, M.; Tachibana, T. and Furuse, M. 2006. Heat production and lipid metabolism in broiler and layer chickens during embryonic development. Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology 143:382-388.

Saw, C. L.; Heng, P. W. and Liew, C. V. 2008. Chick chorioallantoic membrane as an in situ biological membrane for pharmaceutical formulation development: a review. Drug Development and Industrial Pharmacy 34:1168-1177.

Schneider, W. J. 2015. Lipid transport to avian oocytes and to the developing embryo. Journal of Biomedical Research 30:174-180.

Sedaghat, K.; Zahediasl, S. and Ghasemi, A. 2015. Intrauterine programming. Iranian Journal of Basic Medical Sciences 18:212-220.

Shao, Z. H.; Chang, W. T.; Chan, K. C.; Wojcik, K. R.; Hsu, C. W.; Li, C. Q.; Li, J.; Anderson, T.; Qin, Y.; Becker, L. B.; Hamann, K. J. and Vanden Hoek, T. L. 2007. Hypothermia-induced cardioprotection using extended ischemia and early reperfusion cooling. American journal of physiology. Heart and Circulatory Physiology 292:H1995-2003.

Sharma, S.; Ghoddoussi, M.; Gao, P.; Kelloff, G. J.; Steele, V. E. and Kopelovich, L. 2001. A quantitative angiogenesis model for efficacy testing of chemopreventive agents. Anticancer Research 21:3829-3837.

Shell, L.; Burggren, W.; Muirhead, D.; Nelson, T. C. and Dzialowski, E. M. 2016. Circulatory changes associated with the closure of the ductus arteriosus in hatching emu (Dromaius novaehollandiae). Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 191:202-208.

Simsek, H.; Colak, A.; Kaya, S.; Kutlay, M.; Cetinkal, A.; Haholu, A. and Demircan, M. N. 2012. The effects of Diesel exhaust particles on neural tube development in the early stage chicken embryo. Turkish Neurosurgery 22:77-82.

Sirsat, S. K.; Sirsat, T. S.; Faber, A.; Duquaine, A.; Winnick, S.; Sotherland, P. R. and Dzialowski, E. M. 2016. Development of endothermy and concomitant increases in cardiac and skeletal muscle mitochondrial respiration in the precocial Pekin duck (Anas platyrhynchos domestica). Journal of Experimental Biology 219:1214-1223.

Smith, S. M.; Flentke, G. R. and Garic, A. 2012. Avian models in teratology and developmental toxicology. Methods in Molecular Biology 889:85-103.

Spicer, J. I. and Burggren, W. W. 2003. Development of physiological regulatory systems: altering the timing of crucial events. Zoology 106:91-99.

Spicer, J. I. and Rundle, S. D. 2007. Plasticity in the timing of physiological development: physiological heterokairy - what is it, how frequent is it, and does it matter? Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 148:712-719.

Spicer, J. I.; Rundle, S. D. and Tills, O. 2011. Studying the altered timing of physiological events during development: it's about time...or is it? Respiratory Physiology & Neurobiology 178:3-12.

Stangenberg, S.; Chen, H.; Wong, M. G.; Pollock, C. A. and Saad, S. 2015. Fetal programming of chronic kidney disease: the role of maternal smoking, mitochondrial dysfunction and epigenetic modfification. American Journal of Physiology. Renal Physiology 308:F1189-F1196. doi: 10.1152/ajprenal.00638.2014.

Stekelenburg-de Vos, S.; Steendijk, P.; Ursem, N. T.; Wladimiroff, J. W. and Poelmann, R. E. 2007. Systolic and diastolic ventricular function in the normal and extra-embryonic venous clipped chicken embryo of stage 24: a pressure-volume loop assessment. Ultrasound in Obstetrics and Gynecology 30:325-331.

Stern, C. D. 2004. The chick embryo - past, present and future as a model system in developmental biology. Mechanisms of Development 121:1011-1013.

