Diploma Thesis

XENOTRANSPLANATION
Fiction or Reality?

submitted by

Tarik Farahat

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Assoz. Prof. Priv.-Doz. Dr.med.univ. Philipp Stiegler

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Declaration of Authorship

I hereby declare that I have authored this diploma thesis independently, that I have not used other than the declared sources/resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, 28 January 2019 *Tarik Farahat eh.*

Acknowledgement

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Abstract (German)

Die Xenotransplantation beschreibt eine, nicht zwangsläufig humanmedizinische, Methode der Übertragung von Zellen, Geweben oder Organen über Artgrenzen hinweg. Dabei ist nicht nur die Implantationsmethode, sondern auch die Nutzung und Einbindung in die Physiologie des Empfängers von wesentlicher Bedeutung. Im humanmedizinischen Bereich eröffnet die Xenotransplantation neue Ansätze um Patientinnen und Patienten bei akutem Organversagen eine lebensnotwendige Spende zukommen zu lassen oder auch die Lebensqualität, beispielsweise bei chronischem Nierenversagen und der daraus folgenden Dialysepflichtigkeit, zu erhöhen. Die Xenotransplantation erweitert daher die allogene Organspende, welche in vielen Fällen die einzige Methode zur Rettung Schwerstkranker beim Ausfall von Organfunktionen darstellt. Die forschungsrelevante Brisanz der Xenotransplantation wird vorrangig durch den Umstand genährt, dass es einen weltweiten Mangel an allogenen humanen Spenderorganen gibt. Alternative Ansätze, wie z.B. die künstlich erzeugte Organsubstitution oder die molekular-biotechnologische Organwiederherstellung, beschreiben Konzepte der regenerativen Organogenese, beschränken sich derzeit aber auf den Stand der Grundlagenforschung. Infolge des Mangels an menschlichen Spenderorganen verstirbt derzeit etwa jeder dritte transplantationsbedürftige Patient auf der Warteliste. Die allogene humane Organtransplantation weist heute bereits eine hohe Erfolgsquote auf, d.h. der „wartelistenbedingte“ Tod ist vermeidbar, sofern genügend Spenderorgane vorrätig sind. Ist es dann aus ethischer Sicht nicht geboten, Gesellschaft, Wissenschaft und Gesetzgeber dazu zu bewegen, dass die Bedingungen der Organverfügbarkeit verbessert und nach alternativen Möglichkeiten gesucht wird? Es ist wahr, dass die Entwicklung der Xenotransplantation sowie die Bewertung der medizintechnischen Durchführbarkeit und die damit verbundenen Risiken in erster Linie von wissenschaftlichen Erkenntnissen abhängen. Die Berücksichtigung bestehender Rahmenbedingungen und möglicher ethischer, sozialer, ökonomischer und rechtlicher Konsequenzen der Xenotransplantation ist jedoch für die Einführung und klinische Anwendbarkeit des Verfahrens unerlässlich. Aufgrund der aktuellen Forschungslage wird man den künstlichen Organersatz – in absehbarer Zeit möglicherweise zur temporären Überbrückung von Organausfällen – oder die biotechnologische Organogenese nicht zielorientiert als Ersatz für die allogene

Organspende in der Humanmedizin anwenden können. Daher spielt die Xenotransplantation eine wichtige Rolle als alternatives medizinisches Verfahren – und sei es auch nur zu lebenserhaltenden Überbrückungsverfahren bei Notsituationen – um die Patientinnen und Patienten bis zur Bereitstellung eines Allogentransplantates vor dem klinischen Tod zu bewahren.

Abstract (English)

Xenotransplantation describes a general, not necessarily human-medical method of transferring organs, tissues or cells across species borders. Not only the implantation method, but also the adaption and successful integration into the physiology of the recipient are of essential importance. In the field of human medicine, xenotransplantation opens up new approaches to increase the quality of life in patients with acute organ failure. Xenotransplantation therefore extends allogeneic organ donation, which in many cases is the only method of rescuing seriously ill patients in the event of an organ failure. The research-relevant high degree of current relevance of xenotransplantation is primarily fueled by the fact that there is a worldwide shortage of allogeneic human donor organs. Alternative approaches, such as artificial organ substitution or molecular biotechnological organ repair describe concepts of regenerative organogenesis but are currently limited to the state of basic research. Due to the lack of human donor organs about every third patient in need of a transplant is currently on the waiting list. Allogeneic human organ transplantation today already has a high success rate. Eventually, "waitlist-related" deaths are avoidable today if sufficient numbers of donor organs are available.

The introduction and application of xenotransplantation raises a plethora of questions that cannot be studied exclusively within the disciplinary boundaries of the natural sciences. It is ethically of the highest importance to convince society, science and legislators to improve the conditions of organ availability and to look for alternative options. Undoubtedly the development of xenotransplantation as well as the evaluation of feasibility and risks depend first and foremost on scientific advances in knowledge. However, before the method can be tested for clinical applications one has to consider existing scientific and humanistic framework conditions and possible ethical, social, economic and legal consequences of xenotransplantation for patients, organ sources, medical staff and society. Due to the current research situation artificial replacement of organs or biotechnological organogenesis cannot be applied in a goal-oriented manner as a substitute for allogeneic organ donation in human medicine. In view of this, xenotransplantation plays an important role as an alternative medical procedure - even if only for life-support in emergency situations - to save patients from clinical death until a suitable allograft is available.

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1 Introduction

Xenotransplantation is a scientific-medical method of transferring organs, tissues or cells across species borders. In the field of human medicine, xenotransplantation opens up new approaches of providing patients with a vital donation in the event of acute organ failure. Xenotransplantation therefore extends allogeneic organ donation, which in many cases is the only method of rescuing the seriously ill in the case of organ function loss. It appears to be one of the most promising methods to reduce or even remedy the existing shortage of transplantable organs. The scientific relevance of xenotransplantation can be attributed to a worldwide shortage of allogeneic human donor organs. Due to the lack of human donor organs about every third patient in need of a transplant currently dies on the waiting list. The practice of xenotransplantation, however, involves significant medical and technical problems as well as a whole range of ethical, social, economic and legal issues.

The focus of this diploma thesis is the question in which way medical-technical strategies for the implementation of the concept of xenotransplantation have been pursued in the past and to what extent they will be able to cope with the existing scientific-medical problems and risk potentials in the future. The aim of this piece of work is a scientifically founded, comprehensive assessment of the feasibility and risks of xenotransplantation as well as the prerequisites for human use. The study and assessment of the main difficulties will be complemented by the identification of further open questions and research needs. In addition, options for action are proposed, which may possibly help to remove the existing obstacles.

The diploma thesis focuses on the human medical benefits of xenotransplantation and its feasibility and application for humans. Xenotransplantations between other species are not part of the research question.

At the beginning of the thesis (Chapter 2) an overview of the topics and definitions of terms covered by the concept of xenotransplantation will be given. There have been numerous clinical trials over the last 300 years or more to develop a better understanding of the concept and methods of xenotransplantation. These are presented in Chapter 3 with reference to mythological sources and empirical scientific papers: Around 1900, physicians attempted to replace inadequate organ functions of humans by organs of animals such as pigs, goats, lambs or monkeys.

However, all of these xenografts failed within a short time before the scientists could find out possible reasons for this failure. The introduction of immunosuppressive drugs was also the beginning of research and clinical feasibility of allografting. The concept of xenotransplantation and the advantages and disadvantages of choosing the pig as the preferred organ source are presented in Chapter 4. Furthermore, the physiological compatibility of animal and human organs, viz. the ability of the xenograft to maintain life-sustaining functions in humans, is explained. The chapter concludes with alternative treatment concepts for xenotransplantation. The focus of the fifth chapter is the question which scientific strategies are pursued to implement the concept of xenotransplantation and how they are capable of coping with the existing scientific medical problems and risk potentials of this procedure. The analysis focuses on the two major problems that have conflicted with successful and safe xenotransplantation so far: First the mechanisms of immunological transplant rejection reactions and second the risk of infection for the patient and the environment. The results of internationally conducted studies on xenotransplantation are supplemented by a human and animal ethical discourse, which is presented in the sixth chapter of the diploma thesis on the basis of the well-known xenotransplantation case of Stephanie Fae Beauclair, also called “Baby Fae” in 1984.

2 Definitions of organ donation and transplant terms

2.1 Transplantation

The term transplantation refers to the transmission of cells, tissues or organs. In the context of human medicine organ transplant surgeries are performed to replace malfunctioning organs by healthy ones. Transplants are used in terminal organ failure or in irreversible degenerative organ changes as life supportive therapeutic measures. The most frequently transplanted organ is the kidney, followed by liver and heart (Schicktanz 2010, p.4). However, there is a great lack of donor organs for organ transplantation. Thus, the average waiting time for a suitable donor heart is about 200 days. About 10% of patients on the cardiac transplant waiting list die during this time (Cooper 2000, p.1145-1147).

Due to the lack of human donor organs about every third patient in need of a transplant is currently on the waiting list. In Austria, organ donation is regulated by the "Eurotransplant" foundation, founded in 1967, which also operates in the Benelux, Germany, Slovenia, Croatia and Hungary. Potential organ recipients and donors are reported here (Beckmann 2000, p.9-11). According to the mandatory organ allocation criteria, a suitable donor is assigned according to organ, blood group and tissue characteristics (kidney), size, weight and waiting time. Following a detailed evaluation of the patient's medical history, Eurotransplant organizes all potential organ recipients in three classes, those with normal urgency (transplantable), high urgency and not transplantable (Hetzer, Neuhaus, Pfitzmann 2001, p.14).

2.1.1 Allotransplantation

By definition the donor and recipient belong to the same species but are genetically different, as in the case of organ transplants from one human to another. This is the transplantation form most commonly used in human medicine, whereby postmortem and living donations are differentiated. Allogeneic transplants include kidney, liver, pancreas, heart, lung and corneal transplantation. Rejection reactions are to be expected here, so immunosuppression is needed (Burmester 1998, p.176).

2.1.2 Syngeneic transplantation

A subset of allografts, syngeneic transplantation or isograft transplantation means the transplantation of cells, tissues and organs between genetically identical individuals (e.g. identical twins). Singularly among all grafts available, isografts do not trigger an immune response (Souza-Offtermatt 2004, p).

2.1.3 Autotransplantation

Autogenic transplantation means the transplantation of a tissue part in the same organism (e.g. split skin graft). Donors and recipients are identical. Sometimes an autograft is explanted and specifically prepared before it is returned (example: autografting of stem cells and storage of blood before surgery) to its owner (Schlich 1998, 274-275).

2.1.4 Xenotransplantation

Xenotransplantation is a medical, but not necessarily human-medical, method of transferring organs, tissues or cells across species barriers. Not only the implantation method, but also the use and integration into the physiology of the recipient are of considerable importance. In the field of human medicine xenotransplantation opens up new approaches to treat patients with acute organ failure or to increase the quality of life. Xenotransplantation therefore extends the allogeneic donation of organs, which in many cases represents the only therapy of treating the seriously ill in the case of organ function failure (Engelhardt 1998, p.3-4).

2.2 Organ donation

There are two types of organ donors, postmortem donors and living donors. Organ donors are normally characterized as seriously ill patients (e.g. suffering from an intracranial hemorrhage, traumatic brain injury, meningitis, etc.) who are generally treated in intensive care units and despite all therapeutic measures often sustain a progressive brain damage with fatal outcome. The brain death of these patients is characterized by the complete loss of the entire brain functions, including brain stem activities (Hetzer, Neuhaus, Pfitzmann 2001, p.13).

The majority of organs are taken from deceased organ donors when a complete, irreversible brain function failure has occurred. Postmortem organ transplants,

unlike living donations, are also taken from those organs that would lead to the death of a living donor on removal (Stoecker 2012, p.7-9).

In living donation, a distinction is made between "relatives donation" (e.g., parent-child) and "unrelated donation" (which includes emotionally connected and altruistically-affiliated individuals who wish to donate organs on the basis of a donation call). The living donation mainly concerns kidneys as paired organs, as well as liver segments and bone marrow, which is capable of regeneration. Significant benefits include better scheduling of the procedure, which will allow both the donor and the recipient to be psychologically prepared and are less likely to lead to rejection of the allografts due to the higher degree of matching of tissue features (Schröder 2000, p.64-65).

2.3 Chimera and Hybrid

In classical antiquity the stories of mixed creatures that consist of human and animal features experienced a golden age. Homer, for example, describes the chimera as a fearsome fire-spewing monster, composed of body parts of lion, goat and dragon or snake in the sixth chapter of the "Iliad", which threatened the inhabitants of Lycia and that was slain by Bellerophon (Kline 2009, p.127). The term "chimera" subsequently changed from the name of a special monster in Greek mythology to a comprehensive term for all imaginary hybrid beings in which parts of various organisms are combined into a new form. Most uses of the term "chimera" as *terminus technicus* in the biosciences are derived from the chimera in the metaphorical sense - that is, an entity in which properties from different (genetic) sources have been combined. In the biological context, however, the term "chimera", due to its ambiguity and long tradition, holds a high potential for misunderstandings and conflicts, although bioscientists today speak largely neutrally of chimeras as highly helpful tools of their research (Schmidt 2014, p.38-40):

> “[…] the scientific perception of a chimera is essentially that of a tool used by experimental biologists to deepen the undcrstanding of living proc- esses. For non scientists, a chimera is mainly a mental representation frequently linked to the fear that scientists are producing possible monsters as an endpoint.” (Taupitz and Weschka 2009, p.564)

A cinical example of chimerism can be found in the field of hematological oncology. After allogeneic transplantation of blood cells hematopoiesis and

lymphopoiesis are guaranteed by cells of the donor. Since these cells have a genetic origin different from the recipient, a so-called hematopoietic chimerism arises. The condition in which the complete hematopoiesis is conducted by donor cells is termed as complete hyperemia, while a mixed chimerism is the status in which recipient cells are detectable in the bone marrow or in the blood (Gutjahr 2004, p.91).

The recent study of researchers from the Salk Institute for Biological Studies in La Jolla, California, shows another example of chimerism: After mouse embryos had previously been deprived of the ability to develop a particular organ, the foreign stem cells differentiated into the target cells. In the end, the embryos of the mice contained rat cardiac or pancreatic tissue. The researchers could even breed a rat gallbladder in the mice, although its rodent relatives had lost this organ many generations ago. In a second step, the researchers wanted to find out if the procedure also works with human cells. To do so, they inoculated porcine embryos, which were still in their early stages of development, with pluripotent human stem cells, giving those three to four weeks to develop. The published results showed that the contribution of human tissue to the pig embryo remained relatively low during this period.

One of the biggest ethical fears in human-animal chimeras is that the resulting creatures could become too human and one day the clear dividing line between humans and animals will become blurred - for example, by maturing the human stem cells into neurons that participate in building the pig's brain. However, according to the geneticists, this could not have happened within the study. The fact that differentiated cells have formed, including muscle cells and precursors of other organs, is an important step on the way to the breeding of complete human organs (Devlin 2017, p.1).

In contrast, organisms which derived from the reproduction of heterogeneous parents are referred to as hybrids. Thus, hybrids can be defined as the progeny of individuals of different morphospecies but belonging to the same biospecies. In many cases, only the direct descendants are called hybrids, whereas the offspring of the hybrids are no longer hybrids themselves, but form a population (Toepfer 2011, p.74-75).

3 History of Xenotransplantation

3.1 The Lamassu – The symbol of xenotransplantation

In ancient Mesopotamian culture Lamassus were protective deities, first worshipped by common Babylonian people but later becoming guardians of the royal court where they were placed, usually as a pair, at the entrance to deflect humans from chaos and evil (Rosen 2009, p.287). The giant entrance way figures, e.g. guarding the palace gates of Sargon II at Khorsabad, the Gate of All Nations at the ruins of Persepolis in Iran or at the British Museum in London, are depicted in a tetramorphic shape (Fekripour 2016, p.587). As hybrid xenogenic creatures, they are composed of the head of a bearded man, symbolizing high intelligence, the body of a bull and a lion, symbolizing strength and the wings of an eagle, symbolizing freedom (Rosen 2009, p.287).

Figure 3-1 ***Human-headed winged lion (Lamassu),*** **The Metropolitan Museum of Art, http://www.metmuseum.org/toah/images/hb/hb_32.143.2.jpg [Accessed 26 July 2017]**

Figure 3-2 ***Lamassu from Khorsabad (Iraq, Neo-Assyrian),*** **n.d. photograph, https://camel76.files.wordpress.com/2013/03/lamassu-from-the-palace-of-sargon-ii.jpg [Accessed 26 July 2017]**

In modern culture, the mythical figure of a Lamassu served the International Xenotransplantation Association and its scholarly journal “Xenotransplantation” as its logo by the end of 2016 (Cooper 2012, p.49). Due to controversial reactions and discussions, the International Xenotransplantation Association decided to replace its logo with a more stylized and modern image.

Figure 3-3 (right) ***Old logo of the International Xenotransplantation Association showing a Lamassu,*** **IXA, https://www.tts.org/images/stories/newsletter/2014_v11_i3/23-01.jpg [Accessed 26 July 2017]**

Figure 3-4 (left) ***New logo of the International Xenotransplantation Association,*** **IXA, https://confman.ixa2017.org/img/logo.png [Accessed 26 July 2017]**

David K. C. Cooper, Professor of Surgery at the Thomas E. Starzl Transplantation Institute in Pittsburgh (Cooper 2015, p.1310), chose the lamassu for the cover of his book "Xenotransplantation: The Transplantation of Organs and Tissues Between Species" for its more benevolent and protective character compared to other mythical creatures:

> The cover of this book depicts a Lamassu, one of the "fabulous" beasts of mythology. Like many similar creatures, such as the Chimera, Griffon, Hippocamp, and Cockatrice, the body of the Lamassu was clearly a combination of structures derived from several different species - in other words, it provides a highly successful example of xenotransplantation. But in selecting a symbol of xenotransplantation to grace the cover of this volume, why choose the Lamassu in preference to the other ancient beasts? The reason is that the Lamassu appears to have been endowed with a much more benign and desirable character than many of its mythological associates. […] Lamassus, on the other hand, were considered to be kindly figures, specifically acting as guardian divinities of cities. […] Surely, we, the potential xenotransplanters, would prefer our aims and efforts to be associated with an animal of "kindly" disposition and "divine character" rather than with the various alternative ancient mythological beasts. Yes, the Lamassu would definitely seem to be our choice (Cooper 1997, p.VII-IX).

According to Cooper (1997, p.VIII), the mythical chimera, which has recently been used by immunologists when describing an organism that is composed of two or more different genetically distinct cells, symbolizes evil and can therefore not be used for a surgical technique that is supposed to cure illnesses and help mankind:

> […] The Chimera hitherto the animal most commonly selected to symbolize xenografting - killed everyone who came within range of its fiery breath. Perhaps not surprisingly, therefore, the Chimera is variously described as one of the "largest monsters ever born," a "savage creature," and a "symbol of complex evil." With these descriptions in mind, it certainly does not seem to be the ideal choice to represent a field of surgery and science that is intended to be wholly beneficial to the human race (Cooper 1997, p.VIII)!

Besides its usage as a *terminus technicus* in medical genetics, the chimera represents the field of allotransplantation – the transplantation of cells, tissues and organs between two non-genetically identical members of the same species (Cooper 2012, p.49).

3.2 Hybrids and Chimeras in Art, Legends, Folklore and Myths

According to medical literature the first attempts of transplantation refer to allotransplantation experiments about 600 BC (Deschamps 2005, p.1). The idea of transplanting allogenic or xenogenic organs for therapeutic usage, though, may be much older. Various different cultures, e.g. Egyptian, Indian, Greek and prehistoric, include myths and folklore of half-man-half-animal chimeras and the replacement of organs or body parts. These myths often lack proved evidence, but act as a source of inspiration for contemporaneous scientific research of organ replacement. In the last 100 years, especially allotransplantation has become a definitive treatment for terminal organ failure. Although myths can never deemphasize the scientific research and the acknowledged medical value of allotransplantation as a life-saving treatment, they have provided hints for conceptional contributions that are relevant even today, e.g. matching donors and recipients, skilled surgeons and training, preparing the organs, time limits for explanting and replanting of organs (Bhandari 1997, p.495-497) and usage of "magnificent and powerful drugs" (Deschamps 2005, p.2).

3.2.1 Clues from Palaeolithic art

The Palaeolithic cave paintings of Lascaux, located in the Dordogne region of south-western France, are famous for the exceptional quality, variety and size of prehistoric art that prevails until today. The cave complex shows over two thousand different paintings of primarily animal and abstract figures. The only man portrayed is an anthropomorphic figure with human and animal features (Buchanan 2011, p.17). Compared to the other complex images in the caves, the "Birdman" is rather simple. It shows a hybrid silhouette of a man with a bird's head, which is surrounded by a bird on a staff and a disembowelled bison. Although the meaning of this image has not yet been fully elucidated, it is the oldest representation – ca 15.000 BC – of a half man-half animal chimera known (Deschamps 2005, p.1).

