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Maternal Stress and the Neonate's Response to Hypoxia

by

Sandra Young RN MN NNP-BC PhDc

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Abstract

Glucocorticoids promote cellular differentiation over proliferation. This physiological principle inspired the antenatal exogenous glucocorticoid administration in threatened preterm labor to be a standard in practice, as it significantly reduces the incidence of respiratory distress syndrome, intraventricular hemorrhage, and neonatal mortality. The specific aim of this study was to investigate the neonate's response to hypoxia following exposure to a maternal endogenous glucocorticoid release in response to a psychological stressor, as opposed to exogenous glucocorticoid administration. Hence we postulated there may be differences in corticosterone levels measured in rat dams exposed to a psychological stressor versus controls. We then hypothesized that when exposed to hypoxia, rat pups antentally exposed to a maternal endogenous glucocorticoid release, may respond differently, as compared to controls.

We measured the effects on corticosterone levels, in pregnant rat dams on gestational days 17, 18 and 19, following a 30 minute exposure to a simulated open field, and compared these levels to levels obtained from control dams on the same gestational days. Exposure to the open field resulted in significantly increased, non-attenuated, and reproducible corticosterone levels, sustained 120 minutes after exposure to the open field.

Thereafter, spontaneously breathing rat pups on day of life 1, 5, and 10, were placed in a temperature controlled metabolic chamber, and were exposed to either sustained or intermittent hypoxia, during which heart rates and respiratory rates were recorded via 12 channel Polygraph and then analyzed. We found no significant differences between rat

pups exposed to a maternal psychological stressor versus control rat pups on day of life 1, 5, or 10, to withstand hypoxia; as measured by time to last gasp, or number of successful autoresuscitations. However, the total number of gasps in day of life 1 pups exposed to a maternal psychological stressor was significantly less than controls. These findings provide new information regarding factors that influence the neonate's ability to withstand hypoxia: specifically pertaining to intrauterine exposure to an endogenous neuroendocrine stress response in late pregnancy.

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To my Mom, who taught me that just because you come from a small place, doesn't mean you can't dream big.

Turns out, life continues beyond you, while you are consumed in doctoral studies and working in the NICU. Loved ones pass, new are born. Friendships strengthen, while others shift. Life's hiccups remind us we are likely pushing reserves to the limit. But most notable of all, time has morphed my children from pre-K to pre-tweens. I hope to never have a lapse in my attention to their progress again, as it was for them that I tried to compress this time. I hope only to have set an example. Their words, like "Just keep swimming!" and "When you are done can we get a dog?" have been the sweetest of all.

And then there's Colin. Writing about him leaves me without words. There are no words for all he has given. Those three overused words just are not enough. So I will offer him only two, thank-you.

Dedication

For my children,

Abbey McKenna Soutar Young,

and

MacIntyre Harrison Soutar Young.

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"It is highly dishonorable for a reasonable soul to live in so divinely built a mansion as the body she resides in, altogether unacquainted with the exquisite structure and function of it".

Robert Boyle, 1627-1691

CHAPTER I

LITERATURE REVIEW

Hypoxia, literally translated, means deficiency in oxygen. Physiologically, hypoxia refers to inadequate environmental oxygen concentration to meet cellular aerobic demands (Subhi, Smith & Duke, 2009). Fetal and neonatal mammals are especially vulnerable to hypoxia, yet exhibit a multitude of physiologic adaptations to hypoxia (Singer, 1999). An understanding of the neonate's responses to hypoxia requires a background synopsis of fetal and newborn gas exchange and oxygen conformance, a comparison of fetal breathing movements contrasted with continuous postnatal ventilation and an overview of extrauterine transition. Thereafter, a synthesized review of the literature, relevant to factors that influence newborn gasping and autoresuscitation, will be put forth.

A. Gas Exchange

The placenta and the lungs are both organs of gas exchange. The lung is far more efficient than the placenta in oxygen and carbon dioxide exchange for the following reasons: 1) the diffusion distance is greater in the placenta as compared to the lung, 2) the permeability of the blood-blood barrier is lower than the blood-gas barrier of the lung, and 3) the surface area of the placenta is less than the lung (Ross, 1967).

1.0 Fetal Gas Exchange

Fick's Law of diffusion dictates that the oxygen diffusion coefficient, depends upon the surface area and thickness of the diffusion barrier (Fick, 1855). The oxygen diffusion coefficent, or the number of milliliters of a gas, that will diffuse at a distance of 0.001 mm over a square centimeter surface per minute, at 1 atmosphere of pressure, is 1.1

cm2 sec-1 (MacDougall & McCabe, 1967). However, the human hemochorial placental barrier defies the underlying principles of uniform surface area or thickness inherent in Fick's Law (Mayhew, 2006). This results in variable diffusing capacity in different regions of the placenta, due to approximately 20% oxygen consumption by the placenta itself (Power, 1972), the unequal distribution of maternal and fetal blood flow rates (Mayhew, Joy & Haas, 1984; Power, Longo, Wagner, Kuhl & Forster, 1967), the relationship between maternal and fetal blood oxygen and carbon dioxide content (Power, 1972), and the architecture and vasculature of the placenta itself, specifically the spiral arteries (Macara, Kingdom, Kaufmann, Kohen, Hair, More, Lyall & Greer, 1996).

Oxygen and carbon dioxide exchange between mother and fetus is interdependent. The pioneer physiologist, Paul Zweifel, in the year 1876, was the first to report that the fetus consumes oxygen, by recognizing the color differences between the darker blood in the umbilical artery and the brighter blood in the umbilical vein, of fetal rabbits (Barron, 1976). In the placenta, oxygen and carbon dioxide readily diffuse across the gradient between the single cell layer separating terminal villi and intervillous spaces, in response to differences in partial pressure (Longo & Power, 1969). The partial pressure of oxygen in maternal blood is higher than in fetal blood. Consequently, the gradient is such that oxygen readily diffuses across the placenta from maternal to fetal blood (Longo & Power, 1969). Diffusion of carbon dioxide from the maternal blood in the intervillous space shifts the maternal oxyhemoglobin dissociation curve to the right and promotes oxygen off-loading. Conversely, the partial pressure of carbon dioxide is higher in the fetal as compared to maternal blood, due to: 1) relative hyperventilation in pregnancy, and the 2) Double Haldane Effect, whereby deoxygenated blood carries more carbon dioxide. Hence oxygenated fetal hemoglobin releases carbon dioxide and shifts the curve further to the

left enhancing its own affinity for oxygen, and the maternal reduced hemoglobin binds carbon dioxide with greater affinity (Zhang, Kobayashi, Kitazawa, Imai & Kobayashi, 2006).

1.1 Fetal Response to Hypoxia

Oxygen reversibly binds to hemoglobin. The oxygen dissociation curve describes the partial pressure of oxygen at 50% hemoglobin saturation (P50) in a sigmoidal relationship. The unique fetal adaptation to relative hypoxia can be explained by the P50 of fetal blood as 20 torr, closely approximating the theoretical optimal P50 of maximum oxygen delivery to human tissue (Tien, 1979). The fetal oxygen dissociation curve is shifted to the left as compared to the curve for the normal adult (Bunn & Briehl, 1970). 2,3-Diphosphoglycerate (DPG) binds to the beta chains of adult hemoglobin and shifts the curve to the right. Fetal hemoglobin is devoid of beta chains. Hence at the level of the placenta, oxygen unloads readily from maternal hemoglobin, and has a high affinity for fetal hemoglobin (MacLennan, Emerson, Hunter & Darley, 1976).

The relatively low fetal oxygen tension of 18-20 mm Hg in the umbilical artery and 22-26 mm Hg in the umbilical vein, resulting from the high metabolic rate and oxygen consumption of the placenta, does not translate into low fetal oxygen saturation. The fetus compensates for relative hypoxia via the following factors: 1) a relatively higher hematocrit in the fetus as compared to the mother, 2) the aforementioned mechanisms described in the oxygen dissociation curve for maternal and fetal blood, 3) a high cardiac output in the fetus, 4) increasing fetal and maternal blood supply to the placenta with advancing gestation and 5) the Double Bohr Effect (Power, 1972).

An increase in the partial pressure of carbon dioxide, or a decrease in pH in the

blood, shifts the oxygen dissociation curve to the right; the reverse is also true (Zhang, et al, 2006). Therefore there is a proportionate relationship between oxygen, carbon dioxide and pH expressed as the change in log P50 per unit change in pH, known as the Bohr coefficient (Zhang et al, 2006). The Double Bohr effect refers to the cooperative oxygen dissociation curves for maternal adult hemoglobin and fetal hemoglobin moving apart in opposite directions. As the pH of maternal blood falls as it passes through the placenta, more oxygen is released, and a rise in fetal pH increases the uptake of oxygen (Zhang et al, 2006). The increase in the partial pressure of carbon dioxide in the intervillous sinuses promotes oxygen unloading, whereas the decrease in the partial pressure of carbon dioxide in the terminal villi facilitates oxygen loading (Zhang et al, 2006).

1.2 Fetal Oxygen Conformance

Oxygen conformance refers to the ability of the organism to reduce energy demand and oxygen consumption in response to hypoxia, whilst conserving ATP (Gnaiger, 2003). Hypoxia induces a constellation of adaptations in the fetus, including cessation of fetal breathing movements (Boddy Dawes, Fisher, Pinter, & Robinson, 1974), redistribution of cardiac output to the brain, heart and adrenals known as the diving reflex (Peeters, Sheldon, Jones, Makowski & Meschia, 1979), and loss of Rapid Eye Movemnt (REM), postural tone and muscle activity (van Vliet, Martin, Nijhuis, &Prechtl, 1985); thereby conserving energy expenditure and oxygen consumption (Bocking, Gagnon, Milne & White, 1988).

A phylogenic mechanism for reducing oxygen demand in the perinatal period is the defiance of the normal metabolic allometry of mammals. Kleiber's rule (Kleiber & Rogers, 1961), or the allometric non-proportional size relationship of metabolic rate (White & Seymour, 2005), dictates that the smaller the mammal the higher the metabolic

rate. This is because of the larger surface area per body weight; proverbially conceptualized as the hummingbird versus the elephant. However, during perinatal transition, the fetal metabolism is similar to that of the mother's. This suppressed metabolic rate relative to body mass is highly oxygen conservative, and has been described as being akin to acclimatization to high altitude (Singer & Muhfeld, 2007).

Fetal growth comprises 40-50% of energy demand (Singer, 1999). Under chronic hypoxic conditions in utero, energy is allocated to metabolism over growth accretion. Hence growth restriction may be conceived as not only a consequence of chronic hypoxia, but also a compensatory adaptation (Neerhof & Thaete, 2008).

1.3 Fetal Breathing Movements

The purpose of fetal breathing movements remains to be fully elucidated, as these movements do not contribute to gas exchange. However, what is known is that fetal breathing is required for normal lung development (Liggins & Kitterman, 1981; Inanlou, Baguma-Nibasheka & Kablar, 2005); and the absence of fetal breathing movements is associated with impaired lung distention, and results in lung hypoplasia (Liggins, Vilos, Campos, Kitterman & Lee, 1981) as evidenced by decreased lung weight and total lung DNA (Fewell, Lee & Kitterman, 1981). Since the discovery of fetal breathing movements, and the advent of ultrasonic monitoring of fetal well being, the absence of fetal breathing movements or the appearance of gasping like movements, notwithstanding the futility of this reflex; are recognized as ominous indicators of fetal distress and even impending intrauterine demise (Boddy & Dawes, 1975; Liston, Sawchuck & Young, 2007). In contrast, normal fetal breathing movements occur intermittently, approximately 40% of the time (Boddy & Dawes, 1975), primarily during Rapid Eye Movement (REM) sleep (Dawes, Fox, & Richards, 1972), and may serve to prepare the fetus for continuous

ventilation at birth.

Mechanical forces, including those created via fetal breathing movements, balanced with fluid lung volumes and pressures; influence fetal lung development (Kitterman, 1996). The lack of cyclic mechanical stretch that occurs during fetal breathing movements, become translated into the down-regulation of growth factors like Insulin Growth Factor (Hooper, Han & Harding, 1993). While the presence of mechanical stretch up-regulates gene expression and protein synthesis of Platelet Derived Growth Factor (Liu, Liu, Buch, Tanswell, & Post, 1995), thereby mediating fetal pneumocyte proliferation and differentiation. Tracheal occlusion magnifies mechanical stretching and increases fetal lung DNA synthesis and weight (Hashim, Laberge, Chen & Quillen, 1995), while experimental spinal cord transection used to examine the effects of obliterating fetal breathing movements impairs lung growth and fluid production (Liggins et al, 1981).

Phasic stretch, due to fetal lung fluid and fetal breathing movements, induce cell growth and differentiation in type I and II pneumocytes (Inanlou, Baguma-Nibasheka & Kablar, 2005). Alveolar type II pneumocytes synthesize, package, store in the lamellar bodies, and ultimately secrete and recycle surfactant. Surfactant is comprised of approximately 85-90% phospholipid, primarily as dipalmitoyl phosphatidylcholine; 6-8% as proteins A, B, C, and D; and the remainder as cholesterol (Dombrowsky, Tschernig, Vieten, Rau, Ohler, Acevedo, Behrens, Poets, von der Hardt & Bernhard, 2006). As per LaPlace's equation, the ratio of surface tension to radius is equilibrated in the alveolus by surfactant (Hall, Bermel, Ko, Palmer, Enhorning & Notter, 1993), allowing for the establishment of functional residual capacity at birth, normal lung mechanics and uniform

lung expansion thereafter.

Fetal breathing movements in sheep and humans are influenced by many factors. Fetal breathing movements vary in response to diurnal patterns (Visser, Goodman, Levine & Dawes, 1982). Whereas fetal breathing movements increase in response to glucose (Richardson, Hohimer, Mueggler & Bissonnette, 1982), caffeine (McGowan, Devoe, Searle & Altman, 1987) and theophylline (Bissonnette, Hohimer, Chao, Knopp & Notoroberto, 1990); they decrease in amplitude and frequency in response to hypoglycemia (Richardson et al, 1982), and ethanol (Fox, Steinbrecher, Pessel, Inglis, Medvid & Angel, 1978). Nicotine exposure increases the rate of fetal breathing movements, but prolongs periods without fetal breathing movements (Eriksen, Gennser, Lofgren & Nilsson, 1983), and reduces the overall incidence of periods with fetal breathing movements (Manning, Walker & Feyerabend, 1978).

Hypercapnia induces an increase in fetal breathing movements (Boddy & Dawes, 1975), whilst fetal hypoxia results in a marked decrease or absence of fetal breathing movements (Boddy, Dawes, Fisher, Pinter, & Robinson, 1974). Hypoxia inhibits fetal breathing through activation of central adenosine A (2A) receptors (Koos, Mason, Punla & Adinolfi, 1994; Koos, Maeda, Jan & Lopez, 2002). As such, adenosine is thought to be neuroprotective and oxygen-conservative for the fetus during periods of hypoxia.

1.4 Continuous Ventilation

To describe the onset of breathing at the time of birth is a misnomer. Rather, successful extrauterine transition requires the onset of continuous neonatal ventilation as opposed to intermittent fetal breathing movements. Several theories have been proposed

in the literature over time, to explain the switchover from intermittent fetal breathing movements to continuous neonatal ventilation. Early publications simply cite the lungs becoming air filled and are thereby enabled to initiate gas exchange (Boddy & Dawes, 1975). Excitatory mechanisms that may override the inhibitory fetal descending pathways from the lateral pons in the control of breathing of the newborn, have also been proposed, citing the involvement of: serotonin precursors (Quilligan, Clewlow, Johnstone &Walker, 1981), γ-aminobutyric acid (GABA) antagonism (Brown, Lawson, Jansen, Chernick, Taeusch, 1981), releasing hormones such as thyrotropin releasing factor (Umans, Umans, & Szeto, 1986) and corticotrophin releasing hormone (Bennet, Johnstone, Vale & Gluckman, 1990) or prostaglandin synthase inhibitors (Kitterman, Liggins, Clements &Tooley, 1979). Yet others elucidated the role of vagal innervation in the establishment of continuous breathing by underlining the importance of vagal afferent input in modulating respiratory pattern and augmenting phrenic nerve output by demonstrating the impairment of breathing frequency and failure to maintain functional residual capacity, with vagal dennervation (Lalani, Remmers & Hasan, 2001). Still others pointed to the potential contributions of a placental peptide that inhibits continuous breathing until cord clamping (Alvaro, deAlmeida, Al-alaiyan, Robertson, Nowaczyk, Cates & Rigatto, 1993). Finally, convincing evidence is derived from considering the role of oxygen itself, in relation to the relative hypoxia of the fetus in utero inhibiting continuous breathing, contrasted with continuous breathing movements induced in fetal sheep under conditions of hyperoxia (Baier, Hasan, Cates, Hooper, Nowaczyk & Rigatto, 1990; Baier, Hasan, Cates, Hooper, Nowaczyk & Rigatto, 1992); thereby simulating the extrauterine oxygen conditions.

2.0 Extrauterine Transition

Transition from intrauterine to extrauterine life, is generally well tolerated by the neonate, with 90% of newborns requiring no resuscitation (World Health Organization, 1995). However, maladaptation can lead to pathophysiology or even demise, with approximately 19% of newborn deaths worldwide, being associated with asphyxia, thereby underscoring the vulnerability of the neonate to hypoxia at birth (World Health Organization, 1995). Although successful extrauterine transition requires functional adaptations in almost every organ system, the most critical events involve the onset of continuous ventilation as discussed previously, clearance of lung fluid, and the establishment of functional residual capacity.

2.1 Fetal Lung Fluid

The onset of labor stimulates fetal catecholamine release and lung fluid absorption (Brown, Olver, Ramsden, Strang & Walters, 1983). Moreover, increasing fetal cortisol levels are inversely proportionate to decreasing fetal lung fluid volumes near the end of gestation (Kitterman, Ballard, Clements, Mescher & Tooley, 1979). This surge of adrenomedullary catecholamines that takes place, just prior to and at the time of birth, is integral to the adaptation of the newborn to extrauterine life, and arises despite the immaturity of neuronal innervation of the sympathetic nervous system. This non-neurogenic response, as coined by Seidler and Slotkin (1985), is therefore vital to survival. Loss of adrenomedullary catecholamine release or adrenergic receptor function, will impair the neonate's ability to survive periods of hypoxia and stress, such as occurs

at birth or during apnea. There is no inbuilt redundancy in this mechanism; again underlining the neonate's vulnerability to hypoxia during the first hours to days of life.

In the fetus, fluid is secreted by the lung epithelium via active transport of chloride ions and along concentration gradients (Olver & Strang, 1974) at a rate likely slightly greater than functional residual capacity at birth (Harding and Hooper, 1996). Fetal lung fluid is secreted against the resistance created at the level of the glottis and the hydrostatic pressure produced by the amniotic fluid (Kitterman, et al, 1979). The positive intrapulmonary pressure generated by fetal lung fluid is required for lung growth; with accumulation of lung fluid resulting in lung hyperplasia, and on the contrary, deficiency or elimination of lung fluid causing lung hypoplasia (Alcorn, Adamson, Lambert, Maloney, Ritchie & Robinson, 1977; Moessinger, Harding, Adamson, Singh & Kiu, 1990).

Outdated and lay explanations for the clearance of fetal lung fluid by means of the squeeze of the birth canal, do not account for the intricacies of the ion transport mechanisms within the lung epithelium. Whereas fluid lung volume was dependent on chloride secreting membranes, these channels switch to sodium absorbing membranes subsequent to, and at the onset of, labor (Jain & Eaton, 2006). Activation of this switchover is likely dependent upon glucocorticoids (Venkatesh & Katzberg, 1997) and catecholamines (Brown, Olver, Ramsden, Strang & Walters, 1983). Active sodium transport across the lung epithelium moves fluid from the lumen to the cell via the apical membrane, and then from the cell to the interstitium via basolateral membrane. Initially, movement of sodium from the lumen into the cell, across the apical membrane, is mediated via Amiloride-sensitive sodium channels: while the electrochemical gradient, is

maintained by sodium-potassium-ATPase channels, on the basolateral membrane (Matalon, 1991). Amiloride has been shown to block sodium channels and induce respiratory distress in the newborn (O'Brodovich, Hannam, Seear & Mullen, 1990) and thus the sodium transport channels in the lung epithelia are referred to as Amiloride sensitive (Song, Sun, Curstedt, Grossman & Robertson, 1992). The apical membrane, epithelial sodium channel (ENaC) comprised of α, β, and γ sub-units, can be upregulated by glucocorticoids in rat lungs (O'Brodovich, Canessa, Ueda, Rafii & Rossier, 1993). Analogous to the rat lung epithelia, human fetal lung epithelial cells also possess the capacity to transport both sodium and chloride through apical channels (Barker, Boucher, & Yankaskas, 1995).

2.2 Functional Residual Capacity

The initial breaths in a neonate require huge positive opening pressures in order to generate functional residual capacity and tidal volume, and to overcome airway resistance, elastic recoil of the chest wall, and surface tension at the level of the alveoli. These exaggerated inspiratory efforts are much like gasps. Vyas, Field, Milner, & Hopkins (1986) reported inspiratory pressures between 28-105 centimeters (cm) of water pressure (H_2O), and positive expiratory pressure between 18-115 cm H_2O, during the first breath. Similar findings of forceful inspiratory pressures as high as 60 cm H_2O at birth were also detailed by Karlberg, Cherry, Escardo & Koch (1962). Thereafter, with the recruitment of the alveoli, minimal negative intrathoracic pressure is required to maintain functional residual capacity and tidal volumes.

2.3 Cardiovascular Adaptations

Cardiac output and oxygen consumption are never higher than at the time of birth, with newborn cardiac output recorded as high as 200-325 mls/kg/minute, compared to a child with a normal cardiac output of half that value (Walther, Siassi, Ramadan, Ananda & Wu, 1985). In the fetus, blood returning to the right ventricle is largely shunted across the patent ductus arteriosus to the systemic circulation as pulmonary vascular resistance is high, with about 8-13% of the total cardiac output delivered to the pulmonary circulation (Rudolph & Heymann, 1970; Rasanen, Debbs, Wood, Weiner, Weil & Huhta, 1997). Fetal endothelin-1 promotes vasoconstriction of the pulmonary vasculature in this relatively hypoxic environment (Ivy, le Cras, Parker, Zenge, Jakkula, Markham, Kinsella & Abman, 2000). However, in the neonate, pulmonary vascular resistance falls dramatically at birth because of aeration of the lungs, increased oxygen tension, and the release of vascular endothelial mediators such as nitric oxide, prostacyclin and bradykinin (Shaul & Wells, 1994; Boels, Deutsch, Gao & Haworth, 2001). Alas, hypoxia impairs nitric oxide synthase production, thereby inhibiting nitric oxide and upregulating endothelial cyclooxygenase activity, resulting in smooth muscle calcium mobilization and contraction, while inflammatory mediators induce endothelin-1 and thromboxane, causing further constriction (Gordon, Moreira, O'Donnell, Aldinger & Tod, 1997). Thus, the vicious cycle of pulmonary hypertension ensues. Failure of the normal transition from in utero to extra-uterine circulation results in suprasystemic, pulmonary hypertension, increasing right ventricular afterload, right to left intracardiac shunting, hypoxemia and eventual death, if supportive management is not instituted (Dakshinamurti, 2005).

In the near term fetus, right ventricular output comprises 60% of total output, as

compared to left ventricular output of 40% (Rasanen et al, 1997). Accordingly, cardiac myocytes of the right ventricle in the near term fetus, contain more myofibrils than the left ventricle. Postnatally, the fall in pulmonary vascular resistance, and rise in systemic vascular resistance, corresponds with ventricular remodeling, and an increase in myofibrils in the left ventricular myocytes (Smolich, 1995).

3.0 Vulnerability of the Neonate to Hypoxia

The neonate is at greatest risk for hypoxia during apnea, most frequently associated with Rapid Eye Movement (REM) sleep, and during the previously illustrated transition to extrauterine life. REM sleep results in paradoxical inspiratory and expiratory muscle activity potentiating primary apnea. Thus the ability to arouse from sleep during periods of hypoxia is paramount to survival. To compound matters further, neonates spend as many as 14-18 hours per day sleeping, and 50% of this time or greater is spent in REM sleep (Poblano, Haro & Arteaga, 2007).

Anatomically, the neonate is vulnerable to hypoxia. The neonate's upper airway is proportionately narrower as compared to the adult with an elevated hyoid bone and larynx (Gunn & Tonkin, 1989). The chest wall of the newborn is overly compliant contributing to chest wall distortion and paradoxical chest wall movement, which may contribute to diaphragmatic fatigue and apnea (Heldt & McIlroy, 1987). Moreover, when transitioning from quiet to active sleep, the neonate is at highest risk for: 1) paradoxical breathing movements (Henderson-Smart & Read, 1976; Knill, Andrews, Bryan & Bryan, 1976), 2) decline in end-expiratory rib cage circumference (Henderson-Smart & Read, 1979), and

significantly increased oxygen consumption (Stothers & Warner, 1978). Finally, the neonate is predisposed to hypoxemia, due to the relatively low functional residual capacity and oxygen stores, in relation to high metabolic demands, and placement of the neonate on the steep slope of the oxygen dissociation curve (Henderson-Smart & Cohen, 1986).

Additional work of the heart as in hypoxic conditions accentuates the vulnerability of neonatal cardiac performance. Although the number of cardiac myocytes increases throughout gestation, the number remains constant postnatally, with increasing mass throughout the lifespan attributable only to hypertrophy (Austin, Fagan & Mathew, 1995). The physiological properties of the neonatal myocyte are distinct from the child or adult. In the adult the myofibrils, or myofilaments of actin and myosin, are lined up along the long axis of the myocyte, whereas in the fetus and neonate the myofibrils are disorganized and random. Furthermore, there is a relative lack of sarcoplasmic reticulum and t-tubules, coupled with shorter myofibrils; hence the neonatal myocardium is less contractile (Brette & Orchard, 2003). In the fetus the mitochondria are dispersed throughout the cytoplasm, but throughout postnatal life the mitochondria are situated between the myofibrils. The performance of the neonatal heart is therefore working at near maximal capacity, with minimal contractile reserve and a high demand for energy.