Stock, M. K. and Metcalfe, J. 1987. Modulation of growth and metabolism of the chick embryo by a brief (72-hr) change in oxygen availability. The Journal of Experimental Zoology (Suppl. 1): 351-356.

Stoleson, S. H. and Beissinger, S. R. 1999. Egg viability as a constraint on hatching synchrony at high ambient temperatures. Journal of Animal Ecology 68:951-962.

Stow, C. A.; Qian, S. S. and Craig, J. K. 2005. Declining threshold for hypoxia in the Gulf of Mexico. Environmental Science and Technology 39:716-723.

Streit, A.; Tambalo, M.; Chen, J.; Grocott, T.; Anwar, M.; Sosinsky, A. and Stern, C. D. 2013. Experimental approaches for gene regulatory network construction: the chick as a model system. Genesis 51:296-310.

Strick, D. M.; Waycaster, R. L.; Montani, J. P.; Gay, W. J. and Adair, T. H. 1991. Morphometric measurements of chorioallantoic membrane vascularity: effects of hypoxia and hyperoxia. The American Journal of Physiology 260:H1385-1389.

Taylor, L. W. and Kreutziger, G. O. 1966. The gaseous environment of the chick embryo in relation to its development and hatchability 3. Effect of carbon dioxide and oxygen levels during the period of the ninth through the twelfth days of incubation. Poultry Science 45:867-884.

Taylor, L. W. and Kreutziger, G. O. 1969. The gaseous environment of the chick embryo in relation to its development and hatchability. 4. Effect of carbon dioxide and oxygen levels during the period of the thirteenth through the sixteenth days of incubation. Poultry Science 48:871-877.

Tazawa, H. 1973. Hypothermal effect on the gas exchange in chicken embryo. Respiration Physiology 17:21-31.

Tazawa, H.; Andrewartha, S. J. and Burggren, W. W. 2012. Acute regulation of hematocrit and blood acid-base balance during severe hypoxic challenges in late chicken embryos (Gallus gallus). Respiratory Physiology & Neurobiology 184:86-96.

Tazawa, H. and Mochizuki, M. 1978. Oxygen transport in chicken embryos under hypothermal exposure. Respiration Physiology 32:325-334.

Thornburg, K. L. and Louey, S. 2005. Fetal roots of cardiac disease. Heart 91:867-868.

Tintu, A.; Rouwet, E.; Verlohren, S.; Brinkmann, J.; Ahmad, S.; Crispi, F.; van Bilsen, M.; Carmeliet, P.; Staff, A. C.; Tjwa, M.; Cetin, I.; Gratacos, E.; Hernandez-Andrade, E.; Hofstra, L.; Jacobs, M.; Lamers, W. H.; Morano, I.; Safak, E.; Ahmed, A. and le Noble, F. 2009. Hypoxia induces dilated cardiomyopathy in the chick embryo: mechanism, intervention, and long-term consequences. Plos One 4:e5155.

Tissier, M. L.; Williams, T. D. and Criscuolo, F. 2014. Maternal effects underlie ageing costs of growth in the zebra finch (Taeniopygia guttata). Plos One 9:e97705.

Tzschentke, B. and Basta, D. 2002. Early development of neuronal hypothalamic thermosensitivity in birds: influence of epigenetic

temperature adaptation. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 131:825-832.

Tzschentke, B. and Rumpf, M. 2011. Embryonic development of endothermy. Respiratory Physiology & Neurobiology 178:97-107.

Varela-Lasheras, I. and Van Dooren, T. J. 2014. Desiccation plasticity in the embryonic life histories of non-annual rivulid species. EvoDevo 5:16.

Varnagy, L.; Budai, P.; Fejes, S.; Molnar, E. and Fancsi, T. 2001. Teratogenicity testing of dimethoate containing insecticide formulation (BI 58 EC) in chicken embryos. Mededelingen (Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen) 66:879-883.