Figure 3-5 (left) ***Lascaux-Birdman-Bison,*** **n.d. photograph, https://baroquepotion.com/wp-content/uploads/2011/03/Lascaux-Birdman-Bison.jpg [Accessed 26 July 2017]**

Figure 4 (right) ***The bird man Lascaux bull drawing*****, n.d. illustrator,** http://www.midnightsciencejournal.com/wp-content/uploads/2011/05/Lascau-birdman-figure-DC-copyright-version-3.png **[Accessed 26 July 2017]**

3.2.2 Clues from Egyptian Mythology

The ancient Egyptian religion was mainly based on polytheistic beliefs and worship of animal gods and can be traced to 4,000 BC. Animals were not hallowed for their animal being but as symbols of incarnations of particular gods and goddesses. The Egyptians chose an animal for its believed powers or its terrifying character and gave hybrid forms to the formerly abstract deities. Anubis, the jackal god of afterlife and mummification, is usually depicted with a canine head and the body of a man (Remler 2006, p.14-16). Horus, the God of Sky, Kingship and Hunt was worshipped as the sovereign guardian and sentinel of Egypt. His symbol is the lanner falcon and, therefore, he is often depicted with a falcon head and a human body (Remler 2006, p.83).

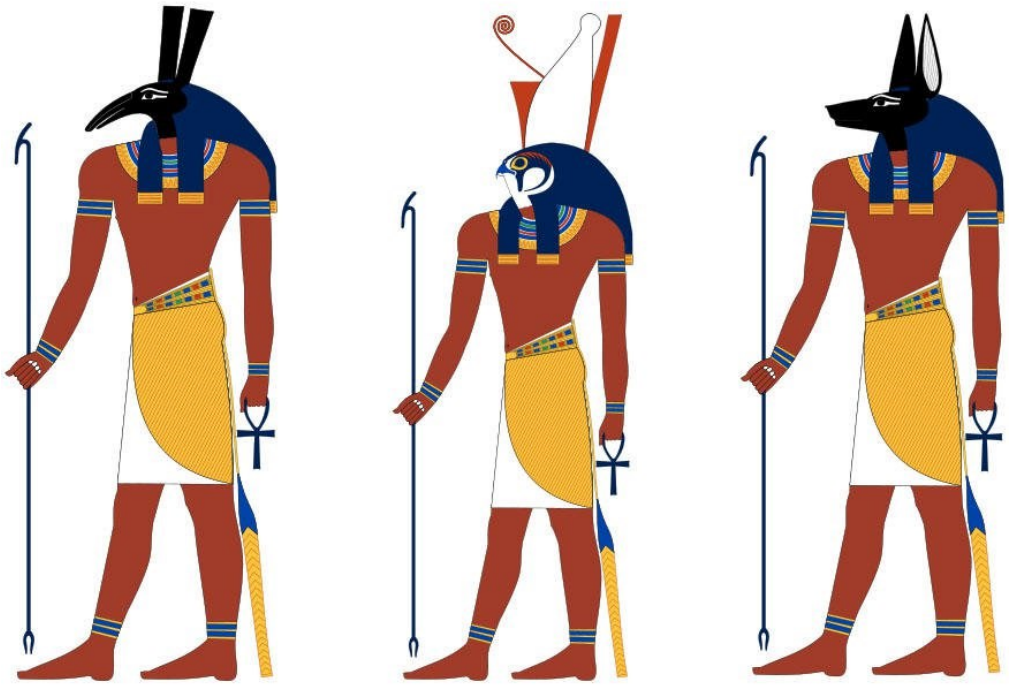

Figure 3-6 *Seth, Horus and Anubis*, n.d. illustrator, https://www.schulbilder.org/bild-seth-horus-und-anubis-i27026.html [Accessed 27 July 2017]

3.2.3 Clues from Indian and Hindu mythology and folklore

The Indian literature compilation, the "Puranas", contains various texts of Indian myths, traditions and folklores. Primarily composed in Sanskrit, it includes the oldest accounts of a xenotransplantation in history, written 300–700 AD. The texts for example recount the non-uterine birth and creation of Ganesha, the Hindu god of beginnings, intellect and wisdom (Brown 1991, p.2-3.). Parvati, the Hindu goddess of love, fertility and divine strength, decides to have a baby without her husband, Lord Shiva, who constantly denies her requests. She forms Vinayaka, Ganesha's name before her transplantation, out of soil and turmeric and performs *Prana Pratistha*, a ceremony held to bring her son to life. Parvati appointed her son as the new guardian of her cave's entrance. One day, Shiva, who occasionally visited his wife, arrived and argued with Vinayaka, who made no exception and constantly blocked Shiva's entry. In the ensuing battle, Shiva beheaded Parvati's son with his trident. Sorrowful and furious, Parvati decided to destroy the Universe if her son was not brought back to life and granted divine powers and status. Shiva, having no option but to accept her conditions, sent out his companions to retrieve the head of the first creature they encounter facing north – an offensive behaviour in the Hindu as the North Pole is believed to disturb peace. Shiva and two other gods combined their powers to attach the retrieved animal's head, an

elephant being used, onto Vinayaka body. Performing this “first documented xenogeneic transplantation” Ganesha, as he is called from that moment on, was brought back to life (Bhandari 1997, p.495-496).

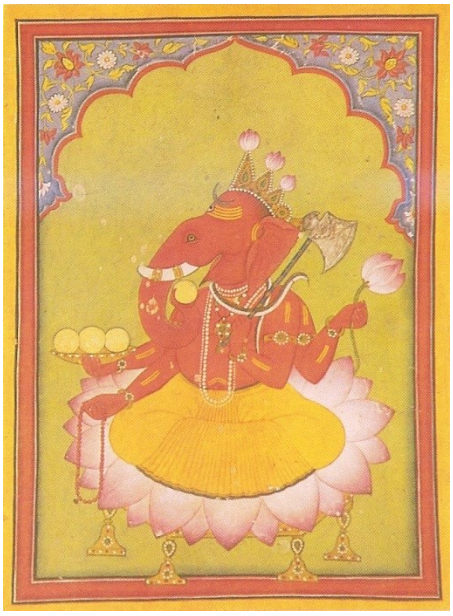

Figure 3-7 (left) ***Ganesha getting ready to throw his lotus,*** **n.d. illustrator, https://upload.wikimedia.org/wikipedia/commons/6/64/Ganesha_Basohli_miniature_circa_1730_Dubost_p73.jpg [Accessed 27 July 2017]**

Figure 3-8 **(right)** ***Six-Armed Dancing God Ganesha,*** **n.d. photograph, http://denverartmuseum.org/sites/default/files/slides/Media%20browser/1968.24_Ganesha.jpg [Accessed 27 July 2017]**

Another example of transplantation can be found in this Indian scripture. Daksha, one of the seven sons of Lord Brama, creator of the Universe in the Hindu religion, had several children. Among them was Sati, the Hindu goddess of marital felicity and longevity, who was very close to Lord Shiva (Williams 2003, p.105-106). Daksha, who disliked this relationship, celebrated a religious ceremony inviting neither his daughter nor his future son-in-law. Against Shiva’s advice, Sati attended the ceremony, ensuing humiliation and insults from her father. Being in disgrace, Sati concludes that she is the cause of the dishonour to her beloved Shiva and therefore throws herself into the sacrificial fire and dies (Bhandari 1997, p.496).

Nowadays “sati” or “suttee” is known as an obsolete Hindu way of self-immolation. A widow immolates herself at the death of her husband on a pyre or commits suicide in another form. Therefore a woman becomes *sati* – Hindi, from Sanskrit *satī* “faithful wife”, from *sat* “good”. – a pure one for her ultimate sacrifice (Hawley 1994, p.3).

Shiva, furious about the death of his beloved Sati, created the wrathful demonic beings Vîrabhadra and Bhadra-Kâlî to destroy Daksha by decapitating him and throwing his head into the fire sacrifice (Williams 2003, p.106). After Daksha's death the other Gods requested Shiva to recall his demons and bring Daksha back to life. Shiva, unable to retrieve the cut off head, attached instead the head of a goat (or ram) to the beheaded trunk of Daksha (Bhandari 1997, p.496).

Figure 3-9 (left) ***Ram-faced Daksha (right) with Virabhadra form of Shiva,*** **n.d. illustrator, http://www.britishmuseum.org/research/collection_online/collection_object_details/collection_image_gallery.aspx?assetId=418696001&objectId=3058344&partId=1 [Accessed 27 July 2017]**

Figure 3-10 (right) ***Shiva Carrying Sati on His Trident,*** **n.d. illustrator, https://upload.wikimedia.org/wikipedia/commons/a/ae/Dakshayani.jpg [Accessed 27 July 2017]**

3.2.4 Clues from Greek Mythology

Among the large variety of Greek mythology and literature sources that detail the lives and heroic deeds of gods, heroes and fabulous creatures, the literary telling of "Daedalus and Icarus" in the 8th book of Ovid's *Metamorphoses* (Ovid 2005, p.316-319) can be interpreted as an ancient attempt of xenotransplantation (Cooper 2012, p.49).

After Daedalus, a skilled and honoured craftsman, killed his more talented nephew in an act of jealousy, he fled to Crete with his son Icarus. There he served King Minos for many years and constructed, among others, the Labyrinth, a prison for the Minotaur, a half man-half bull hybrid. As the story continues, Theseus, a prince of Athens, volunteered to fight the Minotaur and to end the King's cruel death penalty. Among several other men and women, Theseus, who fell in love with King

Minos' daughter Ariadne, was imprisoned in the labyrinth to be slain by the Minotaur. At the request of Ariadne, Daedalus helped Theseus to kill the Minotaur and to find his way back out of the maze and to eventually leave Crete. For his treason, Daedalus was sentenced to life imprisonment together with his unintelligent son Icarus in his own construction. To forestall an escape by sea the king's guards inspected every leaving vessel carefully, Daedalus decided to escape by air route (Hamilton 2013, 144-168). According to Ovid's Metamorphoses, he crafted two pairs of wings from wax and bird's feathers (Ovid 2005, p.316-319). Reemtsma claims this handcraft to be the first successful xenogenic transplant across the species barrier (1991, p.9).

The wings being attached to Daedalus' and Icarus' bodies, they jumped off the prison's highest tower and flew over the sea. Even though Daedalus warned his son not to fly too close to the sun because the heat of the sun would soften the wax that held the wings together, Icarus was too arrogant and drowned eventually in the sea (Hamilton 2013, 144-145). Reemtsma (1991, p.9) called this an "acute graft rejection, attributed to a thermolabile adhesive". To this day the island, where Daedalus landed safely and watched his son drowning is known as Icaria and the sea into which he fell as the Icarian Sea (Reemtsma 1991, p.9).

Figure 3-11 (left) ***Daedalus and Icarus,*** **Charles Le Brun, http://www.wga.hu/frames-e.html?/html/l/le_brun/daedalus.html [Accessed 27 July 2017]**

Figure 3-12 (right) ***La caída de Ícaro,*** **Jacob Peter Gowy, http://www.ancient.eu/img/r/p/750x750/5052.jpg?v=1485682065 [Accessed 27 July 2017]**

3.3 Experimental and clinical trials of xenotransplantation from the 17th century to the 20th century

3.3.1 Xenotransfusion

In the second half of the 17th century English and French scientists researched into the human cardiovascular physiology and the blood functions and its components (Cooper 2015, p.2). By infusing several different agents, chemicals and fluids into an animal organism they proved that blood transports nutrients and materials from and to organs (Winner 2007, p.29-30).

Richard Lower, an English surgeon, is regarded as a pioneer of transfusion medicine for his first successful attempts to transfer blood from a dog (donor) to another dog's bloodstream (recipient) (Roux 2007, p.208). He first published his experiments with allogenic blood transfusions in 1666. Lower's research, though, was primarily limited to cross-animal transfusions, due to ethical (several test animals died) and religious (altering history of creation) concerns (Winner 2007, p.31-32).

The first documented xenotransfusion was performed by Jean-Baptiste Denis, a French surgeon, in 1667. After reading Lower's scientific paper, he transfused an adolescent, who suffered from severe fever, with the blood of a lamb and eventually cured him (Deschamps 2005, p.2-3). At that time, human allogenic blood transfusions were considered to be dangerous for both recipients and donors: human blood was regarded to have bad emotional and tainted characteristics whereas animal blood was thought to restore order and calamity within the patient (Winner 2007, p.33-34).

After a patient received a xenotransfusion with lethal outcome in December 1667, the French court in 1668, along with the Catholic Church and the English Parliament in 1670, declared human allogenic transfusions as unlawful without their previous permission (Roux 2007, p.209-210). Although Denis was finally acquitted due to lack of evidence, few xenotransfusions were performed for the following 150 years (Winner 2007, p.38).

Table 1 Historical review of xenotransfusions according to Deschamps (2005, p.2)

Year (of the report)	Author	Place	Animal source	Number of cases
1667	Denis	Paris, France	Lamb	15 year-old-man
			Lamb	45 year-old-man
			Calf	34 year-old-man
1668	Denis	Paris, France	Lamb	Paralyzed woman
1667	Lower	London, UK	Lamb	22 year-old-man
1872	Albini	Italy	Sheep	Woman (twice)
1874	Hasse	Nordhausen, Germany	Lamb	31 cases
1874	Gradle	Chicago, IL, USA	Lamb	2 men
2000	Baruah	Sonapur, India	Pig	22 year-old-man

Xenotransfusions were often semi-successful, primarily this was due to the lacking knowledge about microbes, infections and the dynamic character of physical-chemical blood properties (Winner 2007, p.43). In 1900, the Austrian physician Karl Landsteiner discovered the specific surface qualities of red blood cells and developed the AB0-system, enabling inter-human transfusions and eventually stopping xenotransfusion research (Roux 2007, p.212). Nowadays medical facilities are faced with a long-term shortage of stored human blood units as comprehensive restrictions of blood donation make many donators unsuitable (Ranucci 2017, p.1). Hence, researchers like Alex Zhu and David Cooper deal with new technologies to overcome cross-species barriers and enable successful xenografts. This gives back priority to the xenotransfusion medicine (Deschamps 2005, p.4). The usage of animal blood products provides a stable and rather safe source of blood supply. The benefits reach from completely reducing the risk of transmitting various infectious human diseases (HIV, hepatitis C, etc.) and porcine endogenous retroviruses (PERVs) as adult red blood cells contain no DNA (Roux 2007, p.213).

3.3.2 Tissue xenotransplantation and testicular xenografts

The first tissue xenotransplantation was published in 1501 by an Iranian physician in his book “The Quintessence of Experience” (Deschamps 2005, p.4). He treated osteomyelitis by replacing sick human bone tissue with healthy animal xenografts. In the second half of the seventeenth century a Russian surgeon used a bone xenograft from a domestic dog (canis lupus familiaris) to implant it into the skull of a soldier. Following several reported - more or less successfully performed - cases of xenotransplantations between animals and humans and animals of different species in the 18th and the beginning of the 19th centuries (Sque 2007, p.153), the Frenchmen Paul Bert and Claude Bernard focused on allogenic and xenogenic tissue experiments. They concluded that transplanting tissues or transfusing peripheral blood from the same species is safer than between subjects of different species (Deschamps 2005, p.4-5).

Three decades before Frederick Banting, Moses Baron and John Macleod isolated the peptide hormone insulin and discovered its therapeutic potential (Madeb. 2005, p.1), Watson Williams, an English physician, transplanted parts of the pancreas of a house sheep into a 15-year-old boy with Type 1 diabetes on the 20th of December, 1893. This operation was the first attempt in medical history to treat a diabetic patient through a transplant. Although the symptoms of the boy improved briefly, he died a few days after the operation due to a severe acidosis (Gruessner 2004, p.39).

In the 1920s testicular xenotransplantations flourished and had a strong cultural marking, e.g. as referred to in a Mary Brother’s song in the movie “The Cocoanuts” (1929): “Let me take you by the hand, Over to the jungle band, If you're too old for dancing, Get yourself a monkey gland.” (Kang 2017, p.241)

Unlike former tissue xenotransplantations, these operations had a high success rate because the testicular gland tissue has a specific immunologic protection. Serge Voronoff (1866 to 1951), a French surgeon from Russia, pioneered this new research area. After opotherapy, a former discipline of endocrinology, established many drugs that contained agents of crushed animal organs, Voronoff had a different aim by establishing endocrine surgery. He focused on rejuvenating men and later women to cure the symptoms of menopause by transplanting testicles of primates, like apes, baboons and chimpanzees. On June 12, 1920 he performed his first successful xenotransplantation by using a testicle allograft from a

chimpanzee (Deschamps 2005, p 5). When the effects promised by Voronoff did not settle in the patients - the short-term successes are today largely attributed to the placebo effect - Voronoff's method of transplantation went out of fashion and largely to oblivion (Friedman 2002, p.270-271). In his lifetime Voronoff performed over 2000 testicular xenotransplantations (Deschamps 2005, p 5). A few years ago, research groups accused Voronoff's testis transplantations to be linked to the HIV/Aids pandemic. Virologists found out that the simian immunodeficiency virus (SIV) is a progenitor virus to HIV and had been transmitted to humans in the early 20th century through primate hunting. However, a study showed that the SIV strain was transferred from a primate species different from the animals which served as organ sources in Voronoff's transplantations and that his operations were performed in the United States and Europe and not in Africa, finally refuting the accusations (Bajic 2012, p.339-340).

Table 2 Historical review of tissue xenotransplantation and testicular xenografts according to Deschamps (2005, p.2)

Year (of the report)	Author	Place	Tissue	Animal source	Number of cases
1501	Baha' al-Dawa	Iran	Bone	Dog	One case report
About 1501	Ala-ul-Din	Herat, Afghanistan	Bone	Dog	One case report
1668	Job van Meeneren	(likely) The Netherlands	Bone	Dog	One case report
1875	Houzé de l'Aulnoit	France	Cheeks	Rabbit	45 case reports
1893	Williams	Bristol, UK	Pancreas segments	Sheep	15 year-old-child
1889	Brown-Séquard	Paris, France	Extract of crushed testicles	Dog and pig	Not known
1920-1951	Voronoff	Paris, France	Testicles, ovaries	Ape	Over 2000 case reports

3.3.3 Organ xenotransplantation

Unlike tissue xenografts, the successful transplantation of organs within and across the species barrier was a rare phenomenon in the early 20th century. The lack of proper surgical techniques, especially performing a vascular anastomosis

which is essential to restore blood flow and ensure normal organ functions, contributed to high failure rates (Deschamps 2005, p.6). In 1902, two French physicians, Carrel and Jaboulay, developed the technique of vascular suture that is still popular today. Carell was therefore awarded the Nobel Prize in Physiology or Medicine in 1912 for pioneering his surgical vascular suturing techniques (Sade 2005, p.2416-2417). Another success factor can be observed in the development of immunosuppressants after the Second World War. In 1933, the Ukrainian surgeon Voronoy carried out the first human-to-human kidney transplant, which, however, failed due to tissue incompatibility. In the following decades, many more unsuccessful attempts were performed: The first successful transplantation of a kidney was achieved in 1954 by the physician Joseph Murray on identical twins. With the help of whole-body irradiation of the transplant recipients and the associated damage to the bone marrow, it was possible to achieve a suppression of the immune system. Since the early 1960s, pharmacological and biological immunosuppressive techniques have been developed that dramatically improve the “survival rates" of transplanted organs. The number of allografts and xenotransplantation trials grew accordingly (De Vito Dabbs 2000, p.419-429).

3.3.3.1 Renal xenografts

Kidney xenotransplantations were performed at a high rate in the 20th century. Due to its single-artery-vascularisation and its proof of physiological function given by steady urine production, transplant surgeons favoured this paired organ (Deschamps 2005, p.6).

In 1902 Emerich Ullmann, a Hungarian-Austrian surgeon, transplanted a dog kidney to the neck of a goat in a hospital in Vienna. Even though his attempt was a failure, Ullman was the first to successfully transplant a solid organ (Margreiter 2014, p.65-66). At the same time, Princeteau in France inserted pieces of a rabbit kidney without vascular anastomosis into the kidney of a child suffering from renal insufficiency. Princeteau wrote at that time that the immediate results had been excellent: “’The immediate results were excellent," he wrote, "The volume of urine increased; vomiting stopped ... On the 16th day the child died of pulmonary congestion ...’” (Cooper 1997, p.9). From today's perspective, this description cannot be considered as realistic (Beckmann 2000, p.84).

