

# **Alternative approaches to newborn resuscitation**

## **-with emphasis on compression to ventilation ratio in an experimental pig model of neonatal asphyxia**

Anne Lee Solevåg

The Department of Paediatrics, Akershus University Hospital

Institute of Clinical Medicine, the Faculty of Medicine, Akershus University Hospital,  
University of Oslo

The Department of Paediatric Research, Oslo University Hospital

Institute for Surgical Research, Oslo University Hospital

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## PAPERS INCLUDED IN THE THESIS

### Paper I

**Solevag AL**, Dannevig I, Nakstad B, Saugstad OD. Resuscitation of severely asphyctic newborn pigs with cardiac arrest by using 21% or 100% oxygen. *Neonatology* 2010 Jun;98(1):64-72.

### Paper II

Dannevig I, **Solevag AL**, Wyckoff M, Saugstad OD, Nakstad B. Delayed onset of cardiac compressions in cardiopulmonary resuscitation of newborn pigs with asphyctic cardiac arrest. *Neonatology* 2011;99(2):153-62.

### Paper III

**Solevag AL**, Dannevig I, Wyckoff M, Saugstad OD, Nakstad B. Extended series of cardiac compressions during CPR in a swine model of perinatal asphyxia. *Resuscitation* 2010 Nov;81(11):1571-6.

### Paper IV

**Solevag AL**, Dannevig I, Wyckoff M, Saugstad OD, Nakstad B. Return of spontaneous circulation with a compression:ventilation ratio of 15:2 versus 3:1 in newborn pigs with cardiac arrest due to asphyxia. *Arch Dis Child Fetal Neonatal Ed* 2011 Mar 10. [Epub ahead of print]

## **ABBREVIATIONS**

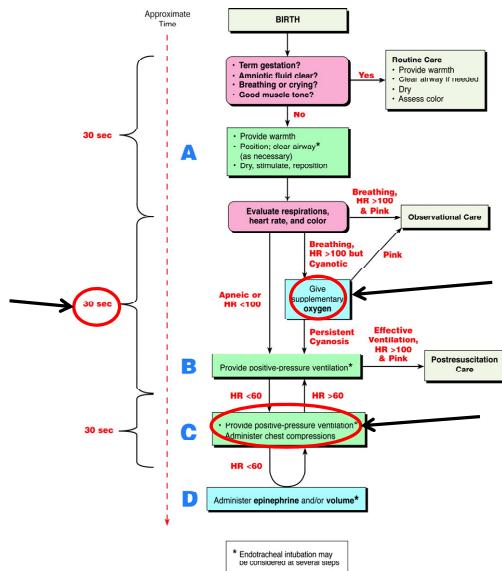
PVR	Pulmonary vascular resistance
NO	Nitric oxide
FRC	Functional residual capacity
PVL	Periventricular leucomalacia
HIE	Hypoxic-ischaemic encephalopathy
ILCOR	The international liaison committee on resuscitation
MRS	Magnetic resonance spectroscopy
EEG	Electroencephalogram
MRI	Magnetic resonance imaging
CPR	Cardiopulmonary resuscitation
ROSC	Return of spontaneous circulation
CPP	Coronary perfusion pressure
C:V ratio	Compression:ventilation ratio
DBP	Diastolic blood pressure
IL	Interleukin
TNF	Tumor necrosis factor
CSF	Cerebrospinal fluid
BALF	Bronchoalveolar lavage fluid
L/P ratio	Lactate/pyruvate ratio
NIRS	Near infrared spectroscopy
rScO <sub>2</sub>	Regional cerebral oxygen saturation
cFTOE	Cerebral fractional tissue oxygen extraction
HR	Heart rate
MABP	Mean arterial blood pressure

## **BACKGROUND**

### **What is this thesis about?**

The idea for the thesis emerged from the fact that many of the recommendations for newborn resuscitation are based on long-standing tradition, expert opinion and experience, rather than scientific evidence. One of the reasons for the lack of research in this area might be significant ethical issues concerned with undertaking clinical intervention studies in this age group. Even though prospective informed consent is impossible to obtain in resuscitation in the delivery room, a so-called “waiver of informed consent” may in some instances be granted by the local ethics committee for emergency research. However, the infrequent occurrence of some resuscitative interventions makes controlled studies difficult to carry out (1). For this reason, most of the data that exist in support of guidelines for newborn resuscitation come from clinical studies in older age groups, manikin studies and experimental animal studies.

We wanted to investigate three aspects of the algorithm for neonatal resuscitation (Figure 1). When we planned our studies there was evidence for harmful effects of 100% oxygen and the as good as or even better effect of air in resuscitation from neonatal asphyxia. However, the existing data were from clinical and experimental studies of predominantly light and moderately asphyxiated newborns, and whether air would be sufficient to resuscitate the most severely affected individuals with cardiac arrest was unknown. This, together with the unexplored area of the optimal ratio of cardiac compressions to ventilations; and the question of whether the recommended 30 seconds of initial positive pressure ventilation before initiation of cardiac compressions can be replaced by a longer ventilation interval, were the focus of our studies.



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 Cardiopulmonary Resuscitation and Emergency  
 Cardiovascular Care,  
 Part 13: Neonatal Resuscitation Guidelines  
 Circulation.2005;112:IV-188 – IV-195  
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Figure 1 Algorithm for newborn resuscitation at the time of our studies. Circles and arrows indicate the specific aspects that we wanted to investigate

Because of the aforementioned difficulties with carrying out clinical research in this field, we chose to explore these questions by using a well-established pig model of neonatal asphyxia (2;3). In order to test our hypotheses, we modified this model developed at the Department of Paediatric Research into an asystole model.

By using newborn pigs with asphyxia-induced cardiac arrest, we argue that we managed to investigate alternatives to the current guidelines for newborn resuscitation. Even though our results give indirect and indicative answers to the questions we asked, we believe that they may provide a starting point for discussions and further studies that can eventually improve the treatment of compromised neonates in the delivery room.

## **INTRODUCTION**

In order to understand the rationale for different resuscitative interventions in the newborn one needs to have knowledge about the complex physiological changes that occur during and immediately after birth.

### **Perinatal transition**

The foetal circulation (Figure 2) is characterised by a relatively parallel flow rather than flow in series (as is the case after completed transition) and gas exchange occurs in the placenta. However, as opposed to what was earlier thought, only about 1/5 (in late gestation) to 1/3 of the combined ventricular output (the total output of the right and left ventricle) passes through the placental vein, meaning that a greater proportion of the blood volume is being recirculated (4;5).

As depicted in Figure 2, the foetal circulation depends on intra- and extracardial shunts: 20-30% of the well-oxygenated blood from the placental vein bypasses the hepatic circulation via the ductus venosus to the inferior vena cava (6). A portocaval pressure gradient across the ductus venosus ensures that this well-oxygenated blood reaches the foramen ovale in order to push its flaps open and fill the left atrium. Interestingly, the blood from the inferior vena cava seems to be directed directly into the left atrium without being passed through the right atrium. This is possible because of a right-deviation of the atrial septum, dilatation of the left side of the inferior vena cava and a higher kinetic energy of the blood flow compared to postnatally (7;8). 11-25% (dependent on gestational age) of the combined ventricular output passes through the lungs via the lung artery and 32-46% of the combined ventricular output passes through the ductus arteriosus (9;10).

In the foetus, pulmonary pressures are equivalent to systemic pressures due to elevated pulmonary vascular resistance (PVR). Pulmonary vasoconstriction is maintained by low oxygen tension and mediators such as catecholamines and other hormones produced both systemically and locally in the lungs (11-15). Low levels of prostacyclin and nitric oxide also contribute to maintaining vasoconstriction in the lungs (16). Mediators of pulmonary vasoconstriction and vasodilatation are presented in Table 1.

<b>Vasoconstriction</b>	<b>Vasodilatation</b>
Low oxygen tension	High oxygen tension
Mechanical compression of the pulmonary vasculature	Shear stress due to blood flow and viscosity in the pulmonary vessels
Endothelin-1	Rhythmical stretching of the lung parenchyma
Leukotrienes (C <sub>4</sub> , D <sub>4</sub> )	17-β-oestradiol
Rho kinase	Prostacyclin (PGI <sub>2</sub> )
Platelet activating factor (PAF)	Nitric oxide (NO)
Thromboxan A <sub>2</sub> (TXA <sub>2</sub> )	Mechanical stretching of the alveoli
PGF <sub>2</sub> , PGE <sub>2</sub> , PGD <sub>2</sub>	Surface tension within the alveoli
Catecholamines and other hormones	Cyclooxygenase

Table 1 Mediators of pulmonary vasoconstriction and vasodilatation, respectively (17)

PG=prostaglandin

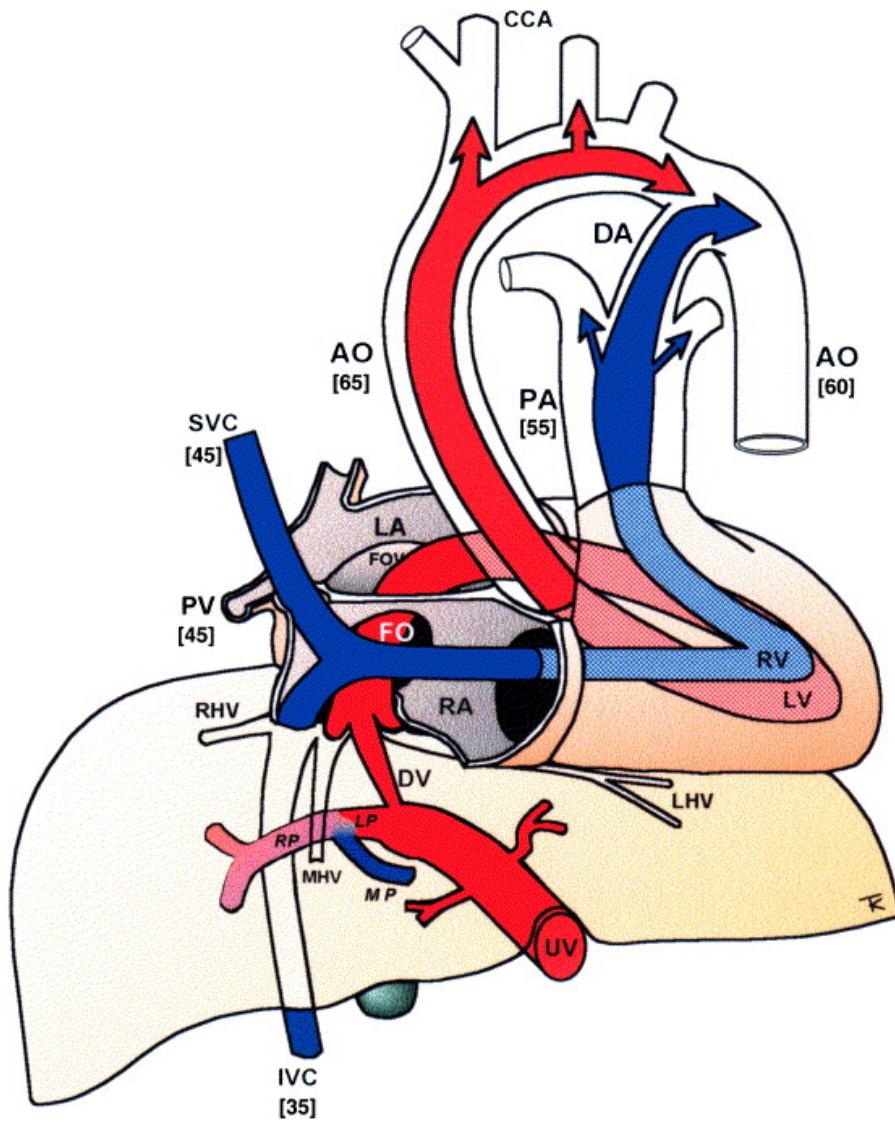


Figure 2

The foetal circulation. The numbers in brackets represent oxygen saturation in % at a particular level of the circulation. Reprinted from Kiserud T. Physiology of the fetal circulation. Semin Fetal Neonatal Med 2005 Dec;10(6):493-503 (18) with permission from Elsevier and the author

A dramatic cardiopulmonary transition occurs at birth in order to establish pulmonary gas exchange and thus make the lungs able to replace the placenta in supplying oxygen to the body. This transition is characterised by a rapid reduction in PVR and pulmonary artery pressure, enabling the entire right ventricular output to circulate through the lungs (9;19) provided that the pulmonary micro-vasculature is mature with a large cross-sectional surface area (20). The process is facilitated by mechanical distension of and increased oxygen tension in the lungs (Table 1). In addition, the foetus prepares for increased production of and sensitivity for the potent vasodilator nitric oxide (NO) by increasing pulmonary expression of nitric oxide synthase and the NO receptor soluble guanylate cyclase in late gestation (21;22).