Varriale, A. 2014. DNA methylation, epigenetics, and evolution in vertebrates: Facts and challenges. International Journal of Evolutionary Biology 2014:475981.

Villamor, E.; Kessels, C. G.; Ruijtenbeek, K.; Van Suylen, R. J.; Belik, J.; De Mey, J. G. and Blanco, C. E. 2004. Chronic in ovo hypoxia decreases pulmonary arterial contractile reactivity and induces biventricular cardiac enlargement in the chicken embryo. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 287:R642-R651.

Villamor, E.; Ruijtenbeek, K.; Pulgar, V.; De Mey, J. G. R. and Blanco, C. E. 2002. Vascular reactivity in intrapulmonary arteries of chicken embryos during transition to ex ovo life. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 282:R917-R927.

Wada, H. and Sewall, K. B. 2014. Introduction to the symposium-uniting evolutionary and physiological approaches to understanding phenotypic plasticity. Integrative and Comparative Biology 54:774-782.

Walter, I. and Seebacher, F. 2009. Endothermy in birds: underlying molecular mechanisms. The Journal of Experimental Biology 212:2328-2336.

Warkentin, K. M. 2011. Environmentally cued hatching across taxa: embryos respond to risk and opportunity. Integrative and Comparative Biology 51:14-25.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.

Westman, O.; Norden, M.; Larsson, M.; Johansson, J.; Venizelos, N.; Hollert, H. and Engwall, M. 2013. Polycyclic aromatic hydrocarbons (PAHs) reduce hepatic beta-oxidation of fatty acids in chick embryos. Environmental Science and Pollution Research International 20:1881-1888.

Willems, E.; Guerrero-Bosagna, C.; Decuypere, E.; Janssens, S.; Buyse, J.; Buys, N.; Jensen, P. and Everaert, N., 2016. Differential expression of genes and DNA methylation associated with prenatal protein undernutrition by albumen removal in an avian model. Scientific Reports 6:20837.

Willems, E.; Koppenol, A.; De Ketelaere, B.; Wang, Y.; Franssens, L.; Buyse, J.; Decuypere, E. and Everaert, N. 2015a. Effects of nutritional programing on growth and metabolism caused by albumen removal in an avian model. The Journal of Endocrinology 225:89-100.

Willems, E.; Wang, Y.; Koppenol, A.; Lesuisse, J.; Franssens, L.; Decuypere, E.; Buyse, J. and Everaert, N. 2015b. Reduced protein availability by albumen removal during chicken embryogenesis decreases body weight and induces hormonal changes. Experimental Physiology 100:1298-1308.

Willems, E.; Wang, Y.; Willemsen, H.; Lesuisse, J.; Franssens, L.; Guo, X.; Koppenol, A.; Buyse, J.; Decuypere, E. and Everaert, N. 2013. Partial albumen removal early during embryonic development of layer-type chickens has negative consequences on laying performance in adult life. Poultry Science 92:1905-1915.

Williams, T. D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biological Reviews of the Cambridge Philosophical Society 69:35-59.

Xu, L. J. and Mortola, J. P. 1989. Effects of hypoxia or hyperoxia on the lung of the chick embryo. Canadian Journal of Physiology and Pharmacology 67:515-519.

Yu, M. H.; Tsunoda, H. and Tsunoda, M. 2011. Environmental toxicology: Biological and health effects of pollutants. 3rd. ed. CRC Press, Boca Raton, FL, USA.

Yuan, Y. J.; Xu, K.; Wu, W.; Luo, Q. and Yu, J. L. 2014. Application of the chick embryo chorioallantoic membrane in neurosurgery disease. International Journal of Medical Sciences 11:1275-1281.

Zhang, H. and Burggren, W. W. 2012. Hypoxic level and duration differentially affect embryonic organ system development of the chicken (*Gallus gallus*). Poultry Science 91:3191-3201.

Zhang, X. and Ho, S. M. 2011. Epigenetics meets endocrinology. Journal of Molecular Endocrinology 46:R11-32.