In 1906, Mathieu Jaboulay in Lyon anastomosed kidneys of a pig and a goat twice to the blood vessels on patients' forearms or lower legs. None of the xenografts functioned properly as the vessels were immediately occluded by venous blood clots. At the end of 1909, Unger, after several attempts, transferred kidneys from a rhesus monkey to a woman. The patient died after 32 hours (Reemtsma 1964, p.384). However, he correctly observed that blood coagulation in the venous blood vessels is less pronounced in xenotransplantation between primates than in organs from other animals (Beckmann 2000, p.84).

In 1923, a physician named Neuhof, attempted to cure a patient who suffered from mercury bichloride poisoning with a xenogenic renal transplantation. The patient died after nine days. Whether the kidney worked is not known (Cooper 1997, p.9). Neuhof maintained, "however, that a heterografted kidney in a human being does not necessarily become gangrenous and the procedure is, therefore, not necessarily a dangerous one, as had been supposed. It also demonstrates that thrombosis or hemorrhage at the anastomosis is not inevitable. I believe that this case report should turn attention anew ..." (Reemtsma 1964, p.384-385).

After the Second World War the first immunosuppressants were developed. With increasing success in the field of allogeneic transplantation the shortage of human organs soon became apparent. Since hemodialysis was not yet generally available and meanwhile the first immunosuppressants had been tested, surgeons resumed work with xenotransplantation (Cooper 1997, p.10).

In 1963, Keith Reemtsma transferred chimpanzee kidneys to a human recipient:

> In our renal allografting program at Tulane University in New Orleans, we had increasing difficulty obtaining donor organs. Attempts to use cadaveric kidneys were inadequate. We were reluctant to press the use of volunteer humans for ethical, scientific, and legal reasons. Chronic dialysis was not available. As this impasse was developing, we decided to explore the use of nonhuman sources for clinical renal transplantation, This decision was prompted, in part, by clinical urgency, Additionally, a regional primate center in this vicinity brought scientists experienced in primatology. Furthermore, an active program in transplantation immunology had been developed to give an added base to the study. (Cooper 1997, p.10-11)

The selection of animal donors in Reemtsma's program was based on blood group compatibility and body size. Both kidneys in the animals were explanted together and transplanted into the thigh of the patients. The first recipient survived nine weeks. Four of the patients died within a week because of lung infections. One patient, however, survived nine months with a functioning kidney. This represents

the longest functional life ever achieved with a xenograft (Beckmann 2000, p.84). Reemsta (1964, p.405), however, considered his surgical interventions as merely experimental: “Under these circumstances only the most stringent precautions will make such work justified and justifiable, and historic experience shows that the field of heterotransplantation may be abused flagrantly.”

Table 3 Historical review of kidney xenotransplantation according to Deschamps (2005, p.3)

Year (of the report)	Author	Place	Animal source	Number of cases	Survival time
1905	Princeteau	Bordeaux, France	Rabbit	1 case report (child)	16 days
1906	Jaboulay	Lyon, France	Pig, Goat	2 Woman (48yr, 50yr)	3 days
1910	Unger	Berlin, Germany	Macaque	Woman (21yr)	32 hours
1913	Schonstadt	Not known	Monkey	Woman	60 hours
1923	Neuhof	New York, USA	Lamb	One case report	9 days
1963	Hitchcock	Minneapolis, MN, USA	Baboon	Woman (65yr)	4 days
1963	Reemtsma	New Orleans, LA, USA	Rhesus monkey	Man (65yr)	63 days
1964	Reemtsma	New Orleans, LA, USA	Chimpanzee	Woman (23yr)	9 months
1964	Reemtsma	New Orleans, LA, USA	Chimpanzees	12 case reports	63 to 270 days
1964	Starzl	Denver, CO, USA	Baboons	6 case reports	19 to 98 days
1964	Hume	Richmond, VA, USA	Chimpanzee	1 case report (man)	1 day
1964	Traeger	Lyon, France	Chimpanzees	3 case reports	<49 days
1966	Cortesini	Rome, Italy	Chimpanzee	Man (19yr)	31 days

3.3.3.2 Cardiac xenografts

The first human heart transplant ever was a xenogeneic transplant and was performed by Hardy in 1964 in Jackson, USA. Hardy transplanted the heart of a chimpanzee on a 68-year-old man with terminal heart failure (Beckmann 2000, p.85). Because of the lack of appropriate human donors and the rapid deterioration of the patient’s health status, he decided to implant the xenograft.

The relatively small organ worked only a few hours, probably because of surgical problems. Previously, Hardy had successfully transplanted hearts and lungs between dogs experimentally (Morris 2017, p.2280-2281). His first attempt of cardiac xenotransplantation led to a discourse within the general public and the medical profession:

> Interestingly, the brief consent form for the procedure was signed by a relative of the semicomatose patient, and although it did state that no heart transplantation had been performed previously, it made no mention that an animal heart might be used. There was an adverse response from both the medical profession and the public to this operation, dissuading Hardy from carrying out any further clinical heart transplantation at that time. (Murthy 2016, p.1605)

In 1968 Denton Cooley published a case-study of a 48-year-old man who suffered from terminal ischemic cardiomyopathy and received a sheep heart in Houston. The xenograft failed after ten minutes due to a hyperacute rejection (Beckmann 2000, p.85).

In 1977, C. Barnard, who had realized the first human to human heart transplantation ten years earlier, attempted two heterotopic heart transplants from baboons to humans as a bridging method. The patients died with the assisting xenografts because no suitable allogenic donor during the bridging time of two to four days could be found in spite of immunosuppression therapy. However, these achieved time spans show that hearts of near-related species can survive for more than just hours (Deschamps 2005, p.8).

L. Baily in Loma Linda, California, used his experience with neonatal patients and suggested that their immune system would provide favorable starting conditions for xenotransplantation (Stoller 1990, p.1-2). The transplantation of a 7-month-old juvenile baboon heart to a newborn baby (Baby Fae, see also Chapter 4) on October 26, 1984 was eventually unsuccessful by causing a lethal multiple organ failure. The reason for the failure was probably the unknown blood group incompatibility (Beckmann 2000, p.85).

In 1996 Dhani Ram Baruah, an Indian surgeon, performed a heart xenotransplantation on a 32-year-old man who suffered from a severe heart defect. After the death of the patient a few days later, Baruah was imprisoned for disregarding the Human Organ Transplantation Act of 1994 (Deschamps 2005, p.10).

Table 4 Historical review of heart xenotransplantation according to Deschamps (2005, p.3)

Year (of the report)	Author	Place	Animal source	Number of cases	Survival time
1964	Hardy	Jackson, MS, USA	Chimpanzee	Man (68yr)	90 minutes
1968	Ross	London, UK	Pig	Man (48yr), second case report	4 minutes, immediately
1968	Cooley	Austin, TX, USA	Sheep	Man (48yr)	10 minutes
1969	Marion	Lyon, France	Chimpanzee	One case report (woman)	“quickly”
1977	Barnard	Cape Town, South Africa	Babbon, Chimpanzee	Woman (25yr), Man (60yr)	5 hours 30minutes, 4 days
1984	Bailey	Loma Linda, CA, USA	Baboon	Girl (14 day old)	20 days
1992	Religa & Czaplicki	Sosnowiec, Poland	Pig	Man (31yr)	23 hours
1996	Baruah	Sonapur, India	Pig	Man (32yr)	7 days

3.3.3.3 Liver and lung xenografts

Liver and lung xenotransplantations have been performed rarely in the history of transplantation surgery due to the highly complex metabolism and physiological function of both organs. In Pittsburgh, Dr. Abouna treated hepatic coma patients with up to 17 consecutively connected livers of various species. In the mid-1960s, more than 460 patients were treated with extracorporeal livers, of which only 45 can be considered long-term survivors. In 1981 Dr. Lie worked primarily with extracorporeal baboon livers and described human survival rates of 66%. Other attempts to improve the condition of pig livers have failed (Beckmann 2000, p.86-87). Xenogenic liver transplants, however, were rare. Starzl was the first to transplant a chimpanzee liver to a human in 1966. The patient died after 24 hours. In 1969, 1970 and 1974, the same group performed three more xenogeneic transplants of chimpanzees on children, unfortunately with survival times of less than two weeks. At the same period of time, Lucien Léger transplanted a baboon liver xenograft to a mid-twenty year old woman who suffered from a severe hepatitis infection. After the liver had functioned normally for two days, the

xenogenic liver was rejected and eventually it had to be removed leading to the death of the patient (Deschamps 2005, p.8). With the approval of an international ethics committee, Starzl was able to transplant baboon livers to patients with end-stage hepatitis B or HIV-infected patients in 1992 and 1993. The two treated patients died after 21 and 70 days (Cooper. 2012, p.52). The last xenogeneic liver transplantation was performed by Makowka in Los Angeles using a pig liver. His patient eventually died within 24 hours of a hyperacute rejection (Ekser 2009, p.1043).

Xenogenic lung transplantations are still in their early stages. The lungs, as an immunologically extremely active organ, are exposed not only to the immunological reaction but also to noxious agents from the outside world. Since lungs as grafts are particularly sensitive to trauma, their number is limited. In 1968, Bryant and his research team experimented with porcine lungs to oxygenate human blood ex-vivo to maintain adequate blood oxygen levels during surgeries. The results showed increased pulmonary vascular resistance and pulmonary oedema (Person 1996, p.749-750). A lung transplantation from animals to humans is not described in historical medical literature concerning clinical trials that took place in or before the 20th century (Beckmann 2000, p.87). Lung xenotransplantation from pigs to humans may serve as a bridging method or an alternative to extracorporeal membrane oxygenation in case of cardiac surgery under the condition that basic research shows consistent positive results. In the meantime, only patients who suffer from severe lung failure and have no chance to be offered an allograft in time and have limited life expectancy without a transplant should receive a lung xenotransplantation (Banner & Polak & Yacoub 2007, p.380-381).

Table 5 Historical review of liver xenotransplantation according to Deschamps (2005, p.3)

Year (of the report)	Author	Place	Animal source	Number of cases	Survival time
1969	Starzl	Denver, CO, USA	Chimpanzee	Child (28 months)	9 days
1969	Bertoye & Marion	Lyon, France	Baboon	Woman (22yr), Boy (7 months)	>4 months, 39 hours
1970	Leger	Paris, France	Baboon	Woman (23yr)	72 hours

1970	Giles & Starzl	Denver, CO, USA	Chimpanzee	Boy (7 months)	26 hours
1971	Pouyet & Bérard	Lyon, France	Baboon	2 Woman (28yr, 24yr)	<2 days
1974	Starzl	Denver, CO, USA	Chimpanzee	1 case report (child)	14 days
1992	Makowka	Los Angeles, CA, USA	Pig	Woman (26yr)	34 hours
1992	Starzl	Pittsburgh, PA, USA	Baboon	Man (35yr)	70 days
1993	Starzl	Pittsburgh, PA, USA	Baboon	Man (62yr)	In a coma for 26 days

4 The concept of xenotransplantation

There are four categories of xenotransplantation: cell xenografts, tissue xenografts, organ xenografts and extracorporeal perfusion. Cell and tissue xenografts use different tissue types or individual cell types to replace non-functional cell complexes in the recipient (e.g., human). In extracorporeal perfusion, the patient's blood is directed outwards through the animal's circulation, such as a liver, kidney, or artificially generated organ system (Bisong 2015, p.2). An area of application for extracorporeal perfusion is e.g. the extracorporeal perfusion for the treatment of acute liver failure by culturing isolated hepatocytes for the use in bioartificial liver (Stockmann 2000, p.461). Xenogenic organ transplantation is a procedure in which individual organs or combined organ systems are transplanted across species barriers (Bisong 2015, p.2). The following subchapters are going to focus on the need for organ xenografts for clinical transplantation in human medicine.

4.1 The choice of donor species

The longest survival times of xenografts in human recipients were observed when animal species were used as organ sources that are phylogenetically related to humans. It was also found that transplants between members of different species, not necessarily human recipients, had different patterns of rejection. Subsequently, the classification of xenografts into two groups, as far as the donor-recipient-constellation is concerned, was proposed: concordant and discordant xenografts. The term concordant is used in a transplant between members of different species, in which the transplant is rejected in a similar time frame as in an allograft to a non-sensitized recipient. In contrast, the term discordant is used in a xenotransplantation in which the graft causes a hyperacute rejection, similar to an allotransplantation to a previously sensitized organ recipient (White 1992, p.1-2). An example of a discordant combination is the transplantation of a porcine organ into a human. An example of concordant xenotransplantation is organ transduction from a baboon or chimpanzee to a human recipient. Concordant xenografts do not cause a hyperacute xenogenic rejection (Hetzer, Neuhaus, Pfitzmann 2001, p.239). As was shown above, researchers have developed different animal models for concordant and discordant xenografts. High success rate in the context of long-time survival rates have been achieved particularly in concordant

xenotransplantation. Transplantations between different animal species, which preceded trials of xenografts in human medicine, were performed to understand the effects of immunosuppressants and the pathways that led to rejections by the human immune system (Fung 1997, p.2).

With respect to immunological compatibility of a human xenograft, a concordant combination in the selection of donor species would be more promising than a discordant combination. Therefore primates were first considered as donor animals for xenotransplantation. In particular, non-human primates (Old World monkeys) have a high compatibility in the anatomy and physiology of their organs and their blood groups (Boneva 2001, p.2). In the first instance chimpanzees were regarded to be the best organ sources for clinical xenografts due to their high degree of relationship with humans. Nevertheless, researchers focused on Baboons instead because they were considered to be a less endangered species but encountered several other problems (Bisong 2015, p.3) as keeping and breeding these animals is difficult and very expensive. In addition, due to the long gestation period and low progeny, a sufficient number of donor animals cannot be guaranteed (Grant 2001, p.244). The small organs of even full-grown baboons cannot maintain adequate circulatory function in an adult, which makes baboons unsuitable as organ donors e.g. heart transplants with their low pressure system are in danger of decompensating (Hammer 1998, p.19-20). Moreover, the blood group O occurs very rarely in baboons and thus already excludes a large part of potential human recipients (Schmoeckel 2010, p.141-142). Another reason against the use of primates is the high risk of transmission of microorganisms due to the phylogenetic proximity of humans and baboons. Thus, the simian foamy virus, as it is related to the better known Human Immunodeficiency Virus, endogenous retroviruses of baboons and the baboon cytomegalovirus were observed in the first clinical transplantation of baboon human livers. The transmission of these and other viruses carried by baboons and other non-human primates let the human population at risk regardless of whether the recipient of the xenografts develops a disease or not (Allan 1998, p.90-95). In 1997 the Advisory Group on the Ethics of Xenotransplantation, founded by the British Government, published a report titled “Animal Tissue Into Humans”. The authors listed a number of arguments against the use of non-human primates as organ sources for xenotransplantation, e.g. close affinity with humans, self-awareness, profound mental capacity and ability of

suffering pain. In contrast, they argued that it would be ethically legitimated to use pigs instead. Even though the authors state clearly that animals have rights and should not be used lightly for xenotransplantation, they also considered that animals differ in their intellect and ability for suffering and eventually rated pigs lower at that scale (Webster 1998, p.281-282):

> "While the pig may be exposed to harm we do not regard it as so unjustifiable as to make the use of the pig unacceptable in principle. Instead, as regards the pig, the issue is one of balancing the rights of the pig to be free from harm, as we understand them, against the rights of the human to who, as have seen could benefit from Xenotransplantation." (Romeo Casabona 2008, p.167)

In 1999 the U.S. Food and Drug Administration published a guidance document on the topic of public health issues by using non-human primate xenografts for clinical applications and recommended that "[…] there is not sufficient information to assess the risks posed by nonhuman primate xenotransplantation. FDA believes that it will be necessary for there to be public discussion before these issues can be adequately addressed" (U.S. Food and Drug Administration 1999, p.5).

From an anatomical and physiological point of view, several mammals are considered as appropriate animal species for xenotransplantation in human medicine. However, these considerations should not overlook the fact that different xenogeneic systems also produce quite different physiological problems. The results are in principle not transferable from one xenogeneic system to another and for this reason, research since the 1990s has focused on the pig as a source of xenografts (with some exceptions) to avoid the considerable amount of research needed to compare different xenogeneic systems. The pig (*Sus Scrofa*) is favored as a potential donor animal for numerous reasons, although organ transmission between pig and primate or between pig and human is an example of the discordant transplantation model (Beckmann 2000, p.136).

The early onset of sexual maturity (4-8 months), short gestation and high reproduction cycle (115 days) with large sized litters (5-12 piglets) guarantees a cost-effective production of donor animals (Grant 2001, p.244). The domestication of the pig over centuries and its agricultural use as a farm animal likewise reduces ethical concerns about organ harvesting for life-sustaining transplants (Rubaltelli 2008, p.162-163). Moreover, porcine xenografts are already used in clinical medicine, e.g. pig heart valves and pig insulin (Cooper 2000, p.1134). To improve the acceptance of animal organs or cells by the patient's immune system, pigs

could be genetically engineered to express particular human genes (Bisong 2015, p.3). Furthermore, there is the possibility of *qualified pathogen free* animal husbandry, whereby known zoonotic and potentially pathogenic pathogens can be largely eliminated from the donor animal breeding (Tucker 2002, p.191-192). However, there are still many hurdles to overcome, as far as physiological incompatibilities, immunological problems and the risk of transmitting porcine endogenous retroviruses (PERVs) and other viruses are concerned, before the first porcine organs can replace human allografts and can be described in written standard operating procedures (Iverson 1998, p.122-123).

4.2 Comparable characteristics of human and porcine organs on anatomy and physiology

There are anatomical differences between humans and pigs, which could be a serious obstacle to xenotransplantation. Some shape, structure and tissue properties, but also mechanical and functional peculiarities exist in the various animal species. These can complicate the surgical techniques (Beckmann 2000, p.119), but according to different anatomical studies on the surgical technique used for xenotransplantations, e.g. (Siepe et al. 2007, Cooper et al. 2016), technical problems can be avoided by considering anatomical particularities of the animal source (Siepe et al. 2007, p.214-215). If the anatomical differences cannot be fully eliminated, xenografts could at least serve as short-term-bridging procedures to allotransplantation (Cooper 2016, p.9). Nevertheless, too large organs will either be compressed or occupy, such as enlarged hearts in the thorax, too much space. On the other hand, too small organs can expand in a short time. They develop oedema and interstitial haemorrhages, which inevitably lead to loss of organ function (Beckmann 2000, p.119-120). Many of the anatomical differences between solid organs of man and pig are probably due to the different posture, because both the upright walk of man and the four-legged posture of the pig have led to a specific adaptation of the organs to their position in the body in the course of evolution. In general, the differences in blood pressure are much more pronounced between the various organs as well as within an organ in upright people than in pigs (Crick 1998, p.116-119).

4.2.1 Porcine blood

Sufficient blood supply to the organs after xenotransplantation is the basic requirement for their function. If an organ is insufficiently supplied, it lacks on the one hand nutrients and oxygen and on the other hand, metabolic degradation products accumulate (Beckmann 2000, p.129). The human red blood cells are the largest among mammals and could theoretically lead to mechanical disturbances of microcirculation. However, all healthy erythrocytes are more elastic than the rigid cell core of white blood cells. In addition, the blood viscosity of the organs, which is particularly influenced by the proportion of cellular constituents (hematocrit) and the protein content, is decisive for the blood circulation of the organs. This is very similar in both species. The normal hematocrit of humans is about 45%, that of the pig at 30% (Hammer 1998, p.21-22). In addition, the blood groups differ. In humans, there is the well-known AB0 system, in pigs, however, 16 blood groups were researched and these are denoted by capital letters from A to P. The rhesus factor of humans is not one of the blood properties of the pig. The oxygen supply of organs in humans differs considerably from that in pigs (Smith 2006, p.186-191). The arterial oxygen partial pressure is 13,3 kPa in humans and only 10,9 kPa in pigs (Douglas 1972, Schmidt 1983, Hannon 1990 and Larsen 2013). Due to the low concentration of hemoglobin in the porcine blood, the oxygen content of the arterial blood in pigs is significantly lower than that of humans. As far as xenotransplantations are concerned, the reduced oxygen level is unlikely to cause problems because the organs are placed in an environment with better oxygen supply (Kulick 2000, p.697-698).

