Medical University of Graz

Diploma Thesis

THE ROLE OF ORGAN-SPECIFIC OXYGEN AND CARBON DIOXIDE PARTIAL PRESSURES AND METABOLISM DURING CARDIOPULMONARY BYPASS:

Implication in a minimal extracorporeal circuit

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Pero Curcic
0311749

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Univ. Prof. Dr. med. univ. Igor Knez
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Zusammenfassung:

Background: Im tierexperimentellen Modell wurden simultan metabolische Parameter und organspezifische pO_2 und pCO_2 Veränderungen im Parieto-temporallappen des Cerebrums, im linksventrikulären Myokard und im rechten Leberlappen während einer standardisierten extrakorporalen Zirkulation (EKZ) erhoben.

Methodik: Fiber-optochemische Sensoren auf der Basis eines „Resonance Energy Transfer" Messprinzips wurden erstmals für die online Gewebsmessung von pO_2 und pCO_2 entwickelt. Hämodynamische und respiratorische Variablen wurden aufgezeichnet, ebenso wie arterielle, zentral-venöse und intrakraniell-venöse metabolische Parameter. 14 Versuchschweine (30.7±2.5 kg) wurden zur Gruppe mini-ECC (Medtronic Resting Heart System®) und zur Gruppe CPB (konventionelle HLM) randomisiert. Nach Baselinemessungen (T1), wurde die EKZ begonnen (T2), dann die Aorta für 60min (T3-T6) geklemmt. Nach Entfernen der Aortenklemme wurde 30 Minuten rezirkuliert (T7-T8), nach Off-Bypass wurden die Versuchstiere 30 Minuten nachbeobachtet (T9- T10).

Resultate: In der Gruppe der konventionellen CPB zeigten sich ein signifikant höherer Bluttransfusionbedarf (1000±823mls vs. 50±36mls) und höhere Verlaufslaktatwerte ($p<0.00001$). Die ANOVA für den Zeitverlauf zeigte signifikant höhere cerebrale pO_2-Werte ($p=0.007$) in der Gruppe mini-ECC, während der pCO_2-Verlauf konstant blieb. Dagegen stiegen Leber- und Myokard-pCO_2 signifikant höher in der Gruppe CPB ($p=0.004$), der pO_2-Kurvenverlauf reagierte aber homogen.

Konklusion: Unter standardisierten Bedingungen besitzt die mini-ECC deutliche Vorteile durch weniger Laktatanschwemmung und zeigte signifikant bessere Resultate betreffend O_2/CO_2 Metabolismus und Transfusionsbedarf

Abstract:

Background: In a multidisciplinary experimental animal model, organ specific parenchymal pO_2/pCO_2 changes were simultaneously evaluated and metabolic variables measured from parieto-temporal lobe of the pigs' brain, left ventricular myocardium and right hepatic lobe.

Materials and Methods: Fibre-optical sensors, combined with a phosphorescent dye were used for pO_2 measurement. PCO_2 was measured using fibre-optical sensors based on phase modulation fluorometry. Sensors were adapted to allow continuous monitoring. The sensors were calibrated for an in-vivo setting. Haemodynamic and respiratory variables were recorded and adjusted to a predefined cardio-surgical perfusion model. Online samples of arterial, central-venous and intracranial venous metabolic variables were recorded at the time points defined by the study protocol. 14 pigs (30.7±2.5kg) were randomized either to the mini-ECC group or controls (conventional cardiopulmonary bypass). The perfusion systems were minimized and adjusted to basic variables comparable to extracorporeal perfusion settings as used in congenital cardiac surgery. After baseline measurements (T_1) cardiopulmonary bypass was established (T_2) and the aorta cross-clamped for 60 min (T_3-T_6). The test animals were reperfused for 30 min (T_7-T_8) and observed for another 30 min. off-bypass (T_9-T_{10}).

Results: There were no differences in pre- and intra-operative variables, except for the significantly higher volume of blood transfusion (1000±823mls vs. 50±36mls, $p \leq 0.00001$*) and higher lactate levels in the CPB group ($p \leq 0.00001$*). ANOVA for repeated measurements at T_1-T_{10} revealed significantly higher cerebral pO_2 levels ($p=0.007$*) in the mini-ECC group, while pCO_2 levels appeared similar. In contrast, both hepatic and myocardial pCO_2 levels were significantly higher in CPB group ($p=0.004$*), while pO_2 levels reacted homogenously.

Conclusion: Under standardized conditions, mini-ECC produced less lactate and showed significantly favourable changes concerning O_2/ CO_2 metabolism.

Declaration in Lieu of an Oath

Declaration in Lieu of an Oath

I herewith declare in lieu of an oath that I have produced the aforementioned thesis independently and without using any other than the aids listed. Any thoughts directly or indirectly taken from somebody else´s sources are made discernible as such.

Graz, the 5th of February Signature

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Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 05. Februar 2010 Unterschrift

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Content

4. Discussion

1. Introduction

1.1. Clinical Background and relevance

The fascination of the human body and anatomy culminates in the complexity and variety of pathology of the human thorax. The living heart, as close to the body surface, remained unreachable for decades to any surgical treatment. Therefore many surgeons were motivated by the symbiosis of simplicity and thrilling efficiency of therapeutic interventions to go beyond these barriers.

The first to perform a successful open heart surgery were Dr. F. John Lewis and Dr. CW Lillehei on September 2, 1952, Minnesota, USA, by closing an atrial septal defect (ASD) in a 5-year old girl under direct vision using inflow stasis and moderate hypothermia [1]. More complex diseases of the heart needed new strategies. One of the keystones of open heart surgery was invention and immense progress in research, especially through the work of Dr. John H Gibbon, Jr., of the cardiopulmonary bypass (CPB) using the extracorporeal circulation (ECC) at the beginning of 1950´s [2]. Nowadays, as the use of CPB, i.e. the heart-lung machine (HLM), in cardiac surgery is considered a safe and effective surgical technique to treat intrathoracical diseases, we face an even bigger urge for development of technology used in cardiac surgery [3]. For example coronary artery bypass grafting (CABG) can be performed with a mortality as low as 3% [4], still heaving only 65% of conventional CABG procedures reported as free of complications. The morbidity of this cardiac procedure is grossly attributed to the adverse effects of the cardiopulmonary bypass. Side effects include excessive hemodilution, haemolysis, contact with foreign surfaces and bubble formation causing among others neurological events, systemic inflammatory response and/or severe organ dysfunction[5].

Therefore several strategies were developed in modern cardiac surgery through lively scientific and clinical debate to face this problem. Abandoning the extracorporeal circulation (ECC) in case of Off-pump coronary artery bypass grafting (OPCAB) is a widely accepted method. OPCAB became an established surgical technique although not representing the gold standard because often total revascularisation of the myocardium is not achieved using this technique [6][7]. OPCAB surgery is definitely a great component

of the armamentarium used to tackle a wide array of clinical challenges, but still there remains the issue of many cardiac diseases which can only be faced by the use of CPB.

On the other hand great endeavours can be recognized to minimize the invasiveness of the heart-lung machine (HLM) [8]. The terminology which is used in literature for these systems is often different: minimized or minimal extracorporeal circuits (MECCs), simplified bypass systems (SBS), minimally invasive extracorporeal circuits (MIECs) or mini-extracorporeal circuits (mini-ECC). There are several systems available at the moment, so as the MECC® (Jostra MECC System; Jostra AG, Hirrlingen, Germany) or the Medtronic Resting Heart System® (RHS; Medtronic, Inc, Minneapolis, MN, USA). All these systems have in common that they are basically simplified HLMs with closed circuits, special coatings on the foreign surfaces and exclude venous reservoirs and cardiotomy suction. The literature about the clinical use of these systems is quite extensive; however the rationale for the clinical benefits of these systems is based only on few small animal experiments [9]. Bearing in mind that lower morbidity of cardiac procedures and shorter intensive care unit stays (ICU) have far-reaching socio-economic consequences, the analysis of pathophysiological mechanisms of the minimally invasive cardio pulmonary bypass systems and conventional CPB could contribute to better patient care.

1.2. Cardiopulmonary bypass: Equipment

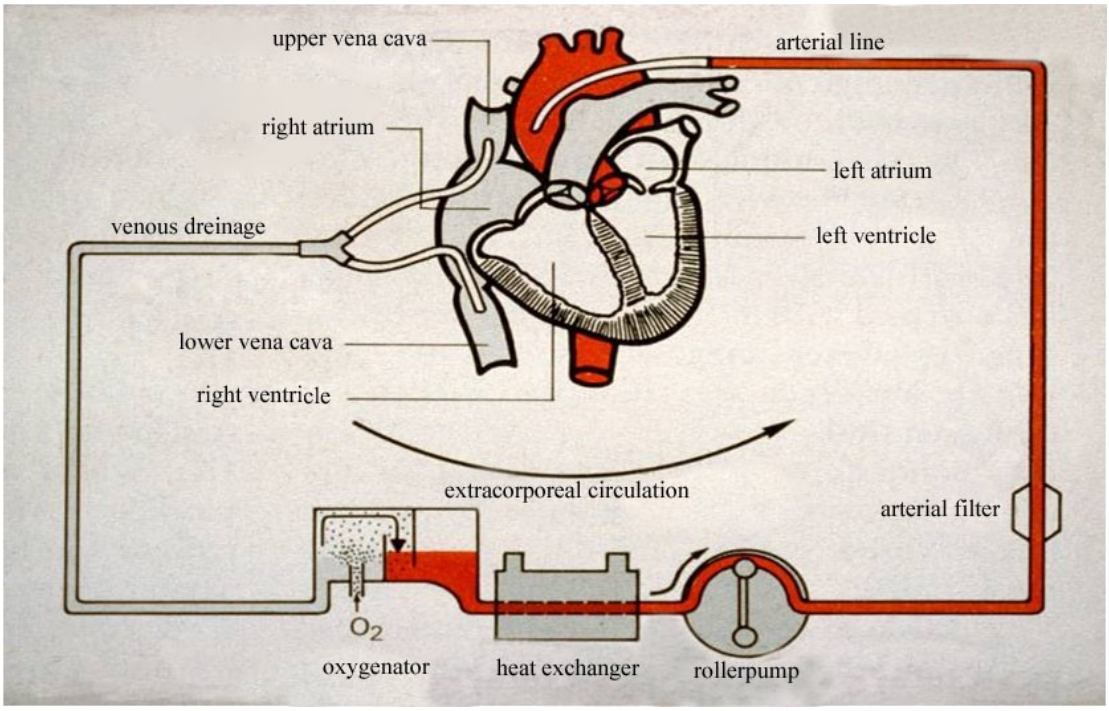

Figure 1: Cardiopulmonary bypass: scheme (by Prof. Rigler)

1.2.1. Conventional heart-lung machine (HLM)

The cardiopulmonary bypass is performed using the heart lung machine (HLM). In the first part of this chapter the conventional heart-lung machine (HLM) with its components is going to be described with its components, in the second the novel concept of minimally invasive cardiopulmonary bypass.

The primary function of the HLM is to replace the propulsion of the heart and the oxygenation of the lungs. Typically, venous blood is drained trough the venous cannula into the venous reservoir usually by gravity either from the superior and inferior vena cava (SVC, IVC) or the right atrium via the right atrial appendage into the venous reservoir. From there the dark coloured blood is propelled into the “artificial“ lungs, the oxygenator (bubble or membrane oxygenator), receives energy through the systemic pump, either centrifugal or roller pump and is then reinfused into the patient´s body through the arterial

cannula placed in the ascending aorta. The temperature can be regulated using the heat exchanger included into the ECC. Parallelly, up to 3 additional roller pumps can be used either as a suction device or for administration of cardioplegia.

More detailed, two different oxygenator systems can be found. The first to name, seldom in use any more, is the bubble oxygenator (bubbler). Desaturated blood is passively entering a mixing chamber where oxygen is pumped against the blood stream causing bubble-formation and carbon-dioxide is eliminated. In the next chamber anti-foaming agents are used to "de-bubble" the blood [10]. On the other hand, membrane oxygenators are mostly used today. The blood passes through a semipermeable membrane, either made of silicone or polypropylene, and gas exchange is performed in the same way as in the lungs. Beside lower risk for gaseous emboli, the membrane oxygenators provide a better gas-exchange rate [11].

Moving along the extracorporeal circuit, the blood passes the „heat-exchanger". It is a device where blood and water are pumped against each other in separate chambers and heat is transferred following the convection principle. Heat exchange is used during cardiac procedures for cooling the patient in order to lower the metabolism rate. Hypothermia has the ability to preserve cell function under low or no perfusion situations [12]. After finishing the procedure re-warming is introduced. The CPB can also be used to re-warm patients following hypothermic accidents [13].

One of the main components of the CPB is the pumping system. In modern HLM´s two main systems are used, the roller pump or the centrifugal pump. The roller pump is longer in use, but also has also some disadvantages compared to the centrifugal pump [14]. The working principle of the roller pump is simple. Two rollers, placed opposite to each other rotate and "roll" the blood trough a soft tube. The blood flow can be regulated by modifying the revolutions per minute (RPM). Conventional HLM systems mostly depend on roller-pumps. On the other hand we can find centrifugal pumps which propel the blood using rotation of an impeller in a rigid housing, creating regions of low and high pressures. The blood is then forced from the in-let to out-let. Different to roller pumps with flow proportional to RPM´s, an in-line flowmeter has to be used here because of their non-occlusive character [14].

To prevent embolic events, several filters are interposed in the circuit. Modern venous reservoirs have build-in filters [15]. Mostly there is also a filter at the end of the circuit, placed just before the blood enters the systemic circuit of the patient. Filters are

made of micro-porous membranes which prevent gas bubbles or fat emboli to enter arterial circulation [16].

One important part of HLM and CPB is the cardiotomy suction. During cardiac surgical procedures large amounts of blood are displaced from the cardiac chambers or big vessels into the pericardium or the operating field. This blood has to be collected and after filtration re-infused into the extracorporeal circuit [17].

1.2.2. Mini- ECC Systems:

As mentioned before, there are large efforts to minimize the side effects of the CPB. Reduction of the priming volume is one of the main ideas of the mini-ECC Systems [8]. The priming volume is the volume of liquid needed to fill all components (tubing, oxygenator/heat exchangers, filters ect.) of the cardiopulmonary bypass prior to installation of the extracorporeal circulation. In order to reduce the priming volume, all parts included in the circuit have to be downsized while the standard of performance is still maintained at a high level. For example, the oxygenators depend on their membrane surface area to be able to provide sufficient gas exchange [18]. Reduction of the tubing´s diameter can be achieved by bringing the CPB unit and the patient closer together [8]. Reduction of diameter can have big influences, as we consider what Poiseuille´s law proves, namely that the flow is proportional to the fourth power of the radius [19]. Even small changes can have influence on, for example, the flow of the venous drainage. To solve this problem in mini-ECC a kinetic-assisted venous drainage (KAVD) is often used. In contrast to the conventional HLM, where the blood is following the force of gravity, in mini-ECC systems active drainage is performed using either the systemic centrifugal pump or a separate venous pump [20].

Another strategy of shortening the circuit as well as reducing foreign surface area, air and blood contact is to abandon the cardiotomy suction and the venous/cardiotomy reservoir. In that case the blood is continuously circulating through the HLM and is not being stored in a reservoir. The blood from operating field has to be aspirated using a cell-processing device. Of course, this strategy shows outstanding advantages but also concerning disadvantages.

Last but not least, all surfaces of the CPB which are in contact with blood are coated with a special, heparin-based substance. We can find different coatings used in mini-ECCs which improve the biocompatibility of the surfaces [21].

1.3. Pathophysiology of the ECC

Unfortunately, the CPB is far from ideal. As it was shown in the previous passages, the extracorporeal circulation is on one hand in its principle very simple. On the other hand during the CPB, a huge number of interactions and influences on body systems are introduced.

1.3.1. Inflammatory response

During and after CPB patients show symptoms of a systemic inflammatory response syndrome (SIRS). As defined by Harrison´s Principles of Internal Medicine, in case of SIRS two out of four following conditions have to be fulfilled:

- Oral temperature >38°C or <36°C;
- Respiratory rate >20 breaths/min or $PaCO_2$ of <32 mmHg
- Heart rate of >90 beats/min
- Leukocyte count of >12.000/µL or <4.000/µL or >10% of immature neutrophils

(This term was first introduced by the American Collage of Chest Physicians/Society of Critical Care Medicine Consensus Conference, 1992 [22])

Because of the special presentation and association with the CPB the term systemic inflammatory response after bypass (SIRAB) was established [18]. It is important to mention in this context that SIRAB does appear with a non-infectious clinical picture, more or less severe. This makes the early diagnosis of SIRAB quite difficult as symptoms could have been caused by poor perfusion or the inflammatory response itself [23].

The pathophysiological mechanisms leading to SIRAB are the following. During CPB the cellular and noncellular (humoral) elements of the blood are exposed to a large number of injurious processes. All contacts with foreign surfaces or air consequently cause a sequence of cytokine-mediated events to be started. In addition, microemboli—common

during CPB—have influence on the sensitive balance of the endothelium allowing further neutrophil-activated inflammatory injury [24].

Injury to red blood cells is very common under the demanding environment of the CPB. Rheologic shear stress forces, occurring during the blood´s passage through a CPB-roller pump or even centrifugal pump, have a negative influence on the membrane integrity of red blood cells. In particular, Na^+/K^+ ionic pump deterioration is initiated and abnormal accumulation of intracellular cations is the consequence. Following this, red blood cell ghosts (parts of membrane after haemolysis) can negatively influence microcirculation [18].

As well as the RBC, the neutrophils play a important role in the systemic inflammatory response following cardiopulmonary bypass. The loss of L-Selectin and the up-regulation of CD11b/CD18 indicate the activation of the neutrophil cell. In connection with that, also the endothelial cell changes its characteristics. Namely, hypoxia, surgical manipulation and cytokines (IL-1b and TNF-alpha) trigger the endothelial cell to express adhesion molecules [22]. After that, neutrophils and other immunological cells adhere to the wall and can emerge into the peripheral tissue causing an inflammatory reaction [19].

Beside the cellular parts of the blood, the humoral system is affected by the CPB [18]. The complement system is mainly activated through the alternative pathway during or shortly after CPB. Contact of blood with non-endothelial surfaces induces this enzymatic cascade [25]. Further activation can be initiated by protamine infusion after completion of CPB through the classical pathway. This in turn, mediates cellular damage, endothelial cell and leukocyte activation, histamine release, increased vascular permeability and systemic inflammatory response. All these mechanisms also do have significant impact on the microcirculation [26].

In cardiac surgery, several cytokines are known to be reliable markers for the inflammatory reaction. Several studies had their emphasis on TNF-α, IL-1β, IL-6 and IL-10 [27-29].

1.3.2. Embolic events

“An embolus is a detached intravascular solid, liquid, or gaseous mass that is carried by the blood to a site distant from its point of origin. Virtually 99% of all emboli represent some part of a dislodged thrombus....” [26]. In case of CPB the situation is to an extent different. We distinguish the following types of emboli in connection with CPB: gaseous emboli, foreign material, and “blood borne” emboli [18].

In the beginning of the CPB-era gaseous emboli were a frightening complication, especially during the use of bubble oxygenators. Today as safety margins are high and older bubble oxygenators have been displaced by membrane oxygenators, there is still an issue about gaseous microembolism (GME) [30]. GME can still occur at oxygenator membranes and air can be sucked up the venous drainage. GME can also be the result of excessive cardiotomy suction [31]. In a conventional extracorporeal circuit one would expect to get rid of gaseous embolism originating from the venous line through the use of a venous reservoir and accompanying filters. Still, studies have shown that despite high safety margins and the use of these technical solutions microbubbles are able to pass into the arterial circulation [32]. Miniaturization of the circuit as in case of mini-ECC, has totally abandoned the venous reservoir so some research groups claim that the rate of GME would be higher [33]. Another source of gaseous embolism is of course the surgical intervention. Opening the chambers to perform reconstructions or cannulating the great vessels can cause air entering the arterial circulation [18].

On the other hand, embolism can have its origin in materials used for CPB and surgery. Foreign materials can enter the circulation, either during the manufacturing process, assembling or while performing CPB, including cotton fibres, plastic or metal particles, filter and tubing materials. The surgical field can also be origin of embolic particles, such as bone wax, muscular particles, bone fragments or surgical thread (Gravlee)[18]. In this context, fat embolism should also be mentioned because it easily can occur during cardiac surgery. Macroembolic and microembolic particles of fat can be generated throughout all surgical procedures, especially by trauma to the fat cells during sternotomy. Fat from the surface of the shed blood can be aspirated by the cardiotomy suction from the surgical field [34]. Using a processing device, like the cell saver, could ameliorate the risk from fat embolism. Another important source of solid particular

embolism is the placement of the arterial cannula and the resulting disengagement of a large atherosclerotic plaque [35].

Postoperative cerebral dysfunction after CPB is mostly attributable to microembolism [36]. Otherwise, it is remarkable how individual differences tend to influence the outcome of embolic events. So for example 15%-20% of people are borne with an incomplete Circulus of Willisi [37]. A patient with a dense capillary bed will react differently compared to a patient with less capillaries confronted with the same amount of microemboli. It is also interesting that in newer research, the stroke rate after CPB is reported rather low, namely 1%- 5%. On the other hand the neurobehavioral deficits rate is somewhere around 30% to 70% in the immediate postoperative period [38].

1.3.3. Haemodilution

Excessive haemodilution results in severe reduction of haematocrit and increase in transfusion requirements. Still haemodilution is one of the strategies to improve the outcome after CPB [18].

In the beginning of the clinical use of CPB whole blood was used for priming of the HLM. The priming volume is the amount of liquid required to pre-fill the tubing, reservoirs, oxygenators or filters. The disadvantage of using whole blood is obvious- very high requirements on the blood bank and high infection risk among others. This made cardiac surgery a very costly and uncertain enterprise.

