Universität Ulm

Klinik für Kinder- und Jugendmedizin

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Accuracy of pulse oximetry during neonatal resuscitation

Dissertation

for the attainment of the

Doctoral Degree of Medicine

at the Faculty of Medicine, Ulm University

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2014.

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Tag der Promotion: 21. 10. 2016

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LIST OF ABBREVIATIONS

ABP	**Arterial blood pressure**
AC	**Alternating current**
CHD	**Congenital heart disease**
CO	**Carbon monoxide**
COHb	**Carboxyhemoglobin**
CPR	**Cardiopulmonary resuscitation**
CTG	**Cardiotocography**
DC	**Direct current**
ECG	**Electrocardiography**
EMS	**Emergency medical services**
FPO or $FSpO_2$	**Fetal pulse oximetry**
FiO_2	**Fractional inspired oxygen**
Hb	**Hemoglobin**
HbO_2	**Oxyhemoglobin**
Hbt	**Total hemoglobin**
HHb	**Deoxyhemoglobin**
ICU	**Intensive care units**
LED	**Light emitting diode**
MetHb	**Methemoglobin**
NICU	**Neonatal intensive care units**
ODC	**Oxyhemoglobin dissociation curve**
$PaCO_2$	**Arterial Carbon dioxide partial pressure**
PaO_2	**Arterial Oxygen partial pressure**
PEEP	**Positive end- expiratory pressure**
PI	**Perfusion index**

PIP	**Peak inspiratory pressure**
RCT	**Randomized controlled trial**
SaO_2	**CO-oximetry estimate of arterial oxygen saturation**
SpCO	**Pulse oximeter estimate of COHb%**
SpHb	**Total hemoglobin measured by pulse oximetry**
SpMet	**Pulse oximetry estimate of MetHb%**
SpO_2	**Pulse oximetry estimate of arterial oxygen saturation**

1. Introduction and review of the topic

1.1. History of blood gas measurement

As oxygen is extremely important to maintain life, the development of a method to monitor oxygen concentration in sick individuals had a great interest of many scientists over periods of scientific history. Before this method, which was called later oximetry, was invented, the adequacy of oxygenation was estimated by looking for change in the skin color and development of cyanosis which was proven later to be an unreliable method for detection of low oxygen saturation, as in good light with adequate hemoglobin concentration a keen observer could detect cyanosis only after 15% of the hemoglobin was desaturated (Comroe and Botelho, 1947).

The measurement of the oxygen saturation in hemoglobin has passed through different stages of inventions and development. The beginning was with inventing the spectrometer when Bunsen and Kirchhoff in 1860 in Germany wanted to measure the exact wavelengths of the emission lines of elements introduced into a colorless flame. This was shortly followed by the discovery by Georg Gabriel Stokes in 1863 that the colored substance in blood was the carrier of oxygen (Stokes, 1863). Felix Hoppe-Seyler in Tübingen, Germany had crystallized the blood pigment and gave it the term "hemoglobin". He demonstrated that hemoglobin was the cause of the absorption of green and blue light from the solar light spectrum, and that this absorption changed when he exposed the solution to air. Then he discovered that oxygen and hemoglobin formed a dissociable compound and named it oxyhemoglobin (Hoppe-Seyler, 1864).

Bunsen and Kirchhoff's spectrometer was applied first to study the spectrum of light transmitted through hemoglobin and oxyhemoglobin in solutions and in finger tissues by Karl von Vierordt in Germany. He showed that after stopping the circulation by a rubber band, the two light bands of oxyhemoglobin disappeared and the light band of deoxygenated hemoglobin appeared. He also measured the oxygen consumption of the living tissues by using reflected light and timing the change from oxyhemoglobin to deoxygenated hemoglobin (Vierordt, 1876). August Krogh and I. Leicht were the first to apply spectrophotometric comparison methods to the measurement of oxygen saturation of blood hemoglobin for physiological experimentation using a cover slip wedge cuvette. However, it had serious problems and needed to be calibrated empirically against other methods (Krogh and Leitch, 1919).

In 1935, David L. Drabkin and James Harold Austin at the University of Pennsylvania tried to avoid these problems by using a glass cuvette with a 0.07-mm liquid path length and they demonstrated that this cuvette had the potential for measuring oxygen saturation because no gas exchange could take place and dilution was not required (Drabkin and Austin, 1935). 10 years later, Drabkin and Schmidt reported the use of this cuvette for directly determining saturation in vivo and in vitro and described, for the first time, the saturation of human and canine arterial hemoglobin in vivo which were 98.5 % and 98.6 % respectively (Drabkin and Schmidt, 1945).

The origin of oximetry was in Germany; when Ludwig Nicolai started the examination of light transmitted through human skin by spectrophotometry to understand the dynamics of tissue oxygen consumption and innovated an apparatus using the blue-green bands of mercury vapor light. By occluding the circulation of the apparatus, he obtained exponential curves for the decay of oxyhemoglobin and the increase in reduced hemoglobin (Nicolai, 1932). Kurt Kramer did not follow the same steps of Nicolai and developed a new apparatus (Figure 1) using bright light and a red filter and he demonstrated that saturation could be measured reproducibly in unopened arteries. However, due to the use of a single red broad band light, there was no compensation for either the concentration of hemoglobin or the changes of light intensity (Kramer, 1935). Karl Matthes built the first apparatus to continuously measure ear oxygen saturation and introduced a second wavelength (green or infrared) insensitive to saturation to compensate for blood volume and tissue pigments (Matthes, 1935).

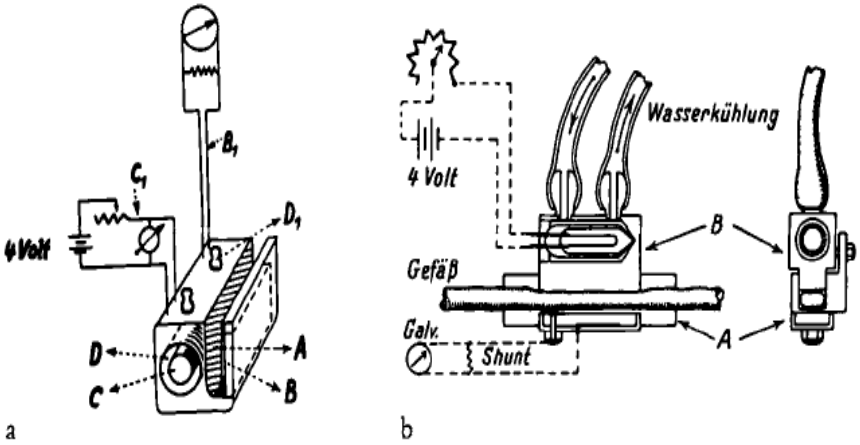


Figure 1: (a) Kramer's apparatus for optically recording oxygen saturation in unopened arteries of experimental animals. The artery was placed in groove (A) against photocell (B), illuminated by light (C), surrounded by cooling water (D) entering port (D1) Light was controlled by (C1) and read by galvanometer (B1). (b) Cutaway view. (Kramer, 1935)

During World War II Millikan and his colleagues were asked for help in developing an oxygen delivery system with a demand valve responsive to altitude and activity, because military pilots lost consciousness at high altitude during fights. By 1940 Millikan and his associates had developed a working oximeter and had arranged for its manufacture by the Coleman Electric Company in which the oximeter reading controlled the oxygen supply to the pilot's mask, which was built with an oximeter attached to it. The oximeter was applied to the ear using a bright, battery operated light along with red and green gelatin filters. They also reported the use of infrared light as the oxygen-insensitive signal stabilizing the oximeter (Millikan, 1942).

The most important next step in oximetry development was in 1948 when Earl H. Wood modified Millikan's earpiece, improving the infrared filter and adding an inflatable balloon with which the ear could be made bloodless for initial zero setting. This oximeter was used with cuvette oximeter in newborn infants, in thoracic surgical operations, in cardiac diagnostic procedures, in exercise testing of congenital heart defect, and in catheterization. Wood and Geraci invented electronic methods of dividing the red by the infrared signal to display saturation continuously. The problem with their oximeter was that the barrier layer photocells were not uniform in spectral sensitivity, variable with time and its output currents could not be measured without some change in voltage, and this altered their responses variably (Wood and Geraci, 1949).

All the previous ideas and applications were based on measuring oxygen saturation by measuring transmission of light. However, measurement of oxygen saturation by reflected light from blood in vitro was first described by Robert Brinkman and W. G. Zijlstra in 1949 in Groningen, Netherlands. They found that the Lambert-Beer law of the logarithmic relationship between light intensity and substance quantity applied to reflected red light. Haemolysis was not needed, thus allowing more rapid analysis and continuous in vivo recording that could be applied to the forehead (Brinkmann and Zijlstra, 1949).

Brinkmann and Zijlstra principles of measuring reflected light were applied by Michael Polanyi to develop the fiberoptic oximetry using optical fibers (Polanyi and Hehir, 1960). It consisted of a cardiac catheter containing a bundle of small and flexible optical fibers. Some of the fibers on its tip illuminated the blood with light, and others conducted the resulting scattered light back through two rotating filters to a photomultiplier tube. Limitation of this fiberoptic oximeter was the rapid failure due to blood clotting at the tip of

the catheter, but this was solved later by Polanyi and Taylor when they designed catheters with side viewing ports (Taylor et al., 1972). Fiberoptic catheter oximeter has been used for (1) intracardiac oximetry in congenital heart disease (Enson et al., 1964); (2) monitoring of mixed venous oxygen saturation (Divertie and McMichan, 1984) and (3) immediate monitoring of newborn infants through an umbilical artery catheter (Wilkinson et al., 1978).

1.1.1. Development of CO-Oximetry

In 1958 Gabriel Nahas designed a special 0.1 mm light path cuvette for the Beckman Company spectrophotometer, and reported a method of measuring oxygen saturation that became the accepted standard (Nahas, 1958). Bjure and Nilsson's developed a technique to modify Nahas' method aiming to distinguish between carboxyhemoglobin, oxyhemoglobin, and hemoglobin (Bjure and Nilsson, 1965). In the 1960s, Instrumentation Laboratories introduced a cuvette oximeter or CO-oximeter, in which additional wavelengths of light were used to permit measurement of carboxyhemoglobin saturation and to eliminate small errors resulting from methemoglobin and other pigments.

Ole Siggaard-Andersen in 1962 (Denmark) and coworkers developed a method to use microsamples of capillary blood. They induced hemolysis by freezing and determined the carboxyhemoglobin percentage after reduction with dithionite (Siggaard-Andersen et al., 1962). The Radiometer company manufactured a commercial version of this device (Radiometer OSM2) and introduced the use of ultrasound for hemolysis (Siggaard-Andersen, 1977). The same Danish investigators investigated the extinction coefficient spectra of oxyhemoglobin, hemoglobin, carboxyhemoglobin, methemoglobin, and sulfhemoglobin to permit their analysis in capillary blood samples. This was the idea behind the development of six-wavelength instrument (Radiometer OSM3) that uses a grating spectrophotometer to identify all these hemoglobin compounds in (Siggaard-Andersen et al., 1972).

1.1.2. Discovery of pulse oximetry

Before 1975 oximeters were applied to measure oxyhemoglobin in vivo, have measured it in tissue but not in arterial blood until Takuo Aoyagi in Japan discovered during his work for measuring the cardiac output by densitometry that he can measure the pulsatile changes in light transmission through living tissues to compute the arterial oxygen saturation. He realized that these changes of light transmission at all wavelengths would

solely be due to pulsatile variations of the arterial blood volume. In that way the unpredictable absorption of light by tissue, bone, skin, and pigments will not disturb the analysis. It was this key idea that permitted the development of instrumentation that required no calibration after its initial setting, as all human blood has essentially identical optical characteristics in the red and infrared bands used in oximetry (Aoyagi, 2003; Aoyagi et al., 1975).

Takuo Aoyagi pulse oximeter was manufactured by Nihon Kohden Corporation by using an incandescent lamp with filters at 630 and 900 nm and analog detection of the pulsatile optical signal ratio at these wavelengths. The first commercial instrument, the OLV-5100 (Figure 2), was made available in 1975 as an ear oximeter. Nakajima, a surgeon, and his colleagues tested this pulse oximeter in patients (Nakajima et al., 1975). In parallel, Minolta Corporation in the United States used the idea of Takuo Aoyagi to manufacture a pulse oximeter called Oximet MET 1471 (Figure 3) as a finger oximeter which was tested also in patients by Nakajima and his colleagues. However, the main problem was its extreme sensitivity to motion (Nakajima et al., 1979).

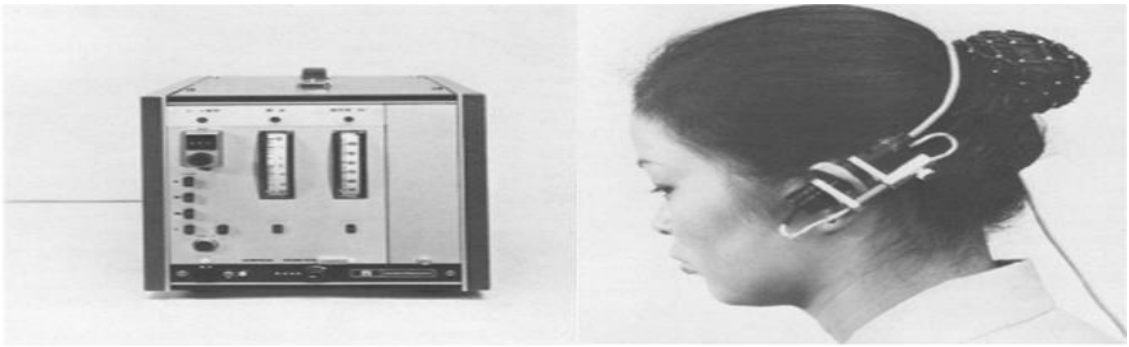


Figure 2: OLV-5100 pulse oximeter; From (Severinghaus and Honda, 1987).

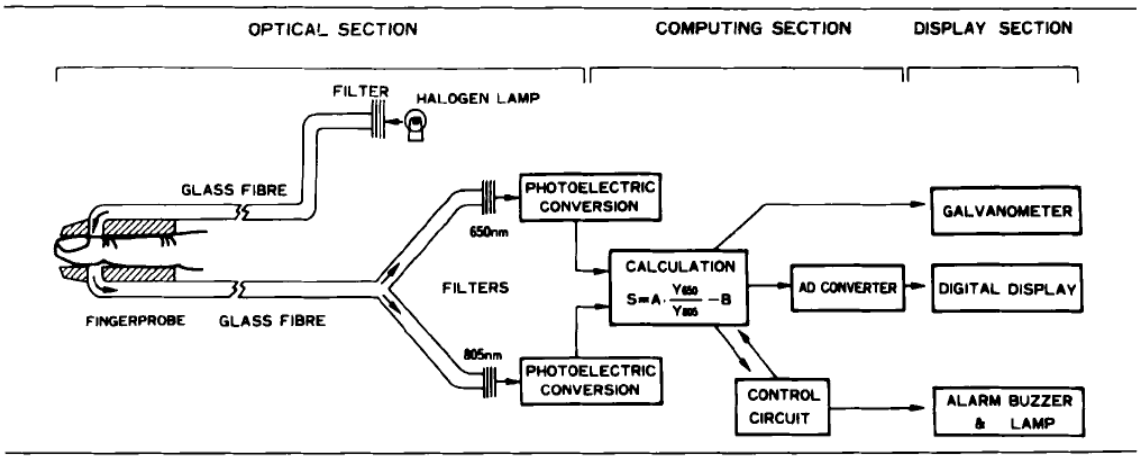


Figure 3: Block diagram of the instrument; Minolta pulse oximeter; from (Yoshiya et al., 1980).

This instrument (Oximet MET 1471), was tested also clinically by anesthesia groups in Japan and was found to provide continuous, convenient, and noninvasive measurements of oxygen saturation (Yoshiya et al., 1980). However, it was overestimating the saturation with average reading of 70% at 50% real saturation (Sarnquist et al., 1980). Shimada and colleagues reported that this overestimation was due to multiple internal scattering, and that the theoretic equations did not apply. All subsequent devices have used empiric algorithms to make the reported saturation fit a certain set of data. However, most of these data were acquired in normal subjects with saturation greater than 70%, so the accuracy has not been documented at lower levels (Shimada et al., 1984).

Mendelson and his colleagues demonstrated the possibility that pulse oximetry could be done using reflected light from skin surfaces as well as transmitted light and this could enable special uses such as fetal scalp monitoring during labor. Their design included the use of light-emitting diodes and a photo diode applied directly on the skin and separated by about 3 mm. They reported excellent accuracy (standard deviation = 0.76% saturation) however they tested it only over a small range (89% to 98%) (Mendelson et al., 1983).

