

## **CHAPTER 3 MATERIALS AND METHODS**

### **3.1 Study design**

This is a single-blind trial with two oxygenators, divided into two equal group size each oxygenators. Patients were approached at the National Heart Institute – Operation Theatre between January 2009 and December 2010. The National Heart Institute is one of the cardiac centres serving a population of approximately twenty eight million (World Bank, World Development Indicators). The National Heart Institute performs approximately 2153 open heart surgery: with 760 congenital heart surgery in 2009. In the study period of January 2009 till December 2010, 2563 open heart surgery was performed, with 896 being congenital. Four paediatric surgeons and 17 perfusionists with the help from anaesthetics participated in this study. This study was approved by National Heart Institute’s internal Ethics Committee [IJNEC/04/2010 (5)]. Refer to IJNEC ethical approval letter in Appendix D.

### **3.2 Patients**

#### **3.2.1 Eligibility**

Eligibility of ages for study is up to 3 year. Patient weight less than or equals to 5 kg and undergoing elective cases are included. Both genders are accepted and no healthy volunteers were included in the study.

#### **3.2.2 Inclusion criteria**

Patients undergoing elective cardiac surgery scheduled as a first time procedure. Consent was obtained from parents or legal guardian on behalf of the child to participate in the study; after have understanding the information sheets (see Appendix B), these

were prepared in English and Bahasa Malaysia. Consent forms were also prepared in both languages (see Appendix C) for parents or legal guardian approval. Bypass time under three hour had been selected as mean point to avoid the time interval during discontinuation of bypass where multiple variables inevitably affect data.

### 3.2.3 Exclusion criteria

Patients undergoing any emergent procedure/surgery are excluded from the study. Patient who are greater than 5 kg of weight are disqualified from this study. Patients with hemoglobinopathies such as sickle cell anaemia and thalassemia, *jaundice* or ECMO usage during surgery, Jehovah's Witnesses case and visible abdominal oedema are excluded from this study. Patients who in the opinion of the investigators should not be included in the study are excluded as well.

## 3.3 Procedure

### 3.3.1 Sample size

A total of 120 patients were studied by separating them into two large groups according to oxygenators; Capiiox<sup>®</sup> RX05 (Terumo Cardiovascular System Co., Tokyo, Japan) or Baby RX<sup>™</sup> (BRX), with a nonheparin biocompatible polymer coating ('X-coating<sup>®</sup>') and Medtronic<sup>®</sup> Minimax Plus<sup>®</sup> Hollow Fiber Oxygenator (Medtronic Inc., Minneapolis, MN, USA) which consist of 60 patients for each oxygenator. Both types of membrane oxygenators were divided into treatment groups of carbogen (5% CO<sub>2</sub>) and control groups (without carbogen) consist of 30 patients each (Figure 3.1). Both treatment and control groups patients were undergone pH-stat management (temperature corrected) throughout the study. In several studies, comparison between pulsatile and nonpulsatile (continues) flow has shown no significant difference in morbidity on patient outcome, so this study employs nonpulsatile flow for patient.