In the foetal lungs, Cl-driven fluid secretion maintains lung distention and is thought to be critical for normal lung development (23). In late gestation, a shift towards Na-driven fluid absorption serves to reduce the size of the airspace in the lungs that needs to be cleared from fluid at birth (24). Fluid absorption after birth is regulated by catecholamines, glucocorticoids, thyroid hormone, arginin vasopressin, somatostatin, dopamine and serotonin (25). Especially  $\beta$ -adrenergic stimulation causes Na-driven fluid absorption (26;27). The first breaths establish the functional residual capacity (FRC) and aerate the fluid-filled alveoli. Stretching of the alveoli causes the initial fall in PVR and distention of the lungs activates cyclooxygenases (COX) with resulting induction of prostaglandins, the most important one being prostglandin I<sub>2</sub> (PGI<sub>2</sub>/prostacyclin) (16). Other contributors to decreasing PVR at birth include establishment of an air-liquid interface, sustained respiration, increased arterial oxygenation, and altered production of vasoactive substances such as endothelin-1, prostacyclin, and NO (17;28) (Table 1).

In the majority of neonates, both term and preterm, the decrease in PVR after birth reverses the shunt flow through the ductus arteriosus to primarily left to right. Constriction of the umbilical vessels in response to increased pO<sub>2</sub> together with external clamping of the umbilical cord increase systemic resistance, raise the pressure in the left side of the heart and contribute to this reversal of flow over the ductus arteriosus and the foramen ovale (29). The flap of the foramen ovale is pushed against the atrial septum within minutes to hours after birth as a result of equalized pressures in the right and left atria (30).

In an ultrasonographic study of fifty healthy term neonates, the ductus arteriosus was closed in most infants within day 3 and in all infants within 168 hours. The ductus venosus was closed in  $\frac{3}{4}$  of the infants on day 7 (31).

With all these changes, leading to the lungs becoming the organ of gas exchange after birth, the systemic and pulmonary circulations separate and the cardiovascular system begins to function as a circulation in series (32).

## **Apgar score**

In 1949 Dr. Virginia Apgar, an American obstetric anaesthesiologist, developed a score to quantify the physiological status (as an effect of the transitional process) of newborn infants shortly after delivery. This tool was based on simple observation, and was thought to be useful for:

1. Comparison of the results of obstetric practices
2. Evaluation of the effects of maternal anaesthesia
3. Evaluation of the effects of neonatal resuscitation

The so-called Apgar score was published in 1953 (Table 2) (33), and since then this scoring system has been widely used as a numeric assessment of the condition of newly born infants. Dr. Apgar also applied the score to:

- a) Judge which neonates needed resuscitative measures
- b) Guide teaching of resuscitation
- c) Predict the probability of survival

The association of the 1 and 5 min Apgar score with neurological disability, as well as mortality has been confirmed in population studies, incl. one of Norwegian newborns 1983-1987 (34-37). However, it is important to keep in mind that the use of the score alone in risk assessment and prognosis is at the statistical level; the score alone is not as useful for predicting individual outcomes. Used together with neonatal severity of illness scores, e.g. the Clinical Risk Index for Babies (CRIB) and Score for Neonatal Acute Physiology (SNAP)-II/ Score for Neonatal Acute Physiology Perinatal Extension (SNAPPE)-II (38), the score might to some degree predict individual outcomes (39). However, it is important to note that the CRIB score has primarily been developed for predicting outcome for preterm babies rather than asphyxiated term infants.

As can be seen from Table 2, four out of five elements of the Apgar score are to a certain extent based on visual inspection of the infant. The fifth, the one found by Apgar to be the most valuable prognostic and diagnostic element of the score: heart rate, was defined as good (equals a score of 2) if over 100 beats per minute. However, as Dawson et al. recently reported that median (IQR) heart rate at 1 minute of age of healthy newborn infants was 96 (65–127) beats per minute (40), Apgar's proposal for a cut-off might not be feasible. Current

international guidelines for newborn resuscitation do, like Apgar, use a heart rate of less than 100 beats per minute as a definition for which infants need intervention at birth (41).

The way the Apgar score is being assigned to preterm babies may cause uncertainty and greater degree of subjectivity of the scoring (O'Donnell (42) referring to an abstract by Hesser, Bhola and Muraskas 2004). It has also been speculated that the element of “reflex response” often is not being formally assessed as many infants are not being actively stimulated (which must be believed to be a prerequisite for a response to occur) (42). As recommendations for routine suctioning of infants (with or without meconium staining of the amniotic fluid) have been abandoned due to insufficient evidence for its benefit, scoring of reflex response might no longer be justified (43). In addition, scoring of infants being resuscitated and in particular after intubation may impose challenges and reduced reliability (44).

Despite concerns regarding subjectivity of Apgar scoring (42), equal weighting of the components, as well as clinical usefulness of the score, the Apgar score is still being used as a diagnostic criterion and in epidemiologic research. E.g. Apgar scores are being used in defining neonatal asphyxia, its severity and need for interventions. However, many argue that immediate resuscitative interventions in the delivery room should no longer be guided by the Apgar score. The American Neonatal Resuscitation Program (NRP) states that “...the Apgar score is not used to determine the need for resuscitation, what resuscitation steps are necessary, or when to use them” (45).

This is in accordance with updated guidelines for neonatal resuscitation where the former recommendation for resuscitative interventions being guided by the colour (in addition to pulse and respiration) of the baby has been removed, as this is an unreliable parameter (46). This change in guidelines is partially based on the work by O'Donnell et al. showing that assessment of colour is highly subjective and that inter-rater variability is high (47). In addition, based on the natural rise in SpO<sub>2</sub> in healthy term neonates where mean time (SD) to SpO<sub>2</sub> > 90% is 5.8 (3.2) min (48), skin colour may not be expected to be “pink” the first minutes of life.

The Apgar score			
	0	1	2
Colour	Pale or blue	Pink body, blue extremities	Pink body and extremities
Heart rate	Absent	<100 beats per minute	>100 beats per minute
Respiration	Absent	Slow and irregular	Good breathing with crying
Reflex response	Absent	Grimace or noticeable facial movement	Cough, sneezes or pulls away
Muscle tone	Absent	Some flexion of extremities	Active and spontaneous movement of limbs

Table 2 The Apgar score (49)

## **Pathological oxygen delivery. Definitions**

Anoxia is defined as the complete lack of oxygen. Hypoxemia refers to insufficient arterial oxygenation, whereas hypoxia is reduced oxygen supply to tissues (due to hypoxemia) with resulting metabolic consequences, e.g. lactic acidosis. Ischaemia is insufficient blood flow to cells or organs to maintain normal function (50).

## **Primary and secondary apnea**

Several investigators have studied the effects of perinatal asphyxia in animals, using both foetal and newborn models. It has been suggested that progressive asphyxia in the newborn results in a series of distinct respiratory patterns: hyperpnea, primary apnea and gasping. If gasps restore oxygenation of the lungs and heart (so-called “autoresuscitation”), cardiovascular function rapidly improves with complete return of function in all organs. However, if hypoxia continues even further, the infant will experience falling heart rate and blood pressure. The infant then takes a last breath and enters secondary apnea. In this stage the infant will not respond to stimulation and death will occur unless resuscitation begins immediately (51). In the uncompensated phase (secondary apnea), the infant develops hypotension because neuroendocrine mechanisms fail to sustain cardiac output. Perfusion of all organs becomes compromised and lactic acidosis develops (52). Profound metabolic acidemia negatively affects resuscitability (53). If treatment is ineffective or delayed, irreversible organ damage develops and shock enters its terminal phase (52).

In the 1950's and 1960's Dawes and coworkers performed a number of experiments on foetal/newborn lambs and monkeys in order to describe physiological changes in normal and pathological perinatal processes. Perhaps one of their most famous theories was the above mentioned model of the progression from primary to secondary apnea during hypoxia in the foetus and newborn (51). Although controversial, the description of the foetus' or newborn individual's ability to respond to different resuscitative measures dependent on the phase of the hypoxic insult still seems valid and reasonable. The biphasic respiratory response to hypoxia in the newborn has later been reproduced in rabbit pups (54;55).

The mechanisms behind the changes in respiratory patterns and change in heart rate during hypoxia have partly been explained by activation of peripheral chemoreceptors. Chemoreceptors in the aortic and carotid bodies show little or no activity at normoxia and

normocarbia. During hypoxia, activity in these bodies leads to vagally mediated sinus bradycardia, peripheral vasoconstriction (in order to maintain blood pressure) and increased pulmonary ventilation (reflex hyperventilation) (56). This hyperventilatory response to hypoxia is attenuated by the use of supplementary oxygen during resuscitation (57). The late response to hypoxia, i.e. the hypoventilatory response has been proposed to happen because of decreased metabolism with resulting reduction in pCO<sub>2</sub> and/or a central depressant effect of hypoxia itself (58).

## **PERINATAL ASPHYXIA**

### **Definitions and criteria**

In 1993 the World Federation of Neurology defined asphyxia as “a condition of impaired gas exchange leading, if it persists, to progressive hypoxemia and hypercapnia” (59). The presence of acidosis is commonly added to this definition (60).

The condition is caused by:

1. Compromised maternal-foetal blood gas exchange before and during delivery
2. Cardio-respiratory complications after delivery or
3. A combination of the two

It is important to separate asphyxia occurring before delivery from that caused by transitional problems and abnormalities in the newborn child after birth, as precise diagnosis and timing are essential for optimal prevention and treatment (61). Also, some evidence suggests that antenatal factors are more important determinants of cerebral palsy than intra- and postpartum factors (62;63).

Criteria to define an acute intrapartum event sufficient to cause cerebral palsy according to The American College of Obstetricians and Gynecologists (ACOG) (64) and International consensus criteria (65):

Essential criteria:

1. Metabolic acidosis in intrapartum foetal, umbilical artery, or very early neonatal blood samples ( $\text{pH} < 7.00$  and base deficit  $\geq 12 \text{ mmol/l}$ )
2. Early onset of moderate to severe neonatal encephalopathy in infants of  $\geq 34$  weeks of gestation
3. Spastic quadriplegic or dyskinetic cerebral palsy
4. Exclusion of other identifiable aetiologies, e.g. trauma, coagulation disorders, infectious conditions or genetic disorders

Criteria that together suggest an intrapartum timing, but by themselves are non-specific:

5. A sentinel (signal) hypoxic event immediately before or during labor

6. Normal foetal monitoring patterns followed by sudden and sustained abnormal patterns (e.g. bradycardia or lack of heart rate variability and late/variable decelerations), usually after a hypoxic sentinel event
7. Apgar scores of 0-3 beyond 5 min
8. Onset of multisystem involvement  $\leq$  72 h after birth
9. Early imaging evidence of acute non-focal cerebral abnormality

## **Aetiology**

Perinatal asphyxia may occur antepartum (20%), intrapartum (35%), postnatally (10%) or as a combination, in particular of ante- and intrapartum (35%) factors (66;67). Maternal risk factors include single civil and/or low socioeconomic status, high age, abnormal weight (over-/underweight), oxytocin use during labor, and severe preeclampsia/eclampsia. Risk factors concerning the foetus/newborn include intrauterine growth restriction or very large for gestational age, pre-/postterm and abnormal presentation (e.g. breech) (68;69).

Common causes for interruption of placental blood flow and/or gas exchange leading to asphyxia include umbilical cord compression (e.g. cord prolapse), maternal anaemia, bleeding, uterine hyperactivity, placental abruption and uterine rupture. These conditions can occur either ante- or intrapartum, or as a combination of the two. Postnatal causes (rare) include pulmonary and/or cardiac abnormalities, airway obstruction, sepsis and respiratory depression due to maternal opiate use (68-75).

## **Pathophysiology**

In global hypoxia in the foetus or newborn, increased shunting across the intra- and extracardial shunts will initially maintain brain, heart and adrenal perfusion. Hence, the lungs, liver, kidneys and intestines are affected by hypoxia first (50).

However, hypoxia will eventually result in bradycardia, hypotension, reduced cardiac output, and combined metabolic and respiratory acidosis. In addition to CNS damage, congestive heart failure and cardiogenic shock, pulmonary hypertension as a result of acute vasoconstriction and later pulmonary arteriole smooth muscle hyperplasia (50;76), respiratory distress syndrome, gastrointestinal perforation, haematuria and acute tubular necrosis are associated with perinatal asphyxia (50).

Under hypoxic circumstances there is a shift from an aerobic to a less efficient anaerobic cell metabolism, and energy depletion (reduced supply of high-energy phosphate compounds) will occur if hypoxia persists. Following hypoxia-ischaemia, reperfusion of tissues leads to an inflammatory response (77), in addition to the generation of free radicals like reactive oxygen and nitrogen species (78). Reactive oxygen species (ROS) can alter gene expression of proteins, as well as damage DNA, lipids and other macromolecules (79).