The use of crystalloid priming solutions has solved some problems and represents the standard in the day-practice of CPB (Han). Blood is a Non-newtonian fluid which when in motion behaves differently to a Newtonian fluid like water for example. The viscosity of blood under low flow is elevated; hence, a higher perfusion pressure is required to achieve sufficient tissue perfusion, as well as tissue oxygenation. Dilution of the blood changes the situation. Lower viscosity allows blood to behave as a Newtonian fluid and low perfusion pressures are possible, having the same effect on tissue perfusion. Lower haemolysis is the consequence of lower perfusion pressures. Since viscosity and haematocrit are directly proportional to each other, the latter is an adequate estimation of the viscosity. Also the fact that reduction of the haematocrit from 40% to 20% result in

only 10% reduction of the oxygen transport, makes it possible to use haemodilution as a salutary strategy in ECC [18].

Haemodilution has certainly important advantages, as mentioned. On the other hand excessive haemodilution and in the end low haematocrits aggravate the outcome postoperatively. Especially in patients with low blood volume or low haematocrits preoperatively or infants, where often the priming volume of the HLM exceeds the blood volume of the infant, excessive haemodilution has deleterious effects. High blood transfusion requirement or coagulopathies after CPB are the resulting consequence of excessive haemodilution.

1.4.4. Other pathopysiological changes

The use of CPB and the accomplishing strategies have many more influences on body systems.

Hypothermia is often used in cardiac surgical procedures. The main reason for application of low temperatures to the body during operations is the ability of hypothermia to preserve cell function for longer periods of low perfusion. The main mechanism behind is the reduction of cell metabolism and oxygen consumption during hypothermia. Also the enzymatic reaction rate is decreased during lower temperature, as well as the acid-base alterations are changed, which should be taken into account when dealing with $PaCO_2$ and pH measurements [39].

Pulsatile perfusion generated by the human heart is absolutely not established by the HLM. The non-pulsatile flow can change the blood distribution to the organs and has for example influence on the hormone secretion during CPB [40].

1.4. Microcirculation, pO_2 and pCO_2

Changes in microcirculation are common during CPB and are associated with many pathophysiological consequences. Possibilities of exact assessment of these changes are limited. Especially the online and continuous measurement of these perioperative

fluctuations is difficult to achieve. Precise and on-time knowledge of these parameters is also crucial for early and satisfying postoperative recovery of the patients undergoing cardiac procedures postoperatively. The measurement of systemic partial pressures of O_2 and CO_2 is routinely performed during cardiac surgery using the blood gas assessment with point of care testing devices. Online and organ-specific control of pO_2 and pCO_2 would give the chance to assess the status of the patient intraoperatively at any time of the procedure and even retrospectively. One possible strategy is the use of near-infrared spectroscopy (NIRS) [41]. At present time, it is used for the measurement of O_2 saturation in cerebral tissue, but also visceral measurements are anticipated. The technology used for NIRS is the two-wave length spectroscopy. A self-adhesive band is attached to the patient´s forehead and the saturated and desaturated haemoglobin is assessed non-invasively [42].

A very different strategy of measuring pO_2 and pCO_2 is the use of opto-chemical sensors. Their benefits like continuous measurement, higher sensitivity, no analyte consumption and less electromagnetic interferences recently have been reported in several publications [43]. The principles used are mainly based on the change of the absorbance or of the fluorescence intensity of an indicator placed at the tip of the optical fibre. The sensitive chemistry is coated onto the sharpened tip of optic cables with core diameters of 400 and 200 µm. A chemical etching process sharpened the fibre tip. The oxygen measuring principle is based on the ability of oxygen to quench luminescence. A phosphorescent dye PtTFPP is coated onto the tip of the optical fibre and pulses of green light are carried through the optical fibre in order to excite the dye at the tip. Because of interaction of the dye with O_2, the luminescence is quenched and sent back to a detection unit. The changes of luminescence are dependent on the pO_2. The pCO_2 was measured in the same manner but using the phase modulation fluorometry as measuring principle [44][45].

Intraoperative, online and exact assessment of pO_2 and pCO_2 could be a unique method to control and improve tissue perfusion and thus compare different CPB techniques or equipments.

1.5. Objectives and aim of the submitted thesis

The following submitted thesis should answer the question whether the mini-ECC system is beneficial in comparison to the conventional heart lung machine regarding O_2 and CO_2 metabolism, distribution of the same and inflammatory response. Furthermore, it should be evaluated whether the fibre-optical sensors are a valid instrument for the detection of CPB induced pathophysiological changes during the conduction of an extracorporeal circulation. An animal model should be established and improved for further investigational procedures.

2. Materials and Methods

2.1. Establishment of the animal model

A novel large animal model was established based on patterns of an already used and proved experimental model at our university. The experimental animal model of this experiment should fulfill the following requirements:

1. Feasibility of standard cannulation as used in adult and congenital cardiac surgery as well as of the positioning of the pO_2/pCO_2 opto-chmical sensors in desired tissues
2. Ability of the organism to survive the period of time defined by the study protocol
3. Comparability and transferability to clinical practice and human physiology and pathophysiology
4. Reproducibility of the experiment at same conditions in different laboratories.

Preliminary to the actual experiment, three pigs were operated on with the aim to establish and optimize our animal model. The weight of the pigs was amounted to 30.7± 2.5 kg and age to 4-5 months. As it was clear that the peripheral venous and arterial cannulation, the central lines, the tracheostomy and anaesthesia, as used in humans, were going to be sufficient and possible to perform, sternotomy was performed. Several problems were faced right at the beginning of the sternotomy [46]. Great care had to be taken of the anatomical structures different to human anatomy [47]. These differences are mainly derived from the different basic body orientation, with the pig having a quadruped stance compared to human orthograde (upright) stance. At opening the thorax we faced following problems. Due to the close position of the porcine heart to the posterior lamina of the sternum difficulties appeared while dividing the sternum. Dissecting the lower part of the sternum and the processus xyphoidalis, which in pigs is made of cartilage, turned to be very simple, the manubrium sterni was recognized as the most difficult part of the sternotomy. The manubrium sterni is quite massive compared to the human manubrium [37]. According to this, an oscillating saw had to be used, but to be able to use it, space had

to be made cranially to the manubrium. After dissecting the platysma, the cervical muscles strictly in the mid-line and passing the thyroid gland, convoluted small venous vessels presented as very vulnerable and a likely origin of haemorrhage. Bleeding from this small vessels, which in this region formed a ´venous confluence´, is difficult to control, especially after full heparinization as it could be shown in this preliminary operation. Comparing to this situation, the human anatomy of this region is simpler with the innominate vein being the main source of concern. After managing to divide the manubrium sterni without compromising the vessels above, the rest of the lamina posterior of the sternum could be divided, preferably using scissors, because the use of the oscillating saw, as shown in one of the preexperimental pigs, can lead to major complication in form of a ventricular injury causing catastrophic haemorrhage. This is because of the porcine right ventricle as well as the apex, are adjacent to the sternum [47]. After resection of the thymus, partially by blunt dissection or by scissors, the anterior face of the heart could be exposed. Because of the porcine anatomy of the thorax and the different body orientation the heart itself presented differently to the human with a striking pulmonary artery entirely covering the ascending aorta. As described by Crick et al. the pig heart had the classic ´Valentine heart´s shape which was quite unlike the human heart where the heart had the distinctly trapezoidal silhouette when seen in frontal projection. The atrial appendages presented at a approximately similar size, whereas the human left appendage is bigger than the right [37]. The ascending aorta appeared short, quickly disappearing into the depth of the thorax, giving only two branches to the upper body. Whereas in men commonly the right carotid artery arises from the truncus brachiochephalicus and the left directly from the aortic arch, the swine has a so called truncus bicaroticus coming from the truncus brachiocephalicus. The second vessel arising from the aortic arch is the left subclavian artery. At that point the plan of cannulation of the heart had to be reconsidered.[48] Because of the hidden and short ascending aorta the purse-string sutures were placed at the prominent truncus brachiocephalicus in order to insert here the arterial cannula. The venous drainage could be established in the common manner by placing the venous cannula in the right atrium through the right atrial appendage. Also the place and the technique of the cardioplegia delivery had to be optimized for our animal model. As well as establishing the anaesthesia, the technique of the sternotomy, the modification of the cannulation site, the delivery of the cardioplegia and the cross-clamping site, the correct and definitive position of our opto-chemical probes

had to be defined. After we have managed to determine the plausibility of the signals in all three preliminary operations, a craniotomy was performed to verify macroscopically the position of the probes and the absence of major parenchymal damage of the brain tissue or serious haemorrhage. Also, the liver was dissected to determine the position of the opto-chemical probe. Furthermore, the use of the PiCCO® for haemodynamical measurements and the BIS® for anaesthesia control were tested and modified. At the end the study protocol was defined and modified as well as the complex course and technique of the operation were established.

2.2. Study design

14 domestic pigs of both sexes were prospectively and randomly assigned by computerized randomization to group A, minimal invasive extracorporeal circulation (mini-ECC) (n=7) and group B, conventional extracorporeal circulation (CPB) (n=7). Furthermore 14 animals served as blood donors. According to randomization either the minimal extracorporeal circulation (Medtronic™ PERFORMA CPB® + Resting Heart Set®) or the conventional cardiopulmonary circulation (Stöckert™ HLM + Dideco® D 905 Oxygenator) were used. After baseline measurements (T1), cardiopulmonary bypass was induced after 15min (T2), the aorta was cross-clamped for 60min (T3- T6), the test animals were re-perfused for 30min (T7-8) and after that observed for another 30min of-bypass (T9-10). The data consisted of the hemodynamic and respiratory variables, as well as arterial, central venous and intracranial venous metabolic variables collected at predefined time points according to the study protocol. At same time online-measurement of the O_2/CO_2 distribution in the parieto-temporal lobe of cerebrum, the myocardium and the right lobe of the liver was performed. After completing the study protocol all animals were sacrificed.

2.3. Study animals

28 domestic pigs were used, 3-4 months of age (13.5 ± 3 weeks) and a mean weight of 30.7 ±2.5. In the morning of the experiment the animals were brought to the surgical vet-lab at the University Clinic of Surgery originating from a local farm with standardized conditions of animal breeding. It was very important that the animals came from the same litter especially because of the blood donors to prevent the high risk of anaphylactic reaction, which can lead to death of the animal. All animals received human care according to the "Principles of Laboratory Animal Care" guidelines stated by the Austrian National Society for Medical Research. The protocols for the experiments were approved by the Medical University of Graz and the Austrian Ministries of Science and Research under the reference number GZ 66.010/16_II/10b/2008. The whole documentation of approval of the experiment is attached in the appendix of this thesis.

2.4. Setting

All operations were performed in the surgical animal laboratory of the University Clinic of Surgery, Medical University of Graz, Austria. The laboratory consists of 3 separate operating theatres and the accompanying facilities. The operating theatres are fully equipped in the same manner as the cardiac surgery theatres of the University hospital. The main operating theatre can be seen in Fig.3, the arrangement of the apparatus, instruments and staff are described in Fig.4. The operating team is in its position, dissecting the neck vessels for i.v. access and the anaesthetist is standing at his place.

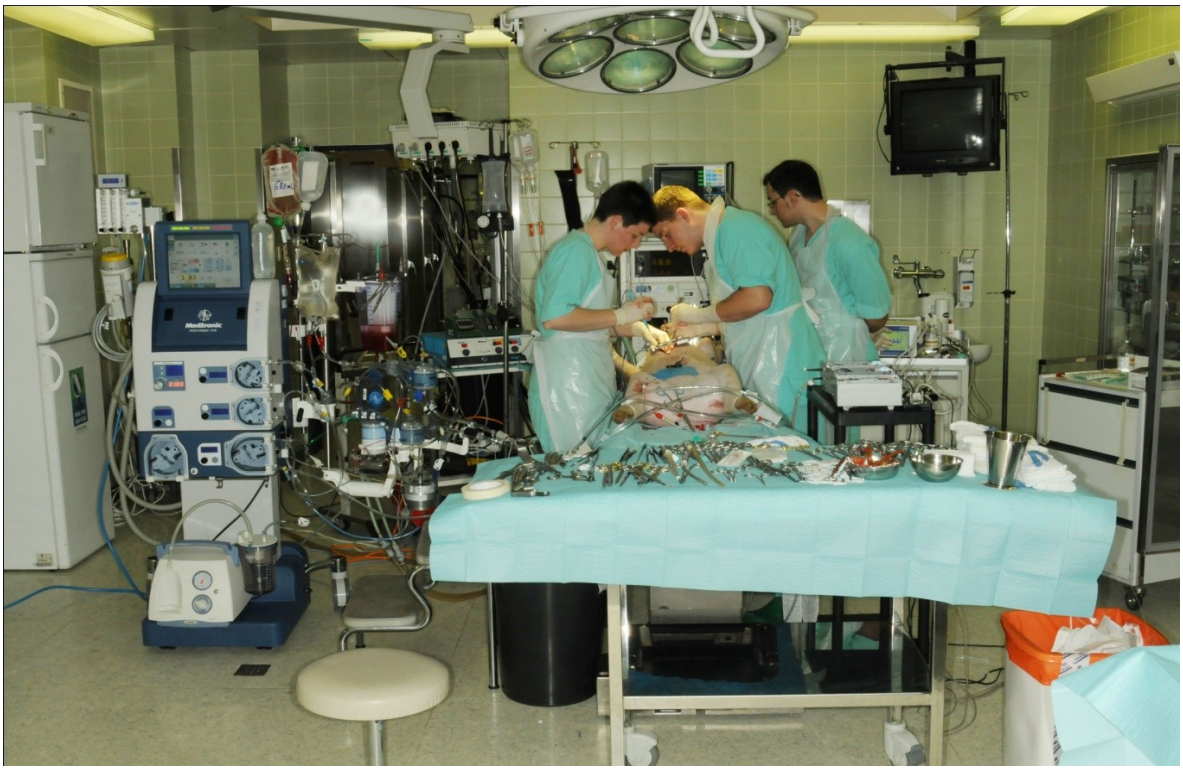

Figure 2: Operating theatre

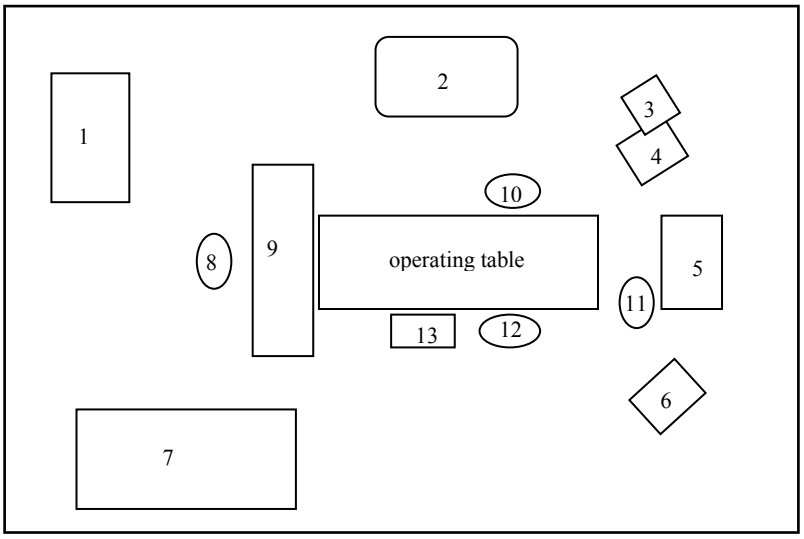

Figure 4: Operating theatre-scheme: (1)point-of-care gas check, (2) mini-ECC/RHS- heart lung machine, (3-4) cautery & suction, (5) anaesthesia devices, (6) PiCCO^{TM} (7), computing station (8) scrub nurse ,(9) instrument table, (10) surgeon, (11)

2.5. Anaesthesiological considerations

At 7 am on each experimental day the animals were delivered in special animal suitable cages to the surgical laboratory. Prior to being taken into the operating theatre the animals

were premedicated with intramuscular Midazolam (0.5mg/kg, Dormicum, Roche, Vienna, Austria) and (S)-Ketamin (5-7.5mg/kg, Ketanest S, Actavis S.P.A., Nerviano, Italy).

20 minutes later the unconscious animals were transferred to the operating theatre and were placed on the operating table in a lateral position to facilitate spontaneous breathing. Following the insertion of a 20 G IV cannula in an auricular vein, the anaesthesia was induced by administration of 2.5-3mg/kg Propofol (Diprivan 1%, AstraZeneca, Luton, UK) and 5µg/kg Fentanyl (Fentanyl Janssen, Janssen Pharmazeutica, Beerse, Belgium). Muscular relaxation was achieved by Cis-atracurium (0.1mg/kg, Nimbex, Glaxo Wellcome Operations LTD, Greenford, UK). Using a special tubed facial mask, the animal was preoxygenated, while being repositioned to a supine position. After performing a quick tracheotomy, the animal was intubated by a common endotracheal tube (4.5-5.5 I.D Mallinckroth Medical, Athalone, Ireland). The correct position of the tube was confirmed by auscultation of both lungs. Using a Sulla 909 V Ventilator (Dräger Medical Austria GmbH, Vienna, Austria) ventilation and inspiratory oxygen concentration (FiO_2) were adjusted individually in order to maintain physiologic values in blood gas analyses (arterial pO_2 from 150 to 200 mmHg). O_2 saturation was controlled by clipping the tail of the animal with an oxymeter. During the entire experiment (also during CPB) anaesthesia was maintained with Sevoflorane 2-2.5% (Sevorane, Abbott Laboratories GB, Queensbrough, UK) and continuous infusion of Fentanyl (10-15µg/kg/h). Anaesthesia depth control was achieved by using the bispectral indexs (BIS®, Aspect Medical Systems International, De Meern, Netherlands) for all animals (Greene).

After dissecting the external as well as the internal jugular vein, the latter one was ligated proximally and a venous central line (Certofix® Mono S 430, Braun AG, Melsungen, Germany) was inserted with the tip resting at the bulbus venae jugularis. A second central line (Arrow® REF CS-15853 Multi Lumen Central Venous Catheterization Set, Arrow International Inc., Reading, PA, USA) was placed in the same manner, but in the external jugular vein, which was ligated distally, for central venous pressure measurements, blood sample collection and fluid administration. A 20 G line for arterial measurements and a Picco-catheter (Pulsiocath®, Pulsion Medical Systems AG, Munich, Germany) were inserted into the left and right femoral artery as it can be seen on the picture below.

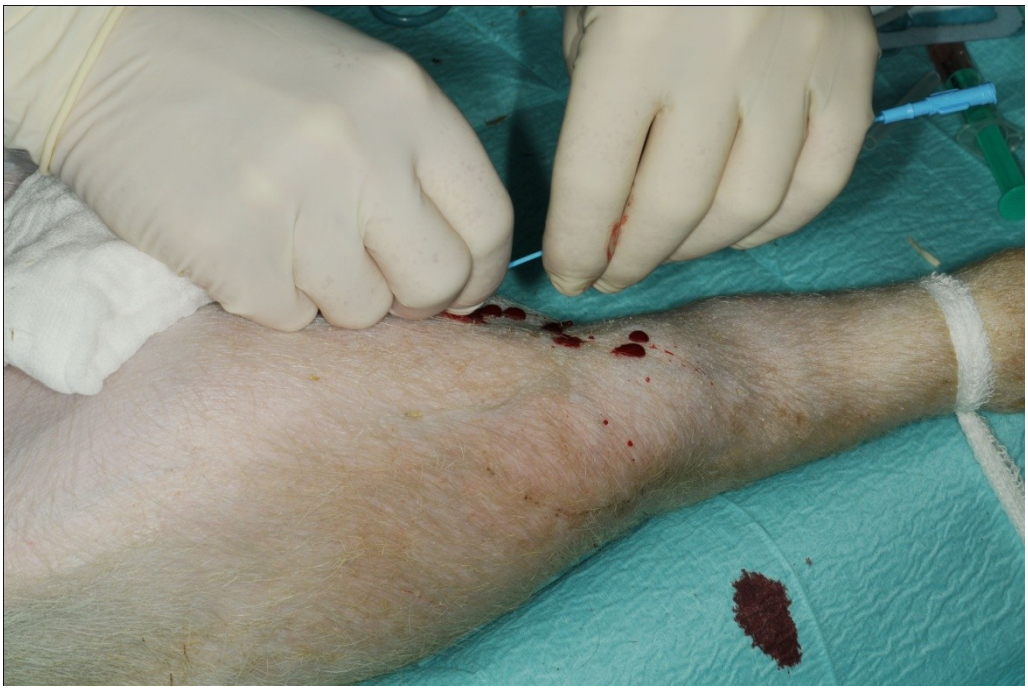

Figure 5: Seldinger technique for puncture of the femoral artery

In the meantime the blood donating pig, which also had been pre-medicated and sedated with Propofol (Diprivan 1%, AstraZeneca, Luton, UK), was placed on a second operating table, oxygenation was maintained via face mask and the ECG and saturation, by pulsoxymetry, were monitored. The auricular vein was punctured with a 20G intra venous cannula, 6000 IU of Heparin and 500ml Ringer-lactate solution were administered as well as additional fluids as the blood donation was proceeding. 20 ml Xylanaest were infiltrated locally.

All blood gas analyses were performed using the GEM Premier 3000® System (Instrumentation Laboratory Company, Lexington, MA, USA). ECG monitoring was done with house-made needle electrodes placed subcutaneously.

2.6. Preoperative Management

The animals were positioned on the operating table in a supine position and strapped securely with dressings. Both fore and back legs were positioned by slight caudal traction,

so best approach either to the operation sites of the neck and the sternum or the femoral arteries was achieved. The temperature was measured by a temperature probe placed in the oesophagus of the testing animal. After completing the anaesthesia the animal was repositioned once again to the side, the head was shaved and the BIS® Quattro sensor (Aspect Medical Systems International, De Meern, Netherlands) attached at the required points of the head. The BIS® sensor was secured by covering the head with op-site transparent adhesive dressing as well as the trepanation site for positioning of the central nervous pO_2/pCO_2 opto-chemical probe was prepared.