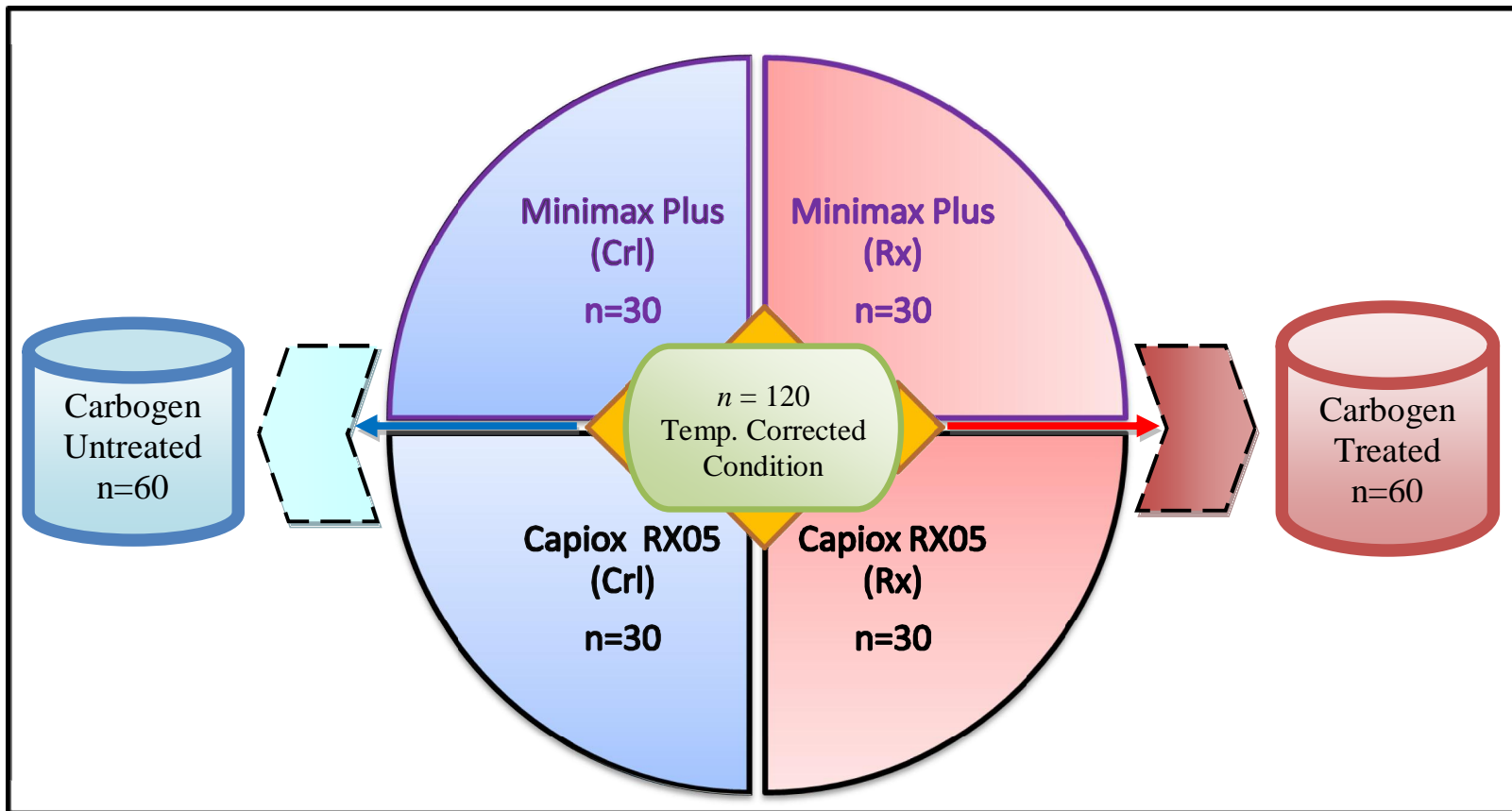


Figure 3.1: Illustration of study design according to oxygenators which further divided to control and treatment. Abbreviation: CrI, Control; Rx, Treatment.

### 3.3.2 Operative measurements

Preoperative data were collected as following: age (days) and weight (kg) at operation, type of oxygenator, preoperative haematocrits and acid-base data after induction of anaesthesia. The following intra- and postoperative data were collected: type of operation performed; haematocrits (%), temperature (°C), PaCO<sub>2</sub> (mm/Hg), peCPBCO<sub>2</sub>, pH, carbogen 5% CO<sub>2</sub> usage (L/min), pump flow (L/min), duration of extracorporeal circulation (min), cross-clamp time (min), bicarbonate (mmol/kg), hemofiltration (ml), bypass urine (ml) and packed-cell blood (ml/kg) administered while priming and during surgery. Refer to data collection form in Appendix A.