Biox Company in Boulder, Colorado which then acquired by Ohmeda made important improvements in the design of Minolta pulse oximeter, and other companies then followed such as Nellcor of Hayward California, Novametrix of Wallingford, Connecticut, and Criticare of Milwaukee, Wisconsin). However, the Biox pulse oximeter was reported to show overestimated saturation in the same way as the Minolta did when actual saturation was less than 75% (Chapman et al., 1986).

There was a subsequent huge growth in the use of pulse oximetry with empiric modifications of the calculation that have tried to correct these errors within reasonable limits. The pulse oximeters then had the advantage that it does not require either calibration by the user or matching to each subject. One of the major advances in pulse oximetry has been the reduction in the size of the light source and the detectors. Photodiodes and light emitting diodes at red and infrared wavelengths have made the probes inexpensive enough to be considered disposable sometimes. These probes can be applied to the ear, the nasal bridge, the nasal septum, the finger, the temple, and on the foot or palm of infants (Severinghaus and Astrup, 1986).

1.2. Definitions

(Ortega et al., 2011; Wukitsch et al., 1988)

- Oxyhemoglobin (HbO_2): Functional hemoglobin with bound oxygen.
- Deoxyhemoglobin (HHb): reduced hemoglobin, without bound oxygen.
- Carboxyhemoglobin (COHb): is nonfunctional hemoglobin which is unable to bind and transport oxygen due to binding to carbon monoxide
- Methemoglobin (MetHb): is nonfunctional hemoglobin which is unable to bind and transport oxygen because it contains ferric iron, $Fe^{3+,}$ an oxidized form of the oxygen-carrying ferrous iron, Fe^{2+}.
- CO-oximeter: a laboratory device that can spectrophotometrically measure hemoglobin, oxyhemoglobin, carboxyhemoglobin, and methemoglobin.
- SaO_2: the percent saturation of oxygen bound to hemoglobin in arterial blood oxygen measured spectrophotometrically by a CO-oximeter: HbO_2/ (HbO_2+ HHb + MetHb + HbCO) x 100.
- SpO_2: Arterial oxygen saturation as measured by pulse oximetry.

1.3. Pulse oximetry principles

The oximetry was based theoretically on Beer-Lambert law. Simply, this law states that the concentration of absorbent in solution can be determined as mathematical function of the amount of light transmitted through the solution, providing that the intensity of incident light, the path length, and the extinction coefficient of a substance at a particular wavelength are known. The absorbance of light by HbO_2 at the red wavelengths (650 to 750 nm) is less than that for HHb. Hence, HbO_2 is more transparent to red light than HHb is and the reverse is true in the infrared region (900 to 1000 nm) Figure 4. This difference enabled the oximeter to calculate the concentration of HbO_2 (Mendelson, 1992).

Applying the Beer-Lambert law to in vivo analysis of blood gases caused many and complex problems as it assumes that incident light = light transmitted + light absorbed. However experiments with human tissue presented that incident light = light transmitted + light absorbed + light scattered + light reflected because, on human light is scattered by the skin surface, tissue, muscle, bone, and blood; light is reflected by the skin surface, which can be a function of skin surface texture and color; light is absorbed by tissue components other than the blood and absorption is also dependent on pigmentation and thickness of the test site; the blood is a nonhomogeneous liquid capable of nonlinear absorption of light,

particularly as hematocrit varies. All these obstacles made the classic oximetry that is not related to the pulse and used two-wavelength, could not effectively be used for in vivo analysis (Wukitsch et al., 1988).

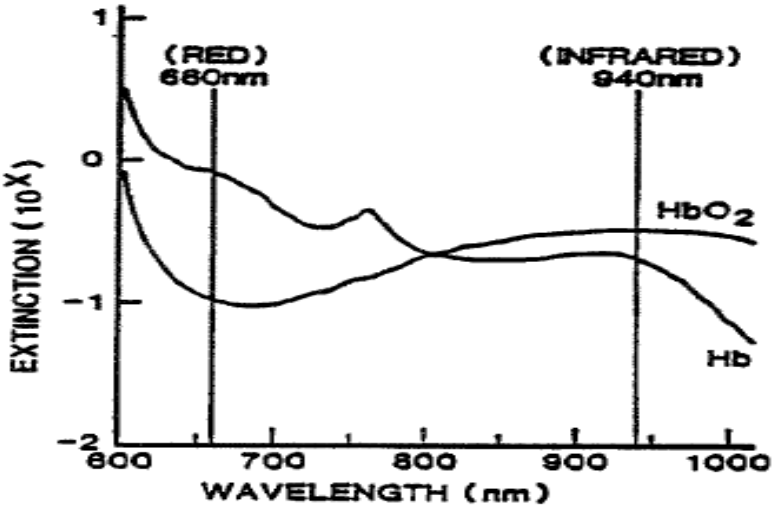


Figure 4: Oxyhemoglobin (HbO_2) and reduced hemoglobin (HHb) absorption (extinction) characteristics relative to red light at 660 nm and infrared light at 940 nm (Wukitsch et al., 1988).

Hewlett-Packard ear oximeter (HP 47201A) introduced the use of eight wavelengths (from 650 to 1,050 nm) instead of the classic two wavelengths to solve the problems found in the two-wavelength approach. This instrument compensated for all the effects of skin pigmentation, ear thickness, or ear probe motion. In addition, applying precalibration, ear vascularization by heat, and a fixed path length, it was able to solve many of the problems of earlier devices (Merrick and Hayes, 1976). Its accuracy was tested in clinically in the field of pulmonology and sleep researchers and it was thought to be the gold standard for oximeters, but later research reported its unstable performance below an arterial oxygen saturation of 70%, and its inability to deal with HbCO (Cissik et al., 1981; Douglas et al., 1979).

The most important step was the recognition that the pulsatile nature of the arterial blood can be employed in oximetry. Hence, pulse oximetry uses the physiologic activity of the cardiac pulse as the basis to determine SpO_2. To have a functioning system, the signals coming from arterial blood must be isolated from those representing venous blood, tissue, and other absorbing material (Figure 5). The light absorption by the various substances is constant which can be defined as direct current (DC) signal, with the exception of arterial blood light absorption which varies with each contraction of the heart as the volume of arterial blood changes and this can be defined as alternating current (AC) signal. The

amplitude of the DC and AC signals are affected by many factors related to the measuring system or the measured sample, therefore the AC signals must be divided by the DC signals at each wavelength to produce a scaled AC level which no longer affected by those factors (Pologe, 1987).

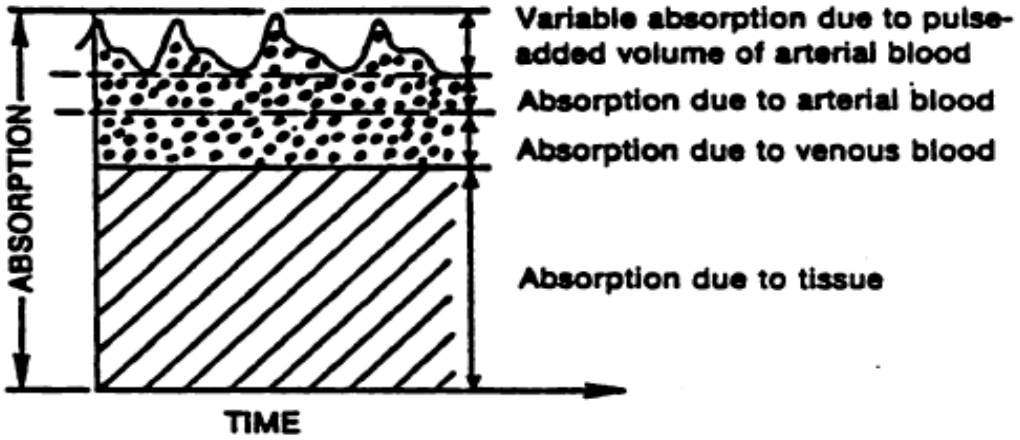


Figure 5: Variations in light absorption by tissue, showing the rhythmic effect of arterial pulsation (Mendelson, 1992).

The current pulse oximeters consist of a peripheral probe contains a photodetector and two light-emitting diodes (LED), analog signal conditioning and/or processing, data acquisition, digital signal conditioning and/or processing, display and control system, and internal system diagnostic functions and a microprocessor unit. Each LED emit light at a different wavelength; one in the red spectrum, at a wavelength of 660 nm and the other in the infrared spectrum, at a wavelength of 940 nm. Due to the different characteristics of light absorption at of HHb and HbO_2 at these wavelengths as described before, the relative concentration of HHb and HbO_2 can be determined. The light emitted by the diodes is absorbed by tissues, and the amount of absorption is determined by the photodetector. Then, percentage of oxyhemoglobin is calculates $=HbO_2/HbO_2+HHb$, and a waveform corresponding to the pulsatile flow in arterial vessels (figure 6), and the pulse rate are displayed (Jubran, 1999; Wukitsch et al., 1988).

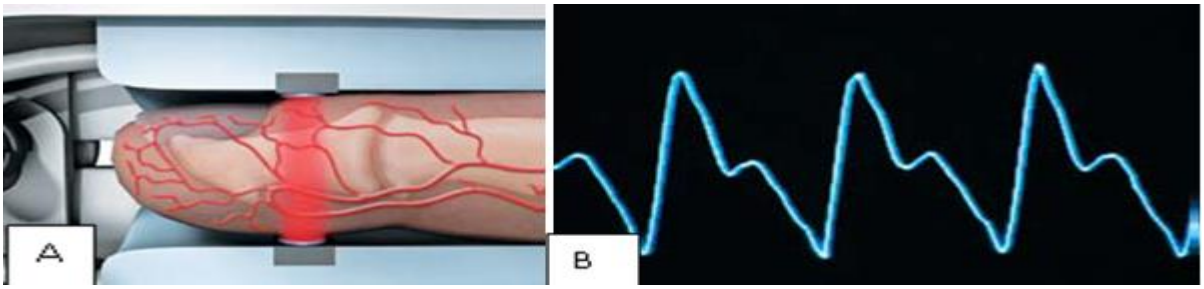


Figure 6: A: Light emitted from the light-emitting diodes (LED) and reaches the photodetector. B: pulse oximetry wave form (Ortega et al., 2011).

The probe is positioned so that the photodetector and light-emitting diodes face each other, with layers of tissue between them (figure 6A). The photodiodes turn on and off several hundred times per second to record the light absorption during pulsatile and nonpulsatile flow. During pulsatile flow, the light absorption of arterial blood, background tissues, and venous blood is detected. During nonpulsatile flow, only the light absorption of background tissues and venous blood is detected. The microprocessor unit compares the light absorption during both pulsatile and nonpulsatile flow phases to isolate the light absorption of arterial blood and thus determine the SpO_2 (Mendelson, 1992; Ortega et al., 2011).

1.4. Applications of pulse Oximetry

Pulse oximetry is indicated in any clinical setting where hypoxemia may occur and it has been suggested that measurement of oxygen saturation should be included in the routine vital signs of temperature, pulse, respiratory rate, and blood pressure. Pulse oximetry can be applied in various clinical settings such as in emergency departments, critical care units, operating rooms, emergency medical services (EMS) systems, postoperative recovery areas, endoscopy suites, sleep and exercise laboratories, oral surgery, cardiac catheterization, conscious sedation, labor and delivery, interfacility patient transfer, and aerospace medicine (Hanning and Alexander-Williams, 1995).

1.4.1 Pulse oximetry in intensive care units

The use of pulse oximetry in the intensive care units (ICU) has the advantages of being real time, continuous, noninvasive and does not need much effort to use. A substantial number of critically ill patients treated in the ICUs suffer from hypoxemic respiratory failure, and adjustments of inspired supplemental oxygen concentration or fractional inspired oxygen (FiO_2) and mechanical ventilation parameters like positive end-expiratory pressure (PEEP), and others benefit from real time evaluation of oxygen saturation. Weaning from mechanical ventilation and other procedures carried out in ICUs, such as upper gastrointestinal endoscopy, hemodialysis, and fiberoptic bronchoscopy, can be monitored effectively using pulse oximetry (Bongard and Sue, 1992). The introduction of pulse oximetry in ICUs has also decreased the number of invasive arterial blood gases analysis and the duration of oxygen therapy, without endangering the patient outcome (Tallon, 1996).

In pediatric practice, the use of pulse oximetry became a standard procedure used in almost all sick neonates. In neonatal intensive care units (NICU), pulse oximetry is used both as an indicator of hypoxia and hyperoxia to reduce the incidence of retinopathy of prematurity. The newborn infants, especially the preterm babies have high concentrations of fetal hemoglobin which cause a lift shift in the oxyhemoglobin dissociation curve (ODC) compared to that of adults hemoglobin (figure 6) so that although the hemoglobin saturation is correctly indicated, the partial pressure of oxygen to which the tissues are exposed at a cellular level may be different. However, the continuous monitoring of oxygen saturation by pulse oximetry plays an important role in the prevention of retinopathy of prematurity (Hartnett and Lane, 2013). Moreover, the current development of automated adjustment of FiO_2 in ventilated preterm infants with the help of pulse oximetry may provide a better protection (Claure et al., 2011).

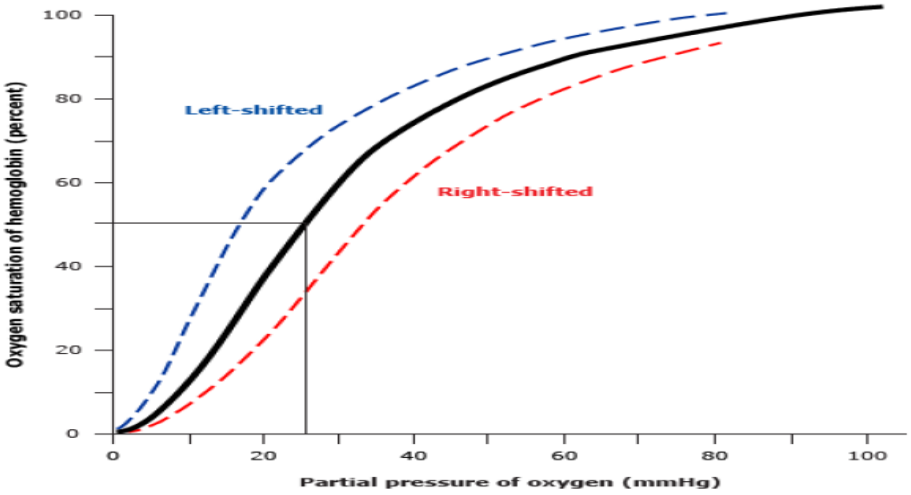


Figure 6: Oxyhemoglobin dissociation curve (ODC). Solid line is the normal adult ODC, blue line is an example of shift to the lift of ODC, and the red line is an example of shift to the right of the ODC (Mechem, 2013).

Pulse oximetry in the ICUs, however, faces many problems that can affect its readings such as low perfusion of the tissue to which pulse oximeter is attached encountered in cases of hypotension and cardiovascular shock and severe hypoxemia which can give misleading oxygen saturations values (discussed later). Moreover, pulse oximetry is unable to assess the efficiency of oxygen transfer across the lungs when the PaO_2 is high enough to place O_2 saturation on the upper horizontal part of the oxyhemoglobin dissociation curve. Arterial PO_2 and the difference between arterial PO_2 and calculated alveolar PO_2 (PAO_2-PaO_2) are the classic tools used for this assessment (Moyle, 1996).

1.4.2. Role of pulse oximetry in detection of congenital heart disease

A common feature of many, but not all, forms of congenital heart disease (CHD) is hypoxemia. Hypoxemia results from the mixing of systemic and venous circulations or parallel circulations as one might see in transposition of the great arteries. Hypoxemia may result in obvious cyanosis. However, generally, 4 to 5 g of deoxygenated hemoglobin is needed to produce visible central cyanosis, independent of hemoglobin concentration (Snider and Roy, 1988). For the typical newborn with a hemoglobin concentration of 20 g/dl, cyanosis will only be visible when arterial oxygen saturation is 80%; if the infant only has a hemoglobin concentration of 10 g/dl, the saturation must be 60% before cyanosis is apparent. Importantly, those children with mild hypoxemia, with arterial oxygen saturation of 80% to 95%, will not have visible cyanosis. Moreover, the clinical identification of cyanosis is particularly problematic in black and Hispanic neonates because of skin pigmentation (Lees, 1970).