These processes are involved in the many mechanisms of brain injury caused by perinatal asphyxia (74;80-82). These include <sup>1</sup>congestion, apoptosis and release of pro-inflammatory cytokines and chemokines (80;81;83). In addition, excitatory amino acids may play an important role (84) by increasing intracellular calcium in neurons leading to activation of proteases, endonucleases and phospholipases with resulting signs of coagulation necrosis and cell death (85).

Magnetic resonance spectroscopy (MRS) is a method for quantification of cerebral metabolites of which phosphorus-31 (<sup>31</sup>P) and proton (<sup>1</sup>H) MRS have been used to investigate cerebral metabolism following perinatal asphyxia (86). In <sup>31</sup>P MRS studies in the 1980's and 1990's three metabolic phases in the progression of injury after perinatal asphyxia were identified (87-92) (Figure 3):

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<sup>1</sup> increased capillary permeability and swelling of endothelial cells

1. Primary energy failure with primary neuronal death
2. Recovery (the “latent phase”) 6-15 hours; and
3. Delayed/secondary energy failure and neuronal death occurring between 6 and 48 hours or even longer (>3 days)

### Phases of Cerebral Injury

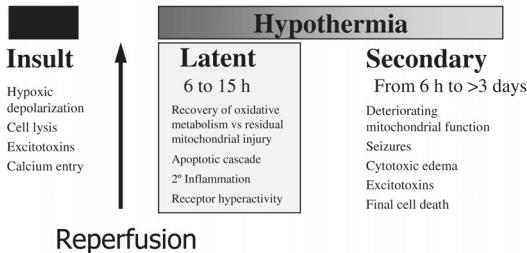


Figure 3 The phases of cerebral injury after hypoxia. Reprinted from Drury PP, Bennet L, Gunn AJ. Mechanisms of hypothermic neuroprotection. Seminars in Fetal and Neonatal Medicine 2010 Oct;15(5):287-92 (93) with permission from Elsevier

Since there is yet no established treatment of primary energy failure in perinatal asphyxia, neuroprotective strategies have until now mainly been targeted at the “latent phase”. This phase has been called “the window of opportunity” (94). Early (1990’s) MRS studies in pigs showed that post-asphyxial hypothermia prevented secondary depletion of high-energy phosphates, reduced lactate levels and reduced injury in the brain (95;96).

If secondary energy failure/depletion is not prevented, mechanisms of delayed neuronal death include hyperaemia, cytotoxic oedema, mitochondrial failure, accumulation of excitotoxins, apoptosis, nitric oxide synthesis, free radical damage and cytotoxic actions of activated microglia (97;98).

The clinical correlate to the CNS lesions is hypoxic-ischaemic encephalopathy (HIE), and was graded at 24 h of age by Sarnat and Sarnat (99), a grading system that was later modified by Levene et al. (100). The score is used to guide treatment and predict outcome (Table 3).

<b>Modified Sarnat and Sarnat score</b>		
HIE Grade I (mild)	HIE Grade II (moderate)	HIE Grade III (severe)
Irritability “Hyper alert”	Lethargic Seizures	Comatose Prolonged seizures
Mild hypotonia	Marked abnormalities of tone	Severe hypotonia
Poor sucking	Requires tube feeding	Failure to maintain spontaneous respiration

Table 3 Modified Sarnat and Sarnat score for hypoxic-ischaemic encephalopathy (100)

## **Distribution of brain injury**

The pattern of brain injury in the asphyxiated newborn depends on

1. The nature of the insult
  - a) Acute
  - b) Prolonged
2. The severity of insult
3. The infant's gestational age at the time of injury

(101;102)

One way of classifying the nature of an asphyxial insult is that of "acute total" and "prolonged partial" (103), each of which leads to characteristic patterns of brain damage. Acute total asphyxia typically gives lesions in the brain stem nuclei, thalami and basal ganglia, whereas prolonged partial asphyxia mainly involves the cerebral cortex, typically in the parasagittal area (104;105).

Prolonged intrauterine hypoxia may also result in periventricular leukomalacia (PVL) (50), i.e. white matter lesions peripheral to the lateral ventricles, especially in preterm infants (23-32 weeks of gestation). Preterm infants demonstrate primarily white matter injury due to PVL, periventricular haemorrhagic infarction (106) and interventricular haemorrhage (50). The periventricular areas are particularly vulnerable due to vascular immaturity, ongoing neuronal differentiation and specific vulnerability to oxidative stress (101).

Neuronal necrosis in the cortex (leading to cortical atrophy) and subcortical white matter, parasagittal ischaemic injury and affection of the basal ganglia (in severe hypoxia-ischaemia) are more often seen in term asphyxiated newborns (50;107-109).

Other white matter injury is also seen in term or near term infants. These lesions are often described as ischaemic; however, important risk factors for white matter disorder in term infants are infection and other inflammatory conditions (110).

Advances in imaging techniques for early detection of hypoxic brain injuries in the newborn have shown to have diagnostic and prognostic importance. Of these is proton MRS the most sensitive technique the first 24 hours of life. Diffusion-weighted imaging might

underestimate the extent of injury if performed the first 24 hours, but is together with T2-weighted spin echo images the preferred modality between 24 hours and 5 days (111).

## **Prevention**

Since there is yet no specific therapy for perinatal asphyxia and HIE, prevention of progressing injury is crucial. To determine the timing of and the degree of birth asphyxia are some of the most challenging evaluations to make in neonatology. There is no direct measurement and definitions tend to depend upon low Apgar scores or respiratory depression, i.e. symptoms rather than causes. Although unspecific, metabolic acidosis might be a good indicator. Features of electronic foetal monitoring (EFM) during labor, e.g. decelerations, are also unspecific for asphyxial conditions (112).

Important questions when developing strategies for injury prevention are:

- When did the hypoxia/ischaemia occur?
- Did the hypoxia/ischaemia initiate the insult/pathology? (112).

Only in a minority of cases, a “sentinel” event such as uterine rupture or cord prolapse leads to acute foetal bradycardia, and a causal link is likely when a depressed neonate is born (107). However, even though the duration and cause of asphyxia is often unknown, imaging techniques such as proton MRS may, as previously mentioned, provide information on timing, as well as prognosis even when utilized in the early acute stages of hypoxia-ischaemia (113).

There is no doubt that good obstetric care and routines for neonatologist’s attendance at high-risk deliveries are important preventative measures. Identifying foetal risk factors, e.g. intrauterine growth restriction might allow for appropriate decisions about timing, mode and place of delivery to be made and hence, prevent intrauterine hypoxic events (114). Routines for foetal surveillance and monitoring of high risk deliveries in developed countries can reduce the number of asphyxia-induced encephalopathies and can perhaps even cause a shift in the predominating causes of perinatal asphyxia (115). E.g. induction of labor, vacuum/forceps or caesarean section at signs of foetal distress might reduce birth asphyxia due to compression of the umbilical cord, placental dysfunction and foetal infection. However, it is difficult to study the effects of preventative measures in different settings (115). Nonetheless, in a Swedish study 177 cases of birth asphyxia were identified from 1990-2005 that were directly caused by delivery-related errors (116). The most common mistakes were that guidelines for foetal surveillance were not followed, signs of foetal

hypoxia were overlooked, oxytocin misuse, and a non-optimal mode of delivery. The authors concluded that there is a need for improving cooperation between professionals in the labor unit and to establish security barriers.

According to clinical practice in obstetrics in Norway, a paediatrician should be present at delivery if asphyxia is anticipated, in breech and instrumental deliveries, in premature deliveries, in acute caesarean section, and immediately when deliveries are complicated by shoulder dystocia. In cases of unexpected asphyxia, a paediatrician should be of assistance as soon as possible: typically at least within 5 minutes after birth and a neonatologist within 20–30 min.

Exceptions from the above are made in smaller delivery units where a paediatrician is not routinely present. In these facilities, trained midwives and anaesthesiologists are responsible for resuscitation of compromised newborns. However, known high-risk deliveries like multiples, abnormal presentation and expected preterm deliveries should be in a hospital with a paediatrician on call.

## **Epidemiology**

Problems in reaching international consensus regarding definitions of perinatal asphyxia make it difficult to accurately estimate its incidence. It has been claimed that infants who require intensive resuscitation (cardiac compressions and/or medications) typically suffer from severe asphyxia (117;118). Hence, the incidence of performance of resuscitative measures may reflect the occurrence of asphyxial events:

An estimated 10% of newborns need help to establish effective ventilation (119), but only about 0.1% of (term and preterm) infants receive cardiac compressions and/or medications (117;120). In preterm infants cardiac compressions have been reported to occur in 2-10% (121;122).

Globally, out of 130 million births approximately 3% (nearly 4 million) develop birth asphyxia requiring resuscitation each year. 900.000 of these die and about 1 million develop sequela (123). In our part of the world severe perinatal asphyxia (causing death or severe neurological impairment) occurs in about 0.1% of all live births. The estimated incidence in developing countries is 5-10 times higher (75).

Out of approximately 60.000 annual births in Norway, 888 (1.4%) had an Apgar score <6 after 5 minutes (the Medical Birth Registry of Norway (MBRN)). However, there are several other reasons for low Apgar scores than asphyxia, for instance preterm birth and congenital malformations.

## **Prognosis**

According to Roberton's Textbook of Neonatology, the risk of death or severe handicap is 1.6% for mild HIE, 24% for moderate HIE, and 78% for severe HIE (124).

Apgar et al. (35) demonstrated an inverse relationship between neonatal mortality and the 1 min Apgar score in 15.348 infants. The same relationship was found for the 5 min Apgar score in a multicenter study of more than 17.000 infants (36). In a Norwegian material Moster et al. found that a 5 min Apgar score of 0-3 markedly increased the risk for both death and cerebral palsy (37). As discussed previously, despite the fact that subjectivity in Apgar scoring and the equal weighting of the components of the score remain a concern (42;125), a predictive relationship between the 5 min Apgar score and neonatal mortality was also found in a retrospective cohort of 145.627 newborns ( $\geq$  26 weeks of gestation) in Dallas, Texas 1988-1998 (34). The Neonatal Resuscitation Program (NRP) recommends assignment of Apgar scores beyond 5 min of age when the Apgar score is <7 (the extended Apgar score) to indicate the response to interventions/resuscitation at birth (126).

The International Liaison Committee on Resuscitation (ILCOR) suggests that it may be reasonable to stop resuscitation if there are no signs of life after 10 min of adequate resuscitation (46). The ILCOR recommendation is based on observational studies of the outcome after prolonged resuscitation of apparently stillborn infants (127-129).

In one of these studies, Laptook et al. found that the 10 min Apgar score provides useful prognostic data before other evaluations are available for infants with HIE. Death or moderate to severe disability is common, but not uniform with 10 min Apgar score < 3 (127). This is in accordance with the conclusion of a review by Harrington et al. that infants with a 10 minute Apgar score of zero almost universally have a poor outcome (130).

In conclusion, despite the recommendations of the NRP (mentioned previously), the Apgar score does (directly or indirectly) still guide decisions about the need for continued resuscitation, partly in accordance with studies that suggest that the duration of resuscitative measures is an important prognostic marker (130;131).

Other factors of prognostic importance include:

1. Gestational age (preterm babies do worse)

2. Severity of encephalopathy (especially stage 3 is associated with poor outcome)
3. Low Apgar score at 20 minutes
4. Persistence of abnormal neurological signs at 2 weeks of age
5. Early electroencephalogram (EEG) and magnetic resonance imaging (MRI) findings.
6. Chest compression >1 minute,
7. Onset of respiration after age 30 minutes
8. Base deficit >16 mmol/L

(132;133)

Amplitude-integrated EEG, aEEG is a feasible method for continuous EEG surveillance in the neonatal intensive care unit after birth (134). The background pattern seen in early (typically within 6 hours) aEEG has been shown to predict neurodevelopmental outcome in term infants with birth asphyxia (134-136). Background patterns/activity in aEEG can be classified as follows, 1. Continuous normal voltage (CNV), 2. Discontinuous normal voltage, (DNV), 3. Burst suppression (BS), 4. Low voltage (LV) and 5. Flat trace (FT).

Abnormal background patterns (3-5), as well as a change from normal to abnormal patterns predict poor neurodevelopmental outcome. Also, the occurrence of early repetitive seizures (clinical and subclinical) might be a negative prognostic factor (134). In addition, prolonged time to normal trace (TTNT) and failure to develop sleep wake cycling (SWC) indicate a negative outcome (136).

Typical early MRI findings after asphyxia are abnormal signal intensity in the basal ganglia, thalamus, corticospinal tract, white matter and cortex. These findings have high predictive values for adverse outcomes, e.g. reduced myelination of the posterior limb of the internal capsule predicts abnormal motor development, whereas abnormal signal intensity in the basal ganglia/thalamus also affects cognitive functions (137;138). Early MRI together with clinical classification according to (modified) Sarnat and Sarnat improve prognostic accuracy, especially for infants classified as having HIE grade 2 (137).

