The effects of declined oxygen levels on hypoxia symptoms and blood gases: An experimental study

To cite this article: A F Sugiharto et al 2018 J. Phys.: Conf. Ser. 1073 042023

View the article online for updates and enhancements.
The effects of declined oxygen levels on hypoxia symptoms and blood gases: An experimental study

A F Sugiharto\textsuperscript{1*}, R A Firdausi\textsuperscript{1}, O Safitry\textsuperscript{1} and Gunanti\textsuperscript{2}

\textsuperscript{1}Department of Forensic Medicine and Medicolegal, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, Indonesia
\textsuperscript{2}Department of Veterinary Clinic, Reproduction and Pathology, Faculty of Veterinary, Institut Pertanian Bogor, Bogor, 16680, Indonesia

*E-mail: selalu_ada_ade@yahoo.com

Abstract. Environmental suffocation is a form of asphyxia. In such cases, an autopsy alone is not sufficient to determine the cause of death, and forensic pathologists need environmental data from the scene and the symptoms of hypoxia from the victim. Considering that the symptoms of hypoxia vary widely, the analysis of gases in the blood is used to assess the level of oxygenation in humans. No research has yet equated a specific level of oxygen in the air with environmental suffocation. This study aimed to determine the correlation among the decreased level of oxygen in the environment, the values of blood gases, and the emergence of the symptoms of hypoxia. Pig animal model (Sus scrofa) was used. This study induced a state of environmental suffocation in the pigs, which were placed in a chamber with oxygen levels decreasing from 21\% to 11\% and then to 7\%. At each oxygen level, the symptoms of hypoxia and the values of arterial blood gases were assessed. It is concluded that the decreased oxygen levels in the chamber resulted in hypoxia symptoms such as changes in the respiratory rate, heart rate, and blood gases, such as the pH, PO\textsubscript{2}, PCO\textsubscript{2}, HCO\textsubscript{3}, BE, and O\textsubscript{2} saturation values. These changes had different percentages that reflect each range of the decreased oxygen levels in the chamber.

1. Introduction
The Center for Disease Control and Prevention (CDC) data from 1999–2004 showed that approximately 20,000 accidental or nonaccidental deaths were associated with asphyxia [1]. Over the past 10 years, the average number of homicides by asphyxia (in addition to that of strangulation) was 107 cases per year, with a relatively consistent number each year [2]. Among the 7,803 homicide cases in the Varanasi region of India, 542 homicide cases by asphyxia were recorded between 2008 and 2011 [3]. These data demonstrated that the most commonly encountered homicide cases worldwide were associated with asphyxiation. For two years, epidemiology in India recorded environmental suffocation (20.5\%) as the leading cause of death by asphyxia and is the second leading cause of the most frequently encountered cases [4]. The recent cases of death in Jakarta, Pulomas, were associated with environmental suffocation [5]. These data showed that the cases of death associated with asphyxia were the most frequently encountered cases worldwide.
The asphyxiation caused by environmental suffocation occurs in the event of a deficiency or inadequacy of oxygen in the environment. Autopsy alone would be insufficient to determine the cause of death in this type of asphyxiation because there would be no specific finding. The nonspecific abnormalities commonly found were the congestion of internal organs. The determination of the cause of death in such cases was performed exclusively [2,6].

According to forensic science, four aspects form the “core of pathology”: (1) cause (etiologies), (2) mechanism (pathogenesis), (3) structural changes that have occurred in the cells and organs of the body (morphologic changes), and (4) functional consequences of the structural changes (clinical significance) [7]. Generally, forensic doctors can determine the causes and mechanisms of death. The analyses of death mechanisms must be based on objective data and analyzed by medical science. The aforementioned analysis correlated all morphological changes on an examined cadaver [6]. The cases of death caused by environmental suffocation need data from the scene environment. Environmental data will then provide information to forensic pathologists about the oxygen concentration that was available. Forensic pathologists require complete data of the crime scene environment and cadaver to accurately determine the correlation between the effect and its cause.

Whether cells adapt, suffer injury, or die depends on the degree of severity of hypoxia. Among the various causes of cell damage and death, hypoxia is the principal cause [7,8]. Currently, the measurement of the oxygenation in the blood requires an examination of blood gases in the laboratory [9].