2.7. Cardio-surgical setup

2.7.1. Blood donor

In the second operating room, the blood donating animal was positioned in a supine position, in the same manner as described above. A 6-8 cm incision was made longitudinally on the top of the pulsation of the carotid artery slightly paramedian. Sharp dissection was used to deepen the incision through the platysma and the superior cervical fascia and a small Weitlaner wound retractor was inserted to enhance the exposure. Haemostatic clips were used for haemorrhage control. After lateral retraction of the sternocleidomastoid muscle and identification the common carotid artery, the thick carotid sheet was dissected longitudinally to the artery, preserving vagus nerve and internal jugular vein. The carotid artery was circumferentially dissected and two ligatures (1 Vicryl Sutupak, Ethicon by Johnson & Johnson Intl, Cincinnati, OH, USA) were placed around it, the distal one pulled tight and the proximal ligature just tightened loose. An intra venous cannula was inserted and the proximal ligature tightened. The cannula was secured by the proximal ligature. Approximately three blood bags (1500 ml) of blood with a mean haematocrit of 22±3% could be retrieved before cardiac fibrillation occurred caused by exsanguinations. At this point the procedure was stopped and the life of the animal terminated by a bolus of 50ml potassium-chloride solution. In case of metabolic deterioration of the animal, due to ischemia during the procedure, a cell salvage system

was used to concentrate the blood and dismiss negative metabolic agents. The blood donated by this animal was used to prime the CPB lines or as transfusion in case of major haemorrhage.

2.7.2. The Neck and the groin

In order to place the central lines and the PiCCO-catheter the neck veins and the femoral artery were dissected as it can be seen in the Fig. 6 where the head is on the left and the rest of the body on the right. Furthermore, the endotracheal tube inserted in the tracheotomy can be seen in the upper part of the picture.

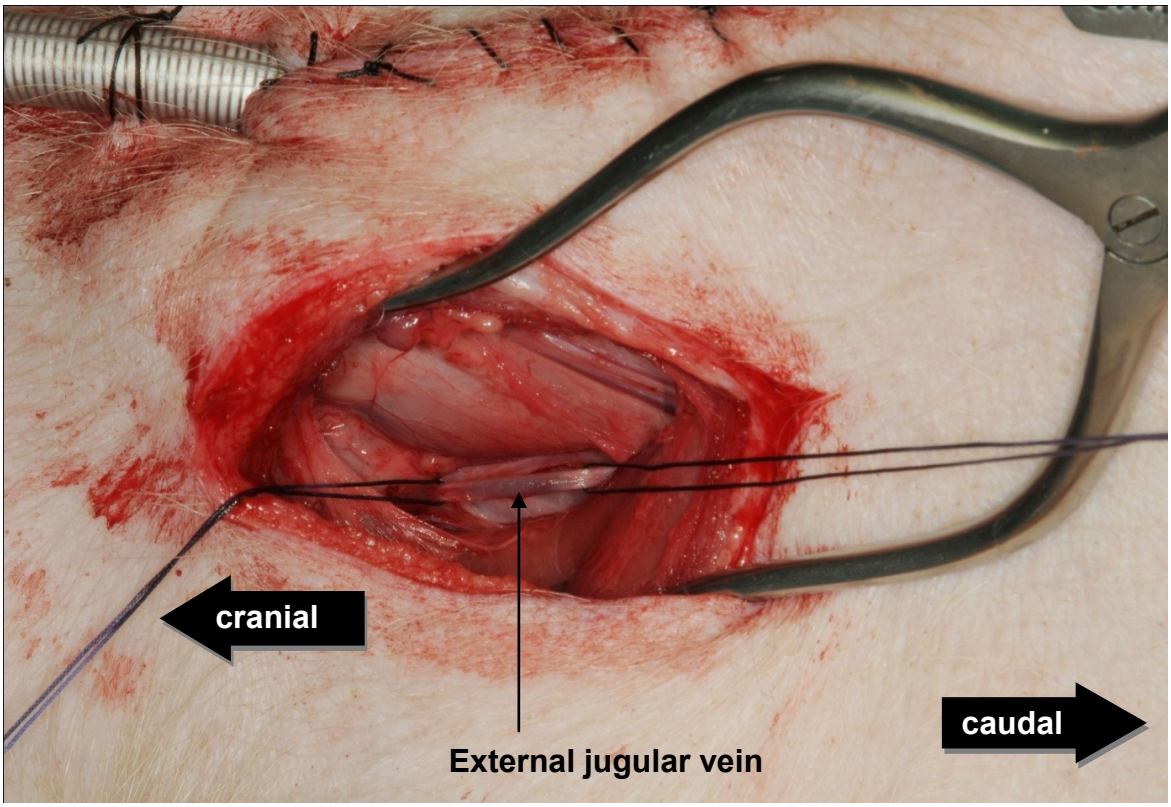

Figure 6: External jugular vein, endotracheal tube, woundretractor

The paramedian incision of approximately 5cm was made medially to the sternocleidomastoid muscle. After sharp dissection of the platysma and the external cervical fascia, the external jugular vein was easily identified. The vein is only exposed anteriorly and two Vicryl 2-0 Sutupak (Ethicon by Johnson & Johnson Intl, Cincinnati, OH, USA) ligatures were placed around with a dissecting clamp. Displacing the

sternocleidomastoid muscle by a small wound retractor anteriorly, the thick carotid sheet was dissected and the internal jugular vein could be visualized. Two ligatures were placed around this vessel. Proceeding, the proximal ligature was tightened. Using an oblique incision of the vein, a tri luminal catheter was inserted with the tip resting at the bulbus of the internal jugular vein. The distal ligature was tightened without occlusion of the catheter. The cut down of the external jugular vein was performed in the same way, with having the tight ligature placed distally and the tip of the triluminal catheter resting in the superior vena cava. The catheters were secured (at the blue part of the catheter shown in the picture below) by sutures and meticulous haemostasis was performed.

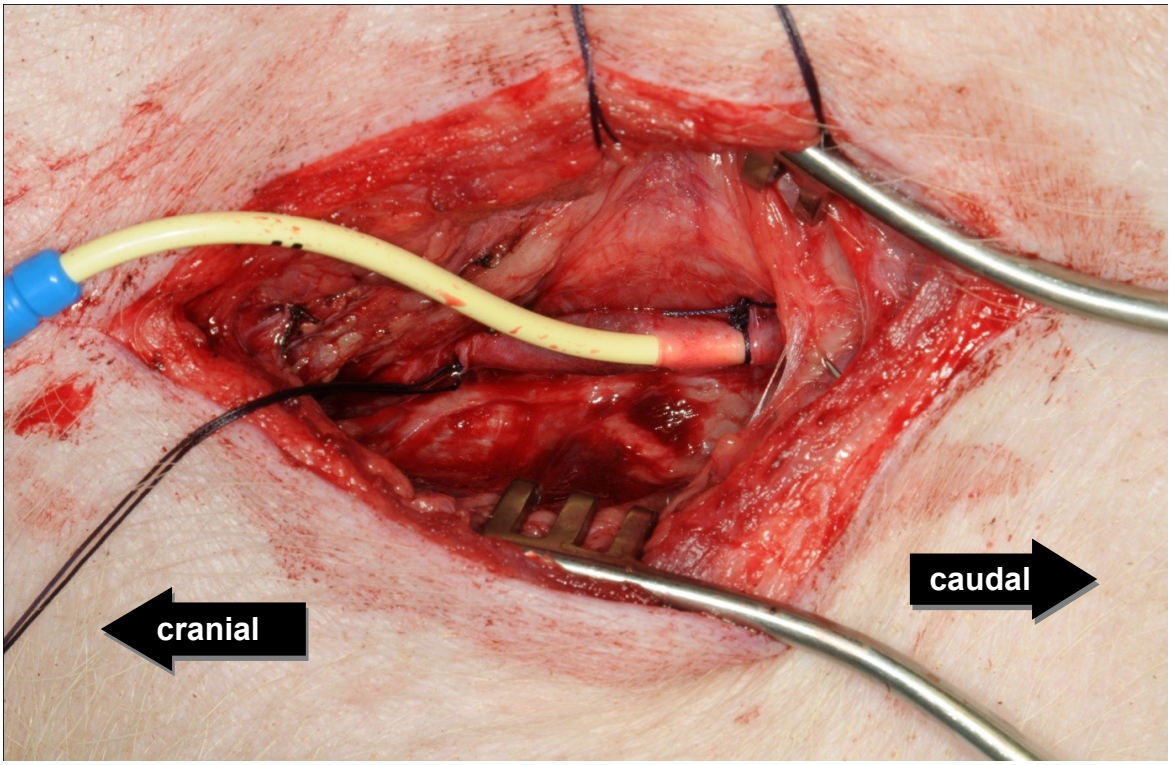

Figure 7: Triluminal catheter inserted in the external jugular vein, placed as central venous catheter

The closure of the incision was completed provisory with Vicryl 2-0 (Ethicon by Johnson & Johnson Intl, Cincinnati, OH, USA). The picture above (Fig. 7) shows the step where the catheter was inserted, the distal ligature tightened (left suture around the vessel) and the proximal only loosely knotted to prevent occlusion of the catheter. Next, arterial access was required. For that purpose the femoral arteries were exposed. After palpating

the pulse of the femoral artery in the groin, a 5 cm long longitudinal incision was performed. By retracting the sartorius muscle laterally, the neurovascular bundle was identified. Careful circumferential dissection of the artery was performed and two Vicryl 2-0 Sutupak (Ethicon™ by Johnson & Johnson, Cincinnati, OH, USA) were passed around the artery. The distal tie was tightened and the proximal placed loose. Using fine Fisher´s scissors a transverse arteriotomy was performed while occluding the artery by gentle traction of the proximal ligature. The arterial line and the PiCCO® catheter (Pulsiocath™, Pulsion Medical Systems AG, Munich, Germany) were introduced either directly or with help of a guide wire. The lines were secured and the skin closed with running sutures.

2.7.3. Sternotomy

The animal lying in supine position, the sternum was palpated. After finding the landmarks of the xiphoid tip and the sternal spike, the midline was accurately identified. A skin incision was performed from the sternal spike to the xiphoid tip and extended 3 cm over the abdomen, in the manner of a proximal laparotomy. Using electrocautery the subcutaneous layers were divided as well as the decussating fibres of the pectoralis major muscle. The midline of the sternum was carefully marked with cautery. The parts of the pectoralis major muscle originating from the sternal spike were dissected, so the spike could be amputated using a rib shears which can be seen in the picture below.

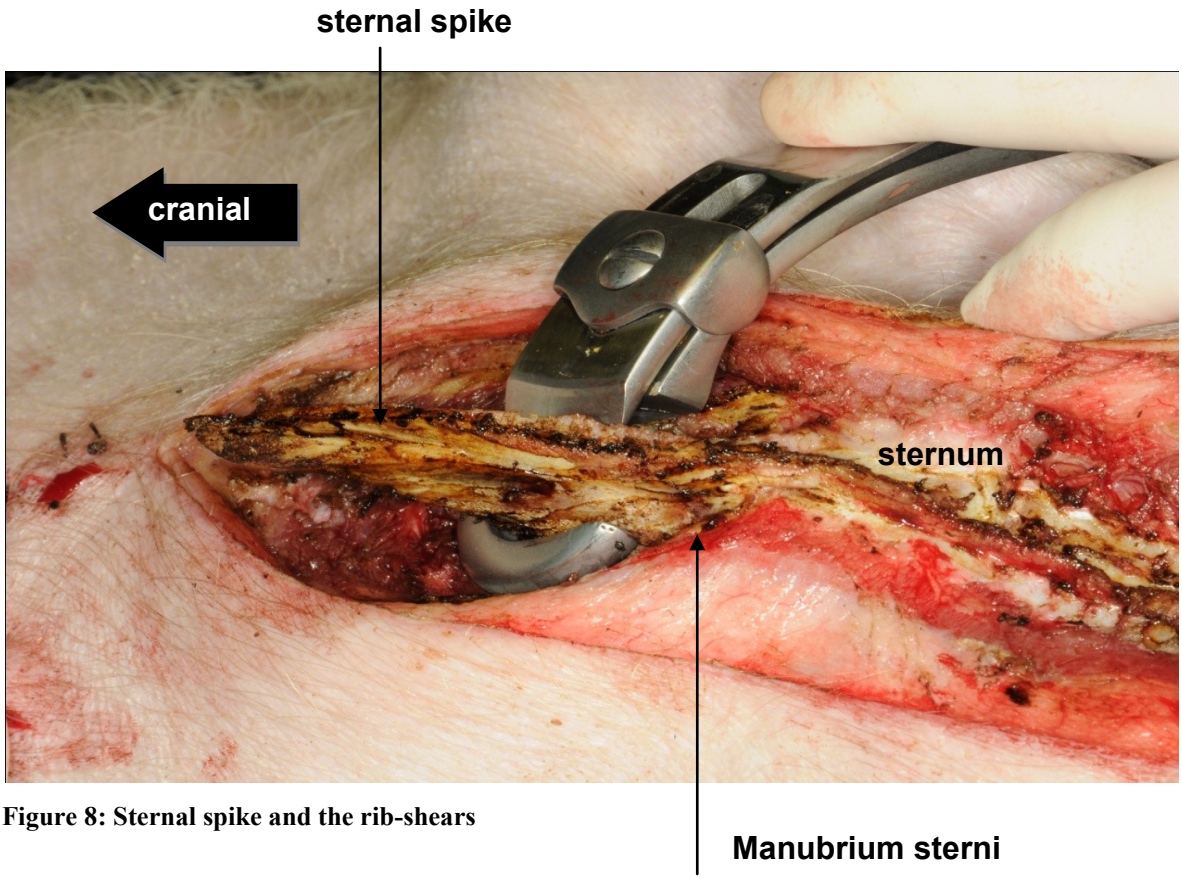

Figure 8: Sternal spike and the rib-shears

Very careful preparation of the space cranial to the sternal notch was required, because haemorrhage from the venous confluence, which is prominent in this region of the animal, was very difficult to deal with. Having the space developed, gentle blunt dissection of the retrosternal space was performed. The xiphoid tip was dissected and again the space posterior to this was developed. The distal third of sternum was divided with strong scissors. Using an oscillating saw, rather than the reciprocating one, the sternum was divided with meticulous control of the cutting depth because in swine the heart is leaning against the sternum, which is also the reason why the pericardium was often opened already during the sternotomy, as it can be seen in the picture below. The bleeding periosteal edges were carefully cauterized, as well as haemostasis of the spongiosa was achieved by bone wax. The sternum is almost divided in the picture below. Lung tissue as well as the left ventricle of the heart can be seen under the fingers of the assistant on the right side of the picture.

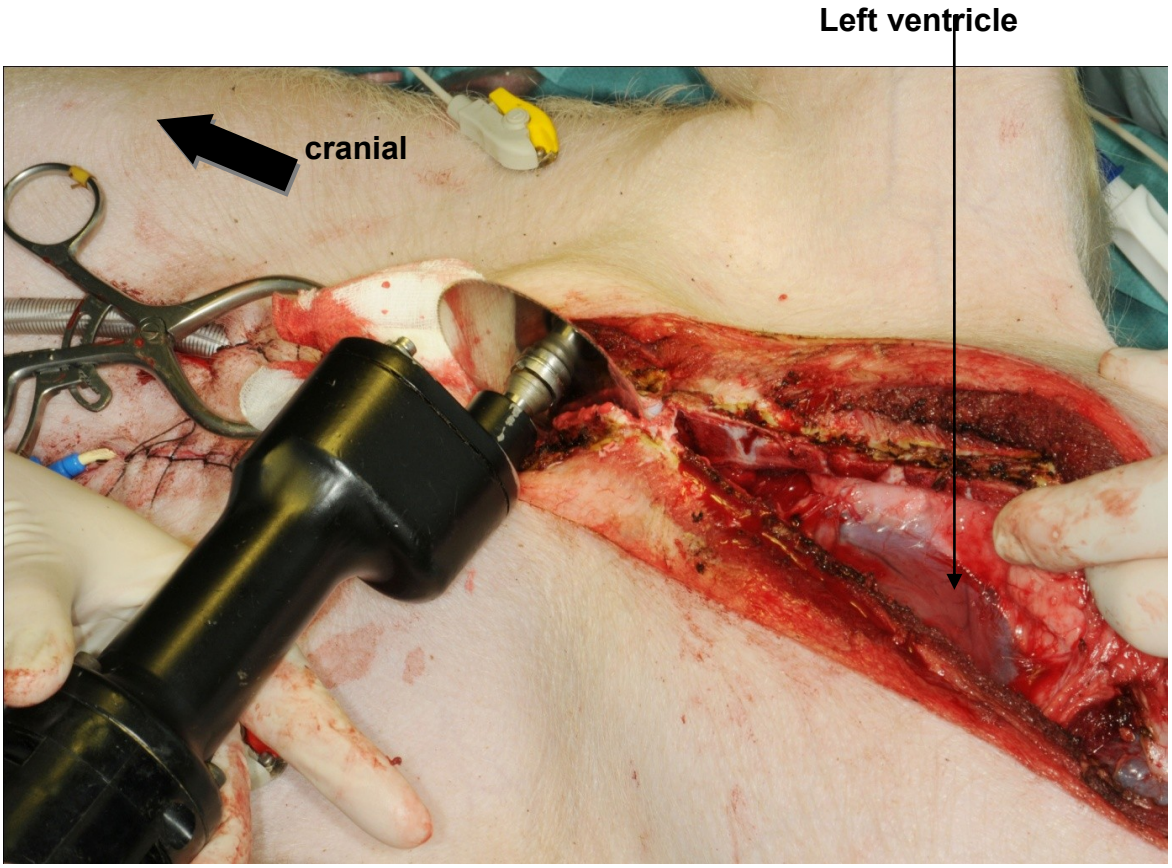

Figure 9: Sternotomy

An appropriate sternal retractor was used to gradually spread the two sternal edges. Special caution was given to the anterior mediastinal space where a partial thymectomy was carried out, the pericardium opened in usual fashion as it can be seen in the picture below and several pericardial traction sutures were placed to provide better exposure but bearing in mind not to kink big vessels. During all manipulations of the position of the heart the haemodynamic parameters of the animal were closely observed. The sternal retractor was brought into final position without compromising the vena cava superior as well as the innominate vein.

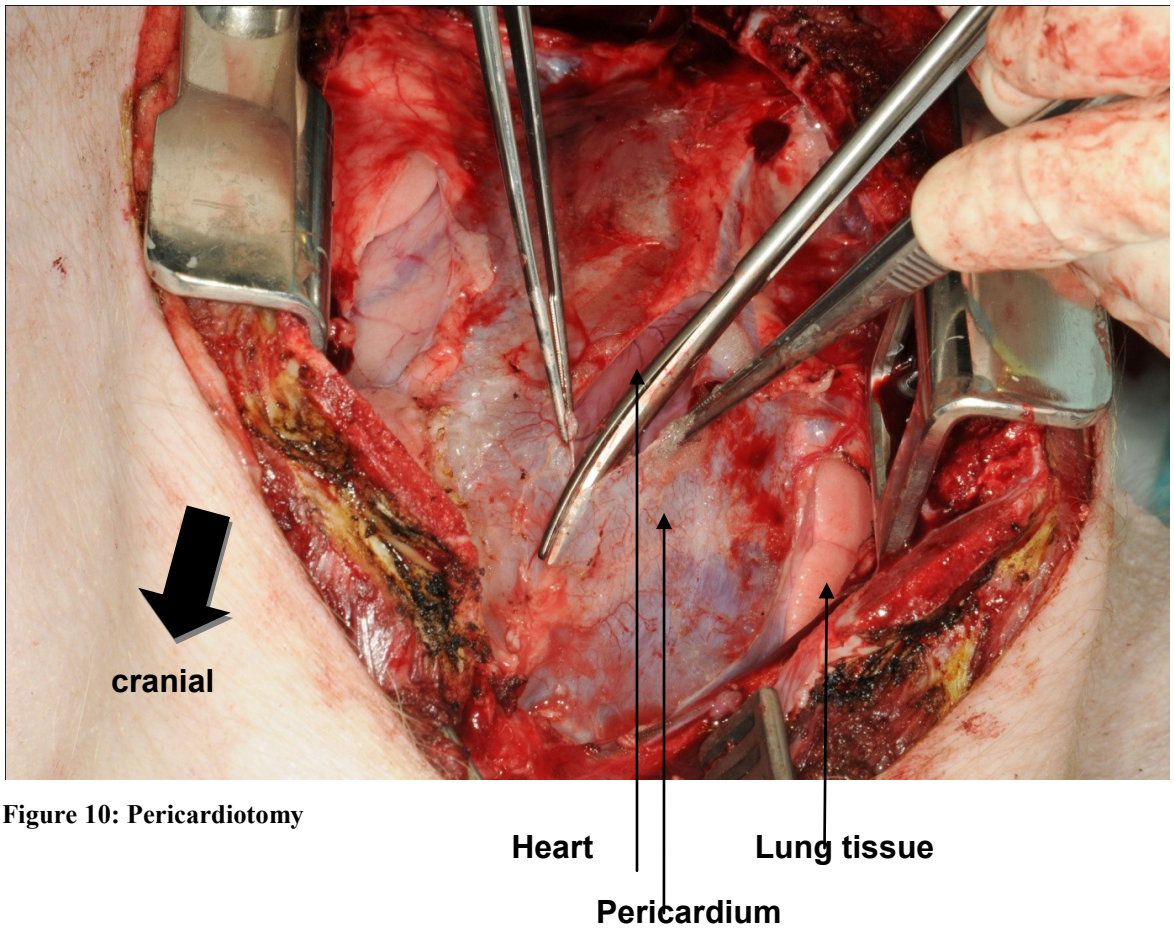

Figure 10: Pericardiotomy

2.7.4 Institution of Cardiopulmonary Bypass

2.7.4.1. Cannulation

In both groups the same cannulation technique was performed. The ascending aorta was identified, a loop placed around it and exposed for arterial cannulation. As described above, the identification of the best cannulation site of the aorta could not always be predicted, so individual intra-operative modifications were necessary without compromising the experimental protocol. For that a two purse-string suture (4-0 RB 1 Prolene®, Ethicon by Johnson & Johnson Intl, Cincinnati, OH, USA) were placed as well as three teflon pledgets each and the sutures were passed through a tourniquet. The right atrial appendage was used for venous drainage. For that the right atrial appendage was clamped with a Satinskiy clamp. Due to the requirements of the closed circuit of the mini-ECC Resting Heart System, the purse-string suture (4-0 RB 1 Prolene, Ethicon by Johnson

& Johnson Intl, Cincinnati, OH, USA) was secured also with several teflon pledgets The surgeon placing the sutures at the right atrial appendage can be seen in the picture below. The orange tubes in the lower right corner of the picture are the tourniquets for the sutures, which are pointing to the head of the animal.