Considering the mortality and marked morbidity of delayed diagnosis of CHD together with the previous facts about clinical detection of cyanosis and its reliability, pulse oximetry has introduced a valuable tool for screening and detection of CHD. With the help of pulse oximetry researchers could determine reference data for oxygen saturation in healthy full-term infants during their first 24 hours of life with median value of 97.8% at 20 to 24 hours of life (O'Brien et al., 2000). Hoke and colleagues used a cutoff of 95% oxygen saturation in lower extremity and they reported that 81% of neonates with critical CHD could be identified (Hoke et al., 2002).

The two major controversies about the use of pulse oximetry in screening for CHD are the postnatal age at which it should be applied and the cutoff oxygen saturation below which screening will be considered abnormal. Because newborns with critical CHD may have clinical deterioration in the first 48 hours of life, one would ideally use oximetry screening soon after delivery. However, arterial oxygen saturation varies considerably in the first 24 hours. Therefore, oximetry screening before 24 hours of life may identify cases with minor transitional respiratory illness and then can result in a significant number of false positive results. The establishment of a cutoff threshold for an abnormal SpO_2 is important because a higher SpO_2 cutoff will increase sensitivity and at the same time decrease specificity leading to decrease the number of false negative screening results and increase the number of false positive screening results. Conversely, a lower SpO_2 cutoff will lower sensitivity and raise specificity (Mahle et al., 2009).

1.4.3. Pulse oximetry role in circulatory monitoring

In addition to the great role of pulse oximetry in detection of hypoxemia, it showed also the possibility to be useful in monitoring of circulatory state and hemodynamic adequacy. Pulse oximetry displays a waveform representing the pulsatile volume of blood in tissue called plethysmogram which used originally to help clinicians distinguish between reliable SpO_2 measurements which are associated with clean, physiologic waveforms and unreliable measurements which are associated with noisy waveforms. The plethysmogram has a waveform similar to an arterial blood pressure waveform (ABP). Because pulse oximetry is noninvasive and nearly available in all hospitals, it is intuitive to seek circulatory information from the plethysmogram, and the extraction of circulatory information related to hypotension, hypovolemia, low cardiac output, vasoconstriction and other circulatory conditions (Pizov et al., 2010; Reisner et al., 2008).

The use of recent technology in pulse oximetry enabled the new Masimo SET pulse oximeter (Masimo Corporation, Irvine, CA) to use the variations of the photoplethysmogram during monitoring for developing indices for hemodynamic monitoring and to guide fluid therapy. Perfusion index (PI), the infrared pulsatile signal indexed against the nonpulsatile signal and reflects the amplitude of the pulse oximeter waveform. PI has been shown to be sensitive sympathetic block (Sebastiani et al., 2012), proximal arterial clamping (Moxey et al., 2006), and neonatal left heart obstruction (Granelli and Ostman-Smith, 2007). Other indices retrieved from pulse oximetry like plethysmography variability index (PVI) and pulse contour analysis derived stroke volume variation (SVV) have been also used to monitor hemodynamic variations in ventilated patients and to predict the response to fluid therapy (Marik et al., 2011; Yin and Ho, 2012).

1.4.4. Pulse oximetry for measurement of dyshemoglobins and total hemoglobin

The conventional pulse oximetry until now uses the two wave length technique to measure HbO_2 concentration. In 2005, Masimo Corporation has introduced the use of eight light wave lengths in the pulse oximetry and developed a new technology called the Rainbow Technology in a new pulse oximeter, Rad-57, and later Radical 7. These eight wave lengths enabled the new pulse oximeter to measure SpO_2 as well as SpCO (pulse oximeter estimate of COHb %) and SpMet (pulse oximeter estimate of MetHb %). The new pulse oximeter was tested in twenty healthy volunteers who were exposed to inspired

carbon monoxide or given i.v. sodium nitrite to induce methemoglobinemia. SpCO and SpMet were monitored with Rad-57 and compared with arterial blood samples simultaneous analysis by laboratory CO-oximetry for COHb% and MetHb% respectively. The bias and precision were 1.22 ±2.19% and 0.00 ±0.45% respectively (Barker et al., 2006).

The next step for this new pulse oximetry was the release of another innovation in the multiwavelength pulse oximetry which is the noninvasive measurement of total hemoglobin (Hbt). This was firstly clinically validated when Macknet and colleagues monitored 30 surgical patients and 18 healthy volunteers by the Radical-7 to measure SpHb (total hemoglobin measured by pulse oximetry). Arterial blood samples were obtained periodically and analyzed for Hbt by CO-oximeter and were compared with the simultaneous SpHb. In a range of Hbt values from 4.4 to 15.8 g/dl, the bias and precision were 0.03 and 1.12 g/dl, respectively (Macknet et al., 2007). This was followed by many clinical research and case reports reporting the use and potential benefits of new pulse oximetry in monitoring both Hbt and dyshemoglobins (Barker and Badal, 2008).

1.4.5. Pulse oximetry during delivery and during neonatal resuscitation

Fetal monitoring during delivery is an important procedure for identification of problems which if not corrected can lead to fetal morbidity and mortality. To ensure good fetal outcome the conventional CTG (cardiotocography) is usually used to monitor the fetal heart rate. In cases of nonreassuring fetal heart rate pattern been identified during labor, a number of additional assessments of fetal well-being may be considered. These aim to improve the intrapartum assessment of fetal well-being to safely reduce operative delivery. They are supposed to improve safety complementary to the CTG. One of these assessments of fetal pulse oximetry (FPO or $FSpO_2$), is the use the reflected light with the light-emitting diodes and photodetector are in the same plane instead of conventional light transmission to measure fetal SpO_2 (East and Colditz, 2007).

Although fetal pulse oximetry monitoring seems to be a promising tool, it has some limitations that can affect its performance. The good contact between the pulse oximetry sensor and the fetal skin is an important requirement for a good signal and accurate reading and this contact can be disrupted by uterine contractions and also the high pressure on the sensor caused by the contractions can affect the accuracy of pulse oximetry (Gardosi et al., 1994). The presence of dark, thick, curly scalp hair may affect the sensor attachment or may be a source of artifact, as it absorbs red light and can affect the signal (Gardosi et al.,

1991) . Caput Succedaneum can also lead to inaccurate FPO readings which can be due to signals coming from venous pulsation, or from artifact as the arterial signal passed through the congested or edematous scalp (Schram and Gardosi, 1994). More over a large RCT demonstrated no difference in cesarean delivery rates with the use of FPO (Bloom et al., 2006).

The first few minutes after delivery are considered critical for the outcome of the newly born baby. Most of the newly born infants do not require intervention after delivery, however considering the large number of newborns (10 % of the newborns who require some degree of resuscitation and the 1% who require extensive resuscitative measures), one can say that a considerable number of the newborn require some degree of postnatal supportive measures (Perlman and Risser, 1995). Traditionally, the most important 3 clinical signs for evaluation of the newborn after delivery were considered to be heart rate, color as indication of oxygenation, and breathing. Because two of these signs can be evaluated and continuously monitored by pulse oximetry, it has been recommended and widely applied more recently to use the pulse oximetry during neonatal resuscitation in the delivery room (Kattwinkel et al., 2010; Leone et al., 2006).

The newborn color as a measure of oxygenation during newborn clinical assessment has been proven to be unreliable; O'Donnell and colleagues showed that the SpO_2 at which observers perceived infants to be pink varied widely, ranging from 10 to 100%. In other words, assessment of color is difficult and therefore is a poor indicator for tissue oxygenation during the first minutes of life and hence can be replaced by pulse oximetry (O'Donnell et al., 2007). Achieving normoxia is an important aim during neonatal resuscitation, although it has been yet clearly identified, Leone and Finer suggested a target SpO_2 of 85 to 90% by three minutes after birth for all infants except in special cases such as diaphragmatic hernia or cyanotic congenital heart disease where lower saturations may be accepted (Leone and Finer, 2005). It is reported that, with the help of pulse oximetry this normoxia can be achieved earlier through the administration of variable concentrations of oxygen guided by pulse oximetry (American Heart Association, 2006; Kattwinkel et al., 2010).

The routine introduction of pulse oximetry during the process of neonatal resuscitation has helped to run many studies to evaluate the percentiles of oxygen saturation of the newborn during the first few minutes after delivery and to define reference saturation values. In most cases blood oxygen levels in uncompromised babies generally do not reach

extrauterine values until approximately 10 minutes following birth and SpO_2 may normally remain in the 70% to 80% range for several minutes following birth. These studies included saturations measured from both preductal and postductal sites, following both operative and vaginal deliveries, those occurring at sea level and at higher altitude, and in term and preterm infants (Altuncu et al., 2008; Dawson et al., 2010; Gonzales and Salirrosas, 2005; Mariani et al., 2007; Toth et al., 2002). These studies were the base of the recommendation to initiate neonatal resuscitation with room air instead of 100% oxygen (Kattwinkel et al., 2010).

The success rate to obtain pulse oximetry readings during neonatal resuscitation was reported ranging between 20% and 100% by 1 min after birth (Dimich et al., 1991; House et al., 1987; Kamlin et al., 2006; Meier-Stauss et al., 1990). By 5 min, the success rate improved to be between 63% and 100%. SpO_2 values were displayed faster when the sensor connected first to the infant then to the device (Meier-Stauss et al., 1990; O'Donnell et al., 2005). The success to obtain SpO_2 measurements can be affected by many factors such as the site where the sensor is applied, the mode of delivery, gestational age, and the type of the device being used (Dawson et al., 2007). The site to apply the sensor has been studied in several studies including placing the over the Achilles tendon (Dimich et al., 1991), the forefoot (Deckardt et al., 1987) or midfoot (Toth et al., 2002). Later studies found that measurements were obtained fastest from the right hand, may be due to better perfusion, higher blood pressure and oxygenation in preductal vessels (Meier-Stauss et al., 1990; Rabi et al., 2006).

1.4.6. Pulse oximetry during cardiopulmonary resuscitation

Most of the previous applications included the use of a pulse oximeter for monitoring oxygen saturation and help in adjustment of inspired oxygen and other respiratory supportive measures. However, during cardiopulmonary resuscitation (CPR) following cardiovascular collapse pulse oximetry encounters a major technical challenge because of the combined effect of profound hypoxemia and low perfusion with reduced pulsatility. In spite of these challenges a potential of correct SpO_2 readings during this critical event may have an impact on the CPR process regarding evaluation of effectiveness of CPR and for decision making at the right time. The evaluation of pulse oximetry during cardiopulmonary collapse has been studied only in a few and mostly adult studies (Spittal, 1993).

Narang reported the utility of pulse oximetry during CPR of 11 months old, 8 kg child who had cardiac arrest during surgery due to an iatrogenic high dose of penicillin G potassium over a short period of time. All the monitoring equipment and procedures like electrocardiography (ECG), peripheral pulse palpation, blood pressure monitoring, and observing the pupil were unreliable. However, pulse oximetry continued to operate with showing a saturation of more than 90% and displayed a pulse rate that corresponded exactly to that of the external cardiac massage. It was concluded that the high saturation displayed by pulse oximetry was an indication of the adequacy of CPR (Narang, 1986). In the field of prehospital management, pulse oximetry also was reported to be important in assessing and treating patients receiving advanced life support (McGuire and Pointer, 1988).

Moorthy and colleagues reported the evaluation of pulse oximetry during CPR in two adult cases. Both cases had lung carcinoma; one of them had cardiac arrest postoperatively diagnosed both clinically and electrocardiographically. The other one suffered from tension pneumothorax shortly after being anesthetized and showed absence of carotid pulsations and difficult ventilation. Pulse oximetry in both cases showed SPO_2 above 90% and pulse rate 72 beats/minute. The authors concluded that pulse oximetry during CPR can lead both to incorrect judgment as to when CPR should be initiated and to the adequacy of CPR. They also emphasized that pulse oximetry cannot replace monitors such as electrocardiograms, the blood pressure cuff, and arterial pulse palpation during CPR (Moorthy et al., 1990).

Spittal tested the benefits of using pulse oximetry during CPR provided to 20 adult patients at the emergency department through a questionnaire to the attending registrar with the sensor applied to the ear initially or to the thumb if there was a signal failure. Three patients suffered from respiratory arrest only and they seemed to benefit from the use of pulse oximetry. In another four patients who had cardiac arrest, pulse oximetry had significantly altered the management and decisions during CPR. Pulse oximetry benefits for the remaining patients were equivocal and sometimes were considered to be hindering (Spittal, 1993). Arguing Spittal work, Griffin and Cooney reported that their use of pulse oximetry during CPR of a younger patient with cardiac arrest was invaluable and markedly helped to improve the outcome (Griffin and Cooney, 1995).

Beyond the scope of the beneficial effect of the use of pulse oximetry for monitoring efficiency of CPR, it may have beneficial effects on the neurological outcome after resuscitation. In an experimental study, pulse oximetry was used after CPR for induced

cardiac arrest in dogs and the dogs were divided into two groups; one group received post-resuscitation 100% FiO_2 and the other one received lower FiO_2 guided by pulse oximetry to keep SpO_2 94 – 96%. The authors found that those in the pulse oximetry group had markedly better neurological outcome when the dogs awakened after 23 hours, which may be due to the neuroprotective effect of avoiding postresuscitative hyperoxia (Balan et al., 2006).

1.5. Limitations of pulse oximetry accuracy

Accuracy of pulse oximetry is defined as the closeness of agreement between a test result (SpO_2) and an accepted reference value (SaO_2) (ISO, 2011). In spite of the wide use of pulse oximetry, it has some limitations that can affect its accuracy due to interference with the proper acquisition of reliable data or the interpretation of pulse oximeter readings of the oxygen saturation values. The following items review some of these limitations:

1.5.1. The effect of motion

Pulse oximeters detect a pulsatile signal that normally is only a small percentage of the whole plethysmographic signal. Therefore, transient motion of the tissues or optics relative to the skin can cause a significant artifact in the optical measurement. Moreover, if these transient artifacts mimic a heartbeat, the instrument may be unable to differentiate between the pulsations that are due to motion artifacts and the normal arterial pulsations, leading to incorrect readings. These artifacts can be reduced by digital signal processing and averaging the SpO_2 values over several seconds before they are displayed. The shape of pulse oximetry waveform and the abnormal displayed heart rate can be helpful in identifying at least some of these motion artifacts. Some manufacturers use the R-wave of the patient's ECG to synchronize the optical measurements, so they can improve the detection of noisy pulsatile signals (Mendelson, 1992). Aoyagi tried to overcome these emotion artifacts by using a three-wavelength system as the effect of tissue movement and optics movement are wavelength-independent (Aoyagi, 2003).

In a trial to study the effect of mechanical motion of the hand of volunteers connected to 3 different devices of pulse oximetry, Barker and Shah reported that these motions markedly affect the performance of pulse oximetry regarding incorrect reading and the rate of signal dropout particularly when the sensor attached after the motion had begun. However, new generation of Masimo pulse oximetry showed better performance due to the

use of a new technology for signal processing leading to marked advance in low signal-to-noise ratio (Barker and Shah, 1997). Other studies showed also similar effects of generated motion of different amplitudes or movements initiated by the tested subject to mimic the movements encountered in the clinical settings like shivering and convulsions (Gehring et al., 2002; Trivedi et al., 1997a).

1.5.2. The effect of ambient light

Ambient light can be a source of artifacts to the performance of pulse oximetry especially considering that ambient light exists either temporarily or continuously in the medical care environment and different sources of ambient light can coexist simultaneously. This believe is due to the fact that pulse oximetry uses the range between two wavelengths (660 and 940) nm for measuring the light absorption of HbO_2 and HHb. Different sources of ambient light can produce wavelengths including the range used by pulse oximeter and hence can theoretically interfere with its measurements (Fluck et al., 2003).

Numerous light sources can be encountered during medical and surgical practice that have been reported to interfere with the accuracy of pulse oximetry increasing the errors compared with arterial measurements of oxygen saturation and increase the rate of signal dropout. These light sources include fluorescent light, incandescent light, quartz-halogen light used in fiberoptic endoscopy, and infrared light used for warming or that emitted from surgical navigation systems (Amar et al., 1989; Block, 1987; Brooks et al., 1984; Trivedi et al., 1997a). However, in a prospective controlled study testing the effect of different light sources on pulse oximeter, the authors concluded that ambient light does not have a significant effect on pulse oximetry readings (Fluck et al., 2003). Two reports demonstrated that foiling the pulse oximetry sensor can diminish the effect of ambient light on the pulse oximetry readings (Siegel and Gravenstein, 1987; Siegmueller, 2013).