This study aimed to ascertain whether a significant correlation exists among the examination of blood gases, the emergent symptoms of hypoxia, and the decreased level of oxygen, which causes environmental suffocation. It is hypothesized that the decreased level of oxygen in the chamber, which potentially causes environmental suffocation, had a significant correlation with the emergent symptoms of hypoxia and the values determined at the examination of blood gases.

2. Methods

This experimental study was performed on pigs by using a comparative design. The Sus scrofa breed of pig from the Faculty of Veterinary Medicine of Institut Pertanian Bogor was used as animal study model. This S. scrofa pig breed is a suitable and well-matched model for investigating human respiratory and circulatory mechanisms. As detailed by Swindle, the organ system of pigs has a 90% similarity to that of humans not only anatomically but also functionally. In this study, castrated male pigs aged two–three months and weighing 15–24 kg were used [10]. The normal heart rate frequency of pigs is between 55 and 80 beats per minute, and their normal respiratory frequency is between 32 and 58 respirations per minute [10,11].

This study used the used the “rule of thumb” sample calculation method from Roscoe (1975). The examiner determined that five pigs will be used for this study by bearing in mind that pigs are categorized as big animals. From these five pigs, the examiner took two samples from each pig at two different periods. Between the two sample taking periods, the pigs were “washed out” to recover and return them to normal [12].

During the recovery process (wash out), the pigs were placed in a cage in a similar manner to that used in the adaptation process. Before being placed into the cage, the pigs were administered intramuscularly with antibiotics (long acting oxytetracycline) at a 1 ml/10 kg body weight dosage and an analgesic (Flunixin meglumin) at a 1.1–2.2 mg/kg body weight dosage. The recovery process took 7–10 days.

This study was conducted at the Experimental Laboratory of the Surgery Division, the Radiology Department of the Reproduction Clinic, and the Pathology of Veterinary Medicine of the Institut Pertanian Bogor. Pig conservation was situated in the Laboratory of Animal Cages (HELAB), Veterinary Medicine, Institut Pertanian Bogor.

Pigs that included all met the following criteria: male pigs (S. scrofa) aged two–three months with weights of 15–24 kg, breathing frequencies between 32–48 breaths per minute, heart frequency of 55–80 beats per minute, normal behavior and activity, no anatomical abnormalities, no apparent
active movement, no hair loss or baldness, good health (according to a veterinarian), and successful adaptation and isolation for 7–10 days. The exclusion criteria were: lack of activity, abnormal exudate from the eyes, mouth, anus, and genitals, weight loss of more than 10% after the adaptation phase in the laboratory, dirty hair, hair loss or baldness, and death during the adaptation process.

The tools that were used in this study are as follows: a laboratory vacuum chamber (modification), 120 cm × 70 × 70 cm in size (833 liters), a portable oxygen analyzer (gas detector) (specifications: Winsen censor (ME2-O2 electrochemical oxygen sensor), ESP8266 NodeMCU V3 WIFI Module, and power supply 2A), a portable AGD analyzer, Abbott and Catridge type G3 + oxygen hose + extension tube, a gas tube filled with nitrogen, and a gas pressure gage for the tube (regulator). The minor surgery instruments and anesthetic instruments used for the animals are as follows: an IV catheter, a three way stop cock, an extension tube, an intravenous catheter, a round blunt needle, a silk and polypropilene suture, a digital thermometer, a hemodynamic monitoring device (PiCCO; PULSION), syringes (1, 3, 5, and 10 cc), a vacutainer, a video camera, and an animal scale. The materials that were used in this study were pig food, blood from pig arteries, ketamine HCL, xylazine HCL, EDTA/heparin, antibiotics, analgesics, and NaCl.

The study began with a seven-day maintenance and adaptation regime of the animal models in a laboratory cage. The animal models were then sedated intramuscularly with ketamine–xylazine. Thereafter, their body weights were recorded, including the results of their physical examination, heart rate frequency, breathing frequency, rectal temperature, and animal behavior. An intravenous access was made into the vein of the ear area (vena auricularis) and intraarterial access into the femoralis arteries and used an oximetry pulse device. The measurements included: pulse and breathing frequency of the pigs, the values of their blood gases; also the oxygen level in the environment (chamber). Thereafter, the pigs and the oximeter were placed into the chamber. The oxygen level in the chamber was then measured again (before closing the chamber) and infused with nitrogen into the chamber slowly (5–10 liters/minute). The oxygen level changes in the chamber were recorded with an oxygen analyzer, the pulse and peripheral O\textsubscript{2} saturation changes were recorded with pulse oximetry, the breathing frequency changes was recorded visually, and the behavior and physical changes were evaluated. When the oxygen level in the chamber reached 11%, the nitrogen gas was stopped, then the oxygen level was maintained for 10 minutes, and artery blood samples were obtained via the intraarterial access at the pig femoralis artery. The measurements were then being carried out again. The nitrogen gas was then infused back into the chamber slowly (5–10 liters/minute). The measurements were then being carried out again. When the oxygen level in the chamber reached 7%, the nitrogen gas was stopped, the oxygen level was maintained for 10 minutes, and artery blood samples were obtained via the intraarterial access at the pig femoralis artery. The measurements were then being carried out again. The pigs were taken out of the chamber, being administered with antibiotics and analgesics, and being allowed to recover before taking them back into their cages.