4.2.2 Porcine hearts

As far as solid organ xenotransplantations are concerned, cardiac xenografts score considerably better with regards to immunological rejection reactions and survival time, suggesting that the physiology of the cardiovascular system is more similar to humans then the immune system. The available data on heart xenotransplantations shows differences and problems concerning anatomy and/or pathophysiology (Dobson 2002, p.99). Porcine and human hearts have different shapes due to their position in the thoracic cavity. The body posture of the pig also places other demands on the orientation of the vessels, e.g. to ensure adequate venous return or output performance. Significant differences between man and pig

also exist in the structure of the right ventricle, especially in the orientation of the pulmonary valve. This anatomic characteristic is attributable to the four-legged posture of the pig, so that the entire outflow system leading to the lungs is constructed differently than in humans. In addition, the thorax of pigs is narrowed laterally, whereas the organs in humans are placed in the direction from the back to the abdomen. Therefore, there is a change in the orientation and position of the porcine organs used as xenografts, possibly leading to a reduction in the performance of the organs (Crick 1998, p.107-115). The cardiac time volume of both porcine and human hearts is nearly identical in relation to the body weight (Beckmann 2000, p.119). However, the construction of heart valves and large cardiac vessels is partly different, e.g. whereas the mitral valve is comparable to the human pendant in size and structure (Lelovas 2014, p.432-433), the aortic valve differs in its opening by approximately 17%. Thus, a pig's heart, when transplanted to humans, would have to do more work for the same ejection volume to overcome the pressure gradient (Beckmann 2000, p.119). Clinically such porcine heart valves prove to be very effective in humans and show in direct comparison to mechanical heart valves similar survival rates of over 20 years (Khan 2001, p.257). Concerning pathophysiologic problems, heart xenotransplantations performed from pigs to non-human primates sometimes caused lethal cardiac arrhythmia. Researchers suggest that this phenomenon is caused by the different intrinsic innervation of the electrical conduction system of the heart (Crick 1999, p.356) but trials over a long timeframe with non-human primates have shown that this acute cardiac event should not pose a problem (Ekser 2015, p.202). Physiological differences in body temperature, blood viscosity, endocrine system and enzymatic balance have been shown in a pig-to-baboon cardiac xenotransplantation and sufficient organ function has been demonstrated in an upright recipient organism (Bauer 2005, p.447-448).

4.2.3 Porcine kidneys

As a central organ in the water and electrolyte balance as well as in the regulation of the acid-base balance, the kidney fulfills a significantly complex function. Similar to heart xenotransplantation, the porcine kidney has been intensively researched and shows similarities to human kidneys in structure and function (Sampaio 1998, p.45-46). For instance, severe physical stress in pigs and humans increases the

blood supply to skeletal muscle at the expense of renal perfusion (Sanders 1976, p.935).

Significant differences are observed concerning blood concentrations of creatinine, urea and uric acid – substances only excreted via the kidneys. Within pigs, the rate of excretion surpasses that of humans. Eventually, xenotransplantation of porcine organs to humans may cause low soluble salts of uric acid with tissue deposits due to the increased uric acid concentrations (Chen 1990, p.126-128).

Differences in electrolyte concentrations and their adaptation to porcine levels after a xenotransplantation of a kidney may lead to potassium, calcium and phosphate concentrations above normal human levels (Schraa 1999, p.332).

The kidney hormone erythropoietin (EPO), which regulates the production of red blood cells in the bone marrow, has the species-specific amino acid sequences. Although human and porcine sequences are 80% homologous (Wen 1993, p.1507), animal xenotransplantation experiments revealed that the porcine EPO does not interact with the bone marrow of non-human primates. This lack of EPO is also known after nephrectomy surgeries and recombinant EPO is able to compensate for this deficiency. This therapeutic approach is also possible within xenotransplantation. However, since porcine erythropoietin is produced simultaneously from the xenogeneic kidney, which is antigenic and causes human antibody production, there is a risk that cross-reacting antibodies directed against porcine EPO could also immunologically eliminate the foreign recombinant EPO (Regulier 1998, p.1019).

Kidney xenografts cause a severe immunological rejection reaction and therefore the recipients need high levels of immunosuppressant therapy. In order to avoid hyperacute rejection, researchers transplant vascularized porcine thymic grafts combined with porcine kidney graft to reduce the recipient's immune response (Sachs 2009, p.4-6):

> The first method involves the preparation of a composite tissue "thymokidney" and the second utilizes the transplantation of an isolated vascularized thymic lobe. Both strategies involve the transplantation of fully vascularized thymic tissue at the time of xenotransplantation, a fact which is crucial for function of the thymic tissue immediately after xenografting and reeducation of recipient T-cells. These strategies have successfully induced tolerance across fully allogeneic models in miniature swine and prolonged graft survival in our pig-to-baboon model of life-supporting xenotransplantation to greater than 80 days with in vitro evidence of donor-specific unresponsiveness. (Costa 2012, p.191)

4.2.4 Porcine livers

The pig liver is surrounded by a strong connective tissue layer, which gives it a more lobe-like appearance than the human liver. Although both organs can be divided into eight different segments, the porcine liver consists of four lobes whereas the human liver has a right and a left lobe. As far as the vascular anatomy and biliary tract are concerned, there are only few differences between the human and the porcine liver, whereby they are especially important for surgical techniques. Considering anatomic species-specific differences, the porcine liver is a suitable candidate for experimental surgery and xenotransplantation (Nykonenko. 2017, p.25-26). Complex organs such as the liver produce numerous enzymes (over 2500) and hormones, whose compatibility and functionality in the human body are questionable (Schön 1999, p.235-238). Many of these enzymes are species-specific, e.g. proteins and enzymes in the blood essential for haemodynamics. An important function of the liver is the production of 95% of proteins for the complement system, which is an essential part of the immune system. In particular, foreign complement proteins are an aggressive mediator and trigger many cell toxic reactions. Pig complement would rapidly destroy the vital transport systems of the microcirculation of the human recipient and thus lead to loss of function of the organ or tissue (Calne 1968, p.1176–1178). Another difference can be seen in the protein albumin, which is responsible for maintaining the colloid osmotic pressure in blood vessels. The amino acid sequence of human and porcine albumin differs markedly and transplanting a porcine liver into humans could lead to a significant reduction in albumin concentration (Rosenoer 1977, p.28-30). This would result in a reduced transport capacity of water-insoluble substances, which may promote the formation of interstitial edema (Beckmann 2000, p.125-126).

Regarding the above selection of existing problems concerning liver xenografts, greater opportunities than permanent liver transplantation are given to transient extracorporeal perfusion of porcine livers and using animal hepatocytes. As for instance patients suffering from liver failure have high blood levels of toxic bile acids. Using a bioartifical liver, perfused with the patients' blood, cleared and excreted the circulating bile acids and reduced toxicity levels (Pazzi 2002, p.1553).

4.2.5 Porcine lungs

Scientific data concerning clinical trials with porcine lung xenografts are limited and usually only veterinary medicine publications and literature provide detailed information concerning porcine lung anatomy and physiology (Judge 2014, p.334): Human left and right lungs consist of two or three lobes, whereas the left porcine lung consists of three and the right one of four lung lobes. In addition, pigs not only have two main bronchi but also an additional main bronchus leading to the cranial lobe of the right lung (Frandson & Wilke & Fails 2009, p.325-327). In the human lung gas exchange normally takes place only in the lower third. However, the pork lung is designed for horizontal ventilation. Therefore, the question arises whether a pork lung xenograft would tolerate an upright position for a long time. Furthermore, breathing is associated with locomotion in a number of animals, as well as humans, in a particular proportion, and therefore respiratory problems may arise in xenotransplantation of lungs from quadrupeds (Beckmann 2000, p.119).
Comparing the porcine and human respiration physiology it is particularly noticeable that the pig, despite its approximately twice as high respiratory time volume, has a lower arterial oxygen partial pressure than a human:

Table 6 Physiological parameters of the lungs of man and pig (Douglas 1972, Schmidt 1983, Hannon 1990 and Larsen 2013)

Paramter	Pig	Human
arterial oxygen partial pressure	10,9 kPa	13,3 kPa
respiratory minute volume	198 ml/min/kg	130ml/min/kg
respiratory rate	10-16/min	7–20/min
tidal volume	10,1 ml/kg	6-8 ml/kg

Porcine lungs, when perfused with human blood, emit neutrophil granulocytes and thrombocytes initiating platelet-coagulation via the coagulation cascade causing severe thrombosis. In addition, severe pulmonary vasoconstrictions may occur in the presence of pulmonary intravascular macrophages in pig lungs (Costa 2012, p.169). These specialised cells are a subgroup of macrophages and have several tasks to fulfil in the porcine organism:

> 1) they are strongly adhered to the capillary walls via desmosome-like intercellular adhesion plaques, which secure stable and lasting direct exposition of the bulk of these cells to the blood stream; 2) their ruffled surface engaged in intense phagocytic activity ensures efficient binding and phagocytosis of nanoparticles; 3) PIM cells express anaphylatoxin receptors, this way C activation can trigger these cells, 4) they also express pattern recognition molecules on their surface, whose engagement with certain coated nanoparticles may also activate these cells or act in synergy with anaphylatoxins and, finally 5) their high metabolic activity and capability for immediate secretion of vasoactive mediators upon stimulation explain the circulatory blockage and other robust physiological effects that their stimulation may cause. (Csukás 2015, p.27)

Nevertheless, the porcine lungs are able to provide enough capability of oxygen and carbon dioxide exchange in non-human primates. The small amount of data, however, allows no definitive conclusions (Ekser 2015, p.202-203). In the meantime the International Society for Heart & Lung Transplantation recommends lung xenotransplantation only for patients that suffer from severe lung failure and have a reduced life expectancy or are unlikely to be offered an adequate allograft in time. In order that clinical lung xenotransplantation become a standard operating procedure, profound basic research and appropriate publications have to show consistent positive results (Banner & Polak & Yacoub 2007, p.380-382).

4.2.6 Porcine pancreatic islet cells

The metabolic disease diabetes mellitus is a chronic disease that can be categorized in several types. Hyperglycemia is the leading symptom of all forms. The characteristic symptoms of an (clinically relevant) increase in blood sugar can be polyuria, polydipsia, unclear weight loss and blurred vision, as well as ketoacidosis and coma. Long-term hyperglycemia, in the context of impaired insulin secretion and / or effect, causes damage to organs and tissues. These disorders mainly affect the kidneys, eyes, nerves, blood vessels and the heart (Baenkler 2001, p.932).

There are essentially two forms of this metabolic disease. Type 1 diabetes mellitus leads to immunological destruction of the pancreatic beta cells which results in absolute insulin deficiency. Diabetes mellitus type 2 develops on the basis of a metabolic syndrome that is a clustering of the following medical conditions: glucose tolerance disorder, visceral obesity, dyslipoproteinemia and essential hypertension. Initial insulin resistance requires more insulin for cellular glucose utilization that leads to an increased appetite, which in turn favors obesity.

In this context, three pathophysiological causes are described (Waldhäusl & Gries & Scherbaum 2014, p.35-40):

- A defect in the insulin receptor of human cells decreases the effectiveness of the hormone. This results in insulin resistance, which eventually leads to elevated blood sugar.
- The disturbance of the postprandial insulin secretion leads to postprandial hyperglycemia and by an additionally increased glucagon secretion to further increase of the blood sugar.
- By apoptosis of the pancreatic beta cells and a loss of 50 percent of the islet cells, a secondary insulin secretion defect occurs and hyperglycemia increases.

As far as the therapy of a diabetes mellitus, especially the therapy of a type 1 diabetes, is concerned, patients, physicians and other healthcare professionals face a great challenge despite continuous developments and optimization in diabetes therapy. The life expectancy of patients with diabetes mellitus type 1 compared to persons without diabetes is still considerably limited (Baenkler 2001, p.932). This underlines above all the need for new and innovative forms of therapy, e.g. xenogeneic islet cell transplantation.

The wild boar, Sus scrofa, hasestablished as a popular donor animal for islet cells of the pancreas. The porcine insulin differs from the human insulin only in one amino acid and is well tolerated by the human organism. Despite this physiological compatibility, differences in the number, size, and morphology of the islet cells in the various pig breeds are evident (Costa 2012, p.213). As far as the medical risk and the transmission of porcine microorganisms are concerned, the expression of PERV mRNA varies in different tissues, with the lowest count in the pancreas. (Akiyoshi 1998, p.4503-4507). Furthermore, there are significant differences in the release of PERV particles between different pig strains (Tacke 2003, p.17-24). The reasons for this are currently the subject of research (Costa 2012, p.214).

The transplantation of isolated, allogeneic pancreatic islet cells – responsible for the insulin production and blood sugar regulation in healthy people – has established as an effective therapy method for patients with Diabetes mellitus type 1, suffering from frequent and severe hypoglycemia. A wider application of this treatment method is limited due to the inadequate availability of donor organs and the need for permanent immunosuppressive therapy. Furthermore, the long-term

results after islet cell transplantation, despite considerable progress, are still unsatisfying. The main reasons for this are a gradual loss of the beta cell function by inflammatory processes, insufficient growth in the donor organism as well as the hardly manageable immunological rejection reactions. One possible strategy to avoid these limitations is the use of cells of alternative origin, such as xenogeneic pancreatic islet cells or stem cell-derived insulin-producing cells. These new approaches presuppose, however, a selective-permeable barrier that allows free diffusion of oxygen, nutrients, and effector molecules while preventing migration of immune cells and cytotoxic, potentially harmful molecules. Considering this, macro- and microencapsulation methods are currently the subject of research and development (Park 2015, p.261–266).

The broad field of cell encapsulation techniques could make an important contribution to the clinical use of xenogeneic islet cell transplantation and could eventually establish a simple and safe therapy for diabetes mellitus. So-called macrocapsules are storage systems in which the entire graft is integrated into a "container" and implanted into a graft recipient. The challenge with these systems is, on the one hand, to ensure a sufficient influx of oxygen and nutrients and the unrestricted outflow of effector hormones and, on the other hand, to maintain a safe barrier to the immune system. By contrast, the benefits of macroencapsulation are their simplified implantation and explantation technique. While in the past hollow fiber designs, as known from modern dialysis methods, were used, today plate-shaped chamber systems are more popular because of their better diffusion properties. An essential aspect for ensuring the long-term survival and maintaining the function of encapsulated pancreatic islet cells is the sufficient supply of oxygen, for example by vascularization of the outer chamber sides. Another approach is to ensure optimal oxygenation of the encapsulated pancreatic islet cells with the help of an integrated oxygen reservoir, which is refilled from outside via special port systems. Eventually a physiological oxygen environment is created for the pancreatic islet cells. The preclinical studies with this system have been very successful in small and large animal experiments. Even a first trial in humans using allogeneic human pancreatic islet cells could prove a stable graft function over ten months with regulated endogenous insulin secretion without the need of an immunosuppressive therapy. Another concept are so-called intravascular implantable chamber systems in which hollow fiber tubes

filled with encapsulated islet cells are connected to the vascular system of the recipient. Unfortunately, these systems appear to cause severe complications, e.g. an increased risk of thrombosis and hemorrhage and are therefore no longer in the focus of current research and clinical testing (Sakata 2012, p.19-26).

In the course of the procedure of islet microencapsulation, the islet cell units are immobilized and implanted in biocompatible hydrogels like alginate or agarose. The most commonly used implantation site is the peritoneal cavity. Over the past decades, numerous scientific studies on islet cell microencapsulation in various rodent models have been published, although a repetition of the promising results in preclinical large animal models and clinical pilot studies has not been successful. Microcapsules have an overall more favorable profile in terms of mechanical stability, surface-volume ratio and immunogenicity compared to the described systems of macroencapsulation. However, inefficient production of intact islet microcapsules and severe complications like post-transplantation fibrosis resulting in impaired hormone diffusion, hypoxia and cell apoptosis, are the main reasons for an unsuccessful clinical use (Opara 2013, p.261-266). Regarding this, approaches using new microencapsulation materials appear more promising. Synthetic materials with high purity and permeability such as polyethylene glycol are placed as thin layers around individual pancreatic islet cells and have shown very promising results in the therapeutic preclinical studies available to date (De Vos 2014, p.28). These special bio-engineered islet cells were transplanted in diabetic baboons, leading to an insulin independence without the need for immunosuppressive therapy (Villa 2016, p.1033-1034).

An essential aspect of islet cell encapsulation technologies is the establishment of a sufficient immune barrier to shield the graft from the recipient’s immune system. A reduction of systemic immunosuppressive therapy for rejection reaction prophylaxis could significantly improve the functional results of islet cell transplantation and expand its clinical use. Transplantating allogeneic or xenogeneic tissue induces complex interactions between the recipient organism and the foreign graft. These include non-immunological factors as well as innate and acquired immunity. These reactions can lead to a gradual or rapid loss of graft functions. In patients with type 1 diabetes mellitus, the situation is even more complex as it is an autoimmune disease (Hering 2016, p.71-72).

4.3 Alternative therapeutic concepts

4.3.1 Dialysis and artificial kidneys

The kidney, with its complex structure and divergent physiological tasks, is an organ that, in case of dysfunction, leads to a variety of pathophysiological events. A permanent reduction of metabolic degradation products, a disturbed elimination of electrolyte and water and a limited hormone secretion constitute the genesis of chronic renal insufficiency. In 28% of the patients with terminal kidney failure diabetes mellitus was the cause of kidney dysfunction. Acute and chronic glomerulonephritis and vascular nephropathies are other diseases that cause chronic kidney dysfunction (Kuhlmann 2008, p.308). The following chart shows the diagnosis of patients with chronic renal failure at the beginning of therapy:

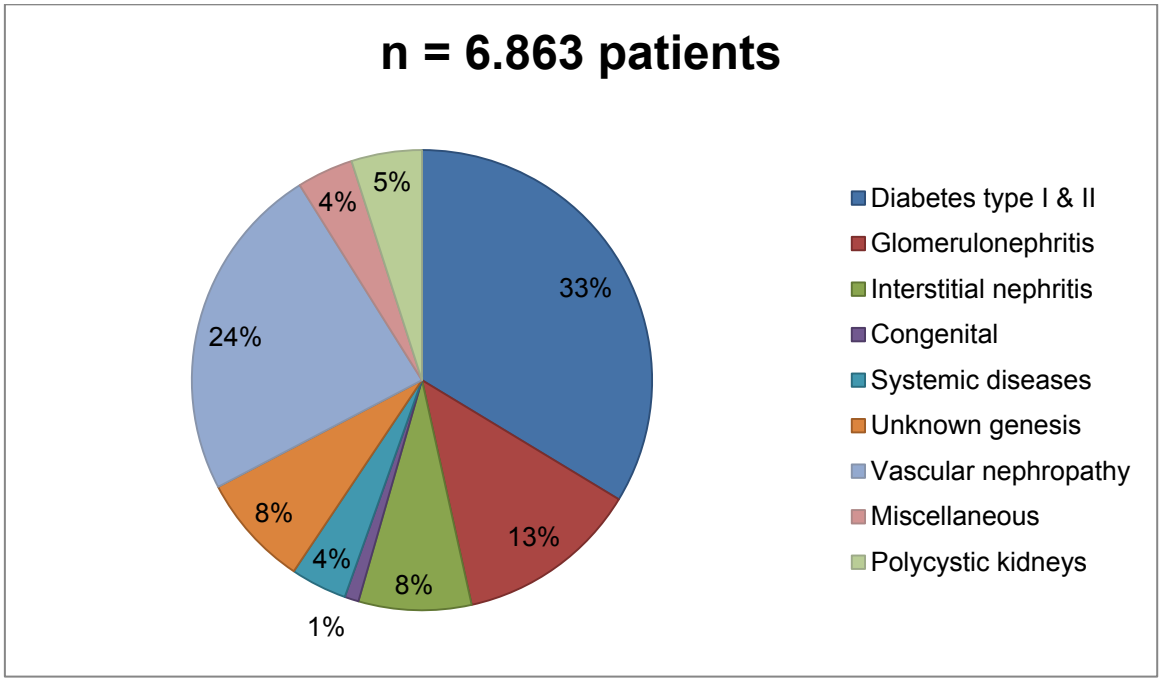

Chart 1 Diagnosis distribution of patients at start of therapy (incidence) in 2006 (Frei 2008, p.35)

In order to assess the function of the kidney, the glomerular filtration rate (GFR) is used in medicine, which is calculated from the total volume of primary urine produced by all the glomeruli of both kidneys and filtered off in a defined time. It provides information about the excretory capacity of the kidneys (clearance) and is around 120 ml/min/1,73m^2 in healthy kidneys (Silbernagl 2007, p.152). According to the National Kidney Foundation, chronic renal insufficiency is present if either of the following conditions applies (International Society of Nephrology 2013, p.6-8):

- GFR <60 ml/min/1,73m^2 over three months, with or without kidney damage

- Deviations from the normal structure or function of the kidneys persist for more than three months and are of health relevance, e.g.: albuminuria, electrolyte and other changes due to tubular disorders, changes in urinary sediment, histologically proven changes, structural changes detected by imaging techniques, kidney transplantation in the patient's anamnesis.

The aim of the treatment of chronic renal insufficiency is to limit the progress of functional deterioration of the organ as far as possible. At the end of the disease process there is the terminal renal insufficiency, the so-called uremia, which makes renal replacement therapy in the form of dialysis or kidney transplantation indispensable (Renz-Polster 2008, p.989).