### 3.3.3 Anatomy of Design

The cardiopulmonary bypass (CPB) circuit consists of a Terumo Capiox RX 05 oxygenator and Medtronic® Minimax Plus® Hollow Fiber Oxygenator with hardshell reservoir, ¼" roller pump arterial raceway with Continues Flow Controller (Stöckert S-V or Stöckert S-III; Stöckert Instrumente, Munich, Germany), CDI 500 in-line blood gas analyser (Terumo Cardiovascular Systems) were used for carbogen treated group and control, Dideco arterial filter D736 (Sorin Group, Italia) with ¼ x ¼" bypass-loop and a 4:1 blood cardioplegia (Sorin Group, Italia). These circuit components and techniques were consistent between the two large groups. The source is 10L aluminium cylinder 5% CO<sub>2</sub> with oxygen balance from a tank (120 bars), connected to the ¼" side of a ¼ x ¼ " male luer lock perfusion adaptor. The male luer end is then connected to the female port of a ¼ x ¼ " luer lock connector. Same model of capnography was used throughout the study (Datex Normocap CO<sub>2</sub> Monitor, Datex Instrumentarium Oy, Helsinki, Finland). The capnography sampling line was connected via a vented

connector to the scavenging port of the oxygenator to monitor mean expired CO<sub>2</sub> discarded from scavenging line from oxygenator. Care was taken to eliminate any leaks of exhaust gas before the sampling point and to prevent air entrainment. Refer to Figure 3.2 schematic layout of the experimental design.