There are two safe and suitable sedation methods for pigs. First, sedating them with the combination of an initial dose of ketamine HCl (20 mg/kg body weight) and xylazine HCL (2 mg/kg body weight), which were administered intramuscularly. The sedation was maintained with the intravenous administration of a maintenance dose comprising a combination of ketamine HCl (10 mg/kg body weight) and xylazine HCL (1 mg/kg body weight). The maintenance dosage was repeated every 30–60 minutes. During sedation, monitoring devices were installed in the animals to follow their pulse frequency and oxygen saturation while observing their respiration visually [13]. The blood samples were collected via the femoralis artery by using the artery catheter installed after sedation and before treatment. 5–10 ml blood was taken before treatment when the oxygen level of the environment reached 11% and when it reached 7%. Therefore, in one session of pig treatment, the blood samples were obtained three times.

The treated pigs were then allowed to recover (washing out). The pigs took approximately one week to recover and were administered with antibiotics and analgesics. For assessing the recovery of the pigs, the following parameters were used: normal activities, body weight increase, no signs of
infection, normal vital signs of pigs, and healed (dried) wounds. Before the pigs were used again for treatment, they were reevaluated to determine if the treatment required an inclusive criterion (Table 1).

Table 1. Initial, postadaptation, and post-wash out pig weights.

|       | Initial weight | Postadaptation weight | Post-wash out weight |
|-------|----------------|-----------------------|----------------------|
| Pig 1 | 21 kg          | 23 kg                 | 24 kg                |
| Pig 2 | 15 kg          | 16 kg                 | 18 kg                |
| Pig 3 | 15 kg          | 16 kg                 | 17 kg                |
| Pig 4 | 15 kg          | 16 kg                 | 17 kg                |
| Pig 5 | 15 kg          | 16 kg                 | 17 kg                |

The primary data such as the blood gas examination values and the records of hypoxia symptoms were obtained, processed with SPSS 11.5 and tested for normality of distribution. Normally distributed data were tested for correlation of variables with the repeated ANOVA test and the pairwise comparison test. Abnormally distributed data were tested for the correlation of variables with the Friedman test and the Wilcoxon signed rank test. The processed data were then presented in an SPSS 11.5.

This study passed the ethical evaluation of the Health Research Ethics Committee of Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital (No. 591/UN2.F1/ETIK/2017) and the Research Ethical Commission, Faculty of Veterinary Medicine, Institut Pertanian Bogor (No. 070/KEH/SKE/IX/2017).

3. Results

Table 2 showed comparison of the characteristic values of treated pigs based on chamber oxygen levels of 21%, 11%, and 7%. Percentage pattern of study parameter value changes on treated pigs based on the chamber oxygen level can be seen in Figure 1. Table 3 and 4 showed correlation between breathing frequency, PO2 level, and BE average of the treated pig artery blood at each chamber oxygen level and at each oxygen level change. Table 5, 6 and 7 showed correlation between pulse frequency, SO2, PCO2, HCO3, O2 saturation of the artery blood and the pH of treated pigs at each chamber oxygen level and each oxygen level change.