If conservative therapy is inadequate, a renal replacement procedure is initiated in the terminal stage because a severe restriction or complete failure of the renal function leads to an accumulation of the blood with urinary substances and as a result to a lethal outcome. Renal replacement therapy is a treatment that takes care of the detoxification and excretory function of the kidneys. This includes hemodialysis, peritoneal dialysis and kidney transplantation (Geberth 2014, p.17-21).

In contrast to the other solid organs an alternative to transplantation is available for patients with chronic renal failure in the form of dialysis (Nowack 2013, p.434). Dialysis, though, cannot completely replace the kidney, but only remove metabolic end products and water from the blood. Regardless of whether hemodialysis or peritoneal dialysis is carried out, longer-term dialysis treatment typically results in metabolic disorders and sequelae, mainly due to the lack of endocrine and metabolic functions of the kidney (Keller 2010, p.263-279).

In hemodialysis the blood is cleaned three times a week by the dialysis machine for four to five hours. To provide easy access to the vascular system of the patient for haemodialysis, a vascular surgeon prepares an artificial connection between an artery and a vein. Eventually, the patient's blood enters a dialyzer with a semi-permeable membrane. There is a difference in concentration between the two sides of the membrane, since the metabolic products are present in high concentration on the blood side. These substances migrate through the pores of the membrane to the opposite side, the dialysate. Due to the semi-permeability of the membrane only small substances can pass, whereas larger molecules (for

example, proteins) or cells cannot. Since the blood is under a higher pressure than the dialysate, the body can also be deprived of water. The purified blood is returned to the body through a shunt. In peritoneal dialysis, the dialysis process takes place within the abdomen. The dialysis membrane used in peritoneal dialysis is the peritoneum, which surrounds all abdominal organs. The dialysis solution is introduced into the abdominal cavity through a catheter and absorbs the molecules there. Three to five times daily, about two liters of sterile dialysis solution are introduced into the abdominal cavity and drained again after a residence time of four to eight hours (Field 2017, p.101-104).

Although dialysis regulates the levels of water, electrolytes, acids and bases in the blood, it only makes a simple correction that guarantees survival. It cannot replace other functions of the kidney, e.g. the production of hormones and regulating blood pressure. In addition, the quality of life of patients requiring dialysis is significantly lower than that of persons who have undergone a kidney transplant. This is attributable to the restrictions in the diet, e.g. in terms of water, sodium and potassium intake and furthermore to the complications that may occur in dialysis treatment, such as e.g. cardiac disorders, hematological problems, endocrine disorders, hypertension, anemia, etc (Hillebrand 2005, p.7 and Keller 2010, p.263-279).

Renal transplantation is the desirable goal of renal replacement therapy for patients with chronic renal failure and is the treatment of choice. It leads to an improvement in the quality of life, reduces mortality and provides an effective renal replacement function that cannot be achieved by hemodialysis or peritoneal dialysis. A functioning graft also leads to an increase in physical performance (Keller 2010, p.293). The first successful kidney transplantation in 1954 marked the beginning of a new era in the treatment of end-stage renal failure. Until the early 1980s a positive effect on long-term survival after kidney transplantation compared to life-sustaining hemodialysis was only observable in living donors. Without adequate immunosuppression no survival benefit could be demonstrated in patients on dialysis compared to kidney graft recipients (Vollmer 1983, p.1556-1558).

Finally, it should be noted that the technical procedures of blood purification only allow replacement of the excretory function of the kidney. The endocrine and paracrine functions of the organ and its considerable capacity for the clearance of

inflammatory mediators cannot yet be replaced. However, this sector has promising approaches. The working group around Humes has been doing research for years on the development of a comprehensive renal support system. They were able to coat the inside of capillaries of high flux polysulfone dialyzers with human tubule cells from non-transplantable kidney donors. This system is connected to a conventional hemofiltration system in which the hemofilter takes over the function of the glomerulus. The ultrafiltrate is then passed through the tubule cell-coated filter to allow a "simulation" of the physiological processes in the tubular system. According to extensive animal studies, a first human application has now been carried out, showing that the cells of this bioartificial kidney support or at least partially compensate for the excretory and endocrine functions of the kidney. This opens up a broader therapeutic spectrum for the future (Humes 2014, p.343-350.

4.3.2 Cardiac assistive devices and artificial hearts

If conventional drug therapy as well as operative measures cannot improve a patient's cardiac condition, transplantation or mechanical circulatory support can be achieved by means of a ventricular assist device (VAD) or even a complete artificial heart (TAH). Artificial long-term heart support systems have been in clinical use since the 1980s and were initially constructed exclusively to bridge a life-threatening condition to a subsequent heart transplant, so only for a foreseeable, limited time. However, systems were developed that remained functional for years, so that for some patients these pumps would inevitably become permanent when faced with lack of donor organs (Kramme 2017, p.574). A univentricular or biventricular pumping assistance for instance is able to temporarily compensate a complete myocardial failure. An indication for this is reversible myocardial damage as well as patients with end stage heart failure to maintain their circulation until a suitable donor heart is available for transplantation.

External and implantable pumping systems can be distinguished. The best known external form of circulatory assistance used in the field of cardiac surgery is the postoperative relief of the left heart by the roller pump of the heart-lung machine. In the simplest case, it is an extension of the final phase of the cardiopulmonary bypass. However, the use of this method is limited by the associated serious

complications (haemolysis, coagulation, severe infection) to usually a few hours. If a centrifugal pump is used instead of the roller pump, such an external pumping system can be used over a period of several days but the mentioned side effects remain. In recent years, various pneumatically or electrically driven pumps have been developed which can be implanted as ventricular heart assist or replacement devices in the body (thorax, abdomen). With such pumps both the left and right ventricles can be assisted or replaced. For pump assistance the blood from the atrium or ventricle is directed into the pump and from there back into the aorta or pulmonary artery. The pumps can work synchronously or asynchronously to the heart, and the delivery volume of the pumps can be controlled by changing the pumping frequency. In the case of complete myocardial failure, the delivery volume is sufficient enough to ensure adequate blood circulation through the pumps alone. In contrast to the use of an extracorporeal assistant, which is limited to a few days, it is also possible to bridge periods of several weeks and months with implantable systems. In rare cases even recovery of the myocardium may occur (Borst 2013, p.40-42). Apart from that, there is on average sufficient time to wait for a suitable donor heart. In addition, after prolonged use of implantable assistance systems, many clinical cases showed that the secondary organ damage, initially caused by the patient's terminal heart failure (e.g. hepatic and pulmonary congestion, oliguria) is reduced (Sen 2016, p.1-2). Moreover, such a system can be used in patients with an acute rejection reaction after heart transplantation if there is a medical need or re-transplantation surgery is considered (Jung 2012, p.116).

The further development of the assistant pumps led to the construction of artificial hearts. The totally artificial heart follows the most radical concept of mechanical heart replacement. After an almost complete removal of the patient's severely diseased and thus insufficient heart two pumping chambers are implanted orthotopically in the thorax and connected via pressure lines to the external pneumatic drive (Antretter 2015, p.431). This results in effective biventricular support with high fluxes and low fill pressures. In contrast to other support concepts, though, weaning from the device is of course not possible. Typical indications for the use of TAH are biventricular pumping failure without a chance of recovery at e.g. severe myocardial damage following myocardial infarction (e.g. extensive anterior infarction). It is licensed in the USA for use as a destination

therapy. However, the system is predominantly used in Europe and the USA to bridge the waiting period until transplantation. Although some patients with TAH have been supported for over four years, long-term experience is limited. The quality of life of patients with TAH is often significantly reduced compared to current left VAD systems due to high noise levels and bulky drive units (Boeken 2017, p.92).

4.3.3 Liver replacement therapy and extracorporeal liver support

Due to its function in the intermediary metabolism and as an immune organ, the liver is of central importance to the course and prognosis of critically ill patients. Disturbances of liver function to the point of liver failure can be caused by diseases of the liver itself, for example in the context of viral hepatitis, or by extrahepatic diseases, as it is the case in severe sepsis with subsequent multi-organ failure. At any rate liver failure is characterised by severe necrosis and apoptosis of hepatocytes. Organ failure occurs when the number of liver cells still functioning becomes so low that liver-specific functions can no longer be sustained. Liver failure is an emergency comparable to acute cardiac failure with cardiogenic shock or acute renal failure. Acute liver failure causes numerous life-threatening complications, such as circulatory failure, cerebral oedema, coagulation disorders and infection. At the stage of terminal liver failure physicians and therapists in charge have to make a decision between a conservative procedure or a liver replacement therapy. Various prognostic parameters concerning a patient's probability of survival are helpful in this decision. If the stage of liver failure has progressed too far, liver transplantation has been established as a life support option for patients with acute liver failure or end-stage chronic liver disease. Due to the lack of enough supply of donor livers there has been a need for artificial liver replacement or artificial liver support with the aim of assisting patients with marginal liver function by either providing a suitable organ for transplantation or, at best, supporting in the regeneration of the diseased liver. An "artificial liver" could also assist patients who are in a phase of organ function recovery, e.g. early stage of post organ transplantation or after extensive surgical procedures, e.g. (partial) liver resections. In recent years various scientific research groups have presented a number of different systems for liver replacement therapy. All mechanical liver replacement procedures pursue two

goals: “Liver dialysis” treatment should compensate the missing detoxification function of the liver whereas artificial liver "bioreactors" aim to replace the entire liver cell function with the help of healthy liver cell cultures (Lodes 2017, p.276-281).

Attempts have been made to develop cell culture systems, so-called "bioreactors", which could at least temporarily take on liver function until their own liver is restored or a suitable graft becomes available.

Even in these approaches there are still many fundamental problems that remain unresolved so far: Human liver cell culture reactors are of questionable value as it is necessary to sacrifice a human liver to load the bioreactor. The question arises whether it would not make more sense to transplant them immediately. When using pig hepatocytes, biocompatibility is unclear. If, on the other hand, immortalized human liver cell lines are used, their differentiated functionality must be demonstrated and a carryover of potentially malignant cells into the organism of the patient must be prevented. Thus, until today, no usable bioreactor for serial production has been available. Research in the field of liver replacement, however, is active and it seems possible that functional concepts will be implemented and put into clinical trials in the near future (Adler 2014, p.484).

Bioreactors include the bioartificial liver, in which porcine hepatocytes are cultivated on the outside of hollow-fiber membranes. In a phase one study 16 out of 18 patients with fulminant liver failure and three out of three patients with a primary non-functional liver after a transplantation were successfully bridged to an urgent liver transplantation surgery. However, the publication provides no comment on the duration of the bridging time. In the group with acute-to-chronic liver failure two out of ten patients survived (Watanabe 1997, p.488-490]. In a similar procedure nine out of twelve patients survived acute hepatitis B induced liver failure (Ding 2003, p.830-831).

The “HepatAssist 2000” also directs separated plasma through an activated carbon filter and then through a hollow fibre unit with porcine liver cells. With this system, ten patients with hepatic encephalopathy stage three and four have been successfully bridged to a liver transplant and shown a significant improvement in the neurological status. The average time to transplant was 46 h (9 hours to 110 hours) and eight patients still lived after one year (Samuel 2002, p.258-263).

The focus of these mechanical therapies lies in the support of the detoxification function of the liver and the maintenance of homeostasis without compensation for the impaired liver synthesis in the sense of a "liver dialysis". For this purpose, classical nephrological procedures, such as haemodialysis, haemoadsorption or haemoperfusion, were initially evaluated, but without showing an improvement of the disease prognosis (Leonhardt 2016, p.279).

The “Molecular Adsorbent Recirculating System (MARS)” is another method of extracorporeal liver support, which was developed at the University of Rostock. It combines methods of haemodiafiltration, adsorption and conventional haemodialysis for the mechanical purification of the blood of toxic substances in case of liver failure. The patient's blood first flows through a filter with a protein-coated polysulfone hollow-fiber membrane. The primary dialysate fluid is an albumin solution, which is designed to absorb protein-bound toxins from the blood. In one cycle, the albumin circulates through an activated carbon filter and an anion exchanger and is thus regenerated. To eliminate water-soluble toxins, the albumin circulation contains another conventional dialysis filter. By this arrangement, not only water-soluble but also albumin-bound substances are eliminated in contrast to conventional dialysis. Due to the pore size of the “MARSFlux” membrane substances with a molecular weight of more than 50.000 dalton are not filtered out. Therefore levels of bilirubin, bile acids, short chain free fatty acids, aromatic amino acids, copper and water-soluble molecules, e.g. creatinine, urea and ammonia are reduced. Generally, depending on the patient's hemodynamic stability, MARS therapy may be either intermittent for six to eight hours or continuous. Continuous therapy requires an interruption to reprocess the system after 24 hours (Wilhelm 2013, p.407-408).

The Prometheus system, like the MARS-system, has a primary, secondary, and dialysate circulation, but differs in structure and in the filters used. The blood filter (“AlbuFlow”) has a larger pore size of 300 kilodalton and is thus permeable to the patient's own albumin, which is guided in the secondary circuit directly over the adsorber. The dialysate circuit is connected in parallel to the bloodstream and contains a high-flux dialyzer. The plasma is first separated by an albumin-permeable filter and then passed through an adsorber in a secondary circuit. In addition, conventional dialysis takes place in this secondary circuit (Burchardi 2011, p.577). Rifai et al. (2003, p.986-987) treated eleven patients with acute and

chronic liver failure with the Prometheus system and were able to show a reduction in bilirubin, bile acids, ammonia, creatinine and urea serum levels.

4.3.4 Extracorporeal membrane oxygenation and artificial lungs

Mechanical extracorporeal heart and lung support systems are nowadays an established intensive care treatment isolated or combined heart and lung failure. There are temporary circulatory and pulmonary support systems that are implanted in the context of acute heart failure or acute respiratory distress syndrome (ARDS) to take over the function of the heart and lungs and to guarantee oxygenation of the blood and the circulatory system. A pump generates blood flow and a system-integrated membrane oxygenator ensures gas exchange in the blood. They function as a modified form of cardiopulmonary bypass for tissue oxygenation in acute cardiopulmonary failure, however, they are transportable, smaller and sealed from the atmosphere. Due to technical and medical progress it is now possible to carry out a prolonged therapy for days to weeks until the heart and lungs recover and regain their function without external support. The optimization of the mechanical equipment and the growing experience are not only reflected in the increasing reliability of the technology, but also in the improvement of clinical outcomes. Temporary support should either result in a recovery of the organs (bridge to recovery) or serve as a bridging measure for permanent supportive systems or a heart and/or lung transplantation (bridge to permanent devices or bridge to transplantation) (Boeken 2017, p.46-47). The first generation of extracorporeal support systems was expensive, difficult to use and limited in use for specially trained cardiac centers and has been used predominantly in the therapy of children. The next generation, on the other hand, is smaller and more manageable, making the material less expensive, safer and more user-friendly to handle and transport in emergency situations (Bartlett 2010, p.538-539). Portable mini-ECMOs were introduced in 2008 and have been used in external hospitals. Patients who are highly catecholamine- and ventilator-dependent and who otherwise would not be able to travel to specialized centers in this state, can now also benefit from extracorporeal membrane oxygenation (Arlt 2011, 691-693). Advances in technology have contributed to the rapid adoption of ECMO worldwide. The Extracorporeal Life Support Organization has established a database of ECMO patient cases, where the survival rate was 0% in the 1980s

and improved to 30-40% in the 1990s and 40-50% in 2000 (Roy 2000, p.1335-1336).

The ECMO, as mentioned above, is a miniaturized heart-lung machine to treat partial or total cardiopulmonary failure. By arterializing the venous blood by an oxygenator, carbon dioxide is eliminated by bypassing the lung. The oxygen-containing blood is actively pumped back into the circulatory system, thus additionally enabling hemodynamic relief of the heart. Basically, an ECMO consists of a vascular access, flexible tubes, a driving force such as a pump (centrifugal pump), a backup battery with charger, a heating system and a gas exchange unit, the oxygenator. It is a mobile unit that can be quickly transported across hospital corridors and elevators on wheels. The difference to the conventional heart lung machine used intraoperatively, which is an open system, is that an ECMO has no permanent venous reservoir and no opportunity of autotransfusion. This makes the ECMO a closed system. The flow control of the ECMO is carried out via a centrifugal pump and in the heart lung machine via a roller pump. The ECMO has only one control pump and generates a non-pulsatile flow. The oxygenator is used to regulate heat at human standard values of body temperature and gas exchange. The venous blood becomes saturated to 95% at the time of flow, but the extent of oxygen transfer depends on the maximum flow rate and the oxygenator used. The thickness of the blood film, the exchange surface, and the thickness and nature of the membrane are important. An oxygenator can be replaced as needed during a treatment session (Larsen 2018, p.340-347).

The indication field for ECMO therapy is wide-ranging. Both primary cardiac patients and those with secondary cardiac decompensation and no specific cardiac history may benefit from this treatment option. The same applies to patients with pre-existing pulmonary disease, as well as patients who develop secondary acute respiratory failure secondary to another disease. The use of ECMO has also become established after heart transplantation with graft failure or in the emergency treatment of other cardiogenic shock forms with low-cardiac output, such as myocarditis, terminal cardiomyopathy, acute coronary syndrome, drug overdose or sepsis (Schmid 2011, p.89-92).

The ECMO is still the only way to support the gas exchange. However, there are still a number of problems that do not allow the use of ECMO as a lung

replacement over a longer period of time. Similar problems exist in the conception of an artificial lung. The focus is on biocompatibility problems that lead to blood coagulation, thrombus formation and membrane function restriction. There are a number of promising approaches for optimizing the biocompatibility of the membranes used in artificial lung systems: chemical modifications (e.g. nitrogen monoxide coatings), physical modification (e.g. silicone coatings), and biological modifications (e.g. colonization with endothelial cells). Biocompatibility and new possibilities of anticoagulation techniques represent a particular challenge for adequate gas exchange due to the large surfaces of artificial lungs (e.g. multimodal strategies, factor Xa inhibitors, etc.). Despite many approaches in scientific research it has not yet been possible to improve biocompatibility to the extent of establishing a suitable method for clinical application. However, advances have been made in miniaturizing the systems to facilitate mobilization and potential implantation similar to a cardiac assistive system (Boeken 2017, p.272-273). To achieve this, alternative membranes (e.g. polydimethylsiloxane, PDMS), smaller hollow fibers and pumpless and tubeless systems are used. Koop et al. developed an oxygenator with an integrated pump and were able to significantly reduce the external surface area (Strauß 2006, p.80-81). Pumpless extracorporeal lung supports (e.g. PECLA or iLA, Novalung) use the patient's circulation by creating an arteriovenous shunt through the femoral vessels with an intermediate oxygenator and are primarily used for decarboxylation with only limited oxygenation (Müller 2013, p.159-160). Since the artificial gas exchange of the potential artificial lung is dependent on the respective activity of the patient, a demand-oriented lung support system (approach: differentiated flow regulation) remains the focus of ongoing research. This would make it possible to offer a patient-specific treatment strategy that is also adapted to individual physical activity and physiological needs. A COPD (chronic obstructive pulmonary disease, characterized by long-term respiring problems and poor ventilation) has a primary need for support in CO2 elimination, while a cystic fibrosis patient (an inherited disorder, characterized by difficult breathing and chronic lung infections) is more likely to be assisted in oxygenation (Boeken 2017, p.274).

Today's oxygenators are in a rigid, inelastic housing. However, the human lung has a certain amount of elasticity and therefore scientists have developed a flexible housing. The basis for the "elastic" artificial lung was the MC3 BioLung. It

is a low resistance oxygenator with a low pressure resistance of 2-3 mmHg / l / min. The commercially available oxygenators have a significantly higher pressure resistance of around 20 mmHg / l / min. The MC3 BioLung has already been successfully tested in a sheep animal model over a period of 30 days. The animal was awake, spontaneously breathing on the artificial lung (Sato 2007, p.1141-1143). The peritoneal artificial lung uses the large capillary network of the peritoneum to perform the gas exchange. Conceptually, oxygenated perfluorocarbon is perfused through the peritoneal cavity. Perfluorocarbon is also called "breathing liquid" is rarely used in liquid ventilation. However, due to the high technical complexity of a permanent peritoneal lung, this approach was dropped again (Matsutani 2010 p.451–445). The paracorporeal artificial lung uses a circuit connected in parallel to the pulmonary circulation with an oxygenator connected in between. A large-lumen vascular prosthesis is anastomosed to the trunk of the pulmonary artery. Furthermore, a likewise large lumen vascular prosthesis is anastomosed to the left atrium. In the usual way oxygen is supplied to the oxygenator. The paracorporeal concept of the artificial lung allows easy monitoring of the oxygenator and easy replacement when needed. Since no additional pump has been integrated into the system, the blood trauma remains very limited (Camboni 2009, p.304-306).