## **Treatment**

Treatment is mainly supportive and focuses on symptoms from affected organs (50). When it comes to neuroprotection, a lot of research has been carried out to develop strategies to reduce brain damage after a hypoxic-ischaemic insult. Both pharmacological and non-pharmacological interventions have been investigated, of which hypothermia (selective head-cooling or whole body) at the present time has proved to be of benefit (139;140).

In the 2010 ILCOR guidelines it is for the first time stated that term or near-term infants with evolving moderate to severe HIE should be offered therapeutic selective head cooling or whole body hypothermia (46).

In general, as mentioned initially, important for performing a successful resuscitation is good understanding of the complex dynamics of foetal and neonatal physiology and perinatal cardiopulmonary transition (141).

For optimal management of the asphyxiated newborn it is also crucial to identify the infants at risk, to facilitate adequate perfusion and nutrients to the brain and maintain glucose and electrolyte homeostasis. As previously mentioned, interventions to interfere with the process of ongoing brain injury (neuroprotection), as well as symptomatic treatment of injury to other organs: lung (e.g. CPAP or ventilator), liver, heart and kidney, and support of endocrine and haematological (e.g. transfusion of blood products) functions might also be necessary (142;143).

## **NEONATAL RESUSCITATION**

### **General remarks**

According to the Merriam-Webster dictionary the word “resuscitate” originates from Latin *resuscitatus*, past participle of *resuscitare*: to reawaken (144). As previously mentioned, transition from intra- to extrauterine life is a complex process, and most infants do not really require “reawakening”, but rather just assistance to facilitate the transition from placental to pulmonary gas exchange. This is important to remember when we study “neonatal resuscitation”.

### **Guidelines**

“Since intubation and positive pressure ventilation were first recommended (...) a pattern of resuscitation has evolved based on extrapolation and assumption rather than clinical measurement. There can be few areas of medicine where the potential benefit is so great but which have been subjected to so little evaluation”(145).

As previously mentioned, Dr. Virginia Apgar (1909-1974) made a substantial effort in order to objectify evaluation and need for interventions in the newly born. The development of the Apgar score was based on a study of more than 15.000 infants and can be said to be one of the earliest attempts in modern times at guiding decisions to perform resuscitative measures in the newborn, and later studies showed that the score could provide valuable criteria for newborn resuscitation (146).

The International Liaison Committee on Resuscitation (ILCOR) was formed in 1992 as a forum for liaison between resuscitation organisations from different parts of the world (147). At present representatives of the American Heart Association (AHA), European Resuscitation Council (ERC), Heart and Stroke Foundation of Canada (HSFC), Australian and New Zealand Committee on Resuscitation (ANZCOR), Resuscitation Councils of Southern Africa (RCSA), Inter American Heart Foundation (IAHF), and the Resuscitation Council of Asia (RCA - current members Japan, Korea, Singapore, Taiwan) form the ILCOR.

Shortly after the ILCOR was established, the committee made its first recommendations for resuscitation, mainly based on consensus (148). Seven years of extensive evidence evaluation followed, and in 1999 An Advisory Statement From the Pediatric Working Group of the

International Liaison Committee on Resuscitation came (147). These efforts paved the way for international guidelines for neonatal advanced life support, and in 2000 the American Heart Association/American Academy of Pediatrics published International Guidelines for Neonatal Resuscitation (149). Revised guidelines were published in 2005 and 2010, respectively (46;150).

Even though we now have formalised guidelines for neonatal resuscitation, the basis for which are laid at regular intervals by international consensus meetings, the evidence underlying the recommendations are of varying quality, and in some instances completely lacking. Also, the frequency of performance of resuscitative measures is not the same in different parts of the world, and even not amongst institutions and resuscitators (151;152). Compliance with the algorithm with regards to timing of the different interventions has been reported to be low (153); e.g. Perlman and Risser reported in a retrospective study that one infant was first auscultated after 12 minutes (117). In a multicenter observational study of term newborns with suspected perinatal asphyxia and need for resuscitation, Laptook et al. reported the following delivery room interventions: 100% of infants received oxygen, 97% received bag and mask ventilation, 96% of infants were intubated, 63% had chest compressions, and 57% of infants received resuscitative medications (127).

## **Ventilation**

While in adults asystole is often of primarily cardiac origin, it is more commonly the result of decreased oxygenation and/or failure of ventilation in newborns, or respiratory disorder or failure in older children. Physiological respiratory rates in infants and children are also faster than in adults. Acknowledging this, international consensus statements emphasise ventilation during paediatric cardiopulmonary resuscitation (CPR) (154;155).

On the other hand, the results of experimental animal studies have suggested that excessive ventilation impairs cardiac output and decreases coronary and cerebral perfusion pressures (156;157). The mechanism behind this is increased intrathoracic pressure as a result of ventilation, leading to decreased venous return and thus, reduced cardiac output (158).

However, ventilation during CPR is necessary in order to maintain tissue oxygen delivery and CO<sub>2</sub> removal. Even studies in adult ventricular fibrillation models indicate that assisted ventilation improves return of spontaneous circulation (ROSC) (159;160) as hypoxia and hypercarbic acidosis profoundly decrease myocardial function and adversely affect resuscitability (161-164).

Apnea and bradycardia after birth are in the majority of cases caused by hypoxia of the brain stem and myocardium, respectively. Newborn infants who do not breathe adequately need assistance in order to clear fluid from the alveoli, aerate the lungs and form a functional residual capacity (FRC). When oxygenation of the myocardium improves, the heart rate and blood pressure increase, the brain stem recovers and spontaneous breathing commences. Hence, neonatal resuscitation has traditionally been guided by heart rate, respiration and colour (165). The American Academy of Pediatrics states in their 2010 guidelines for neonatal resuscitation that pulse oximetry should be used as a supplement to cardiac auscultation (Kamlin et al. have shown that cardiac auscultation alone might be unreliable in measuring heart rate (166)), as evaluation of colour is a too subjective measure of response to resuscitation (41)

Most apneic newborn infants respond well to aeration of the lungs. If the heart rate does not increase quickly, ventilation might not be effective or adequate (167).

In newborn resuscitation, assisted ventilation rates of 40-60 breaths per minute are commonly used, but evidence supporting this is lacking. As previously mentioned, the primary measure

of adequate initial ventilation is a rapid increase in heart rate. If the heart rate does not improve, then chest wall movement should be assessed. As such, the initial peak inflating pressures should be sufficient to achieve an increase in heart rate and/or chest wall movement. An initial inflation pressure of 20 cm H<sub>2</sub>O may be effective, but ≥ 30 to 40 cm H<sub>2</sub>O may sometimes be required. The optimal inflation time is unknown (46).

## **Coronary perfusion pressure**

Coronary perfusion pressure (CPP) is defined as the pressure gradient between the ascending aorta and the right atrium during the decompression phase of chest compressions and can be regarded as the driving force of blood flow to the myocardium (168). In other words, CPP is the mid-diastolic aortic pressure (AoP) minus mid-diastolic right atrial pressure (RAP). In CPR one might argue that CPP does not take into account interruptions for ventilation. Therefore, some investigators calculate CPP as the difference between the ascending aorta and right atrium during the entire CPR period (the total time of pulsatile diastolic pressure). This so-called integrated CPP will then account for pauses in chest compressions (169).

In cardiac arrest, morbidity and mortality might be affected by the quantity and quality of chest compressions (170) as interruptions of chest compressions negatively affect coronary as well as cerebral perfusion and may hinder or delay ROSC (160). In accordance with this, CPP is said to be a determinant of ROSC (170-172) and neurological outcome (169)

## **Cardiac compressions**

Cardiac compressions in neonatal resuscitation should be initiated if the heart rate remains below 60 per minute and is not increasing despite approximately 30 seconds of effective ventilation. Cardiac compressions should be given at a ratio of 90 compressions to 30 ventilations (equals a ratio of 3:1) per minute until a heart rate  $\geq$  60 beats per minute is achieved (46).

The cardiac component of CPR was developed during the second half of the 19th century (173-175). In the first half of the 1960's closed-chest cardiac compressions replaced the open-chest method (176-178).

The purpose of cardiac compressions is to provide blood flow to vital organs (i.e. heart and brain). Under optimal conditions, chest compressions may maintain from 30-40% to even more than 50% of normal cerebral and myocardial blood flow due to preferential circulation of these organs (179-181). However, the prognosis of infants receiving CPR due to cardiac arrest is poor as survival to discharge has been reported to be only 2.4-21% (182). In a British study of 105 infants, 52% of those receiving CPR, including adrenaline and/or atropine died within 12 hours of treatment (183), and optimising chest compressions might be a way of improving outcome (184). Infants who require prolonged CPR with no signs of life beyond 10 minutes have been reported to have an 83% mortality and 77% of survivors suffer from severe disability, 15% from moderate disability, 8% from mild disability, with no normal survivors (130).

The exact mechanism by which chest compressions generate forward blood flow has yet to be identified. Two commonly referred theories are the “cardiac pump” and the “thoracic pump” theories. Being the oldest of the two, the cardiac pump theory is explained by compression of the heart between the chest (sternum) and the spine, resulting in blood flow due to a direct mechanical effect on the left ventricle (185). A bit more complex is the thoracic pump theory which proposes that forward blood flow is driven by a pressure gradient between the arterial and venous compartment resulting from collapse of the veins during compression of the thorax (186;187).

In newborns, mainly two methods for providing cardiac compressions are being applied: the two-thumb encircling hands technique and the two-finger technique (Table 4). The two-

thumb technique might cause less resuscitator exhaustion and higher quality compressions as indicated by a greater depth, less variability with each compression and more correct positioning of the compressions over a longer time period than the two-finger method (188). Also, this technique may generate higher peak systolic pressure and CPP than the two-finger technique (189;190). Thus, the two-thumb technique is recommended for performing chest compressions in the newborn. However, despite the notion that the two-thumb method is the better, the two-finger technique might be more appropriate in single rescue because of reduced ability to adequately manage the airways with the two-thumb technique in this setting (191). Also, the two-finger technique may be preferable during umbilical catheter insertion (Table 4).

<b>Two-thumb encircling hands</b>	<b>Two-finger</b>
Less rescuer exhaustion	Single rescuer
Higher quality compressions over time	Umbilical catheter insertion
Higher peak systolic and coronary perfusion pressure	

Table 4 Advantages of the two-thumb encircling hands and the two-finger technique, respectively during neonatal cardiopulmonary resuscitation

Even though current recommendations for performing cardiac compressions in the newborn might seem clear and concise, one should bear in mind that much of the knowledge about methods for and haemodynamics of closed chest cardiac compressions in the newborn comes from cadaver studies and case reports from the 1960's (192;193). The methods used for performing cardiac compressions the following two decades were to a large extent based on these studies. However, radiographic studies and one small clinical cross-over trial published in the mid 1980's (194-196), lead to recommendations for placing external pressure over the lower third rather than over the middle third of the sternum. A cardiac compression in the newborn is therefore today defined by the ILCOR as external pressure applied to the center of the chest, aimed at moving the lower third of the sternum 1/3 of the anteroposterior diameter of the chest towards the thoracic spine, followed by rapid decompression and passive

expansion of the thorax. However, the question of which is the most effective way of performing cardiac compressions in asphyxiated neonates remains to be elucidated (197).

## **Compression to ventilation ratios**

The rationale for maintaining age-specific compression:ventilation (C:V) ratios is that the aetiology of cardiac arrest in the newborn, paediatric and adult populations differs.

Aetiology-based CPR suggests that initial C:V ratios should emphasise chest compressions in adults with primary ventricular fibrillation events (primary cardiac origin), whereas newborns with primary hypoxic hypercapneic cardiac arrest need more ventilations (198-203).

The main purposes of the circulation are to deliver oxygen and remove metabolic waste products, and circulation may be of benefit even if little or no oxygen is delivered. The effectiveness of the circulation can be viewed as the product of some function of blood flow multiplied by some function of oxygen delivery (204). Theoretically (apart from the small amount of ventilation generated by chest compressions themselves), neither compression only nor ventilation only CPR can sustain systemic oxygen delivery (205). In addition, the results of a study of infant pigs indicated that continuous cardiac compressions caused grave impairment of myocardial and cerebral perfusion, especially if resuscitation was prolonged (206).

Because a single C:V ratio for all age groups would be easier to teach, learn, remember and perform, national and international guideline developers have considered recommending a single C:V ratio. Such a universal ratio should address the minimum number of compressions and ventilations delivered and rescuer fatigue (207). Available data indicate that assisted ventilation rates are typically higher and chest compression rates are often lower than recommended (156). Interrupting chest compressions for ventilations results in fewer chest compressions and can result in less blood flow per minute of CPR (208;209). Also, the neonatal ratio of 3:1 might be more difficult to perform than resuscitation at higher C:V ratios (207).