Table 2. Comparison of the characteristic values of treated pigs based on chamber oxygen levels of 21%, 11%, and 7%.

| Variable                                      | O2 level | 21%     | 11%     | 7%     |
|----------------------------------------------|----------|---------|---------|--------|
| initial weight [kg; average ± SB]            | 17.9 (15-24) | 78 (50-80) | 94.3 ± 10.3 | 104.4 ± 10.5 |
| pulse frequency [times per minute; average ± SB; median (min–max)] | | 49.3 ± 6.9 | 87.6 ± 14.9 | 51.6 ± 16.7 |
| breathing frequency [times per min; average ± SB] | | 92.1 ± 1.9 | 70.9 ± 7.2 | 49.5 ± (48-63) |
| oxygen saturation on pulse oximetry [%; average ± SB; median (min–max)] | | | | |
Table 2. Continue  

| Variable | O₂ level | 21% | 11% | 7% |
|----------|----------|-----|-----|----|
| PCO₂ [mmHg; [%; average ± SB; median (min–max)] | 45 ± 3.7 | 45.4 (41.4-48.3) | 41.9 ± 2.6 |
| PO₂ [mmHg; [%; average ± SB] | 86.8 ± 8.7 | 37.3 ± 5.9 | 20.7 ± 2.3 |
| HCO₃ [mmHg; [%; average ± SB; median (min–max)] | 31.3 ± 2.6 | 31.4 ± 2.4 | 27.4 (24.9-28) |
| O₂ saturation [%; average ± SB; median (min–max)] | 97 (94-98) | 74 (60-81) | 36.2 ± 7.2 |
| BE [mmol/L; average ± SB] | 8.6 ± 3.2 | 5.8 ± 1.9 | 2.9 ±1.1 |
| pH [average ± SB; median (min–max)] | 7.45 ± 0.2 | 7.44 (7.42–7.48) | 7.44 (7.39–7.45) |

Figure 1 Percentage pattern of study parameter value changes on treated pigs based on the chamber oxygen level.

Table 3. The correlation between breathing frequency, PO₂ level, and BE average of the treated pig artery blood at each chamber oxygen level.

| Oxygen level | N  | Breathing freq (times/minute) | P value | PO₂ (mmHg) | P value | BE (mmol/L) | P value |
|--------------|----|-------------------------------|---------|------------|---------|-------------|---------|
| 21%          | 10 | 49.3 ± 6.9                    | < 0.001*| 86.8 ± 8.7 | < 0.001 | 8.6 ± 3.2   | 0.001*  |
| 11%          | 10 | 7.6 ± 14.9                    |         | 37.3 ± 5.9 |         | 5.8 ± 1.9   |         |
| 7%           | 10 | 1.6 ± 16.7                    |         | 20.7 ± 2.3 |         | 2.9 ± 1.1   |         |

*Repeated ANOVA test ; *p<0.05
### Table 4. The correlation between breathing frequency, PO$_2$ level, and BE average of the treated pig artery blood at each oxygen level change.

| Oxygen level change | N  | Average difference | 95% IC        | P value |
|---------------------|----|---------------------|----------------|---------|
|                      |    |                     | min           | max     |         |
| Breathing frequency  |    |                     |               |         |
| 21% → 11%           | 10 | -38.3               | -50.4 -26.2   | < 0.001*|
| 21% → 7%            | 10 | -2.3                | -15.8 11.2    | 0.709   |
| 11% → 7%            | 10 | 36                  | 28 44        | < 0.001*|
| PO$_2$              |    |                     |               |         |
| 21% → 11%           | 10 | 49.5                | 41.5 57.5     | < 0.001*|
| 21% → 7%            | 10 | 66.1                | 60 72.2      | < 0.001*|
| 11% → 7%            | 10 | 16.6                | 12.9 20.3    | < 0.001*|
| BE                  |    |                     |               |         |
| 21% → 11%           | 10 | 2.8                 | 1.4 4.2     | 0.002   |
| 21% → 7%            | 10 | 5.7                 | 3.7 7.7     | < 0.001*|
| 11% → 7%            | 10 | 2.9                 | 1.9 3.9     | < 0.001*|

*Pairwise comparison test ; *p<0.05

### Table 5. Correlation between pulse frequency, SO$_2$, and PCO$_2$ of the artery blood treated pigs at each chamber oxygen level.

| oxygen level | N  | Pulse frequency (times/minute) | P value | SaO$_2$ (%) | P value | PCO$_2$ (mmHg) | P value |
|--------------|----|-------------------------------|---------|-------------|---------|----------------|---------|
| 21%          | 10 | 78 (50-80)                    | < 0.001*| 92          | < 0.001 | 44 (40.7-49.7) | 0.13    |
| 11%          | 10 | 97 (76-112)                   |         | 71.5        | (55-80) | 44.5           | (41.4-48.3)|
| 7%           | 10 | 103.5 (83-123)                |         | 49.5        | (48-63) | 41.6           | (36.2-45.3)|