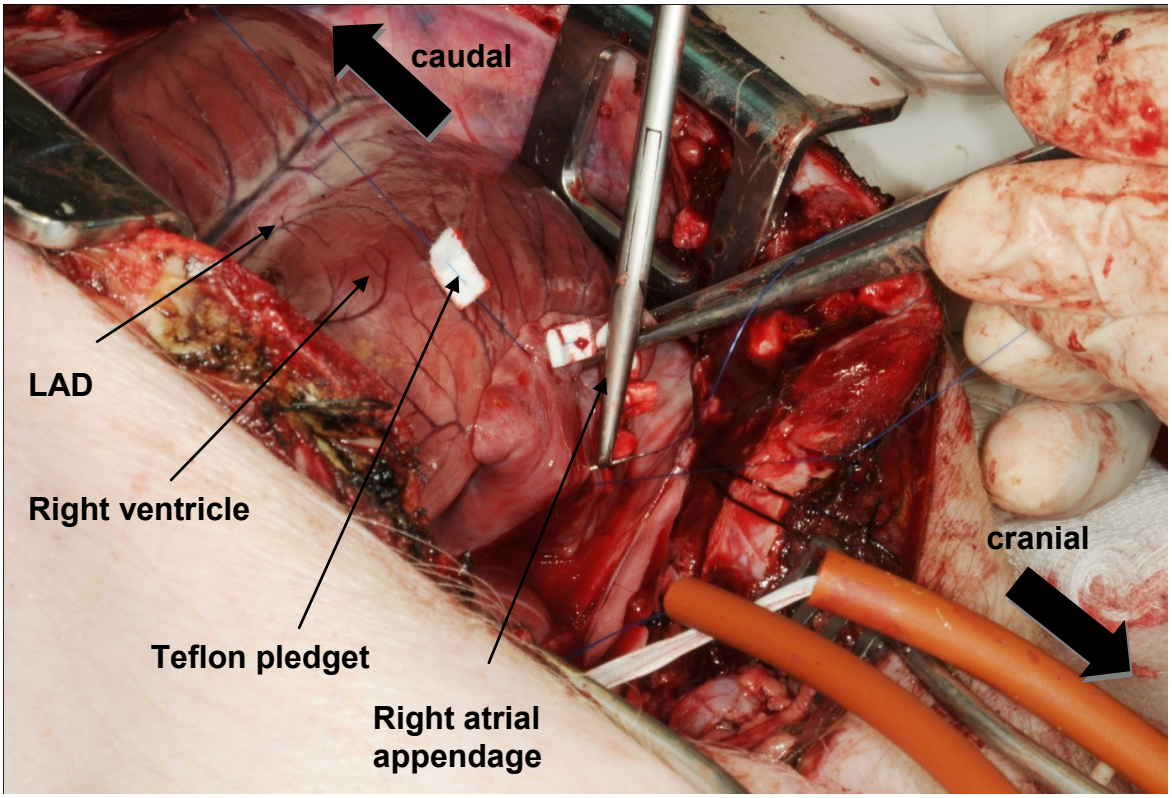

Figure 11: Right atrial appendage, cannulation sutures

At that point heparin was administered. The dose of 300 IU per kg of animals' body weight was sufficient to achieve systemic anticoagulation, which was confirmed by the "activated clotting time" (ACT® II Automated Coagulation Timer System, Medtronic, Inc, Minneapolis, MN, USA). ACTs between 400 and 500 seconds were targeted.

Having reached the targeted ACT, cannulation was continued. First, the arterial cannula was inserted. For that, an incision was made with the knife in between the purse-string suture. A diffusion tipped angled cannula (12 Fr, Medtronic Inc. Minneapolis, MN; USA) was inserted, de-aired and turned into the right position and secured. Having drawn the purse-string sutures in their tourniquets tight, the same were secured to the cannula with heavy sutures and the cannula connected to the arterial line after de-airing. The right arterial venous two-stage cannula (28 Fr, Medtronic Inc, Minneapolis, MN, USA) was inserted through the right atrial appendage into the inferior vena cava and secured in the

same way. At that point baseline (T1) measurements were taken. Finally, cardiopulmonary bypass was instituted and cooling to 32°C core temperature was performed followed by aortic cross-clamping using an aortic clamp. The picture below shows the arterial cannula placed in the ascending aorta (left upper corner). To the right from that one can see the prominent pulmonary artery. The two-stage venous cannula is placed blow that in the right atrial appendage.

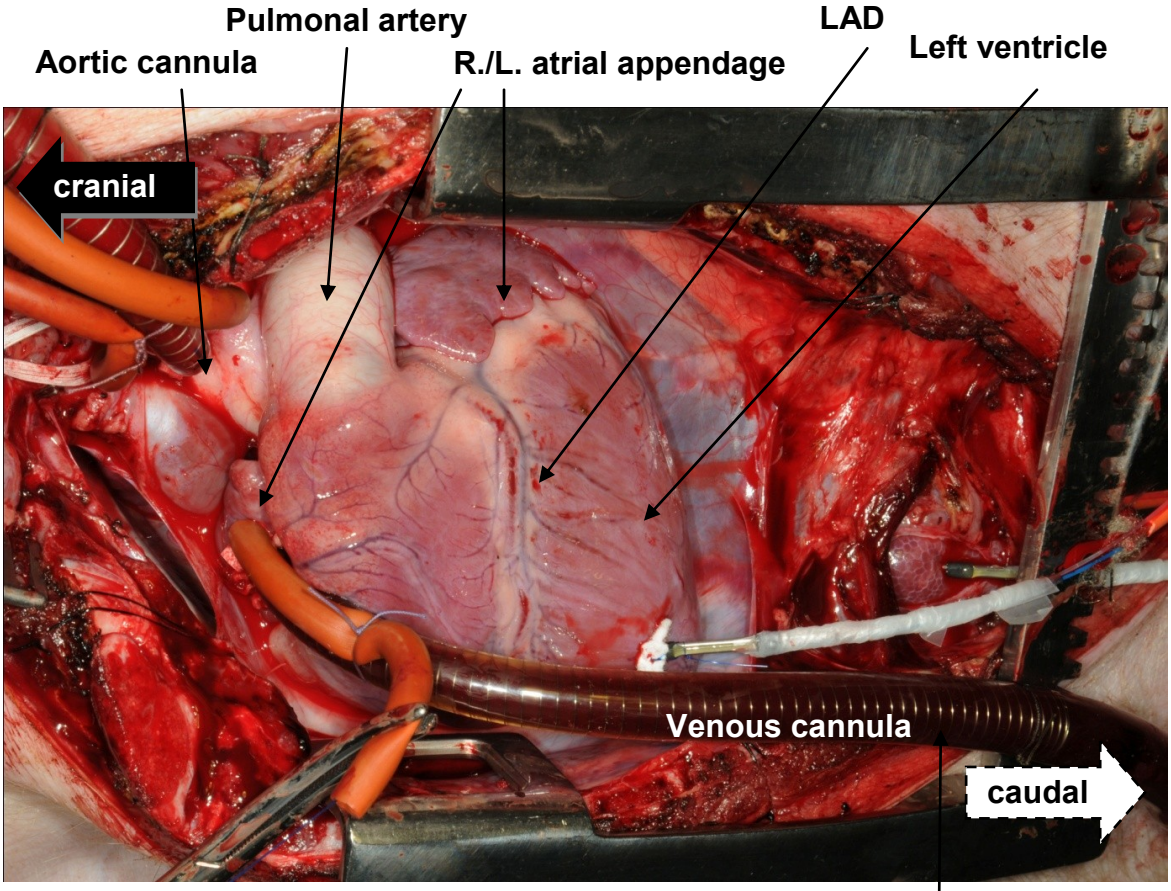

Figure 12: Operation site, cannulation, arterial and venous cannula

2.7.4.2. Cardioplegia

In both groups an antegrade cardioplegia was used. For that purpose, an antegrade aortic-root cannula was placed high in the ascending aorta, still leaving enough space to place the aortic clamp. A 4-0 mattress suture (4-0 Prolene®, Ethicon by Johnson & Johnson, Cincinnati, OH, USA) with a tourniquet was used to secure this cannula. 500ml of crystalloid St. Thomas cardioplegia solution, cooled to 5°C, were delivered at a rate of 200

ml/min and a pressure not exceeding 70 mmHg. Cardiac arrest was confirmed visually by the surgeon as well as by the ECG. If spontaneous heart action was detected during the defined clamping time, additional cardioplegia was delivered, which occurred only in two experimental animals.

2.7.4.3. Group A: mini- ECC

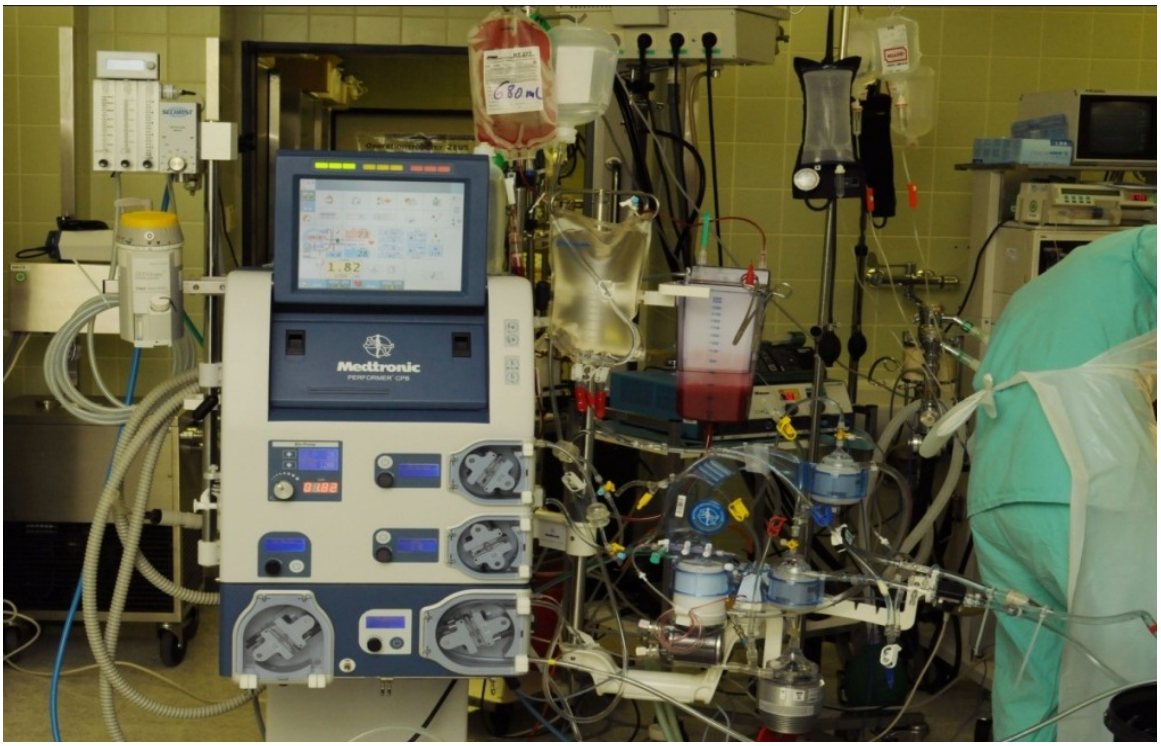

Figure 13: mini-ECC: Performer console® and Resting Heart System®

According to randomization, for animals in group A the mini-ECC Resting Heart System® (RHS®; Medtronic™, Inc, Minneapolis, MN, USA) in connection with the Performer® CPB console (Medtronic™, Inc, Minneapolis, MN, USA) was used (both can be seen at the picture above, figure 6). Resting Heart System is an integrated, low-prime arrested, semi-closed loop CPB-System offering minimal air blood interface and elimination of anti-foam agents. RHS provides a membrane surface area of 2.5m^2 and keeps the priming volume as low as 1000 ml. Primary blood surface areas are coated with a bio-compatible coating (Carmeda® Bioactive Surface, Carmeda, Stockholm, Sweden). Furthermore, the RHS can provide a blood flow from 1 to 6 litres per minute as well as a second circuit for venting the heart. This blood is then re-infused into the main circle. The absence of a

cardiotomy reservoir limits the foreign surface area. Cardiotomy suction was excluded in this group, so an erythrocyte salvage system was used instead (Cell saver®, Medtronic™, Inc, Minneapolis, MN; USA).

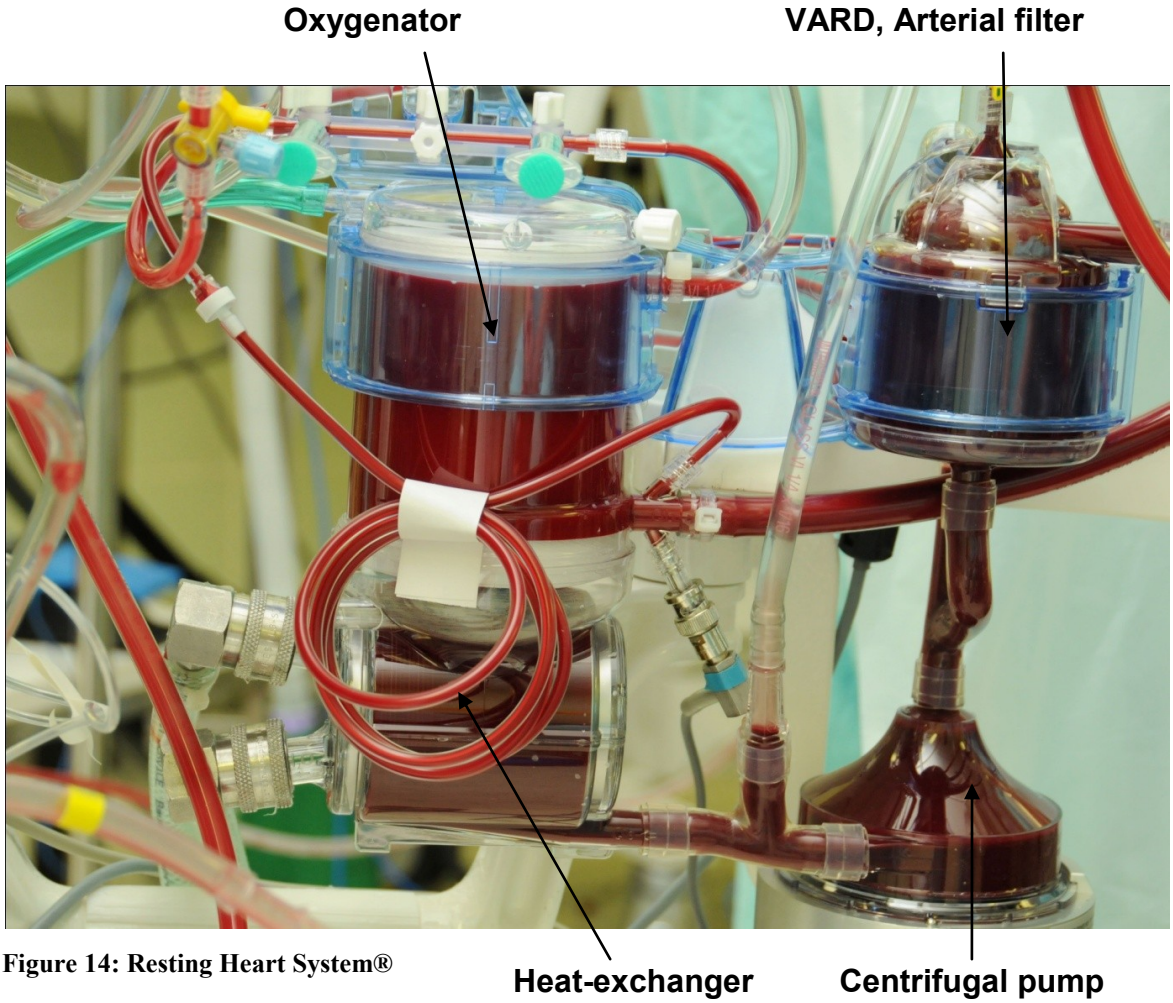

Figure 14: Resting Heart System®

Because of the closed circuit nature of this system a special device is used to detect and exclude bubbles. Two pairs of ultrasonic fluid sensors detect air in the Carmeda® Affinity® venous air removal device (VARD) which is coming from the venous line. If air enters the VARD an evident signal is given and the air is automatically removed in the upper part of the device. A heat-exchanger and a centrifugal pump are also connected into the circuit. As described above cannulation was performed for both groups in the same fashion. Cardiopulmonary bypass was maintained for 60 minutes and measurements were recorded every 15 minutes according to study protocol. After 45 minutes re-warming was initiated.

2.7.4.4. Group B: conventional CPB

In group B a conventional cardiopulmonary bypass system was used. The priming volume was precalculated with 2000mls. Standard lines without heparin coating were used; a matched oxygenator (Dideco D905 EOS, Sorin Group, Modena, Italy) and a standard roller pump (Stöckert CPB, Sorin Group, Modena, Italy) were included also using standard arterial filters, intrapericardial suction and a cardiotomy reservoir. All this components can be seen in the picture below

Figure 15: conventional HLM

2.7.4.5. Reperfusion and off-bypass observation period

After 45 minutes of cross-clamp time the animals were gradually re-warmed to 37°C (the arteriovenous gradient never exceeded 10°C to prevent bubble formation). Cooling and heating was accomplished by the heat exchanger in the bypass circuit. 60 minutes after institution of cardio-pulmonary bypass the aortic clamp was released. In 12 experimental animals heart action was restored spontaneously, only in 2 animals the ventricular fibrillation was terminated by delivery of charge of 5-20 Joule using the internal paddles and a defibrillator (Lifepak TM 9 B, Medtronic Physio-control, Medtronic, USA). Lidocaine and Dobutamine were used after defibrillation. In case of bradycardia or atrio-ventricular conduction block an epicardial pacemaker was brought in position. After 30 minutes of reperfusion, termination of cardio pulmonary bypass was initiated. For that reason, manual ventilation and oxygenation was started already few minutes before. After adjustment of the rate and rhythm, partial occlusion of the venous line started to fill the heart. At the same time the arterial flow was reduced so the heart could start to eject. If the arterial blood pressure could be maintained around 50mmHg, the venous line was occluded completely and the arterial pump stopped. The venous cannula was removed and the purse string suture tightened in its tourniquet. At this point the systemic anticoagulant effect of heparin was neutralized by administration of Protamine, using the standard dose of 1mg of Protamine per 100U of total heparin dose. The arterial cannula was left in its position for volume replacement. After termination of the cardiopulmonary bypass the animal was observed for additional 30 minutes where metabolic data and haemodynamical parameters were controlled closely and if required corrected. At the end of the off-bypass observation period the experiment was terminated and the animal was sacrificed by intra-aortic infusion of 80- 100 ml of KCL solution through the cardioplegia cannula.

2.8. Measurements

2.8.1. O_2/CO_2 Opto-chemical sensors: placement of the opto-chemical sensors

In order to standardize the measurements, the opto-chemical sensors were tested in all positions of in preliminary experiments.

After shaving the forehead, placing the BIS® electrodes and covering by op-site adhesive dressings, the right place of the trepanation was marked. As it can be seen in the picture, the trepanation hole was made over the left parieto-temporal lobe. A neuro-surgical drilling device was used to drill the skull. To maintain always the same position of the sensor a neurosurgical stereotactical method was used. The sensor was inserted intracranially in a depth of two centimetres and secured by heavy sutures. Precise haemostasis was performed. Caution was given to the positioning of the head in supine position to prevent kinking of the optical cable which would have destroyed the sensor.

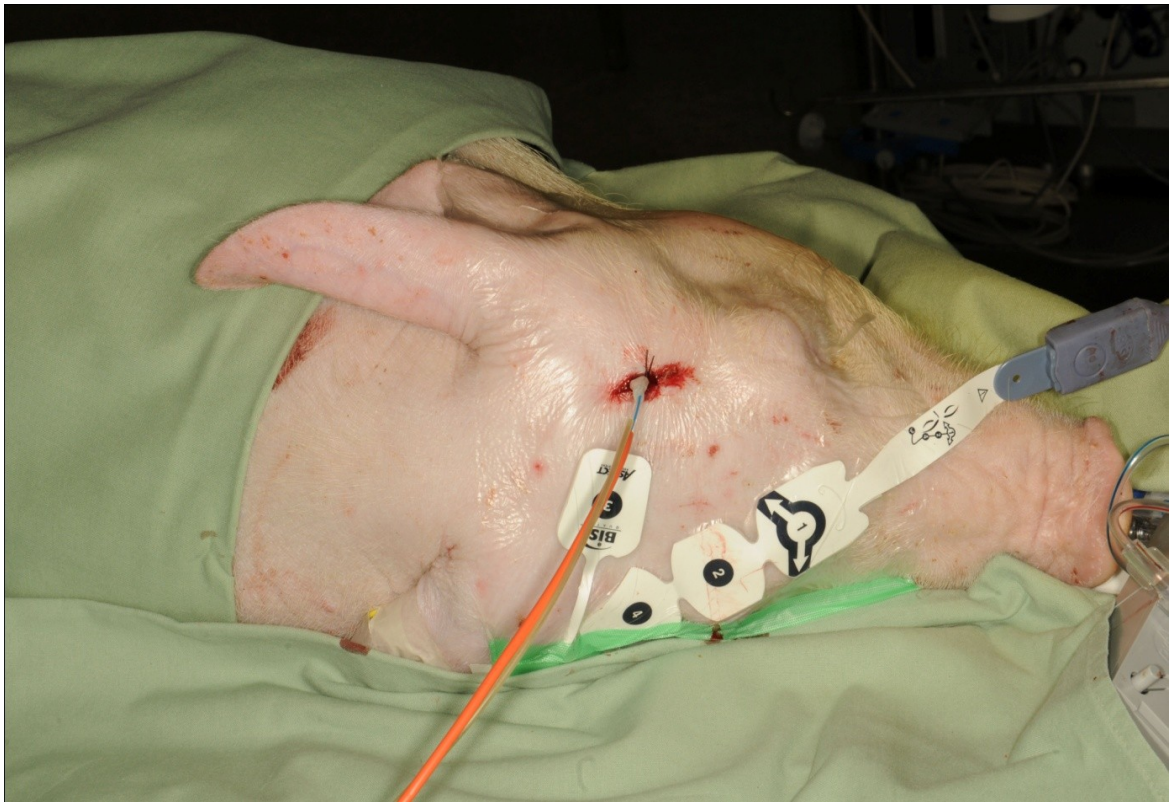

Figure 16: opto-chemical probe and BIS

The myocardial sensors were constructed slightly differently. The tip was only 0.5- 1 centimetre long in order to be sure that it is placed in the myocardium and not penetrating into the left ventricle. For that, a 5-0 mattress suture (Prolene®, Ethicon by Johnson & Johnson, Cincinnati, OH, USA) was placed at the apex, the sensor covered by a needle (22G) stabbed into the myocardium and then secured by the suture.