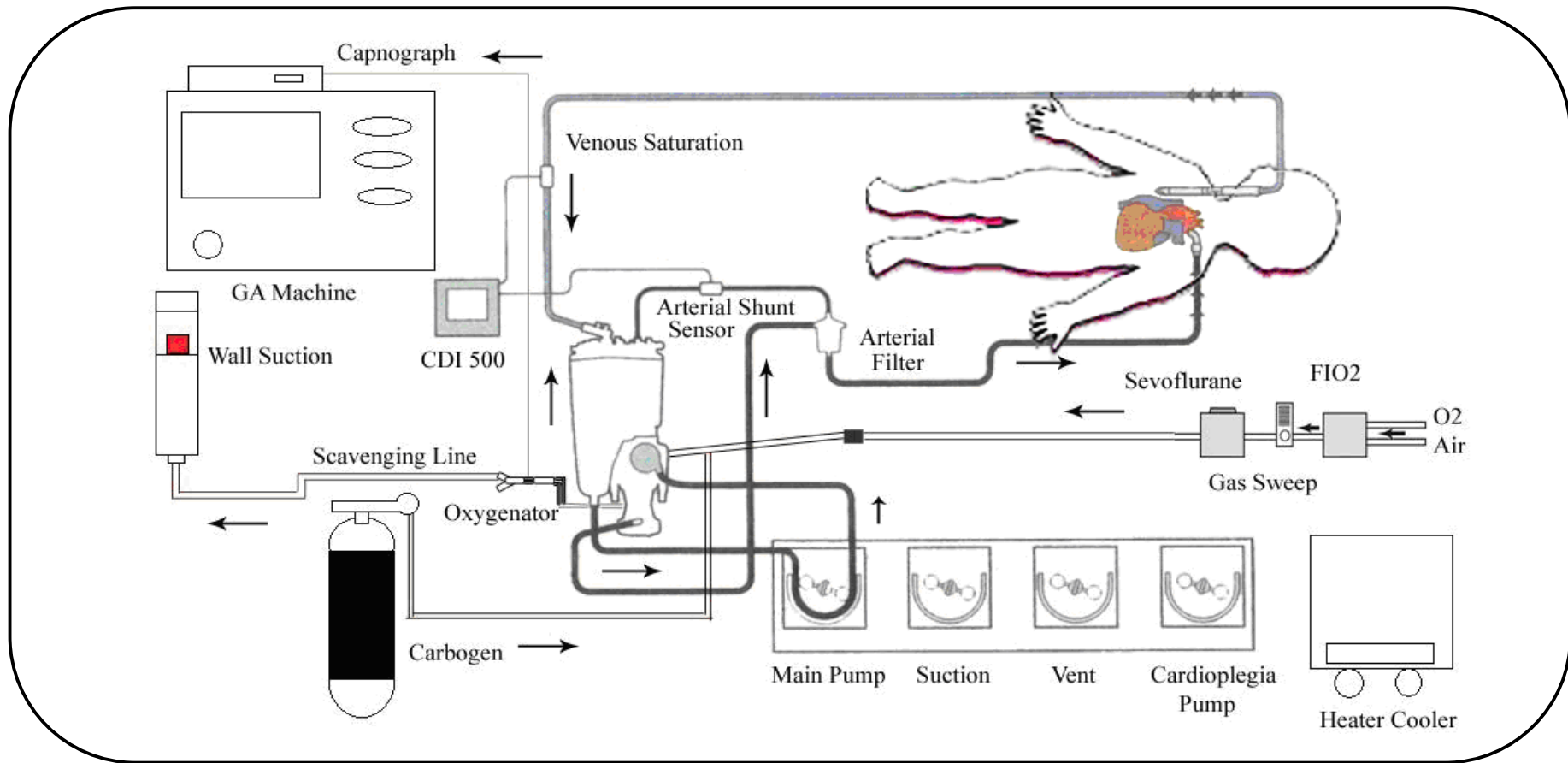


Figure 3.2: Schematic diagram of cardiopulmonary bypass circuit with carbogen set-up to regulate the pH-stat management. Desaturated blood drains from the right atrium to venous reservoir *via* the venous line and is oxygenated. Carbogen were also introduced whereby passive transfer of gas exchange takes place. Oxygenated blood then travels through the arterial filter to the aorta and reaches the systemic circulation.

### 3.3.4 ECC Priming

CO<sub>2</sub> flush procedure was never performed on both oxygenators before priming the circuit. A gas-to-blood flow ratio of 0.5:1 are recommended when initiating bypass for Terumo<sup>®</sup> Capiox<sup>®</sup> RX 05 and Medtronic<sup>®</sup> Minimax Plus<sup>®</sup> Hollow Fiber Oxygenator. FiO<sub>2</sub> were adjusted according to blood gas measurements however while initiating bypass, FiO<sub>2</sub> gas supply set to 100% for Terumo<sup>®</sup> Capiox<sup>®</sup> RX 05 and 80% for Medtronic<sup>®</sup> Minimax Plus<sup>®</sup> Hollow Fiber Oxygenator as recommended. After sterile assembly of the CPB circuit, priming begins with crystalloid and colloids solution. The priming solution consists of Plasmalyte-Lyte 148 Injection (Baxter Healthcare; Deerfield, IL), 3500 units/L beef lung heparin, sodium bicarbonate 2 meq/kg/L prime, 10 gm 20% albumin, mannitol 0.5mg/kg, calcium chloride 20mg/kg plus approximately 200 to 300ml/unit packed red blood cells. All blood is screened, cross-match, and added to keep the dilute haematocrit greater than 25%.

### 3.3.5 Preoperative management

The patients were anesthetized with Ketamine (1.2 to 2mg/kg) bolus/infusions. Additional Tranexamic acid (10mg/kg), Pancuronium (0.3mg/kg) and Methylprednisolone (30mg/kg) are added to the CPB circuit during priming. The heat-exchanger to the oxygenator is connected to heater-cooler and temperature regulation is done accordingly. One blanket was placed under the child and one thermo blanket was placed over the child's lower extremities for adjunctive surface warming. The blanket warmer (WarmTouch by Tyco Healthcare Group LP) is to maintain normothermia until the patient is heparinised. CPB was initiated with a minimum activated clotting time (ACT) of 450 seconds. After heparinization, ACT will be commenced and cannulation will be performed by surgeons (Figure 3.3) meanwhile perfusionist will initiate

cardiopulmonary bypass. Circulating heparin levels and ACT's are measured three minutes after initiation of bypass and every 60 minutes thereafter.