4.0 Oxygen Conformance in the Neonate

Hypoxia induces preferential shunting of blood away from the non-essential periphery; like the skin and gut, due to a catecholamine surge (Langercrantz & Slotkin, 1986), resulting in circulatory redistribution to essential central organs such as the brain,

heart and adrenals (Shah, Riphagen, Beyene, & Perlman, 2004). This adaptive physiological response was first identified in marine mammals, and is therefore known as the diving reflex (Butler & Jones, 1997). Anaerobic metabolism ensues in conditions of hypoxia, and with recovery and reperfusion, there is a transient rise in lactate, entitled the wash out effect (Chiu & Bindon, 1987).

During hypoxia, the neonatal myocardium maintains electrical activity for longer periods than the mature myocardium, due to increased glycogen stores and a slower decline in ATP (Jarmakani, Nakazawa, Nagatomo & Langer, 1978). In fetal lambs neither an acute nor chronic reduction in aortic blood oxygen content by as much as 50%, changed myocardial oxygen consumption nor cardiac performance; the only difference noted was that lactate continued to be consumed rather than produced (Fisher, Heymann & Rudolph, 1980). In fetal guinea pigs, ATP concentration and heart rate were unaltered by decreased oxygen levels as low as 60% of normal (Rolph & Jones, 1983). Consistently, fetal and neonatal rabbit hearts can tolerate almost total oxygen deprivation with not only increased production of ATP from glycolysis, but also preservation of ATP (Parrish & Farrar, 1990).

5.0 The Neonate's Response to Hypoxia

The response of the fetus to hypoxia is principally by means of inhibitory influence over the control of breathing, as evidenced by periodicity and even apnea during sleep, prolongation of expiratory time, and an exaggerated reflex stimulation of the larynx. Up-regulation of inhibitory neurotransmitters such as denosine, GABA, prostaglandin, endorphins and serotonin, and the down-regulation of

excitatory neurotransmitters, like glutamate, may contribute to the mechanisms underlying this predominantly inhibitory input (Abu-Shaweesh, 2004).

In contrast to the fetus, the neonate, across species, initially responds to hypoxia with hyperpnea. Cross and Warner (1951) were the first to describe the respiratory response of the neonate to hypoxia with an initial increase in minute ventilation, followed by a decrease in respiratory rate or apnea, by the second minute of exposure to hypoxia. Subsequently, this biphasic response to hypoxia has been deemed the augmentation and depressive stages (Bissonnette, 2000). In lambs, both tidal volume and respiratory rate increase in the augmentation phase, whereas tidal volume declines significantly more than frequency, in the depressive phase (Bureau, Zinman, Foulon & Begin, 1984).

5.1 Chemoreceptors

Chemoreceptors are present both centrally and peripherally. Central chemoreceptors located just below the medulla, detect changes in the partial pressure of carbon dioxide (PCO_2) and the acidity (pH) of the cerebrospinal fluid (CSF) (Walker, 1984). As per the Henderson-Hasselbach equation that relates to plasma, the same is true of CSF; that is, an increase in PCO_2 will result in a decrease in pH at a given bicarbonate concentration, where 0.03 is the solubility coefficient of carbon dioxide in mmol/liter torr and pk is the negative log of the dissociation constant for carbonic acid (pk=6.1) (Henderson, 1908). The blood brain barrier is relatively impermeable to hydrogen and bicarbonate ions. Hence changes in PCO_2 are transmitted readily to the CSF, and thus to the extracellular fluid surrounding the central chemoreceptors of the ventrolateral medulla

(Hanson & Torrance, 1981). Hypercarbia stimulates the central chemoreceptors and results in hyperpnea. Hyperventilation serves to normalize the PCO_2 in the plasma and CSF. Hence central chemoreceptors are exquisitely sensitive to changes in PCO_2, but not to changes in oxygen tension (PaO_2).

Peripheral chemoreceptors are located in the aortic and carotid bodies and respond primarily to changes in the partial pressure of oxygen, and secondarily to increases in PCO_2 and decreases in pH (O'Regan & Majcherczyk, 1982). The aortic bodies transmit this information afferently via the vagus nerve, and the carotid bodies via the glossopharyngeal nerve to the medulla. Type I Glomus Cells within the peripheral chemoreceptors are dense with mitochondria, hence their unique metabolic sensitivity to oxygen tension (O'Regan et al, 1982). Glomus cells also contain many synaptic vesicles for dopamine, acetylcholine, norepinephrine and neuropeptides (Milsom & Burleson, 2007). This redundancy in neurotransmitters ensures a highly sensitive mechanism to minute changes in oxygen tension.

The sensitivity of chemoreceptors to oxygen at birth, is reset from the relative hypoxic conditions in utero, to the rapid rise in arterial oxygen tension extra-utero. This resetting takes place over the course of the first 2 weeks of life in rats (Eden & Hanson, 1987). However the mechanism therein remains to be elucidated. Hertzberg, Hellstrom, Langercrantz & Pequignot (1990) correlated the sensitivity of the carotid bodies to oxygen, with the rapid decline in dopamine content, and the rate-limiting enzyme in catecholamine synthesis, tyrosine hydroxylase, in the carotid bodies of near term rat fetuses, as compared to rat pups of one week of life. Additionally, down regulation of Hypoxic Inducible Factor -1α during the first week of life, may also be involved in the

maturation of the peripheral chemoreceptors to hypoxia (Roux, Brismar, Asperia & Langercrantz, 2005).

Hypoxia activates oxygen sensitive neurosecretory cell catecholamine release not only in the type I glomus cells of the carotid and aortic bodies (Acker, 1989), but also in the lung parenchyma (Youngson, Nurse, Yeger, & Cutz, 1993) and in the neonate, the adrenal medullary chromaffin cells (Thompson, Jackson, & Nurse, 1997). Excitation of these aforesaid chemoreceptive cells by hypoxia is dependent upon inhibition and subsequent closure of oxygen sensitive potassium channels, which leads to membrane depolarization and calcium influx, and neurosecretory release of catecholamines (Lopez-Barneo, Lopez-Lopez, Urena & Gonzalez, 1988). Moreover, neurons within the rostral ventrolateral medulla itself are chemosensitive to hypoxia (Solomon, Edelman & Neubauer, 2000), but how these neurons may affect apneusis or how they are activated is yet to be determined.

An intra-adrenal portal vascular system within the adrenal gland is shared between the adrenal cortex and medulla. Therefore, responses to stress via hormonal release of adrenocroticotropic hormone, and the release of cortisol from the adrenal cortex, may induce the enzyme, phenylethanolamine-N-methyltransferase and thereby stimulate synthesis of norepinephrine from the adrenal medulla (Wurtman, 2002). Hence, this mechanism may influence the neonatal adreno-medullary response to hypoxia, and hypoxia itself may impact steroidogenesis in the neonatal adrenal cortex (Raff, Hong, Oaks & Widmaier, 2003).

5.2 Gasping

Medial to the nucleus ambiguus, and ventral to the nucleus of the tractus solitarius, is the lateral tegmental field, where the neurogenesis of gasping originates, but not eupnea (St. John, 1990; Fung, Wang, & St.John, 1995; Fung, Huang & St. John, 1997). Lumsden, 1923, was the first to report the induction of gasping after pontomedullary transection and exposure to anoxia (Lunsden, 1923). Hypoxia suppresses the pontomedullary control of eupnea, thereby inducing apneusis, via suppression of inhibitory synaptic transmission via GABA, augmentation of extracellular potassium, and increased conductance via sodium channels (St. John, 1996).

When exposed to unrelenting hypoxia, neonatal mammals present with primary apnea, followed by a period of latency (Gozal & Torres, 1997; Fewell, Smith, Ng, Wong & Wang, 2000), and then a triphasic pattern of gasping (Gozal, Torres, Gozal & Nuckton, 1996). There are two types of gasps noted within the three phases of gasping (Gozal et al, 1996). Type I gasps are comprised of both inspiratory and expiratory components, whereas Type II gasps are primarily inspiratory. The first phase (P1) of gasping consists of frequent type I gasps. Phase two (P2) characteristically is comprised of infrequent gasps, of less than two per minute. Phase three (P3) classically appears as the onset of frequent type II gasps of decrementing amplitude, ending in terminal apnea (Gozal et al, 1996). This triphasic gasping pattern is not detectable by the third week of life in rats (Gozal et al, 1997).

5.3 Autoresuscitation

Neonatal mammals exposed to hypoxia undergo a well-described series of responses: hyperpnea, agitation, primary apnea coupled with bradycardia, then three phases of gasping, and finally, secondary or terminal apnea (Jacobi & Thach, 1989; Saiki, Ikeda, Nishikawa, Tanimoto, Yoshida & Matsumoto, 2001). Autoresuscitation is the final physiological mechanism used by mammals to survive, following exposure to anoxia or hypoxia, after failure of other mechanisms by gasping (Fewell, 2005). Without gasping, autoresucitation cannot occur. The term autoresuscitation, albeit somewhat informal, was derived from self-resuscitation, and coined autoresuscitation by Guntheroth & Kawabori (1975).

Gasping comprises the first stage of autoresuscitation (stage I) (Fewell et al 2000), and occurs when PaO2 reaches approximately 8-10 torr (Jacobi & Thach, 1989). Three to four gasps are usually required to increase heart rate, marking the second stage of autoresuscitation (stage II) or cardiac resuscitation (Gershan, Jacobi & Thach, 1990). Potentially, gasping coupled with sufficient pulmonary circulation despite bradycardia, serves to reperfuse atrial pacemaker cells resulting in an increase in heart rate (Fewell, 2000). An increase in heart rate and cardiac output results in reperfusion of medullary centers and a gradual return to eupnea, known as stage III of resuscitation, or respiratory resuscitation (Fewell, 2000). Therefore autoresuscitation failure results from inadequate gasping, or a failure in reperfusion; and autoresuscitation success necessitates that stages I and II precede stage III, in step-wise and sequential order.

Rat pups exposed to hypoxia present with heightened arousal and tachypnea, followed by a period of agitation, seemingly running to escape the environmental threat. Within about approximately one minute of exposure to hypoxia, opisthotonus is noted concomitant with primary apnea. During primary apnea the pup loses postural muscle tone and is unresponsive to somatosensory stimulation. If the pup is then exposed to normoxia, respiratory effort resumes in the form of gasping, within about 10-20 seconds, followed by tachycardia, and a gradual stabilization to baseline heart rate and eupnea. This aforesaid process is deemed successful autoresuscitation (Gershan et al, 1990; Fewell, 2005).

5.4 Successful Autoresuscitation in Humans

There is a dearth of literature that describes successful or failed autoresuscitations in humans. Sridhar, Thach, Kelly & Henslee (2003) analyzed home monitoring tracings of 24 infants. Sridhar et al (2003) described the onset of the upstroke to peak amplitude in inspiration to be significantly shorter in infants undergoing home monitoring as compared to controls. The reverse was true regarding expiration; the peak amplitude to the onset of the next upstroke was significantly longer in study subjects as compared to controls. Whereas previous authors asserted gasping in humans was rare (Guntheroth & Kawabori, 1975), Sridhar et al (2003), noted that akin to animals subjects, the human subjects who failed autoresuscitation, demonstrated hyperpnea, followed by an initial primary apneic phase, then a period of gasping that gradually decreased in amplitude, eventually ending in terminal apnea. Atypically, 11 of 24 subjects presented with double or triple gasps near the onset of gasping, with an unfortunate 4 of 5 infants who succumbed to sudden infant death (SIDS) showing double or triple gasps (Sridhar et al, 2003). This study confirms the

work of Poets, Meny, Chobanian & Bonofiglo (1999) who also described foreshortened inspiration and prolonged expiration in 7 of 9 infants who died of SIDS.

Thach (2008) emphasizes two fundamental mechanisms essential to the survival of developing infants enduring hypoxemia. The first being the hypoxic gasping of autoresuscitation, the second, being the laryngeal chemoreflex (LCR). Upon exposure to hypoxia, chemoreceptor firing results in hyperpnea, followed by apnea and arreflexia, and then gasping. Hypoxic gasping begins at an arterial oxygen tension of 8 to 10 torr (Lawson & Thach, 1977). Thereafter, reoxygenation via gasping, results in a return to normal heart rate, and gradual return to eupnea. The LCR is a brainstem response to fluid in the larynx resulting in swallowing, apnea, cough, hypertension and arousal (Perkett & Vaughan, 1982). The major stimuli for the LCR is the absence or reduced concentration of chloride ions in the laryngeal fluid (Boggs and Bartlett, 1982). The LCR protective reflex to aspiration is active even during primary apnea, previously thought to be a period of arreflexia (Khurana & Thach, 1996).

Leiter & Bohm (2007) put forth a more generalized hypothesis regarding the underlying mechanism of successful versus failed autoresuscitation. A reduced sensitivity to excitatory respiratory stimuli, coupled with enhanced inhibitory neurotransmission, may lead to an inhibition of regular and sustained respiration and a prolongation of apnea. This model focuses on how multiple factors may serve to create an excitatory-inhibitory imbalance, as opposed to concentrating on any one given risk factor. This hypothesis is further developed within the framework of the ontogeny of cardiorespiratory reflexes; whereby the inhibitory effects, namely bradycardia and apnea, or the laryngeal chemoreflex, dominate in the neonatal period, and give way to excitatory effects with

maturation. Leiter et al (2007) propose that inhibitory reflexes in the early neonatal period are reflective of intrauterine life, whereby the fetus is highly conservative and adaptive to periods of hypoxia, and that failed autoresuscitation or SIDS may be the result of delays in physiologic maturation, or incomplete transitioning from fetal to neonatal cardiorespiratory reflex pathways.

5.5 Factors Influencing Autoresuscitation in Animals

The abovementioned studies by Sridhar et al (2003) and Poets et al (1999) cover the entire body of published literature pertaining to the analysis of failed autoresuscitative cardiorespiratory tracings in humans. While there is a paucity of human literature, there exists a substantial body of published animal research regarding autoresuscitation. Hence, the forthcoming critical appraisal of the literature will review animal research on the subject of autoresuscitation, inclusive of the influences of: 1) core temperature, 2) prior exposure to hypoxia and 3) maturation; the effects of metabolic substrates such as 4) glucose and 5) glycogen; the impact of toxins such as 6) nicotine; the relevance of neurotransmitters and gasotransmitters such as 7) serotonin, 8) glutamate and 9) nitric oxide as well as signal transduction via 10) adenosine receptors and their relationship with 11) caffeine. Finally, the important role of 12) the adrenal gland in the newborn rat, relative to autoresuscitation, will be emphasized.

5.5.1 Temperature

Elevated body temperature curtails (Serdarevich & Fewell, 1999), whereas reduced body temperature prolongs, autoresuscitative efforts (Adolph, 1948). Serdarevich et al (1999) analyzed the ability of 5-6 day old rat pups to autoresuscitate from hypoxia

amidst forced changes in core temperature brought about by randomly selected changes in ambient temperatures of 33, 35, 37, 39, and 41°C. Core temperature significantly impaired the ability of the rat pups to autoresuscitate from primary apnea during repeated exposure to hypoxia in an inversely proportionate manner; with increasing core temperature, the number of successful autoresuscitations decreased.

Kahraman & Thach (2004) examined the effects of hyperthermia on the autoresuscitation rate in 21-23 day of age C57BL/6 mice. Successful autoresuscitation following repeated exposures to hypoxia, were adversely effected at body temperatures of 41.5 degrees and greater. In addition, total number of gasps, and time to last gasp, were significantly decreased in hyperthermic animals following exposure to continuous anoxia. Unlike the findings of Serdarevich et al (1999), mice exposed to only one episode of hypoxia demonstrated a significant risk to fail autoresuscitation above body temperatures of 41.5 degrees Celsius (Kahraman et al, 2004). Finally, dehydration and moderate heat stress did not significantly affect autoresuscitation success rate (Kahraman et al, 2004).

5.5.2 Antecedent Hypoxia

Gozal, Gozal, Reeves & Lipton (2002) demonstrated that intermittent and prior exposure to hypoxia, negatively impacts the ability of the neonatal rat to generate gasps and autoresuscitate. The three experimental groups within this study consisted of: 1) prenatal exposure to alternating hypoxia and normoxia every 90 seconds from day 5 of gestation forward, 2) postnatal exposure of litters to the same regimen, and 3) control litters. At 5 days of age each group was exposed to anoxia, and time to last gasp and autoresuscitation experiments were conducted. Mean total gasp duration, total number of

gasps, and the ability to autoresuscitate from phase three of gasping, was significantly reduced in both the antenatal and postnatal antecedent hypoxia exposed groups, as compared to controls.

Fewell, Ng & Zhang (2005) hypothesized that prior exposure to 1, 2, 3, 4, 9 and 14 autoresuscitation cycles would effect the onset, duration and number of autoresuscitative gasps in 5-6 day old rat pups. Results demonstrated prior exposure to autoresuscitative intervals did not significantly alter the time to first gasp (p, 0.344), but did decrease the time to last gasp ($p<0.001$) after two cycles, and the total number of gasps ($p<0.001$) after three cycles, following exposure to unrelenting hypoxia.

5.5.3 Maturation

Time to last gasp, and the number of successful autoresuscitations, decreases proportionately, with advancing postnatal age. Gershan, Jacobi, & Thach (1992) compared the ability of BALB/c and SWR mice on day of life 5, 10, 17-22 and greater than 40, to autoresuscitate. Number of gasps declined significantly, as did successful autoresuscitations, with advancing postnatal age. Overall, BALB/c mice autoresuscitated far more robustly, and in significantly greater numbers (63/63), following the first hypoxic exposure, as compared to SWR mice (14/24). Finally, failure in cardiac resuscitation, or the second stage of autoresuscitation, accounted for autoresuscitation failure in 86% of BALB/c and 100% of SWR mice. From this study, we can conclude that gasping and the ability to autoresuscitate decreases with advancing postnatal age, and with repeated exposure to hypoxia the mode of death is likely to be cardiac arrhythmia.

Further evidence relative to the effects of postnatal maturation on autoresuscitation, is offered by Gershan, Jacobi, Thach & Bradley (1990) who examined the process of autoresuscitation following a hypoxic challenge in adult, 18-22 days of age, and 5-10 days of age BALB/c mice, by recording electrocardiogram and respiration tracings during a hypoxic challenge of 97% nitrogen and 3% carbon dioxide. Time to tachycardia and tachypnea was longer in the adult mice, but gasp frequency was lower in the 5-10 day old mice. Moreover, bradycardia at the onset of gasping was more pronounced in the pups. A 30 or 60 second delay in administration of oxygen after the onset of primary apnea dramatically impaired the ability of adult mice to autoresuscitate, without a difference in the ability of the pups to autoresuscitate. Gasp frequency was significantly different in room air versus 97% nitrogen/3% carbon dioxide in the pups, but not in the weanling or adult mice. The authors concluded that infant mice possessed a greater tolerance of hypoxia, with a potentially energy-conservative autoresuscitative pattern of lower heart rate and gasp frequency.

5.5.4. Glucose

Yuan, Blennow, Runold & Langercrantz (1997) sought to test the effects of glucose administration on energy utilization and gasping in day of life 1 and 8 rat pups exposed to anoxia. Glucose administration was associated with significantly prolonged gasping in 8 days of age rat pups, with no difference in gasping duration in 1 day of age pups, exposed to anoxia. There was no correlation between gasping and lactate accumulation in either age group. These aforesaid results are weighed against the findings of Moore, Lione, Regen, Tarpley & Raines (1971), who demonstrated glucose utilization in newborn rat brains to be approximately one-tenth that of an adult rat. Hence, under

conditions of hypoxia, rat pups on day of 1, may more effectively minimize the mismatch between energy demand and utilization as compared to day of life 8 pups.

5.5.5 Glycogen

Deshpande, Khurana, Hansen, Wilkins & Thach (1999) analyzed cardiac and hepatic glycogen stores, and rates of depletion of these stores, in mice exposed to anoxia, and related the same to autoresuscitation failure of a cardiac origin. Cardiac glycogen content was reported to be significantly lower in SWR as compared to BALB/c mice after one exposure to hypoxia. However, thereafter glycogen stores stabilized and were not depleted. As glycogen utilization is a reflection of cardiac work, lower cardiac glycogen stores, may explain the significantly higher failure rate in SWR mice to autoresuscitate. Finally, cardiac glycogen levels reached their nadir by 21 days of life, which correlates with a period of vulnerability to hypoxia from 17-23 days of life previously reported (Jacobi, Gershan & Thach, 1991). The authors speculate that these findings may bear clinical relevance to the age related peak incidence in sudden infant death syndrome (Deshpande et al, 1999).

5.5.6 Nicotine

Adrenomedullary catecholamine release is integral to successful autoresuscitation from hypoxia in the first week of life (Seidler & Slotkin, 1985). Fetal exposure to nicotine impairs adrenomedullary catecholamine release and adrenal cell function (Slotkin, Lappi, McCook, Lorber & Seidler, 1995; Wong, Wickstrom & Holgert, 2003). When exposed to hypoxia newborn rats secrete as much as 40% of the entire catecholamine content of the adrenal medulla, whereas nicotine-exposed rats secrete almost no catecholamines (Slotkin et al, 1995). Relatively, Buttigieg, Brown, Zhang, Lowe, Holloway & Nurse (2007)

reported a significant suppression in the acute hypoxia sensitivity in adrenal chromaffin cells after intrauterine nicotine exposure and activation of nicotinic acetylcholine receptors.

Fewell, Smith & Ng (2001) studied the effects of prenatal exposure to nicotine on the ability of rat pups on days of 1-2, 5-6 and 10-11 of life, to autoresuscitate from primary apnea during repeated exposures to hypoxia. Prenatal exposure to nicotine decreased the time to last gasp in the 1-2 day old pups, yet significantly increased the total number of gasps in this age group, and significantly decreased the total number of autoresuscitations in the1-2 and 5-6 day old pups. Hence, fetal nicotine exposure may increase the neonate's vulnerability to hypoxia via impaired hypoxic sensitivity in adrenal chromaffin cells, and insufficient adrenomedullary catecholamine release.

5.5.7 Serotonin

Controversial, and sometimes contradictory evidence exists in the literature, regarding in vivo versus in vitro studies, focused on the pacemaker activity underlying eupnea versus gasping. Whereas consensus has been reached regarding the the pre-Botzinger complex as the respiratory rhythm generator for eupnea (Smith, Ellenberger, Ballanyi, Richter & Feldman, 1991); and the lateral tegmental field, as the cite for neurogenesis of gasping (St. John, 1990; Fung et al, 1995; Fung et al, 1997); evidence supporting the roles of a variety of neurotransmitters in both processes is less certain. One such neurotransmitter is serotonin, or 5-hydroxytryptamine (5-HT), with low levels implicated in the occurrence of SIDS (Kinney, Richerson, Dymecki, Darnall & Nattie, 2009).

Serotonin, acting on 5-HT2 receptors, may modulate conductance through persistent sodium channels in medullary pacemaker neurons, resulting in the initiation

and generation of gasping (St. John & Leiter, 2008). However, evidence for 5-HT2A receptor activation as a requirement for gasping rhythmogenesis is discrepant. While in vitro preparations support the notion that 5-HT2A receptor activation is essential for the generation of gasping rhythm following exposure to hypoxia (Tryba, Pena & Ramirez, 2006); in vivo studies have countered these findings (St.John, Li & Leiter, 2009); showing phrenic discharge during eupnea and gasping, was no different in wild-type mice versus knock-out mice with a genetic reduction of 85-90% in serotonergic neurons.

5.5.8 Glutamate

Gasping is modulated by the glutamate-nitric-oxide pathway, such that gasping is dependent upon activation of central glutamate NMDA receptors (Gozal & Torres, 1997). Glutamate, an excitatory neurotransmitter, is released from the pre-synaptic nerve terminal following neuronal depolarization, then binds to, and activates post-synaptic glutamate receptors such as *N*-methyl-*D*-aspartate (NMDA) receptors (Johnston, 2005). Hypoxia impairs the function of astroglial glutamate transporters in the synaptic cleft creating an accumulation of glutamate, which is associated with perinatal brain injury and inhibition of the hypoxic ventilatory response (Silverstein, Buchanan & Johnston, 1986).

Activation of NMDA receptors, in the solitary tract nucleus, where afferent neuronal projections of the carotid bodies terminate, modulates the ventilatory response and onset of gasping, following exposure to hypoxia in adult rats (Mizusawa, Ogawa, Kikuchi, Hida, Kurosawa, Okabe, Takishima & Shirato, 1994). Gozal & Torres (1997), corroborated the findings of Mizusawa et al (1994), in adult rats, by studying the effects of NMDA channel antagonist, MK801, versus vehicle in randomly assigned rat pups of 2, 5, 10 and 15 days of age. MK801 significantly prolonged the onset of the first gasp

following primary apnea across all age groups, and eliminated type I gasping with both inspiratory and expiratory components in 2 and 5 day old pups. Thus, NMDA receptor blockade alters the types and phases of gasping in neonatal rats when exposed to hypoxia.

5.5.9 Nitric Oxide

Nitric oxide, a signaling molecule and neuronal excitatory gasotransmitter, is biosynthesized from L-arginine in the presence of Nicotinamide Adenine Dinucleotide Phosphate-Oxidase (NADPH) and dioxygen, via neuronal nitric oxide synthase (Paternotte, Gacher, Labrude, Stoltz & Menu, 2008). Hypoxia causes glutamate accumulation, activation of NMDA receptors, intracellular calcium influx and activation of neuronal nitric oxide synthase (Espey, Pluta & Colton, 1997). Nitric oxide expression in the lateral tegmental field modulates the timing and phasing of hypoxic gasping (Gozal, Torres, Gozal & Nuckton, 1998). Just as neonatal rats demonstrate triphasic gasping when exposed to hypoxia, Gozal & Torres (2001) reported an associated triphasic pattern in brainstem nitric oxide concentrations, paralleling the triphasic pattern of gasping in 5 days old rat pups. Moreover, brainstem nitric oxide production was associated with earlier gasping, more frequent gasping, but shorter duration of gasping (Gozal et al, 2001).