1.5.3. The effect of skin pigmentations and nail polish

The available data about the effect of skin pigmentation on the pulse oximetry readings are conflicting. Theoretically, different skin pigmentation should not disturb pulse oximetry measurements because it measures the ratio of the pulsatile to the total transmitted red light divided by the same ratio for infrared light which should be dependent only on arterial saturation, making pulse oximetry independent of skin color. Many large controlled studies, including one comparing 284 African American and Caucasian subjects reported no

significant pigment related increase in bias or decrease in precision in pulse oximeter measurements at normal SaO_2 values (Adler et al., 1998; Bothma et al., 1996).

However, deoxyhemoglobin and melanin are the primary light absorbers in skin at the wavelength used for hemoglobin absorbance, hence the effective light path for red light through the tissue will vary with skin pigmentation. Many studies reported that darkly pigmented skin markedly affect the pulse oximetry accuracy particularly at low SaO_2 values causing overestimation of the real saturation when compared with moderately dark or lightly pigmented skin (Bickler et al., 2005; Feiner et al., 2007). Another report of a baby with bronze baby syndrome showed inconsistencies between pulse oximetry values and pulse and arterial partial pressure of oxygen (Hussain, 2009).

Nail polish can also interfere with pulse oximetry readings. The fingers are often the primary site for applying the pulse oximetry sensor and in emergency situations there may be no enough time for cleaning nail polish from the fingers. Therefore nail polish has been studied in volunteers and in critically ill patients in many studies as a confounding factor for pulse oximetry readings. Studies reported also the effect of different colors of nail polish and some concluded that the red color produced the lowest influence while for black, purple, and dark blue nail polish had the largest influence (Coté et al., 1988; Hakverdioğlu Yönt et al., 2013; Sütçü Çiçek et al., 2011). However, the data available is conflicting as some reports showed that, nail polish does not affect the pulse oximeter readings (Chan et al., 2003; Hinkelbein et al., 2007; Rodden et al., 2007; Yamamoto et al., 2008).

1.5.4. The effect of anemia

Theoretically, anemia should not alter the measurements of pulse oximetry because pulse oximetry measures functional saturation of oxyhemoglobin at two wavelengths (660 and 940 nm) through calculating the ratio of absorption A660:A940. This ratio is not significantly affected by anemia until absorbance changes of HbO_2 become too small to be detected, in other words; until the signal to noise ratio of the surrounding soft tissue masks them. The critical hemoglobin concentration where this masking occurs has been controversial. 5 g/dL was reported as the lower limit in hemoglobin concentration at which SpO_2 is still measurable by pulse oximetry (Jay and Renzi, 1992).

Several studies and case reports have shown that anemia can have an effect on pulse oximetry readings increasing errors in the measured SpO_2. Lee and colleagues reported marked increase in bias and decrease in precision with SpO_2 underestimating the SaO_2 values in dogs with hemodilutional anemia (Lee et al., 1991). Shibata and colleagues also reported a case with sever dilutional anemia (Hg 2g/dl) after hemorrhage and showed SpO_2 underestimating 100% SaO_2 (Shibata et al., 2002). However, other studies reported an overestimation of the real saturation when measured by pulse oximetry in cases of anemia particularly with low oxygen saturations (Jay et al., 1994; Severinghaus and Koh, 1990).

1.5.5. The effect of dyshemoglobinemia

Dyshemoglobinemia describe the presence of abnormal hemoglobin in the blood including carboxyhemoglobinemia and methemoglobinemia. Carboxyhemoglobin (COHb) result from carbon monoxide (CO) poisoning which can be due to exposure to a variety of sources including propane-powered engine, natural gas, automobile exhaust, portable generators, gas log fireplaces, kerosene heaters, fire smoke, and paint strippers and spray paints. CO has a 240 times oxygen affinity to hemoglobin, so it combines with Hb forming COHb causing tissue hypoxemia. COHb can absorb the red light at wavelength 660 nm similar to HbO2 (figure 7), therefore conventional pulse oximetry cannot differentiate between COHb and HbO_2 leading to abnormally high or normal SpO_2 which overestimates the fractional HbO_2 (Mendelson, 1992; Michaelis et al., 1988).

Methemoglobinemia results from formation of methemoglobin due to exposure to oxidizing chemicals or drugs such as nitrites, nitrates, aniline dyes, aniline derivatives (phenacetin, dapsone), sulfonamides, and lidocaine. These agents oxidize the iron (Fe) in the hem moiety from Fe^{+2} to Fe^{+3}, the resulting MetHb bind less to oxygen and causes a shift to the left in ODC causing sever tissue hypoxia. MetHb can alter pulse oximetry readings because it absorbs red light in the same way as HHb and absorbs infrared light more than both HHb and HbO_2 (figure 7), therefore high levels of MetHb leads to a trend of SpO_2 towards 85% and can overestimate or underestimate SaO_2 in case of hypoxemia or normoxemia respectively (Barker et al., 1989; Chan et al., 2013). Recently produced pulse oximetry by Masimo Corporation can measure both MetHb and COHb accurately by using up to 12 wavelengths (Barker et al., 2006; Roth et al., 2011).

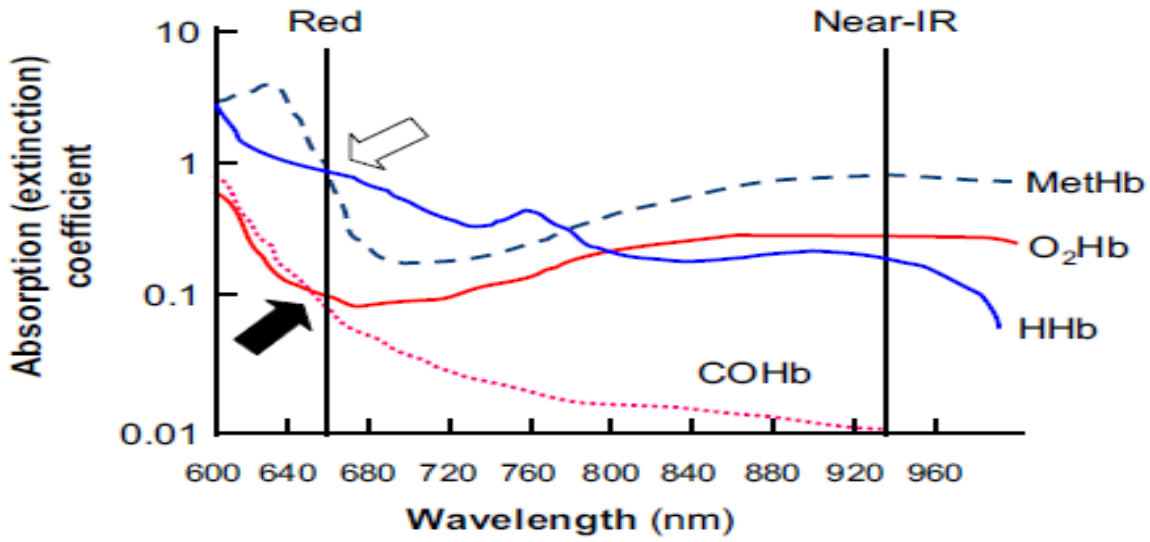


Figure 7: Absorption spectra for Oxyhemoglobin (HbO_2), reduced hemoglobin (HHb), carboxyhemoglobin (COHb), and methemoglobin (MetHb). Arrows point to the sites where dyshemoglobins red light absorption is similar to normally occurring hemoglobin (Chan et al., 2013).

1.5.6. The effect of hypoxemia and hypoperfusion

Although, many clinicians argue that the trend of pulse oximetry measurements is more important than absolute values, the accuracy of pulse oximetry is clinically relevant especially if it can result in an undetected hypoxemia. Many studies have tested the accuracy of pulse oximetry during hypoxemia either in volunteers through the use of low FiO_2 gas (Trivedi et al., 1997b), in critically ill patients with hypoxemia (Carter et al., 1998), or in children with congenital cyanotic heart disease (Schmitt et al., 1993; Tachibana et al., 1996). The ranges of hypoxemia were almost reported as above or below 75% as measured by CO-oximetry. Most of the studies reported SpO_2 overestimating SaO_2 (Thrush and Hodges, 1994), but some reported SpO_2 underestimating SaO_2 during hypoxemia (Severinghaus et al., 1989; Trivedi et al., 1997b).

Solevåg et al tested the accuracy of pulse oximetry before and during resuscitation of 22 newborn piglets with asphyxia induced cardiac arrest. They reported decreased pulse oximetry accuracy at low saturations particularly those below 70% as measured by co-oximeter (Solevåg et al., 2013). Dawson et al. reported also decreased accuracy of pulse oximetry measured by two recent devices of Masimo and Nellcor when SaO_2 below 70% in

17 ventilated and anaesthetised newborn lambs with hypoxemia induced by successive reduction in the respiratory support (Dawson et al., 2014).

Pulse oximetry signal relies on adequate arterial pulsation. A significant decrease in peripheral vascular pulsation due to low peripheral perfusion can produce a signal too small to be processed reliably by the oximeter. The effect of hypoperfusion on the accuracy and performance of pulse oximetry has been studied in several studies revealing the increase in errors of pulse oximetry readings or different degrees of poor performance comparing different devices. These studies used different ways to simulate the state of hypoperfusion such as induced sepsis (Hummler et al., 2004) or hemorrhagic hypotension (Barrington et al., 1986) in experimental studies. Studies included volunteers used either pneumatic compression on the brachial artery (Trivedi et al., 1997a) or lowering room temperature (16 – 18ºC) with exposure of the subject's arm (Shah et al., 2012). Patients receiving vasoconstrictive drugs in ICU were also included in some studies (Ibáñez et al., 1991).

In summary, in spite of the known limitations, the use of pulse oximetry can be of great help in different clinical situations to monitor patient oxygenation and guide oxygen supplement therapy. Neonatal resuscitation is a frequently encountered procedure in the delivery room, and giving the large number of deliveries, a considerable number of the newborn infants may need extensive cardiopulmonary resuscitative efforts. Pulse oximetry is being used now as a routine during CPR which usually encounters severe hypoxemia and hypoperfusion. However, the reliability of pulse oximetry during this severely adverse condition in newborn infants is not well defined. In this study we aimed to assess the accuracy of pulse oximetry measurements in comparison with simultaneous CO-oximetry as the gold standard during cardiopulmonary resuscitation in newborn piglets' model of neonatal cardiac arrest.

2. Materials and methods

2.1. Animal preparation

All animals were cared for according to the current version of the German law on the protection of animals and to NIH guidelines for the care and use of laboratory animals, and the protocol was approved by the Animal Care Committee of the state government agencies. The study subjects were newborn piglets because the advantage that they have a size that allows the insertion of invasive devices, as well as the presence of a similar pulmonary vascular response as in the human. The newborn piglets before the experiment were fed and cared by its own mother until the day of the experiment then they were separated from the mother immediately before the experiment.

2.1.1. Premedication, venous access and anaesthesia

The newborn piglets were given atropine 0.02 mg/kg, ketamine 15 mg/kg and midazolam 0.5 mg/kg intramuscular as a premedication. All procedures and CPR process was done while the animal lied down on his back on an open care unit with its four limbs fixed with soft bandages to the care unit (Figure 8). Once the effect of premedication was most effective (usually after 5 – 10 minutes), a venous access with 24G cannula was placed in an ear vein to start and maintain the anaesthesia. After i.v. injection of 0.5 mg atropine, the animal was given midazolam (0.5 mg/kg), fentanyl (50mcg/kg) and propofol (5 mg/kg) i.v. to start anaesthesia then the animal was intubated with a 3.5 mm (internal diameter) endotracheal tube. The anaesthesia was maintained by a continuous infusion of propofol (10 mg/kg/h). Additional doses of fentanyl (25–50 mcg/kg) i.v. were used during instrumentation; whenever the vital signs or behaviour of the animal pointed to any pain e.g. increase in heart rate and/or blood pressure.

2.1.2. Mechanical ventilation

The animals were placed on pressure-controlled ventilation using a Stephanie Ventilator (Stephanie, Fa. Stephan Medizintechnik GmbH, Gackenbach, Germany) with the following setting: FiO_2: 0.3, PIP (peak inspiratory pressure): 20 cmH_2O, PEEP: 5 cmH_2O, inspiratory time: 0.4s, respiratory rate: 30/min. The rate was adjusted to maintain a $PaCO_2$ within the target range of 35 – 45 mmHg. Vecuronium 0.15 mg/kg i.v was given if necessary and immediately prior to the resuscitation procedure. Dextrose 2.5 % with Na 94.5mmol/l, K 9 mmol/l and 1 U Heparin/ml were administered at 8 ml/kg/h into the peripheral vein.

2.1.3. Central catheterization

For the central vascular catheter insertion, the inguinal region was disinfected. Under sterile conditions, a transverse incision (about 3 cm) in the inguinal region skin was performed then the subcutaneous tissue was bluntly dissected from the muscle compartment. Then the muscle layer overlying the vessels was splitted longitudinally and thereby exposed the femoral vessels (Figure 9). A 3.5F single lumen arterial catheter (commercial umbilical catheter for premature babies) was introduced into the femoral artery and used for continuous blood pressure measurement and blood sampling for blood gas analyses and other parameters. This arterial access was continuously flushed with heparinised (1 IU / ml) 0.9% saline solution at an infusion rate of 5 ml/h. 3.5F dual lumen catheter was inserted into the femoral vein and advanced into the inferior vena cava . One limb was used to measure the central venous pressure, and the second was used for injection of KCl to induce cardiac arrest and used also to secure venous access for anaesthesia during resuscitation. Both limbs of this central were continuously flushed with heparinised (1 IU/ml) 0.9% saline solution at a rate of 2 ml/h.

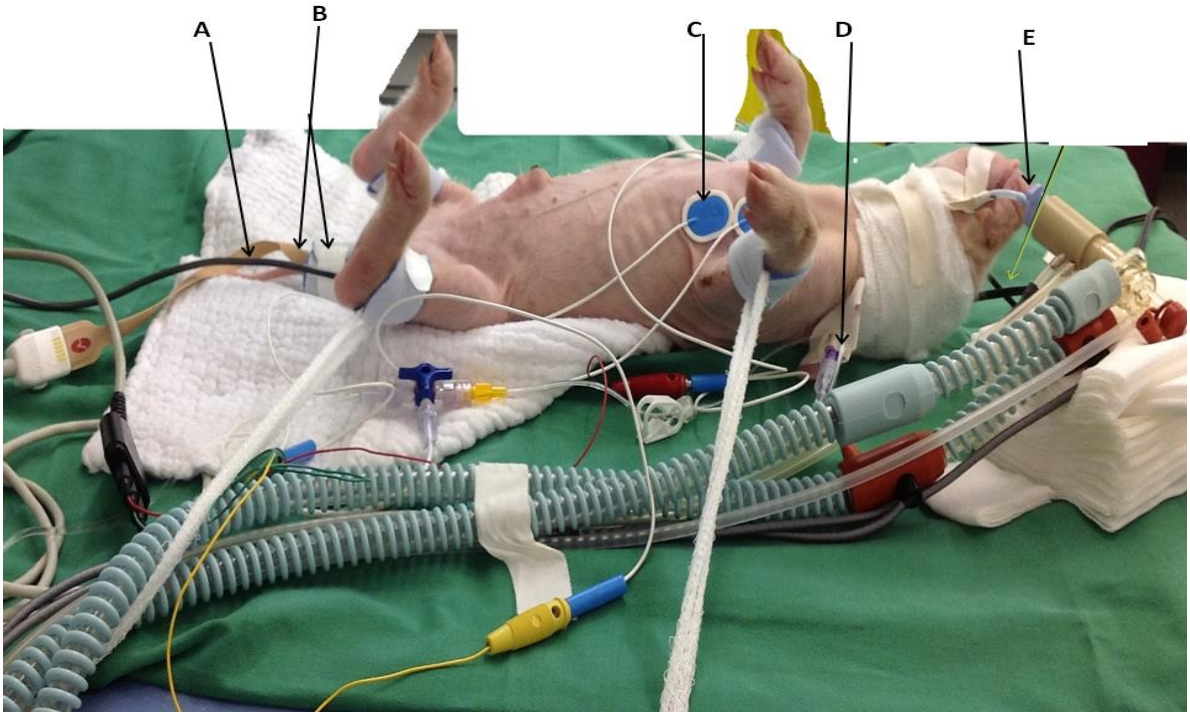


Figure 8: The newborn piglet lying and fixed to the open care unit with rectal temperature probe A, pulse oximetry sensor and its shield B, electrocardiogram (ECG) electrodes C, ear intravenous line D, and endotracheal tube fixed and connected to ventilator circuit E.