## **Adrenaline**

Adrenaline is used in CPR in an attempt to increase CPP via peripheral vasoconstriction (210). In newborns, adrenaline administration is recommended if the heart rate remains less than 60 beats per minute despite effective positive pressure ventilation with 100% oxygen together with cardiac compressions for 30 seconds (46). The need for CPR and/or adrenaline has been reported to be 1.2 in 1000 live births (117).

Adrenaline exerts its effects through  $\alpha$ - and  $\beta$ -adrenergic receptors, and is used in CPR mainly because of the  $\alpha_2$ -adrenergic effect on peripheral vessels (211-213) with resulting increase in blood flow to the brain and heart (214;215). On the other hand, the  $\alpha_1$ -adrenergic and  $\beta$ -adrenergic actions might be detrimental due to associated myocardial dysfunctions (216;217). Also, cerebral microcirculation might be negatively affected by i.v. adrenaline administration (218). In adult animal models of hypoxic bradycardia, adverse effects including tachycardia, hypertension and increased mortality have been described from the inappropriate administration of standard dose i.v. adrenaline. Also, uncertainty exists on whether adrenaline in adult resuscitation improves survival to hospital discharge (219;220). However, extrapolating data for newborns from ventricular fibrillation arrest models and clinical studies in adults might not be appropriate (221).

When it comes to the optimal dosage and route of administration, few studies from the paediatric population exist (222). Until such studies are undertaken, the recommended i.v. dose is 0.01 to 0.03 mg/kg, and endotracheal administration of adrenaline should be avoided. The concentration of adrenaline should be 0.1 mg/ml (46). Higher doses might cause hypertension, as well as decreased myocardial and neurologic function (223-225).

## **Inspired oxygen fraction**

During the past 30 years, the increased cell and tissue injury resulting from high oxygen exposure of hypoxic tissues, and the role of free radical production and oxidative stress with resulting apoptosis and reperfusion injury have been explored (226-228).

There is now considerable evidence from animal and human studies that hyperoxia is damaging to the neonatal brain and other organs. Randomised studies in asphyxiated newborn babies also strongly suggest that air is at least as effective as 100% oxygen in the short term (227;229;230). Based on this evidence, the ILCOR recommended in October 2010 that ventilation of term infants in need for resuscitation should be initiated with air (46).

When it comes to the depressed preterm infant, there seems to be a need for at least some supplementary oxygen, but the exact amount is not known (227). The risk of hyperoxia if 100% oxygen is used (231;232), has lead to suggestions that oxygen be titrated to achieve a certain SpO<sub>2</sub> (233;234). Recently, Dawson et al. made a substantial effort in establishing cut-off values for preterm infants based on the natural rise in SpO<sub>2</sub> in healthy preterm infants the first minutes after delivery (235).

So, even though the optimal oxygen concentration during resuscitation is not yet entirely known, the resuscitator needs to be aware of the toxic effects of both hypoxia and hyperoxia. Choosing the right FiO<sub>2</sub> is also difficult because we usually do not know the severity or duration of the hypoxic-ischaemic event in a particular individual (227). However, neonatal transition is a gradual process (healthy babies born at term may take  $\geq 10$  minutes to achieve an oxygen saturation  $>90\%$ ) (236), and a low saturation at birth should not automatically lead to oxygen supplementation.

In conclusion, it seems as if though there is now substantial evidence that most infants requiring resuscitation in the delivery room (regardless of gestational age) benefit from a lower concentration of oxygen (preferentially air). However, there are some newborns that might require at least some extra oxygen:

1. Premature infants with surfactant deficiency and other primary lung pathology (237).
2. The most severely asphyxiated individuals in secondary apnea (237).

## METHODOLOGY

### Pro-inflammatory cytokines

Ischaemia and reperfusion induce an inflammatory response in tissues, including the brain (238). Cytokines, including interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, transforming growth factor- $\beta$  (TGF- $\beta$ ) and fibroblast growth factor (FGF) are more abundantly expressed by activated leukocytes (239;240); in the central nervous system by activated microglia (241-243).

Cytokines mediate extravasation of inflammatory cells into reperfused tissue. Increased expression of adhesion molecules such as P- and E-selectins and Inter-Cellular Adhesion Molecule-1 (ICAM-1) on endothelial cells (244;245); and of integrins on leukocytes (246), mediates neutrophil adherence to the endothelium. The neutrophils then migrate through the vessel wall and accumulate in the interstitium (247-250). Neutrophils are activated by several inflammatory mediators released during ischaemia (e.g. cytokines, platelet-activating factor). They produce O<sub>2</sub><sup>-</sup> (superoxide anion), other oxygen metabolites and hypochlorous acid which in turn alter proteins, carbohydrates, DNA and lipid membranes leading to cell and organ dysfunction (251).

Over-expression of pro-inflammatory cytokines has been observed in areas of leukomalacia in the brain (252), and hypoxia-ischaemia and inflammation therefore share several pathogenetic mechanisms (253). Concomitant hypoxia-ischaemia and infection are thought to augment each other.

Results from clinical and experimental studies are somewhat conflicting with regards to which cytokines that are up- or downregulated in perinatal asphyxia and at what time. In experimental animal studies a huge range of models of perinatal asphyxia, as well as differences in tissue- and fluid preparation make comparisons between the different studies difficult. We therefore did not find the available data sufficient for us to make a choice on which cytokine to measure in our studies and in what time frame specimens should be collected. We therefore tested methods for cytokine analysis of tissue homogenates and fluids (cerebrospinal fluid (CSF), bronchoalveolar lavage fluid (BALF) and plasma) from two asphyxiated and two control pigs. The control animals had underwent instrumentation (intubation and placement of venous and arterial catheters), but not asphyxia. Fully aware of

the small sample size, we reached the following conclusions (cut from our laboratory protocol from the autumn of 2007):

#### CNS

IL-1 $\beta$  was expressed more abundantly in the brain of the asphyxiated piglets compared to the control animals. The levels of IL-1 $\beta$  in the CSF of both the asphyxiated and control animals were not detectable.

As for IL-6 and TNF- $\alpha$ , these could only be detected in the brain of one of the control animals. IL-6 could be detected in the CSF of one of the asphyxiated and one of the control animals.

IL-8 was detectable at low levels in the brains of the control animals. The control animal with the highest amount of IL-8 in brain tissue, also had detectable levels in CSF. IL-8 was not detectable in the CNS of the asphyxiated animals.

#### RESPIRATORY ORGANS

IL-1 $\beta$  was detectable in relatively high amounts in the lungs of the asphyxiated animals. There were also detectable levels of IL-1 $\beta$  in BALF of these animals. No IL-1 $\beta$  was found in the lungs of the control animals.

TNF- $\alpha$  was expressed more abundantly in the lungs of the control animals than in the asphyxiated ones. This also seemed to be the case for IL-6.

#### LIVER

The amounts of all the cytokines measured in this study were comparable between the groups.

#### KIDNEY

IL-1 $\beta$  and IL-6 were measured at considerable higher levels in the asphyxiated animals. TNF- $\alpha$  and IL-8 were detectable at comparable levels in the two groups.

#### PLASMA

IL-1 $\beta$  was not detectable in the plasma of any of the animals.

As for the other three cytokines, levels were higher in the control animals.

#### LIMITATIONS

The use of other biological fluids and tissue homogenates in place of serum samples in the porcine ELISA kits from R&D Systems has not been thoroughly investigated.

Sera from Yorkshire and Chester-White pigs have been validated for use in this assay. Other strains of swine have not been tested and consequently their use has not been validated.

These results, showing an increase in IL-1 $\beta$  in the brain and lungs of asphyxiated animals, together with neonatal rat data indicating that the expression of mRNA and bioactive protein in brain extracts for the pro-inflammatory cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$  increase 1-4 hours after hypoxia-ischaemia (254;255), made us decide on measuring IL-1 $\beta$  in brain and

lung 4 hours after asphyxia. Later we experienced problems with homogenisation of tissues (giving undetectable levels of cytokines in the Quantikine porcine IL-1 $\beta$  enzyme-linked immunosorbent essay (ELISA) (R&D Systems Europe, Ltd., Abingdon, UK)). We therefore decided to analyse CSF and BALF, possibly reflecting expression of IL-1 $\beta$  in the organs of interest, even though IL-1 $\beta$  had not been detectable in CSF in our pilot animals.

## **Lactate/pyruvate**

Pyruvate is formed as a product of glucose metabolism in the cells. Under aerobic conditions most of the pyruvate formed during the glycolytic process is further metabolised through the tricarboxylic acid cycle. Under anaerobic conditions, pyruvate serves as hydrogen acceptor for NADH<sub>2</sub> and is reduced to lactate: pyruvate + NADH<sub>2</sub> → lactate + NAD<sup>+</sup>.

Hence, hypoxia leads to accumulation of lactate which can be used as an indirect measure of tissue hypoxia. There is a strong correlation between umbilical cord pH, base deficit and lactate, and several investigators claim that measurement of lactate is more feasible than that of pH in surveillance of foetal distress (256). However, as lactate can be elevated by any condition or substance that increase blood glucose and glycolysis (e.g. β-agonists or catecholamines) (257), the lactate/pyruvate (L/P) ratio is suggested to be a more specific marker of tissue hypoxia in the newborn (258). The L/P ratio has been used as an index of oxygen availability to tissues (259).

Since most cell membranes are permeable to lactate and pyruvate, measurements of extracellular L/P ratios can be used for detecting tissue hypoxia. In experimental and clinical conditions of cerebral hypoxia, the L/P ratio in cerebrospinal fluid (CSF) seems to reflect changes in the cerebral NADH<sub>2</sub>/NAD<sup>+</sup> system (260-263).

## Near Infrared Spectroscopy (NIRS)

Oxygen saturation can be determined by the ratio of oxyhaemoglobin (HbO) to total haemoglobin (Hb + HbO)

One way to measure oxygen saturation is spectrophotometry of haemoglobin. Common forms are pulse oximetry, tissue oximetry and vascular oximetry. Haemoglobin (Hb) is the primary oxygen carrier in blood, and both Hb and HbO have their distinct colour spectra (fingerprints). Pulse oximetry is, as the name indicates, dependent on pulse. Thus, pulse oximetry measures oxygenation in arteries, whereas tissue oximetry measures oxygenation in a mixed vascular bed, approximately 30% arterial and 70% venous (venules). This technology takes advantage of the fact that some near infrared wavelengths readily penetrate human tissue, including the bone of the infant skull (264;265).

Tissue oximetry, near infrared spectroscopy (NIRS) has been used for cerebral, splanchnic, gastrointestinal and renal oxygenation monitoring (266;267). Reported use of cerebral tissue oximetry include, in addition to HIE (and hypothermia), organ transplantation, extracorporeal membrane oxygenation (ECMO), high frequency oscillatory ventilation (HFOV), inhaled nitric oxide (iNO) therapy and cranial trauma (268).

A probe is placed on the skin overlying the tissue of interest. Near-infrared light (730 nm and 810 nm) is emitted from the probe, and the light penetrates the tissue below. As previously mentioned, HbO and Hb (deoxygenated Hb) absorb light at these wavelengths, each with their own respective absorption and reflection properties. From the amount of light reflected back to the probe, the oximeter determines the percentage of HbO and Hb in the underlying vascular bed. As previously mentioned, these values are displayed as a proportion of HbO in comparison to the total amount of haemoglobin, i.e. HbO/Hb + HbO (total Hb). This percentage represents the regional tissue oxygen saturation ( $rSO_2$ ), and is also being referred to as the tissue oxygenation index (269;270)

Brain tissue oximetry measures brain tissue oxygen tension reflecting the combined effect of blood flow, arterial oxygenation and metabolic activity (271).

Cerebral fractional tissue oxygen extraction (cFTOE) can be calculated from  $rScO_2$  and  $SpO_2$  values. A ratio of  $(SpO_2 - rScO_2)/SpO_2$  represents the balance between oxygen delivery and oxygen consumption (272).

Previous reports indicate that reduction of ventilation lowers brain perfusion and cerebral tissue oxygen tension during CPR (160;273)

## **AIMS OF THE THESIS**

We wanted to compare some of the current guidelines for neonatal resuscitation to alternative measures. By doing this we hoped to initiate a discussion and stimulate to further work in support of an evidence-base that until now has been missing when it comes to the parts of the neonatal resuscitation algorithm we chose to investigate:

1. We find that at the time of study, there was in fact substantial evidence that air is as good as or better (less harmful) than 100% oxygen in resuscitation of asphyxiated newborns. However, clinical and experimental studies to date have mostly studied individuals with mild or moderate asphyxia and compromise. We therefore wanted to investigate whether air could be used also in severely compromised newborn pigs with cardiac arrest (Paper I).
2. The recommendations for initiation of cardiac compressions if the heart rate continues to be less than 60 beats per minute after 30 seconds of positive pressure ventilation have not been subject to scientific evaluation (Paper II).
3. A compression:ventilation ratio of 3:1 has been chosen according to expert opinion and long-standing tradition based on the aetiology of cardiac arrest (and bradycardia) in the newborn. This ratio emphasises ventilation, but whether 3 compressions in a series can generate sufficient coronary perfusion pressures in order to achieve return of spontaneous circulation is unknown (Paper III and IV).