Gozal, Torres, Gozal, Nuckton, Dixon, Gozal & Hornby (1998) exposed 2 to 15 day old rat pups to hypoxia, following administration of N-nitro-*L*-arginine methyl ester (*L*-NAME), a nitric oxide synthase (NOS) blocker, *L*-arginine (*L*-Arg), a NO precursor, or vehicle. Gasping duration was curtailed after *L*-arginine administration, and prolonged after *L*-NAME administration. NOS inhibition prolonged gasping duration, yet decreased

gasping frequency, with increasing postnatal age. Furthermore, western blots from the lateral tegmental field, involved in gasp generation, revealed proportionately increased neuronal NOS concentration with postnatal maturation. Hence, nitric oxide concentrations in the lateral tegmental field, may be associated with initial autoresuscitation success, with earlier onset of gasping, but may be associated with detrimental outcomes in the face of prolonged hypoxia.

5.5.10 Prostaglandins

Prostaglandins, derived from arachidonic, eicosapentaenoic and gamma-linolenic fatty acids, via oxidation by cyclooxygenases COX-1 and COX -2, mediate the inflammatory cascade (Englmon, Ek, Saha, Ericsson-Dahlstrand, Jakobsson & Blomqvist, 2002). Further, prostaglandins modulate the central ventilatory effects of Interleukin – 1 Beta (IL-1β) (Hofstetter, Saha, Siljehav, Jakobsson & Herlenius, 2007). IL-1β is a cytokine, produced by macrophages, monocytes and dendritic cells in the inflammatory cascade. Furthermore, IL-1β increases the expression of adhesion factors on endothelial cells, facilitating the transmigration of leukocytes (Church, Cook & McDermott, 2008). IL-1β binding to IL-1 receptors on endothelial cells at the level of the blood brain barrier induces cyclooxygenase – 2 (COX-2) and prostaglandin E synthase –1 activity in the synthesis of prostaglandin E2 (PGE2) (Englmon, et al, 2002).

Whilst studying the mechanism underlying the central respiratory effects of IL-1β, Olsson, Kayhan, Langercrantz & Herlenius (2003) examined the response of 7 day old rat pups to anoxia, and reported that IL-1β does not directly inhibit autoresuscitation, rather, its effects are mediated via the prostaglandin pathway. Moreover, Indomethacin, a

pharmaceutical COX inhibitor, moderates the respiratory depression associated with IL-1β (Olsson et al, 2003). Earlier reports by Kitterman, Liggins, Fewell & Tooley (1983) had established the relationship between prostaglandins and depression of fetal breathing movements in sheep. Then Guntheroth (1989) proposed the connection between respiratory infection, interleukin and SIDS. Hofstetter & Herlenius (2005), demonstrated that intraperitoneal injection of IL-1β, significantly depressed the duration of phases II and III of gasping, and significantly impaired autoresuscitation, in response to anoxia in 9 day old mice. More recent evidence, by Hofstetter, Saha, Siljehav, Jakobsson & Herlenius, (2007), in 9 day old mice, solidifies the link between autoresuscitation failure, IL-1β activation of PGE2, and resultant in PGE2 binding to EP3 prostaglandin receptors in the brainstem.

5.5.11 Adenosine

Although sympathetic innervation in the rat pup is not functional until the end of the first week of life, with catecholamine release originating from the adrenal medulla; parasympathetic innervation is functional at birth in rats (Robinson, 1996). Moreover, adenosine, a neuromodulator ubiquitously present in all cells, is not dependent on the presence of mature synaptic structures for release or function, and adenosine A1 receptors are among the earliest receptors expressed in the brain and heart of the fetus and neonate. Both parasympathetic overdrive and adenosine accumulation are associated with bradycardia in the neonate. Humoral adenosine increases under conditions of hypoxia or stress, and serves to decrease myocardial workload, by reducing afterload and heart rate. Under extreme conditions of ischemia, the cardiovascular effects of adenosine, via A1 receptors, are atrioventricular node block and hypotension due to arterial endothelial

smooth muscle relaxation (Rivkees, Zhao, Porter & Turner, 2001). Accordingly, Fewell, Zhang & Gillis (2007) investigated the effects of vagotomy and adenosine A1 receptor blockade on gasping, and the bradycardia associated with primary apnea, in 1-2, 5-6 and 10-11 day old rat pups when exposed to hypoxia. Vagotomy did not influence heart rate, time to last gasp, nor total number of gasps in rat pups exposed to hypoxia under 10 days of age (Fewell et al, 2007). However, upon exposure to hypoxia, administration of a selective adenosine A1-receptor antagonist significantly attenuated the heart rate response, decreased the time to last gasp, and significantly increased the total number of gasps, in all three age groups.

5.5.12 Caffeine

Caffeine, a xanthine alkaloid, readily crosses the blood brain barrier where it is has been described as an antagonist to both A1 and A2A adenosine receptors. However, caffeine binds to adenosine receptors without activating them, therefore it is more correctly termed a competitive inhibitor of adenosine (Somani & Gupta, 1988). Although caffeine readily crosses the placenta (Browne, 2006), and caffeine administration is a standard drug of choice in the treatment of apnea of prematurity (Henderson-Smart & Steer, 2001); there exists a paucity of studies that have investigated the in vivo effects of antenatal caffeine exposure on neonatal hypoxia tolerance.

Picard, Guenin, Larnicoi & Perrin (2008) provided rat dams with caffeine in their drinking water throughout gestation, to approximate human caffeine consumption in pregnancy, then tested the neonate's response to hypoxia. Under conditions of normoxia, neither respiratory rate nor tidal volume differed between caffeine exposed versus control animals. In contrast, when 0-2days of age pups were exposed to 11% oxygen, caffeine

exposure in utero, attenuated the hyperventilation phase and blunted the respiratory depression phase, as compared to controls. In addition, A2A adenosine receptor and α2 GABA receptor mRNA expression were increased, in the medulla of caffeine exposed pups. Hence, caffeine may affect receptor expression in the fetal and neonatal medulla, and may alter the neonate's response to hypoxia.

5.5.13 Adrenal Gland

Yuan, Runold & Langercrantz (1997) demonstrated that the duration of gasping in 1 and 8 day old rat pups, was adversely effected by adrenalectomy, as compared to sham operated pups. In addition, the ability of 8 days old pups to autoresuscitate following secondary apnea, was also significantly impaired in the adrenalectomized group. Of note, to our knowledge, this laboratory is the only researcher group to publish successful autoresuscitation following secondary or terminal apnea.

Seidler & Slotkin (1985) established the adrenomedullary origin of catecholamine release, in response to hypoxia, in 1 day old rat pups, compared to the neurogenic source of catecholamine release per splanchnic innervation, in day of life 8 rat pups. Antagonism of the adrenal release of catecholamines, as opposed to sympathetic neural release of catecholamines, significantly decreased survival after exposure to hypoxia in 1 day old rats. To ascertain whether the increased mortality rate in the group who had undergone adrenalectomy was related to loss of adrenomedullary function, as opposed to adrenocorticol function, the rat pups were administered desoxycorticosterone replacement therapy prior to hypoxic challenge. Steroid replacement did not significantly change survival rates following exposure to hypoxia in either group. However, maternal stress induced via serial injections of saline over the course of the last 5 days of pregnancy, did

serve to accelerate sympathetic innervation, and resulted in a functional catecholamine response of neurogenic nature as evidenced via insulin challenge ($p<0.001$).

Although the significance of the adrenal medulla, as a source of catecholamine release in the first week of life, relative to successful autoresuscitation, has been established; Seidler & Slotkin's (1985) aforementioned work, shifted attention to the importance of adrenocorticol, as compared to adrenomedullary, function in the newborn. Thus the forthcoming critical appraisal of the literature segues into the induction and attenuation of the stress response in pregnancy, inherent physiologic mechanisms to protect the fetus from the maternal stress response, and the effects of endogenous and exogenous glucocorticoids on the fetus and newborn.

B. The Maternal Response to Stress

1.0 The HPA Axis

The Hypothalamic-Pituitary-Adrenal (HPA) axis is integral to the physiological stress response. When the HPA axis is excited via catecholamines, the signal is mediated by hypothalamic paraventricular alpha-adrenoreceptors. Corticotropin Releasing Hormone (CRH), a 41 amino acid polypeptide, is synthesized within the parvocellular neurosecretory cells of the paraventricular nucleus (PVN) of the hypothalamus, with neurons extending to the median eminence, where CRH can be stored in neurosecretory terminals, before its release into the hypophyseal portal system and anterior pituitary (Lightman, 2008; Venkataraman, Munoz, Candido & Witchel, 2007).

Corticotropin Releasing Hormone (CRH) and Corticotropin Releasing Factor (CRF) are used interchangeably in the literature. As the term "...corticotropin-releasing

factor more appropriately designates the function of this peptide. It is recommended that corticotropin releasing factor (CRF) rather than corticotropin-releasing hormone (CRH) should be used in keeping with the nomenclature for other important factors ... that exhibit a multiplicity of biological actions" (Hauger, Grigoriadis, Dallman, Plotsky, Vale & Dautzenberg, 2003); the abbreviation CRF will be referenced heretofore within this document.

The paraventricular nucleus also synthesizes and stores Vasopressin (AVP) within magnocellular neurosecretory cells, with neurons extending to the posterior pituitary, but AVP also augments the regulation of the synthesis of ACTH from the precursor polypeptide POMC in corticotrope cells, and subsequent release, from the anterior pituitary, in synergy with CRF. However, without CRF, AVP exerts very little secretagogue activity (Lightman, Windle, Ma, Harbuz, Shanks, Julian, Wood, Kershaw & Ingram, 2002).

There are two CRF receptors in humans, types I and II each encoded by a separate gene (Hauger et al, 2003). The CRF receptor is expressed in the central nervous system with major expression sites in cortex, cerebellum, hippocampus, amygdala and pituitary. In the anterior pituitary, CRF binds to CRF Receptor-1, a Guanine Nucleotide binding protein (G-protein-coupled receptor), on the cell membrane of the corticotropes, activating $G_{\alpha s}$, and stimulating adenylyl cyclase (AC), which increases intracellular cyclic adenosine monophosphate levels (cAMP), activating protein kinase A (PKA), stimulating L-type calcium channels, increasing intracellular calcium, resulting in exocytosis of pre-formed Adrenocorticotropic hormone (ACTH), and gene transcription of ACTH (Aguilera, Rabadan-Diehl & Nikodemova, 2001).

ACTH, a 39 amino acid peptide, is synthesized in the anterior pituitary corticotrophs, derived from the pre-hormone, pro-opiomelanocortin (POMC) (Venkataraman et al, 2007). ACTH binds to melanocortin-2 receptors, coupled to a heterotrimeric G protein on the membrane of the fasciculata and reticularis cell layers of the adrenal cortex, thereby stimulating AC, which in turn increases intracellular cAMP, activating PKA, causing down stream phosphorylation leading to the first step in the synthesis of cortisol, the conversion of cholesterol to pregnenolone (Simpson & Watterman, 1988).

There are five reactions required in the synthesis of cortisol from cholesterol: 1) side chain cleavage of the long side chain from the carbon at position 20 producing pregnenolone, 2) the hydroxyl group at position 3 is then oxidized to generate progesterone, 3) a hydroxl group is then added to position 17 to form 17αhydroxyprogesterone, 4) a hydroxyl group is added at carbon 21 to make deoxycortisol, 5) finally a hydroxyl is added at position 11 to form cortisol. Four of these five aforementioned reactions are catalyzed by P450 cytochrome oxidases (Guayee & Auchus, 2007).

Only 3% of cortisol circulates unbound, with a half-life of approximately 90 minutes, and the remaining 80-90% is bound to corticosteroid-binding globulin and 7% is bound to albumin (Venkataraman et al, 2007). All nucleated cells have cytoplasmic glucocorticoid receptors. Cortisol binds to the E domain located towards the C terminus of the glucocorticoid receptor (GRE). The cortisol-receptor complex then translocates from the cell membrane to the nucleus for transcription (Heitzer, Wolf, Sanchez, Witchel & DeFranco, 2007).

Cortisol is required for normal sensitivity of adrenergic receptors resulting in positive inotropic effect and increased blood pressure; behavioural changes; metabolic consequences including glycogenolysis, lipolysis, gluconeogenesis, and increased free amino acids; increased gastric acid secretion; renal effects such as increased diuresis, inhibition of sodium loss, but increased potassium loss and relative alkalosis; as well as immuno-suppressive effects via inhibition of T-cell proliferation and phagocytosis, decreased IgA and interleukin -1, as well as leukocyte redistribution (Weber, 1998).

Cortisol provides negative feedback to both the anterior pituitary and the hypothalamus, resulting in the inhibition of the synthesis and secretion of both CRH and AVP, as well as inhibiting the cleavage of POMC into ACTH. Cortisol is the primary regulator of the transcription of POMC, and chief inhibitor of the release of pre-synthesized ACTH and CRH (Miller & O'Callaghan, 2002). Furthermore, adrenergic neurons receive stimulatory innervation from the serotoninergic and cholinergic systems, and inhibitory input from gamma-aminobutyric acid, opioids and glucocorticoids (Calogero, Bagdy, Szemeredi, Tartaglia, Gold & Chrousos, 1990).

The Locus Ceruleus (LC/NE) is a nucleus made of medium sized neurons within the rostral pons of the brainstem that also serves to coordinate the physiological response to stress via the synthesis and release of norepinephrine. The prefrontal cortex, hypothalamus, cingulate gyrus, the nucleus paragigantocellularis, the raphe nuclei and amygdala innervate the LC/NE; therefore both psychological and physical stressors will induce a noradrenergic response. More specifically, there is emerging evidence that the LC/NE creates crosstalk between the HPA axis and the SNS primarily via CRF1 and α-1-adrenergic receptors. The activation of the LC/NE by a stressor results in the release of

norepinephrine, enhancing attention and cognition via the prefrontal cortex, boosting motivation and fear via the nucleus accumbens, and stimulating the secretion of CRF from the PVN (Sved, Cano, Passerin & Rabin, 2002). ACTH is then released from the anterior pituitary via the hypothalamic-hypophysial portal system, and promotes the synthesis and secretion of cortisol from the adrenal cortex.

2.0 Attenuation of the HPA Axis

Responsiveness of the HPA axis is altered by pregnancy (Lightman, Windle, Wood, Kershaw, Shanks & Ingram, 2001). By the latter stage of pregnancy the HPA axis becomes less responsive to stressful stimuli, and has thereby been described as attenuated, relative to the hypo-responsiveness to both physical and psychological stressors during pregnancy, across species. Proposed mechanisms underlying the attenuation of the HPA in pregnancy are: a decrease in the expression of CRF mRNA (Johnstone, Wigger, Douglas, Neumann, Langraf, Seckl & Russell, 2000), a decrease in the sensitivity and density of CRF receptors in the corticotroph (Neumann, Johnstone, Hatzinger, Liebsch, Shipston, Russell, Landgraf, & Douglas, 1998) and the reversal of the effects of endogenous opioids, whereby opioids actually inhibit rather than stimulate the HPA axis (Wigger, Lorscher, Oehler, Keck, Narou & Neumann, 1999), and the dampening of excitatory input to the HPA axis secondary to a reduction in the expression of noradrenergic α1A-adrenoceptors in the hypothalamic PVN, during pregnancy in rats (Douglas, Meddle, Tosch, Bosch & Neumann, 2005).

It is postulated that the attenuation of the HPA axis in pregnancy may be protective against programming effects on the developing fetus. Furthermore down-

regulation of the stress response continues during lactation, however it is highly dependent upon the continued suckling stimulus, thus underlining a role for oxytocin. It is further hypothesized that oxytocin release from the posterior pituitary may crosstalk with the anterior pituitary and modulate adaptive maternal behavior in the peripartum period (Neumann et al, 1998; Lightman et al, 2001).

2.1 Attenuation of the HPA Axis in Human Pregnancy

Although there is a plethora of literature that examines the attenuation of the HPA axis in pregnancy in animals, hypo-responsiveness of the HPA axis in humans is less well described. The majority of studies examining the hypo-responsiveness of the HPA axis, in relation to psychological stress in pregnancy, are limited in humans by: participant recall, the inherent subjectivity of the experience of stress, a lack of standardization of inventory and survey tools, as well as type, duration, and timing of the stressor.

The paucity of literature that address the experimental induction of either physical or psychological stressors in human pregnancy is likely reflective of the ethical optics of the same. However, neuroendocrine testing of the HPA axis does offer one clinical model in humans worthy of exploration. Notwithstanding the small sample size of seven, none of the late gestation women in a study by Shulte, Weisner & Allolio (1990) demonstrated a significant rise in cortical levels following ACTH stimulation testing. These results were attributed to increased CRF binding protein in pregnancy, desensitization of the corticotrophs to CRF, and negative feedback to CRF and ACTH secondary to elevated maternal cortisol levels during pregnancy (Shulte, et al, 1990).

The phenomenon of prenatal stress, the mechanisms of inherent in the attenuation of stress in pregnancy, and the sequelae of stress in pregnancy for both mother and offspring, have not yet been fully elucidated. Whereas there is a dearth of prospective experimental models of stress in pregnancy in humans, in contrast, the literature is replete with both physical and psychological stress models in animals that demonstrate the highly plastic nature of the HPA axis, most especially during late pregnancy.

2.2 Attenuation of the HPA Axis in Rat Pregnancy

Douglas, Meddle, Tosch, Bosch & Neumann (2005) reported significant attenuation of noradrenergic input to the PVN in late gestation pregnant rats, in response to a physical stressor, namely forced swimming, as compared to virgin controls. Forced swimming increased noradrenaline levels in virgin rats 3.6 fold above baseline levels (mean pre swim 760 + 258 pg/ml; mean post swim 1992 + 929 pg/ml, $p<0.01$), with minimal to no change in noradrenaline levels in pregnant rats (p, 0.899).

Douglas, Johnstone, Hatzinger, Neumann, Landgraf and Russell, (1996) have studied glucocorticoid release relative to both physical and psychological stressors in pregnant dams. This group reported corticosterone levels in pregnant dams approaching term gestation, exposed to an elevated plus maze and forced swimming, to be significantly lower (263 ± 21 ng ml and 409 ± 30ng ml respectively), as compared to non-pregnant rats (371 ± 39 ng ml and 692 ± 58 ng ml) ($p<0.01$) (Douglas et al, 1996).

Similarly, Neumann, Johnstone, Hatzinger, Liebsch, Shipston, Russel, Landgraf & Douglas, (1998) measured: 1) the responses of the HPA axis during pregnancy in rats, to the elevated plus maze followed by forced swimming, 2) the response to exogenous CRF

administration in both pregnant and non-pregnant rats, and 3) cAMP production in pregnant and non-pregnant rats in response to CRF administration. An increasingly blunted neuroendocrine response to elevated plus maze was noted with advancing gestation, whereby ACTH levels increased 6 fold in non-pregnant rats, but only 3.1 and 1.9 fold in gestational day 18 and 21 pregnant rats respectively, ($p<0.0001$). Forced swimming induced a 15 fold increase from baseline in corticosterone levels in non-pregnant rats, compared to 9.5 and 8.3 fold increase in gestational day 18 and 21 pregnant rats respectively, ($p<0.0001$). Secretion of corticosterone in response to both elevated plus maze and forced swimming was significantly lower on gestational days 18 and 21 of pregnancy, as compared to non-pregnant rats ($p<0.01$). Intravenous administration of exogenous CRF resulted in significantly lower ACTH levels in gestational day 22 pregnant rats, with a 1.5 fold increase from baseline, as compared to virgin rats with a 4.4 fold increase from baseline ($p< 0.05$). Moreover, cAMP accumulation was significantly lower in gestational day 21 pregnant rats, as compared to non-pregnant rats ($p<0.05$). Albeit these findings support other aforementioned studies in pregnant rats, Neumann et al (1998) put forth a novel finding, as they have identified that the attenuation of the HPA axis in pregnant rats begins on gestational day 15.

A final example comes from exposure of a laboratory animal to a novel environment, specifically the simulated open field, as an established model to induce a psychological stress response in rats. Smith, Fewell and Abu-Amarah (1997) carried out experiments on Sprague-Dawley rats on days 10, 15 and 20 of gestation. Rats were handled and familiarized with the guillotine for three sequential days prior to the day of the experiment. On the fourth consecutive day, rats were individually carried in their cage

to the experimental room. Rats were randomized to one of four groups: 1) control for 30 minutes (remaining in home cage), 2) control for 120 minutes (remaining in home cage), 3) open field for 30 minutes, and 4) open field for 120 minutes. Thereafter rats were decapitated and truncal blood was collected. Exposure to an open field significantly increased corticosterone levels in both pregnant and non-pregnant rats ($p<0.001$). Levels returned to baseline in pregnant rats on days 10 and 15 of gestation and in non-pregnant rats. However, corticosterone levels were sustained at elevated levels in pregnant dams of 20 days gestation.

C. Effects of Glucocorticoids on the Fetus

1.0 11 ß-Hydroxysteroid Dehydrogenase

Excess prenatal exposure to glucocorticoids has been associated with adverse outcomes such as decreased birthweight and a phenomenon known as fetal programming (Seckl, 2004). Fetal programming has been theorized to increase susceptibility to chronic adult diseases such as hypertension, diabetes and mental illness later in life (Barker, 2004).

Target cell expression of 11 ß-hydroxysteroid dehydrogenase influences the specific action of glucocorticoids (Venkataram et al, 2007). This modulation of glucocorticoid activity plays a pivotal role in the protection of the fetus from the potential harmful effects of glucocorticoids. During fetal development, glucocorticoids are held to a finite balance between normal fetal cellular genesis, proliferation and differentiation; whilst protecting the fetus from premature cellular differentiation, disruption and apoptosis. Placental 11-β hydroxysteroid dehydrogenase type 2

(11βHSD2) is the enzyme that safeguards the fetus from excess maternal glucocorticoid levels by catalyzing the unidirectional conversion of active cortisol to 11-keto metabolites, cortisone and 11-dehydrocorticosterone (Benediktsson, Calder, Edwards & Seckl, 1997). The placenta in both the human and rat highly express 11β-HSD2 until near term gestation, maintaining a controlled gradient of cortisol from maternal to fetal circulation (Waddell, Benediktsson & Seckl, 1996).

There are two known 11- ß-HSD isoforms: 1) a low-affinity NADP-dependent dehydrogenase/oxoreductase 11- ß-HSD1, and 2) a high-affinity NAD-dependent dehydrogenase 11- ß-HSD2. The Type I enzyme acts as an oxoreductase in the conversion of inactive cortisone to active cortisol, and is expressed mainly in the liver, but also in adipose tissue, skeletal and smooth muscle, gonads, lung and the central nervous system. The type II enzyme inactivates active cortisol to cortisone and is highly expressed in the human kidney and placenta as well as the gonads, liver, and pancreas (Albiston, Obeyesekere, Smith, & Krowowski, 1994). Low levels of placental 11- ß-HSD2 results in increased exposure of the fetus to glucocorticoids and may be associated with decreased birth weight (Edwards, Benediktsson, Lindsay & Seckl, 1996).

There are distinct differences across species in the expression of placental 11- ß-HSD2. Prenatal glucocorticoid administration in sheep reduces placental 11- ß-HSD2 expression and activity (Kerzner, Stonestreet, Wu, Sadowska & Malee, 2002), whilst expression of both Types 1 and 2 11- ß-HSD enzymes are present in rat placenta and decidua (Burton, Smith, Krozowski & Waddell, 1996). In humans, van Beek, Guan, Julan & Yang (2004) were the first researchers to demonstrate that glucocorticoids stimulate

placental 11 ß-HSD2 expression in cultured trophoblasts through increased 11 ß-HSD2 gene transcription and mRNA stability.

Stewart, Murry & Mason (1994) showed that in human fetal tissues, from mid gestation abortions between 16-19 weeks, Type 2 11 ß-HSD was readily present in fetal tissues with a high degree of mineralocorticoid and glucocorticoid activity, similar to the kidney, lung, gonad and liver. In contrast, no Type 1 11 ß-HSD activity was detected. From this study we may infer that distribution of Type 2, high affinity, 11 ß-HSD in mid gestation fetal tissues, may be highly protective for the developing fetus from the potentially harmful effects of glucocorticoids.

Conversely, Type 1 isoform activity may be more pronounced in late gestation or in the neonatal period, thereby regulating maturational changes required for extrauterine transition, such as surfactant production. Murphy & Clifton (2003) demonstrated placental 11 ß-HSD2 enzymatic activity decreased significantly between 38 and 40 weeks gestation, whilst placental 11- ß-HSD1 mRNA expression increased significantly with the onset of labor. However, conversion to Type 1 11 ß-HSD in late gestation, from the expression of Type 2 11- ß-HSD in early to mid gestation, is not well described.

Placental 11ß-HSD2 is an efficient, but probably incomplete barrier to maternal glucocorticoids. ACTH stimulation is associated with inhibition of placental 11ß-HSD2 and impaired conversion of cortisol to cortisone (Walker, Campbell, Fraser, Stewart & Edwards, 1992). Moreover, dexamethasone is a poor substrate for the enzyme 11ß-HSD, as compared to endogenous glucocorticoids. Van Beek, Guan, Julan & Yang, (2004) elegantly demonstrated that treatment of human trophoblasts with dexamethasone

increased 11β-HSD2 activity in a time-dependent manner, with an approximately 1.5-fold increase after 12 hours, and a 2-fold increase by 24 hours. In comparison, endogenous cortisol increased 11β-HSD2 activity in a concentration-dependent manner, requiring a 10 fold increase in its concentration in order to elicit a similar response to dexamethasone (van Beek et al, 2004). These results potentially hold implications for the exposure of the fetus to the endogenous release of glucocorticoids by the mother, versus the exogenous administration of synthetic glucocorticoids antenatally to the mother.