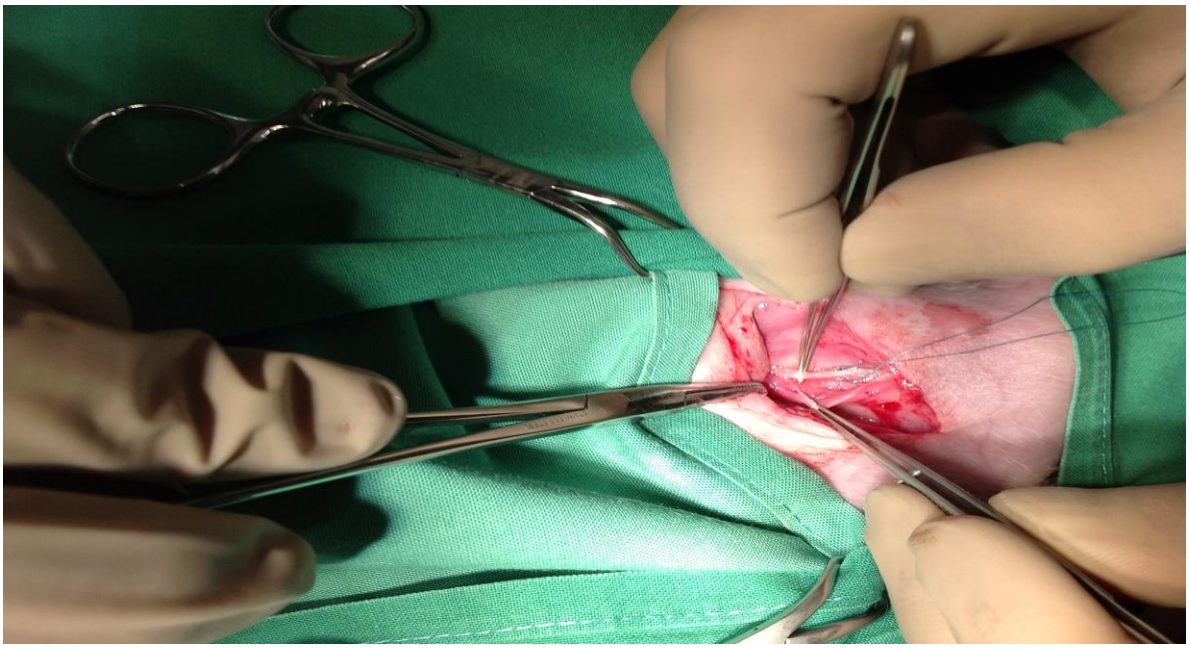


Figure 9: The incision through the skin and dissection of the muscles to reach the femoral vessels.

2.1.4. Vital and study parameters monitoring

A rectal temperature probe (Siemens Sirecust 302, Erlangen, Germany) was placed and a core temperature of 39.0-39.5° C was maintained using an open care unit with a heating gel mattress and an overhead radiant warmer (Babytherm 8000, Dräger, Lübeck, Germany). Air way, arterial and central venous pressure were measured with Sorenson Transpac pressure transducers (Transpac 4, Abbott Critical Care Systems , North Chicago, IL) which were coupled with a respective amplifiers (Gould, Valley View , OH). All pressure sensors were calibrated before the experiment with a water manometer. The flow signal was derived from the ventilator and calibrated by means of a flowmeter (Model 8270 Gas Products, Matheson, Montgomeryville, PA).

A self-adhesive pulse oximetry sensor (LNOP NeoPt-L, Masimo SET, Irvine, CA) (Figure 10) was applied to the tail and was shielded with a thick bandage then it was connected to the pulse oximetry device (Radical, Masimo SET V4.6.0.2, Irvine, CA) for continuous monitoring of SpO_2. Furthermore, 3 ECG electrodes were placed on the shaved chest wall. All signals were digitized at a frequency of 100 Hz and recorded simultaneously using a computerized data system (DATAQ Instruments, Inc., Akron, OH).

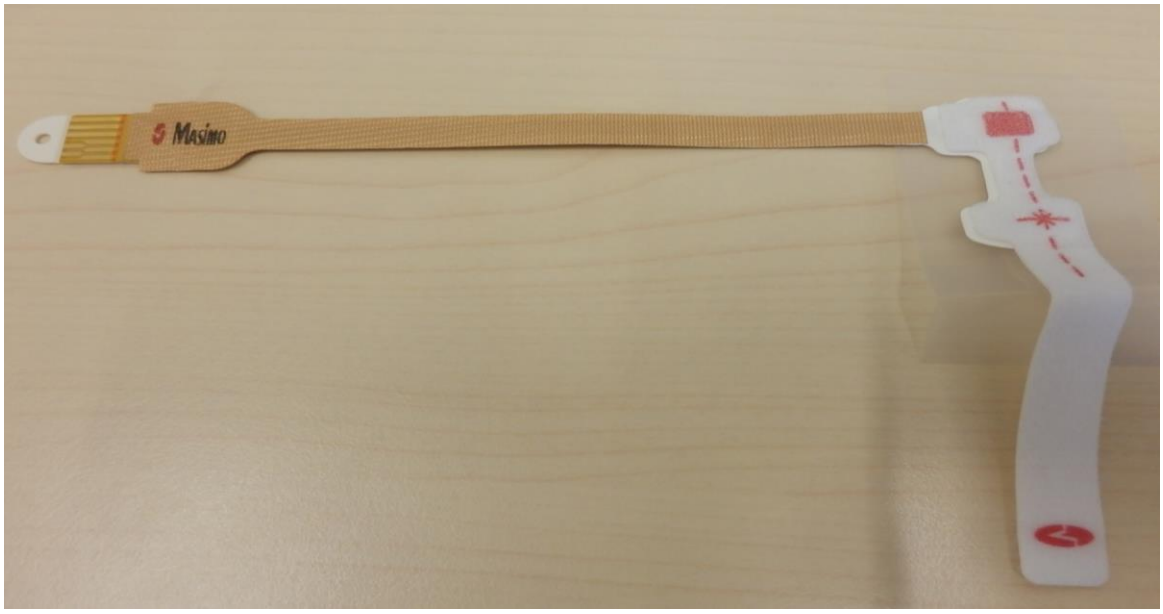


Figure 10: The pulse oximetry sensor used during the experiment.

2.2. Experimental procedure

Figure 11 shows the experimental stepwise protocol. After instrumentation the FiO_2 was reduced to 0.21 and after 15 minutes the data recording was started and an arterial blood gas sample was withdrawn to be analysed by co- oximetry (Radiometer ABL 700 series, Copenhagen) to measure the arterial oxygen saturation (SaO_2) by, PaO_2, PCO_2, pH, lactate, potassium measure and ionised calcium. These measurements were considered the baseline measurements. Then a bolus of KCl 2 mmol/kg was administered via the central venous catheter and flushed with 2 ml of 0.9% NaCl. This dose can raise the intravascular K^+ concentration to at least 3-4 times the normal value and lead within seconds to cardiac arrest. Furthermore, continuous intravenous infusion of KCl 3 mmol/kg/h was administered, to maintain the hyperkalemic state during the experiment. Mechanical asystole was defined as a loss of pulsation in arterial blood pressure measurement curve and simultaneously loss of regular ECG activity. At this time, the ventilation was disconnected and then started again after 30s, where the animals were randomized into one of the following three groups:

- Group 1 received the respiration by the newborn ventilator with PIP/PEEP of 20/5 cm H_2O and a fixed frequency of 30 ventilation strokes /minute (inspiratory time 0.40 s) with a rectangular airway pressure curve and cardiac compressions (CCs) were then administered at a rate of 120/min , without paying attention to synchrony with the ventilator .
- Group 2 received mechanical ventilation with a T- piece with a PIP / PEEP of 20/5 cm H_2O and a respiratory rate of 30/minute. CCs were applied with a ratio of 3 CC: 1 breath.
- Group 3 were ventilated by using a conventional manual ventilation handbag trying to keep having a PIP of 20 cm H_2O (as measured and monitored by observing the air way pressure curve) without PEEP, the frequency at a rate of 30/min between cardiac compressions with a 3:1 ratio was maintained.

The randomly assigned respiratory support was started using a FiO_2 of 0.21 according to current guidelines (Perlman et al., 2010). CC was started 30 seconds after adequate respiratory support (according to the randomization); at this time the 1st

arterial blood sample was withdrawn for analysis in the same way as the baseline sample. The appropriate rate of CC was guided using a metronome with a rate of 120/minute and the depth of CC was "goal directed", i.e. it was adjusted to keep a systolic arterial blood pressure of 50 mmHg as measured and continuously monitored throughout the experiment (figure 12). CCs were applied by means of two-thumb method, since this method in comparison to the 2-finger method in neonatal resuscitation was proven to be more effective (Christman et al., 2011). After 10 minutes the FiO_2 was increased to 1.0. Resuscitative efforts were continued for a total time of 20 minutes after beginning of asystole. Arterial blood samples were taken every 2 minutes and processed immediately.

The working team included at least 4 members; one observer who monitored the experiment process and made sure that everything was going according to the planned protocol; a second person was responsible for the respiratory support given to the animal during the experiment; a third person was responsible for starting and continuing the cardiac compression; and a fourth person was responsible for regular withdrawal and immediate analysis of arterial blood gas samples. After the end of the resuscitation process, all i.v. fluids and anaesthetics were discontinued. Recording of the vital signs, including cerebral oxygen saturation was carried out for a further 10 min after the end of resuscitation to ensure the animal death.

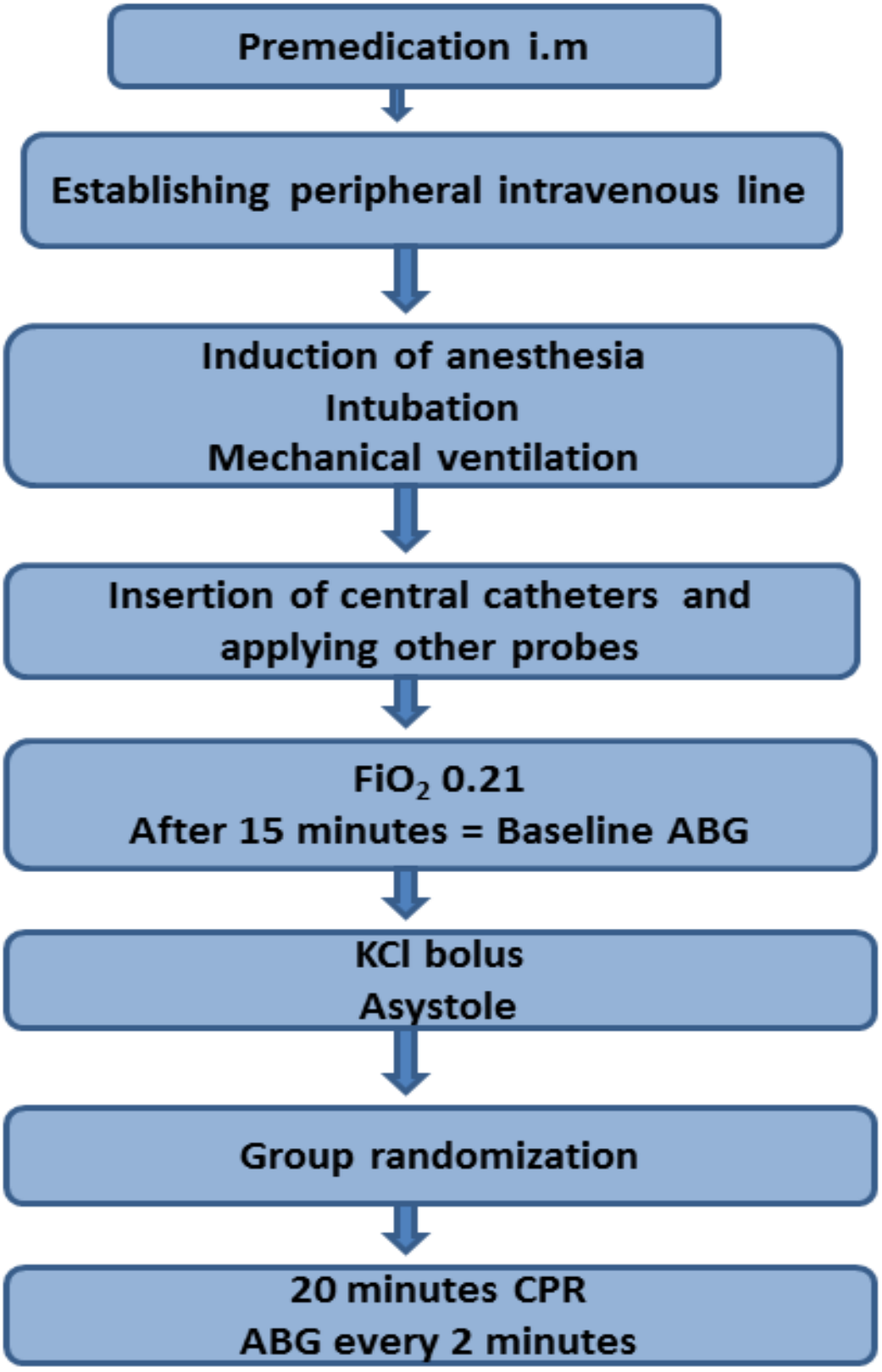


Figure 11: Experimental protocol. i.m: intramuscular; FiO_2: fraction of inspired oxygen; KCl: Potassium chloride; CPR: cardiopulmonary resuscitation; ABG: Arterial Blood Gas analysis.

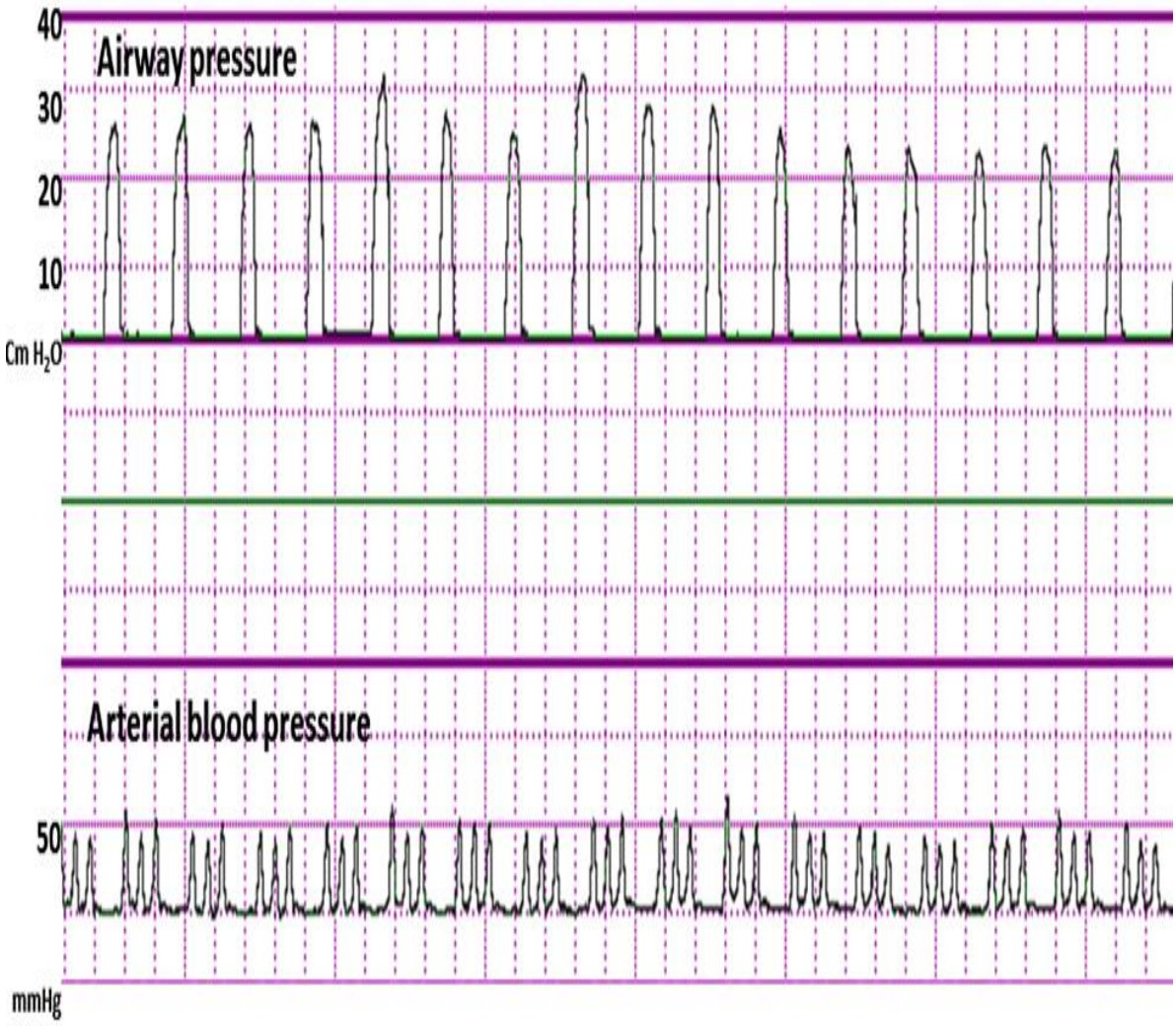


Figure 12: A compressed trace during cardiopulmonary resuscitation with airway pressure curve (upper panel) and arterial blood pressure curve (lower panel) showing the 3:1 CC rate and that most of the generated blood pressure waves peaks are reaching the 50 mmHg.