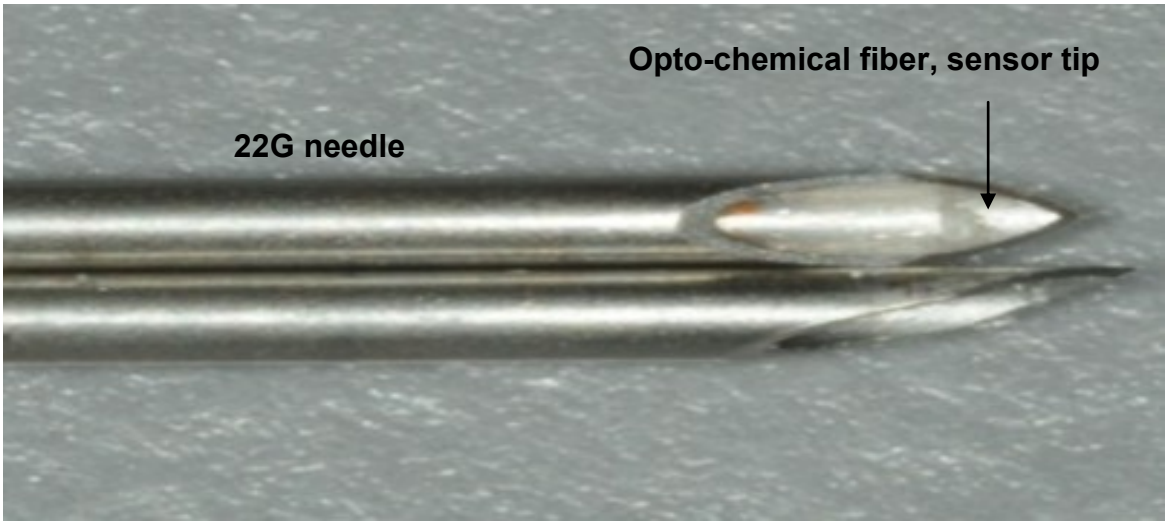

Figure 17: The tip of the opto-chemical sensor, brain.

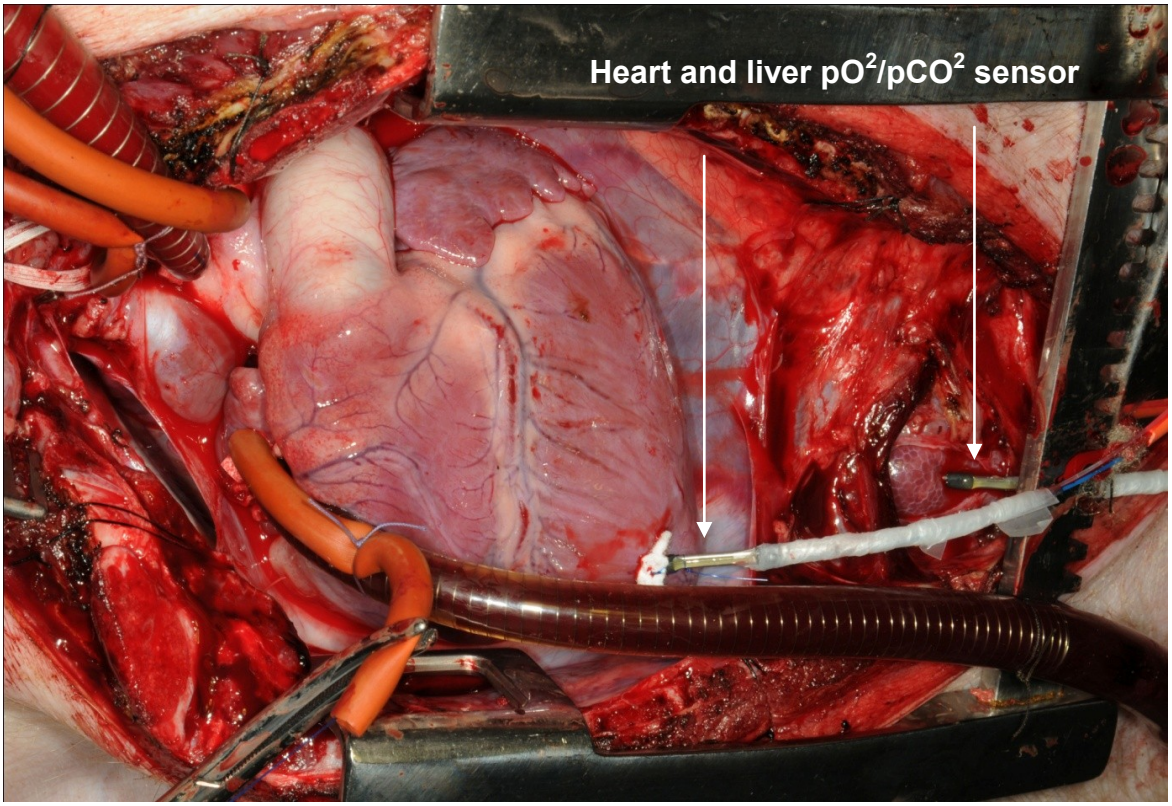

Figure 18: Sensors in heart and liver

Finally, a proximal laparotomy was performed and the hepatic sensor was brought in position by inserting the optical fibres into the right hepatic lobe. In the picture above (Fig.18) the sensor can be seen at the right side below the sternal retractor. Sensors were secured to any stable and bordering structure, mostly the xiphoid tip, to prevent dislocation. Convincing oneself that no bleeding was caused by placing the hepatic sensor was crucial because after full heparinisation haemorrhage from the liver could have been catastrophic. The opto-chemical sensors were connected to the detecting devices placed next to the operating table as seen in fig. 19 as well as in fig. 2.

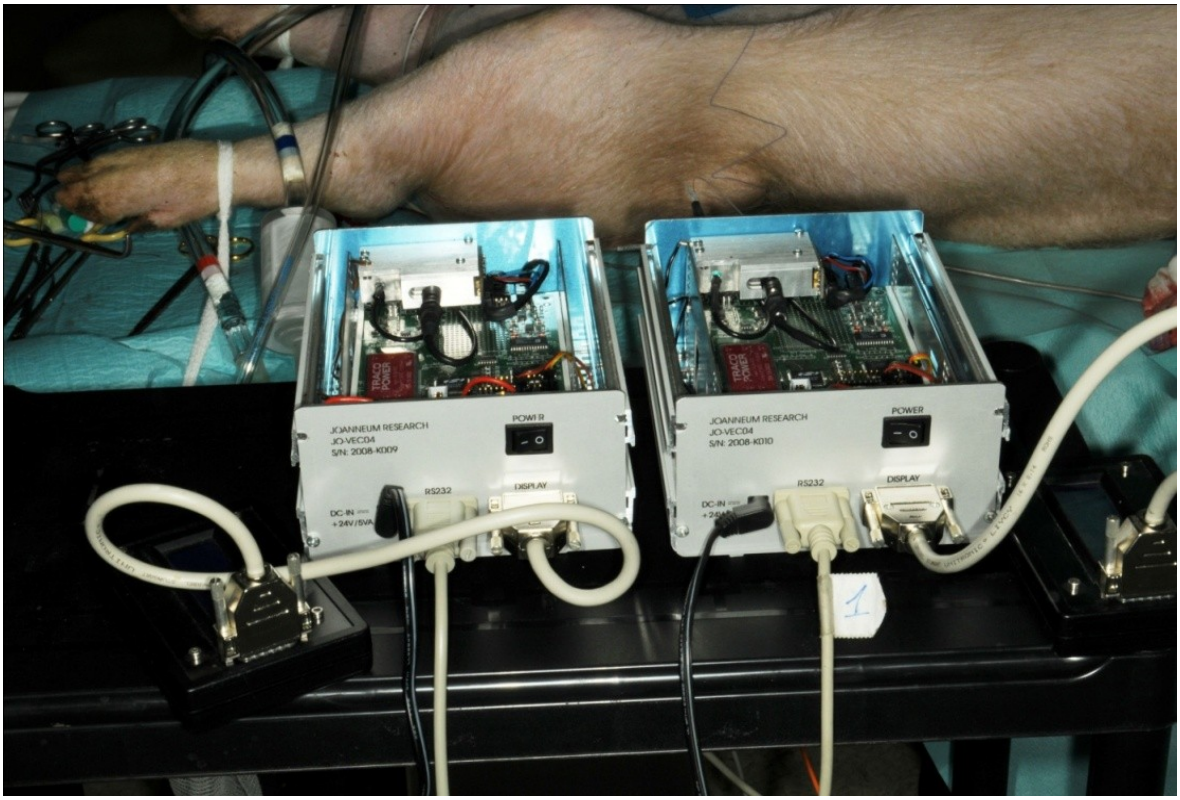

Figure 19: Fibre-optical detection devices

They were also connected to a computing system placed in the operating room where online and continuous measurement of the parenchymal pO_2 and pCO_2 was conducted.

Figure 20: The calibration laboratory next to the operating room. The computing system which was transfered to the operating theatre after calibration.

The opto-chemical sensors were produced and the measurements of the pO_2 and pCO_2 were performed with help of the Joanneum Research team

2.8.2. Haemodynamic variables

Measuring the haemodynamic variables was achieved by the PiCCO-catheter (Pulsiocath^{TM}, Pulsion Medical Systems AG, Munich, Germany) placed in the femoral artery as described above and connected to the PiCCO Plus Monitor (Pulsion Medical Systems AG, Munich, Germany). The circuit contained also a central venous line with the injectant temperature sensor housing (Pulsion Medical Systems AG, Munich, Germany). The PiCCO technology is based on transpulmonary thermodilution. In order to gain deep insight into the physiological and pathophysiological changes during the whole procedure, recording of cardiac index (CI), stroke volume (SV) and systemic vascular resistance (SVR) was necessary. On the other hand, these variables were used to perform a high quality perfusion of the animal during the procedure and to be able to react on time if changes occurred.

2.8.3. Inflammatory markers:

Blood samples of arterial blood were taken from every animal at the initiation of CPB as well as at the end. All samples were brought to the Institute of Nuclear Medicine at the University of Medicine, Graz. It was arranged that inflammatory markers would be determined. TNF-α, IL-1β, IL-6 and IL-10 should be assayed in quantitative sandwich ELISA.

2.8.4. Metabolic variables

At time points defined in the study protocol venous and aterial blood was sampled and analysed on-site using the GEM Premier 3000 TM System (Instrumentation Laboratory Company, Lexington, MA, USA). At each time point three samples of blood were taken, two venous and one arterial. For each sample a fresh syringe was used. One was taken from the central line resting at the bulbus of the jugular vein, one from the superior vena cava and the third sample from the femoral artery. All samples were immediately processed.

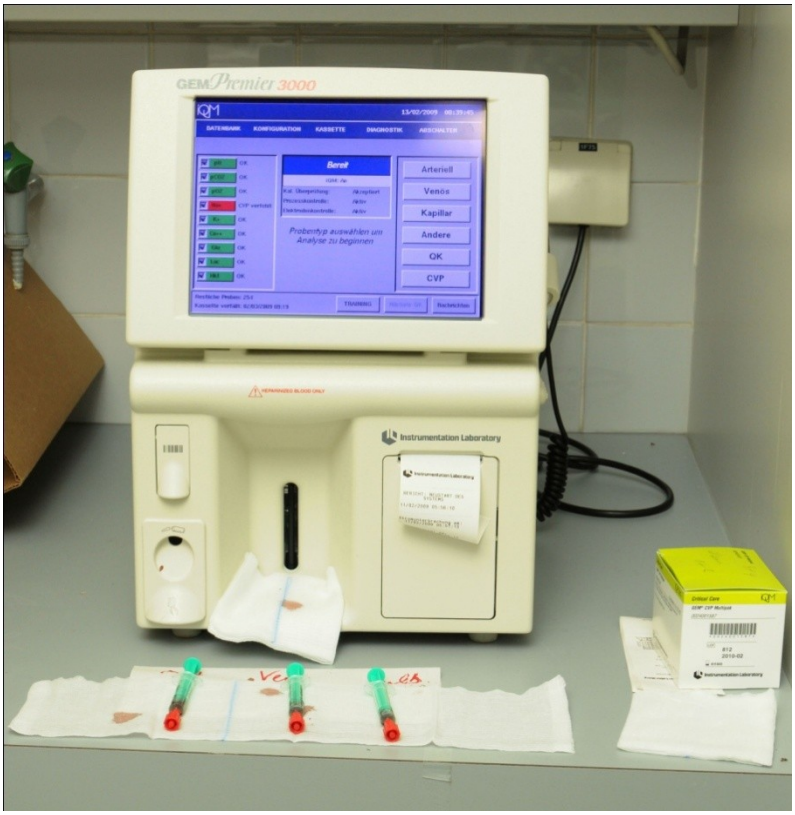

Figure 21: On-site gas check

2.9. Study protocol

Haemodynamics, blood gases, haematocrit and other red cell parameters, parenchymal pO_2 and CO_2 metabolism variables and temperature were recorded online and analysed at the following time points (T_X):

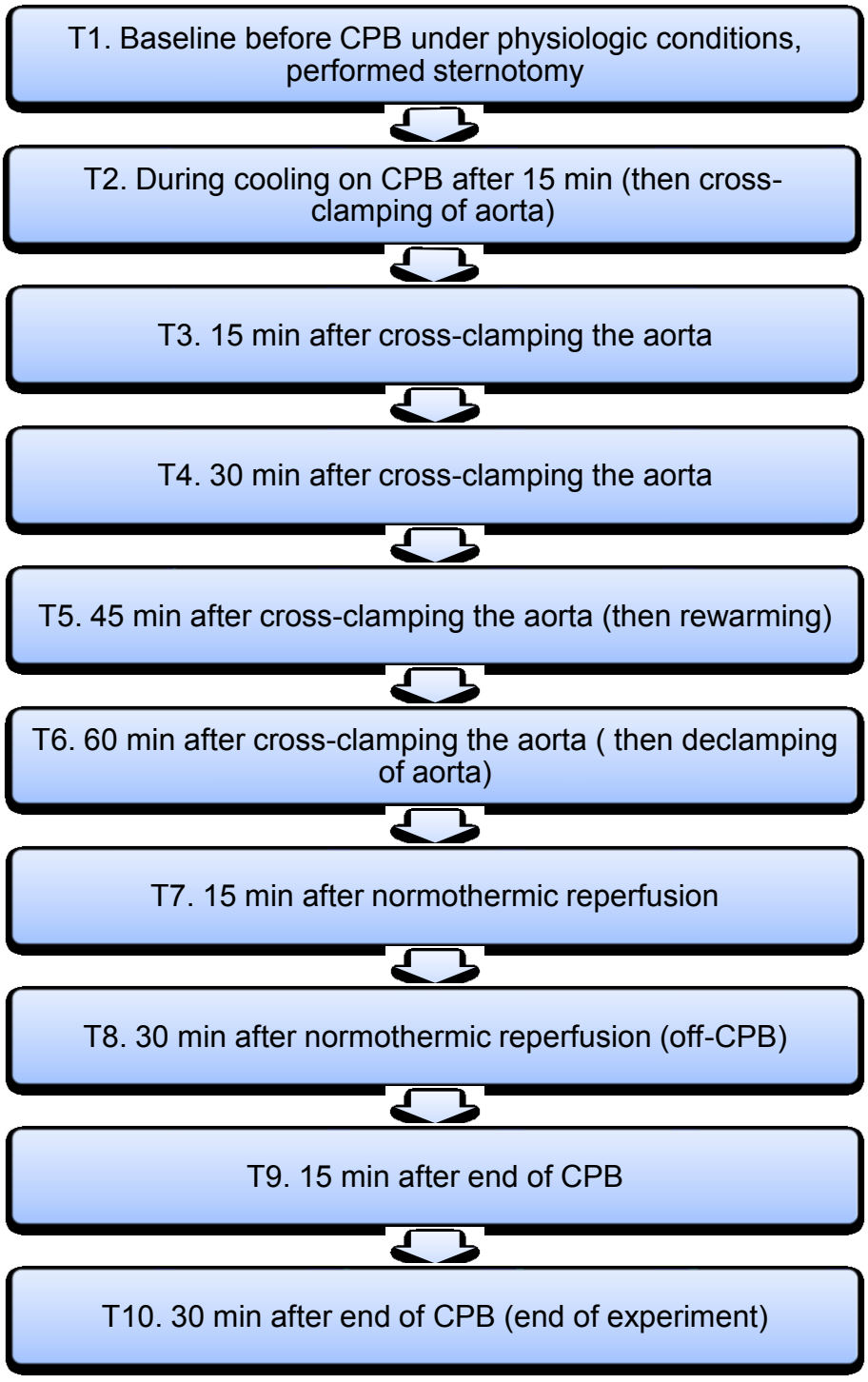

Figure 22: Study protocol

2.10. Statistical Analysis

Obtained data were entered in an Excel spreadsheet and further statistical analyses were performed using SPSS® software (version 15.0; SPSS Inc, Chicago, IL).All data are presented as mean values ± SD and as median values (25th percentile, 75th percentile). Subject characteristics and baseline data between the two groups were compared by two-sample reports for quantitative variables (i.e. Mann-Whitney U/Wilcoxon Rank-Sum tests for difference in medians with respect to Kolmogorow-Smirnow test for different distributions). To compare time and group-dependent differences in haemodynamics, blood gases and continuing organ-specific pO_2 and pCO_2 variables a repeated two-way analysis of variance (ANOVA) model was used. Correlation of the variables were analysed with Pearson- and with Spearman correlation analysis. Only p-values less than 0.05 were considered statistically significant [49].

3. Results

All experimental animals were operated and observed as it was defined in the study protocol. There were no animals which deceased before the end of the observational period. The results are presented as comparison of the two groups to each other. When data are presented graphically, the abscissa shows the measuring time points as stated by the study protocol and the ordinate the respective variables with the corresponding units. Data presented are means ± standard deviation. The data collected from the opto-chemical probe measurements of specific tissue (pO_2, pCO_2) showed high variance of the initial values, so the parameters were normalized to the initial values at a level of 100%.

3.1. Baseline Measurements

Table 1: Demographic and haemodynamic baseline variables

Variable:	Group A_conv. CPB (n=7):	Group B_mini ECC/RHS (n=7):	Probability:
Mass: kg	30.1 ± 2.6	31.2 ± 3.8	n.s.
BSA: m^2	0.8 ± 0.1	0.8 ± 0.1	n.s.
Pump flow: mls/min	2565.4 ± 126.1	2623.8 ± 179.2	n.s.
Priming volume: mls	1928.5 ± 188.9	964.2 ± 94.4	$p < 0.00001$*
Cardioplegia: mls	500.1 ± 57.7	500 ± 57.7	n.s.
HR: beats/min	78.2 ± 15.2	83 ± 7.2	n.s.
MAP: mmHg	65.7 ± 13.1	72.8 ± 11.8	n.s.
CVP: mmHg	11.5 ± 3.1	8.29 ± 2.6	n.s.
SVR: dyn*sec/cm5	1573.6 ± 576.8	1214.1 ± 404.6	n.s.
SVV: %	10.9 ± 5.9	15 ± 5.7	n.s.
Hct: %	28.3 ± 5.09	29 ± 4.6	n.s.
Hb: g/dl	8.9 ± 1.7	9.1 ± 1.4	n.s.
Temperature: °C	35.4 ± 1.2	36.2 ± 0.5	n.s.

Data are presented as mean ± SD.
miniECC=minimal extracorporeal circulation, RHS= Resting Heart System®, BSA=body surface area, HR=heart rate, MAP=mean arterial pressure, CVP=central venous pressure, SVR=systemic vascular resistance, SVV=stroke volume variation, Hct=haematocrit,

Baseline demographic and hemodynamic variables are shown in Table 1. The measurements include variables retrieved directly from the animal, measured by the PiCCO^{TM} or the GEM Premier 3000^{TM} System. Baseline variables of blood gases are shown in Table 2. Both groups were absolutely comparable except for a statistically significant difference in priming volume (1928.57 mls±188.98 mls in group A vs 964.29 mls±57.74 mls in group B, $p< 0.00001$*).

Table 2: Blood gas baseline variables:

Variable:	Group A_conv. CPB (n=7):	Group B_mini ECC/RHS (n=7):	Probability:
Arterial			
pH-a	7.5 ± 0.1	7.5 ± 0.1	n.s.
paO_2: mmHg	149.4 ± 48.7	173.3 ± 79.4	n.s.
$paCO_2$: mmHg	38.6 ± 5.5	39.6 ± 3.5	n.s.
saO_2: %	98 ± 3	99.4 ± 0.5	n.s.
Lactate-a: mg/dl	1.8 ± 1.2	1.7 ± 1.2	n.s.
Venous			
pH-v	7.5 ± 0.1	7.4 ± 0.1	n.s.
pvO_2	47.2 ± 20.6	41.9 ± 13.8	n.s.
$pvCO_2$	42.9 ± 3.8	45.9 ± 5.7	n.s.
svO_2	75.6 ± 29.4	75.6 ± 10.6	n.s.
Lactate-v	1.76 ± 0.4	1.8 ± 1.3	n.s.
Bulbo-venous			
pH-b	7.4 ± 0.1	7.4 ± 0.1	n.s.
pbO_2	51.7 ± 25.5	51.3 ± 36.7	n.s.
$pbCO_2$	45.6 ± 4.1	47.6 ± 7.2	n.s.
sbO_2	83.3 ± 12.9	74.71 ± 14.65	n.s.
Lactate-b	2.1 ± 0.8	2.1 ± 1.1	n.s

3.2. Haemodilution and Haematocrit

The main goal was to keep the haematocrit higher than 20% throughout the entire procedure (see Fig.22).