*Friedman test

### Table 6. Correlation between HCO$_3^-$, O$_2$ saturation of the artery blood, and the pH of treated pigs at each chamber oxygen level

| oxygen level | HCO$_3^-$ (mmol/L) | P value | O$_2$ Saturation (%) | P value | pH     | P value |
|--------------|---------------------|---------|----------------------|---------|--------|---------|
| 21%          | 31.4 (28.3-36.1)    | 0.001*  | 97 (94-98)           | <       | 7.45   | 0.020*  |
| 11%          | 31.2 (27.2-36.3)    |         | 74 (60-81)           | 0.001   | 7.44   | (7.42-7.48)|
| 7%           | 27.4 (24.9-28)      |         | 36 (27-48)          |         | 7.44   | (7.39-7.45)|

*Friedman test ; *p<0.05
Table 7. Correlation between pulse frequency, \( \text{SO}_2 \), \( \text{PCO}_2 \), \( \text{HCO}_3 \), \( \text{O}_2 \) saturation of the artery blood and the pH of treated pigs at each oxygen level change.

| Oxygen level change | N | P value |
|---------------------|---|---------|
| Pulse frequency     |   |         |
| 21\% → 11\%        | 10| 0.005*  |
| 21\% → 7\%         | 10| 0.005*  |
| 11\% → 7\%         | 10| 0.005*  |
| SaO2 pulse oximetry |   |         |
| 21\% → 11\%        | 10| 0.005*  |
| 21\% → 7\%         | 10| 0.005*  |
| 11\% → 7\%         | 10| 0.005*  |
| HCO3                |   |         |
| 21\% → 11\%        | 10| 0.878   |
| 21\% → 7\%         | 10| 0.005*  |
| 11\% → 7\%         | 10| 0.005*  |
| \( \text{O}_2 \) saturation |   |         |
| 21\% → 11\%        | 10| 0.005*  |
| 21\% → 7\%         | 10| 0.005*  |
| 11\% → 7\%         | 10| 0.005*  |
| pH                  |   |         |
| 21\% → 11\%        | 10| 0.575   |
| 21\% → 7\%         | 10| 0.017*  |
| 11\% → 7\%         | 10| 0.028*  |

*Wilcoxon signed rank test ; *p<0.05

4. Discussion
After the oxygen level in the chamber had been decreased, the emerging hypoxia symptoms in the pigs were a change in skin color from pink to a purplish color, a darkening of blood color, a change in the saturation of oxygen value on pulse oximetry, and changes in pulse and breathing frequencies. The theoretical interpretation of a change in skin color to purple is that of cyanosis symptoms. A darkened blood color is a sign of low blood oxygen. Blood color depends on the hemoglobin count in the red blood cells and oxyhemoglobin. The color of blood darkens when it lacks oxygen [8,14].

With the decreasing level of oxygen in the chamber from 21\% to 11\%, breathing frequency increased significantly from an average of 49.3 breaths per minute to 87.6 breaths per minute (Table 2). The normal breathing frequency of pigs aged 2–3 months is 32–48 breaths per minute.[10] According to the distributive data, the increase in breathing frequency was as high as 78\%. An adult human has a normal breathing frequency of 16–24 breaths per minute. Breathing less than 16 times per minute is called bradipnea, and breathing more than 24 times per minute is called takipnea [14]. An increase of 78\% occurred on the basis of a breathing frequency of 20 breaths per minute. A breathing frequency of 36 breaths per minute is called takipnea. Theoretically, a deficit in the body of oxygen in the blood evokes a respiratory system response of increased breathing frequency. The peripheral chemoreceptors respond by sending afferent impulses to the medulla inspiratory neuron to increase ventilation. The increase in breathing frequency is regulated by the brain respiratory system, which is located in the medulla oblongata [8-14].

When the oxygen level decreased from 11\% to 7\%, the breathing frequency decreased significantly from an average of 87.6–51.6 breaths per minute (see Figure 2). On the basis of the distributive data, the breathing frequency decreased by 41\%. An adult human has a normal breathing frequency of 16–24 breaths per minute. A 41\% decrease on a breathing frequency of 20 breaths per minute indicates that the breathing frequency will decrease to 8 breaths per minute. The changes in the breathing frequency pattern with changes in the oxygen level in the chamber from 21\% to 11\% to
7% revealed that the pattern in the respiratory system responded accordingly. The respiratory response pattern was a frequency increase until the oxygen level in the chamber reached 11% and then a breathing frequency decrease down to 7%. Such a change in the breathing frequency pattern would evoke a physiological response [14]. According to the literature, the respiratory system will fail when the respiratory center cannot compensate for the lack of oxygen in the blood [14,15]. When the respiratory system is not able to supply enough oxygen and remove enough carbon dioxide, respiratory failure ensues. Such an event will trigger hypoxemia, hypercapnia, or a combination of both. Respiratory failure is divided into two types: type I and type II [16].