2.0 Endogenous Glucocorticoids

Fetal cortisol serves to optimize neonatal survival, with rising concentrations near the end of gestation. At physiological levels, cortisol plays two major roles in preparation for the transition from intrauterine to extrauterine life: 1) the promotion of cell differentiation over proliferation, and 2) the activation of multiple enzymatic pathways that serve little to no functional role in fetal life, but are immediately required for neonatal extrauterine transition and ultimate survival.

Thyroid function is maintained at a basal level in utero, however birth requires an acute change in metabolic demand and thermoregulation. Fetal triiodothyronine (T3) concentrations increase as cortisol increases. Cortisol switches type III iodothyronine monodeiodinase activity to type I activity in preparation for extrauterine metabolic demands (Wu, Klein, Chopra, & Fisher, 1978). Cortisol also regulates surfactant production in type II pneumocytes, as the rate limiting enzyme in the phosphatidlylcholine pathway, cholinephosphate cytidyltransferase, is highly receptive to glucocorticoids, as is mRNA for the synthesis of surfactant proteins (Rooney, 1985). In

addition, cortisol plays a role in the increasing glycogen stores of late gestation, required in the immediate postnatal period, via the stimulation of glycogen synthetase (Schwartz & Rall, 1975). The adrenergic response in the brown fat for thermoregulation, liver for gluconeogenesis, heart for increased peripheral vascular resistance, and lung for fetal lung fluid resorption, are all caused by the catecholamine surge of the immediate neonatal transition period; but it is the action of glucocorticoids that upregulates adrenergic receptors (Barnes, Jacobs, & Roberts, 1984).

3.0 Exogenous Glucocorticoids

Exogenous administration of glucocorticoids, present the fetus with combined positive and negative effects. Exogenous glucocorticoids, administered antenatally, in threatened preterm labor, significantly reduce the incidence of respiratory distress syndrome, intraventricular hemorrhage and neonatal mortality (Baud, Foix-Helias, Kaminski, Audibert, Jarreau, Papiernik, Huon, Lepercq, Dehan, Lacaze-Masmonteil, 1999). Antenatal steroid therapy has become a standard in the management of preterm labor between 24-34 weeks gestation as published by the Society of Obstetricians and Gynaecologists of Canada (Crane, 2003). Antenatal glucocorticoid therapy optimizes neonatal lung volume and compliance; the mechanisms therein are as follows: 1) accelerating differentiation of Type I pneumocytes by flattening epithelial cells and thinning the saccular septae, thereby improving gas exchange; and by 2) accelerating cellular differentiation in Type II pneumocytes, by hastening enzymatic pathways in the production of surfactant, and 3) stimulating not only the synthesis of all active surfactant proteins: A, B, C, and D; but also key enzymes in phospholipid synthesis (Robertson, 1993).

Navarro, Kudlacz, Eylers & Slotkin (1989) evaluated the switchover from cell replication to differentiation involving adrenoreceptors in the lung, and the enzyme ornithine decarboxylase (ODC) responsible for the synthesis of polyamines in growing tissue. If cellular polyamine synthesis is inhibited, cell growth is halted. Rat dams were administered 0.2 mg/kg of dexamethasone on gestational days 17, 18 and 19.

Lung DNA concentration and content as indicators of cell density, and total numbers of cells respectively, were measured in fetuses on the final day of gestation, and in neonatal pups on postnatal days 2, 5, 10, 18, 25, 31, and 39. The results were as follows: 1) antenatal dexamethasone administration significantly decreased cell number and density of cells in the lung, from gestational day 21 through to the end of postnatal week 4, and 2) antenatal dexamethasone administration accelerated the conversion from proliferation to differentiation, as evidenced by the premature coupling of lung ODC to adrenergic receptors. Therefore, antenatal glucocorticoids may induce alterations in trophic signaling and may accelerate cell differentiation over replication.

Fetal dexamethasone exposure, and its effects on sympathetic activity in the cardiac tissue of rats, was studied by Bian, Seidler, Olsen, Raymond & Slotkin (1993). As sympathetic innervation is not functional at birth in rats, the authors postulated that while endogenous glucocorticoids signal normal differentiation of adrenergic neurons, exogenous glucocorticoids might disrupt adrenergic neuronal maturation. Rat dams were administered dexamethasone, at doses of 0.05, 0.2 or 0.8 mg/kg, on gestational days 17, 18 and 19. Norepinephrine levels were measured in pups on postnatal days 2, 4, 7, 10, 14, 18, 23, 28, 35, and 42. Dexamethasone resulted in decreased total body and heart weight, resulting in relative cardiomegaly. Dexamethasone also altered noradrenergic innervation,

as evidenced by a dose dependent effect on deficient norepinephrine levels in neonatal rat hearts from the immediate postnatal period through to 6 weeks of life.

Kauffman, Seidler and Slotkin (1994) noted antenatal glucocorticoids accelerate cell differentiation in type II cells and promote surfactant production and therefore postualated that other cells may undergo comparable changes in differentiation, under the influence of glucocorticoids. Hence, they investigated the capacity of the neonate exposed to glucocorticoids in utero, to maintain cardiac function when exposed to hypoxia. In this experiment, dams were administered 0.05 mg, 0.2 mg or 0.8 mg of dexamethasone, on gestational days 17, 18 and 19. The pups of these litters were compared to controls relative to survival after exposure to 5% oxygen for 120 minutes.

Prenatal administration of dexamethasone resulted in a greater impairment in survival in all dexamethasone exposed pups, as compared to controls. Moreover, there was a dose dependent response, whereby the higher the dose of dexamethasone, the fewer pups survived hypoxia, with a 6% mortality rate in the 0.05 mg/kg group, 17% mortality rate at 0.2 mg/kg ($p<0.002$), and a 26% mortality rate at 0.8 mg/kg ($p<0.0002$); as compared to a 2% mortality rate in controls.

Whereas control animals maintained normal sinus rhythm throughout exposure to hypoxia, with no deaths associated with arrhythmia; death in pups exposed to dexamethasone was preceded by atrioventricular conduction arrhythmias, and occurred 3-4 times more frequently in the group administered the highest dose of dexamethasone. Moreover, under conditions of normoxia, pups exposed to dexamethasone demonstrated significantly lower resting heart rates with prolonged PR intervals. Finally, as

sympathetic innervation is not functional in the first week of life in neonatal rat pups, adrenal catecholamine content was also measured following exposure to hypoxia. Total adrenal catecholamine content was found to be significantly decreased in a dose dependent manner in the dexamethasone exposed group (<0.0001). In summary, Kauffman et al (1994) demonstrated that prenatal exogenous dexamethasone exposure adversely effected neonatal survival and tolerance of hypoxia, with death being primarily attributable to arrhythmia.

D. Aims of the Study

Accordingly, the premise of the study design heretofore, is predicated on the principle that the potential promotion of cell differentiation in the fetus, via intrauterine glucocorticoid exposure, will affect the functional cardiorespiratory physiology of the neonate when exposed to hypoxia. A key difference in the forthcoming research design, as opposed to research published by Kauffman et al (1994), and Seidler et al (1985), is that the design is based on endogenous corticosterone release in the mother, as stimulated via a psychological stressor, rather than exogenous, potentially supraphysiologic dosing of a combined synthetic mineral-glucocorticoid, namely dexamethasone. What is more, this psychological stressor was introduced in late pregnancy, during a period of gestation when the maternal HPA axis was previously reported to be hyporesponsive (Johnstone, Wigger, Douglas, Neumann, Landgraf, Seckl & Russell, 2000). Since cellular differentiation in the fetus has been reportedly accelerated due to intrauterine exposure to exogenously administered glucocorticoids to pregnant dams, and has effected the neonate's response to hypoxia; the specific aim of this study was to investigate the effects

of maternal endogenous glucocorticoid release in response to a psychological stressor, introduced in late pregnancy, on the neonate's response to hypoxia.

E. Research Questions and Null Hypotheses

1.0 What are the differences in corticosterone levels amongst pregnant rats of similar gestation exposed to a psychological stressor versus control?

Null hypothesis: There are no differences in corticosterone levels measured in rat dams exposed to a psychological stressor versus control.

2.0 What are the differences in time to last gasp, number of gasps and frequency of gasps in rat pups at 1, 5, and 10 days of life, after intrauterine exposure to maternal stress versus control when exposed to hypoxia?

Null Hypothesis: There are no differences in time to last gasp, number of gasps or frequency of gasps in rat pups at 1, 5, and 10 days of life, after intrauterine exposure to maternal stress versus control, when exposed to hypoxia.

3.0 What are the differences in successful autoresuscitation rates in rat pups at 1, 5, and 10 days of life after intrauterine exposure to maternal stress versus control when exposed to hypoxia?

Null Hypothesis: There are no differences in the ability of rat pups at 1, 5, and 10 days of life, to autoresuscitate following intrauterine exposure to maternal stress versus control when exposed to hypoxia.

CHAPTER II

EXPERIMENTAL DESIGN AND METHODOLOGY

A. Open Field Experiments

1.0 Subjects

Experiments were carried out on time-mated pregnant Sprague Dawley rat dams, undergoing their first pregnancy, as supplied from Charles River Breeding Laboratories. The dams arrived to the laboratory on day 7 of gestation.

2.0 Animal Care and Use

Dams were housed individually in plexiglas cages lined with wood chip laboratory bedding. Rats were kept in a controlled environmental chamber at a constant humidity of approximately 20 % and temperature of 25 °C, the optimal neutral thermal zone for mid to late pregnancy and lactating dams (Gordon, 1989). The chamber was controlled on a 12:12 h light–dark cycle with lights on from 0700 h to 1900hrs and off from 1900 to 0700 h. All animals had continuous access to food, Labdiet®, and tap water ad libitum.

3.0 Ethics

All experimental procedures were carried out in accordance with the Guide to the Care and Use of Experimental Animals provided by the Canadian Council on Animal Care (Olfert, Cross & McWilliam, 1993), and with the approval of the Animal Care Committee of the University of Calgary (University of Calgary, 2008).

4.0 Conditions of Observations

Experiments on pregnant dams were conducted on gestational days 17, 18 and 19 of gestation, with full term being approximately 22 days. The first priority was to establish elicitation of a psychological stress response in the pregnant dam in late pregnancy. For this purpose, four dams per group, were randomly assigned to 7 groups, for a total of 28 dams. Four dams per group were studied in the simulated open field (OF) on: 1) day 17, 2) another four dams on day 17 and 18, 3) another four dams on day 17 with a latent period to day 18, and finally 4) four more dams on day 17, 18, and 19. Four dams per group were also assigned as matched controls on 5) day 17, 6) day18 and 7) on day 19. As per Table 1, corticosterone levels were collected 120 minutes after exposure to the open field. We studied corticosterone levels in late pregnancy, in sequence and with latent periods built into the design, to ascertain as to if the effect of a psychological stressor would be reproducible, sustained, or habituated over the course of gestational days 17, 18 and 19 of gestation.

Table 1

Experimental Groups for Open Field and Corticosterone Levels

Group Number	Gestational Day 17	Day 18	Day 19
1	Open Field + LEVEL		
2	Open Field	Open Field + LEVEL	
3	Open Field	Open Field	Open Field + LEVEL
4	Open Field	LEVEL	
5	CONTROL + LEVEL		
6		CONTROL + LEVEL	
7			CONTROL + LEVEL

After ascertaining the elicitation of a psychological stress response in the pregnant rat dam during late pregnancy, we then employed the open field as a means to expose the developing pups to an endogenous intrauterine stressor. For this second phase of experiments, 14 dams were studied, with 7 in the experimental group, placed in the simulated open field on day 17, 18 and 19 of gestation, and 7 serving as controls. Dams in this study were delivered to the lab in pairs on gestational day 7. Each week, one dam was randomly assigned to the experimental group, and the other to the control group.

5.0 Protocol

Rat dams in the control group were not handled beyond required care guidelines. Home cages for open field dams were carried one at a time from the environmental chamber to the experimental room. The dam was then placed in the center of the open field for 30 minutes. Noise and activity in the laboratory were minimized to near absent, as to avoid extraneous stressors. Observations of animal activity were documented in 5 minute intervals. After 30 minutes, the animal was returned to its home cage and then to the environmental chamber. The open field was thoroughly cleaned between dams with 70% alcohol to control for scent or tracking of urine or feces. All open field experiments were conducted between 1000-1200hrs and corticosterone levels were obtained between 1200-1400 hours to coincide with the diurnal nadir of pregnant rats as reported by Atkinson & Waddell (1995).

6.0 Apparatus

The psychological stress model used in our experiments was a simulated open field, composed of a 30 x 60 x 24 inch white acrylic finish box illuminated by a dual tube white flourescent light (Fewell & Tang, 1997; Smith, Fewell, & Abu-Amarah, 1997). Use

of the simulated open field, in an experimental model, as a means to induce psychological emotionality or stress, was first reported by Hall (1934). Validation of novel exposure to the simulated open field as a reliable test of psychological stress in rats has been established for decades (Roth & Katz, 1979).

7.0 Measurements and Calculations

7.1 Corticosterone Levels

Corticosterone levels were obtained 120 minutes after exposure to the open field. Smith et al (1997) had established that although corticosterone levels returned to baseline 120 minutes after exposure to the the simulated open field in pregnant rats on day 10 and 15 of gestation, corticosterone levels remained significantly elevated after 120 minutes in pregnant rats on day 21 of gestation. Therefore, we measured corticosterone levels 120 minutes after exposure to the the simulated open field in pregnant rats on days 17, 18 and 19 of gestation, as a sustained effect at this gestation had not been previously reported.

Trunk blood was immediately obtained via guillotine decapitation to expedite blood collection, void of extraneous stressors such as cannulation or vascular access. Blood was collected into heparinized polypropylene tubes and then placed on ice. Samples were then centrifuged for 10-20 minutes and plasma was removed and stored immediately at -80°C.

Samples were later thawed and measured at room temperature. Serum corticosterone levels were measured using a Coat-A-Count Rat Corticosterone Solid Phase Radioimmunoassay (RIA) Kit from Siemens Medical Solutions and Diagnostics (Los Angeles, CA). Sensitivity of the corticosterone RIA was 5.7ng/ml and the intra-

assay and inter-assay coefficients of variation averaged 8% and 10% respectively (University of Iowa Laboratories).

7.2 Statistical Analysis

A two-way analysis of variance (ANOVA) was conducted to evaluate the effect of a psychological stressor in late pregnancy, namely exposure to the open field, and the day of gestation on maternal corticosterone levels. The between subject factors, with two levels, were exposure to the open field and control, and time at three levels, gestational days 17, 18 and 19. The alpha level was 0.05. With detection of a significant group, by day interaction effect, a Scheffe post hoc testing was also conducted.

8.0 Sample Size Calculations

Effect size was estimated relative to variances reported in the literature examining corticosterone levels after exposure to simulated open field experiments in rat dams (Smith, Fewell & Abu-Amarah, 1997). Sample size calculations for a specified power of 0.80 and a level of significance of 0.05, as well as a conservative 0.25 effect size, were calculated as per Lachin (1981). Hence, four dams per group, were randomly assigned to 7 groups, for a total of 28 dams.

B. Time to Last Gasp and Autoresuscitation Experiments

1.0 Subjects

All neonatal experiments were conducted using the spontaneously breathing rat pups on day of life 1, 5, and 10, born at full term via spontaneous vaginal delivery, to Sprague-Dawley dams undergoing their first pregnancy.

2.0 Animal Care and Use

Each pup was housed in 12 hour light/dark cycles, with their respective dam and siblings, in a neutral thermal environment of 25 °C and 20% relative humidity. Although 25°C is below the neutral thermal zone of the newborn rat, pups would nest with their mother and siblings ensuring behavioral thermoregulation. Pups were permitted uninterrupted access to maternal contact and lactation.

3.0 Ethics

All experimental procedures were carried out in accordance with the Guide to the Care and Use of Experimental Animals provided by the Canadian Council on Animal Care (Olfert, Cross & McWilliam, 1993), and with the approval of the Animal Care Committee of the University of Calgary (University of Calgary, 2008).

4.0 Animal Preparation

Each pup was implanted with electrodes for electrocardiogram (ECG) analysis. A bipolar lead II electrocardiogram was recorded from multi-stranded stainless steel wire electrodes (AS 633, Copper Wire) sewn on the right shoulder and the left hind leg as described by Osborne (1981). Generally lead II provides the largest and most easily discernible R wave, therefore lead II is preferred for heart rate computations (Osborne, 1981). Moreover lead II records the most observable P wave of all standard extremity leads, because the main vector of atrial depolarization is parallel to lead II, allowing for ease of detection of arrhythmia in the mammalian heart (Osborne, 1981). In the instrumented pups, respiratory movements were recorded via a mercury-in-silicone

rubber strand gauge (Model HgPC, DM Davis Incorporated) placed around the chest. Following instrumentation the pup was placed in the metabolic chamber for stabilzation.

5.0 Protocol

On day of life 1, 5, and 10 respectively, one pup from the open field dam, and one pup from the control dam, were randomly removed from the litter, weighed, and then placed in a beaker housed in a water bath of 37 °C (Endocal Refrigerated Circulating Bath RTE-8DD, Neslab). TLG experiments would be undertaken after 15 minutes of temperature stabilization. Thereafter two more additional pups would be removed from their litters, one pup from the open field dam, and one pup from the control dam, weighed, and placed in a beaker housed in a water bath of 37 °C. After 15 minutes of temperature stabilization, autoresuscitation experiments would then be initiated. Stable baseline measurements of ECG and respiratory tracings were obtained prior to experimental interventions. As per Walker, Curtis, Hearse, Campbell Janse, Yellon, Cobbe, Coker, Harness, Harron, Higgins, Julian, Lab, Manning, Northover, Parratt, Riemersma, Riva, Russell, Sheridan, Winslow & Woodward (1988), the presence of a clear P wave was established prior to initiating experimental protocols.

5.1 Time to Last Gasp

For Time to Last Gasp (TLG) experiments, the gas flow was changed from 0.21 FiO_2, at a rate of 1 LPM, to 97% N2-3% CO_2; and was sustained under conditions of environmental hypoxia. During a TLG experiment, stages of the respiratory response to hypoxia, namely hyperpnea, primary apnea, phases of gasping, and terminal apnea, were directly observed and recorded on the polygraph tracing.

5.2 Autoresuscitation

For autoresuscitation experiments, the gas flow was changed from 0.21 FiO_2 at a rate of 1 LPM, to 97% Nitrogen (N2) and 3% Carbon Dioxide (CO_2), until primary apnea was evidenced by the absence of respiratory movements, associated with bradycardia; at which time the gas flow was changed back to room air or 0.21 FiO_2. When the gas mixture was changed, the flow rate was increased to 5 LPM until the gas concentrations in the chamber had stabilized; the flow rate was then lowered to 1 LPM. The aforementioned procedure was repeated every 5 minutes until autoresuscitation failure was noted, as evidenced by arrhythmia with non-conducting electrical activity, or terminal apnea.

6.0 Apparatus

Each pup, assigned to either Time to Last Gasp (TLG) or Autoresuscitative experiments, was individually placed into a metabolic chamber, consisting of a double-walled plexiglas cylinder, with controlled temperature of 37 $\pm$0.1 degrees Celsius, filled with 0.21 FiO_2 at a continuous gas flow at a rate of 1 Litre per minute (LPM). Chamber ambient temperature was regulated to 37.0 +/- 0.1°C by circulating water from a temperature controlled bath (Endocal Refrigerated Circulating Bath RTE-8DD, Neslab) through the space between the walls.

7.0 Measurements and Calculations

7.1 ECG and Respiratory Movements

ECG electrodes were connected to a model 7HIP5 high-impedance probe coupled to a model 7P5 wide-band EEG alternating-current preamplifier (Grass Instrument).

Respiratory movements recorded via a mercury-in-silicone rubber strain gauge (Model HgPC, DM Davis Incorporated) were connected to a bridge amplifier (BTS) coupled to a Grass Model 7Po3 Adapter Panel. ECG and respiratory movements were continuously recorded via Grass Medical Instruments 12 Channel Polygraph, at a paper speed of 10 mm/s. Carbon dioxide was measured using an applied electrochemistry carbon dioxide analyzer (AMetek) coupled to a Grass Model 7Po3 adapter panel.

7.2 Visual Analysis

Tracings for TLG experiments were visually analyzed and the following variables were calculated: 1) Time to Primary Apnea (TPA), 2) Frequency of gasps per minute, following primary apnea, 3) Time to Last Gasp (TLG), and 4) baseline heart rate, and heart rate every minute for five minutes following exposure to hypoxia. Tracings for autoresuscitation experiments were visually analyzed for the following parameters: 1) number of successful autoresuscitations, and 2) mode of death, via arrhythmia or terminal apnea.

7.3 Statistical Analysis

An independent t-test was conducted to test for a significant difference in the number of pups delivered to dams exposed to the open field, versus the number of pups delivered to control dams.

Two-way ANOVA calculations were conducted to determine the intervention effect of endogenous stress versus control, and day of life, on: 1) time to last gasp, 2) total number of gasps, and 3) number of successful autoresuscitations. The alpha level was

0.05. With detection of a significant group by day interaction effect, then Scheffe post hoc testing was also conducted.

A three-way Repeated Measures ANOVA was conducted to determine the effect of endogenous stress versus control, and day of life, on pre and post heart rate measurements relative to exposure to hypoxia.

8.0 Sample Size Calculations

Effect size was estimated relative to variances reported in the literature examining autoresuscitation and time to last gasp in neonatal rat pups (Fewell et al, 2005; Fewell et al, 2000). Sample size calculations for a specified power of 0.80 and a level of significance of 0.05, as well as a conservative 0.25 effect size, were calculated as per Lachin (1981). Hence, a total of 84 pups were studied, one pup from the 7 experimental dams, and one pup from the 7 control dams, on day of life 1, 5, and 10 respectively.

CHAPTER III

RESULTS

A. Induction of a Psychological Stressor During Pregnancy

There were significant differences in corticosterone levels measured in rat dams in exposed to a psychological stressor in late pregnancy, namely the simulated open field, as compared to controls. Hence the null hypothesis was rejected.

1.0 Maternal Corticosterone Levels

In the control groups, corticosterone levels ranges from 71-191 ng/ml on day 17 of gestation, with a mean of 109 ng/ml (SD = $\pm$ 55 ng/ml), 69-123 ng/ml on day 18 of gestation, with a mean of 95 ng/ml (SD = $\pm$ 30 ng/ml), and 58-195 ng/ml on day 19 of gestation, with a mean of 121 ng/ml (SD = $\pm$ 58 ng/ml).

In contrast, corticosterone levels were significantly elevated after exposure to the open field. Corticosterone levels ranged from 305-466 ng/ml on day 17 of gestation, with a mean of 371 ng/ml (SD = $\pm$ 74 ng/ml), 370-607 ng/ml on day 18 of gestation, with a mean of 528 ng/ml (SD = $\pm$261 ng/ml), and 427-488 ng/ml on day 19 of gestation, with a mean of 527 ng/ml (SD = $\pm$77 ng/ml).

Corticosterone levels were sustained at significantly higher levels, 120 minutes after exposure to the open field. Corticosterone levels were not attenuated with increasing gestation, nor were the responses habituated with serial exposure to the open field, rather the effect was reproducible.

Corticosterone levels were measured on day 18 of gestation, after dams were exposed to the open field on day 17, to examine if the effect of the open field was sustained over

time. Corticosterone levels had returned to baseline on day 18 of gestation and were comparable to control levels, with results ranging from 71-135 ng/ml, with a mean of 111 ng/ml (SD = ± 31 ng/ml).

Two Way ANOVA yielded a significant effect for group ($F(1, 18) = 68.7$, $p<0.001$, but not for day ($F(2, 18) = 2.4$, $p=0.118$, nor an interaction effect of group by day ($F(2, 18) = 2.9$, $p=0.081$; such that the average corticosterone level for the open field group (M = 515, SD = ±188), was significantly higher than the control group (M = 108, SD = ±46).

Post Hoc Scheffe testing revealed the following 2 homogeneous subsets; Subset 1: Control day 18 = 95, Control day 17 = 109, OF Day 17/Latent Day 18 = 111, and Control Day 19 = 121; Subset 2: OF Day 17 = 371, OF Day 19 = 527, OF Day 18 = 646. Therefore, after testing for all possible contrasts among the factor level means using a highly conservative multiple comparison procedure, 1) the mean corticosterone levels for control dams on day 17, day 18, day 19, combined with the latent group, and 2) the mean corticosterone levels for open field dams on day 17, day 18 and day 19; grouped together.

1.1 Number of Pups

Significantly more pups were delivered to dams in the control group (M = 13, SD ± 1.2), as compared to pups delivered to dams in the open field group (M = 11, SD = ± 1.2), $t(18) = 2.9$, $p<0.011$.

Table 2

Mean weights of rat pups

	Control	Open Field
Day of life 1	7.5g	7.1g
Day of lie 5	12.8g	12.9g
Day of life 10	20.5g	21.6g

B. The Ability of the Neonate to Withstand Hypoxia

There were no significant differences in the ability of rat pups on day of life 1, 5, or 10 to withstand hypoxia as measured by: 1) number of successful autoresuscitations, 2) time to last gasp, and 3) time to primary apnea, following intrauterine exposure to a maternal psychological stressor. Therefore the null hypothesis was accepted.

1.0 Autoresuscitation

The mean number of autoresuscitations for the day of life 1, control group was 35.3 (SD = $\pm$ 13.5), and 26.3 (SD = $\pm$ 10.2) for the group delivered to dams exposed to the open field. The mean number of autoresuscitations for the day of life 5, control group was 15.6 (SD = $\pm$ 1.8), and 14.7 (SD = $\pm$ 4.1) for the group delivered to dams exposed to the open field. The mean number of autoresuscitations for the day of life 10, control group was 11.6 (SD = $\pm$ 2.3), and 12.4 (SD = $\pm$ 1.9) for the group delivered to dams exposed to the open field.