The SpO_2 measurements with pulse oximetry were analyzed using WINDAQ Data Acquisition software taking the average of the 10 seconds that coincided with the arterial blood sample withdrawal every 2 minutes. The arterial blood sampling onset were recognized in the software by the temporarily loss of arterial blood pressure curve due to blood withdrawal from the arterial catheter (Figure 13). Whenever there was a pulse oximetry signal dropout at the onset of arterial blood sampling a period of 1 minute before or after that onset was searched for the closest 10 seconds with a recorded pulse oximetry signal.

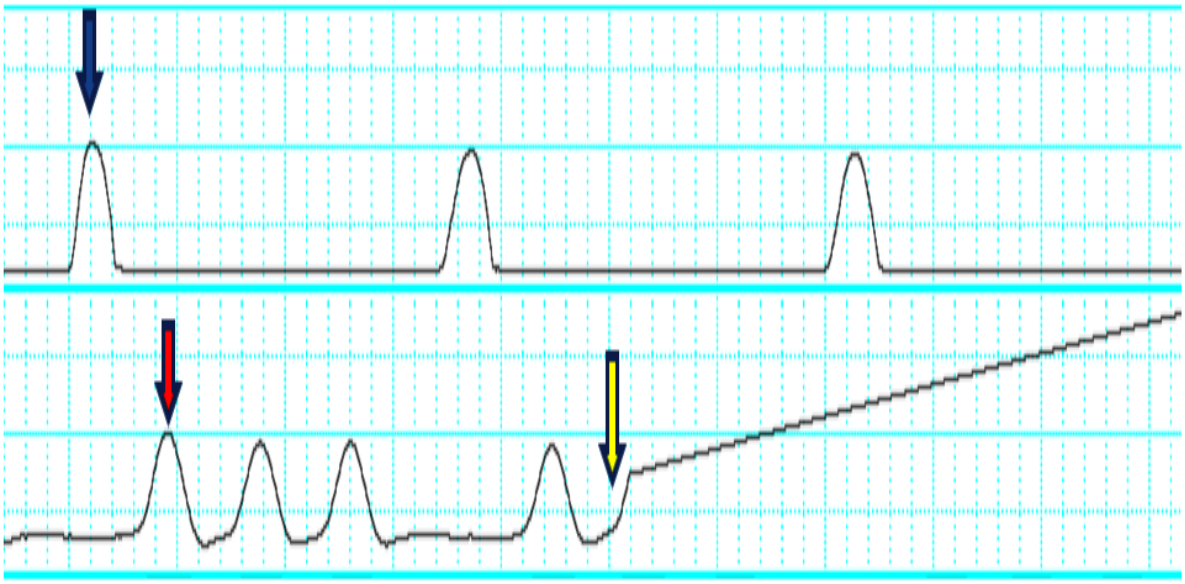


Figure 13: Recorded signals of respiratory support (blue arrow) and arterial blood pressure (red arrow) showing loss of arterial pressure curve coincide with blood withdrawal (yellow arrow). Notice the 3 cardiac compression: 1 breath ratio used during resuscitation.

The bias was calculated as the average difference between oxygen saturation as measured by pulse oximetry (SpO_2) and CO-oximetry (SaO_2) at the base line and throughout the experiment time and it represents the total systematic error. An acceptable bias was defined arbitrarily for this condition as ± 5%. The accuracy of pulse oximetry measurements was defined according to the international standards (ISO, 2011) as the closeness of agreement between the test value (SpO_2) and the reference value (SaO_2). It is a combination of systematic and random errors and it was calculated as the root-mean-square (A_{rms}) difference ($SpO_2 - SaO_2$) where higher values of A_{rms} reflect larger Bias and lower accuracy. Precision was defined as the closeness of agreement among independent test results obtained under stipulated conditions. It can be recognized as the scatter of data points about the best fitting curve of agreement and indicates the random error. It was calculated as standard deviation of residuals (S_{res}) from a linear regression model where higher values reflect low precision (ISO, 2011). For the calculations of precision we used the ISO standards

instead of using the ±SD of the bias values which have been used by other studies (Carter et al., 1998; Fanconi, 1988).

Modified Bland- Altman plots were used to compare time dependent changes of the accuracy of pulse oximetry measurements across the experimental time using the mean ± 2SD (or more accurately mean ± 1.96 SD) values of the difference (SpO_2 - SaO_2) measured at each time point. The original Bland- Altman plot for assessing the agreement between methods of clinical measurement were used to assess the agreement between SpO_2 and SaO_2 using the 95% limits of agreement (Bland and Altman, 1986, 1999). Paired *t*-test or Wilcoxon signed ranks tests were used where appropriate to compare the measurements by pulse oximetry and co- oximetry. Analysis of variants (Anova) test was used to compare the 3 different groups. Statistical level of significance was considered p-value less than 0.05. Statistical analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Study subjects

Thirty healthy newborn piglets were included in the study in the period from February 2012 to the end of June 2012. Subject details are given in table 1. Table 2 shows Potassium, sodium, glucose, and lactate levels at baseline and across the experiment. Potassium levels were maintained >10 mEq/L across the experiment. Lactate levels were increasing throughout the experiment suggesting the presence of low peripheral perfusion during CPR (Figure 14).

Table 1: Demographic data of study subjects.

	Median (Minimum - Maximum)	Mean ± SD
Age (days)	5 (2 – 11)	5.30 ± 2.29
Weight (g)	1875 (1200 – 2980)	1982 ± 422

SD: standard deviation.

Table 2: Sodium (Na), Potassium (K), Glucose, and Lactate levels at the baseline measurements and across the experiment. Values are mean ±SD

	Na	*K*	*Glucose*	*Lactate*
Baseline	132 ± 4	4 ± 1	115 ± 31	2.3 ± 1.2
2 min.	124 ± 6	20 ± 2	113 ± 30	2.8 ± 1.3
4 min.	127 ± 5	18 ± 3	145 ± 38	4.4 ± 1.5
6 min.	128 ± 5	16 ± 4	168 ± 43	5.7 ± 1.6
8 min.	128 ± 6	15 ± 3	183 ± 51	6.7 ± 1.6
10 min.	130 ± 5	15 ± 3	199 ± 56	7.5 ± 1.8
12 min.	130 ± 5	15 ± 2	213 ± 62	8.2 ± 2.1
14 min.	131 ± 5	15 ± 2	223 ± 71	8.5 ± 2.1
16 min.	131 ± 5	15 ± 2	232 ± 75	8.9 ± 2.2
18 min.	132 ± 5	14 ± 2	235 ± 80	9.3 ± 2.4
20 min.	132 ± 5	15 ± 2	239 ± 84	9.5 ± 2.5

SD: standard deviation.

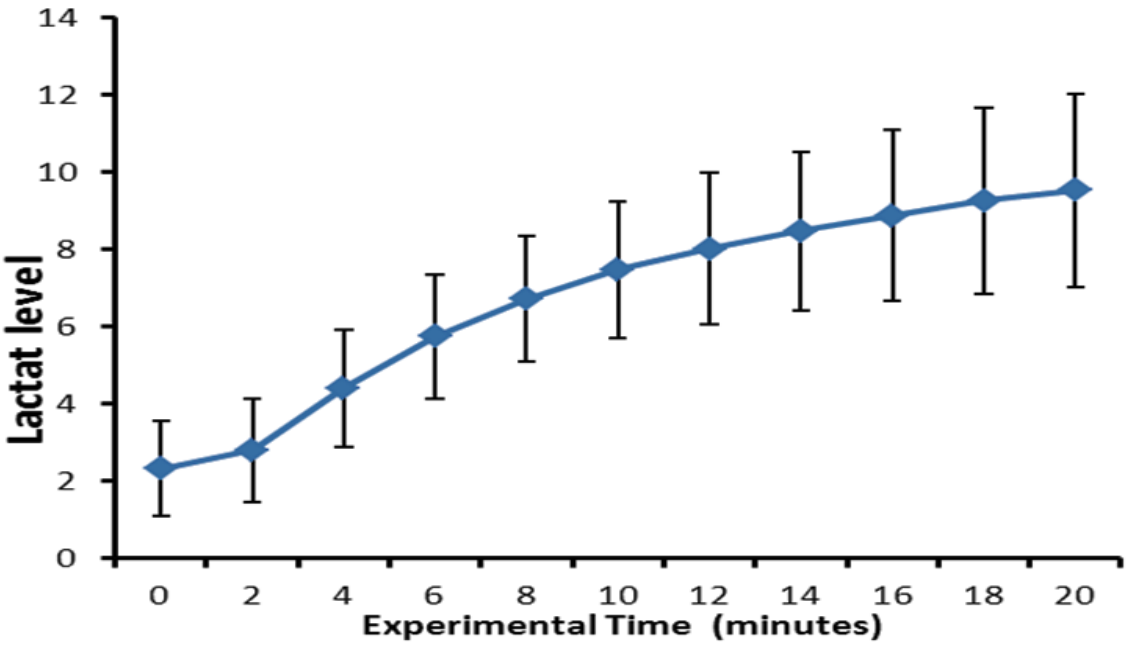


Figure 14. Increasing mean lactate levels (mean ± standard deviation.) across the experimental time

3.2. Signal dropout

From the expected 330 SpO_2 measurement points (1 baseline and 10 during the experiment in 30 subjects) we had 313 measurement points due to 17 events of signal dropout at the onset of arterial blood gas withdrawal ± 1 minute. Table 2 shows the total dropout time during which there was no recorded pulse oximetry signal during the experimental time. The median total dropout time was low (1.44 minutes); however it showed a wide range reaching a maximum of 19.89 minutes in one piglet i.e. during almost the whole experiment. Every subject had at least one episode of signal dropout and most of them had an episode with the onset of the cardiac arrest when for a short period of time no CPR was provided. The total number of episodes of signal dropout was low with a median of 3.5 episodes; however it showed also a wide range reaching the maximum of 31 episodes with a total time of 9 minutes in one subject. Table 3 shows also the signal drop out of the pulse rate during CPR. Pulse rate during CPR was of 120 ±10 beats/ minute which coincided with the rate of CC in the mechanically ventilated group, and it seems that the pulse oximetry picked up the individual breaths between CCs in the other two groups.

Table 3: The median and range of the total time, number of episodes of signal dropout, and duration of single episodes of signal dropout for SpO_2 and pulse rate.

	SpO_2	**Pulse rate**
	Median (Minimum–Maximum)	Median (Minimum–Maximum)
Total Dropout time (minutes)	1.44 (0.29 - 19.89)	1.48 (0.61 – 19.68)
Number of episodes of signal dropout	3.5 (1 – 31)	2 (1 – 15)
Duration of episodes of signal dropout (minutes)	0.34 (0.03 - 14.65)	0.85 (0.13 – 15.35)

SpO_2: pulse oximetry measured arterial oxygen saturation.

3.3. SpO_2 compared with SaO_2

Figure 15 shows the mean ± SD of measurements of pulse oximetry and corresponding values measured by CO-oximetry across the experimental time. Both SpO_2 and SaO_2 are close to each other at the base line measurement before the onset of cardiovascular collapse. Two minutes after asystole during CPR, there was already a mean difference of 13% between SpO_2 (71.1 ± 22.7%) and SaO_2 (58.1 ± 20.6%) and the difference continued to increase throughout the experimental time. SaO_2 showed a minimal increase after 10 minutes that coincided with the increase of the FiO_2 from 21% to 100%, while SpO_2 continue to overestimate SaO_2. The difference between the measurements of SpO_2 and SaO_2 is strongly statistically significant (p value < 0.001; paired t- test) for all measurements across the 20 minutes of the experiment including the baseline measurements (p value 0.009; Wilcoxon signed rank tests).

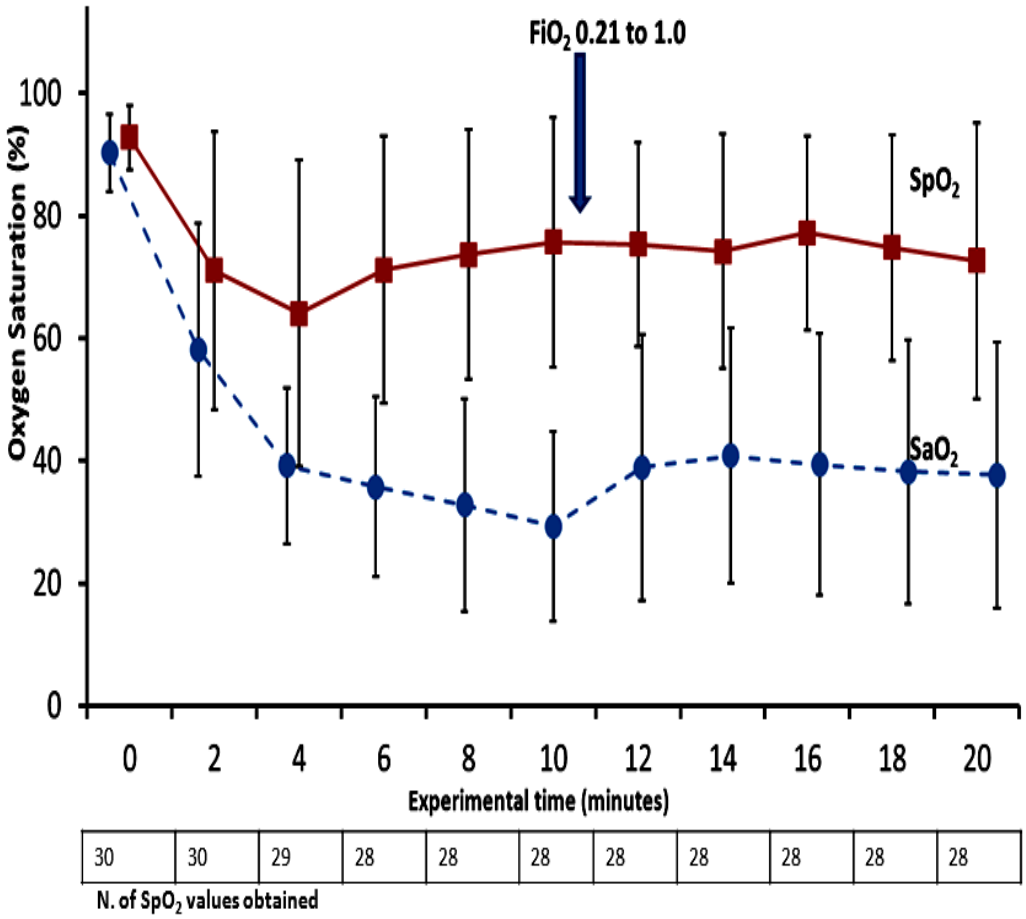


Figure 15. Mean ± standard deviation of SpO_2 (pulse oximetry measured arterial oxygen saturation) and SaO_2 (CO-oximetry measured arterial oxygen saturation) across the experimental time with table of the number of SpO_2 values in coincide with experimental time. 0 is the baseline measurements.

3.4. Bias, accuracy, and precision

Table 4 and figure 16 show the mean bias and variability of individual bias values throughout the experimental time. Although the SpO_2 was statistically significantly higher than SaO_2, the mean bias and variability were small at the baseline measurements (Fig. 15, time 0), then increased both during the experiment until the mid-time of the experiment with a little change in the mean bias afterwards. The individual bias values were 2.49 ±9.2% (mean ±2SD) at baseline and increased dramatically to 12.94 ±67.7% after 2 minutes of resuscitation. During the 20 minutes during the experiment, bias increased further.