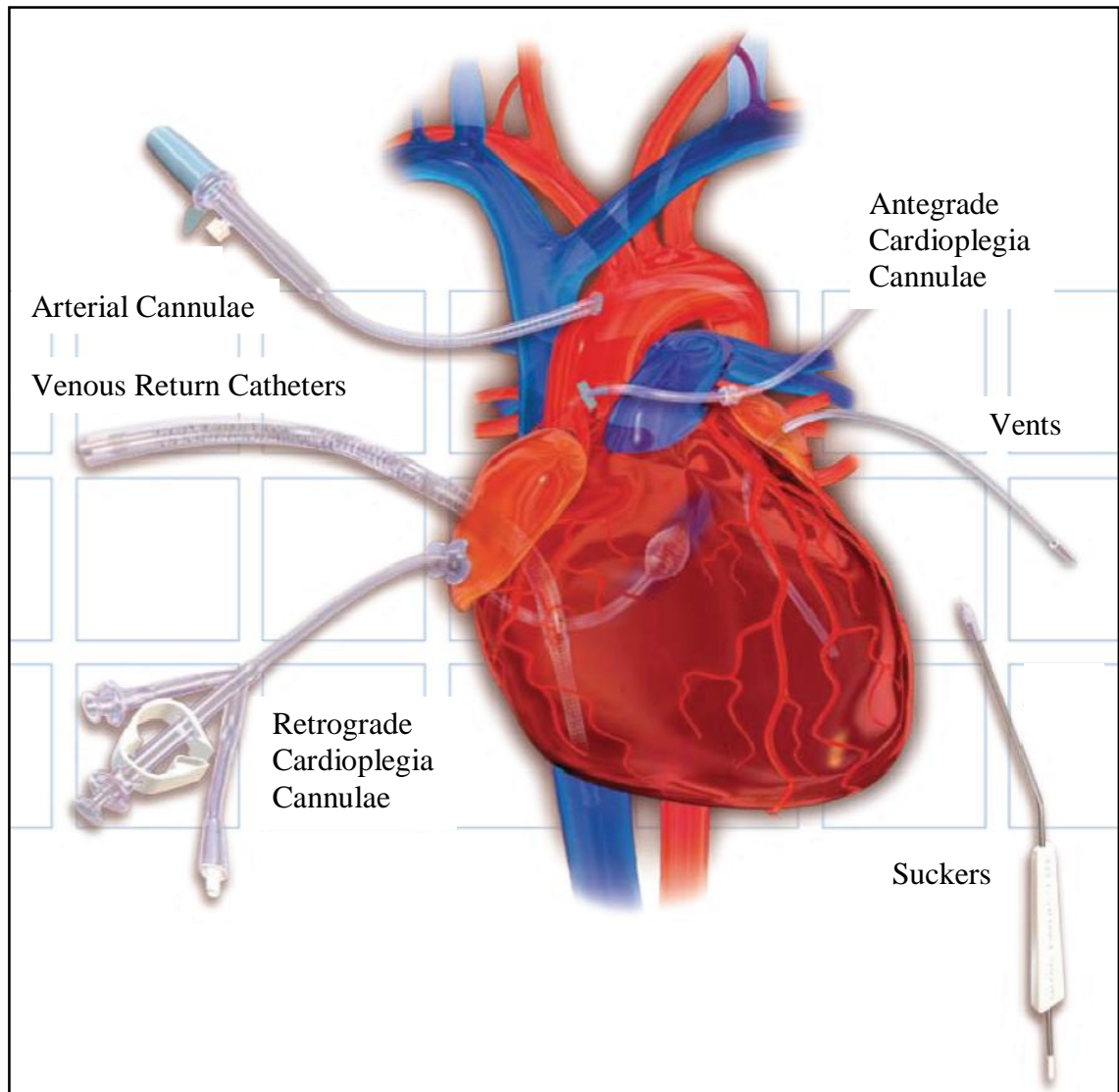


Figure 3.3: Cannulation position (Adapted from cannulae, catheters, and sternotomy products catalogue, July 2008 Terumo Corporation).

### 3.3.6 Temperature Management

Nasopharyngeal temperatures were measured. Core cooling with the heater-cooler set at 33°C was immediately begun after the start of bypass to cool the entire