## **MATERIALS AND METHODS**

The experiments were performed in a modified asystole version of a well-established porcine model of neonatal asphyxia in accordance with The Norwegian Animal Welfare Authority Act and approved by The Norwegian Animal Research Authority.

### **Animal preparation and sedation/analgesia**

Healthy newborn Noroc pigs weighing 2.0-2.7 kg were anaesthetised with Sevoflurane gas 5% prior to a bolus of i.v. midazolam, fentanyl and pentobarbital. The animals were tracheostomised and placed on a mechanical ventilator before surgical instrumentation. After establishment of an intravenous access in the left external jugular vein, sedation and analgesia were maintained by a continuous infusion of midazolam and fentanyl.

### **Haemodynamic measurements**

A 1.5-2 cm incision was made on the right side of the neck (approx. 2 cm lateral to the trachea), and the common carotid artery was found through dissection. A 22GA venflon-catheter was then introduced for continuous blood pressure and heart rate (HR) recordings using the Biopac 150 module-based acquisition system and the Acqknowledge® software. From the pressure tracings we could read DBP and mean arterial blood pressure (MABP) for comparisons between the different interventions. Because of some drift in the baseline of the invasive blood pressure curve due to the different positioning of the animals throughout the experiment (the positioning of the animals changed between the prone and supine position at least thrice during each experiment), we had some trouble with calibration of the equipment. However, we expect this drift to have been equal between the treatment arms, and DBP calculations focused on changes in DBP, not absolute values.

### **Near Infrared Spectroscopy (NIRS)**

A paediatric cerebral tissue oximetry probe was placed on the left side of the head in half of the pigs. Because we resuscitated two pigs each day of the experiments, but only had one cerebral oximeter, we were not able to obtain NIRS-values for the other half of the pigs. The daily animal we obtained NIRS-values for was the one placed on the bench in the operating room where the cerebral oximeter was installed. Since randomisation to intervention was

made after stabilisation for all animals, systematic allocation to NIRS-registration within each group of interventions was not made.

rSO<sub>2</sub> was continuously recorded (with registrations every 4 seconds) on an external computer hard disk. After the experiments cerebral fractional tissue oxygen extraction (cFTOE) was calculated using the formula (SpO<sub>2</sub>-rScO<sub>2</sub>)/SpO<sub>2</sub>.

### **End-tidal CO<sub>2</sub>**

We measured end-tidal CO<sub>2</sub> with the Datex Normocap Oxy (Datex, Helsinki, Finland). We used the values in order to:

- a) Follow progression of asphyxia and targeting of pCO<sub>2</sub>
- b) Confirm endotracheal tube in the trachea

### **ECG**

The three ECG leads were placed behind the left and right ear and in the perineum, respectively. ECG was continuously registered through the Biopac 150 and saved to a computer hard disk using the Acqknowledge® software. The ECG tracings turned out quite coarse, and could not be used for detailed rhythm analysis. However, the ECG was used to confirm a heart rate of zero during the experiments and retrospectively.

### **Arterial blood sampling**

Arterial specimens (0.2 ml) for temperature corrected blood gas analysis (and glucose and lactate) were drawn from the catheter in the common carotid artery immediately following surgical instrumentation (i.e. at the beginning of the 60 min stabilisation period), after 20 min, 40 min and 60 min (i.e. at the end of the stabilisation period, defined as “baseline”). Arterial blood gases were also analysed every 5 min throughout asphyxiation and each of the first five minutes following ROSC. The blood gas data presented in the respective papers as “at asystole” were from the last blood gas obtained before HR=0, i.e. a maximum of 5 minutes before actual asystole. Blood gas data presented as “immediately after/following ROSC” are from the sample taken 1 min after ROSC. Five minutes after ROSC, blood gases were analysed with gradually increasing intervals, and the last three hours of postresuscitation observation, arterial blood gases were analysed half-hourly.

In addition, 4-5 ml of EDTA-blood were preserved from baseline (immediately before initiation of the asphyxiation process), 2 hours after ROSC, and at the end of the experiment (4 hours after ROSC)

In the first, second and third study (corresponding to Paper I, II and III, respectively), 1 ml of whole blood was drawn at the end of the experiment for analysis of lactate and pyruvate at the Department of Clinical Biochemistry at Oslo University Hospital Rikshospitalet.

### **Cerebrospinal and bronchoalveolar lavage fluid**

At the end of the experiment, immediately before killing of the animals, CSF was collected through a lumbar puncture (using a 23 G syringe). Immediately after death, bronchoalveolar lavage was performed using 30 ml/kg warm saline. Aspirated samples were immediately centrifuged at  $2000\times g$  and 4 °C for 20 min to remove cells. The supernatant was transferred to polypropylene tubes (NUNC) and frozen at -80 °C for later cytokine analysis.

In the first, second and third study, 0.5-1 ml of CSF was preserved for analysis of lactate and pyruvate, in addition to cytokine analyses.

### **Chest compressions**

Chest compressions were in papers I-III administered by the same resuscitator. In paper IV, chest compressions were performed by two different investigators.

Compressions were performed with the two-thumb encircling hands technique at a metronome-guided rate of 90 per min, and were aimed to generate a MABP of approximately 20 mmHg.

Post-mortem examination of the position of the heart in the chest of 13 pilot animals determined the point on the pigs' chests to which we applied pressure during CPR in the main studies.

## **Experimental protocol**

The studies underlying paper I, II and III were performed in the same experimental series, with randomisation to one of 5 group:

- 21% oxygen, 30 seconds of initial ventilation, compression:ventilation ratio 3:1 (n=15)
- 100% oxygen, 30 seconds of initial ventilation, compression:ventilation ratio 3:1 (n=16)
- 21% oxygen, 60 seconds of initial ventilation, compression:ventilation ratio 3:1 (n=16)
- 21% oxygen, 90 seconds of initial ventilation, compression:ventilation ratio 3:1 (n=8)
- 21% oxygen, 30 seconds of initial ventilation, compression:ventilation ratio 9:3 (n=16)

Randomisation was made by pulling folded cards from a bowl, and stratification was made with respect to gender. In the analysis of the results, group number 1 served as reference group for comparison against the other groups.

Paper IV is based on experiments performed about one year later. In this series, randomisation to intervention (C:V ratio 15:2) or control (C:V ratio 3:1) was made by drawing sealed envelopes, and stratification was also in this series made with respect to gender. Power analysis for this series was made based on results from the first experiments.

The animals were brought to us from a local farm on the day of the experiments. Before leaving the farm the animals were fed by the sow. Transportation was done in a warm incubator, and prior to the experiment the animals were weighed. Anaesthesia was induced by inhalation of Sevoflurane 5% for two minutes. A Neoflon was then placed in an ear vein, through which midazolam, fentanyl and pentobarbital were given as bolus injections. The animals were then tracheostomised with a 3.5 endotracheal tube and placed on a Babylog ventilator with  $\text{FiO}_2$  0.21, PIP 25 and PEEP 5 mmH<sub>2</sub>O. Initial rate was set to 25 breaths per minute and adjusted according to pCO<sub>2</sub> values. Following 60 minutes of stabilisation, asphyxiation was initiated by reducing  $\text{FiO}_2$  to 0.08 and adding CO<sub>2</sub> to inspiratory gases to achieve a pCO<sub>2</sub> > 7 kPa (titrated after end-tidal CO<sub>2</sub> values and blood gas analyses).

Asphyxia was maintained and the ventilator rate reduced by 10 breaths per minute every 10 minutes until the heart rate was zero as judged by ECG, invasive pressure tracings and

cardiac auscultation. At asystole we turned the animal from the prone to the supine position and CO<sub>2</sub> was discontinued at the same time as we switched the ventilator settings to FiO<sub>2</sub> 0.21 (except for the animals randomised to resuscitation with 100% oxygen –Paper I), and rate of 40 breaths per minute. The positioning and ventilator adjustments took approximately 20-30 seconds, mimicking the initial stimulation and positioning part of the algorithm for newborn resuscitation. This was followed by providing 30, 60 or 90 seconds (Paper II) of positive pressure ventilation (PIP 25 cm H<sub>2</sub>O, PEEP 5 cm H<sub>2</sub>O) before initiation of cardiac compression at the ratio assigned by randomisation (Paper III and IV). If cardiac compressions together with ventilation failed to induce ROSC after 30 seconds, 0.02 mg/kg of i.v. adrenaline was administered and chest compressions and ventilations were continued at the assigned ratio. As per ILCOR guidelines, i.v.adrenaline administration was repeated every third minute of CPR until ROSC, defined as a heart rate  $\geq$  100 beats per minute. If ROSC did not occur within 15 minutes of resuscitation the animal received an overdose of i.v. pentobarbital and the experiment was ended. If successful resuscitation, the animal was observed on the ventilator for 4 hours prior to lumbar puncture and pentobarbital-induced death. In the animals resuscitated with 100% oxygen, FiO<sub>2</sub> was switched to 0.21 after 30 minutes of observation and the animals were ventilated with air for the remaining 3.5 hours.

Throughout the experiment a continuous infusion of midazolam and fentanyl was given, together with Salidex.

## Statistical analysis

Based on <sup>2</sup>a pilot experiment comparing the effect of 21% and 100% oxygen in this newborn pig model of neonatal asphyxia and cardiac arrest, the studies underlying paper I through III were powered to detect a difference in 50 s in time to ROSC between the groups with a type I error rate of 0.05.

We had no comparable outcome data, so we had to determine what would be a “clinically significant difference” in our studies. Based on the pilot data, we set this difference to be 50 seconds. By using the following formula:

$$n = 2\left(\frac{\sigma}{\Delta}\right)^2 \cdot k$$

Where  $\sigma$ =standard deviation and  $\Delta$ =clinical significant difference and  $k$  representing a significance level (type I error rate) of 0.05 and a power of 80% (0.80) as shown in Table 5, the number of pigs needed ( $n$ ) in each study arm (group) was 16.

Significance level	Power			
	0.95	0.90	0.80	0.50
0.10	10.8	8.6	6.2	2.7
0.05	13.0	10.5	7.9	3.8
0.01	17.8	14.9	11.7	6.6

Table 5 Estimation of  $k$  as a function of significance level and power

As the experiments reported on in paper I through III were undertaken in parallel (in the same series in order to be able to utilise the “control” animals for comparison against several alternative interventions), this power calculation determined the number of pigs in each group reported on in paper I-III.

<sup>2</sup> The pilot experiment included 13 asphyxiated pigs with times to ROSC ranging from 30 to 200 seconds (the times to ROSC being expressed as even “tens” in the pilot study), mean time to ROSC being 131.2 seconds with a standard deviation (SD) of 46.9 seconds.

The experiment reported on in paper IV was undertaken with 11 animals in each group after a renewed power estimate based on the ROSC-data from the pigs resuscitated with 100% oxygen and air (C:V ratio 3:1, initial ventilation for 30 seconds), data for which are presented in paper I.

Most descriptives were given as mean and standard deviations (SD), and comparisons between groups were mostly made using parametrical tests (student's test). However, due to skewness of the data, times to ROSC were given as medians and interquartile ranges (IQR), and the Mann-Whitney test was used for comparing this parameter between the groups. In paper I, II and III this was also done in order to accommodate for the animals that did not achieve ROSC and were given the arbitrary ROSC-value of 1000 s.

### **Repeated measures analysis of variance (ANOVA)**

In general, ANOVAs test quality of means. Repeated measures ANOVA is used when all members of a random sample are measured under a number of different conditions; the measurement of the dependent variable (e.g. blood pressure) is repeated. As opposed to standard ANOVAs, the repeated measures ANOVA accommodates for correlation (dependence) between the repeated measures.

In this particular setting the repeated measurements model is an analysis of variance accommodating for interdependence within different points of time in each animal and independence between pigs.

All statistical analyses were performed using the software package SPSS for Windows version 14.0 (Paper I-III) or version 15.0 (Paper IV).

## **SUMMARY OF RESULTS**

### **Paper I**

#### **Resuscitation of severely asphyctic newborn pigs with cardiac arrest by using 21% or 100% oxygen**

In this study median time to ROSC (IQR) was 150 s (115-180) for the group resuscitated with air (n=15), whereas animals in the 100% group (n=16) achieved ROSC after 135 s (113-168) ( $p=0.80$ ). The results indicate that the effect of air is similar to that of 100% oxygen in resuscitation from perinatal asphyxia, even when the animals were in the state of complete cardiac arrest, as opposed to resuscitation of mild to moderately asphyxiated individuals with less severe depression. We were not able to demonstrate a difference in markers of hypoxia and inflammation in the animals exposed to air and 100% oxygen, respectively. Four out of 16 animals resuscitated with 100% oxygen died during the 4 hour postresuscitational observation, whilst all pigs resuscitated with air survived to completion of the experiment.