With the non-acceptable bias (SpO_2 -SaO_2) defined ≥ ±5%, the individual bias values were beyond these limits at 269/ 313 measurements (86%) with 37/313 (12%) underestimated values and 232/313 (74%) overestimated values. The values of these bias were far beyond the ± 2% bias limit commonly considered acceptable for pulse oximetry devices designed for clinical use. The bias (SpO_2 -SaO_2) was 2.4% ±4.58% at the baseline. Accuracy and precision were high at the baseline measurements (A_{rms} 5.1% and precision 3.8%) then dramatically decreased after 2 minutes of resuscitation (A_{rms} 35.7% and precision 22.5%) and across the experiment (Table 4).

Table 4: Mean ± standard deviation of SpO_2 and SaO_2 values; mean ± standard deviation of bias, accuracy (A_{rms}), and precision across the experimental time. Mean SpO_2 was significantly higher than mean SaO_2 ($p < 0.01$; paired t- test or Wilcoxon signed rank tests when appropriate).

	Mean SpO_2±SD	**Mean SaO_2±SD**	**Mean bias ± SD**	**Accuracy (A_{rms})**	**Precision (S_{res})**
Base line	92.79 ± 5.27	90.31 ± 6.30	2.49 ± 4.58	5.15	3.84
2 min.	71.08 ± 22.73	58.14 ± 20.62	12.94 ± 33.85	35.71	22.58
4 min.	64.03 ± 25.03	39.22 ± 12.72	24.06 ± 25.29	34.59	24.84
6 min.	71.20 ± 21.81	35.79 ± 14.74	35.58 ± 27.05	44.40	22.20
8 min.	73.67 ± 20.36	32.77 ± 17.30	40.24 ± 28.12	48.81	20.66
10 min.	75.68 ± 20.32	29.31 ± 15.44	45.58 ± 28.31	53.39	20.16
12 min.	75.39 ± 16.63	38.99 ± 21.72	35.20 ± 32.51	47.52	15.50
14 min.	74.26 ± 19.14	40.85 ± 20.90	32.43 ± 30.38	44.06	19.33
16 min.	77.27 ± 15.86	39.43 ± 21.30	36.94 ± 28.25	46.20	16.04
18 min.	74.82 ± 18.46	38.26 ± 21.52	36.79 ± 31.07	47.79	18.45
20 min.	72.74 ± 22.52	37.7 ± 21.71	34.96 ± 33.02	47.68	22.78

SpO_2: pulse oximetry measured arterial oxygen saturation; SaO_2: CO-oximetry measured arterial oxygen saturation; A_{rms}: accuracy root mean square; S_{res}: standard error of residuals; SD: standard deviation.

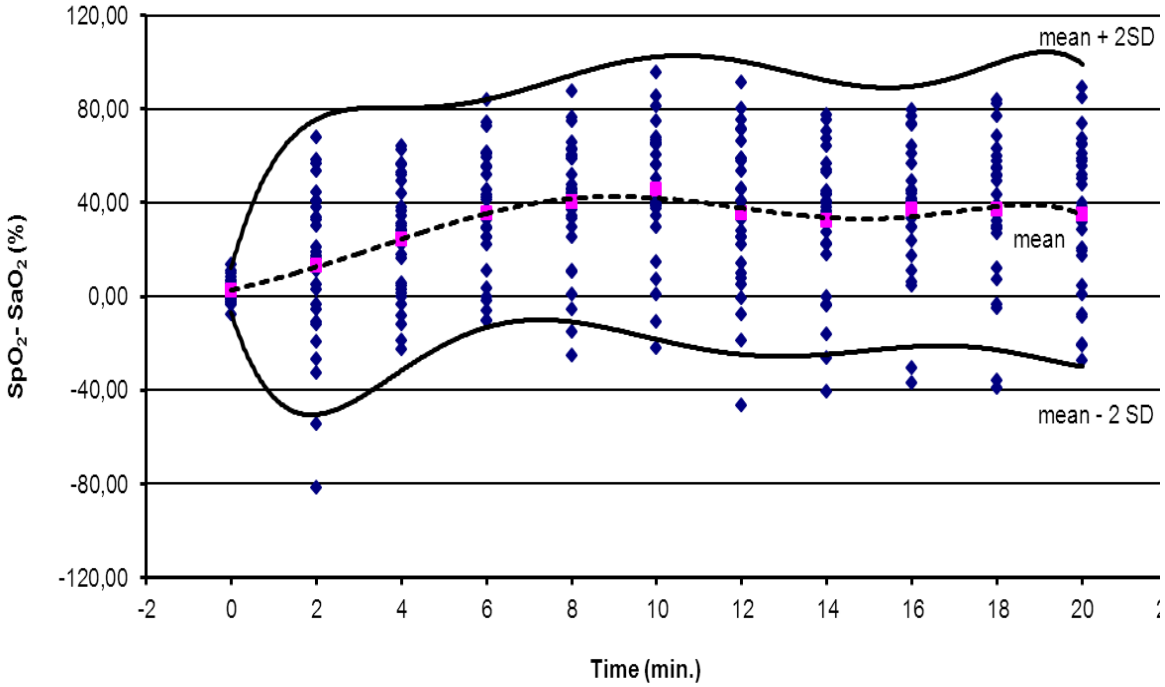


Figure 16: Modified Bland-Altman plot of the measured bias values (SpO_2 -SaO_2) across the experimental time. Each dot refers to one measurement at each time point. Mean bias along with the mean ± 2SD values of these individual bias are also plotted. The y axis has been extended to include all the individual values including the outliers. Time 0 is the baseline. SpO_2: pulse oximetry measured arterial oxygen saturation; SaO_2: CO-oximetry measured arterial oxygen saturation; SD: standard deviation.

3.5. Limits of agreement

The original Bland- Altman plots for assessing the agreement between methods of clinical measurements were used to assess the agreement between SpO_2 and SaO_2 using the 95% limits of agreement at the base line measurements, 10 minutes after the onset of the experiment, and at the end (at 20 minutes) of the experiment (Figure 17 A, B, C). At baseline the variability of individual SpO_2 -SaO_2 difference was small, i.e. precision was high with limits of agreement (±2SD of bias) that SpO_2 can be 11.5 % above or 6.5 % below SaO_2. After 10 minutes SpO_2 was overestimating SaO_2 values in most animals dramatically and precision was much lower with limits of agreements at 10 minutes that SpO_2 can be almost 100 % above or 10 % below SaO_2 and at 20 minutes can be 99 % above or 30 % below SaO_2. Bias levels did not systemically depend on the actual oxygenation status as measured by SaO_2.

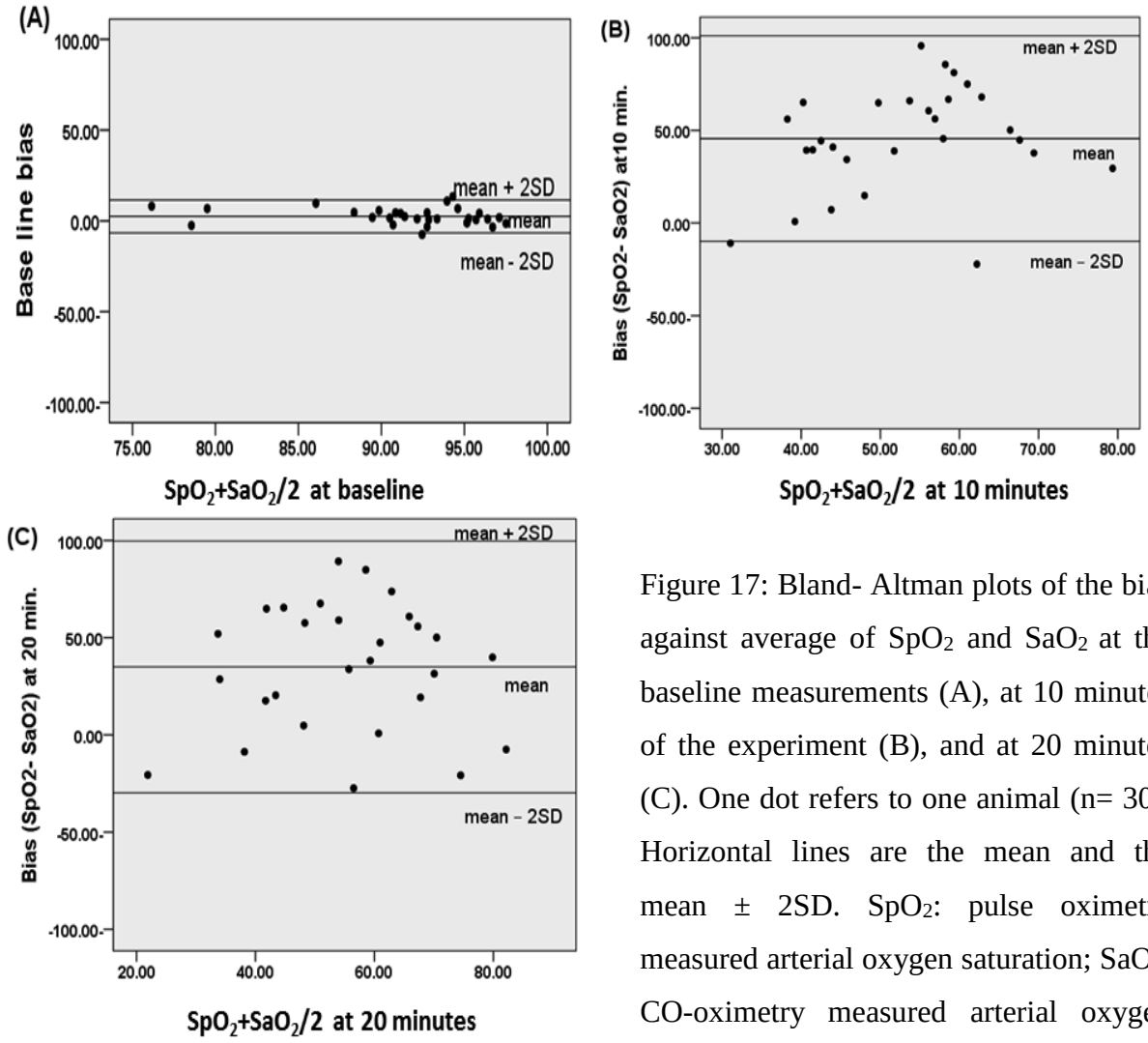


Figure 17: Bland- Altman plots of the bias against average of SpO_2 and SaO_2 at the baseline measurements (A), at 10 minutes of the experiment (B), and at 20 minutes (C). One dot refers to one animal (n= 30). Horizontal lines are the mean and the mean ± 2SD. SpO_2: pulse oximetry measured arterial oxygen saturation; SaO_2: CO-oximetry measured arterial oxygen saturation; SD: standard deviation.

3.6. Performance in different subject groups

We repeated the analysis dividing the whole 30 piglets into 3 groups according to the randomization for the 3 different respiratory support methods during CPR. Figure 18 shows the averages of SpO_2 and SaO_2 throughout the experimental time of the three different study groups. There was a trend towards a larger bias with the use of handbag for resuscitation than Stephanie or T- piece ventilation. However, there was no statistically significant difference between the bias of the three groups (analysis of variance, Post Hoc test), p values were 0.3, 0.5 and 0.6 at 2 minutes; 0.9, 0.07 and 0.09 at 10 minutes; and 0.3, 0.3 and 0.9 at 20 minutes comparing Stephanie ventilator group to T-piece group, Stephanie ventilator group to handbag ventilation group, and T-piece group to handbag ventilation group respectively.

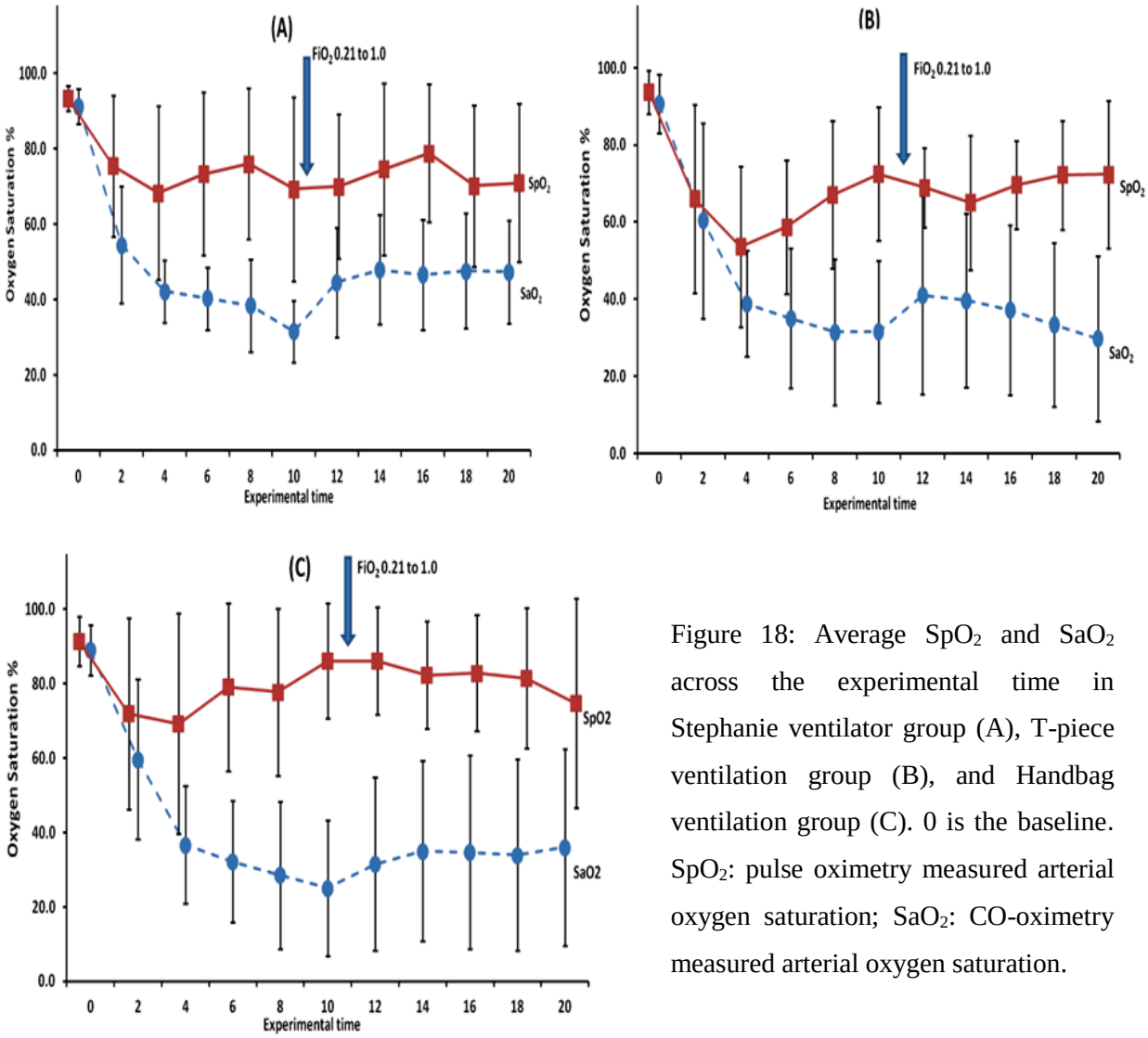


Figure 18: Average SpO_2 and SaO_2 across the experimental time in Stephanie ventilator group (A), T-piece ventilation group (B), and Handbag ventilation group (C). 0 is the baseline. SpO_2: pulse oximetry measured arterial oxygen saturation; SaO_2: CO-oximetry measured arterial oxygen saturation.

4. Discussion

Pulse oximetry is widely used in intensive care and emergency situations and it is considered by some authors as the fifth vital sign along with heart rate, respiratory rate, temperature, and blood pressure and its use can result in important changes in the clinical management of pediatric patients (Mower et al., 1997). Furthermore, it can be used not only for monitoring during routine care but also in emergency situations such as during resuscitation (Spittal, 1993). In spite of the wide and advantageous use of pulse oximetry, it faces many challenges which can be met in different clinical situations and can affect its reliability especially in emergency situations where rapid and precise decisions are needed. These limitations include the effects of ambient light, motion, and low peripheral perfusion (Trivedi et al., 1997a).