body. The patient was cooled gradually to core temperature as surgeon requires. Optimal CO<sub>2</sub> then needs to be added for management on bypass during hypothermic condition. Two different hypothermic conditions were randomly investigated namely moderate and deep hypothermia; mild hypothermia temperature ranging from 33°C to 35°C are excluded from this study; moderate hypothermia temperature range from 25°C to 32°C and deep hypothermia temperature ranging from 15°C to 20°C. Although two hypothermic conditions were chosen for this study temperature were selected according to surgeon request and randomly assigned to control and treatment groups for both oxygenators. There were five different stages namely pre-operation, cooling, stable, warming and post-operation (Figure 3.4). Cooling stage is continued for a minimum of twenty minutes, or longer if needed, to achieve global cooling. Temperatures are monitored carefully not only for their absolute values, but for the gradients between all the temperatures monitored. Blood flows are maintained at full flow during the entire cooling phase except when requested by surgeon. Metabolic acidosis may be sometimes treated with sodium bicarbonate. Adequate flows and good perfusion of all vascular beds are achieved with adequate vasodilation. Blood cardioplegia is administered to arrest the heart. Once the repair or replacement takes place rewarming process will commence. A warming gradient of 8°C between the venous blood temperature and the heater-cooler temperature is diligently maintained, keeping the warming time to less than or equal to 1°C every three minutes. Warmer or blower utilized during rewarming for infant to adjuvant warming process. Additional mannitol (0.25 to 0.50 mg/kg), sodium bicarbonate, Magnesium Sulphate and Midazolam are added as warming progresses. Two additional arterial blood gases samples are obtained during rewarming at 34°C and off bypass at 35.5 to 36°C.