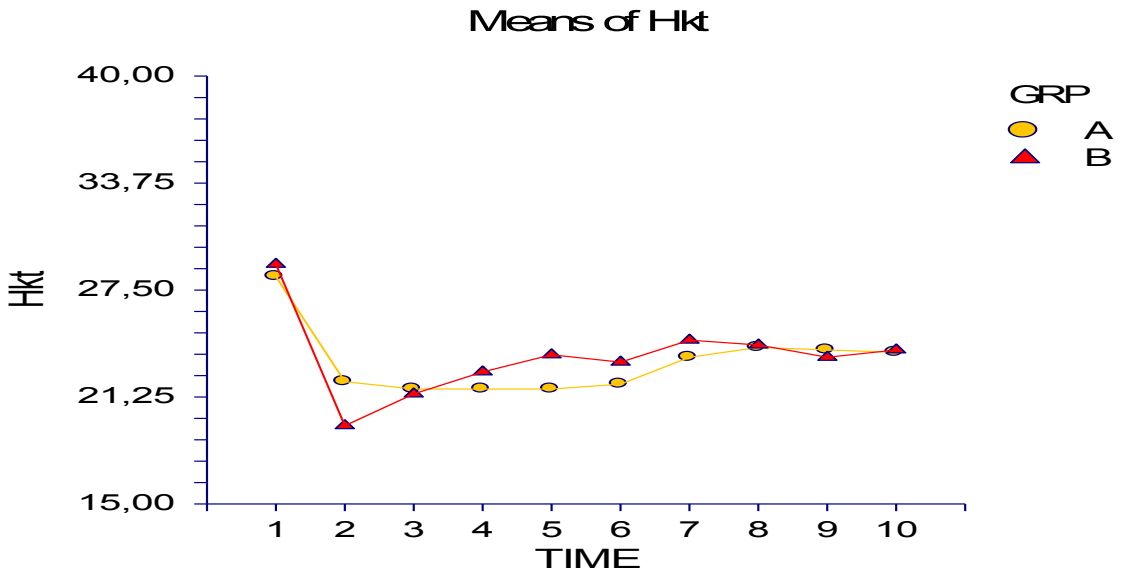

Figure 23: Means of haematocrit

An obvious drop of the haematocrit curve as well as of the curve of the haemoglobin is due to the installation of the CPB between the time point 1 and 2 and the consequent hemodilution of the animal. In order to keep the haemoglobin and haematocrit constant , a total demand for blood transfusion was excessively higher in group A (1000±823 mls vs 50±36 mls in group B, p<0,00001*).

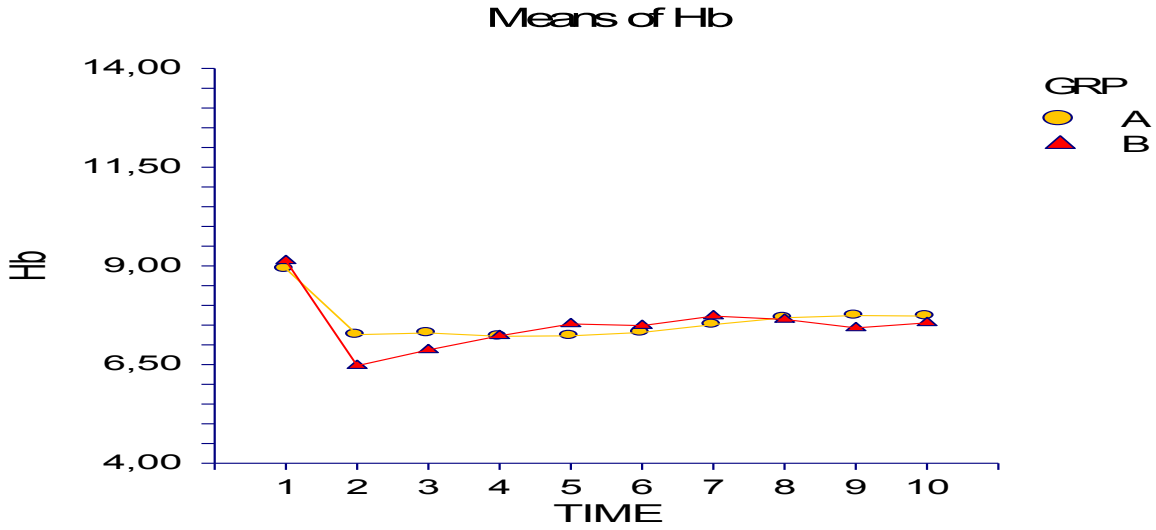

Figure 24: Means of haemoglobin

3.3. Arterial Lactate

ANOVA of arterial lactate for the factor interaction of groups/time also showed a clear tendency without being significant (Fig. 24).

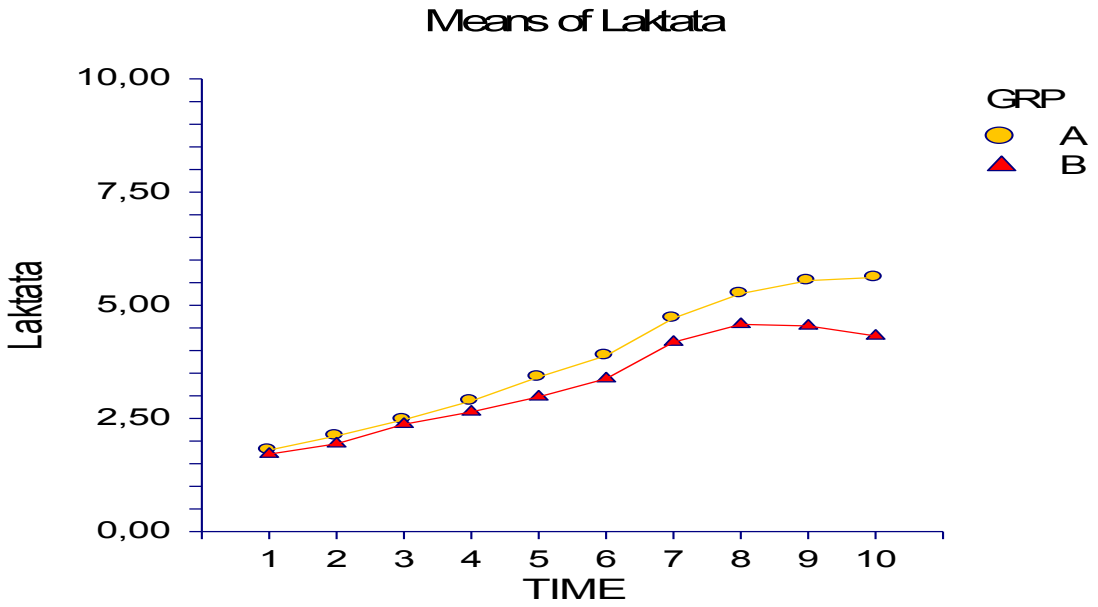

Figure 25: Means of arterial lactate

3.4. Tissue Derived Partial Pressure of O_2/CO_2

3.4.1 The heart

Differences in ANOVA concerning myocardial pO_2 (Fig. 25) could not be found, even though immediate reactions at time points T_2/T_3 (15 minutes after crossclamping of aorta and delivery of crystalloid cardioplegia) and T_6/T_7 (15 minutes after declamping of aorta and reperfusion) turned out to be very impressive.

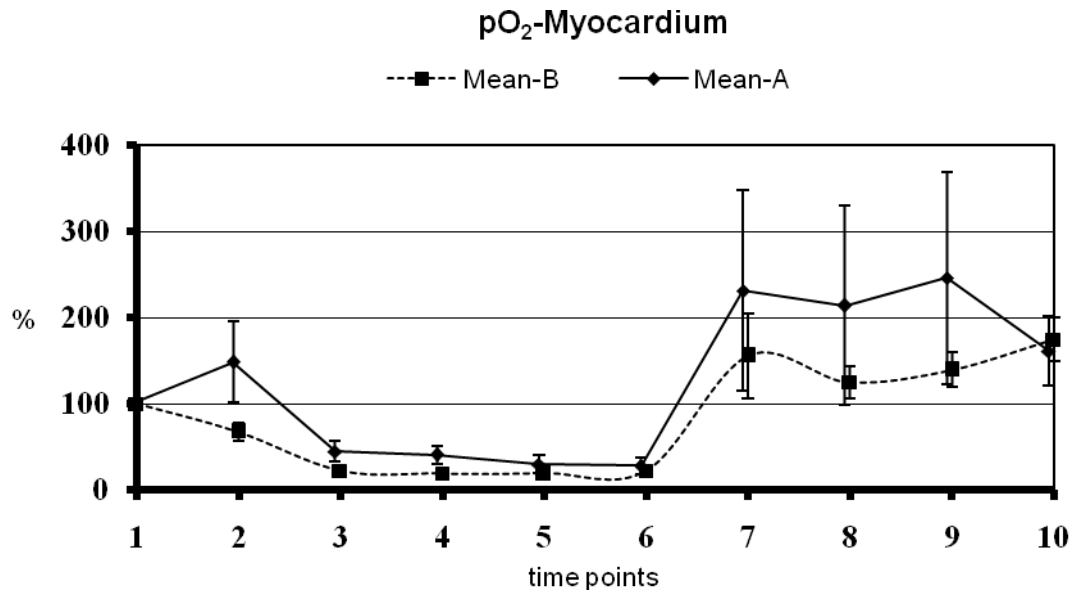

Figure 26: pO_2- Myocardium

Same pattern, only in the opposite direction, could be recognized in case of the distribution of the pCO2 in the heart muscle measured over time; nevertheless no significance was found (Fig. 26).

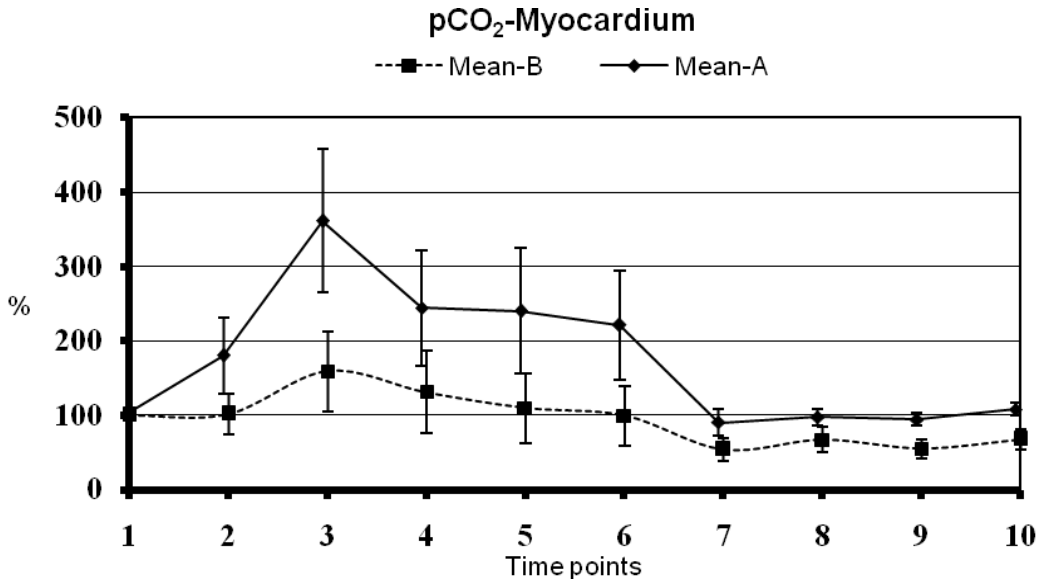

Figure 27: pCO_2- heart muscle

The liver pO_2 curves of both groups showed similarities, without being significant. The pO_2 of the liver stayed quite constant throughout the whole procedure, but showed a tendency at the end of the experiment.

3.4.2 The Liver

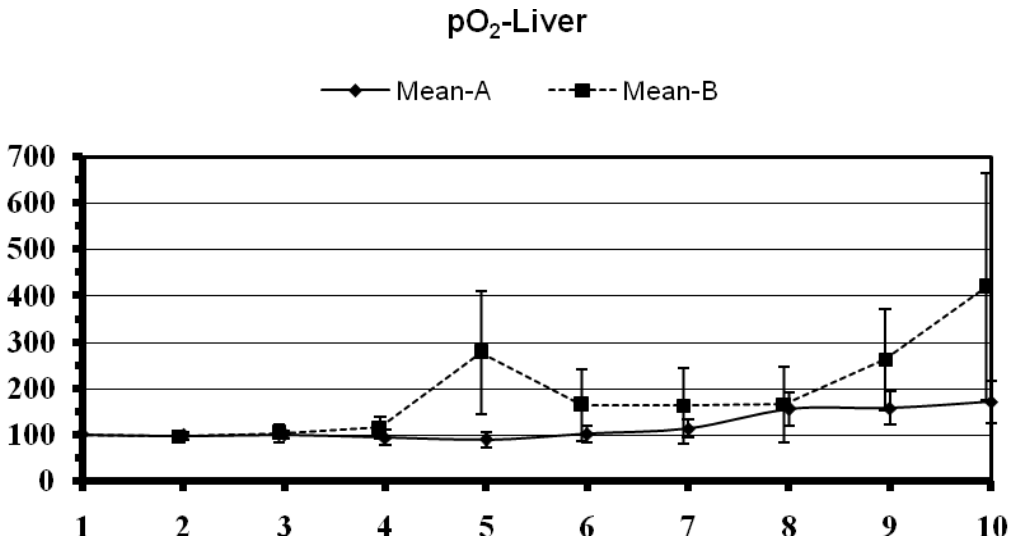

Figure 28: pO_2 of the liver

Liver pCO_2 curve was significantly higher in group A (p=0.008*) throughout the measuring period.

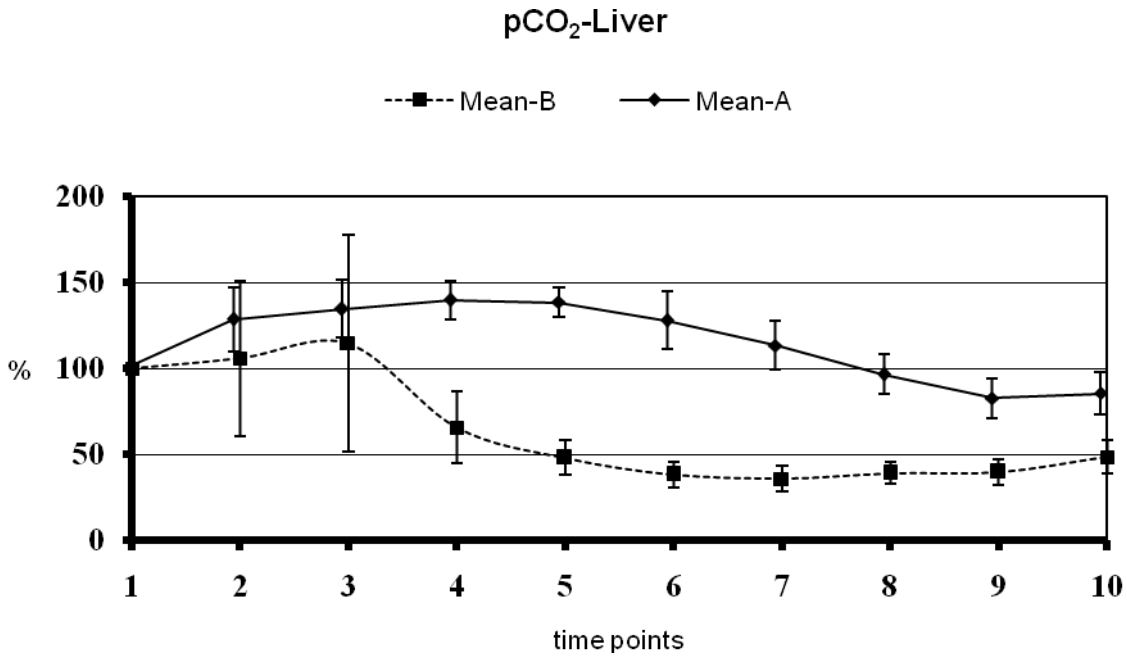

Figure 29: pCO_2 measured by opto-chemical probes in the liver

3.4.3. The Brain

Brain derived pO_2 curves showed significantly higher in group B (p=0.007*) for the factor interaction between groups in time. Furthermore Spearman analysis reported highly significant correlations between pO_2 in brain with arterial and bulbovenous derived lactate.

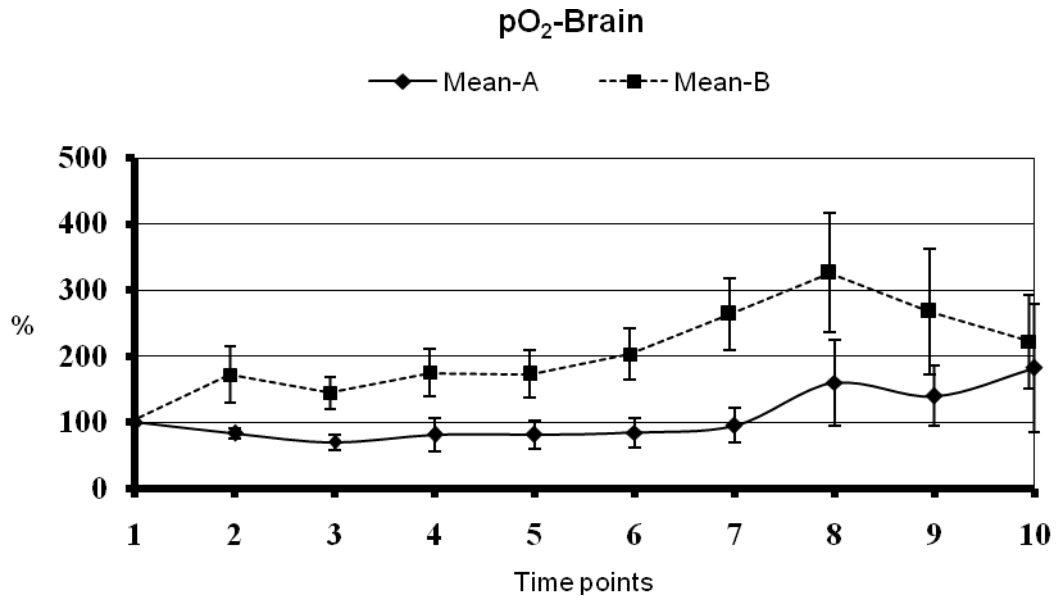

Figure 30: pO_2 in the central nervous system

The pCO_2 of the brain didn´t show any statistical differences between the groups but showed a clear tendency and thus behaves in accordance to the bulbovenous lactate.

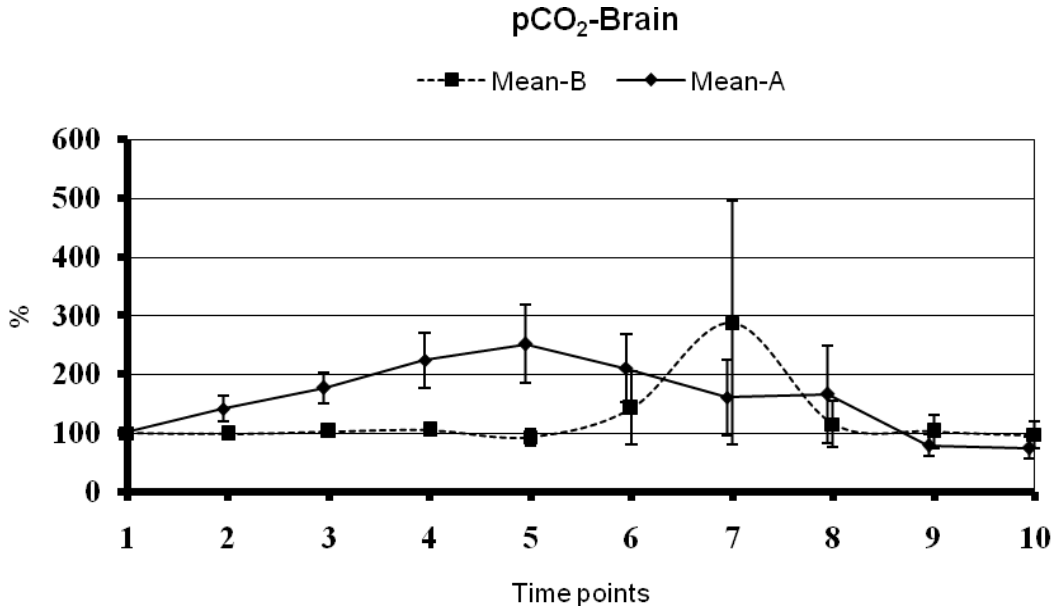

Figure 31: pCO_2 of the cerebrum

3.5. Inflammatory Markers:

Measurement of the inflammatory markers (TNF-α, IL-1β, IL-6, IL-10) before initiating the cardiopulmonary bypass and after cessation of the same showed values mostly under the threshold of the test due to the dilution of the samples. All data obtained from the experiment on the behaviour of the inflammatory response during the cardiopulmonary bypass were unusable.

4. Discussion

4.1. Swine as a model for cardio-surgical research

The swine could be considered as a suitable model for research in cardiac surgery. As it was shown in chapter 2 the anatomy of the swine is- to a high extent- similar to the anatomy of the human [47]. Not only the anatomical circumstances, but also the physiology of the swine allows us to consider the swine as a suitable model for research in cardiac surgery. For example, pigs weighing between 25 and 30 kg have the same heart-bodyweight ratio as the human [50] and the central nervous circulatory regulation is sufficiently developed at the age of three months [51]. Other large animal models would fulfill the same requirements of comparability as the porcine model does, but in regard to animal housing, costs and animal protection, these models turn out to be rather unsuitable for our experiment. Of course, there are many smaller animal models in the research of cardiopulmonary bypass, which probably are more interesting from the economical and statistical point of view [52]. Still, as the cardio-surgical procedure is that complex, the use of standardized anaesthesia and peri- and intraoperative management in regards of comparability is inevitable.