Two Way ANOVA for Autoresuscitation revealed a main effect for day of life ($F(2, 36) = 94.9$, $p<0.001$), no main effect for intervention ($F(1,36) = 0.8$, $p=0.384$), and no interaction effect for intervention by day of life ($F(2,36) = 0.2$, $p=0.813$).

2.0 Time to Last Gasp

The mean Time to Last Gasp (TLG), as reported in seconds, for day of life 1 control group was 1900.4 seconds (SD = ± 293.6), and 1714.0 seconds (SD = ± 672.5) for the group delivered to dams exposed to the open field. The mean for day of life 5 control group was 1069.4 seconds (SD = ± 179.8), and 1070.9 seconds (SD = ± 126.5) for the group delivered to dams exposed to the open field. The mean for day of life 10 control group was 339.7 seconds (SD = ±84.6), and 431.6 seconds (SD = ± 127.3) for the group delivered to dams exposed to the open field.

Two Way ANOVA for Time to Last Gasp revealed a main effect for day of life ($F(2, 36) = 26.1$, $p<0.001$), no main effect for intervention ($F(1,36) = 1.3$, $p=0.272$), and no interaction effect for intervention by day of life ($F(2,36) = 1.1$, $p<0.341$).

3.0 Total Number of Gasps

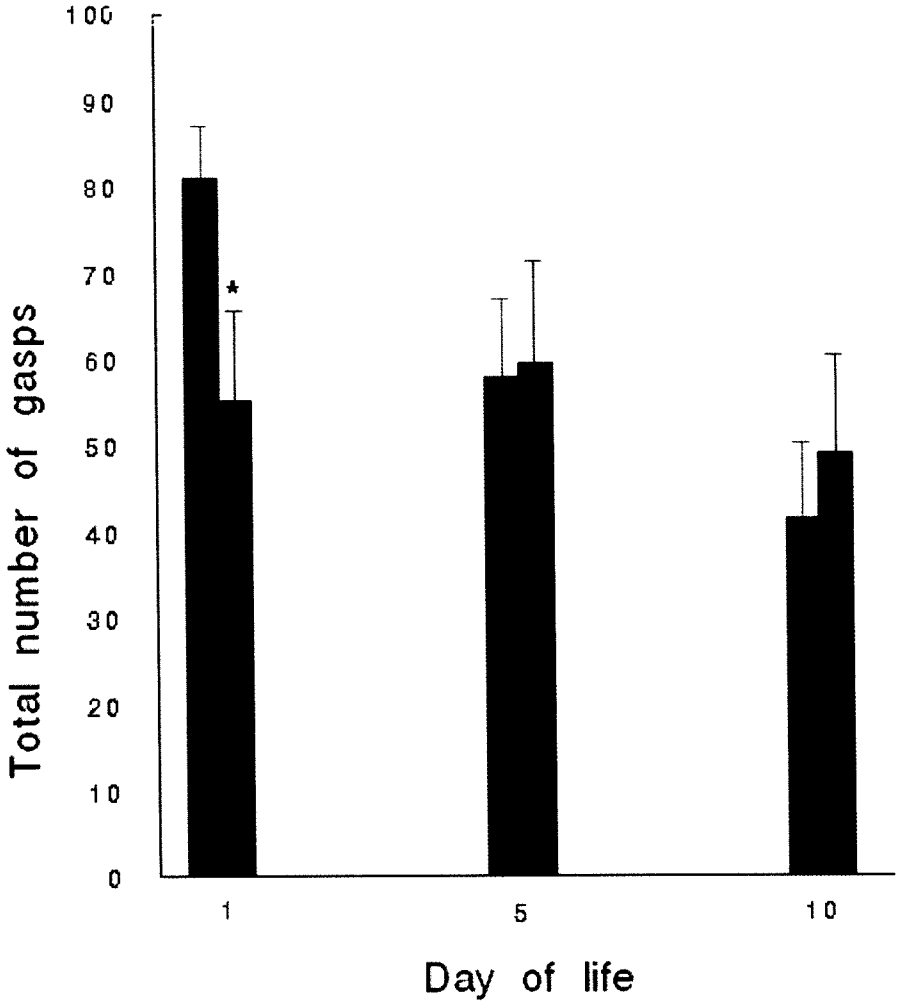
The mean total number of gasps for day of life 1 pups in the control group was 81 (SD = ± 6.1), and 55 (SD = ± 10.2), for day of life 1 pups delivered to dams exposed to the open field. The mean total number of gasps for day of life 5 pups in the control group was 58 (SD = ± 9.1), and 60 (SD = ± 11.7), for day of life 5 pups delivered to dams exposed to the open field. The mean total number of gasps for day of life 10 pups in the control group was 42 (SD = ± 8.8), and 49 (SD = ± 11.4), for day of life 10 pups delivered to dams exposed to the open field.

Two Way ANOVA for Total Numberof Gasps revealed a significant main effect for day of life ($F(2, 36) = 14.308$, $p<0.004$), no main effect for intervention ($F(1,36) = 2.532$, $p=0.112$), and a significant interaction effect for intervention by day of life ($F(2,36) = 7.49$, $p<0.002$) (see Figure 1).

Figure 1

Total Number of Gasps

Influence of exposure to an intrauterine stressor (red solid bars) verus control (blue solid bars) on the total number of gasps following exposure to unrelenting hypoxia in 1, 5 and 10 day old rat pups. Values are represented as means $\pm$ SD. $P < 0.002$ by ANOVA.



4.0 Heart Rate

Mean baseline heart rate per minute, on day of life 1, for the control group was 261.8 (SD = ± 15.3), and at 1 minute post hypoxia was 152.5 (SD = ± 24.4). Mean baseline heart rate per minute, on day of life 1, for pups delivered to dams exposed to the open field was 252.0 (SD = ± 63.6), and at 1 minute post hypoxia was 144.5 (SD = ± 52.7).

Mean baseline heart rate per minute, on day of life 5, for the control group was 332.0 (SD = ± 38.5), and at 1 minute post hypoxia was 104.4 (SD = ± 14.1). Mean baseline heart rate per minute, on day of life 5, for pups delivered to dams exposed to the open field was 320.4 (SD = ± 26.1), and at 1 minute post hypoxia was 118.6 (SD = ± 34.9).

Mean baseline heart rate per minute, on day of life 10, for the control group was 391.7 (SD = ± 30.8), and at 1 minute post hypoxia was 114.0 (SD = ± 18.5). Mean baseline heart rate per minute, on day of life 10, for pups delivered to dams exposed to the open field was 386.6 (SD = ± 36.7), and at 1 minute post hypoxia was 113.0 (SD = ± 36.6).

There were no significant differences in heart rates between control and experimental groups. A Repeated Measures, Three Way ANOVA revealed: a significant within subjects, main effect for time ($F(2, 36)=11.2$, $p<0.002$); a significant between subjects, interaction effect for time by day of life ($F(2,36)=49.3$, ($p<0.001$). There was no main effect for time by intervention ($F(2,36)=0.4$, $p=0.540$), nor time by day of life by intervention effect ($F(2,36)=0.5$, $p=0.603$).

CHAPTER IV

DISCUSSION

Our experiments offer new information about factors that influence the neonate's ability to withstand hypoxia; specifically related to the neuroendocrine response to stress in late pregnancy. Novel findings of our study were: 1) exposure to a novel environment in late pregnancy elicited a reproducible, non-attenuated, and sustained maternal stress response, as measured by plasma corticosterone levels, 2) exposure to a novel environment in late pregnancy did not alter the time to last gasp, but did alter the total number of gasps, and thus the pattern of gasping in an age dependent manner, and 3) exposure to a novel environment in late pregnancy did not alter the ability of the the neonatal rat to autoresuscitate.

A. Psychological Stress in Late Pregnancy

In this study, we demonstrated significant differences in corticosterone levels in late pregnancy, between dams exposed to a psychological stressor as compared to controls. Hence we rejected the null hypothesis.

1.0 Strengths

In our study design we strived to minimize extraneous variables that may have resulted in stimulation of the maternal HPA axis outside of the experimental condition of the simulated open field. In so doing, we endeavoured to reduce the risk of committing a Type I error, or observing a difference when in fact there was no difference (Polit, 1996). We also attempted to design our study such that the effects of the experimental condition

would be detected, thereby reducing the risk of committing a Type II error, or failing to observe a difference when in fact there was a difference (Polit, 1996). For these aforesaid reasons, we attempted to control for the following factors: 1) physical stressors, 2) habituation, 3) animal model, 4) circadian rhythm, 5) blood sampling technique, 6) timing of blood sampling, and 7) environment.

1.1 Physical stressors

The psychological stress stimulus in our study was the simulated open field with intense illumination, as first described by Hall (1934, 1936), and validated as the reliable standard of a psychological stressor in rats by Roth & Katz (1979). The simulated open field induces a strictly psychological stress response in rats, thereby increasing the relevance and applicability of the results obtained. Comparatively, a multitude of other psychological stressors have also been described in the literature, but may be misrepresentative, in that frequently the apparent psychological stressor is confounded by the concurrent introduction of a physical stressor. Examples of combined psychological and physical stressors are: maternal defeat by an aggressive lactating co-resident (Neumann, Kromer & Bosch, 2005), restraint (Neumann et al, 2005), elevated plus maze (Neumann et al, 1998), inescapable footshock (Darnaudery & Maccari, 2008), and forced swimming in cold water (Douglas, Johnstone Wigger, Landgraf, Russell & Neumann, 1998).

1.2 Habituation

The only intervention our dams were exposed to was the open field, whereas in Smith's et al (1997) study, the animals were permitted to explore and become familiar

with the guillotine. Although this design was intended to train the animal, a learned behavior does add a confounding variable that may have influenced corticosterone levels, specifically via habituation, as compared to the stress response elicited when exposed to the open field alone.

1.3 Animal Model

In our experiments we utilized Sprague-Dawley rat dams. Whereas assessment of the maternal HPA axis is problematic in human pregnancy, because the placenta also produces a CRF–like peptide (Sasaki, Tempst, Liotta, Margioris, Hood, Kent, Sato, Shinkawa, Yoshinaga, Kreiger, 1988), the rat placenta does not produce this peptide (Brunton, Russell & Douglas, 2008). Hence, the rat dam serves as an ideal animal model in which to investigate factors that may influence the maternal HPA axis in pregnancy, void of the endocrine role of the placenta.

1.4 Circadian Rhythm

Atkinson & Waddell (1995) studied the corticosterone profile of pregnant rats, obtained every 2-3 hours for 24 hours, on days 2, 6, 10, 14, 18, and 22 of pregnancy. Results were presented in a cosine curve with nadirs reached consistently at noon each day (Atkinson et al, 1995). Therefore, to limit the confounding variable of peaks in circadian rhythm, all open field experiments were conducted between 1000-1200hrs, and corticosterone levels were obtained between 1200-1400 hours.

1.5 Blood Sampling Method

We collected blood samples via decapitation using a guillotine. This method affords three major advantages: 1) an ethically sound means to instantly euthanize the animal; whilst 2) optimizing blood sampling void of stimulation of the HPA axis via extraneous stressors, such as chronic cannulation or tail vein sampling, nicking or cutting; and 3) allowing the animal to be freely moving. Therefore we mitigated the following confounding variables of chronic vascular cannulation: prolonged immobilization or restraint, anesthesia, surgery, potential infection or pain (Lestage, Vitte, Rolinat, Minot, Brousoll & Bobillier, 1985; Vahl, Ulrich-Lai, Ostrander, Douglas, Elfers, Seeley, D'Alessio & Herman, 2005). Although decapitation without prior anesthesia results in the most reliable measurements of ACTH and corticosterone, (Vahl et al, 2005) this method is obviously limited as it does not allow for serial measurements.

1.6 Timing of Blood Sampling

Serum corticosterone levels reported by Smith et al (1997), collected at 30 minutes following exposure to the open field, were comparable to levels reported in our study. However, a key difference is that levels obtained in our study were obtained 120 minutes following exposure to the open field. As described by Smith et al (1997), corticosterone levels returned to baseline by 120 minutes, following exposure to the open field, in all but gestational day 21 dams. Similarly, plasma corticosterone levels peaked at 15 minutes post restraint stress, and had returned to baseline by 60 minutes as reported by Johnstone et al, (2000). Yet our results suggest the stress response we elicited as evidenced by significantly elevated corticosterone levels 120 minutes after exposure to

the open field, were either sustained over time, or potentially peaked to much higher levels and then decayed to still significantly elevated levels. To our knowledge, sustained corticosterone levels, collected at 120 minutes following exposure to the open field, on gestational days 17, 18 and 19, provides a novel contribution to the literature.

1.7 Environment

Lestage et al (1985) described significantly elevated plasma corticosterone levels within 15 minutes of changing the cage that normalized within 24 hours. In our lab, cages are changed on Tuesdays and Fridays, whilst open field experiments were conducted serially from Saturday through to Monday, or Wednesday through to Friday. Hence cage changes did not confound our corticosterone level experiments.

Lestage et al (1985) also reported changes in the experimental environment may also contribute to elevated corticosterone levels. In our study, animals arrived on gestational day 7, and were individually housed in a metabolic chamber for 10 days prior to introduction of the experimental conditions. These aforesaid measures are consistent with previously reported recommendations to control the experimental conditions, and in so doing, maintain corticosterone levels to near baseline (Burt, Arbeit, & Brennan, 1980).

2.0 Limitations

2.1 Home Cage

One limitation of our study, was that we carried the cages of animals randomized to the open field out of the metabolic chamber, while the control animals were not handled, nor moved, any more than that required during routine animal care. In contrast,

Smith et al (1997), removed the home cage from the metabolic chamber to the experimental room, and then randomly assigned the animal to one of four groups. Although moving the animal to the experimental room is likely to elicit a minimal stress response (Lestage et al, 1985), there is strength in the design by Smith et al (1997), whereby all animals were treated consistently until the independent variable was introduced.

2.2 Control Subjects

Basal plasma corticosterone levels in late pregnancy as documented in our study were consistent with those reported by Smith et al (1997) and Johnstone et al (2000). Also consistent with our findings, Dupouy, Coffigny & Magre, (1975) reported a marked rise in plasma corticosterone levels on day 18 and 19 of gestation in non-adrenalectomized dams. In contrast to Dupouy et al, (1975), and Smith et al (1997), we did not find an incremental increase in corticosterone levels in pregnant rat dams in late pregnancy, in either our control group, nor our experimental group. An advantage to our study was that we measured corticosterone levels in both experimental and control animals. In contrast, Dupouy et al, (1975), compared corticosterone levels in adrenalectomized dams in late pregnancy across different gestational ages, but no control group was utilized; thereby limiting the potential inferences that could be made from their data. As per Smith et al (1997) who not only studied pregnant control animals across gestational ages, but also non-pregnant control animals; our results measuring the effects of a psychological stressor in late pregnancy, may have carried greater weight had we included a group of non-pregnant controls.

2.3 Attenuation of the HPA Axis

In the rat, secretion of ACTH and corticosterone have been reported as being attenuated, as a hyporesponsive means to minimize exposure of the fetus to excess glucocorticoids (Douglas et al, 1998; Neumann et al, 1998; Wigger et al, 1999; Johnstone et al, 2000; Brunton et al, 2000). However, we did not report an attenuation, nor habituation in the stress response; as evidenced by consistently elevated and reproducible corticosterone levels following exposure to a psychological stressor in late pregnancy. We restricted our design to the measurement and analysis of corticosterone levels on gestational days 17, 18 and 19, in order to establish an endogenous glucocorticoid response, over the same time period Kauffman et al (1994) administered an exogenous glucocorticoid to pregnant dams. However, had we obtained corticosterone levels over the course of gestational days 20 and 21 as well, we may have more readily detected a significant attenuation of the HPA axis in pregnant rat dams.

We may have gleaned a more complete reflection of the maternal stress response, and further explanation as to why our results did not reflect stress hyporesponsiveness during pregnancy, had we measured HPA activity further up the axis. For example, mRNA expression for the ACTH precursor POMC (Ma, Shipston, Morilak, & Russell, 2005), expression of the immediate early gene nur77 mRNA as a regulator of CRF gene expression, (Chan, Brown, Ericsson, Kovacs & Sawchenko, 1993), as well as the density and sensitivity of CRF receptors in the anterior pituitary (Neumann et al, 1998); have been reported as being reduced or blunted in late pregnancy in rats.

2.4 ß-Hydroxysteroid Dehydrogenase

We investigated the premise that endogenous exposure to glucocorticoids *in utero*, may potentiate fetal glucocorticoid excess, and possibly alter the neonate's physiologic response to hypoxia. We extrapolated this theory from a body of published research that reported that prenatal exogenous glucocorticoid exposure, may induce cellular differentation at the cost of proliferation, in fetal pneumocytes (Phelps & Floros, 1991), and myocytes (Slotkin, Seidler, Kavlock, & Bartolome, 1991; Kauffman, et al, 1994). As pups in both our experimental and control groups generally responded homogeneously to hypoxia, we may assume that the placental 11 ß-HSD2 feto-placental barrier to glucocorticoids was intact, and that 11 ß -HSD2 levels were within normal levels. The differing responses of the neonatal pups in our study to hypoxia, when compared to those in studies published by Slotkin's laboratory, may be explained by van Beek et al (2004), who showed that endogenous glucocorticoids increase 11 ß -HSD2 activity in a concentration dependent manner. Moreover, dexamethasone is a poor substrate for 11 ß -HSD2, as evidenced by a 10 fold increase in endogenous glucocorticoid required to elicit the same response, as compared to dexamethasone.

In future studies, measurements of the effects of maternal stress on the physiologic responses of the offspring, would be more comprehensive if not only levels of corticosterone were obtained, but if they were interpreted in relation to 11 ß -HSD2 bioactivity assays (Edwards et al, 1996). Welberg, Thrivikraman & Plotsky (2005) demonstrated that acute exposure to stress on gestational day 20 up-regulated placental 11 ß -HSD2 activity by 160%, whereas chronic stress from gestational age 14-19 did not significantly increase 11 ß -HSD2 activity. As our study was an investigation of stress in

late pregnancy, a greater appreciation of the mechanisms at play may have been revealed had 11 ß -HSD2 levels been assayed.

3.0 Recommendations for Future Research

We and other aforementioned investigators have established a stimulatory effect on the HPA axis, as evidenced by elevated corticosterone levels in the pregnant rat dam, following exposure to a psychological stressor (Smith et al, 1997). Cytokines, involved in the mediation of the inflammatory response to infection, such as interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF α), also produce hypothalamo-pituitary-adrenal (HPA) axis activation (Yurnball & Rivier, 1995). Therefore, comparing the effects of a maternal psychological stressor in the rat dam, as in the simulated open field, versus a physical stressor, as per an antental injection of an endotoxin, on the neonate's ability to withstand hypoxia, may potentially add to our understanding of the effects of the comparable human experiences of perinatal stress and chorioamnionitis on the fetus and neonate. Moreover, an examination of the effects of early versus late gestation stress, as well as acute versus chronic stress, on both the mother and offspring, would also be meaningful contributions to the current literature and may offer direction for future research.

Long-term follow-up of the offspring from our open field experiments, may have afforded us opportunities to learn more about the responsiveness of the offspring's HPA axis in adulthood. A multigenerational approach to examining stress responsiveness, maternal behavior, and offspring birth weights, may be suggested areas of focus in the field of fetal programming (Barker, 2004).

4.0 Number of Pups

The number of pups counted per dam was significantly less in the psychologically stressed group as compared to controls. These results are consistent with Euker & Riegle (1973), and Baker, Chebli, Rees, Lemarec, Godbout & Bielajew (2008), who also reported fewer pups delivered to antenatally stressed dams, attributed to fetal loss and increased progeny mortality rate.

Cannibalism may explain the fewer pups per litter with the stressed dams as compared to controls. Cannabalism may be assumed to be a maladaptive behavior correlated with a stress response in the experimental setting (Day & Galef, 1977), but it may be highly adaptive in the natural setting, in which sick or weak pups may put the remainder of the litter at risk for infection or vulnerability to predators (DeSantis & Schmaltz, 1984).

5.0 Maternal Behavior

Although not directly relevant to the original research questions, maternal behavior between the dams exposed to a psychological stressor as compared to controls, was notably different. Dams exposed to the open field were frequently found with pups scattered and unattended within the cage, whereas control dams were consistently found nesting with or feeding all of their pups. Such alteration in maternal rodent behavior following exposure to an antenatal stressor was also described by Pardon, Gerardin, Joubert, Perez-Diaz & Cohen-Salmon (2000). As oxytocin is involved in the regulation of maternal behavior, and has been found to be anxiolytic in pregnant and lactating rats presented with physical and psychological stressors (Neumann et al, 2005; Neumann,

2008); our study may have been strengthened by measuring oxytocin levels in response to stress.

In human studies, adverse pregnancy outcomes associated with psychological stress as quantified using Life Inventories, are dependent upon the timing of the stressor and more importantly, the woman's perception of the stressor (Hobel, Goldstein, & Barrett, 2008). Life Inventories can be skewed by a multitude of factors such as relying on the subject recall, the willingness of the subject to reveal less socially accepted stressors like domestic violence or drug use, the subjectivity of self-reporting, and the difficulty in measuring influences such as culture, or overarching risk factors such as depression. Although animal studies cannot be inferred to represent the human condition, the pregnant rat dam exposed to the simulated open field, provides a pure model of the effects of a psychological stressor in late pregnancy, devoid of confounding variables such as previous life experiences.

Furthermore, the relatively mild stressor introduced in the laboratory setting as in our study, may be more representative of moderate daily stress experienced during normal life. As opposed to a more severe noxious stressor, such as unpredictable introduction of a predator, that may more closely replicate a major life event, or post-traumatic stress disorder (Zoladz, Conrad, Fleshner & Diamond, 2008). Therefore our study may be more generalizable to the more commonly encountered human experience of psychological stress and anxiety in pregnancy.

6.0 Clinical Relevance

Published research has drawn associations between maternal psychosocial stress during pregnancy and negative obstetrical, fetal and neonatal outcomes; including an increased risk for stillbirth (Wisborg, Barklin, Hedegaard & Henriksen, 2008), decreased fetal weight (Diego, Jones, Field, Hernandez-Reif, Schanberg, Kuhn & Gonzalez-Garcia 2006), and preterm delivery (Mancuso, Schetter, Rini, Roesch & Hobel, 2004). Clearly the gravity of these findings confirms the need for therapeutic counseling and social support for women at risk. However, self-reporting, and a lack of consensus in the definitions and the degrees of distress, are not highly reliable. Moreover, sole measurements of blood or salivary cortisol may only indicate present day stress. A new technology, involving maternal hair cortisol measurements, may trace a more complete trajectory of peaks in stress correlated with specific gestations, and a cumulative measure of stress, over the course of the entire pregnancy (Kramer, Lydon, Seguin, Goulet, Kahn, McNamara, Genest, Dassa, Chen, Sharma, Meaney, Thomson, van Uum, Koren, Dahhou, Lamoureaux & Platt, 2009).

B. The Neonate's Response to Hypoxia

1.0 Gasping

We found no differences in time to primary apnea, time to last gasp, nor number of successful autoresuscitations after exposure to hypoxia, amongst rat pups at 1, 5, and 10 days of life, delivered to mothers exposed to a psychological stressor as compared to control animals. Hence we accepted the null hypotheses.

To our knowledge, this is the first study to report a significantly decreased total number of gasps in neonatal rats on day of life one, following exposure to a psychological stressor in late pregnancy. Gasps represent opportunities for successful autoresuscitation. Logically, the greater the number of gasps, the greater potential for successful autoresuscitation. However, Gershan et al (1992), offer a sound counterpoint by questioning the significance of gasp frequency, when gasping is sustained for such long periods of time, and may exceed 75 gasps despite unrelenting hypoxia. Could a decrease in the number of gasps actually be adaptive or energy conservative?

Nitric oxide expression in the lateral tegmental field modulates the timing and phasing of hypoxic gasping (Gozal et al, 1998). Brainstem nitric oxide production has been reported as being associated with more frequent gasping in rat pups (Gozal et al, 2001). In contrast, we reported a decreased number of total gasps in day of life 1 pups, exposed to an antenatal psychological stressor. Future studies examining the effects of exposure to antenatal endogenous and exogenous glucocorticoids, on the expression of nitric oxide in the lateral tegmental field, may be warranted.

1.1 Postnatal Age

Consistent with Stafford & Weatherall (1960), Gershan et al (1990), Jacobi et al (1991), Gershan et al (1992), Fewell et al (2000), Gozal et al (2003), and Fewell et al (2007); we found a maturational difference in the time to last gasp, as well as number of successful autoresuscitations; whereby time to last gasp, and the number of successful autoresuscitations, decreases proportionately with advancing postnatal age. Although the definitive explanation for the inverse relationship between postnatal age and gasping

duration in neonatal rats exposed to hypoxia has not been established, nitric oxide has again been shown to mediate gasping duration with advancing postnatal age (Gozal et al, 1998) with western blots from the lateral tegmental field of the medulla, involved in gasp generation, revealling proportionately increased neuronal nitric oxide synthase concentration with postnatal maturation in neonatal rats.

1.2 Phases of Gasping

Comparable to Gozal et al (1996), Gozal et al (1997), Yuan et al (1997), and Serdarevich et al (1999); we noted three phases in gasping. Frequent gasping with a predominant inspiratory phase, or Phase 1 gasping, generally transpired within the first 3 minutes after exposure to hypoxia in pups across all postnatal ages. In contrast, the period of infrequent gasping of <2 gasps per minute, or Phase 2 gasping, was markedly foreshortened with advancing postnatal age in both experimental and control groups. Finally, a return to frequent gasping with decrementing amplitude, or phase 3 gasping, was also significantly shorter in duration with increasing maturation.

1.3 Secondary Apnea

As first described in neonatal rabbits by Godfrey (1968), neonatal rats in our study demonstrated a predictable response to a single unrelenting hypoxic challenge comprised of hyperpnea, primary apnea, gasping, and terminal apnea. As per Guntheroth & Kawabori (1975) no spontaneous recovery was noted following secondary apnea, in our time to last gasp experiments. In contrast, Yuan et al (1997) reported that secondary was not tantamount to terminal apnea in either 1 or 8 day old rat pups exposed to hypoxia. Hence, future experiments, aimed at replicating Yuan et al (1997) findings, may be

warranted. Such studies may be strengthened, by measuring the optimal oxygen concentration from which spontaneous recovery from secondary apnea is observed.