The concept of longer-term, artificial organ replacement already works very well in the area of other organ systems (see heart and kidney). Unfortunately, this does not apply to lung replacement. A bridging lung replacement in the range of a few weeks can nowadays be realized very well by means of an ECMO under intensive medical conditions. A longer-term, even ambulatory lung replacement over months or years is currently not available (Boeken 2017, p.275).

4.3.5 Artificial pancreas and closed-loop systems

The basis of the independent treatment of diabetes is the control of the glucose level by the patients themselves by measuring at least four to six times daily. The value measured in capillary blood can be used to correct hyperglycemia and to calculate an insulin bolus to correct carbohydrate intake. In case of continuous glucose measurement, a glucose sensor is inserted into the subcutaneous fat tissue, which continuously measures the blood sugar level. These data are

transmitted to a receiver device and thus allow a constant overview of the tissue concentration (Bequette 2012, p.262-263).

As a further development of this measurement method, so-called closed-loop artificial pancreas systems have been tested on humans since 1970. The essential components of an artificial pancreas are a continuously measuring glucose sensor for measuring blood glucose level, a pump for controlled insulin delivery and a miniaturized computer that evaluates the sensor's measurement data and controls the pump through an algorithm. With this system it is possible to simplify the treatment of type 1 diabetes and moreover to provide diabetics with a higher quality of life. The idea of such a form of therapy has existed for several decades, but the technology of a combined continuous blood glucose measurement and an insulin pump has not been sophisticated enough until recently. The artificial pancreas has been the subject of various clinical studies in recent years, the results of which have shown great advances in the development and usability of the systems. A closed-loop system could help diabetics, who often suffer from hypoglycemia and younger children who cannot yet treat diabetes themselves (Kudva 2014, p.1188-1189). Programming an algorithm to correctly deliver insulin in response to the measured blood glucose level, however, is a challenging task. The problem needed to be solved is the subcutaneous insulin delivery in consideration of the physiologically occurring "time lag" in the measurement of glucose in the interstitium, since closed-loop system show a slower response under standard conditions. The artificial pancreas was able to maintain normal levels of blue sugar in a state of fasting and physical rest. After food intake, however, this compensation was not sufficiently possible and increased blood sugar levels were detected (Doyle 2014, p.1196).

The use of closed-loop systems represents a major advance in glucose metabolism control. There was a reduction in hypoglycemia compared to conventional insulin therapies but also associations with higher average glucose levels. It is possible to reduce acute and chronic complications of diabetes mellitus and the burden of standard therapies. Previous studies have shown promising results, but it will only be possible to assess the full benefits and long-term effects by long-term routine use and studies (Boughton 2018, p.1-8).

5 Analysis of medical risk and solutions concerning xenotransplantations

5.1 Immunological obstacles

Between 1943 and 1945 researchers proved that immune responses after transplantation (allograft) are systemic immune reactions. Since then, transplantation medicine has become an essential part of the treatment of patients with organ failure and tumour diseases in recent years. But just this short time interval makes organ or tissue transplantation a process with which the immune system is usually not confronted - even from an evolutionary point of view (Land 2006, p.37). The only contact that could cause the immune system to come into contact with foreign tissue is pregnancy in a female organism. Thereby the maternal immune system is able to tolerate the father's foreign genes of the fetus and the placenta (Rink 2015, p.178). After a (xeno-)transplantation, the immune system only recognizes cells that are not part of endogenous tissue and repels the transplant through an immune reaction. This is due to a number of genetic differences. Certain genes significantly influence tissue compatibility during transplantation (Kaufmann 2014, p.105).

On human tissue, there are a number of molecules that present antigens on the cell surface. These so called major histocompatibility complexes (MHC) are responsible for tissue compatibility and play a crucial role in transplantation immunology. In humans, they are called HLA (human leukocyte antigen). Class I MHC molecules are found on all nucleated cells in the human body and on thrombocytes. Due to a polygenism on the sixth chromosome, several genes for MHC-I molecules are present in humans, of which HLA-A, HLA-B and HLA-C are essential for antigen presentation and transplant medicine. In addition, class II HLA is expressed on antigen-presenting cells (B-cells, dendritic cells and macrophages), activated T-cells, epithelial cells and endothelial cells. These cells have the polygenic subtypes of the human leukocyte antigen HLA-DP, HLA-DQ and HLA-DR. Human leukocyte antigens often have a large number of polymorphisms, i.e. that different allelic variants of these genes occur. As a result, a transplanted donor organ is likely to have foreign human leukocyte antigens. The immune system of the recipient can recognize these antigens as foreign, eventually triggering a rejection reaction of the transplant. A complete match, a so-

called "full house match," between non-genetically related individuals, is statistically improbable. This is only possible through regional accumulations of HLA types, which occur more often than average in certain population groups. The likelihood that a graft will be rejected is the higher the smaller the accordance between donor and recipient (Doerr 2010, p.289-290).

Although the large number of different HLA molecules is a hindrance to successful transplantation, it offers advantages in the defense against pathogens. Not every HLA molecule is able to bind to all possible peptides. Polygenism allows an organism to have several different HLA molecules per specific class, thereby covering a wider variety of presentable antigens. The polymorphisms have a similar function for the entire species. Thus, in case of disease, a pathogen whose key antigens are poorly presented by a particular HLA molecule cannot destroy the entire population; because there are also individuals with HLA molecules that cope better with the pathogen, securing the survival of the species (Rink 2015, p.179).

5.2 Mechanisms of immunological transplant rejection reactions

5.2.1 Allogenic graft rejection in transplant recipients

The rejection reaction is an immune-induced process mediated by T-lymphocytes (TH1-CD4 cells and cytolytic CD8 cells). The response is directed against human leukocyte antigens (HLA) in humans. The rejection of an allogeneic graft is thus an expression of an immune response: If the transplanted organ carries MHC molecules that are not identical to the recipient's MHC molecules, it will induce a specific T-cell response. The human HLA region contains at least seven genes, each of which codes for a transplantation-relevant HLA-anti gene. For each of these genes there is a large number of alleles; a particular gene product thus occurs in the human species in 10-40 different types. In graft rejection, the recipient's T-lymphocytes recognize the foreign MHC molecule on the cells of the graft. The CD4 T-lymphocytes recognize the MHC class II molecules, while the CD8 T-lymphocytes react with the MHC class I molecules. Both T-cells induce inflammation. Once this immunological pathway is initiated, effector cells (e.g. mononuclear phagocytes) that do not recognize the foreign antigen are attracted. The initiation of the rejection process is antigen-specific, the demarcating inflammation itself is secondary and nonspecific (Suerbaum 2012, p.102).

There may be two different types of rejection reactions. First, the recipient's immune system can be directed against the transplanted tissue. This is called “host versus graft disease”. On the other hand, it is also possible that transplanted immune cells are directed against the recipient's organism, which is called “graft versus host disease”. Graft versus host disease is of particular importance in bone marrow transplantation, in which immunocompetent cells are transferred. The rejection of allografts is based mainly on T cells. In most cases, however, antibodies are also involved in the immune reaction and, in the further course, additional cell types, in particular macrophages, are activated. The various reactions by which the immune system induces graft rejection can be divided into several categories. This classification is based primarily on the time course of the immune reaction. The immunological processes in the later rejection reaction (accelerated to chronic) are not always the same, and the strength of the involvement of the individual components of the immune system may differ significantly between individual cases (Häcker 2014, p.249-250).

5.2.2 Xenogenic graft rejection in transplant recipients

Even though the domestic pig has many advantages concerning its role as a donor species for xenogen transplants, the significant phylogenetic differences between the porcine donor and the human recipients cause enormous immunological problems after transplantation (Boksa 2015, p.181). The rejection reactions occurring in discordant xenotransplantation of vascularized solid organs are classified according to the time course of transplant rejection: the hyperacute rejection within minutes to hours, the acute vascular or delayed rejection within a few days, the cell-mediated rejection within days to weeks as well as the chronic rejection, which begins at the earliest after a few months (Cascalho 2001, p.440).

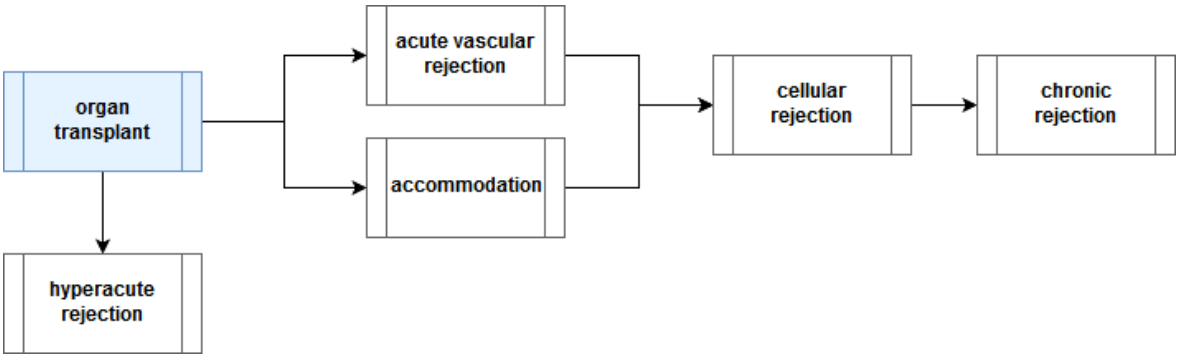

Figure 5-1 Sequence of xenotransplant rejection reactions of vascularised organs

5.2.2.1 Hyperacute rejection reaction

Hyperacute rejection of an organ occurs within minutes or hours after transplantation. If the organ recipient has already formed antibodies against antigens of the donor, e.g. after previous transplants or transfusions, it reacts with antigens on the vascular endothelium. The cause is the binding of preformed xenoreactive antibodies of the recipient organism to endothelial cells of the organ transplant, followed by rapid activation of the endothelial cells and the classical pathway of the complement cascade via recognition of antigen-antibody complexes. This rejection reaction is based on preformed natural antibodies of the recipient against antigens on the endothelium of the transplant (Denner 2016, p.718). The majority of the preformed natural antibodies are directed against galactose α (1,3) galactose epitopes - in the following named GAL. The GALs are synthesized by the α (1,3) galactosyltransferase (α1,3GT), which is expressed by lower mammals and New World monkeys, but not Old World monkeys, apes and humans (Vadori 2014, p.2). The natural xenoreactive anti-α (1,3) Gal antibodies are formed in the first weeks of life as an immunological response to microorganisms of the intestinal flora, which carry the α (1,3) Gal epitope as a cell wall component. The transplantation of pig hearts into neonatal baboons, in which natural anti-α (1,3) Gal antibodies are not yet measurable, thus does not lead to a hyperacute rejection reaction (Itescu 1997, p.4879-481). Species that do not express the α1,3GT show high levels of antibodies (AK) to GAL. These antibodies mainly belong to the IgM and IgG classes, and the ratio of these AKs can vary from individual to individual. Xenoreactive IgM isotype antibodies make up about 0.1%-4% of the circulating IgG level and are more important for the initiation of hyperacute xenogen rejection than the IgG isotype, which accounts for about 1% of the circulating IgG level. They bind to about 80% via alpha-galactosyl epitopes to the endothelial cells. Other relevant antigenic structures are e.g. membrane-bound, endothelial glycoproteins (gpl 15/135 complex), which have a similar structure to integrins (Pfitzmann 2001, p.239-240).

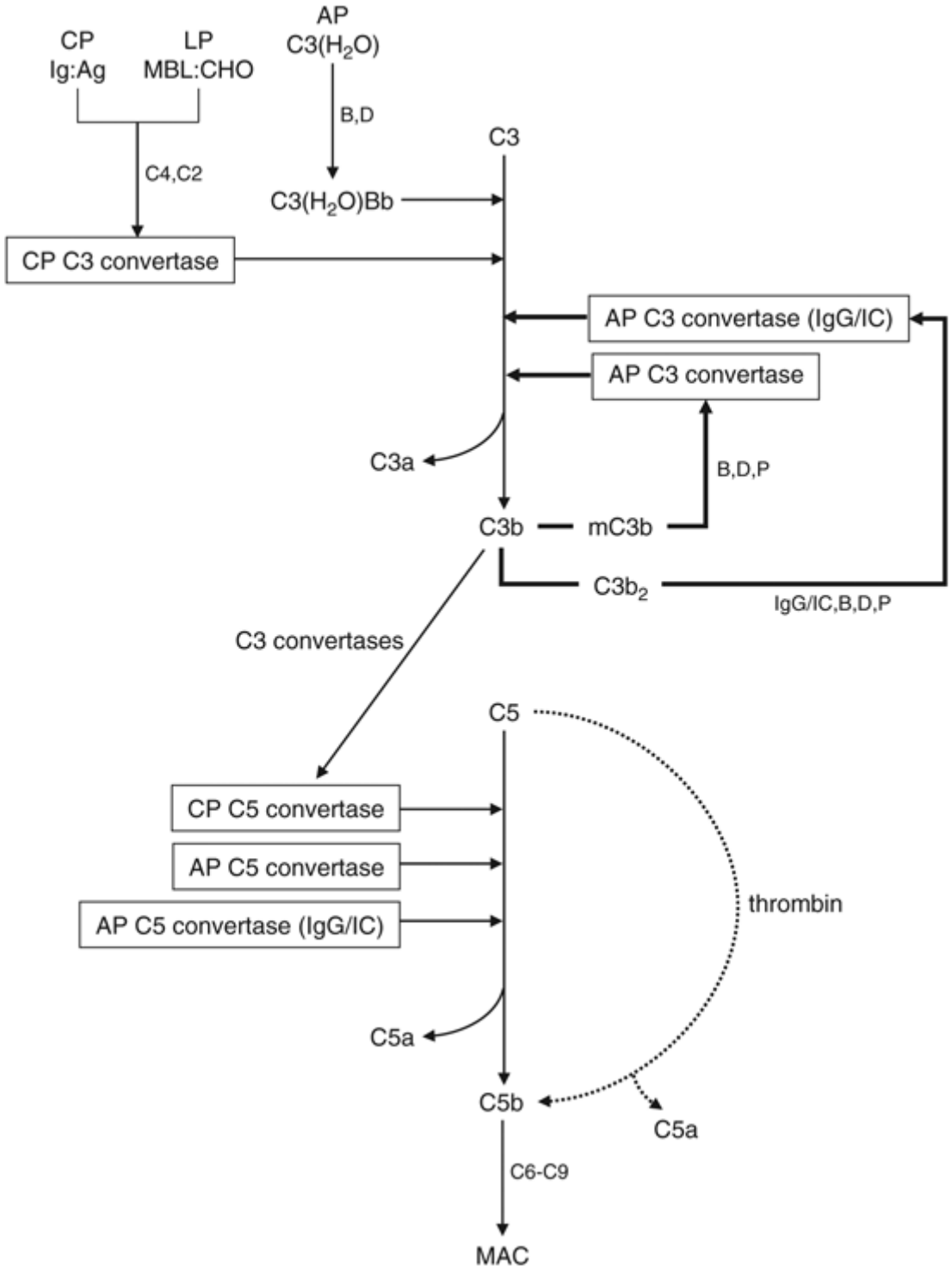

Figure 5-2 Pathways of complement activation in xenotransplantation, Peter J Cowan and Anthony JF d'Apice https://www.nature.com/articles/icb2008107/figures/1 [Accessed 13 March 2018)

At the end of the complement cascade the membrane attack complex, in the following called MAC, forms, which contributes a major part to the hyperacute rejection reaction. The MAC can lead to necrosis in the graft either through direct cell lysis or through the formation of pores in the cell surface. In addition, the MAC can also initiate the apoptosis of cells. Furthermore, the xenogen cell complex

loses its integrity through exposure to xeno-antibodies, complement factors and the formation of intercellular gaps that may be responsible for the observed vascular leakage. Moreover, there is a loss of heparan sulfate on the cell surface and increased expression of thromboplasmin or tissue factor, creating a highly pro-coagulatory surface and promoting the formation of intravascular clots. Another component is the proinflammatory effect of the MAC, which, by binding to the cell surface, leads to the activation of a signaling cascade and the transcription of genes, which ultimately results in the up-regulation of cell adhesion molecules and the production of cytokines and chemokines. In this way, the adhesion of leukocytes and the infiltration of the tissue with inflammatory cells is promoted (Griesemer 2014, p.247).

In addition to the MAC, however, earlier complement factors also contribute to proinflammatory effects in the context of HAR. The factors C3a, C4a and C5a (so-called anaphylatoxins) act in e.g. chemotactic way and can bind on their cell surface receptors that are expressed by eosinophils, neutrophils and mast cells thus leading to pro-inflammatory effects. In addition, xenoreactive AKs of the IgG class interact with and activate the Fc-receptors of phagocytes (membrane receptors for various immunoglobulin isotypes) and natural killer cells, which also leads to graft destruction. In particular, a significant procoagulatory stimulation is important here (Boksa 2015, p 182-183).

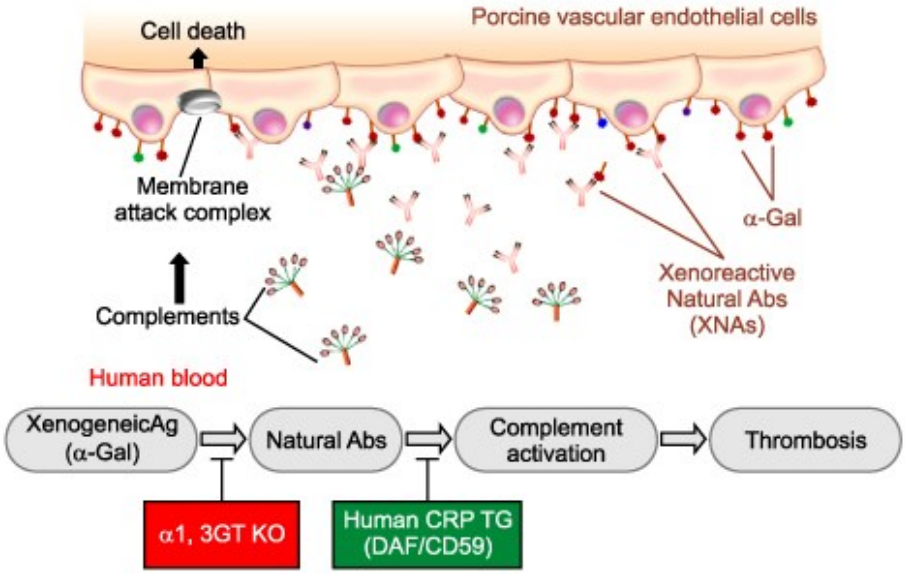

Figure 5-3 Hyperacute rejection, The Korean Society for Transplantation, J Korean Soc Transplant. 2009 Dec;23(3):214-226. https://synapse.koreamed.org/ViewImage.php?Type=F&aid=379346&id=F2&afn=83_JKSTN_23_3_214&fn=jkstn-23-214-g002_0083JKSTN (Accessed 21 March 2018)

As far as the implantation and reperfusion of a porcine graft is concerned, the natural antibodies can react with the alpha-(1,3)-Gal epitopes of porcine endothelia. This leads to a 2-phase activation of the classical path of the complement cascade in the organ recipient. In the first rapid phase of this reaction, the endothelial cells lose their protective mechanisms. Destruction of the blood-tissue barrier initiates blood clotting. This leads to thrombosis in the second phase, first in the capillaries and later, these blood clots also appear in the large blood vessels. In parallel, white leukocytes remain attached to the damaged endothelium, leading to hemorrhage and loss of organ functions (Krukemeyer 2005, p.335).

Histologically thrombotic occlusions, diffuse haemorrhages, edematous swellings, inflammatory infiltrates, spotty necrosis areas, as well as deposits of complement factors, immunoglobulins, and fibrin are found in the capilars of the graft during a hyperacute rejection reaction. (Stevens 1992, p.417-419).

5.2.2.2 The acute vascular (humoral) rejection reaction

The acute vascular (humoral) rejection reaction can start in the first 24 hours after xenotransplantation and lead to graft failure within a few days to weeks. It is caused by endothelial cell activation, which leads to proinflammatory and procoagulatory changes of the surface (Lin 1998, p.1745). The exact mechanisms of endothelial cell activation are not yet fully understood. But most likely several factors play a role. By binding of natural and high affinity induced anti-α (1,3) Gal-IgG antibodies, but even more by anti-pig antibodies against non-α (1,3) Gal cell surface antigens lead to a direct complement-independent type II endothelial cell activation besides activating the complement cascade (Shimizu 2010, p.11-13). This is in contrast to the type I endothelial cell activation associated with the synthesis and expression of various adhesion molecules (E-selectin, P-selectin, ICAM-1 and VCAM-1), cytokines (TNF-α and IFN-γ), chemokines (IL -1, IL-6, IL-8 and MCP-1 = monocyte chemoattractant protein-1) and procoagulant molecules (TF and PAI-1 = plasminogen activator inhibitor-1) (Knosalla 2009, p.1013-1014). Natural killer cells, like macrophages and neutrophils, bind granulocytes to the endothelium and migrate into the graft. Molecular differences between the killer cell immunoglobulin-like receptors of the recipient's natural killer cells and the porcine MHC class I molecules lead to direct cell lysis. In addition, natural killer

cells and monocytes can bind to xenoreactive immunoglobulins and lyse porcine endothelial cells via antibody dependent cellular cytotoxicity (Saadi 2004, p.1077-1079).