4.2. Experimental Results

4.2.1. Haemodilution

One of the findings of this experiment was the increased transfusion requirements in group A (conventional CPB) compared to the requirements in group B (Resting Heart System®). In order to keep the haematocrit above 20%, allogenic blood transfusion was necessary in almost all animals of group A. It is important to stress that the technique of haemodilution is necessary for high quality perfusion. Haemodilution, as it was mentioned in chapter 1,

allows the blood flow not to be connected to perfusion pressure, as it would be in case of whole blood—hence allowing same perfusion with less rheological stress [18]. Excessive haemodilution, which is occurring in case of large priming volumes, low BSA (Body surface Area) or low preoperative haematocrits is the concerning matter [53].

Immer et al. showed in their study similar behaviour regarding the transfusion requirements, as they compared patients (n=1053) who underwent CABG procedure on mini-ECC and those having a CABG on conventional CPB in a prospective, but non randomised study. Their data showed significantly lower need for inotropic support and blood transfusion six hours after ICU admission [54].

The same conclusion could be reported by van Boven et al. where 184 patients underwent CABG either with the conventional or the minimized heart lung machine. He stated statistically significant differences between the blood requirements in favour of minimized cardiopulmonary bypass [55].

It is obvious that excessive hemodilution is associated with worse perioperative vital organ dysfunction, increased use in blood products, as well as increased short-and intermediate term mortality of patients undergoing cardiac surgery procedures, the same as it could be shown in a retrospective analysis conducted by Habib et al. [56]. This author could only speculate about the causes of the adverse effects of low haematocrit, such as the inadequate oxygen delivery, which is resulting in ischemic and/or inflammatory organ distress. Furthermore DeFoe et al. could show in their multicenter prospective cohort study that the lowest haematocrit (<19%) on-pump is significantly associated with increased risk of in-hospital mortality, risk of intra-or postpoperative intra-aortic balloon pump and return to cardiopulmonary bypass after attempted separation. They also showed that the nadir haematocrit is affected by the gender, the body mass index (BSA) and the preoperative haematocrit levels as well as the priming volume [53]. Wiesenack et al. reported also less blood product requirements by the use of a mini-ECC system [57].

On the other hand Nollert et al. have admitted benefits of a mini-ECC regarding blood transfusion and blood products requirements in 15 consecutive patients undergoing elective, isolated CABG procedures, but after stating safety concerns in the exploitation of the system, they cancelled further investigations. They argued that the same reduction of priming volume would be achievable as well in conventional cardiopulmonary bypass by shortening the bypass circuit. Excessive haemodilution and consequent drop of haematocrit would be avoided in the same manner without loss of safety margins [28]. It is plausible

that there is room for shortening the circuit length of the conventional heart lung machine, but still the idea of having a closed circuit, where the patient serves as his own cardiotomy reservoir, is measure decreasing the volume needed for priming to an extent as no other measure. Reduction of haemodilution in order to maintain higher intra-operative haematocrit levels is a reasonable strategy of avoiding intra-and postoperative complications. The abandoning of the cardiotomy reservoir, shortening the circuit length, as well as using blood cardioplegia as it is the case in the Resting Heart System® used in our experiment, resulted in reduced haemodilution and furthermore less blood products needed.

4.2.2. Immunological Reaction

Haemodilution during our experiment had an adverse effect on another aspect of the present theme. During the conduction of cardiopulmonary bypass almost all body systems are affected. Especially blood contact with foreign surfaces, such as the tubing of the HLM, initiates a whole body inflammatory reaction, i.e. systemic inflammatory response syndrome [18].

Beside cellular reactions, humoral answers of the immunological system are the most deleterious for various organs. Such as the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-10 are mostly reported to be valuable indicators of the immunological status of the body. It is the question, whether the mini-ECC is capable of reducing the systemic inflammatory response or not. Several studies tried to answer this question. Frommes and associates reported a prospective study, where a mini-ECC system was compared to conventional CPB on 30 patients in each group with regard to the cytokine release. They could conclude that there was less IL-6, TNF-α and Elastase secretion in the mini-ECC group than in the conventional-CPB group [29]. On the other hand Bical et al. stressed in a consecutive study that only TNF-α would be a reliable marker in this context and IL-6 would be activated mainly by the surgical procedure and not the CPB. They as well concluded that mini-ECC attenuates the inflammatory response [27]. The main idea of the mini-ECC, beside minimization of the circuit, is the usage of biocompatible materials

(heparin-coated), the exclusion of cardiotomy suction and blood-air contact. It is interesting, that Defraigne et al. reported from a large randomized cohort study (n=200) that heparin-coated circuits (conventional HLM) do not influence the release of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-8 or complement products. They admitted that the difference to other studies could result from a different patient population [58].

On the other hand, it seems plausible that in a multi-factorial setting like a cardiac surgical procedure definitely is, modification of only one parameter (surface-coating) cannot influence the whole system. In comparison to a later published study, a mini-ECC—where not only heparin-coated surfaces but also the various strategic components of a mini-ECC system are used—can lead to a reduction of circulating pro-inflammatory mediators [59]. One of the endpoints of our study was to show the influence of a mini-ECC system on the inflammatory reaction in a swine. In most of the experimental animals, the results were below the sensitivity of the used test in both groups. The cytokines were measured in human quantitative sandwich ELISA and not in a porcine which could have had influence on the accuracy of the measurement. As well as the correct test, it should also be taken in account that haemodilution has an influence on the cytokine concentration after cardiac surgery [60].

Subsequent experiments should as well concentrate on procalcitonin as one of the markers of the inflammatory reaction following CPB or mini-ECC. Several studies have reported procalcitonin to be a useful parameter in distinguishing between sepsis and SIRS as well as stressed out that not the absolute value of PCT but the dynamical changes would be clinically valuable in identifying critically ill patients in cardiac surgery [61][62]. Franke et al. showed in their study that IL-6 is elevated in all thoracic procedures and recommended procalcitonin as an inflammatory marker which is closely associated to the usage of CPB and not only to the surgical procedure [63].

4.2.3. Tissue pO_2 and pCO_2

4.2.3.1. Brain

Data of this thesis do confirm the findings of these authors to a high degree, but there is a lack of understanding of the underlying mechanisms behind the positive effects of the mini-ECC [54]. Measurement of the immunological response through the inflammatory mediators does not reflect to which extent organs are affected during the cardiopulmonary bypass. This preclinical experiment tried to explain possible pathways of O_2 and CO_2 metabolism during cardiopulmonary bypass by the use of specified opto-chemical probes. Partial pressure of O_2 and CO_2 can be measured online and directly in the tissues, in this proper case in the myocardium, the liver and brain. It is the microcirculation of an organ, which is reflected by these measurements.

On the other hand, Brattli et al. described the regional distribution of blood flow during proximal aortic cross clamping by means of coloured microspheres in an intensive animal laboratory experiment [64]. Coloured microspheres served as an alternative to the radioactive microspheres and were used to detect regional blood flow [65].

Another investigational method was used by Gelman et al. during their investigation on aortal cross clamping. They observed by means of transcranial Doppler that during aortic crossclamping the CBF significantly rose in the middle cerebral artery [66].

CBF (cerebral blood flow) alone is not a sufficient parameter to determine whether the cerebral metabolic rate for oxygen ($CMRO_2$) is affected, because as well mean arterial pressure (MAP) and haematocrit (Hct) as well have an influence on the metabolism of O_2 [18]. Our measuring method on the other hand reflects the real O_2 delivery in the tissue. In this context one important investigational method should not be missed which is the near-infrared spectroscopy (NIRS). This method uses haemoglobin as it undergoes a characteristic near-infrared absorption shift when binding to O_2. This shift can be measured transcutaneously and non-invasive [67]. It would be interesting to compare our fibre-optical measurement of tissue pO_2 to NIRS because there are several points where NIRS has its weaknesses. So for example it is not clear where exactly the region of interrogation is and how the measurement is influenced by the scalp and skull blood [18]. Measuring

tissue derived pO_2 and pCO_2 by fibre-optical probes could be a chance to calibrate the NIRS.

Comparing fibre-optical tissue pO_2 and pCO_2 of the brain to jugular bulb venous pO_2 and pCO_2 as it is in this thesis is to a high extant reasonable. Still both measurements are different to that degree that measuring jugular bulb pO_2 reflects the global O_2 metabolic situation of the cerebrum and the opto-chemical tissue measurement is absolutely focused on the one region of the cerebrum, where the probe is stereotactically placed.

Nevertheless, our findings suggest that significantly higher pO_2 values, measured by the fibre-optical probes, in the mini-ECC group have a connection to the reduced induction of the inflammatory response produced by the Resting Heart System® compared to the conventional CPB. Boyle et al. showed in their publication that cytokines and pro-inflammatory mediators are harmful to the endothelial cell of the cerebral vasculature thus stimulating the endothelium to bind circulating blood elements more actively [68].

4.2.3.2. The Liver

Cardiac surgery has a great impact on the organ liver through various detrimental effects of the cardiopulmonary bypass. Liver dysfunction occurs in up to 3.2% of operated patients, which significantly increases mortality and morbidity [18].

As we look more detailed, the liver is supplied with blood as following: from the portal vein, which drains most of the gastro-lienal venous blood, and from the hepatic artery. The portal vein contributes about three fourth of the blood amount and is responsible for almost 50% of O_2 saturation of the liver whereas 25% are derived from the hepatic artery [69]. On the other hand it is obvious that this proportions show variability and a deficit in one source of blood can be compensated by an increase in the other, nevertheless the total liver blood flow should remain constant. In this context one question seems to be reasonable. To which extent is this fragile system influenced by an auto-regulatory mechanism?

Chetty et al. found that there was no alteration in liver blood flow with induction of cardiac anaesthesia and consecutive positive pressure ventilation. They explained that their results were expected because of the self-buffering system of the liver. Furthermore, an

anaesthetic agent during cardiac surgery had little effect on the liver system, reflecting to a certain extent an absence of auto-regulation of the system [70].

Because there is no clear consensus on the effect of CPB on splanchnic or liver blood flow, in this experiment the hypothesis was followed—stated in many studies—that the delivery of the liver oxygen is decreased by hypothermic CPB. Although the rationale for this statement is disputable, the loss of perfusion pressure during cardiopulmonary bypass seems to be the main factor. Loss of pulsatility of flow and redistribution of the blood during CPB could influence the oxygen supply of the liver as well [18].

Mathie et al pointed out that loss of pulsatility in a group of patients undergoing CABG is a consequence of hypothermic CPB. They explored furthermore, that a fall in perfusion pressure during the period of CPB was combined with a fall in liver blood flow during the same period. The decrease of liver blood flow was higher than the decrease in perfusion pressure, suggesting that this loss of perfusion pressure alone cannot be the only factor of the reduction of the oxygen supply in liver during hypothermic CPB [71].

On the other hand, Desai et al. published a dog experiment that an increase in liver blood flow was higher during hypothermic CPB comparing to normothermic CPB. They concluded that the fall in liver blood flow might be disconnected from the temperature of the patient and explained this contradictive finding with the question whether there might be discordance between the physiology of the dog and the human.

Another hypothesis which was brought by Desai et al. was if there were possibilities of opening of arteriovenous shunts in the intestinal vasculature during CPB with the effect of more or less metabolically active liver systems [72].

As well as the liver blood flow is affected by the temperature, also the interaction between immune system and the endothelium of liver vasculature could play an important role in this system. CPB-induced inflammatory response probably affects the endothelial surface of the vessels in the same way as it does in the central venous system with the consequence of even more deterioration of oxygen supply to the liver. Patients with borderline liver function and after prolonged cardiopulmonary bypass or even bad perfusion techniques could be pushed into liver failure by such a period of hypoxia.

Consequently, emphasis should be put on the maintenance of a sufficient perfusion pressure during CPB, especially in patients with pre-existing organ impairment and risk of developing severe post-operative complications.

Prasser et al. reported a study on elective CABG patients that the mini ECC offers satisfactory clinical benefits but they failed to demonstrate superiority of mini-ECC over conventional CPB in patients without pre-existing liver injury. Better microcirculatory perfusion of the liver could be one of the positive effects of the closed loop system in the mini-ECC compared to the conventional CPB [73]. In accordance to that, the results of this animal experiment show absolutely higher pCO_2 curves and significant correlation of pCO_2 and arterial and venous lactate in the group A (conventional CPB) under standardized conditions in the liver.

4.3. Limitations

Although a small number of animals are included in this experiment, the results are statistically clear and the conclusion appears reasonable. In following experiments, a larger number of animals could be considered to improve statistical power of the study.

Swine model is similar to human in-vivo testing to a limited extent, but because of the invasiveness of the experiment, a swine model had to be used. However, one has to expect adverse haemodynamic reactions while using a swine model.

4.4. Conclusion

The data of this thesis support the hypothesis that the mini-ECC is beneficial compared with the conventional cardiopulmonary bypass system. Higher brain derived pO_2 levels in association with lower arterial and bulbovenous lactate and lower volume of blood transfusion in the mini-ECC group support the hypothesis. The use of fibre-optical sensors for measuring tissue derived pO_2 and pCO_2 showed to be a valid research tool. The invasiveness of the method, however, restricts its use to research settings.

5. References

(1) Lewis FJ, Varco RL, Taufic M. Repair of atrial septal defects in man under direct vision with the aid of hypothermia. Surgery 1954 Sep;36(3):538-556.

(2) Gibbon JJ. The development of the heart-lung apparatus. Am.J.Surg. 1978(135):686-700.

(3) Edwards FH, Clark RE, Schwartz M. Coronary artery bypass grafting: the Society of Thoracic Surgeons National Database experience. Ann.Thorac.Surg. 1994 Jan;57(1):12-19.

(4) Fitzgibbon GM, Kafka HP, Leach AJ, Keon WJ, Hooper GD, Burton JR. Coronary bypass graft fate and patient outcome: angiographic follow-up of 5,065 grafts related to survival and reoperation in 1,388 patients during 25 years. J.Am.Coll.Cardiol. 1996 Sep;28(3):616-626.

(5) Kirklin JK. Prospects for understanding and eliminating the deleterious effects of cardiopulmonary bypass. Ann.Thorac.Surg. 1991 Apr;51(4):529-531.

(6) Cleveland JC,Jr, Shroyer AL, Chen AY, Peterson E, Grover FL. Off-pump coronary artery bypass grafting decreases risk-adjusted mortality and morbidity. Ann.Thorac.Surg. 2001 Oct;72(4):1282-8; discussion 1288-9.

(7) Diegeler A., Doll N., Rauch T., Haberer D-, Walter T., Falk V., et al. Humoral Immune Response During Coronary Artery Bypass Grafting: a Comparison of Limited Approach, "Off-Pump" Technique, and Conventional Cardiopulmonary Bypass. Circulation 2000;102(3):95-100.

(8) von Segesser LK, Tozzi P, Mallbiabrrena I, Jegger D, Horisberger J, Corno A. Miniaturization in cardiopulmonary bypass. Perfusion 2003 Jul;18(4):219-224.

(9) Gourlay T, Stefanou DC, Asimakopoulos G, Taylor KM. The effect of circuit surface area on CD11b(mac-1) expression in a rat recirculation model. Artif.Organs 2001 Jun;25(6):475-479.

(10) Lim MW. The history of extracorporeal oxygenators. Anaesthesia 2006 Oct;61(10):984-995.

(11) Dapper F, Neppl H, Wozniak G, Strube I, Boldt J, Hehrlein FW, et al. Influence of 4 different membrane oxygenators on inflammation-like processes during extracorporeal circulation with pulsatile and non-pulsatile flow. Eur.J.Cardiothorac.Surg. 1992;6(1):18-24.

(12) Rajek A, Lenhardt R, Sessler DI, Kurz A, Laufer G, Christensen R, et al. Tissue heat content and distribution during and after cardiopulmonary bypass at 31 degrees C and 27 degrees C. Anesthesiology 1998 Jun;88(6):1511-1518.

(13) Saxena P, Shehatha J, Boyt A, Newman M, Konstantinov IE. Role of extracorporeal circulation in the management of accidental deep hypothermia. Heart Lung Circ. 2009 Dec;18(6):416-418.

(14) Wahba A. Centrifugal blood pump use in routine cardiac surgery. Interact.Cardiovasc.Thorac.Surg. 2006 Jun;5(3):299-300.

(15) Nielsen PF, Funder JA, Jensen MO, Nygaard H. Influence of venous reservoir level on microbubbles in cardiopulmonary bypass. Perfusion 2008 Nov;23(6):347-353.

(16) Whitaker DC, Newman SP, Stygall J, Hope-Wynne C, Harrison MJ, Walesby RK. The effect of leucocyte-depleting arterial line filters on cerebral microemboli and neuropsychological outcome following coronary artery bypass surgery. Eur.J.Cardiothorac.Surg. 2004 Feb;25(2):267-274.

(17) Jewell AE, Akowuah EF, Suvarna SK, Braidley P, Hopkinson D, Cooper G. A prospective randomised comparison of cardiotomy suction and cell saver for recycling shed blood during cardiac surgery. Eur.J.Cardiothorac.Surg. 2003 Apr;23(4):633-636.

(18) Gravlee GP. Cardiopulmonary bypass : principles and practice. 3rd ed. Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.

(19) Guyton AC, Hall JE, Guyton-Hall . Textbook of medical physiology. 10th ed. Philadelphia, Pa. u.a.: Saunders; 2000.

(20) Hayashi Y, Kagisaki K, Yamaguchi T, Sakaguchi T, Naka Y, Sawa Y, et al. Clinical application of vacuum-assisted cardiopulmonary bypass with a pressure relief valve. Eur.J.Cardiothorac.Surg. 2001 Sep;20(3):621-626.

(21) Hussaini BE, Treanor PR, Healey NA, Tilahun D, Srey R, Lu XG, et al. Evaluation of blood components exposed to coated arterial filters in extracorporeal circuits. Perfusion 2009 Dec 11.

(22) Harrison TR, Fauci AS. Harrison's principles of internal medicine. 14th ed. New York u.a.: McGraw-Hill; 1998.

(23) Sablotzki A, Muhling J, Dehne MG, Zickmann B, Silber RE, Friedrich I. Treatment of sepsis in cardiac surgery: role of immunoglobulins. Perfusion 2001 Mar;16(2):113-120.

(24) Asimakopoulos G. Systemic inflammation and cardiac surgery: an update. Perfusion 2001 Sep;16(5):353-360.

(25) Moat NE, Shore DF, Evans TW. Organ dysfunction and cardiopulmonary bypass: the role of complement and complement regulatory proteins. Eur.J.Cardiothorac.Surg. 1993;7(11):563-573.

(26) Cotran RS, Kumar V, Collins T. Robbins Pathologic basis of disease. 6th ed. Philadelphia u.a.: Saunders; 1999.

(27) Bical OM, Fromes Y, Gaillard D, Fischer M, Ponzio O, Deleuze P, et al. Comparison of the inflammatory response between miniaturized and standard CPB circuits in aortic valve surgery. Eur.J.Cardiothorac.Surg. 2006 May;29(5):699-702.

(28) Nollert G, Schwabenland I, Maktav D, Kur F, Christ F, Fraunberger P, et al. Miniaturized cardiopulmonary bypass in coronary artery bypass surgery: marginal impact on inflammation and coagulation but loss of safety margins. Ann.Thorac.Surg. 2005 Dec;80(6):2326-2332.

(29) Fromes Y, Gaillard D, Ponzio O, Chauffert M, Gerhardt MF, Deleuze P, et al. Reduction of the inflammatory response following coronary bypass grafting with total minimal extracorporeal circulation. Eur.J.Cardiothorac.Surg. 2002 Oct;22(4):527-533.

(30) Miller A, Wang S, Myers JL, Undar A. Gaseous microemboli detection in a simulated pediatric CPB circuit using a novel ultrasound system. ASAIO J. 2008 Sep-Oct;54(5):504-508.

(31) Myers GJ, Voorhees C, Haynes R, Eke B. Post-arterial filter gaseous microemboli activity of five integral cardiotomy reservoirs during venting: an in vitro study. J.Extra.Corpor.Technol. 2009 Mar;41(1):20-27.

(32) Rodriguez RA, Rubens F, Belway D, Nathan HJ. Residual air in the venous cannula increases cerebral embolization at the onset of cardiopulmonary bypass. Eur.J.Cardiothorac.Surg. 2006 Feb;29(2):175-180.

(33) Liebold A, Khosravi A, Westphal B, Skrabal C, Choi YH, Stamm C, et al. Effect of closed minimized cardiopulmonary bypass on cerebral tissue oxygenation and microembolization. J.Thorac.Cardiovasc.Surg. 2006 Feb;131(2):268-276.

(34) Brooker RF, Brown WR, Moody DM, Hammon JW,Jr, Reboussin DM, Deal DD, et al. Cardiotomy suction: a major source of brain lipid emboli during cardiopulmonary bypass. Ann.Thorac.Surg. 1998 Jun;65(6):1651-1655.

(35) Calafiore AM, Di Mauro M, Teodori G, Di Giammarco G, Cirmeni S, Contini M, et al. Impact of aortic manipulation on incidence of cerebrovascular accidents after surgical myocardial revascularization. Ann.Thorac.Surg. 2002 May;73(5):1387-1393.

(36) Clark RE, Brillman J, Davis DA, Lovell MR, Price TR, Magovern GJ. Microemboli during coronary artery bypass grafting. Genesis and effect on outcome. J.Thorac.Cardiovasc.Surg. 1995 Feb;109(2):249-57; discussion 257-8.

(37) Hafferl A, Thiel W. Lehrbuch der topographischen Anatomie : mit den Pariser und Jenaer Nomina anatomica. 3 Aufl ed. Berlin u.a.: Springer; 1969.

(38) Newman MF, Mathew JP, Grocott HP, Mackensen GB, Monk T, Welsh-Bohmer KA, et al. Central nervous system injury associated with cardiac surgery. Lancet 2006 Aug 19;368(9536):694-703.