2.0 Strengths

2.1 Environmental Temperature

Environmental temperature significantly influences the neonate's tolerance of hypoxia. Both neonatal and adult rats will demonstrate behavioral thermoregulation by selecting an ambient temperature within their respective thermoneutral zone (Gordon, 1987; Fewell, Kang & Elison, 1997). Consequently, we allowed pups unlimited access to nesting with their mothers, and then optimized the environmental temperature for neonatal pups during experiments, by ensuring a neutral thermal environment via a constantly monitored water bath set to 37 degrees Celsius.

2.2 Monitoring Respiratory Movements

In our study, respiratory movements were recorded via a mercury-in-silicone rubber strain gauge (Model HgPC, DM Davis Incorporated) placed around the chest, connected to a bridge amplifier (BTS) coupled to a Grass Model 7Po3 Adapter Panel, and recorded via Grass Medical Instruments 12 Channel Polygraph. Regardless of movement, agitation or positioning, this method consistently provides reliable tracings via polysomnograpy. Numerous other studies examining gasping and autoresuscitation employ barometric plethysmography (Mizusawa et al, 1994; Gozal et al, 1997; Yuan et al 1997). This method of measuring respirations can be prone to error.

Barometric plethysmography was first described by Chapin in 1954 as a non-

invasive method used to measure ventilation in hamsters. The plethysmograph system is comprised of a double walled box with a known internal volume that may have a single chamber, or may be divided into upper and lower chambers. Pressure changes within the chambers are measured via a transducer. Air enters the system at a constant flow rate, temperature and humidity, while the same volume of air is removed from the chamber via a vacuum system. The constant airflow in and out of the chamber is monitored via high resistance needle valves adjusted to maintain a set gradient across the valves. Temperature and humidity are monitored by means of a hygrometer. Measurements of ventilation are made based on calculations of tidal volume and expired carbon dioxide, generated from the original calibration of pressure within the system (Fleming, Levine, Goncalves & Woolard, 1983). However, the system is limited by the need for ongoing calibration, a propensity for undetected leaks, artifact, and finally reported risks of carbon dioxide accumulation within the chamber itself (Fleming et al, 1983). The aim of a study by Fleming et al (1983) was to provide evidence that the barometric plethysmograph was a reliable and practical method to measure ventilation in both clinical and research settings via a non-invasive apparatus. However, in so doing, all subjects underwent tracheostomy, potentially increasing airway resistance, and were connected to four lead impedence pneumography, potentally contributing to chest wall impedence. Hence these researchers confounded their results with the very invasive modalities they were arguing against.

3.0 Cardiac

3.1 Arrythmia as Mode of Death

We reported the mode of death in the autoresuscitation experiments as being predominantly via atrioventricular (AV) dissociation, or failure of stage II of autoresuscitation, cardiac resuscitation (Gershan et al, 1992). In one animal, of 5 days of age, from the control group, gasping ceased prior to cardiac resuscitation. In two additional animals, both 10 days of life, and both in the control group, autoresuscitation failure was due to the absence of gasping. Although, these aforesaid findings are consistent with Fewell, Smith, Ng, Wong & Wang, 2000, who reported terminal apnea preceding arrythmia in only one 5 day old subject; our results differ from Fewell et al, (2000), who reported terminal apnea as the mode of death in the majority of animals greater than 10 days of age.

While Kauffman et al (1994) reported respiratory arrest as the mode of death in the one control animal that died after exposure to hypoxia for 2 hours, arrythmia was the mode of death in all of the dexamethasone exposed pups who presented with a significantly higher, and dose dependent mortality rate. In contrast, we did not ascertain a difference between control or experimental groups in relation to the incidence of arrythmia as the mode of death.

As reported by Gershan et al (1992) in mice, we also noted that arrythmia occurred with increasing frequency towards the latter cycles of successful autoresuscitations. Furthermore, a return to sinus rhythm, as described by Gershan et al (1992), consistently

preceded successful autoresuscitations in all animals, both experimental and control and across all age groups.

Albeit the mechanism therein was not formally investigated, AV dissociation was likely attributable to adenosine acting via adenosine A1 receptors, as previously reported by Fewell et al (2007). Adenosine accumulates during hypoxia and induces bradycardia or heart block in the atrioventricular node, which is likely protective, serving to decrease myocardial workload under hypoxic conditions. Arrhythmia is mediated by Adenosine 1 receptors (A1), which are densely expressed in the newborn rat myocardium (Cothran, 1995); inhibiting adenyl cyclase, thereby reducing cyclic AMP, and resulting in hyperpolarization of the AV node cells via efflux of potassium ions.

3.2 Postnatal Age and Sympathetic Innervation

We demonstrated a significant increase in baseline heart rate with advancing postnatal age in both experimental and control groups. We reported mean baseline heart rate on day of life 1, approximating the mid to upper 200 beats per minute (bpm), the low to mid 300 bpm on day of life 5, and approaching 400 bpm on day of life 10. These baseline findings are consistent with other investigators (Adolph, 1967; Tucker & Domino, 1988; Fewell, Zhang & Gillis, 2007). The increase in baseline heart rate can be ascribed to the non-functional sympathetic neurotransmission and innervation in the neonatal myocardium within the first week of life, and the subsequent development of functional sympathetic neurotransmission by the second week of life (Lau & Slotkin, 1981).

Synthetic exogenous glucocorticoids have been reported to modulate the development of sympathetic neurotransmission. Whereas Kauffman et al (1994) reported a difference

in baseline rhythm between control versus dexamethasone with prolonged PR intervals, we did not detect a difference in rhythm or rate at baseline in either our control or experimental groups. Similarly, Lau & Slotkin (1981) found that dexamethasone given for 5 days starting on the first day of life, did not alter baseline heart rate as compared to controls, however chronotropic responses to tyramine and isoproterenol, were deficient from day 3 forward in dexamethasone treated rat pups. In addition, despite cessation of dexamethasone administration, catecholamine content and biosynthesizing enzymes were deficient, and did not reach normal levels until 3-5 weeks of age. This inhibition of adrenomedullary maturation may potentially be explained by the intimate venous supply of the adrenal cortex with the medulla, and the dependence of the enzyme, phenylethanolamine N-methyltransferase (PNMT), on physiologic concentrations of endogenous glucocorticoids for catecholamine synthesis. This mechanism can be inhibited by supraphysiologic exogenous glucocorticoid administration via negative feedback mechanisms (Wurtman & Axelrod, 1966, Wurtman, 2002).

4.0 Strengths

4.1 Lead II

In our study, each pup was implanted with electrodes and a bipolar lead II electrocardiogram was recorded. Lead II provides the largest and most easily discernible R wave, therefore lead II is preferred for heart rate computations in rodents (Osborne, 1981). However, in other published studies examining the tolerance of rat pups to hypoxia, with heart rate as the dependent variable, neither lead I, II or III are specified. (Gershan et al, 1992; Gozal et al, 1996; Deshpande et al, 1999)

4.2 P Wave

Lead II records the most observable P wave of all standard extremity leads. As per Walker et al (1988), the presence of a clear P wave was established prior to initiating experimental protocols in our study. This fundamental baseline measurement was not reported in other studies where rat pups were exposed to hypoxia, and heart rate was a primary outcome (Gershan et al, 1992; Gozal et al, 1996; Deshpande et al, 1999).

5.0 Clinical Relevance

5.1 Room Air Resucitation

We demonstrated that newborn rat pups can successfully and repeatedly autoresuscitate from primary apnea, induced by exposure to hypoxia, once exposure to room air was reinitiated. The first report of room air resuscitation in animals, was put forth by Campbell, Cross, Dawes & Hymen (1966). The common denominator between both human and animal data is that likely ventilation is more integral to resuscitation, as opposed to what percent of oxygen concentration is delivered during ventilation. Whereas our study relied upon the spontaneous initiation of ventilation of the animal itself following primary apnea, Campbell et al, (1966) studied varying modes of resuscitation following last gasp, or apparent terminal apnea. Campbell et al (1966) demonstrated no difference in survival, nor time from last gasp to the beginning of spontaneous ventilation, in asphyxiated and intubated newborn rabbits immersed in saline, and receiving positive pressure ventilation, via room air versus 100% oxygen. Moreover, exposure to hyperbaric oxygen was profoundly less effective than positive

pressure ventilation with 100% oxygen, in the resuscitation of the asphyxiated newborn rabbit.

Albeit Bookatz, Mayer, Wilson, Vento, Gelfand, Haxhiu & Martin (2007) reported significant delays in the onset of respiration in hyperoxic versus normoxic conditions, following exposure to hypoxia; replication of this study design in non-anesthetized, non-intubated rat pups, within the first week of life, may be more germane to the human experience. Future research in rat pups undergoing autoresuscitation experiments, comparing the effectiveness of a range of concentrations of oxygen, coupled with measurements of oxidant stress versus hypoxic injury, may be additive to the current body of literature on room air resuscitation.

Consensus is building in the human literature pointing to the superiority of room air over the administration of supplemental oxygen during neonatal resuscitation, relative to lower mortality rates at 1 week and 1 month overall, with no significant difference in Stage II or III hypoxic ischemic encephalopathy (Rabi, Rabi & Yee, 2007). However, in humans, long-term neurodevelopmental studies are needed, as are studies that are stratified per gestational age.

5.2 Glycogen

We describe an inverse relationship between the younger the rat, the greater the number of successful autoresuscitation trials. There is evidence to explain this maturational difference relative to: 1) the non-neurogenic, hypoxia-sensing mechanism in adrenal chromaffin cells that precedes sympathetic splanchnic innervation within the first week of life (Seidler et al, 1985; Slotkin et al, 1995; Kirby & McCarty, 1987; Thompson,

Jackson & Nurse, 1997Slotkin et al, 1998); and 2) the relatively high level of cardiac glycogen stores in the neonatal rat that vary inversely with maturity, theorized to be due to the lack of mobility and insulating hair in the neonatal rat as compared to other mammals (Shelley, 1961). Quantification of cardiac glycogen utilization and depletion, and the relationship of the same to successful autoresuscitation, albeit in mice, has been reported by Deshpande, et al (1999). Herein lies an opportunity for future studies to measure cardiac glycogen utilization and depletion not only in the immediate neonatal period, but also over the course of the first weeks of life, as a means to explain the relationship between maturation and the peak incidence of SIDS in humans between 2-3 months of age (Richardson, Walker & Horne, 2008).

5.3 Preterminal Gasping

The presence of gasping has been reported to increase venous return (von Planta, Weil, van Planta, Bisera, Bruno, Gazmuri & Rackow, 1988), as well as aortic pressures and coronary perfusion (Xie, Weil, Sun, Yu & Tang, 2004). Moreover, the presence of gasping was associated with improved outcomes post cardiopulmonary resuscitation (CPR), according to an analysis of pre-hospital conditions in a registry of 4548 patients across the life span (Mullie, Lewi, and van Hoeyweghen, 1989). In view of these findings, it may be noteworthy to track the presence or absence of gasping, by first responders to cases of apparent SIDS or drownings in infants. Collation of this data may reveal important information regarding prognostication. In addition, perhaps a non-invasive Doppler probe placed over the superior vena cava (Grives, Kuschel, Knight & Skinner, 2008) during neonatal resuscitations, would provide us with hemodynamic

information as to how to continually improve upon our current resuscitation standards of ventilation and chest compressions in the severely depressed patient.

C. Summary

In this study, we demonstrated the following novel findings:

1) Significantly elevated corticosterone levels in late pregnancy, in dams exposed to a psychological stressor, as compared to controls. These results were obtained some 120 minutes after exposure to the open field, suggesting a sustained stress response.

2) We did not report a significant attenuation, nor habituation in the stress response; as evidenced by consistently elevated and reproducible corticosterone levels following exposure to a psychological stressor in late pregnancy.

3) We found a significantly decreased frequency of gasps in day of life 1 neonatal rats exposed to an intrauterine stressor. This finding is noteworthy as gasps represent opportunities for successful autoresuscitation.

We have also replicated previously published findings by reporting the following:

1) We demonstrated that repeated cycles of hypoxia will ultimately result in autoresuscitation failure.

2) All pups in our study demonstrated a predictable response to a single unrelenting hypoxic challenge comprised of hyperpnea, primary apnea, gasping and terminal apnea.

3) We found a maturational difference in the time to last gasp, as well as number of successful autoresuscitations; whereby time to last gasp and the number of successful autoresuscitations, decreases proportionately with advancing postnatal age.

4) Frequent gasping with a predominant inspiratory phase, or Phase I gasping, generally transpired within the first 3 minutes after exposure to hypoxia in pups across all postnatal ages. In contrast, the period of infrequent gasping of <2 gasps per minute, or Phase II gasping, was markedly foreshortened with advancing postnatal age in both experimental and control groups. A return to frequent gasping with decrementing amplitude, or Phase III gasping, was also significantly shorter in duration with increasing maturation.

5) We reported the mode of death in the autoresuscitation experiments as being predominantly via atrioventricular (AV) dissociation, or failure of stage II of autoresuscitation, cardiac resuscitation. We also noted that arrythmia occurred with increasing frequency towards the latter cycles of successful autoresuscitations.

6) A return to sinus rhythm, consistently preceded successful autoresuscitations in all animals, both experimental and control, and across all age groups.

7) We demonstrated a significant increase in baseline heart rate with advancing postnatal age in both experimental and control groups.

The experiments put forth in this dissertation provide us with a greater understanding of the endocrine responses of the rat dam in late pregnancy to a psychological stressor,

and the neonate's response to hypoxia following intrauterine exposure to a maternal psychological stressor. Although these data represent a significant addition to the field, more questions have been raised, and may be pursued in future research endeavors.

BIBLIOGRAPHY

Abu-Shaweesh, J. (2004). Maturation of respiratory reflex responses in the fetus and neonate. Seminars in Neonatology, 9: 169-180.

Acker, H. (1989). PO2 chemoreceptors in arterial chemoreceptors, Annual Review in Physiology, 51: 835-844.

Adolph, E. (1948). Tolerance to cold and anoxia in infant rats. American Journal of Physiology, 155(3): 366-377.

Adolph, E. (1967). Ranges of heart rates and their regulations at various ages (rat). American Journal of Physiology, 212(3); 595-602.

Aguilera, G., Rabadan-Diehl, C. & Nikodemova, M. (2001). Regulation of pituitary corticotropin releasing hormone receptors. Peptides, 22(5): 769-774.

Albiston, A., Obeyesekere, V., Smith, R. & Krowzowski, Z. (1994). Cloning and tissue distribution of the human 11beta-hydroxysteroid dehydrogenase tpe 2 enzyme. Molecular and Cellular Endocrinology, 105: R11-R17.

Albrecht, G., Sartore, G., Connor, L., Higginbotham, N., Freeman, S., Kelly, B., Stain, H., Tonna, A. & Pollard, G. (2007). Solastalgia: the distress caused by environmental change. Australasian Psychiatry, 15(Supplement 1): S95-S98.

Alcorn, D., Adamson, T., Lambert, T., Maloney, J., Ritchie, B. & Robinson, P. (1977). Morphological effects of chronic tracheal ligation and drainagae in the fetal lamb lung. Journal of Anatomy, 123(Part 3): 649-660.

Alvaro R, de Almeida V, al-Alaiyan S, Robertson M, Nowaczyk B, Cates D, Rigatto H. (1993). A placental extract inhibits breathing induced by umbilical cord occlusion in fetal sheep. Journal of Developmental Physiology, 19(1): 23-28.

Atkinson, H. & Waddell, B. (1995). The hypothalamic-pituitary-adrenal axis in rat pregnancy and lactation: Circadian variation and interrelationship of plasma adrenocorticotropin and corticosterone. Endocrinology, 136 (2): 512-520.

Austin, A., Fagan, D., Mayhew T. (1995). A stereological method for estimating the total number of ventricular myocyte nuclei in fetal and postnatal hearts. Journal of Anatomy, 187(Part 3): 641-647.

Baier RJ, Hasan SU, Cates DB, Hooper D, Nowaczyk B, Rigatto H. (1990). Effects of various concentrations of O2 and umbilical cord occlusion on fetal breathing and behavior. Journal of Applied Physiology, 68(4): 1567-1604.

Baier, R., Hasan, S., Cates, D., Hooper, D., Nowaczyk, B. & Rigatto, H. (1992). Effects of various concentrations of O2 and umbilical cord occlusion on fetal breathing and behavior. Journal of Applied Physiology, 68(4): 1597-1604.

Baker, S., Chebli, M., Rees, S., Lemarec, N., Godbout, R. & Bielajew, C. (2008). Effects of gestational stress: 1. Evaluation of maternal and juvenile offspring behavior. Brain Research, 1213: 98-110.

Barker, P., Boucher R., Yankaskas, J. (1995). Bioelectric properties of cultured monolayers from epithelium of distal human fetal lung. American Journal of Physiology, 268(2 Part 1):L270-L277.

Barker, D. (2004). The developmental origins of adult disease. Journal of the American College of Nutrition, 23(6 Supplement): 588S-595S.

Barnes, P., Jacobs, M., & Roberts, J. (1984). Glucocorticoids preferentially increase fetal alveolar B-adrenoreceptors: autoradiographic evidence. Pediatric Research, 18: 1191-1194.

Barron, D. (1976). Paul Zweifel, pioneer fetal physiologist. Archiv fur Gynakologie, 221: 1-4.

Baud, O., Foix-L'Helias, L., Kaminski, M., Audibert, F., Jarreau, P., Papiernik, E., Huon, C., Lepercq, J., Dehan, M., Lacaze-Masmonteil, T. (1999). Antenatal corticosteroid glucocorticoid treatment and cystic periventricular leukomalacia in very premature infants. New England Journal of Medicine, 341(16): 1229-1231.

Benediktsson, R., Calder, A., Edwards, C. & Seckl, J. (1997). Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clinical Endocrinology, 46(2): 161-166.

Bennet, L., Johnstone, B., Vale, W. & Gluckman, P. (1990). The effects of corticotrophin-releasing factor and two antagonists on breathing movements in fetal sheep. Journal of Physiology, 421: 1-11.

Bian, X., Seidler, F. & Slotkin, T. (1993). Fetal dexamethasone exposure interferes with establishment of cardiac noradrenergic innervation and sympathetic activity. Teratology, 47: 109-117.

Bian, X., Seidler, F., Olsen, C., Raymond, J., Slotkin, T. (1993). Effects of fetal dexamethasone exposure on postnatal control of cardiac adenylate cyclase: beta adrenergic receptor coupling to Gs regulatory protein. Teratology, 48(2): 169-177.

Bissonnette, J., Hohimer, A., Chao, C., Knopp, S. & Notoroberto, N. (1990). Theophylline stimulates fetal breathing movements durin hypoxia. Pediatric Research, 28(2): 83-86.

Bissonnette, J. (2000). Mechanisms regulating hypoxic respiratory depression during fetal and postnatal life. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 278: R1391-R1400.

Bocking, A., Gagnon, R., Milne, K. & White, S. (1988). Behavioral activity during prolonged hypoxemia in fetal sheep. Journal of Applied Physiology, 65: 2420-2426.

Boddy, K., Dawes, G., Fisher, R., Pinter, S. & Robinson, J. (1974). Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. The Journal of Physiology, 243(3): 599-618.

Boddy, K. & Dawes, G. (1975). Fetal breathing. British Medical Bulletin, 31(1): 3-7.

Boels, P., Deutsch, J., Gao, B., & Haworth, S. (2001). Perinatal development influences mechanisms of bradykinin-induced relaxations in pulmonary resistance and conduit arteries differently. Cardiovascular Research, 51(1): 140-150.

Boggs, D. & Bartlett, D. (1982). Chemical specificity of a laryngeal apneic reflex in puppies. Journal of Applied Physiology, 53(2): 455-462.

Bookatz, G., Mayer, C., Wilson, C., Vento, M., Gelfand, S., Haxhiu, M. & Martin, R. (2007). Effect of supplemental oxygen on reinitiation of breathing after neonatal resuscitation in rat pups. Pediatric Research, 61(6): 698-702.

Brette, F. & Orchard, C. (2003). T-tubule function in mammalian cardiac myocytes. Circulation Research, 92(11); 1182-1192.

Brown, E., Lawson, E., Jansen, A., Chernick, V. & Taeusch, W. (1981). Regulation fetal breathing induced by pilocarpine infusion in the near-term fetal lamb. Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology, 50(6): 1348-1352.

Brown, M., Olver, R., Ramsden, C., Strang, L. & Walters, D. (1983). Effects of adrenaline and of spontaneous labour on the secretion and absorption of lung liquid in the fetal lamb. Journal of Physiology, 344:137-152.

Browne, M. (2006). Maternal exposure to caffeine and risk of congenital anomalies: A Systematic Review. Epidemiology, 17(3): 324-331.

Bruce, E. (1981). Control of breathing in the newborn. Annals of Biomedical Engineering, 9: 425-437.

Brunton, P., Russell, J. & Douglas, A. (2008). Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis during pregnancy and lactation. Journal of Neuroendocrinology, 20: 764-776.

Bunn, H. & Briehl, R. (1970). Th interaction of 2,3-Diphosphoglycerate with various human hemoglobins. The Journal of Clinical Investigation, 49: 1088-1095.

Bureau, M., Zinman, R., Foulon, P. & Begin, R. (1984). Diphasic ventilatory response to hypoxia in newborn lambs. Journal of Applied Physiology, 56(1): 84-90.

Burt, M., Arbeit, J. & Brennan, M. (1980). Chronic arterial and venous access in the unrestrained rat. American Journal of Physiology, 238(4): H599-H603.

Burton, P., Smith, R., Krozowski, Z. & Waddell, B. (1996). Zonal distribution of 11beta-Hydroxysteroid dehydrogensase Types 1 and 2 messenger ribonucleic acid expression in the rat placenta and decidua during late pregnancy. Biology of Reproduction, 55: 1023-1028.

Butler, P. & Jones, D. (1997). Physiology of diving birds and mammals. Physiological Reviews, 77(3): 837-899.

Buttigieg, J., Brown, S., Zhang, M., Lowe, M., Holloway, A. & Nurse, C. (2008). Chronic nicotine in utero slectively suppresses hypoxic sensitivity in neonatal rat adrenal chromaffin cells. The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology, 22: 1317-1326.

Calogero, A., Bagdy,G., Szemeredi, K., Tartaglia, M., Gold, P. & Chrousos, G. (1990). Mechanisms of serotonin receptor agonist-induced activation of the hypothalamic - pituitary-adrenal axis in the rat. Endocrinology, 126: 1888-1894.

Calogero, A., Gallucci, W., Chrousos, G. & Gold, P. (1988). Interaction between GABAergic neurotransmission and rat hypothalamic corticotropin-releasing hormone secretion in vitro. Brain Research, 463(1): 28-36.

Campbell, A., Cross, K., Dawes, G. & Hymen, A. (1966). A comparison of air and oxygen in a hyperbaric chamber or by positive pressure ventilation, in the resuscitation of newborn rabbits. The Journal of Pediatrics, 68(2): 153-164.

Chan, R., Brown, E., Ericsson, A., Kovacs, K. & Sawchenko, P. (1993). A comparison of two immediate-early genes, c-fos and NGFI-B, as markers for functional activation in stress-related neuroendocrine circuitry. Journal of Neuroscience, 13(12): 5126-5138.

Chapin, J. (1954). Ventilatory responses of the unrestrained and inanesthetized hamster to CO_2. American Journal of Physiology, 179: 146-148.

Chiu, R. & Bindon, W. (1987). Why are newborn hearts vulnerable to global ischemia? The lactate hypothesis. Circulation, 76(supplement V): V146-V149.

Church, L., Cook, G. & McDermott, M. (2008). Primer: inflammasomes and interleukin 1B in inflammation. Nature Clinical Practice Rheumatology, 4: 34-42.

Cothran, D., Lloyd, T., Taylor, H., Linden, J. & Matherne, G. (1995). Ontogeny of rat myocardial A1 adenosine receptors. Biology of the Neonate, 68(2): 111-118.

Crane, J. (2003). Antenatal corticosteroid therapy for fetal maturation. Journal of Obstetrics and Gynaecology Canada, 25(1); 45-48.

Cross, K. & Warner, P. (1951). The effect of inhalation of high and low oxygen concentrations on the respiration of the newborn infant. Journal of Physiology, 114: 283-295.

Dakshinamurti, S. (2005). Pathophysiologic mechansisms of eristent pulmonary hypertension of the newborn. Pediatric Pulmonology, 39(6): 492-503.

Darnaudery, M. & Maccari, S. (2008). Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Research Reviews, 57(2): 571-585.

Dawes, G., Fox, H., Leduc, B., Liggins, G. & Richards, R. (1970). Respiratory movements and paradoxical sleep in the foetal lamb. Journal of Physiology, 210(1): 47P-48P.

Dawes, G., Fox, H. & Richards, R. (1972). Variations in asphyxial gasping with fetal age in lambs and guinea-pigs. Quarterly Journal of Experimental Physiology and Cognate Medical Sciences, 57(2); 131-138.

Day, C. & Galef, B. (1977). Pup cannibalism: One aspect of maternal behavior in golden hamsters. Journal of Comparative and Phsyiological Psychology, 91(5): 1179-1189.

Denenberg, V. Open field behavior in the rat: what does it mean? Annals of the New York Academy of Sciences, 159(3): 852-859.

DeSantis, D. & Schmaltz, L. (1984). The mother-litter relationship in developmental rat studies: cannibalism vs. caring. Developmental Psychobiology, 17(3): 255-262.

Deshpande, P., Khurana, A., Hansen, P., Wilkins, D. & Thach, B. (1999). Failure of autoresuscitation in weanling mice: significance of cardiac glycogen and heart rate regulation. Journal of Applied Physiology, 87(1): 203-210.

de Weerth, C., Zijl, R. & Buitelaar, J. (2003). Development of cortisol circadian rhythm in infancy. Early Human Development, 73 (1-2): 39-52.