![Breathing Frequency Change](image)

**Figure 2.** Pattern of breathing frequency change in treated pigs in the chamber with oxygen levels of 21%, 11%, and 7%.

In our study, changes in the pattern of breathing frequency combined with changes in the values of artery blood gases suggest environmental suffocation with respiratory failure type I, which is characterized by a low PO$_2$ level and a normal or low PCO$_2$ level. Respiratory failure begins with a compensation stage accompanied by increasing respiration. Decompensation then occurs with a decrease in respiration activity. The analysis of blood gases is one of the examinations used to determine a diagnosis of respiratory failure [17]. Hence, the analysis of blood gases results could be used in cases of environmental suffocation.

Pulse frequency changes, which are one the signs of hypoxia, also occurred when the chamber oxygen level decreased. Pulse frequency increased significantly with every decrease in oxygen level. The decreasing level of oxygen in the chamber from 21% to 7% triggered an increase in pulse frequency by 41% (see Figure 3). The normal pulse frequency in humans is 60–100 pulses per minute. Pulse rates of less than 60 pulses per minute and more than 100 pulses per minute are defined as bradycardia and tachycardia, respectively [14]. A 41% increase on a pulse frequency of 80 pulses per minute would mean that the pulse frequency would reach 113 pulses per minute, that is, tachycardia.

The observed increase in the pulse frequency was a response of the cardiovascular system toward a lack of oxygen in the blood [12]. The cardiovascular response may be divided into two phases. The first phase or acute phase is a vasoconstriction mechanism that increases the resistance of the blood vessels. The second phase occurs if the hypoxia condition persists longer (at least one week); this condition will trigger the remodeling of the blood vessels [11]. In this study, the observed hypoxia was of the first phase or acute phase.

The correlation between the decrease in the chamber oxygen level and the changes in the values of the blood gases could be explained simply.
There was a significant correlation between the decrease in oxygen level in the chamber from 21% to 7% and the changes in the values of blood gases for every parameter (pH, PO\textsubscript{2}, PCO\textsubscript{2}, HCO\textsubscript{3}, BE, and O\textsubscript{2} saturation in the blood artery). This event showed that the step-by-step decrease in the oxygen level had a direct effect on the changing values of blood gas examination. Theoretically, this direct effect would manifest symptoms such as an acute physiological response, which could present as emergent hypoxia symptoms (e.g., the cardiovascular response of an increase in pulse frequency), and a respiratory response (e.g., an increase in breathing frequency and a decrease in peripheral oxygen saturation) [12-14].

The changes in blood gases with decreasing oxygen level in the chamber from 21% to 7% were a pH decrease of 0.13%, a PO\textsubscript{2} decrease of 76%, a PCO\textsubscript{2} decrease of 7%, an HCO\textsubscript{3} decrease of 13%, a BE decrease of 66%, and a decrease in O\textsubscript{2} saturation in the artery blood of 62%. The changes in blood gases in pigs treated with environmental suffocation followed a certain pattern: PO\textsubscript{2} decreased by 76% to an average of 20.7 mmHg at a chamber oxygen level of 7%, and O\textsubscript{2} saturation in the artery blood decreased by 62% to an average of 36.2% at a chamber oxygen level of 7%. The correlation between PO\textsubscript{2} and SaO\textsubscript{2} is shown in Figure 4 below.

The SaO\textsubscript{2} value in the analysis of blood gases shows the percentage of dissolved oxygen in the artery blood hemoglobin. According to Larkin and Zimmanck, the oxygen saturation value in hemoglobin is directly related to the PO\textsubscript{2} value. A PO\textsubscript{2} value of <60% was a critical value that described the condition of hypoxia (see Figure 5) [18]. The range of normal values of SaO\textsubscript{2} is between 80% and 100%. SaO\textsubscript{2} values of <80% could prevent the oxygen from penetrating the tissue, particularly for organs requiring high oxygen consumption, such as the brain, liver, and kidney. Hypoxemia is the condition in which there is a lack of SaO\textsubscript{2} and PO\textsubscript{2} and is classified as mild, medium, or severe [19].