(39) Duebener LF, Hagino I, Sakamoto T, Mime LB, Stamm C, Zurakowski D, et al. Effects of pH management during deep hypothermic bypass on cerebral microcirculation: alpha-stat versus pH-stat. Circulation 2002 Sep 24;106(12 Suppl 1):I103-8.

(40) Poswal P, Mehta Y, Juneja R, Khanna S, Meharwal ZS, Trehan N. Comparative study of pulsatile and nonpulsatile flow during cardio-pulmonary bypass. Ann.Card.Anaesth. 2004 Jan;7(1):44-50.

(41) Murkin JM. NIRS: a standard of care for CPB vs. an evolving standard for selective cerebral perfusion? J.Extra.Corpor.Technol. 2009 Mar;41(1):P11-4.

(42) Tobias JD, Russo P, Russo J. Changes in near infrared spectroscopy during deep hypothermic circulatory arrest. Ann.Card.Anaesth. 2009 Jan-Jun;12(1):17-21.

(43) Bizzarri A, Konrad C, Čajlaković M, et al. New developments of an optochemical measurement system for the continuous monitoring in subcutaneous tissue by microdialysis. IEEE Sensors October 2007.

(44) Čajlaković M, Bizzarri A, Suppan M, Konrad C, Ribitsch V. Continuous monitoring of pO_2 and pCO_2 by microdialysis indicates physiologic status of the critically il patients. Sens&Act B 2009(1):181-186.

(45) Bizzarri A. Continous oxygen monitoring in subcutaneous adipose tissue using microdialysis", Anal. Chim. Acta Vol. 573-574, pp. 48-56, 2006. Anal. Chem. Acta 2006;573.

(46) Fischer JE, Bland KI, Ovid Technologies I. Mastery of surgery. 2007.

(47) Crick SJ, Sheppard MN, Ho SY, Gebstein L, Anderson RH. Anatomy of the pig heart: comparisons with normal human cardiac structure. J.Anat. 1998 Jul;193 (Pt 1)(Pt 1):105-119.

(48) Kaiser LR, Kron IL, Spray TL, et al. Mastery of cardiothoracic surgery. 2007.

(49) Harms V. Biomathematik, Statistik und Dokumentation : eine leichtverständliche Einführung ; nach den Gegenstandskatalogen für den 1. und 2. Abschnitt der ärztlichen Prüfung. 6, völlig neubearb Aufl ed. Kiel: Harms; 1992.

(50) Hughes HC. Swine in cardiovascular research. Lab.Anim.Sci. 1986 Aug;36(4):348-350.

(51) Verdouw PD, van den Doel MA, de Zeeuw S, Duncker DJ. Animal models in the study of myocardial ischaemia and ischaemic syndromes. Cardiovasc.Res. 1998 Jul;39(1):121-135.

(52) Dong GH, Wang CT, Li Y, Xu B, Qian JJ, Wu HW, et al. Cardiopulmonary bypass induced microcirculatory injury of the small bowel in rats. World J.Gastroenterol. 2009 Jul 7;15(25):3166-3172.

(53) DeFoe GR, Ross CS, Olmstead EM, Surgenor SD, Fillinger MP, Groom RC, et al. Lowest hematocrit on bypass and adverse outcomes associated with coronary artery bypass grafting. Northern New England Cardiovascular Disease Study Group. Ann.Thorac.Surg. 2001 Mar;71(3):769-776.

(54) Immer FF, Ackermann A, Gygax E, Stalder M, Englberger L, Eckstein FS, et al. Minimal extracorporeal circulation is a promising technique for coronary artery bypass grafting. Ann.Thorac.Surg. 2007 Nov;84(5):1515-20; discussion 1521.

(55) Gerritsen WB, van Boven WJ, Wesselink RM, Smelt M, Morshuis WJ, van Dongen HP, et al. Significant reduction in blood loss in patients undergoing minimal extracorporeal circulation. Transfus.Med. 2006 Oct;16(5):329-334.

(56) Habib RH, Zacharias A, Schwann TA, Riordan CJ, Durham SJ, Shah A. Adverse effects of low hematocrit during cardiopulmonary bypass in the adult: should current practice be changed? J.Thorac.Cardiovasc.Surg. 2003 Jun;125(6):1438-1450.

(57) Wiesenack C, Liebold A, Philipp A, Ritzka M, Koppenberg J, Birnbaum DE, et al. Four years' experience with a miniaturized extracorporeal circulation system and its influence on clinical outcome. Artif.Organs 2004 Dec;28(12):1082-1088.

(58) Defraigne JO, Pincemail J, Larbuisson R, Blaffart F, Limet R. Cytokine release and neutrophil activation are not prevented by heparin-coated circuits and aprotinin administration. Ann.Thorac.Surg. 2000 Apr;69(4):1084-1091.

(59) Panday GF, Fischer S, Bauer A, Metz D, Schubel J, El Shouki N, et al. Minimal extracorporeal circulation and off-pump compared to conventional cardiopulmonary bypass in coronary surgery. Interact.Cardiovasc.Thorac.Surg. 2009 Aug 11.

(60) Roth-Isigkeit A, Borstel TV, Seyfarth M, Schmucker P. Perioperative serum levels of tumour-necrosis-factor alpha (TNF-alpha), IL-1 beta, IL-6, IL-10 and soluble IL-2 receptor in patients undergoing cardiac surgery with cardiopulmonary bypass without and with correction for haemodilution. Clin.Exp.Immunol. 1999 Nov;118(2):242-246.

(61) Sponholz C, Sakr Y, Reinhart K, Brunkhorst F. Diagnostic value and prognostic implications of serum procalcitonin after cardiac surgery: a systematic review of the literature. Crit.Care 2006;10(5):R145.

(62) Arkader R, Troster EJ, Lopes MR, Junior RR, Carcillo JA, Leone C, et al. Procalcitonin does discriminate between sepsis and systemic inflammatory response syndrome. Arch.Dis.Child. 2006 Feb;91(2):117-120.

(63) Franke A, Lante W, Fackeldey V, Becker HP, Kurig E, Zoller LG, et al. Pro-inflammatory cytokines after different kinds of cardio-thoracic surgical procedures: is what we see what we know? Eur.J.Cardiothorac.Surg. 2005 Oct;28(4):569-575.

(64) Brattli OS, Nystuen K, Saether OD, Aadahl P, Gronbech JE, Myhre HO. Regional distribution of blood flow during proximal aortic cross-clamping: an experimental study using coloured microspheres. Scand.J.Clin.Lab.Invest. 2007;67(5):526-535.

(65) Hale SL, Alker KJ, Kloner RA. Evaluation of nonradioactive, colored microspheres for measurement of regional myocardial blood flow in dogs. Circulation 1988 Aug;78(2):428-434.

(66) Gelman S. Carbon dioxide and hepatic circulation. Anesth.Analg. 1989 Aug;69(2):149-151.

(67) Murkin JM. NIRS: a standard of care for CPB vs. an evolving standard for selective cerebral perfusion? J.Extra.Corpor.Technol. 2009 Mar;41(1):P11-4.

(68) Boyle EM,Jr, Pohlman TH, Johnson MC, Verrier ED. Endothelial cell injury in cardiovascular surgery: the systemic inflammatory response. Ann.Thorac.Surg. 1997 Jan;63(1):277-284.

(69) Chetty G, Sharpe DA, Nandi J, Butler SJ, Mitchell IM. Liver blood flow during cardiac surgery. Perfusion 2004 May;19(3):153-156.

(70) Chetty G, Sharpe DA, Nandi J, Butler SJ, Mitchell IM. Liver blood flow during cardiac surgery. Perfusion 2004 May;19(3):153-156.

(71) Mathie RT, Ohri SK, Batten JJ, Peters AM, Keogh BE. Hepatic blood flow during cardiopulmonary bypass operations: the effect of temperature and pulsatility. J.Thorac.Cardiovasc.Surg. 1997 Aug;114(2):292-293.

(72) Desai J., Mathie R., Taylor K. Hepatic blood flow during cardiopulmonary bypass in the dog: a comparison between pulsatile and non-pulsatile perfusion. Life Support Syst 1984;2:303-305.

(73) Prasser C, Abbady M, Keyl C, Liebold A, Tenderich M, Philipp A, et al. Effect of a miniaturized extracorporeal circulation (MECC System) on liver function. Perfusion 2007 Jul;22(4):245-250.

6. Appendix:

Animal experiment aproval:

BMWF

Bundesministerium für Wissenschaft und Forschung

Herrn
Ao.Univ.-Prof.Dr. Igor Knez
p.A. Universitätsklinik für Chirurgie
Klin.Abt. für Herzchirurgie
Medizinische Universität Graz
Auenbruggerplatz 29
8036 Graz

mit dem Ersuchen um Kenntnisnahme und Information.

Beilage

Wien, 12. März 2008
Für den Bundesminister:
SektChef Dr. Wolf Frühauf

Elektronisch gefertigt

Geschäftszahl: BMWF-66.010/0016-II/10b/2008
Sachbearbeiter/in: Manuela Putz
Abteilung: II/10b
E-Mail: manuela.putz@bmwf.gv.at
Telefon/Fax: (+43) 01/53120-7211 / 53120-817211
Ihr Zeichen:

Antwortschreiben bitte unter Anführung der Geschäftszahl.

Minoritenplatz 5, 1014 Wien
www.bmwf.gv.at

DVR 0064301

BMWF

Bundesministerium für Wissenschaft und Forschung

Medizinische Universität Graz
Rektorat
Auenbruggerplatz 2/4
8036 Graz

Universitätsklinik für Chirurgie,
Klin.Abt. für Herzchirurgie,
Genehmigung eines Tierversuches gemäß
Tierversuchsgesetz (BGBl. Nr. 501/1988 i.d.g.F.)

BESCHEID

Das Bundesministerium für Wissenschaft und Forschung hat über Ihren mit do. Schreiben vom 26. Februar 2008 übermittelten Antrag auf Genehmigung eines Tierversuches gemäß Tierversuchsgesetz (TVG, BGBl.Nr. 501/1989, zuletzt geändert durch BGBl. I Nr. 162/2005) wie folgt entschieden:

1. Gemäß dem ergänzten Antrag (s. Beilage) wird der Medizinischen Universität Graz die Genehmigung gemäß § 8 TVG zu dem in der Beilage umschriebenen Tierversuch (Akutversuch) – und gemäß § 10 Abs. 1 TVG bis 31. März 2009 befristet - erteilt.
   Die Beilage stellt somit einen integrierten Bestandteil dieser Genehmigung dar.
2. Dem Leiter des Tierversuches Ao.Univ.-Prof.Dr. Igor Knez wird die Genehmigung gemäß § 7 TVG für den in der Beilage umschriebenen Tierversuch erteilt. Tierversuche dürfen nur von Personen oder unter Verantwortung oder Aufsicht von Personen vorgenommen werden, denen dafür die Genehmigung erteilt worden ist. Der Leiter des Tierversuches hat über die Tierversuche Aufzeichnungen gemäß § 15 TVG zu führen.
3. Die in Punkt 11 der Beilage angeführte Tierversuchseinrichtung wird gemäß § 6 TVG für den in der Beilage umschriebenen Tierversuch genehmigt. Der Träger der Tierversuchseinrichtung hat bis zum 1. März eines jeden Jahres die im vorangegangenen Kalenderjahr verwendeten Versuchstiere gemäß § 16 TVG dem Bundesministerium für Wissenschaft und Forschung bekannt zu geben.

Geschäftszahl: BMWF-66.010/0016-II/10b/2008
Sachbearbeiter/in: Manuela Putz
Abteilung: II/10b
E-Mail: manuela.putz@bmwf.gv.at
Telefon/Fax: (+43) 01/53120-7211 / 53120-817211
Ihr Zeichen:

Antwortschreiben bitte unter Anführung der Geschäftszahl.

Minoritenplatz 5, 1014 Wien
www.bmwf.gv.at

DVR 0064301

An das
Bundesministerium für Bildung,
Wissenschaft und Forschung
Bereich Gentechnik und Tierversuche
Ref. II/10b (Gentechnik und Tierversuche)
Im Dienstweg

Antrag auf Genehmigung von Tierversuchen

gemäß Tierversuchsgesetz, BGBl. Nr. 501/1989,
i.d.F. BGBl. I Nr. 169/1999

1. **Institut/Klinik (Stempel)**

Kurztitel des Projekts: Minimal invasive extrakorporale Zirkulation (MECC) versus konventionelle extrakorporale Zirkulation („Herz-Lungen Maschine/HLM"): Unterschiede in der organbezogenen Messung von Sauerstoff- und Kohlendioxidpartialdruck – eine experimentelle herzchirurgische Studie am Tiermodell

2. **Verantwortlicher Leiter des Tierversuchs**

Name/Vorname/Titel: Ao. Univ. Prof. Dr. Igor Knez

Tel/Fax/email: 0316/385 80527, Fax: 0316/385 4672, igor.knez@meduni-graz.at

Fachliche Qualifikation:

☐ Veterinärmedizin	☐ Biologie
☐X Humanmedizin	☐ Pharmazie
☐ sonstige:	

Spezialkenntnisse gem. § 7: FA für Chirurgie, FA für Herzchirurgie

.....

3. **Personen, die mit der Durchführung des beantragten Tierversuches betraut werden:**

Name, Vorname, Titel, fachl. Qualifikation: Ao. Univ. Prof. Dr. Igor Knez, FA f. Chirurgie, FA für Herzchirurgie

Name, Vorname, Titel, fachl. Qualifikation: Univ. Ass. Dr. Elisabeth Beran, in Ausbildung Chirurgie

Name, Vorname, Titel, fachl. Qualifikation: Univ. Ass. Dr. Ismar Ovcina, in Ausbildung Chirurgie

Name, Vorname, Titel, fachl. Qualifikation: Ao. Univ. Prof. Dr. Drago Dacar, FA für Chirurgie, FA für Herzchirurgie, FA für Chirurgische Intensivmedizin

Name, Vorname, Titel, fachl. Qualifikation: Dr. Jakub Krumnikl, FA für Anästhesiologie und Intensivmedizin

Name, Vorname, Titel, fachl. Qualifikation: Ao. Univ. Prof. Dr. Gerfried Zobel, FA für Kinderheilkunde, FA für Intensivmedizin

Name, Vorname, Titel, fachl. Qualifikation: Univ. Ass. Dr. Klaus Pfurtscheller, FA für Kinderheilkunde

Name, Vorname, Titel, fachl. Qualifikation: VR Univ. Prof. Dr. Karlheinz Tscheliessnigg, FA für Chirurgie, FA für Herzchirurgie, FA für Gefäßchirurgie, FA für Chirurgische Intensivmedizin

Name, Vorname, Titel, fachl. Qualifikation: Ao. Univ. Prof. Peter Leber, FA für Neurochirurgie, FA für Neurochirurgische Intensivmedizin

4. Durch wen erfolgt die veterinärmedizinische Betreuung der Versuchstiere:

Name, Vorname, Titel, fachl. Qualifikation: Mag. Dr. Birgit Gutmann, Leiterin Biomedizinische Forschung, Graz

Mag. Heimo Kren, Veterinärmediziner

5. Fachkundiges Hilfspersonal zur Betreuung der Versuchstiere (Tierpfleger):

Name, Vorname: Rodler Reinhard, staatl. Geprüfter Facharbeiter für Tierpflege, Tierhaltung

D´Alonzo Norbert, staatlich geprüfter Tierpfleger,

fachl. Qualifikation, nachgewiesen durch[1]: Mitarbeiter der Sektion Chirurgische Forschung

6. Art und Anzahl der beantragten Versuchstiere sowie nach Herkunft aufgegliedert[2]:

Art (Stamm)	Gesamt	gem. 1.3[2]	gem. 1.4[2]	gem. 1.5[2]	gem. 1.6[2]	gem. 1.7[2]	transgen:
Hausschweine	20				20		☐ja ☒nein
							☐ja ☐nein
							☐ja ☐nein

bei Verwendung mehrerer Arten die Begründung: .....

Herkunft der Versuchstiere (und Name der Zuchteinrichtung): FINK JOSEF

Name und Anschrift des Vorbesitzers (bei Hunden und Katzen): Oberretenbach 19 8212 Pischelsdorf

7. Art und Anzahl der beantragten Versuchstiere nach Versuchszweck[3]:

Art (Stamm)	gem. 2.2[3]	gem. 2.3[3]	gem. 2.4[3]	gem. 2.5[3]	gem. 2.6[3]	gem. 2.7[3]	gem. 2.8[3]	gem. 2.9[3]	Gesamt
Hausschweine	20								20

8. sofern ein Tierversuch im Zusammenhang mit Krankheiten von Mensch und Tier beantragt wird, ist die Krankheit[4] anzugeben:

4.2. Herz-Kreislauferkrankungen

[1] z.B. Tierpfleger gem. Tierpfleger-Ausbildungsordnung, BGBl. II Nr. 64/1997
[2] Tierversuchsstatistik-Verordnung (TVSt-V), Anh. I Tab. 1 nach Herkunft aufgeschlüsselte Anzahl der verwendeten Tiere
[3] TVSt-V, Anh. I, Tab. 2 nach Versuchszweck aufgeschlüsselte Anzahl der verwendeten Tiere
[4] TVSt-V, Anh. I, Tab. 4 Tierversuche im Zusammenhang mit Krankheiten von Mensch und Tier

9. **sofern ein Tierversuch zur Herstellung und Qualitätskontrolle von Produkten und Geräten beantragt wird (siehe Punkt 7, Spalte 2.4 und 2.5), ist die hierfür maßgebende Rechtsvorschrift[5] anzugeben:**

   .....

10. **sofern ein Tierversuch für toxikologische und sonst. Unbedenklichkeitsprüfungen beantragt wird (siehe Punkt 7, Spalte 2.6), ist (a) die zu testende Produktkategorie[6], (b) die hierfür maßgebende Rechtsvorschrift Produktkategorie[7], (c) die Versuchsart Produktkategorie[8] und (d) die Versuchsart/Produkt Produktkategorie[9] anzugeben:**

    (a) .....
    (b) .....
    (c) .....
    (d) .....

11. **Wo wird der Versuch durchgeführt (Tierversuchseinrichtung)?**

    Operationssaal der Chirurgischen Forschung der Univ. Klinik für Chirurgie, Graz

12. **Wo werden die Versuchstiere untergebracht?**

    Akutversuch, INSTITUT FÜR BIOMED. FORSCHUNG

13. **Zweck des Tierversuchs (BEILAGE)**

    Ausführliche Beschreibung und Begründung des Versuchszieles, Literaturhinweise, Versuchsplan und vorgesehene statistische Auswertungsverfahren

14. **Art des Eingriffes bzw. der Behandlung (BEILAGE)**

    Ausführliche Beschreibung der experimentellen Methode (Operationstechnik, Narkose, Analgesie, Tötung usw.); Dauer des Eingriffes bzw. der Behandlung

15. **Zucht- und Liefereinrichtung gem. Tierversuchs-Verordnung § 3 Abs. 2**

    ☐ Die Zulassung und Registrierung der folgenden Zucht- und Liefereinrichtung wird beantragt:

    ☐ Folgende Änderung der Zulassung wird beantragt:

    ☐ Die Zulassung wurde bereits erteilt. (GZ. ....., Datum .....)

    - Name, Sitz (Adresse) der Einrichtung:

      .....

    - Unternehmensgegenstand bzw. Angaben über Zucht und Lieferung von Versuchstieren (Art und Umfang):

      .....

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[5] TVSt-V, Anh. I, Tab. 5 Rechtsvorschrift für TV zur Herstellung und Qualitätskontrolle von Produkten und Geräten der Human,- Zahn- und Veterinärmedizin

[6] TVSt-V, Anh. I, Tab. 3 nach Produkt/Art aufgeschlüsselte Anzahl der bei toxikolog. und sonst. Unbedenklichkeitsprüfungen verwendeten Tiere

[7] TVSt-V, Anh. I, Tab. 6 nach Rechtsvorschrift/Art aufgeschlüsselte Anzahl der bei toxikolog. und sonst. Unbedenklichkeitsprüfungen verwendeten Tiere

[8] TVSt-V, Anh. I, Tab. 7 nach Versuchsart/Art aufgeschlüsselte Anzahl der bei toxikolog. und sonst. Unbedenklichkeitsprüfungen verwendeten Tiere

[9] TVSt-V, Anh. I, Tab. 8 nach Versuchsart/Produkt aufgeschlüsselte Anzahl der bei toxikolog. und sonst. Unbedenklichkeitsprüfungen verwendeten Tiere

- Bezeichnung (Name/n) der für die Zucht- und Liefereinrichtung sachkundigen Person/en, die für die in der Einrichtung gezüchteten oder gehaltenen Tiere Pflege und Betreuung Sorge zu tragen hat/haben:

Name, Vorname: .....

fachl. Qualifikation, nachgewiesen durch[1]: .....

Name, Vorname: .....

fachl. Qualifikation, nachgewiesen durch[1]: .....

Die Unterzeichneten bestätigen, dass das angestrebte Versuchsziel nicht durch andere Methoden erreicht werden kann und dass die erforderlichen Anlagen, Geräte und Räumlichkeiten zur tierschutzgerechten Haltung und Wartung der Versuchstiere zur Verfügung stehen.

Datum: 15.02.2008

Unterschrift des Leiters der Zucht-/Liefereinrichtung[10]

Ao. Univ. Prof. Dr. Igor Knez

Univ. Prof. Dr. Tscheliessnigg
Suppl. Rektor, VR f. Klinischen Bereich, Vorstand der
Medizinischen Univ. Klinik für Chirurgie

Unterschrift des Versuchsleiters

Unterschrift des Rektors

KLINIK FÜR CHIRURGIE
UNIV.PROF. D.r.
KH.TSCHELIESSNIGG

UNIVERSITÄTSKLINIK
für CHIRURGIE GRAZ
Sektion für
CHIRURGIE + CHE FORSCHUNG
Auenbruggerplatz 29
A – 8036 GRAZ

MEDIZINISCHE UNIVERSITÄT GRAZ
Rektor
A-8036 Graz, Austria, Auenbruggerplatz 2/4

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Institut für
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[10] Nur im Falle eines Antrags zu Punkt 15 erforderlich