Diaz, R., Brown, R. & Seckl, J. (1998). Distinct ontogeny of glucocorticoid and mineralocorticoid receptor and 11beta-Hydroxysteroid dehydrogenase types I and II mRNAs in the fetal rat brain suggest a complex control of glucocorticoid actions. The Journal of Neuroscience, 18(7): 2570-2580.

Diego, M., Jones, N., Field, T., Hernandez-Reif, M., Schanberg, S., Kuhn, C. & Gonzalez-Garcia, A. (2006). Maternal psychological distress, prenatal cortisol and fetal weight. Psychosomatic Medicine, 68(5): 747-753.

Dombrowsky, H., Tschernig, T., Vieten, G., Rau, G., Ohler, F., Acevedo, C., Behrens, C., Poets, C., von der Hardt, H. & Bernhard, W. (2006). Molecular and functional changes of pulmonary surfactant in response to hyperoxia. Pediatric Pulmonology, 41(11): 1025-1039.

Douglas, A., Johnstone, H., Hatzinger, M., Neumann, I., Landgraf, R., & Russell, J. (1996). Pregnancy reduces hypothalamo-pituitary-adrenal (HPA) axis secretory responses to stressors in the rat. Journal of Physiology, 495P: 109P.

Douglas, A., Johnstone, H., Wigger, A., Landgraf, R., Russell, J. & Neumann, I. (1998). The role of endogenous opioids in neurohypophysical and hypothalamo-pituitary-adrenal axis hormone secretory responses to stress in pregnant rats. Journal of Endocrinology, 158: 285-293.

Douglas, A., Brunton, P. Bosch, O., Russell, J. & Neumann, I. (2003). Neuroendocine responses to stress in mice: Hyporesponsiveness in pregnancy and parturition. Endocrinology, 144(12): 5268-5276.

Douglas, A., Meddle, S., Tosch, N., Bosch, O. & Neumann, I. (2005). Reduced activity of the noradrenergic system in the paraventricular nucleus at the end of pregnancy: implications for stress hyporesponsiveness. Journal of Neuroendocrinology, 17(1): 40-48.

Dupouy, J., Coffigny, H. & Magre, S. (1975). Maternal and foetal corticosterone levels during late pregnancy in rats. Journal of Endocrinology, 65: 347-352.

Eden, G. & Hanson, M. (1987). Maturation of the respiratory response to acute hypoxia in the newborn rat. Journal of Physiology, 392: 1-9.

Edwards, C., Benediktsson, R., Lindsay, R. & Seckl, J. (1996). 11 beta-Hydroxysteroid dehydrogenases: key enzymes in determining tissue-specific glucocorticoid effects. Steroids, 61(4): 263-269.

Eliason, H. & Fewell, J. (1997). Thermoregulatory control during pregnancy and lactation in rats. Journal of Applied Physiology, 83(3): 837-844.

Englmon, D., Ek, M., Saha, S., Ericsson-Dahlstrand, A., Jakobsson, P. & Blomqvist, A. (2002). Prostaglandins as inflammatory messengers across the blood-brain barrier. Journal of Molecular Medicine, 80(1): 5-15.

Eriksen, P., Gennser, G., Lofgren. O. & Nilsson, K. (1983). Acute effects of maternal smoking on fetal breathing and movements. Obstetrics and Gynecology, 61(3): 367-372.

Euker, J. & Riegle, G. (1973). Effects of stress on pregnancy in the rat. Journal of Reproduction and Fertility, 34: 343-346.

Feldman, J. & Negro, C. (2006). Looking for inspiration: new perspectives on respiratory rhythm. Nature Reviews. Neuorscience, 7(3): 232-242.

Fewell, J., Lee, C. & Kitterman, J. (1981). Effects of phrenic nerve section on the respiratory system of fetal lambs. Journal of Applied Physiology, 51(2): 293-297.

Fewell, J. & Tang, P. (1997). Pregnancy alters body-core temperature response to a simulated open field in rats. Journal of Applied Physiology, 82(4): 1406-1410.

Fewell, J. & Smith, F. (1998). Perinatal nicotine exposure impairs ability of newborn rats to autoresuscitate from apnea during hypoxia. Journal of Applied Physiology, 85(6): 2066-2074.

Fewell, J., Smith, F., Ng, V., Wong, V. & Wang, Y. (2000). Postnatal age influences the ability of rats to autoresuscitate from hypoxia-induced apnea. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology , 279(1): R39-R46.

Fewell, J., Smith, F. & Ng, V. (2001). Prenatal exposure to nicotine impairs protective responses of rat pups to hypoxia in an age-dependent manner. Respiratory Physiology, 127(1): 61-73.

Fewell, J., Ng, V. & Zhang, C. (2005). Prior exposure to hypoxic-induced apnea impairs protective responses of newborn rats in an exposure-dependent fashion: influence of normoxic recovery time. Journal of Applied Physiology, 99(4): 1607-1612.

Fewell, J. (2005). Protective responses of the newborn to hypoxia. Respiratory Physiology and Neurobiology, 149(1-3): 243-255.

Fewell, J. Zhang, C. & Gillis, A. (2007). Influence of adenosine A (1)-receptor blockade and vagotomy on the gasping and heart rate response to hypoxia in rats during early postnatal maturation. Journal of Applied Physiology, 103(4): 1234-1241.

Fick, A. (1855). On liquid diffusion. Philosophical Magazine, 10: 31-39.

Fisher, D., Heymann, M. & Rudolph, A. (1980) Myocardial oxygen and carbohydrate consumption in fetal lambs in utero and in adult sheep. American Journal of Physiology, 238(3): H399-H405.

Fleming, P., Levine, M., Goncalves, A. & Woollard, S. (1983). Barometric plethysmograph: advantages and limitations in recording infant respiration. Journal of applied Physiology, 55(6): 1924-1931.

Fox, H., Steinbrecher, M., Pessel, D., Inglis, J., Medvid, L. & Angel, E. (1978). Maternal ethanol ingestion and the occurrence of human fetal breathing movements. American Journal of Obstetrics and Gynecology, 132(4): 354-358.

Fung, M., Wang, W. & St.John, W. (1995). Medullary loci critical for expression of gasping in adult rats. Journal of Physiology, 480 (Part 3): 597-611.

Fung, M., Huang, Q., Zhou, D. & St. John, W. (1997). The morphology and connections of neurons in the gasping centre of adult rats. Neuroscience, 76(4): 1237-1244.

Gershan, W., Jacobi, M., Thach, B. (1990). Maturation of cardiorespiratory interactions in spontaneous recovery from hypoxic apnea. Pediatric Research, 28(2): 87-93.

Gershan, W., Jacobi, M. & Thach, B. (1992). Mechanisms underlying induced autoresuscitation failure in BALB/c and SWR mice. Journal of Applied Physiology, 72(2): 677-685.

Gnaiger, E. (2003). Oxygen conformance of cellular respiration. A perspective of mitochondrial physiology. Advances in Experimental Medicine and Biology, 543: 39-55.

Godfrey, S. (1968). Respiratory and cardiovascular changes during asphyxia and resuscitation of fetal and newborn rabbits. Experimental Physiology, LIII(2): 97-118.

Goldstein, D. & Kopin, I. (2007). Evolution of concepts of stress. Stress, 10(2): 109-120.

Gordon, C. (1990).Thermal biology of the laboratory rat. Physiology & Behavior, 47(5): 963-991.

Gordon, J., Moreira, G., O'Donnell, D., Aldinger, A. & Tod, M. (1997). Relative effects of cyclooxygenase and nitric oxide synthase inhibition on vascular resistances in neonatal lamb lungs. Pediatric Research, 42(6): 738-743.

Gozal, D., Torres, J., Gozal, Y. & Nuckton, T. (1996). Characterization and development aspects of anoxia-induced gasping in the rat. Biology of Neonate, 70: 280-288.

Gozal, D. & Torres, J. (1997). Maturation of anoxia –induced gasping in the rat: Potential role for N-Methyl-D-Aspartate glutamate receptors. Pediatric Research, 42: 872-877.

Gozal, D., Torres, J., Gozal, E., Nuckton, T., Dixon, M., Gozal, Y & Hornby, P. (1998). Nitric oxide modulates anoxia-induced gasping in the developing rat. Biology of the Neonate, 73(4): 264-274.

Gozal, D. & Torres, J. (2001). Brainstem nitric oxide tissue levels correlate with anoxia-induced gasping activity in the developing rat. Biology of the Neonate, 79: 122-130.

Gozal, D., Gozal, E., Reeves, S. & Lipton, A. (2002). Gasping and autoresuscitation in the developing rat: Effect of antecedent intermittent hypoxia. Journal of Applied Physiology, 2: 1141-1144.

Greisen, M., Bolwig, T. & Wortwein, G. (2005). Cholecystokinin tetrapeptide effects of the HPA axis function and elevated plus maze behavior in maternally separated and handled rats. Behavioural Brain Research, 161(2): 204-212.

Groves, A., Kuschel, C., Knight, D. & Skinner, J. (2008). Echocardiographic assessment of blood flow volume in the superior vena cava and descending aorta in the newborn infant. Archives of Diseases in Children, Fetal and Neonatal Edition, 93(1): F24-F28.

Guayee, H. & Auchus, R. (2007). Basic concepts and recent developments in humansteroid hormone biosynthesis. Reviews in Endcrine and Metabolic Disorders, 8(4): 289-300.

Gunn, T. & Tonkin, S. (1989). Upper airway measurements during inspiration and expiration in infants. Pediatrics, 84(1): 73-77.

Guntheroth, W. & Kawabori, T. (1975). Hypoxic apnea and gasping. The Journal of Clinical Investigation, 56(6): 1371-1377.

Guntheroth, W. (1989). Interleukin-1 and intermediary causing prolonged sleep apnea and SIDS during reposiratory infections. Medical Hypotheses, 28: 121-123.

Hall, C. (1934). Emotional behavior of the rat. I. Defaecation and urination as measures of individual differences in emotionality. Journal of Comparative Psychology, 18: 385-403.

Hall, C. (1936). Emotional behavior of the rat. III. The relationship between emotionality and ambulatory behavior. Journal of Comparative Psychology, 22: 345-352.

Hall, S., Bermel, M., Ko, Y., Palmer, H., Enhorning, G. & Notter, R. (1993). Approximations in the measurement of surface tension on the oscillating bubble surfactometer. Journal of Applied Physiology, 75: 468-477.

Hanson, M. & Torrance, R. (1981). The location of carbonic anhydrase in relation to the blood-brain barrier at the medullary chemoreceptors of the cat. Journal of Physiology, 320: 113-125.

Harding, R. and Hooper, S. (1996). Regulation of lung expansion and lung growth before birth. Journal of Applied Phsyiology, 81(1): 209-224.

Hashim, E., Laberge, J., Chen, M. & Quillen, E. (1995). Reversible tracheal obstruction in the sheep: effects on tracheal fluid pressure and lung growth. Journal of Pediatric Surgery, 30(8): 1172-1177.

Hauger, R., Grigoriadis, D., Dallman, M., Plotsky, P., Vale, W. & Dautzenberg, M. (2003). International Union of Pharmacology. XXXVI. Current Status of the Nomenclature for Receptors for Corticotropin-Releasing Factor and Their Ligands. Pharmacological Reviews, 55(1): 21-26.

Heitzer, M., Wolf, I., Sanchez, E., Witchel, S. & DeFranco, D. (2007). Glucocorticoid receptor physiology. Reviews in Endocrine & Metabolic Disorders, 8: 321-330.

Heldt, G. & McIlroy, M. (1987). Distortion of chest wall and work of diaphragm in preterm infants. Journal of Applied Physiology, 62(1): 164-169.

Henderson, L. (1908). Concerning the relationship between the strength of acids and their capacity to preserve neutrality. American Journal of Physiology, 21(2): 173-179.

Henderson-Smart, D. & Read, D. (1976). Depression of respiratory muscles and defecrive responses to nasal obstruction during active sleep in the newborn. Australian Pediatric Journal, 12(4): 261-266.

Henderson-Smart, D. (1980). Vulnerability to hypoxemia in the newborn. Sleep, 3: 331-342.

Henderson-Smart, D. & Cohen, G. (1986). Apnea in the newborn infant. Australian Pediatric Journal, 22(Supplement 1): 63-66.

Henderson-Smart, D. & Steer, P. (2001). Methylxanthine treatment for apnea in preterm infants. Cochrane Database Systematic Reviews, 3: CD000140.

Herman, J. (1993). Regulation of adrenocorticosteroid receptor mRNA expression in the central nervous system. Cellular and molecular neurobiology, 13(4): 349-372.

Hertzberg, T., Hellstrom, S., Langercrantz, H. & Pequignot, J. (1990). Development of the arterial chemoreflex and turnover of carotid body catecholamines in the newborn rat. Journal of Physiology, 425: 211-225.

Hobel, C., Goldstein, A. & Barrett, E. (2008). Psychosocial stress and pregnancy outcome. Clinical Obstetrics and Gynecology, 51(2); 333-348.

Hofstetter, A. & Herlenius, E. (2005). Interleukin -1B depresses hypoxic gasping and autoresuscitation in neonatal DBA/11acJ mice. Respiratory Physiology & Neurobiology, 146: 135-146.

Hofstetter, A., Saha, S., Siljehav, V., Jakobsson, P., & Herlenius, E. (2007). The induced prostaglandin E2 pathway is a key regulator of the respiratory response to infection and hypoxia in neonates. Proceedings of the National Academy of Sciences of the United States of America, 104(23): 9894-9899.

Hooper, S., Han, V. & Harding, R. (1993). Changes in lung expansion alter pulmonary DNA synthesis and IGF-II gene expression in fetal sheep. American Journal of Physiology, 265(4 Part 1): L403-L409.

Inanlou, M., Baguma-Nibasheka, M. & Kablar, B. (2005). The role of fetal breathing-like movements in lung organogenesis. Histology and Histopatholy, 20: 1261-1266.

Jacobi, M., Gershan, W., & Thach, B. (1991). Mechansim of failure of receovery from hypoxic apnea by gasping in 17-23 day old mice. Journal of Applied Physiology, 72(2): 677-685.

Jacobi, M. & Thach, B. (1989). Effect of maturation on spontaneous recovery from hypoxic apnea by gasping. Journal of Applied Physiology, 66(5): 2384-2390.

Jain, L. & Easton, D. (2006). Physiology of fetal lung fluid clearance and the effect of labor. Seminars in Perinatology, 30(1): 34-43.

Jarmakani, J., Nakazawa, M., Nagatomo, T., & Langer, G. Effect of hypoxia on mechanical function in the neonatal mammalian heart. American Journal of Physiology, 235(5): H469-H474.

Johnstone, H., Wigger, A., Douglas, A., Neumann, I., Landgraf, R. Seckl, J & Russell, J. (2000). Attenuation of hypothalamic-pituitary-adrenal axis stress responses in late pregnancy: changes in feed-forward and feedback mechanisms. Journal of Neuroendocrinology, 12(8): 811-822.

Johnston, M. (2005). Excitotoxicity in perinatal brain injury. Brain Pathology, 15: 234-240.

Kahraman, L. & Thach, B. (2004). Inhibitory effects of hyperthermia on mechanisms involved in autoresuscitation from hypoxic apnea in mice: a model for thermal stress causing SIDS. Journal of Applied Physiology, 97: 669-674.

Karlberg, P., Cherry, R., Escardo, F. & Koch, G. (1962). Pulmonary ventilation and mechanics of breathing in the first minutes of life, including the onset of respiration. Acta Paediatrica Scandinavica, 51:121-136.

Kauffman, K., Seidler, F., & Slotkin, T. (1994). Prenatal dexamethasone exposure causes loss of neonatal hypoxia tolerance: Cellular mechanisms. Pediatric Research, 35(5): 515-522.

Kahraman, L. & Thach, B. (2004). Inhibitory effects of hyperthermia on mechanisms involved in autoresuscitation from hypoxic apnea in mice: a model for thermal stress causing SIDS. Journal of Applied Physiology, 97(2): 669-674.

Keller–Wood, M. & Dallman, M. (1984). Corticosteroid inhibition of ACTH secretion. Endocrine Reviews, 5(1): 1-24.

Kerzner, L., Stonestreet, B., Wu, K., Sadowska, G. & Malee, M. (2002). Antenatal dexamethasone: effect on ovine placental 11beta-hydroxysteroid dehydrogenase type 2 expression and fetal growth. Pediatric Research, 52(5): 706-712.

Khurana, A. & Thach, B. (1996). Effects of upper airway stimuli on swallowing, gasping and autoresuscitation in hypoxic mice. Journal of Applied Physiology, 80: 472-481.

Kirby, R. & McCarty, R. (1987). Ontogeny of functional sympathetic innervation to the heart and adrenal medulla in the preweanling rat. Journal of the Autonomic Nervous System, 19: 67-75.

Kinney, H., Richerson, G., Dymecki, S., Darnall, R. & Nattie, E. (2009). The brainstem and serotonin in the sudden death syndrome. Annual Review of Pathology, 4: 517-550.

Kitterman, J., Ballard, P., Clements, J., Mescher, E. & Tooley, W. (1979). Tracheal fluid in fetal lambs: spontaneous decrease prior to birth. Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology, 47(5): 985-989.

Kitterman, J., Liggins, G., Clements, J. & Tooley, W. (1979). Stimulation of breathing movements in fetal sheep by inhibitors of prostaglandin synthesis. Journal of Developmental Physiology, 1(6): 453-466.

Kitterman, J., Liggins, G., Fewell, J. &Tooley, W. (1983). Inhibition of breathing movements in fetal sheep by prostaglandins. Journal of Applied Physiology, 54(3): 687-692.

Kitterman, J. (1996). The effects of mechanical forces on fetal lung growth. Clinics in Perinatology, 23(4); 727-737.

Kleiber, M. & Rogers, T. (1961). Energy metabolism. Annual Review of Physiology, 23:5-36.

Knill, R., Andrews, W., Bryan, A. &Bryan, M. (1976). Respiratory load compensation in infants. Journal of Applied Physiology, 40(3): 357-361.

Kobayashi, S., Onimarau, H., Inoue, M., Inoue, T. & Sasa, R. (2005). Localization and properties of respiratory neurons in the rostral pons of the newborn rat. Neuroscience, 134(1): 317-325.

Koos, B., Mason, B., Punla, O. & Adinolfi, A. (1994). Hypoxic inhibition of breathing in fetal sheep: relationship to brain adenosine concentrations. Journal of Applied Physiology, 77(6): 2734-2739.

Koos, B., Maeda, T., Jan, C. & Lopez, G. (2002). Adenosine A(2A) receptors mediate hypoxic inhibition of fetal breathing in sheep. American journal of Obstetrics and Gynecology, 186(4):663-668.

Kramer, M., Lydon, J., Seguin, L., Goulet, L., Kahn, S., McNamara, H., Genest, J., Dassa, C., Chen, M., Sharma, S., Meaney, M., Thomson, S., van Uum, S., Koren, G., Dahhou, M., Lamoureaux, J. & Platt, R. (2009). Stress pathways to spontaneous preterm birth: The role of stressors, psychological distress and stress hormones. American Journal of Epidemiology, 169(11): 1319-1326.

Lachin, J. (1981). Introduction to sample size determination and power analysis for clinical trials. Controlled Clinical Trials, 2: 93-113.

Lalani, S., Remmers, J., & Hasan, S. (2001). Breathing patterns, pulmonary mechanics and gas exchange: role of vagal innervation in noenatl lamb. Experimental Physiology, 86(6): 803-810.

Lagercrantz, H. & Slotkin, T. (1986). The stress of being born. Scientific American, 254(4): 100-107.

Lau, C. & Slotkin, T. (1981). Maturation of sympathetic neurotransmission in the rat heart. VII. Suppression of sympathetic responses by dexamethasone. The Journal of Pharmacology and Experimental Therapeutics, 216(1): 6-11.

Lavezzi, A. & Matturri, L. (2008). Functional neuroanatomy of the human pre- Botzinger complex with particular reference to sudden unexplained perinatal and infant death. Neuropathology, 28(1): 10-16.

Lawson, E. & Thach, B. (1977). Respiratory patterns during progressive asphyxia in newborn rabbits. Journal of Applied Physiology, 43(3): 468-474.

Leiter, J. & Böhm, I. (2007). Mechanisms of pathogenesis in the Sudden Infant Death Syndrome. Respiratory Physiology and Neurobiology, 159(2): 127-138.

Lestage, P., Vitte, P., Rolinat, J., Minot, R., Broussolle, E. & Bobillier, P. (1985). A chronic arterial and venous cannulation method for freely moving rats. Journal of Neuroscience Methods,13(3-4) 213-222.

Liggins, G. (1976). Adrenocortical-related maturational events in the fetus. American Journal of Obstetrics and Gynecology, 126(7): 931-941.

Liggins, G. & Kitterman, J. (1981). Development of the fetal lung. CIBA Foundation Symposium, 86: 308-330.

Liggins, G., Vilos, G., Campos, G., Kitterman, J. & Lee, C. (1981). The effect of spinal cord transection on lung development in fetal sheep. Journal of Developmental Physiology, 3: 267-274.

Liggins, G. (1994). The role of cortisol in preparing the fetus for birth. Reproduction, Fertility, and Development, 6: 141-150.

Lightman, S., Windle, R., Wood, S., Kershaw, Y., Shanks, N. & Ingram, C. (2001). Peripartum plasticity within the hypothalamo-pituitary-adrenal axis. Progress in Brain Research, 133: 111-129.

Lightman,S., Windle, R., Ma, X., Harbuz, M., Shanks, N., Julian, M., Wood, S., Kershaw, Y. & Ingram, C. (2002). Hypothalamic-pituitary-adrenal function. Archives of Physiology and Biochemistry, 110(102):90-93.

Lightman, S. (2008). The neuroendocrinology of stress: a never-ending story. Journal of Neuroendocrinology, 20(6): 880-884.

Liston, R., Sawchuck, D. & Young, D. (2007). Fetal health surveillance: Antepartum and intrapartum consensus guideline. Journal of Obstetrics and Gynecology Canada, 29(9, Supplement 4): S3-S56.

Liu, M., Liu, J., Buch, S., Tanswell, A. & Post, M. (1995). Antisense oligonucleotides for PDGF-B and its receptor inhibit mechanical strain-induced fetal lung cell growth. American Journal of Physiology: Lung Cellular and Molecular Physiology, 269(2): L178-L184.

Longo, L. & Power, G. (1969). Analyses of PO_2 and PCO_2 differences between maternal and fetal blood in the placenta. Journal of Applied Physiology, 26(1): 48-55.

Lopez-Barneo, J., Lopez-Lopez, J., Urena, J. & Gonzalez, C. (1988). Chemotransduction in the carotid body: K+ current modulated by PO2 in Type I chemoreceptor cells. Science, 241(4865): 580-582.

Lumsden, T. (1923). Observations on the respiratory centres in the cat. Journal of Physiology, 57(3-4): 153-160.

Ma, S., Shipston, M., Morilak, D. & Russell, J. (2005). Reduced hypothalamic vasopressin secretion underlies attenuated adrenocortciotropin stress responses in pregnant rats. Endocrinology, 146(3): 1626-1637.

Macara, L., Kingdom, J., Kaufmann, P., Kohen, G., Hair, J., More, I., Lyall, F. & Greer, I. (1996). Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. Placenta, 17(1): 37-48.

MacDougall, J. & McCabe, M. (1967). Diffusion coefficient of oxygen through tissues. Nature, 215: 1173-1174.

MacLennan, A., Emerson, P., Hunter, D. & Darley, J. (1976). Tissue oxygenation and red cell 2,3-Diphosphoglycerate in normal and abnormal pregnancy. British Journal of Obstetrics and Gynecology, 83: 378-382.

Majzoub, J. & Karalis, K. (1999). Placental corticotropin-releasing hormone: Function and regulation. American Journal of Obstetrics and Gynecology,180(1 Pt 3): S242-246.

Makrigiannakis, A., Zoumakis, E., Kalantaridou, S. & Chrousos, G. (2004). Endometrial and placental CRH as regulators of human embryo implantation. Journal of Reproductive Immunology, 62(1-2): 53-59.

Mancuso, R., Schetter, C., Rini, C., Roesch, S. & Hobel, C. (2004). Maternal prenatal anxiety and corticotropin-releasing hormone associated with timing of delivery. Psychosomatic Medicine, 66(5): 762-769.

Manning, F, Walker, D. & Feyerabend, C. (1978). The effect of nicotine on fetal breathing movements in conscious pregnant ewes. Obstetrics and Gynecology, 52(5): 563-568.

Mastorakos, G. & Ilias, I. (2003). Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. Annals of the New York Academy of Sciences, 997: 136-149.

Matalon, S. (1991). Mechanisms and regulation of ion transport in adult mammalian alveolar type II pneumocytes. American Journal of Physiology: Cell Physiology, 261(3): C727-C738.

Mathew, O., Thach, B., Abu-Osba, Y., Brouillette, R. & Roberts, J. (1984). Regulation of upper airway maintaining muscles during progressive asphyxia. Pediatric Research, 18(9): 819-822.

Mayhew, T. (2006). Allometric studies on growth and development of the human placenta: growth of tissue compartments and diffusive conductances in relation to placental volume and fetal mass. Journal of Anatomy, 208(6): 785-794.

Mayhew, T., Joy, C. & Haas, J. (1984). Structure-function correlation in the human placenta: the morpholometric diffusing capacity for oxygen at full term. Journal of Anatomy, 139: 691-708.

McGowan, J., Devoe, L., Searle, N. & Altman, R. (1987). The effects of long and short term maternal caffeine ingestion on human fetal breathing and body movements in term gestations. American Journal of Obstetrics and Gynecology, 157(3): 726-729.

Miller, D. & O'Callaghan, J. (2002). Neuroendocrine aspects of the response to stress. Metabolism, 51(6 Supplement 1): 5-10.