### **Paper II**

#### **Delayed onset of cardiac compressions in CPR of newborn pigs with asphyctic cardiac arrest**

Pigs ventilated for 30 s before initiation of cardiac compressions (n=15) achieved ROSC in 150 s (115-180), whereas animals ventilated for 1 minute (n=16) and 1.5 minute (n=8) initially achieved ROSC after 163 s (124-177) and 282 s (199-364), respectively (median (IQR)). There was no difference in time to ROSC when we ventilated for 30 s compared to 1 minute before initiation of cardiac compressions. However, when comparing 1.5 minute of ventilation to the currently recommended 30 s, time to ROSC was significantly longer ( $p<0.001$ ). There were no differences in markers of hypoxia and inflammation between the groups.

### **Paper III**

#### **Extended series of cardiac compressions during CPR in a swine model of perinatal asphyxia**

We were not able to achieve a higher DBP during resuscitation when we administered 9 cardiac compressions in a series, as opposed to 3. Oxygenation and ventilation, evaluated by

arterial blood gases were similar whether we resuscitated asphyctic animals with compressions:ventilations 3:1 or 9:3. These factors together possibly resulted in a comparable time to ROSC when newborn pigs with cardiac arrest were resuscitated with a compression to ventilation ratio of 3:1 (n=15) and 9:3 (n=16), respectively.

#### **Paper IV**

##### **Return of spontaneous circulation with compression to ventilation ratio 15:2 versus 3:1 in newborn pigs with cardiac arrest due to asphyxia**

When we increased the number of cardiac compressions delivered in a series to 15, mean increase in DBP during compression cycles was higher as compared to the standard of 3 compressions between ventilations. However, in both groups the mean (SD) DBP (mmHg) we were able to achieve with cardiac compressions was low: 7.1(2.8) versus 4.8(2.6), in the 15:2 and 3:1 group, respectively ( $p=0.004$ ).

Hence, the main outcome parameter, time to ROSC was not different between pigs resuscitated with the C:V ratio of 3:1 (n=9) versus 15:2 (n=9).

## **DISCUSSION OF THE RESULTS**

### **Paper I: Oxygen**

The primary outcome parameter in this study, time to return of spontaneous circulation, was not different between asystolic pigs resuscitated with 21% or 100% oxygen. This is in accordance with the results of a study of asystole following severe asphyxia in newborn pigs by Linner et al. (274).

Experimental and clinical studies indicate that resuscitation with 100% oxygen increases inflammation and oxidative stress (275;276). We did not find a difference in expression of IL-1 $\beta$  at the protein level in CSF and BALF, possibly due to lack of sensitivity or wrong timing of sample collection. We did not measure markers of oxidative stress in this study, but the relatively prolonged exposure to oxygen (30 min) in our model might have increased the extent of hypoxic-ischaemic injury both through these mechanisms and by decreased cerebral blood flow caused by hyperoxia. This might in part explain why 25% of the animals resuscitated with 100% oxygen did not survive to completion of the protocol, although overall survival was not statistically different between the two groups.

With regards to the results of this study, we argue that it is safe to initiate ventilation with air, even in severely asphyxiated newborns with cardiac arrest.

After this study was undertaken, new guidelines for neonatal resuscitation was published, and recommendations are now that one should initiate ventilation with air in term newborns requiring resuscitation (46).

### **Paper II: Ventilation**

The results of this study investigating different durations of positive pressure ventilation before initiation of cardiac compressions indicate that 30 seconds of effective ventilation might be too short. One can therefore argue that at least in the case of bradycardia ( $HR < 60$  beats per minute) one should be more patient before giving up on ventilation only and cardiac compressions are initiated.

The assumption is that after delivery of an infant it is difficult to differentiate between primary and secondary apnea (277). Therefore, present guidelines are aimed at treating apnea as secondary.

However, as cases with asystole are by far outnumbered by bradycardia in newborns requiring resuscitation at their birth, our results are likely to be in the “conservative” end when it comes to changes in practice. However, customising guidelines is obviously at the expense of simplification, and retention of resuscitation skills is easier if guidelines are simplified. The changing of guidelines is therefore a complex issue.

Considering the potential physical trauma of receiving cardiac compressions, these factors should, however, be taken into account when new guidelines and algorithms are made. In fact, as effective ventilation is sufficient to resuscitate almost all apneic neonates, updated ILCOR guidelines suggest that adequate ventilation should be ensured before proceeding with chest compressions. The neonatal resuscitation flow algorithm does no longer suggest a definite duration of ventilation before chest compressions should be started (46).

In our model, the animals had completed transition, and there is no doubt that the respiratory and circulatory responses of these animals to resuscitation were different from the responses of a newborn where the most crucial part of resuscitation is to clear fluid from and fill the lungs with air.

After publication of the paper, renewed calculations for the parameters pH, BE and pCO<sub>2</sub> at ROSC were made because the distribution of these parameters turned out to be skewed (not normally distributed). Non-parametrical tests for comparisons between groups were made with the result that the higher pCO<sub>2</sub> at ROSC in both groups with prolonged initial ventilation (1 and 1.5 min) compared to the group ventilated for 30 s became statistically significant.

The clinical relevance of the results indicating higher pCO<sub>2</sub>-values with prolonged intervals of initial ventilation (1 min and 1.5 min, respectively) is uncertain, but might be subject to further studies. However, animal data indicate that higher pCO<sub>2</sub> following asphyxia restores cerebral microcirculation faster (2) and that mild hypercapnia is associated with less severe brain injury following hypoxia-ischaemia (278). Moderately high pCO<sub>2</sub> might also reduce cellular oxygen demand and facilitate oxygen delivery (279).

### **Paper III and IV: Cardiac compressions**

Since neither the C:V ratio of 9:3 nor 15:2 proved to be better than the ratio of 3:1, our results are not in favour of change in current resuscitation practices in suspected asphyxia. However,

updated guidelines for neonatal resuscitation state that a higher C:V ratio than 3:1 can be considered when the cause of cardiac arrest is suspected to be of cardiac origin (46).

Pigs have become increasingly popular models in CPR studies. A large number of studies on chest compressions have been performed on adult pigs (208;280;281), and a smaller number of paediatric and neonatal CPR-studies in pigs have been reported (274;282). In the interpretation of the results, not many authors express concerns that the shape of the porcine chest is different from that of a human, but they usually argue that effectiveness of chest compression is adequate, also when performed by compressing the V-shaped chest of the pig in the anteroposterior direction. However, Steen et al. problematised this after having performed anteroposterior chest compressions in adult pigs (283). Based on post-mortem examination of their animals, they claim that the shape of the pig's chest makes compressions generate forward blood flow only by means of the "thoracic pump" mechanism and not by means of the "cardiac pump" mechanism (described on p. 41 in this synopsis).

In a study by Neurauter et al., human and porcine chest stiffness and viscosity were compared, and they conclude that the human and porcine chest do not differ much with regards to stiffness. However, differences in chest viscosity at medium and deep chest compression depth, possibly partly due to the shape of the porcine chest and the structure of the sternum, should be taken into account when extrapolating porcine results to humans (284).

Even though these considerations call for caution when interpreting the results of our CPR studies, we argue that the relative differences found between interventions in this model may be relevant to human infants, and might highlight mechanisms of CPR from cardiac arrest in asphyxia.

In our study, we defined effective chest compressions as compressions that resulted in a MABP of at least 20 mmHg. As we can see from representative arterial pressure tracings, we succeeded in reaching this target (Figure 4). However, one might argue that this target might have been set too low, as DBP not at any C:V ratio by means of compressions alone, reached the threshold of 15 mmHg assumed to be necessary to achieve ROSC in adults (285).

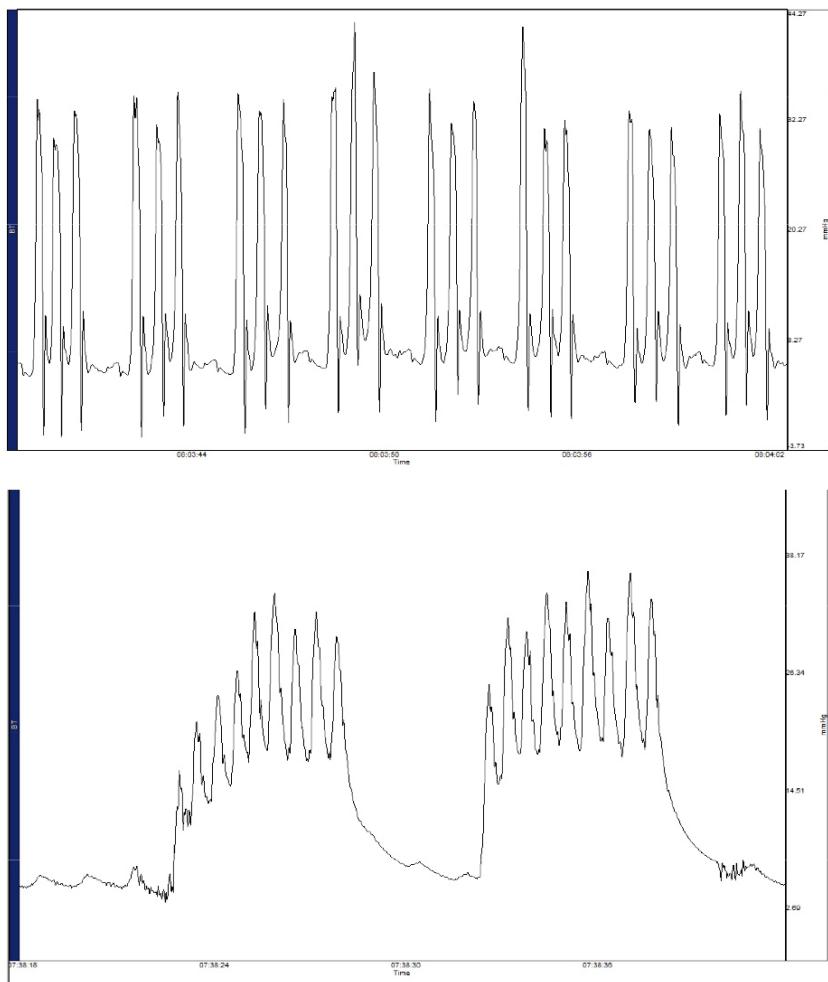


Figure 4 Arterial pressure tracings at a compression:ventilation ratio of 3:1 (top panel) and 9:3 (lower panel)

As we were not able to generate adequate DBP during either intervention (including increasing the number of cardiac compression delivered in series), and as nearly all animals required at least one dose of i.v. adrenaline in order to achieve ROSC (see data in the individual papers), one might wonder if the benefit of adrenaline in severe acidæmia (as is often the case in perinatal asphyxia) at the present time is being underestimated. A recent randomised study in adults indicates that the occurrence of ROSC in asystole or pulseless

electrical activity (PEA) is higher after adrenaline administration. However, there was no difference in short-term outcome in adults whether adrenaline was administered or not when the patient was having ventricular fibrillation or pulseless ventricular tachycardia (286). The authors propose that the degree of benefit or harm of adrenaline might be dependent on the cardiac rhythm at the time of administration. An interesting question is therefore whether the effect of adrenaline is different in newborns with asystole versus bradycardia. Our study was not designed to explore this.

However, as adrenaline, especially in high doses, might increase the risk of intracranial haemorrhage and myocardial damage (287;288), changes in guidelines should be preceded by more studies on appropriate timing, doses and routes of administration.

The impact of an open ductus arteriosus on haemodynamics might also influence the response to CPR. With an open ductus arteriosus, the effect will presumably trend towards the need for higher pressures in order for ROSC to occur as compared to in an individual that does not have this left-to-right shunt. However, asphyxia may induce pulmonary hypertension and reopening of the ductus arteriosus with a right-to-left shunt, the effect in CPR of which to my knowledge has not been studied. Fugelseth et al. showed in a study of asphyxiated newborn pigs reversible pulmonary hypertension measured by increasing tricuspid regurgitation during hypoxia, and a small proportion (approximately 10%) of initially closed ducts reopened during asphyxia (289).

## **GENERAL REMARKS**

### **Animal welfare and ethics**

The use of animals in research in Norway is regulated by the Norwegian Animal Welfare Act. Since they were first described in 1959 (290), the so-called “Three R’s” have had a major impact on legislations and ethical guidelines concerning animal research worldwide. The three R’s are: Refinement, Reduction and Replacement.

Each of these should be accounted for in all applications for approval of animal research. This means that in the case where the researcher does not find a feasible alternative to doing animal experiments (replacement), even though the researcher feels comfortable that the animals do not suffer more than necessary (refinement), the number of animals used for experiments should be kept to the absolute minimum required for the experiments (reduction).