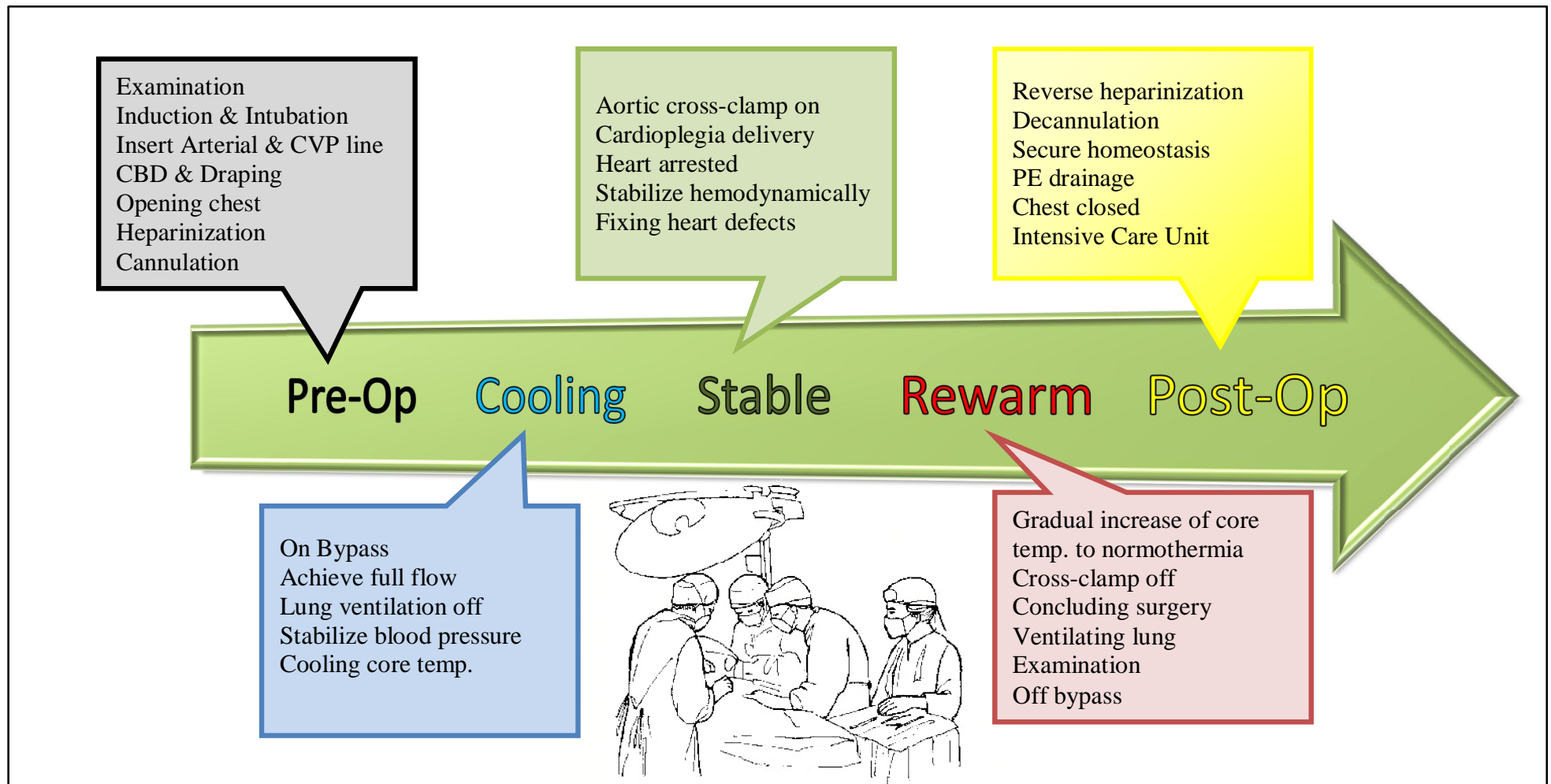


Figure 3.4: Stages during cardiopulmonary bypass. Abbreviations: CVP, Central venous line; CBD, Continuous Bladder Drainage; temp., temperature; PE, Pericardial effusion.

### 3.3.7 Blood sampling and oxygenation

CPB was initiated with a gas: blood flow of 0.5:1. After assuring adequate gas transfer across the membrane oxygenator, the  $FiO_2$  was immediately adjusted down to as low as 0.21 to 0.30. Carbogen delivery was started according to mean CPB pump expired carbon dioxide ( $peCPBCO_2$ ) reading at capnography. The gas flow was immediately decreased to 0.1 LPM, keeping the  $pCO_2$  35 to 40mmHg at the actual measured temperature according to in-line monitoring system. The  $FiO_2$  and gas flow were finely adjusted; and additional  $CO_2$  5% introduced to keep the  $pCO_2$  35 to 40 mmHg at the actual temperatures during the entire cooling phase. By keeping the gas flow low at the initiation of bypass,  $CO_2$  usually only needed to be added to the circuit briefly till the hypothermia temperature was achieved. As soon as a change in the  $paCO_2$  was seen through blood gas analysis,  $CO_2$  flows from tank were kept constant. However, additional carbogen overshoot required minimal regulation with the gas flow to the circuit. With careful titration, it was not often necessary to blow off  $CO_2$  to maintain a balanced  $pCO_2$ . The CDI 500 allowed for real-time data collection. Blood gases analyses were done after approximately ten minutes of CPB to calibrate the CDI 500 and for electrolytes confirmation. The blood-gas analyser (Cobas b 221 system, Roche Diagnostics, Mannheim, Germany) was calibrated to one point every 60 min and two points every 12h, for everyday usage. The  $pCO_2$  electrode used the Severinghouse principle, based on the potentiometric measurement of the pH change in the electrode caused by  $CO_2$ . Data were collected according to corrected temperature versus  $pCO_2$  and pH. Corrected temperature results were recorded; temperature correction was performed automatically by the analyser using the following formula (Burtis *et al.*, 2006):

$$PCO_2^t \text{ (corrected)} = PCO_2 \text{ (measured)} \times 10^{0.019(t-37)}$$

where  $t$  = patient's measured body temperature. At the same time, the capnography reading, inline ABG monitoring reading, pump flow, gas flow, and nasopharyngeal temperature were recorded.

### 3.3.8 Weaning from CPB

Weaning from CPB was attempted once the patient was evenly rewarmed, ionized calcium and electrolytes corrected, and inotropes or vasoactive infusions commenced where indicated. Conventional ultrafiltration (CUF) model Dideco DHF02 (Sorin Group) was utilized in all cases. Ultrafiltration was initiated from beginning of bypass and continued until CPB was terminated (zero balance ultrafiltration). The practice in this institution was to wean from bypass at a haematocrit  $>30\%$ . If using ultrafiltration, cannot be achieve the target Hb, additional packed red blood cells are added. The blood in the pump is salvaged and returned to the patient. This blood protocol is very patient specific. Any combination of packed cells, fresh frozen plasma, platelets, and cell salvage blood were used, attempting to keep donor blood minimal and haematocrit optimal. Blood gases were based on pH-stat strategy throughout the procedure. Bypass was terminated when the nasopharyngeal temperatures were about the range of 35.5 to 36°C.