Miller, W. (2008). Steroidogenic enzymes. Endocrine Development, 13: 1-8.

Milsom, W. & Burleson, M. (2007). Peripheral arterial chemoreceptors and the evolution of the carotid body. Respiratory Physiology and Neurobiology, 157(1): 4-11.

Mizusawa, A., Ogawa, H., Kikuchi, Y., Hida, W., Kurosawa, H., Okabe, S.,Takishima, T. & Shirato, K. (1994). In vivo release of glutamate in nucleus tractus solitarii of the rat during hypoxia. Journal of Physiology, 478(1): 55-66.

Moessinger, A., Harding, R., Adamson, T., Singh, M. & Kiu, G. (1990). Role of lung fluid volume in growth and maturation of the fetal sheep lung. Journal of Clinical Investigation, 86(4): 1270-1277.

Moore, T., Lione, A., Regen, D., Tarpley, H. & Raines, P. (1971). Brain glucose metabolism in the newborn rat. American Journal of Physiology, 221(6): 1746-1753.

Mullie, A., Lewi, P. & Van Hoeyweghen, R. (1989). Pre-CPR conditions and the final outcome of CPR. Resuscitation, 17(Supplement):S11-S21.

Murphy, V & Clifton, V. (2003). Alerations in human placental 11 beta-hydroxysteroid dehydrogenase type 1 and 2 with gestational age and labour. Placenta, 24(7): 739-744.

Navarro, H., Kudlacz, E., Eylers, J. & Slotkin, T. (1989). Prenatal dexamethasone administration disrupts the pattern of cellular development in rat lung. Teratology, 40: 433-438.

Neerhof, M. & Thaete, L. (2008). The fetal response to chronic placental insufficiency. Seminars in Perinatology, 32(3): 201-205.

Neumann, I., Johnstone, H., Hatzinger, M., Liebsch, G., Shipston, M., Russell, J., Landgraf, R. & Dougals, A. (1998). Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes. The Journal of Physiology, 508:289-300.

Neumann, I., Kromer, S., & Bosch, O. (2005). Effects of psycholosocial stress during pregnancy on neuroendocrine and behavioral parameters in lactation depend on the genetically determined stress vulnerability. Psychoneuroendocrinology, 30(8); 791-806.

Neumann, I. (2008). Brain oxytocin: a key regulator of emotional and social behaviors in both females and males. Journal of Neuroendocrinology, 20(6): 858-865.

O'Brodovich, H., Hannam, V., Seear, M. & Mullen, J. (1990). Amiloride impairs lung water clearance in newborn guinea pigs. Journal of Applied Physiology, 68(4): 1758-1762.

O'Brodovich, H., Canessa, C., Ueda, J., Rafii, B., Rossier, B. & Edelson, J. (1993). Expression of the epithelial Na+ channel in the developing rat lung. American Journal of Physiology: Cell Physiology, 265(34): C491-C496.

Olfert, E., Cross, B. & McWilliam, A. (1993). Guide to the Care and Use of Experimental Animals. Volume I. Canadian Council on Animal Care.

Olsson, A., Kayhan, G., Langercrantz, H. & Herlenius, E. (2003). IL-1B depresses respiration and anoxic survival via a prostaglandin-dependent pathway in neonatal rats. Pediatric Research, 54(3): 326-331.

Olver, R. & Strang, L. (1974). Ion fluxes across the pulmonary epithelium and the secretion of lung liquid in the foetal lamb. Journal of Physiology, 241: 327-357.

O'Regan, R. & Majcherczyk, S. (1982). Role of peripheral chemoreceptors and central chemosensitivity in the regulatioin of respiration and circulation. Journal of Experimental Biology, 100: 23-40.

Osborne, B. (1981). The electrocardiogram of the rat. In The Rat Electrocardiogram in Pharmacology and Toxicology. R. Budden, D. Detweiler & G. Zbinden, Eds. Pergamon Press, New York, NY: pp. 15-28.

Pardon, M., Gerardin, P., Joubert, C., Perez-Diaz, F. & Cohen-Salmon, C. (2000). Influence of prepartum chronic ultramild stress on maternal pup behavior in mice. Biological Psychiatry, 47(10): 858-863.

Parrish, M. & Farrar, S. (1990). Force and oxygen consumption in the immature rabbit heart. Pediatric Research, 27(5): 476-482.

Paternotte, E., Gacher, C., Labrude, P., Stoltz, J. & Menu, P. (2008). Review: Behavior of endothelial celss faced with hypoxia. Biomedical Materials and Engineering, 18: 295-299.

Paulose, C. & Dakshinamurti, K. (1987). Chronic catheterization using vascular access port in rats: blood sampling with minimal stress for plasma catecholamine determination. Journal of Neuroscience Methods, 22: 141-146.

Peeters, L., Sheldon, R., Jones, M., Makowski, E. & Meschia, G. (1979). Blood flow to fetal organs as afunction of arterial oxygen content. American Journal of Obstetrics and Gynecology, 135(5): 637-646.

Perkett, E. & Vaughan, R. (1982). Evidence for a laryngeal chemoreflex in some human preterm infants. Acta Pediatrica Scandanvica, 71(6): 969-972.

Perrin, M. & Vale, W. (1999). Corticotropin releasing factor receptors and their ligand family. Annals of the New York Academy of Sciences, 885: 312-328.

Phelps, D. & Floros, J. (1991). Dexamethasone in vivo raises surfactant protein B mRNA in alveolar and bronchiolar epithelium. American Journal of Physiology, 260(2 Part 1): L146-L152.

Picard, N., Guenin, S., Larnicol, N. & Perrin, Y. (2008). Maternal caffeine ingestion during gestation and lactation influences respiratory adatation to acute alveolar hypoxia in newborn rats and adenosine A2A and GABAa receptor mRNA transcription. Neuroscience, 156: 630-639.

Poblano, A., Haro, R., & Arteaga, C. (2007). Neuorphysiologic measurement of continuity in the sleep of fetuses in the last week of pregnancy and in newborns. International Journal of Biological Sciences, 4(1): 23-28.

Poets, C., Meny, R., Chobanian & Bonofiglo, R. (1999). Gasping and other cardiorespiratory patterns during sudden infant deaths. Pediatric Research, 45: 350-354.

Polit, D. (1996). Data Analysis and Statistics. Prentice Hall International. London: UK.

Power, G., Longo, L., Wagner, H., Kuhl, D. & Forster, R. (1967). Uneven distribution of maternal and fetal placental blood flow, as demonstrated using mcroaggregates and its response to hypoxia. The Journal of Clinical Investigation, 46(12): 2053-2063.

Power, G. (1972). Some aspects of O_2 and CO_2 transfer in the placenta. Chest, 61(2): 22S- 24S.

Quilligan, E., Clewlow, F., Johnston, B., & Walker, D. (1981). Effect of 5-hydroxytryptophan on electrocortical activity and breathing movements of fetal sheep. American Journal of Obstetrics and Gynecology, 141(3): 271-275.

Rabi, Y., Rabi, D. & Yee, W. (2007). Room air resuscitation of the depressed newborn: A systematic review and meta-analysis. Resuscitation, 72: 353-363.

Raff, H., Hong, J., Oaks, M. &Widmaier, E. (2003). Adrenocortical responses to ACTH in neonatal rats: effect of hypoxia from birth on corticosterone, StAR, and PBR. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 284(1):R78-R85.

Ramirez, J. & Viemari, J. (2005). Determinants of inspiratory activity. Respiratory Physiology and Neurobiology, 147(2-3): 145-157. Annual Review of Physiology, 60: 385-405.

Rasanen, J., Debbs, R., Wood, R., Weiner, S., Weil. S. &Huhta, J. (1997). Human fetal right ventricular ejection force under abnormal loading conditions during the second half of pregnancy. Ultrasound in Obstetrics and Gynecology, 10(5):325-332.

Richardson, B., Hohimer, A., Mueggler, P. & Bissonnette, J. (1982). Effects of glucose concentration on fetal breathing movements and electrocortical activity in fetal lambs. American Journal of Obstetrics and Gynecology, 142(6 Part 1):678-683.

Richardson, H., Walker, A. & Horne, R. (2008). Sleep position alters arousal processes maximally at the high-risk age for sudden infant death syndrome. Journal of Sleep Research, 17(4):450-457.

Richter, D., Ballanyi, K. & Schwarcher, S. (1992). Mechanisms of respiratory rhythm generation. Current Opinion in Neurobiology, 2(6): 788-793.

Rivkees, S., Zhao, Z., Porter, G. & Turner, C. (2001). Influences of adenosine on the fetus and newborn. Molecular Genetics and Metabolism, 74: 160-171.

Robertson, B. (1993). Corticosteroids and surfactant for prevention of neonatal RDS. Annals of Medicine, 25(3): 285-288.

Robinson, B., Emanuel, R., Frim, D. & Majzoub, J. (1988). Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. Proceedings of the National Academy of Sciences United States, 85: 5244-5248.

Robinson, R. (1996). Autonomic receptor-effector coupling during postnatal development. Cardiovascular Research, 31: E68-E76.

Rolph, T. & Jones, C. (1983). Regulation of glycolytic flux in the heart of the fetal guinea pig. Journal of Developmental Physiology, 5(1): 31-49.

Rooney, S. (1985). The surfactant system and lung phospholipid biochemistry. American Review of Respiratory Disease, 131: 439-460.

Rosenfeld, P., Suchecki, D. & Levine, S. (1992). Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. Neuroscience and Behavioral Reviews, 16(4): 553-568.

Ross, B. (1967). Comparative properties of the lungs and the placenta: A graphical analysis of placental gas exchange. Development of the Lung. CIBA Foundation.

Roth, K. & Katz, R. (1979). Stress, behavioural arousal, and open field activity. A reexamination of emotionality in the rat. Neuroscience & Biobehavioral Reviews, 3(4): 247-263.

Roux, J., Brismar, H., Asperia, A. & Langercrantz, H. (2005). Developmental changes in HIF transcription factor in carotid body: Relevance for O2 sensing by chemoreceptors. Pediatric Research, 58(1): 53-57.

Rudolph, A. & Heymann, M. (1970). Circulatory changes during growth in the fetal lamb. Circulation Research, XXVI: 289-299.

Saiki, C., Ikeda, M., Nishikawa, T., Tanimoto, T., Yoshida, S. & Matsumoto, S. (2001). The process of cardiorespiratory autoresuscitation in intact newborn rats. Canadian Journal of Physiology and Pharmacology, 79(12): 1036-1043.

Sasaki, A., Tempst, P., Liotta, A., Margioris, A., Hood, L., Kent, S., Sato, S., Shinkawa, O., Yoshinaga, K. & Krieger, D. (1988). Isolation and characterization of a corticotropin-releasing hormone-like peptide from human placenta. The Journal of Endocrinology and Metabolism, 67(4):768-773.

Schulte, H., Weisner, D. & Allolio, B. (1990). The corticotropin releasing hormone test in late pregnancy: lack of adrenocorticotrophin and cortisol response. Clinical Endocrinology, 33(1): 99-106.

Schwartz, A. & Rall, T. (1975). Hormonal regulation of glycogen metabolism in human fetal liver. Regulation of glycogen synthase activity. Diabetes, 24(12): 1113-1122.

Seckl, J. (2004). Prenatal glucocorticoids and long-term programming. European Journal of Endocrinology, 151 (Supplement 3): U49-U62.

Seidler, F. & Slotkin, T. (1985). Adrenomedullary function in the neonatal rat: responses to acute hypoxia. Journal of Physiology, 358:1-16.

Serdarevich, C. & Fewell, J. (1999). Influence of core temperature on autoresuscitation during repeated exposure to hypoxia in normal rat pups. Journal of Applied Physiology, 87(4): 1346-1353.

Shah, P., Riphagen, S., Beyene, J. & Perlman, M. (2004). Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. Archives of Diseases in Childhood, Fetal and Neonatal Edition, 89(2): F152-F155.

Shaul, P. & Wells, L. (1994). Oxygen modulates nitric oxide production selectively in fetal pulmonary endothelial cells. American Journal of Respiratory Cell and Molecular Biology, 11(4): 432-438.

Shelley, H. (1961). Glycogen reserves and their changes at birth and in anoxia. British Medical Bulletin, 17(2): 137-143.

Silverstein, F., Buchanan, K., Johnston, M. (1986). . Perinatal hypoxia-ischemia disrupts striatal high-affinity [3H] glutamate uptake into synaptosomes. Journal of Neurochemistry, 47(5): 1614-1619.

Simpson, E. & Watterman, M. (1988). Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Annual Review of Physiology, 50: 427-440.

Singer, D. (1999). Neonatal tolerance to hypoxia: a comparative physiological approach. Comparative Biochemistry and Physiology, Part A, 123: 221-234.

Singer, D. & Muhfeld, C. (2007). Perinatal adaptation in mammals: The impact of metabolic rate. Comparative Biochemistry and Physiology, Part A, 148: 780-784.

Slotkin, T., Seidler, F., Kavlock, R. & Bartolome, J. (1991). Fetal dexamethasone exposure impairs cellular development in neonatal rat heart and kidney: effects on DNA and protein in whole tissues.Teratology, 43(4):301-306.

Slotkin, T., Lappi, S., McCook, E., Lorber, B. & Seidler, F. (1995). Loss of neonatal hypoxia tolerance after prenatal nicotine exposure: Implications for sudden infant death syndrome. Brain Research Bulletin, 38(1): 69-75.

Slotkin, T. (1998). Fetal nicotine or cocaine exposure: Which one is worse? The Journal of Pharmacology and Experimental Therapeutics, 285(3): 931-945.

Slotkin, T., Kreider, M., Tate, C. & Seidler, F. (2006). Critical prenatal and postnatal periods for persistent effects of dexamethasone on serotonergic and dopaminergic systems. Neuropsychopharmacology, 31(5); 904-911.

Slotkin, T., Seidler, F., Wood, C. & Lau, C. (2008). Development of glucocorticoid receptor regulation in the rat forebrain: Implications for adverse effects of glucocorticoids in preterm infants. Brain Research Bulletin, 76(5): 531-535.

Smith, J., Ellenberger, H., Ballanyi, K., Richter, D. & Feldman, J. (1991). Pre-Botzinger Complex: A brainstem region that may generate respiratory rhythm in mammals. Science, 254: 726-729.

Smith, F., Fewell, J. & Abu-Amarah, I. (1997). Endocrine effects of pregnancy and exposure to a simulated open field in rats. American Journal of Physiology, 273(3): R1053-R1057.

Smith, J., Butera, R., Kohiya, N., Del Negro, C., Wilson, C. & Johnson, S. (2000). Respiratory rhythm generation in neonatal and adult mammals: the hybrid pacemaker-network model. Respiratory Physiology, 122(2-3): 131-147.

Smolich, J. (1995). Ultrastructural and functional features of the developing mammalian heart: a brief overview. Reproduction, Fertility and Development, 7(3): 451-461.

Solomon, I., Edelman, N & Neubauer, J. (2000). Pre-Botzinger complex functions as a central hypoxia chemosensor for respiration in vivo. Journal of Neurophysiology, 83: 2854-2868.

Somani, S. & Gupta, P. (1988). Caffeine: a new look at an age-old drug. International Journal of Clinical Pharmacology, Therapy, and Toxicology, 26(11): 521-533.

Song, G., Sun, B., Curstedt, T., Grossman, G. & Robertson, B. (1992). Effect of amiloride and surfactant on lung liquid clearance in newborn rabbits. Respiratory Physiology, 88(1-2): 233-246.

Sridhar, R., Thach, B., Kelly, D. & Henslee, J. (2003). Characterization of successful and failed autoresuscitation in human infants, including those dying of SIDS. Pediatric Pulmonology, 36: 113-122.

St. John, W. (1990). Neurogenesis, control, and functional significance of gasping. Journal of Applied Physiology, 68(4): 1305-1315.

St. John, W. (1996). Medullary regions for neurogensis of gasping: noed vital or noeds vitals. Journal of Applied Physiology, 81(5): 1865-1877.

St. John, W. & Leiter, J. (2008). Maintenance of gasping and restoration of eupnea after hypoxia is impaired following blockers of alpha1-adrenergic receptors and serotonin 5-HT2 receptors. Journal of Applied Physiology, 104 :665-673.

St.John, W., Li, A. & Leiter, J. (2009) Genesis of gasping is independent of levels of serotonin in the PET-1 knockout mouse. Journal of Applied Physiology, ahead of print.

Stafford, A. & Weatherall, J. (1960). The survival of young rats in nitrogen. Journal of Physiology, 153(3):457-472.

Steptoe, A. (1998). Coping, control and health risk. Annals of the New York Academy of Sciences, 851(1): 470-476.

Stewart, P., Murry, B. & Mason, I. (1994). Type 2 11- ß-Hydroxysteroid Dehydrogenase in human fetal tissues. Journal of Clinical Endocrinology and Metabolism, 78(6): 1529-1531.

Stothers, J. & Warner, R. (1978). Oxygen consumption and neonatal sleep states. Journal of Physiology, 278: 435-440.

Subhi, R., Smith, K., & Duke, T. (2009). When should oxygen be given to children at high altitude? A systematic review to define altitude-specific hypoxemia. Archives of Diseases in Children, 94(1): 6-10.

Sun, K., Adamson, S., Yang, K. & Challis, J. Interconversion of cortisol and cortisone by 11β-hydroxysteroid dehydrogenases Type 1 and 2 in the perfused human placenta. Placenta, 20(1): 13-19

Sved, A., Cano, G., Passerin, A. & Rabin, B. (2002). The locus coeruleus, Barrington's nucleus, and neural circuits of stress. Physiology & Behavior, 77(4-5) 737- 742.

Thach, B. (2008). Some aspects of clinical relevance in the maturation of respiratory control in infants. Journal of Applied Physiology, 104: 1828-1834.

Thach, B. (2001). Maturation and transformation of reflexes that protect the laryngeal airway from liquid aspiration from fetal to sdult life. The American Journal of Medicine, 111 (Supplment 8): S69-S77.

Thompson, R., Jackson, A. & Nurse, C. (1997). Developmental loss of hypoxic chemosensitivity in rat adrenomedullary chromaffin cells, Journal of Physiology, 498(2): 503-510.

Tien, Y. (1979). The optimal position of the oxyhemoglobin dissociation curve. Anesthesiology, 51: S375.

Trippenback, T. (1994). Pulmonary reflexes and control of breathin during development. Biology of the Neonate, 65(3-4): 205-210.

Tryba, A., Pena, F. & Ramirez, J. (2006). Gasping activity in vitro: a rhythm dependent on 5-HT2A receptors. The Journal of Neuroscience, 26(10): 2623-2634.

Tucker, D. & Domino, J (1988). Balance among autonomic controls of heart rate in neonatal spontaneously hypertensive and borderline hypertensive rats. Journal of the Autonomic Nervous System, 22: 11-21.

Turnball, A. & Rivier, C. (1995). Regulation of the HPA axis by cytokines. Brain, Behavior and Immunity, 9(4): 253-275.

Umans, J., Umans, H. & Szeto, H. (1986). Effects of thyrotropin-releasing hormone in he fetal lamb. American Journal of Obstetrics and Gynecology, 155(6): 1266-1271.

University of Calgary (2008). Animal Care and Use Policy. Effective Date January 29, 1993, last revision June 26, 2008.

Vahl, T., Ulrich-Lai, Y., Ostrander, M., Dolgas, C., Elfers, E., Seeley, R. & D'Alessio, D., Herman, J. (2005). Comparative analysis of ACTH and corticosterone sampling methods in rats. American Journal of Physiology: Endocrinology and Metabolism, 289: E823-E828.

van Beek, J., Guan, H., Julan, L. & Yang, K. (2004) Glucocorticoids stimulate the expression of 11B-Hydroxysteroid dehydrogenase Type 2 in cultured human placental trophoblast cells. The Journal of Clinical Endocrinology & Metabolism, 89(11): 5614-5621.

Van Dam, L. & Fox, J. (1998). Adolf Fick (1829-1901), Physiologist: a heritage for anesthesiology and critical care medicine. Anesthesiology, 88(2): 514-518.

van Vliet, M., Martin, C., Nijhuis, J. & Prechtl, H. (1985). The relationship between fetal activity and behavioral states and fetal breathing movements in normal and growth retarded fetuses. American Journal of Obstetrics and Gynecology, 153(5): 582-588.

Venkataraman, S., Munoz,R., Candido, C. & Witchel, S. (2007). The hypothalamic-pituitary-adrenal axis in critical illness. Reviews in endocrine & metabolic disorders, 8: 365-373.

Venkatesh, V. & Katzberg, H. (1997). Glucocorticoid regulation of epithelial sodium channel genes in human fetal lung. American Journal of Physiology, 273(17): L227-L233.

Visser, G., Goodman, J., Levine, D. & Dawes, G. (1982). Diurnal and other cyclic variations in human fetal heart rate near term. American Journal of Obstetrics and Gynecology, 142(5): 535-544.

Von Planta, I., Weil, M., von Planta, M., Bisera, J., Bruno, S., Gazmuri, R. & Rackow, E. (1988). Cardiopulmonary Resuscitation in the rat. Journal of Applied Physiology, 65(6): 2641-2647.

Vyas, H., Field, D., Milner, A. & Hopkin, I. (1986). Determinants of the first inspiratory volume and functional residual capacity. Pediatric Pulmonology, 2: 189-193.

Waddell, B., Benediktsson, R. & Seckl, J. (1996). 11 beta-Hydroxysteroid dehydrogenase type 2 in the rat corpus luteum: Induction of messenger ribonucleic acid expression and bioactivity coincident with luteal regrssion. Endocrinology, 137(12): 5386-5391.

Walker, D. (1984). Peripheral and central chemoreceptors in the fetus and newborn. Annual Review of Physiology, 46: 687-703.

Walker, M., Curtis, M., Hearse, D., Campbell, R., Janse, M., Yellon, D., Cobbe,S., Coker, S., Harness, J., Harron, D., Higgins, A., Julian, D., Lab, M., Manning, A., Northover, B., Parratt, J., Riemersma, R., Riva, E., Russell, D., Sheridan, D., Winslow, E. & Woodward, B. (1988). The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. Cardiovascular Research, 22(7): 447-455.

Walker, B., Campbell, J., Fraser, R., Stewart, P. & Edwards, C. (1992). Mineralcorticoid excess and inhibition of 11 beta-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. Clinical Endocrinology, 37(6): 483-492.

Walther, F., Siassi, B., Ramadan, N., Ananda, A. & Wu, P. (1985). Pulsd Doppler determinations of cardiac output in neonates: normal standards for clinical use. Pediatrics, 76(5): 829-833.

Weber, C. (1998). Cortisol's purpose. Medical Hypotheses, 51(4): 289-291.

Welberg, L., Thrivikraman, K. & Plotsky, P. (2005). Chronic maternal stress inhibits the capacity to upregulate placental 11B-hydroxysteroid dehyrogenase type 2 activity. Journal of Endocrinology, 186: R7-R12.

White, C. & Seymour, R. (2005). Allometric scaling of mammalian metabolism. The Journal of Experimental Biology, 208(Part 9): 1611-1619.

Wigger, A., Lorscher, P., Oehler, I., Keck, M., Naruo, T. & Neumann, I. (1999). Nonresponsiveness of the rat hypothalamo-pituitary-adrenocortical axis to parturition-related events: inhibitory action of endogenous opioids. Endocrinology, 140(6):2843-2849.

Wisborg, K., Barklin, A., Hedegaard, M., & Hendriksen, T. (2008). Pscyhological stress during pregnancy and stillbirth: prospective study. BJOG : An International Journal of Obstetrics and Gynaecology, 115(7): 882-885.

Wong, T., Wickstrom, R. & Holgert, H. (2003). Chronic prenatal nicotine exposure alters enkephalin mRNA regulation in the perinatal rat adrenal medulla. Pediatric Research, 53(5): 814-816.

World Health Organization (1995) Neonatal Health in the Context of Maternal, Newborn and Child for the Attainment of the Millenium Development Goals of the United Nations Millenium Declaration.

Wu, S., Klein, A., Chopra, I. & Fisher, D. (1978). Alterations in tissue thyroxine-5'-monodeiodinating activity in perinatal period. Endocrinology, 103(1): 235-239.

Wurtman, R. & Axelrod, J. (1966). Control of enzymatic synthesis of adrenaline in the adrenal meduall by adrenal cortical steroids. The Journal of Biological Chemistry, 241(10): 2301-2305.

Wurtman, R. (2002). Stress and the adrenocortical control of epinephrine synthesis. Metabolism, 51(Supplement 1): 11-14.

Xie, J., Weil, M., Sun, S., Yu, T. & Tang, W. (2004). Spontaneous gasping generates cardiac output during cardiac arrest. Critical Care Medicine, 32(1): 238-240.

Yang, L., Weil. M., Noc, M., Tang, W., Turner, T. & Gazmuri, R. (1994). Spontaneous gasping increases the ability to resuscitate during experimental cardiopulmonary resuscitation. Critical Care Medicine, 22(5): 879-883.

Youngson, C., Nurse, C., Yeger, H. & Cutz, E. (1993). Oxygen sensing in airway chemoreceptors. Nature, 365: 153-155.

Yuan, S., Blennow, M., Runold, M. & Langercrantz, H. (1997). Effects of hyperglycemia on gasping and autoresuscitation in newborn rats. Biology of the Neonate, 72: 255-264.

Yuan, S., Runold, M. & Langercrantz. H. (1997). Adrenalectomy reduces the ability of newborn rats to gasp and survive anoxia. Acta Physiologica Scandinavica, 159: 285-292.

Zhang, Y., Kobayashi, K., Kitazawa, K., Imai, K. & Kobayashi, M. (2006). Contribution of cooperativity and the Bohr effect to efficient oxygen transport by hemoglobins from five mammalian species. Zoological Science, 23: 49-55.

Zoladz, P., Conrad, C., Fleshner, M. & Diamond, D. (2008). Acute episodes of predator exposure in conjunction with chronic social instability as an animal model of post-traumatic stress disorder. Stress, 11(4): 259-281.