These principles lead to the decision that we would conduct the experiments in a manner where several alternative approaches to resuscitation were compared to the same reference group. Also, the decision to stop inclusion in one of the groups (initial ventilation for 1.5 minute, paper II) because the intervention seemed to us like an “obvious flaw” (time to ROSC was approximately the double of what we had experienced in the other groups) was guided by the principle of reduction. However, it is important to note that included in this principle lies that reduction should be done without compromise of the scientific output and the quality of the research (291), and some might argue that the decision made by us in this case made our conclusions less reliable.

### **Oxygen**

The results from a pilot study indicated that resuscitation with air was as good as 100% oxygen in this pig model of perinatal asphyxia and cardiac arrest. Results from clinical resuscitation studies of asphyxiated human newborns combined with our pilot data lead to the decision that air was used as the standard inspiration gas during resuscitation of the animals in our studies. This raises the question of whether the main outcome parameter, time to ROSC would have been different resulting from the different interventions if other oxygen fractions were used. However, evidence of supplementary oxygen causing oxidative stress and inflammation when used for reoxygenation of the newborn is increasing, and as we

measured parameters of hypoxic/reoxygenation damage, we wanted to reduce the impact of oxygen itself on these factors.

## **Ventilation**

In delivery room resuscitation, a resuscitation bag or a T-piece is routinely used for ventilation of the newborn. In this study, we used the ventilator for this purpose because this would give us more standardised ventilations in terms of pressure and duration of individual ventilations. Also, because of limited number of hands (the experiments were performed by two resuscitators), we found the use of the ventilator a feasible way of also managing cardiac compressions, administer adrenaline and coordinate the events. A limitation to performing the resuscitation in this manner is that sometimes ventilations and compressions inevitably would coincide. However, pauses in cardiac compressions were made in order for effective ventilation(s) to occur after each compression cycle. The ventilations that did occur during the compressions are not believed to have affected the effectiveness of the compressions, as can be seen from arterial pressure tracings showing that inflations do not affect pressures substantially (Figure 4, top panel).

## **Ventilator settings**

During the experiments, the animals were ventilated with pressures that most likely would have contributed to over-ventilation of a healthy human neonate. However, our experience is that the porcine lungs are less compliant than those of humans, and a PIP of 25 cm H<sub>2</sub>O and PEEP of 5 cm H<sub>2</sub>O have been chosen after years of experience with these pigs in our department. The pCO<sub>2</sub> measured throughout the experiment, including the one hour stabilisation period indicates normoventilation with pCO<sub>2</sub> being >4.5 kPa (see data in the individual papers).

## **CO<sub>2</sub>**

The studies described in this thesis were preceded by a pilot study where we initially induced cardiac arrest by clamping the endotracheal tube. Perhaps surprisingly, these animals had a low pCO<sub>2</sub> at asystole, and we were not able to resuscitate them. We therefore started to hypoventilate them by stepwise reduction of the ventilator rate, and at the same time adding CO<sub>2</sub> to inspiratory gases. Unfortunately, we did not test whether adding CO<sub>2</sub> really was necessary in order to achieve “asphyxial” levels of CO<sub>2</sub> in the hypoventilation model. After

our experiments were finished, Linner et al. published a paper where asphyxiated newborn pigs were disconnected from the ventilator after 20 min of hypoventilation and achieved a pCO<sub>2</sub> of about 20 kPa (274).

### Cytokines

The data supporting our choice of cytokines to measure in our studies and at what point of time was rather scarce. However, a considerable amount of time and effort was put into trying to find answers in the literature, and even by contacting some researcher that had done similar work. However, since the published data and the memory of the investigators we contacted proved out to be imperfect, we had to make our own experiences in analysing cytokines at the protein level in our pigs. Tissue homogenisation for the purpose of ELISA analysis turned out to be difficult, and plasma did not give detectable levels in our model (probably due to wrong timing of blood collection). CSF and BALF turned out to be easy to handle and gave measurable and what we saw as reliable results. IL-1 $\beta$  was the one cytokine we “believed” in based on the literature and our own data. Hence, this was our choice. As we were unable to detect differences in this biomarker in our interventional groups, one might argue that this choice may have been inappropriate.

### ROSC

ROSC was defined as a heart rate  $\geq$  100 beats per minute. However, we were able to catch the exact moment where the heart rate reached 100 per minute only in a minority of animals. As can be seen from invasive pressure tracings (Figure 5), heart rate and blood pressure at ROSC rose in a matter of just a few seconds to higher levels than baseline levels (before induction of asphyxia). Hence, heart rate “at ROSC” is reported as being substantially higher than 100 beats per minute. This also explains why the time it took for the heart rate to increase from approximately 60 per min to  $\geq$  100 beats per minute was fairly short (as reported in paper I as being 12 and 17 seconds in the two groups, respectively).

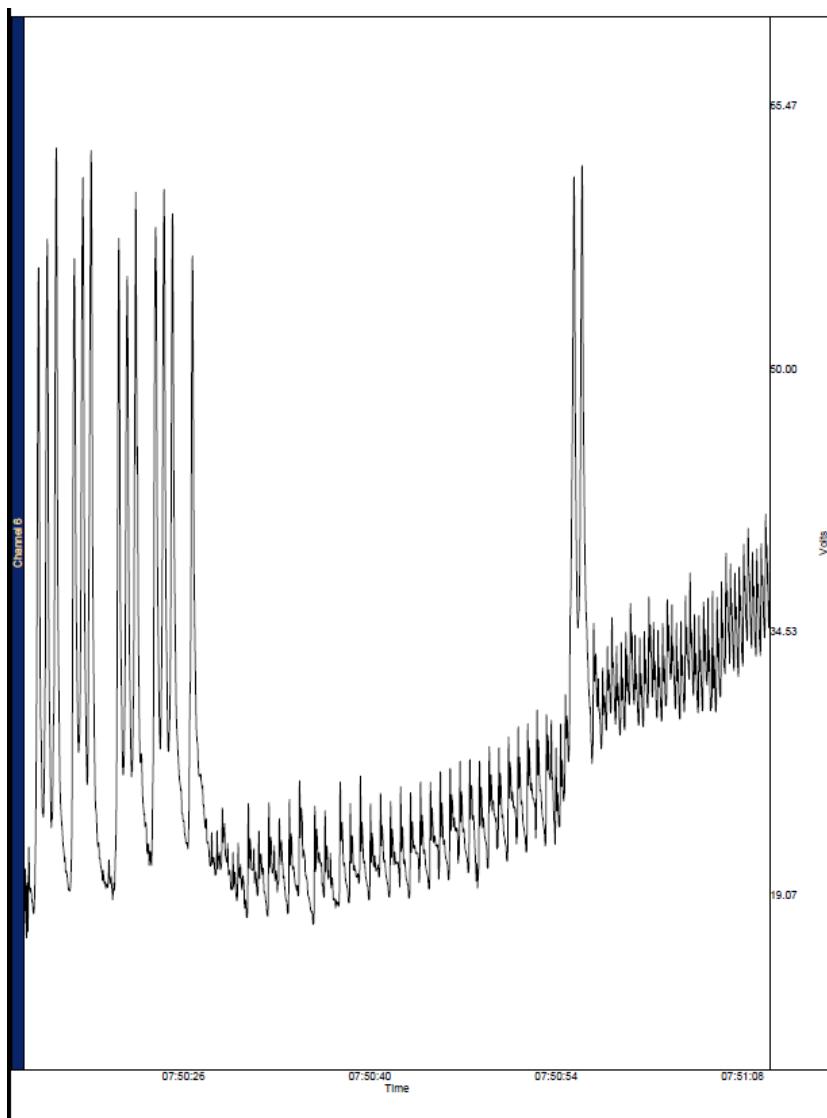


Figure 5 Arterial blood pressure tracing showing rapid changes in heart rate and blood pressure at ROSC. Note that the unit on the y-axis is volts, not mmHg

## LIMITATIONS

To find out more about what is the optimal initial treatment of asphyxiated newborns imposes challenges to the researcher in this field. Special circumstances around the birth of a baby and the fact that the need for resuscitation is often not anticipated, make the ethics surrounding research on newborn resuscitation particularly difficult (1). Hence, different models have been used in order for researchers to gain more knowledge about this group of patients. When the aim is to investigate the effect of ventilation and cardiac compressions in the newborn in order to facilitate transition from intra- to extrauterine life, it is of considerable importance that the model in use resembles the newborn in the first minutes of life. Different animal models of newborn transition have been developed (292;293), however, we chose to use a well-established model of perinatal asphyxia, the advantages and disadvantages of which have been considered thoroughly throughout the years, in the work of this thesis. Even though the model traditionally does not take the animals as far as cardiac arrest as was the case in this thesis, the advantages of experience and years of trying and failing were valued when we decided on using the one day old pigs.

However, as stated in the AHA guidelines: -The following guidelines are intended for practitioners responsible for resuscitating neonates. They apply primarily to neonates undergoing transition from intrauterine to extrauterine life. The recommendations are also applicable to neonates who have completed perinatal transition and require resuscitation during the first few weeks to months following birth (46).

However, something that might have influenced our results is that the ECG tracings unfortunately could not be used for a detailed rhythm analysis, and some kind of electrical activity at a clinical heart rate of zero (asystole) can not be entirely ruled out. If this was the case, the animals might have been more easily resuscitated. However, to my knowledge, the electrical activity in the hearts of pulseless asphyctic human infants has not been studied either. It would therefore be very interesting to perform an echocardiographic study in this model.

## **CLINICAL APPLICATION**

### **Term versus preterm**

The gestational age of the newly born influences pulmonary pathology and resuscitation physiology (147). Amount of and quality of surfactant, as well as risk of meconium aspiration, are dependent on gestational age and might call for specific resuscitative measures that have not been discussed in this thesis.

In addition, preterm babies have immature lungs that may be more difficult to ventilate and more vulnerable to injury by positive-pressure ventilation (165). Data from a retrospective study indicate that current guidelines for intensive resuscitation need to be adjusted for extreme premature infants (294).

The experiments underlying this thesis were performed in a model of a newborn at term; the level of development of the neonatal pig's brain is comparable with that of a term human newborn (295), and our findings do not apply to other gestational ages. Special considerations around preterm infants have not been subject for discussion in this thesis.

## **FURTHER RESEARCH**

As previously mentioned, intensive resuscitation involving chest compressions and medications is an infrequent event, and randomised controlled studies are difficult to undertake (117). Also, ethical considerations surrounding the treatment of an acutely compromised newborn make clinical studies in this setting difficult to carry out.

However, studies investigating the use of adrenaline with regards to doses, administration route, and importantly: time of administration should be carried out. With regards to the results of our experiments, cardiac compressions alone might not be the way to achieve adequate coronary perfusion pressures in the severely asphyxiated and systemically vasodilatated newborn. We would therefore be interested in studying the effect of adrenaline given prior to initiation of cardiac compressions in bradycardia versus asystole in our model of perinatal asphyxia.

A question that remains largely unanswered after our studies is whether ventilation is significantly compromised by increasing the compression to ventilation ratio in the newborn. Even though the ventilator we used for resuscitating the pigs offers the opportunity to measure and control tidal volumes, this was not done in the studies underlying this thesis. However, our group is planning to perform further studies on the optimal compression to ventilation ratio by performing cardiopulmonary resuscitation in a newborn manikin and at the same time measuring tidal and minute volumes. Also, the efficacy of various ventilation rates (at the present time 40-60 per min) can be studied in this model.

Studies comparing the two-thumb encircling hands technique with the two-finger technique are scarce in number. Also, not all these studies performed chest compressions in combination with ventilation breaths and in none was the quality of the ventilations analysed (191). This question can also be addressed in a manikin study, together with the effect on ventilation of continuous cardiac compressions (uncoordinated compressions and ventilations).

## **CONCLUSIONS**

1. As there was no difference in time to return of spontaneous circulation when newborn pigs with severe neonatal asphyxia and cardiac arrest were resuscitated with air or 100% oxygen, air can be used for resuscitation of even the most compromised neonates. This is in accordance with another study of asystole following severe asphyxia in newborn pigs by Linner et al. (274).
2. A slight prolongation of positive pressure ventilation, i.e. ventilation for 60 s, before initiation of cardiac compression does not affect short term outcome compared to standard treatment (adequate ventilation for 30 s prior to cardiac compression if persisting bradycardia/asystole). However, ventilation for 90 s before cardiac compressions might be too long.
3. Newborn pigs with cardiac arrest following severe asphyxia tolerate longer compression cycles between ventilations than the recommended three compressions to one ventilation. However, even though results from adult studies suggest beneficial haemodynamic effects of increasing the number of compressions in a series, neither the compression to ventilation ratio of 9:3 nor 15:2 provided the increase in coronary perfusion pressure needed for a faster return of spontaneous circulation compared to the ratio of 3:1. We speculate that the reason for this is that the hypoxia and profound metabolic acidosis preceding cardiac arrest in the asphyxiated newborn make adequate coronary and systemic pressures difficult to achieve.

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I



**II**



III



IV