In this study we tried to overcome many of these known limitations by keeping core temperature of 39.0-39.5°, using dim light throughout the experiment, good fixation of the animal and shielding of the pulse oximeter sensor, continuous sedation and paralysis of the animal, maintaining a systolic arterial blood pressure of 50 mmHg by standardized CPR. Furthermore, we used Masimo SET pulse oximetry which is the most recently available technique; which uses a constellation of advanced techniques including radiofrequency, light-shielded optical sensors, digital signal processing, and adaptive filtration in comparison with the conventional pulse oximetry. These advanced techniques should enable the device to measure SpO_2 accurately during challenging clinical conditions and limit the effects of motion and low perfusion on pulse oximetry readings (Goldman et al., 2000). In summary, the study setting was standardized to provide an environment and other circumstances to optimize consistent CPR for all study subjects.

We demonstrated that the mean bias of SpO_2 values measured by pulse oximetry were 2.4% ±4.58% at baseline with a SaO_2 saturation of 90 % or above with normal hemodynamics. Although these bias values comparing SpO_2 with SaO_2 values was significant from the statistical point of view, they are somewhat close to values reported before and considered acceptable at normal conditions with the mean bias < 2% and a standard deviation of < 3% when SaO_2 is 90% or above (Jubran, 1999).

However, during CPR bias increased dramatically to 13 ±34% after 2 minutes and reached a maximum of 45.58 ±28.31% after 10 minutes over a range of mean SaO_2 between 29.31% and 58.14%. We also reported that the limits of agreement (±2SD of bias) at mean SaO_2 90% were 2.5 ± 9 (mean ± 2SD); this means that SpO_2 can be 11.5 % above or 6.5 % below SaO_2. This range is already beyond the acceptable range of bias at mean SaO2 90%, and the limits of agreement recorded in this study during CPR with severe hypoxemia are clearly not acceptable for clinical conditions in humans.

Accuracy of pulse oximetry calculated as A_{rms} should be ≤4% at SaO_2 over the range of 70 –100% (ISO, 2011) which is close to our A_{rms} calculated at the baseline. During CPR A_{rms} range was 34.59–53.39% over SaO_2 ranging 29.31–58.14% indicating low accuracy over this range. Precision reported in our study using the ISO definition was high at the baseline measurement (3.84%) and poor during CPR (15.5–24.84%).

Several studies reported that pulse oximetry becomes inaccurate at low arterial oxygen saturation with an increase in bias and decrease in precision as arterial saturation decreases. Some authors have reported SpO_2 overestimating SaO_2 (Schmitt et al., 1993), others reported SpO_2 underestimating SaO_2 (Carter et al., 1998; Trivedi et al., 1997b), and others reported over- and underestimation comparing two pulse oximeters (Hummler et al., 2004). In our study the mean SpO_2 was overestimating SaO_2 with the onset of cardiac arrest, with constantly applied cardiac compression, and throughout the experimental time in most measurements. These differences can be due to the use of different devices and the profound hypoxemia encountered in our study reaching to mean arterial saturation as low as 29 % with wide limits of agreement recorded during CPR while in most of the previous studies the SaO_2 was around 75%.

Over a similar range of SaO_2 encountered in our study during CPR, Solevåg et al. reported a better accuracy with A_{rms} 11.7– 21% and reported a total precision of 17.3% using the same definition (Solevåg et al., 2013). Corresponding to previous studies which reported precision as SD of the bias (Carter et al., 1998; Fanconi, 1988), our data showed much higher SD of the bias which may be due to much lower SaO_2 as low as 29% encountered in our study. In a recent study with induced hypoxemia in 17

newborn lambs through successive reduction of respiratory support, Dawson et al. reported that the mean difference (±SD) of Nellcor pulse oximeter SpO_2-SaO_2 was 17% (−12% to 46%) and that of Masimo pulse oximeter was 13% (−19% to 45%) with a SaO_2 <70% (Dawson et al., 2014).

The reason for this large difference between SpO_2 and SaO_2 is currently not clear. However, a number of explanations have been proposed for the poor performance of pulse oximeters at low oxygen saturations. One of these explanations is the use of software "look-up" tables to empirically determine saturation from the available data which were derived from studies done to healthy volunteers with induced hypoxemia, but for ethical reasons this was limited to a SpO_2 around 75%. Extrapolation is used below this level (Kelleher, 1989). Additional reasons for limited performance at low saturation can be the slight variations in the output wavelength of the LEDs which can cause more errors during hypoxemia, and the generation of proportionally larger errors in the measurement of transmitted red light than for infrared light at low saturations because of the large extinction coefficient of reduced hemoglobin at the red light wave length (Carter et al., 1998).

Several studies also tested the accuracy of pulse oximetry during conditions associated with low peripheral perfusion. Trivedi and colleagues used pneumatic compression on the brachial artery to reach mean arterial blood pressure of 50 – 60 mmHg and showed that at this level the failure of pulse oximetry to obtain readings was 30 – 100% within different devices (Trivedi et al., 1997a). Barrington and colleagues also simulated the state of hypoperfusion by repeated blood sampling in adult rabbits inducing hemorrhagic hypotension. They reported that below a mean arterial blood pressure of 44 mmHg, pulse oximetry failed to display measurements (Barrington et al., 1986). In our study most of the animals were considered to have severely impaired hemodynamics with low peripheral perfusion as suggested by increasing lactate level towards the end of the study (Fig. 14); however pulse oximeter continued to have false readings until the end of the experiment without many episodes of signal dropout.

CPR is a clinical situation during which pulse oximetry faces the two major challenges of severe hypoxemia and low peripheral perfusion. Few studies reported systematic evaluation of pulse oximetry during CPR. Moorthy and colleagues reported false pulse oximetry readings at the onset of diagnosis of cardiac arrest and claimed that these incorrect and misleading readings were due to the 8 – 10 seconds delay required by pulse oximetry to start displaying asystole. However, they did not report on the pulse oximeter behavior during the whole CPR process and if CPR has been improved with pulse oximetry in the reported case with successful resuscitation and they also did not report simultaneous SaO_2 values (Moorthy et al., 1990). On the other hand Narang was satisfied by the high saturation values indicated by pulse oximetry during CPR and considerd them an indicator of the effective CPR, but did not report simultaneous SaO_2 values as the SpO_2 values could be clearly an overestimation of the real saturation (Narang, 1986). Although his patient had pink skin color during CPR ,depending on patient color is not a good indicator for the true arterial oxygen saturation (O'Donnell et al., 2007).

Pule oximertry during CPR was also evaluated by Spittal, but he used a questionaire of the attending regestrars and documented only the lowest and average of SpO_2 measurements during the whole CPR. He did not report simultaneous SaO_2 as he was testing the benefit not the accuracy of pulse oximetry during CPR (Spittal, 1993). In a similar study to ours, Barrington and colleagues have induced asystole in 6 adult rabbits by KCl injection, attempted to keep systolic blood pressure 40 mmHg during CPR, and had 4 arterial blood samples during the 20 minutes CPR for ABG analysis. However they used the correlation to report that there was no relation between SaO_2 and SpO_2 during CPR (Barrington et al., 1986). In our study we have tested SpO_2 with simultaneous SaO_2 every two minutes during CPR in addition to a baseline measurement; we maintained a systolic blood pressure of 50 mmHg; and used the limits of agreements as recommended to compare SpO_2 and SaO_2 (Bland and Altman, 1986).

One limitation of our study is that we did not aim to restore the spontaneous circulation and breathing, therefore the performance of pulse oximetry during recovery of spontaneous circulation was not tested. However, we intended to evaluate gas

exchange and SpO_2 response with complete cardiac arrest and this model allowed us to collect more blood samples during persistent cardiac arrest. An asphyxia model would have been more preferable to simulate the real circumstances of neonatal resuscitation after asphyxia, but with asphyxia model the return of spontaneous circulation usually does occur which may interfere with standardized CPR. Another potential limitation is the site of pulse oximeter sensor (proximal Tail). It may be that during adverse hemodynamic conditions vasoconstriction in the piglet's tail affects the capability to detect an accurate signal. This also might be the reason why Solevåg et al. had somewhat better accuracy and precision with using the thigh as the recording site (Solevåg et al., 2013). Obviously other sites should be tested in the future. One more limitation is that the Masimo has developed the "blue sensors" for low SpO_2 conditions with proven accuracy at saturations as low as 60%. These sensors may perform better (Masimo Corporation, 2013).

All piglets studied in this experiment were beyond the early neonatal transitional period (median age 5 days). Therefore, they may have pathophysiologic differences compared to a human newborn during the early transitional period such as absence of circulatory shunts and residual lung fluids. However, the piglet model has been used in several studies on neonatal resuscitation (Solevåg et al., 2011, 2013). Moreover, it has been suggested that newborn piglets can provide a relevant model of human preterm physiology during neonatal age (Eiby et al., 2013). We did not correct the CO-oximetry for porcine blood. However, correction would have resulted in increased systematic bias due to shift of the human oxyhemoglobin dissociation curve to the left in comparison with pigs (Serianni et al., 2003).

These false SpO_2 readings encountered in the critical condition of neonatal cardiopulmonary resuscitation may lead to clinically inappropriate FiO_2 adjustments and may give the health provider a false impression of the true gas exchange. There seems to be a substantial risk for false interpretation of measured data if the same errors occur in human beings. Arterial blood gases evaluation can be used if the judgment of gas exchange is considered important.

5. Summary

Oxygen is extremely important to maintain life for the human being; hence one can argue that monitoring of oxygen level in blood during patient care and especially in life threatening conditions carries the same importance. It has been a long time in the medical history until we reached the current techniques to measure arterial oxygen saturation noninvasively and even without calibration which is now named pulse oximetry. Pulse oximetry is one of the medical devices who improved overtime with successive scientific discoveries and developments; staring from the prism, passing through the spectrophotometer until reaching the radiofrequency. The main principle of pulse oximetry is the use of transmitted light with different wave lengths across the living tissue and based on the fact that oxygenated hemoglobin and reduced hemoglobin have different degrees of light absorption at red and infrared spectra at specific wave lengths, the device can determine the concentration of both and display the oxyhemoglobin concentration. Takuo Aoyagi who discovered the pulse oximetry used the changing character of the arterial pulsation during cardiac cycle to enable the device to detect and measure only the arterial oxygen saturation.

Although pulse oximetry is widely used in many clinical situations to monitor oxygen saturation and adjust oxygen therapy, it has its own limitations which can affect its performance. These limitations are of importance because they are frequently encountered during patient care and can include the effect of motion, ambient light, anemia, skin pigmentations, nail polish, low peripheral perfusion, and sever hypoxemia. Neonatal cardiopulmonary resuscitation (CPR) is continuously advancing and frequently encountered practice due to the increase in the newborn number worldwide. The performance of pulse oximetry during CPR is critical because pulse oximetry can face many challenges during CPR including motion, low peripheral perfusion, and hypoxemia. Moreover, CPR is a condition during which rapid decisions are needed, which ideally should be based on precise measurements.

Our objective was to study the accuracy of pulse oximetry measurements of oxygen saturation (SpO_2) in comparison with simultaneous CO-oximetry oxygen saturation measurements (SaO_2) as the gold standard during cardiopulmonary resuscitation in newborn piglets' model of cardiac arrest.

Our study was designed as prospective cohort study. The study included thirty healthy, anesthetized, ventilated newborn piglets with induced cardiac arrest. The animals were anaesthetized and ventilated during the preparation period of the experiment. Then cardiac arrest was induced by using bolus KCl injection. Thereafter, animals were randomized into three groups according to the type of respiratory support and cardiopulmonary resuscitation was started and continued for 20 minutes with continuous monitoring of oxygen saturation by pulse oximetry. Arterial blood was sampled and analyzed by CO-oximetry every two minutes.

Oxygen saturation was continuously monitored by pulse oximetry (SpO_2) before the onset of cardiac arrest and throughout the CPR process with the sensor applied to the proximal tail. SpO_2 showed very few episodes of signal dropout. SpO_2 was analyzed during the time coinciding with oxygen saturation measured by CO-oximetry (SaO_2) every 2 minutes and bias considered whenever the difference (SpO_2 - SaO_2) was $\geq \pm 5\%$. Comparison of SpO_2 and SaO_2 revealed decreased bias and higher precision with SaO_2 above 90% at the baseline measurements with mean bias of 2.4% ±4.58% as compared to $SaO_2 \leq 90\%$. The onset of cardiac arrest was accompanied by marked decrease in SaO_2 which continued across the experiment and a marked increase in bias up to 13 ±34% after 2 minutes and reaching a maximum of 45.58 ±28.31% after 10 minutes over a range of mean SaO_2 between 29.31% and 58.14%. This was accompanied with a decrease in accuracy and precision after the onset of cardiac arrest and throughout the experiment.

There are very few studies evaluating the performance of pulse oximetry during CPR and most of them were adult studies, case reports, or did not control their results with the true arterial oxygen saturation measured by CO-oximetry. We tried to overcome these limitations and have carried out a well-designed study during which we tried also to limit the factors that can affect the performance of pulse oximetry through keeping the conditions optimal for CPR.

In conclusion pulse oximetry may show a wide range of bias and decreased precision in a model of profound hypoxemia and low perfusion after cardiopulmonary collapse and during CPR despite using recent pulse oximetry technique with high sensitivity during low perfusion. We recommend further studies to clarify the exact mechanisms of these false readings. We also recommend that clinician shouldn't rely only on pulse oximetry readings at adverse conditions with low perfusion for FiO_2 adjustment.

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7. Curriculum vitae

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Last Name: Hassan **First name**: Mohammad **Middle name**: Ahmad

Date and Place of Birth: November 20, 1980, Assiut, Egypt.

Gender: Male

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Other Languages: English (TOEFL score: 589), German (B2 level)

Present Employment: Assistant lecturer of Pediatrics, Sohag Faculty of Medicine, Sohag University, Egypt.

(II) DEGREES:

1- M.B.B.Ch.: Sohag Faculty of Medicine, South Valley University, Sohag, Egypt; September 2003, General grade: Excellent with honor

2- M.Sc. (Pediatrics): Sohag Faculty of Medicine, Sohag University, Sohag, Egypt; January 2009, General Grade: Excellent

(III) PRESENT POSITIONS:

1- Assistant lecturer of Pediatrics, Sohag Faculty of Medicine, Sohag University, Egypt, March 2009.

2- Attending neonatologist in the NICU, Sohag Faculty of Medicine Hospital, January 2009

(IV) PREVIOUS POSITIONS:

Resident in the Pediatric Department, Sohag Faculty of Medicine Hospital, (March, 2005 – February, 2008)

(V) TRAINING:

1. House Officer (Rotating internship): Sohag Faculty of Medicine Hospital, Sohag, Egypt (March, 2004 – February, 2005)
2. Residency (Pediatrics): Sohag Faculty of Medicine Hospital, Sohag, Egypt (March, 2005 – February, 2008)

3. Visiting physician and researcher at neonatal and pediatric intensive care unit, Children hospital, Ulm university, Germany (April 2012 – April 2014)
4. Neonatal Resuscitation Instructor Course, Assiut, Egypt (January 20-21, 2008) by the American Academy of Pediatrics.

(VI) RESEARCH AND SCINTIFIC ACTIVITIES:

1. Accuracy of pulse oximetry during neonatal resuscitation in a piglet model of cardiac arrest. **Hassan M**, Mendler M, Huang L, Waitz M, Maurer M, Hummler HD; PAS, 2013

2. Effects of Synchronization during Noninvasive Intermittent Mandatory Ventilation in Preterm Infants with RDS immediately after Extubation. Huang L, Mendler M, Waitz M, Schmid M, **Hassan M**, Hummler H. PAS, GNPI 2013.

3. Respiratory support during cardiopulmonary resuscitation in newborn piglets. Mendler M, Maurer M, **Hassan M**, Huang L, Waitz M, Hummler HD. PAS & GNPI, 2013.

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8. Acknowledgment

I would like to take this opportunity to express my limitless thanks to many people who contributed to the completion of this research work. First and foremost, I would like to express my deepest respect and gratitude to Prof. Dr. Helmut D Hummler who undertook to act as my research supervisor despite his many other academic and professional commitments. He is not only a great professor with deep vision, but a very kind, warm-hearted, and easy-going person as well. In the course of this animal trial, he provided guidance to all the members of the team of the study and he acted also as one of the team in most of the experimental sessions. His generous help and suggestive discussion have been benefiting me so much during this study, and still in my future clinical and research work. My thanks go out to Dr. Marc Robin Mendler and Dr Li Huang who provided me the support for the trial and helped me in discussing important points in this thesis.

It is no doubt that I must thank University of Ulm, especially the Medical Faculty and the facility of animal experiment laboratory, for providing the appropriate place and circumstances to accomplish this study and achieve our goals.

Last but not least, I deeply appreciate the great encouragement and love from my dear wife and lovely daughter because their unconditional support and love gave me the strength and confidence to stay in Germany and finish this thesis.