As shown in Figure 7, the PO\textsubscript{2} and SaO\textsubscript{2} curves had parabolic shapes. However, the parabolic shape of the PO\textsubscript{2} curve was more pronounced than that of the SaO\textsubscript{2}. These two are related; however, at a lower value of PO\textsubscript{2}, the PO\textsubscript{2} and SaO\textsubscript{2} curves did not have the same degree of change when the SaO\textsubscript{2} value decreased. The presence of oxygen dissolved in the hemoglobin caused a delay or time lag in the lowering of the SaO\textsubscript{2} value in the blood. Theoretically, oxygen (gas) movement into the hemoglobin uses a difference of the principal gradient pressure [20]. The difference of gradient pressure in our study triggered the time lag. The shifting pH value of the artery blood in this study was also related to changes in the PO\textsubscript{2} and SaO\textsubscript{2} values. The correlation between the three could be interpreted as the oxyhemoglobin dissociation curve. Considering that pH influences the ability of red blood cells to transport O\textsubscript{2} to all tissues and remove CO\textsubscript{2} from all tissues, the pH value plays a major role in determining the degree of oxygen saturation in the red blood cell [20].

![Figure 3. Pulse frequency change in pigs treated in the chamber with oxygen levels of 21%, 11%, and 7%](image-url)
**Figure 4.** Correlation between PO$_2$ and SaO$_2$ from pigs’ artery blood exposed to environmental suffocation.

**Figure 5.** Oxyhemoglobin dissociation curve (Frankel LR, 2007) [18].

If the shifting values of the pH, PO$_2$, and SaO2 were read by the oxyhemoglobin dissociation curve, the value changes would show signs of hypoxia.
During this study, we did not observe the compensating mechanism described in the literature, which could be seen from the changing values of the blood gases. This mechanism could have been proved by the absence of pH and HCO₃ changes, which led to metabolic compensation. On the basis of literature, the main organ regulating the buffer of H+ and HCO₃ is the kidney, which does so via urine excretion; metabolic compensation appears after three days to five days [21].

We found in our study results that the oxygen saturation values from the pulse oximetry differed from those in the artery blood (from the blood gases examination). The oxygen saturation on pulse oximetry decreased by 44% when the chamber oxygen level decreased from 21% to 7%. If human oxygen saturation in a normal chamber was assumed to be 100%, the oxygen saturation in a chamber with an oxygen level of 7% would be 56%. The peripheral oxygen saturation of 56% is categorized as severe hypoxia [22]. On the other hand, the oxygen saturation measured from the blood gas examination decreased by 62%. If human oxygen saturation in a normal chamber was assumed to be 100%, the oxygen saturation in a chamber with an oxygen level of 7% would be 38%. The peripheral oxygen saturation of 38% is categorized as extreme hypoxia [22]. Human body tissues have various time windows of tolerance to hypoxia: brain tissues can tolerate less than 3 minutes, the liver and kidney can tolerate between 15 and 20 minutes, and skeletal muscles can tolerate between 60 and 90 minutes. In the case of patients with acute respiratory distress syndrome, the oxygenation level should be maintained at 85% of oxygen saturation, and the patient should be kept in the intensive care unit [23]. For humans with an oxygen saturation level of 38%, if they are not immediately given intensive care, their hypoxic condition can cause the death of vital organs such as the brain, liver, and kidneys.

In our study, the decreasing oxygen level in the chamber, which mimicked environmental suffocation, was proved to cause hypoxia and artery blood gas changes and showed the shifting pattern explained above. These changes were statistically significant. The shifting pattern identified here could be used by forensic pathologists in solving death cases caused by environmental suffocation. Environmental suffocation cannot be determined as the cause of death by autopsy only, and forensic pathologists need additional data from the crime scene.

In this study, the volume of the chamber that was used and the decrease in oxygen level were measured; the chamber was 588 liters in volume, and the duration of the decrease in the oxygen level was 45 minutes. Therefore, in solving the death cases caused by suspected environmental suffocation, data from the crime scene, such as the volume of the chamber or the room, are important in assisting the forensic pathologist. If the volume of the room were measured, the pathologist could determine how long the victim had been exposed to the lack of oxygen in the room. According to the kinetic theory of gases, Boyle’s law and Charles’ law state that the gas volume is linearly proportional to the room volume and pressure. A greater room volume leads to a greater oxygen level.