Disseminated intravascular coagulation (DIC) enhances the progressive coagulation cascade because porcine thrombomodulin cannot bind and activate human protein C to a sufficient extent. As a result, the coagulation factors V and VIII, which ultimately promote the conversion of fibrinogen to fibrin, are not sufficiently inactivated. Intravascular thrombosis is promoted. At the same time, the subendothelial matrix (including collagen and von Willebrand factor) is also released, which also promotes platelet aggregation. All this leads to thrombotic vascular occlusions and immune cell-mediated cell lysis and eventually to an infarction of the donor organ (Cozzi 2009, p.292-293).

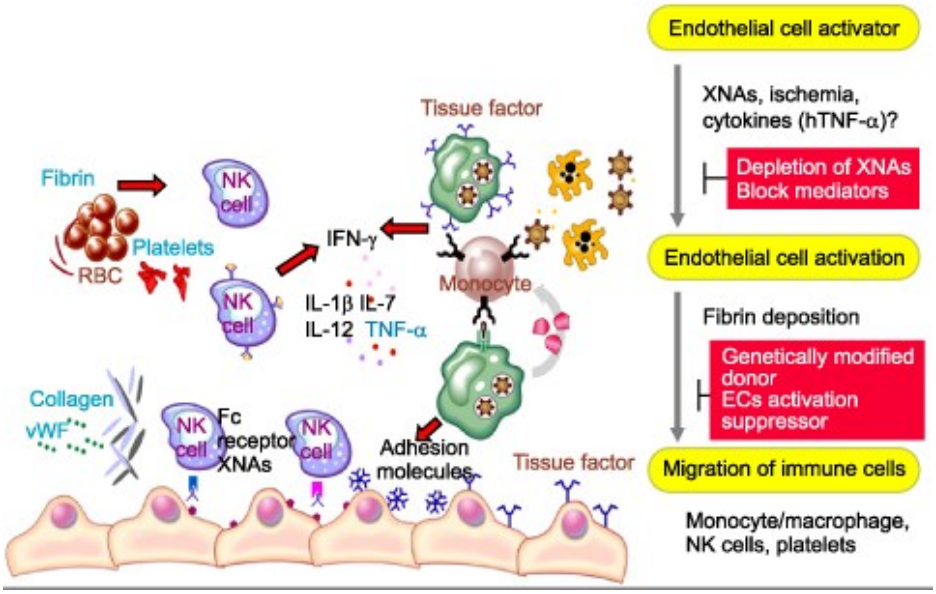

Figure 5-4 Acute vascular (humoral) rejection reaction, The Korean Society for Transplantation, J Korean Soc Transplant. 2009 Dec;23(3):214-226. https://synapse.koreamed.org/ViewImage.php?Type=F&aid=379346&id=F2&afn=83_JKSTN_23_3_214&fn=jkstn-23-214-g002_0083JKSTN (Accessed 21 March 2018)

Histopathologically the picture of acute vascular (humoral) rejection reaction differs little from that of hyperacute reaction. There are endothelial cell ruptures, interstitial edema, hemorrhages, immunoglobulin and complement deposits, infiltration of mononuclear cells and thrombus formation (Samstein 2001, p.185).

5.2.2.3 The acute cellular rejection reaction

If the hyperacute and acute vascular (humoral) rejection reaction can be overcome, acute cellular rejection (ACR) occurs within a few weeks after

transplanting a xenogenic organ. ACR after xenotransplantation is believed to be very similar to ACR after allografting. The key mechanism of ACR is the T-cell-mediated immune response to the foreign surface proteins of the xenograft. The T cells can be activated either directly by MHC (Major Histocompatibility Complex) class I molecules on antigen presenting cells or indirectly after antigen processing by MHC class II molecules. In contrast to allografts other proteins, beside MHC molecules, also seem to play a role in immunological pathways in xenografts. As a result a greater variety of T cells can be activated. It is therefore assumed that the xenogeneic ACR is at least as strong as the allogeneic ACR or even stronger (Yang 2007, p.521-522). A more pronounced cellular immune response after xenotransplantation compared to allografting is expected for the following reasons: First, a xenograft has a much greater number of foreign peptides than an allograft, which can elicit a strong T cell response via the indirect pathway. Second, the humoral response to xenografts can enhance the cellular response. Third, both the direct and indirect pathway of T cell activation by porcine stimulator cells induces a strong human T cell proliferative response via different signaling pathways. These pathways mediate the migration of cytotoxic cells into the graft. However, this form of rejection in xenografts is not as well understood compared to hyperacute and acute vascular rejection reactions and can be well controlled by immunosuppressants (Pierson 2009, p.267-268).

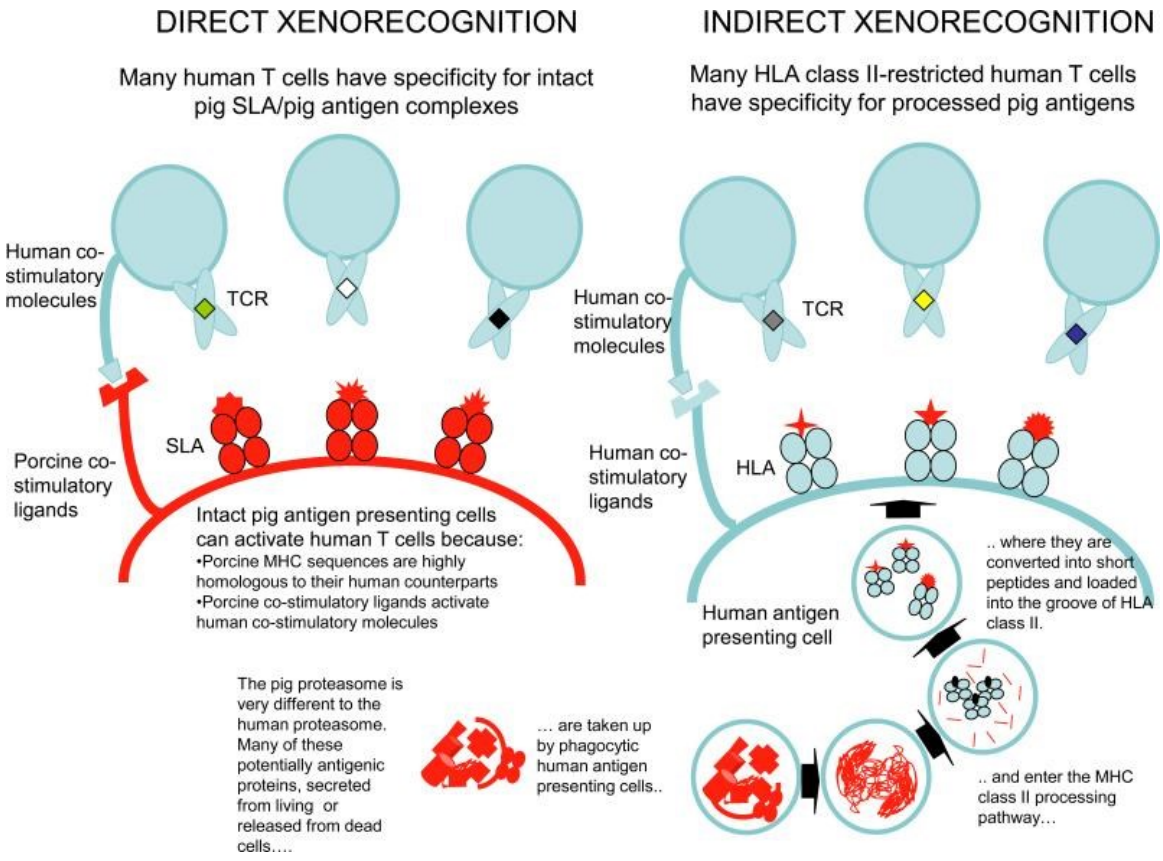

Figure 5-5 T cell-dependent anti-xenograft responses, Pierson RN, Dorling A, Ayares D, et al. Current Status of Xenotransplantation and Prospects for Clinical Application. *Xenotransplantation*. 2009;16(5):263-280.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2866107/figure/F4/?report=objectonly (Accessed 16 April 2018)

Histologically ACR is characterized by multifocal lymphocyte infiltration of T and B lymphocytes, macrophages and few natural killer cells, as well as direct myocyte damage. Vascular thrombosis and interstitial hemorrhage are typically absent. Likewise, vascular deposits of immunoglobulins, complement or fibrin are rather low or absent (Cooper 2015, p.213).

5.2.2.4 The chronic rejection reaction

The chronic rejection reaction (CR) occurs weeks to months after xenotransplantation and is still poorly understood, as it is rarely seen in the xenogeneic model. However, in 2005 chronic xenogenic vasculopathy was first described under continuous immunosuppressive therapy. In a pig-to-baboon heterotopic heart transplantation model three primates received αGalT hearts and survived for 78, 110, and 179 days. The humoral and cellular rejection could be linked to the development of chronic vasculopathy. This was characterized by thickening of the intima, fibrin exudate, complement and immunoglobulin

deposition in the tissue and cellular infiltration. Furthermore, a chronic xenograft rejection in a hamster-to-rat model was performed and showed similar features as in the allograft. There were differences in the type of arterial injury, antibody deposition (particularly IgM instead of IgG) and the type of cell infiltrates. Immunosuppressive therapy with cyclosporine and leflunomide provided positive effects on the chronic rejection reactions (Cozzi 2005, p.103).

5.3 Possibilities and strategies for the prevention of rejection reactions in xenografts

Through an increasing understanding of the mechanisms of xenogeneic rejection reactions and the possibility of genetically engineered pigs the hyperacute rejection reaction was successfully overcome as the first immunological hurdle. There are several ways to prevent or attenuate graft rejection, depending on what type of rejection reaction is present.

5.3.1 Strategies to overcome the HAR

There are several ways to remove the xenoreactive immunoglobulins from the serum of the recipient. Probably the most commonly used methods are to remove the antibodies by means of extracorporeal immunoadsorption or plasmapheresis preoperatively. In the recipient's plasma natural xenogenic antibodies can be absorbed and eliminated by extracorporeal immunoadsorption by flooding past polyclonal sheep antibodies. In this method, however, antibodies are nonspecifically eliminated, resulting in a weakening of the immune system of the recipient. A more specific way of removing preformed natural antibodies is by infusing soluble GAL epitopes coupled to a carrier and binding the antibodies (Leventhal 1997, p.360-361).

Processes for the regulation or inhibition of the complement system are observed under physiological conditions by membrane-bound complement regulators and also discussed for clinical use in xenotransplantations. Genetically engineered modification of donor pigs is based on the assumption that complement regulators are species specific, i.e. membrane-bound porcine complement regulators cannot adequately control the human complement cascade through molecular incompatibilities. By using transgenic pigs that overexpress a human complement regulator on the endothelial cells, the complement cascade can be effectively

inhibited and a HAR successfully prevented. Alternatively, by the administration of soluble complement regulators such as complement receptor type I (sCR1), Cobra venom factor (CVF), decay accelerating factor (DAF), CD59 (membrane inhibitor of reactive lysis), C1-esterase inhibitor or MCP (membrane cofactor protein), the complement system is also inhibited (Mohiuddin 2007, p.431-432).
After successfully overcoming the hyperacute rejection of xenogeneic transplants, therapeutic strategies are needed to control the delayed acute vascular rejection.

5.3.2 Strategies to overcome the AVR

The removal of the induced antibodies can be done by regular immunoadsorption of the recipient's blood or regular administration of soluble GAL epitopes. Another approach is to eliminate B cells to minimize antibody production. Described methods for this are the splenectomy and the irradiation of lymphatic tissue. Drug therapies would include the administration of antibodies against CD20 (rituximab) or cyclophosphamide, a cytostatic agent. In addition, a co-stimulation blockade can prevent T cell-dependent activation of B cells (Ekser 2009, p.88-91).

5.3.3 Strategies to overcome the ACR and CR

Immunosuppressants are substances that diminish the functions of the immune system and are already an integral part of the prophylaxis of rejection reactions in the allografting of organs and tissues. In xenograft models, they are also tested on porcine organs to examine the effectiveness and side effect profile of the drugs and eventually increase the graft survival. Already in use are abatacept (selective costimulation modulator of T cells), anti-CD154 antibodies (TNF superfamily), antithymocyte globulin (mixture of polyclonal antibodies), belatacept (inhibits the costimulation of T lymphocytes), cyclosporin (inhibits helper T cell production of growth factors), everolimus (mTOR inhibitor), fingolimod (sphingosine 1-phosphate analog), mycophenolate mofetil (selective, non-competitive and reversible inhibitor of inosine monophosphate dehydrogenase), rapamycin (mTOR inhibitor with macrolide structure) and tacrolimus (calcineurin inhibitor) (Boksa 2015, p.187). The use of immunosuppressive drugs has already been shown to enhance survival after xenogeneic transplantation (Griesemer 2009, p.7-8 and Mohiuddin 2012, p.6-7)

In transplantation medicine, immunological tolerance offers the possibility of triggering no immune response to graft antigens and thus avoiding immunosuppressant therapies. In detail, this is called “the specific absence of a destructive immune response to the graft in the absence of immunosuppressive therapy” (Sachs 2017, p.220). In the case of xenotransplantation, although the risk of hyperacute rejection (HAR) could be reduced by a-1,3-galactosyl-transferase knockout (GalT-KO) in pigs, natural antibodies cause rejection reactions. The immunological tolerance is achieved by a deletion of T cells, which are able to react with the graft. This can be done on the one hand by a thymic transplantation or by the "mixed chimerism". Both methods are targeted for deletion tolerance by exposing anew emerging T cells to negative selection in the thymus of the host and donor. In mixed melanism, the patient receives bone marrow stem cells from the donor, and ideally, his immune system is also tolerant of the new organ. In contrast to the lifelong drug immunosuppression of organ transplant patients, which is required today, the immune system is not impaired in its other function and can therefore continue to react unhindered against infections and tumors. Regulatory mechanisms for inducing immunological tolerance include the activation or production of regulatory T lymphocytes by the activity of the transcription factor forkhead box protein P3 (FoxP3), also called scurfin (Vagefi 2015, p.292-294). FoxP3 binds to the DNA and thus stimulates the expression of proteins that are used in the regulatory T cells of the immune system. Regulatory T cells prevent the development of autoimmune diseases in a healthy organism (Passerini 2014, p.129-130).

5.4 Transmission of porcine microorganisms

A major problem with xenotransplantation is the risk of transmitting pathogens and microorganisms to the organ recipient. Xenotransplantation bypasses natural barriers such as the skin and mucous membranes and places potentially pathogens in direct contact with the recipient's organs and bloodstream. Because the patient is highly immunosuppressed, infections caused by bacteria, parasites or viruses have a chance to spread. Many of the infectious diseases known today have resulted from zoonoses, e.g. human immunodeficiency virus (HIV) causing AIDS and severe acute respiratory distress syndrome induced by pathogenic coronaviruses (Denner 2012, p.321-322).

The majority of non-viral human pathogenic agents of the pig, such as Trichinella spiralis, Mycobacterium avium, Toxoplasma gondii, Listeria, Campylobacter coli, Strepptococcus suis, Leptosira interogans, Erysipelothrix rhusiopathiae, Cryptosporidium parvum and Brucella suis can be removed from the pig by regarding specific-pathogen-free (SPF) conditions (Onions 2000, p.144).

However, viruses play a greater role in the risk assessment of clinical transplantation of xenogenic organs and tissue. The main barrier to viral infections is physical. In order to reach the target cells, viruses must overcome this barrier without losing their risk of infection. In the case of enveloped viruses, the risk of dehydration leading to loss of infectivity is acute. In comparison, the non-enveloped viruses are mostly stable and can survive for longer periods of time in severe conditions. Normally, viruses can overcome physical barriers in humans only after injury or through use of active entrapment structures (eyes, skin, respiratory tract, genitourinary tract, digestive tract and anus). This forms an effective defense mechanism against most viral infections. In xenotransplantation, however, the barrier is bypassed and viruses reach the immediate vicinity of the target cells. Also, dehydration and concomitant loss of infection risk of enveloped viruses is overridden by xenotransplantation and contact with the recipient's blood (Beckmann 2000, p.200-201).

In recent years, numerous viruses of the pig have been identified, which have a potentially human pathogenic potential. Pigs, for example, are hosts of the influenza viruses. It has been shown that porcine influenza viruses can overcome the species barriers and also infect humans - especially in patients with weakened immune systems such as children, the elderly or immunosuppressed patients after transplantation. Transmission of the swine influenza virus is thought to be responsible for the Spanish flu, an influenza pandemic, which killed over 20 million people in 1918 (Schuurman 2009, p.216-217).

Potentially transmissible viruses, such as foot and mouth disease virus, herpes viruses, porcine hepatitis viruses, swine disease virus (SDV), porcine papovirus (PPV), vesicular stomatitis virus (VSV), porcine encephalomyocarditis virus, rabies virus and swinepox virus, may be considered more likely to be harmless under conditions of normal immune competence or of receiving prophylactic vaccination. Human pathogens, however, are better adapted to the physiology of humans than porcine microorganisms. For only a few of the porcine viruses a zoonotic risk is

described. E.g. Hepatitis E genotype 3 viruses induce fulminant infections in immunosuppressed and / or individuals with pre-existing liver failure (Denner 2017, p.3-4). Furthermore, these viruses can be eliminated by intensive breeding and keeping of pigs under SPF conditions and strict controls (Tucker 2002, p.191-192).

A particular challenge is the removal of circoviruses, which are among the smallest and most resistant viruses. The circoviruses are very important, as they are distributed worldwide in pig populations and can be transmitted both horizontally and vertically and an elimination of this virus cannot be achieved with simple means. As a result, one of the main goals of current research is to evaluate the transmission risk of PCV to humans and the susceptibility of different human cell types to PCV1 and PCV2. If two related viruses from different species meet, partial homology and recombination events can be the result, eventually leading to the formation of chimeric viruses. Regarding their pathogenicity and their host specificity, these recombinant viruses cannot be predicted in advance and therefore represent an incalculable risk. For this reason, another important aspect of the assessment of PCV with regard to the virus safety of xenotransplantation should be the question of whether there is a human circovirus that could recombine with the porcine circoviruses in the event of xenotransplantation (Denner 2017, p.1-3). Furthermore, there is a risk of unknown viruses, which are difficult to identify due to lack of pathogenic features. The consequences of infection with hitherto unknown viruses can be understood using the example of the Ebola and Marburg virus as well as the SARS and HI virus (Boneva 2001, p.3). Another risk of xenotransplantation is the transmission of porcine endogenous retroviruses (PERV), which are integrated into the genome of all pigs and cannot be removed by breeding and keeping under SPF conditions (Denner 2013, p.62).

5.4.1 Porcine endogenous retroviruses (PERV)

Porcine endogenous retroviruses are part of the porcine genome of all porcine species. These viruses were described for the first time in the early seventies of the last century by Breese: *Virus-like Particles Occurring in Cultures of Stable Pig Kidney Cell Lines* (Breese 1970, p.403). One characteristic that is common to all retroviruses and is reflected in the naming is the enzyme reverse transcriptase. It enables retroviruses to reverse the conventional genetic flow of DNA from RNA to

protein by transforming the retroviral single-stranded RNA genome into double-stranded DNA. The PERV are integrated in the porcine genome at different loci, depending on the breed of pigs. However, many of these viruses have mutations and therefore cannot form intact infectious virus particles. The intact, particle-forming proviruses are classified into the subtypes PERV-A, PERV-B and PERV-C. PERV-A and B, unlike PERV-C, are present in the genome of all pigs. The expression of PERV mRNA varies in different tissues, with the lowest expression of PERV mRNA in the pancreas and the highest expression rate in the kidneys (Bittmann 2012, p.329-330). PERV-A and PERV-B are polytropic viruses that are able to infect cells of various species including humans and non-human primates. The porcine endogenous retroviruses show no pathogenic symptoms in their natural host, the pig. However, they have high homologies with other known γ-retroviruses. These viruses are pathogenic and cause leukemia in both gibbons (Gibbon ape leukemia virus, GALV) and koalas (endogenous koala retrovirus, KoRV) (Wood 2009, p.705-707), as well as feline and murine γ-retroviruses (FeLV, MuLV), which have already been described as capable of inducing immunodeficiencies and inducing tumors in their host. This is of central importance for the clinical use of xenotransplantation since PERV transmission could also lead to immunodeficiency and increased tumor formation due to the integration of the proviruses into the cellular genome (Denner 2003, p.39-41)

PERV transmission to humans in future xenotransplantations cannot be ruled out. Although PERV-C is not human-tropic, the highly infectious PERV-A / C resulting from the recombination between PERV-A and PERV-C poses a particular risk for xenotransplantation. For this reason, only PERV C-free animals should be used. The risk increases e.g. by the use of transgenic pigs, in which, for improved acceptance of the porcine organ in the human organism, the α-gal epitope was removed by knockout of α1,3-galactosyl transferase. Although this reduces the likelihood of rejection of the graft, it increases the protection of the porcine endogenous retroviruses from the human immune response. Due to the lack of Gal epitopes in the porcine cell-derived envelope membrane, the xenoreactive antibodies to the Gal epitope are ineffective (Fischer 2016, p.1-3).