5. Conclusion
The decreasing oxygen level in the chamber had a significant correlation with the changing values of the blood gases. These changes occurred for pH, PO₂, PCO₂, HCO₃, BE, and O₂ saturation in the artery blood. The percentages of the changes differed over the range of decreasing oxygen levels in the chamber. The decreasing level of oxygen in the chamber was significantly correlated with the emergent signs and symptoms of hypoxia. The pulse and breathing frequencies and peripheral oxygen saturation changed significantly and were measured on the basis of percentages. The change in pulse frequency showed an increasing pattern throughout the range of decreasing oxygen levels in the chamber. The decrease in breathing frequency was in agreement with respiratory failure theory.

References
[1] Center for Disease Control and Prevention (CDC). Multiple Cause of Death File 1999-2004, series 20, No. 2J. 2007. Diambil dari http://wonder.cdc.gov/mcd.html pada 3 Maret 2017.
[2] DiMaio V J, DiMaio D 1941 Forensic Pathology. Boca Raton: C R C Press; 244-245.
[3] Chaurasia N, Pandey S K and Mishra A 2012 An Epidemiological Study of Violent Asphyxial Death in Varanasi Region (India) a Killing Tool J. Forensic. Res. 3 174
[4] Maddileti G B, Mohanty S K, Kumar V, Reddy K B, Bhuvan V and Yamini K 2015 An epidemiological study of suffocation deaths In twin Cities of South India J. Indian Forensic Med.37 232–6
[5] Sugiharto A F 2017 Laporan Analisa Medikolegal. Departemen Ilmu Kedokteran Forensik dan Medikolegal FKUI-RSCM: Jakarta.
[6] Sawaguchi A, Sawaguchi T. Asphyxia -The Physiopathology-. Tokyo: Koyo Printing; 41–2
[7] Kumar V, Cotran R S and Robbins A L 2003 Basic Pathology. Philadelphia: W B Saunders Company 2–3
[8] Knight B 2006 Forensic Pathology. (New York: Oxford University Press) p 357
[9] Danckers M. 2016 Arterial Blood Gas Sampling. Medscape Journal
[10] Smith A C and Swindle M M 2006 Preparation of Swine for the Laboratory Ilar J. 47 358–63
[11] Swindle, et al. 2012 Swine as models in biomedical research and toxicology testing. Veterinary Pathology 49 2 ;https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/ilarjournal pada 20 April 2017.
[12] Roscoe J T. Fundamental Research Statistic for The Behavior Sciences (2nd, ed). New York: Holt, Rinehart and Winston; 1975.
[13] Gunanti. 2008 Pemanfaatan Hewan Babi Dalam Teknik Operasi Colesistektomi Dengan Metode Endo-Laparoskopik Sebagai Model Untuk Manusia. Fakultas Kedokteran Hewan Institut Pertanian Bogor. Proceedings KIVNAS
[14] Sherwood L. Fisiologi Manusia (Introduction to Human Physiology). Jakarta: EGC p 523-527.
[15] Liere E J V. Hypoxia. Toronto: The university of chicago press. 1993; 276-277.
[16] Nitu ME, Elger H. Respiratory failure. Ped Rev. 2009;30.
[17] Frankel L R. Respiratory distress and failure. Kliegman R, Behrman R and Jenson H. Nelson textbook of pediatrics. 18th ed. Philadelphia: Saunders Elsevier. 2007:59-62.
[18] Larkin B and Zimmanck R 2017 Interpreting Arterial Blood Gases Succesfully AORN journal. 102 343–57
[19] Davis J W, Shackford S R, Mackersie RC and Hoyt DB 1988 Base Deficit as A Guide to Volume Resuscitation J. Trauma. 28 1464–7
[20] Larkin B G and Zimmanck R J 2015 Interpreting Arterial Blood Gases Succesfully AORN J. 102 343–57.
[21] Hall J E 2015 Acid-base regulation. Pocket Companion to Guyton and Hall Textbook of Medical Physiology Philadelphia. Elsevier Health Sciences
[22] Woerleen G M. Anesthesia and Hypoxia. 2005. Diambil dari http://www.anesthesiaweb.org/hypoxia.php pada 3 Maret 2017.
[23] Leach RM and Treacher DF 1998 ABC of Oxygen : Oxygen Transport-2. Tissue Hypoxia BMJ. 317 1370