5.4.2 Strategies to overcome the AVR

In order to limit the transmission of porcine endogenous retroviruses in a xenotransplantation in advance, promising strategies have been developed.

In the case of viremia, antiretroviral therapy is a suitable first measure for the prophylaxis or treatment of the infection. There is a whole range of active ingredients that are already approved for the treatment of HIV-1 and also have an inhibiting effect on PERV. Most promising are reverse transcriptase inhibitors (RTI). These drugs competitively inhibit the virus-specific reverse transcriptase and thus prevent the reverse transcription of the viral genome into DNA. The integration of the virus into the genome of the target cell and the subsequent replication are thus prevented (Shi 2009, p.201-202). Argaw et al. (2016, p.5-7) demonstrated that zidovudine should be the first-line therapy for the clinical use of PERV infections as well as prophylaxis in patients exposed to xenogeneic tissues (for example in the context of extracorporeal perfusion with porcine livers or liver cells). Of the HIV-1 drugs, dolutegravir and raltegravir, as IN inhibitors, were the most effective at suppressing PERV replication.

Since the virus is located with more than 100 copies at different loci of the porcine genome, the use of the classical gene knockout method is extremely difficult. Thus, the possibility of RNA interference offers itself to protect a transmission, with which one can block the replication of the PERVs at the molecular level (Denner 2017, p.1-8). RNA interference (RNAi) plays an important role in posttranscriptional gene regulation. As a mediator of RNAi short complementary RNA molecules have been identified (anti-sense RNA, siRNA), which hybridize via homologous base pairing with the target RNA and initiate an enzymatically controlled degradation or translational blockade. RNAi is one of the ways to reduce the transmission risk of PERV. Initial studies identified effective siRNAs whose transfection into PERV-producing cells resulted in a reduction in PERV expression (Popp 2012, p.110-111).

Another promising way to prevent transmission of PERV to the xenograft recipient is to immunize the patient before transplantation. In the event that virions are released by the xenograft, they should be specifically bound and neutralized by the induced antibodies before transmission can take place. Successful vaccines against HIV-1 and HIV-2 have failed to date, but there are existing vaccines against murine leukemia virus and feline leukemia virus. Since PERVs do not

infect non-human primates and small mammals, the efficacy of vaccines and their neutralizing antibodies can only be assessed by reference to infections of related gammaretroviruses (e.g. infection of cats with feline leukemia virus). In addition to its relevance for xenotransplantation, the development of effective PERV vaccines could also be beneficial for the development of an HIV vaccine. HIV and gammaretroviruses have structural similarities of the envelope proteins (Denner 2012, p.334).

In addition to the described strategies, further methods are being tested. Since none of the possibilities that have been investigated so far promise one hundred percent transmission protection, it is conceivable that a combination of the methods could ultimately lead to success. Denner et al. (2012, p.331-335) has described, inter alia, the following strategies:

- Development of PERV-inhibiting ribozymes
- The generation of transgenic pigs with extended spectrum of different restriction factors (e.g. hAPOBEC3G)
- PERV knockout using specific zinc finger nucleases
- The alteration of the glycosylation pattern of porcine cells and thus reduction of the infectivity of PERV
- The mechanical encapsulation of porous cells and tissue by polymers

All of the strategies listed here are still unclear in the field of basic research and their clinical application in the context of xenogenic transplantation.

6 Medical-ethical aspects of xenotransplantation

6.1 Applied ethics and medical ethics

As a subarea of applied philosophy, applied ethics has set itself the goal of finding and justifying evaluable arguments on moral issues for human action. Applied ethics makes use of normative-ethical approaches and aims to find an answer to the question of which actions are morally right or wrong. It is important to find universal and morally valid provisions to assess specific actions and individual decisions. The ethical principles of this normative theory are primarily intended to be used for specific conflict and problem situations in various areas of human action and life. The various fields of applied ethics range from environmental ethics, medical ethics and animal ethics to technology and science ethics, political ethics, legal ethics and professional ethics for different professions (e.g. technicians, doctors, lawyers, psychologists, journalists, etc.) to economic ethics. Each of these sub-disciplines deals with different concrete moral problems and offers moral actors a great potential for discussion and reflection. In the context of the applied ethical discourse, however, no "ad hoc" solution should be offered for any moral problem that arises. Rather, applied ethics considers itself as a tool to question these different statements of moral positions and to offer an at least consistent, if possible coherent number of moral theories as the basis for such discussions. New experiences in the ethical discussion are intended to create different perspectives and exchange experiences, which, if necessary, will allow existing positions to be modified and revised. By excluding an "ad-hoc" opinion of moral actors, a reasoned opinion can be clarified and understood and there is a real, critical examination and consideration of competing moral principles. In addition to the occupation with moral principles, applied ethics also deals with relevant empirical hypotheses for the respective field of application. Depending on the fields of application, concrete empirical data from the fields of medicine, psychology, anthropology, biology, sociology, etc. are used (Morscher 2001, p.12-15).

Bioethics, and in particular medical ethics, deals with the field of health care and makes use of two competencies: normative ethics as an implementation strategy and the field of medicine as the area of application in which it has to be carried out. A requirement for the understanding and use of ethics for moral actors is

sound expertise and close cooperation between ethics experts and specialists in the field of application. Within bioethics, these experts are in particular physicians and medical-scientific professionals. In the field of medical ethics, questions of medical research, the organization of health care in national and international comparison and the specific requirements of different medical professions are discussed. As a result, anyone working in a medical profession is bound to have at least rudimentary skills in the field of bioethics (Morscher 2004, p.30-32).

6.2 A case study – the surgeon Leonard Bailey and the case of Baby Fae

Dr. Leonard L. Bailey is a renowned pediatric surgeon and works as a chief physician at the Loma Linda Children's Hospital near Los Angeles (Loma Linda University Medical Center 2017). More than 20 years ago, he became internationally recognized by the world's first xenogenic heart transplantation. On October 26, 1984, Bailey transplanted a baboon heart into the breast of twelve-day-old Stephanie Fae Beauclair, known as "Baby Fae". She suffered from a severe congenital heart defect (hypoplastic left heart syndrome), and had, according to the state of the medicine at that time, no survival chance (Petechuk 2006, p.98). Surgically, the procedure was a success. The heart took over the physiological functions, but the infant developed a strong immunological rejection reaction to the xenograft. Baby Fae died twenty-five days after the operation of immune-related necrosis of the baboon heart. The death of the infant did not come as a surprise to many scientists and physicians who had actively and successfully been researching in the field of human immunology. Successfully performed "cross-over-species" transplantations were conceived as "utopian" because the knowledge about the immune system of mammals did not exceed basic research (Mak 2005, p.908).

The case of "Baby Fae" was preceded by Bailey's long-standing research on xenotransplantation. His scientific pieces of work on xenografts, initially assumed useful in human medicine, were largely unpublished - primarily due to many negative results. In the seven years before the "Baby Fae" case, Bailey and his team carried out almost 160 inter-species organ transplants, with no donor animal - mostly goats and sheep - surviving for more than six months. When Bailey presented his research in the context of a surgical conference, there were again

critical voices not entirely satisfied with his results. His findings were classified as too risky for human use. Bailey argued that an immature immune system, like that of an infant of a few days, would not reject an allogeneic or xenogeneic transplant. There was already scientific evidence showing that the part of human immune defense which is responsible for the recognition and destruction of foreign cells is available at the time of birth. Bailey, however, had ignored that fact (Stoller 1990, p.1-2).

The use of a baboon's heart was another controversial fact concerning the "Baby Fae" case, because the evolutionary relationship between humans and baboons is more distant than that between other primates. Furthermore, there are no documented antigens which are present on the surface of baboon cells similar to humans (Mak 2005, p.908). Bailey's decision to use a baboon heart was discussed in an interview between Bailey and an Australian radio presenter. Since the interviewer was forbidden to ask detailed questions concerning the operation, they confined themselves to the question of why he preferred a baboon as an organ donator despite the known biological and evolutionary differences (Stoller 1990, p.1-2). Bailey answered: "I find that difficult to answer. You see, I do not believe in evolution." (Prothero 2007, p.356)

In addition, Bailey argued that the death of "Baby Fae" had nothing to do with the choice of the organ donor. Bailey ignored both scientifically proven evolutionary relationships and published data on basic immunological research in order to carry out the xenotransplantation (Stoller 1990, p.2).

The basic precondition for carrying out experimental medical interventions or studies on humans is the documented approval of local specialist committees and/or commissions. In 1984, Bailey received a bioethically questionable written confirmation for five inter-species transplantations of baboon hearts to human recipients, although no empirical data was available at that time confirming that allogeneic and/or xenogeneic transplantations in children are possible and safe. In an endeavor to protect patients from untested experimental medical procedures, permits of the "Secretary of Health and Human Services" are required and the guidelines of the "California's Protection of Human Subjects in the Medical Experimental Act" (PHSMEA)" are to be observed (Stoller 1990, p.1). The following extract is from the Californian Law, Health and Safety Code (HSC),

Division 20. Miscellaneous health and safety provisions: Ch.1.3. *Human Experimentation [24175]*, added by Stats. in 1978:

> "Informed consent given by a person other than the human subject pursuant to subdivisions (b) through (d), inclusive, of this section shall only be for medical experiments related to maintaining or improving the health of the human subject or related to obtaining information about a pathological condition of the human subject."

In 1979 Dr. William Imon Norwood, Jr. developed a multistage heart surgery to create a new systemic circulation in (pediatric) patients with hypoplastic left heart syndrome (Ziemer 2017, p.585). The survival rate of the Norwood operation is between 50 to 75 percent (Vijayalakshmi. 2013, 679). Bailey did not consider this surgical treatment or an allogenic heart transplant for "Baby Fae". In the informed consent to the xenotransplantation procedure, Bailey presented the case in the following way:

> "Temporizing operation to extend the lives of babies like yours by a few months have generally been unsuccessful. We believe heart transplantation may offer hope of life for your baby. Laboratory research at Loma Linda University over the past seven years, including over 150 heart transplants in newborn animals, suggest that long term survival with appropriate growth and development may be possible following heart transplantation during the first week of life." (Stoller 1990, p.1-2)

After the death of "Baby Fae", the "National Institutes of Health" reviewed the case in 1985. In their report, they published several legal weaknesses concerning the approval procedure. In particular, the Committee noted that the chance of "long-term survival" had been overestimated. The reason why Bailey did not require approval from a higher authority to carry out this experimental procedure or did not obey the Californian PHSMEA guidelines was not mentioned in the report (Dommel 1986, p.3-4). Here the question arises why Baily was not prosecuted. Although the case of "Baby Fae" attracted vocal criticism among the general public and medical circles asking for the exclusion of Bailey from the medical profession, the San Bernandino prosecutor's office had not provided sufficient evidence to justify such an accusation. The facts, however, indicate that "Baby Fae" had to die to advance Leonard Bailey's career and scientific progress. Concerning the medical knowledge at that time, Bailey should have reconsidered his plan and look for alternative methods. Rules and laws to protect the very young patient were violated by those to whom she had been entrusted and who had vowed to defend her interests (Stoller 1990, p.1-2)

6.2.1 Reflections concerning animal and human ethics – perspective of the parents

Although both parents consented to the xenotransplantation, inadequate information on other medical treatment options for their child was provided. Parents usually make medical decisions on the basis of the best therapeutic treatment for their child (Palmer 2005, p.88-89). At the same time, the necessary realistic options must also be provided for a fair decision-making process. For an allogenic heart transplant operation, the Loma Linda Hospital demanded a high money sum or the proof of an adequate health insurance. At the time of the birth of "Baby Fae" her parents were divorced, not insured and financially weak. The Norwood method was also a cost-intensive therapy and the parents had to pay the transport and stay of their child in Philadelphia. As a result, "Baby Fae's" parents had actually two options: either not to use surgical corrective treatment and consequently let their daughter die from the consequences of hypoplastic left heart syndrome, or choose an experimental, cost-free, xenotransplant operation and enable their child a chance for cure - or, in simple terms: do something or do nothing. Faced with this physically and mentally stressful situation, parents normally decide upon the survival of their child. But is "taking action" always the most responsible answer? Do unorthodox and experimental procedures, with minimal scientifically proven evidence and little prospect of success, justify the mere attempt to heal a seriously ill infant, without reflecting the ethical, medical and psychological consequences of therapy (Devettere 2010, p.412)? Even today, thirty years after the "Baby Fae" case, it still seems irresponsible to agree to a xenotransplantation, especially in pediatrics. The evidence-based research of complex animal organs and their use as xenografts in human medicine is still at the level of basic research (Gericke 2014, p.1).

“Baby Fae's” father signed the consent form for carrying out the xenotransplant operation. Despite an almost seven-hour discussion between the medical directors and Bailey's team, which prepared the operation, a detailed medical information / education about potential risks, side effects, alternatives and prognoses was not passed on to the parents. This is clearly a violation of the physician profession's code of ethics. Like any other patient and family collective, parents must be fully informed of a medical intervention concerning their child in order to give them

sufficient time to understand the medical procedure and to reflect the advantages and disadvantages (Devettere 2010, p.412).

6.2.2 Reflections concerning animal and human ethics – perspective of the attending physicians

Bailey, who was not present when Baby Fae was hospitalized, contacted her parents a few days later and offered to operate on their child at the Loma Linda Hospital. In addition to a slide show and films, Bailey explained to Baby Fae's mother why a xenotransplantation of a baboon heart was the best option. In an interview after the operation, Bailey explained that there was no effort to find a suitable human donor for Baby Fae (Palmer 2005, p.88-89). In fact, the evidence of his research should be confirmed:

> We were not searching for a human heart. We were out to enter the whole new area of transplanting tissue-matched baboon hearts into newborns who are supported with antisuppressive drugs. I suppose we could have used a human heart that was outsized and that was not tissue-matched, and that would have pacified some peo-ple, but it would have been very poor science (Pence 2000, p.280)

There is a risk for physicians, particularly for those working in the scientific field, that secondary interests of a personal or institutional nature jeopardize primary interests. This conflict of interests can have a negative impact both on patient treatment and on scientific work. Medical care and action, which is goal-oriented and value-oriented, finds its basic formulation of a medical ethics in the Hippocratic Oath or in the Declaration of Geneva (Physician's Oath) adopted by the World Medical Association. In summary, the goals of medicine are disease prevention and health promotion, alleviating pain and suffering caused by disease, curing diseases and caring when healing is not possible, and preventing premature death and striving for a peaceful death. Scientific work, on the contrary, is presented as profound, value-free and evidence-based research, which is intended to expand knowledge and present new therapy options (Hucklenbroich 2011, p.205-207).

First and foremost, medical action should ensure good patient care. The extension of medical knowledge must therefore not be carried out at the expense of the physical and mental integrity of patients. For example, a doctor could argue for any type of transplant surgery, maintaining that there is sufficient evidence that treatment success prevails on the trauma of surgery. In the "Baby Fae" case, the

performed xenotransplantation did not fulfill these criteria. Bailey, motivated by the idea to test his operation method in the human model, acted to improve his research. His primary goal was not to find the best possible treatment option for his patient but perform the world's first xenogenic heart transplantation (Devettere 2010, p.412). The medical ethos - the health of my patient will be my first consideration (World Medical Association (WMA) 2006) – was deliberately ignored by Bailey's unilateral approach. The missing education talks with both parents about the possible risks, side effects, alternative treatment options and prognoses of the procedure and as well as the conflict of interest of his xenotransplantation research entailed unethical actions and moreover a violation of the medical ethos (Palmer 2005, p.88-89).

6.2.3 Reflections concerning animal and human ethics – perspective of the Ethics Committee

In the "Baby Fae" case, the Ethics Committee (Institutional Review Board, IRB) is an important moral authority. The reasons why the ethics committee justified the killing of the baboon and its use as an organ source are not known. It is, however, important that ethics committees should advise and supervise scientists on the ethical and legal aspects of their research – especially when they intend to research on living, deceased and, above all, vulnerable groups of persons who cannot give an informed consent (Palmer 2005, p.89-90).

In March 1985, a committee was set up to examine the consent on the "Baby Fae" case. It was found that the elucidation of the parents did not indicate any alternative treatment options (e.g. allogenic heart transplantation, Norwood surgery), and the prognosis of xenotransplantation was described too positively. In general, the whole informed consent process was classified as tenuous and questionable. The question why the IRB agreed to the xenotransplantation remains open. None of the members commented on the details of the report (Devettere 2010, p.413).

6.2.4 Reflections concerning animal and human ethics – perspective of the animal

Concerning xenotransplantation, essential questions can be formulated regarding the discourse between animal ethics and bioethics: From which ethical point of view is human organ donation to be preferred to xenotransplantation? Should

xenotransplantation present itself as medically equivalent to allogenic transplantation in the future, will xenografts be established as the new professional standard in transplant surgery? A semantic, animal-relevant nuance presents itself in the terminology of "organ donor". The idea of donation in general refers to a voluntary human service, i.e. a person allows his/her organ to be removed legally by consent in life or after death, without awaiting or receiving a consideration, but with the dedicated purpose for the organ to be transplanted into another person. Animals can neither be informed adequately about the organ donation because of linguistic and comprehension barriers nor can they communicate their consent or refusal towards those methods. The term "organ donor" does not apply in the context of xenogenic transplants. Rather, the medical term of “organ source” should be used (Haniel 2002, p.46).

Organ donation in the human medicine sector comprises several types. It is possible to perform a live donation, whereby organ lobes, tissue or paired organs are removed and transplanted. The organ donors only have limited life restrictions after the surgery. Unpaired organs that are necessary for life may only be removed after death (Beckmann 2000, p.44-49). The situation is different in xenotransplantation. Animals which serve as organ sources are killed for the extraction of xenografts:

> The idea of using animals as a source for organ donation is an example of speciesism, premised as it is on the idea that animals are things for us to use as best suits our own interests, without much concern for the interests of the animals themselves. Perhaps the easiest way to see this is to ask yourself the following question; why should we be prepared to accept the use of organs from animals, but not be prepared to take them from human infants who are, and always will be, less intellectually developed than the nonhuman animals? (Singer 1992, 730)

In the context of the animal assessment of xenotransplantations, the question arises when and under what circumstances the indisputable right of animal life underlies the human right to live. In his essay “Recent Studies in Animal Ethics” Nelson postulated that not only do humans not have the right to exploit animal life in such ways, to a greater degree they have the responsibility to protect them from such abuse (Nelson 1985, p.13-24).

Animal life is often variously assessed, depending on what role and status the animal in question has in the human world view. Generally humans can reason their interests and their wish for preserving human life towards other species, which is a phenomenon uniquely found among Homo sapiens, since animals do

not treat each other according to duties or legal principles, but according to the type of behavior typical of their kind. Therefore animals do not have a responsibility to preserve life, however, a right to the preservation of the animal life can be asserted against mankind (Birnbacher 2001, p.9-14).

The animal ethical assessment of the case "Baby Fae" differs depending on the underlying ethical view and what value is attributed to animal life and human existence: The anthropocentric view of the world gives mankind an independent value. Through their existence, plants, animals and inanimate matter have the only purpose to serve humans (Bradshaw 2007, p.149-151). The strong anthropocentrism sees the use of xenografts, the killing of animals and the subsequent transplanting of the organs into humans as unproblematic. Animals are to be understood as consumer objects and they are not awarded a genuine moral value. The weak anthropocentrism, on the other hand, was i.a. shaped by Kant and Schopenhauer. Animals have a human-derived moral status. Killing animals or torturing animals violates human duty and empathy. Consequently, the killing of animals and the use of organs as a xenograft are ethically justified only if no excessive suffering has been inflicted (Wissenschaftlicher Beirat der Bundesärztekammer 1999, p.1924).

Pathocentrism, as advocated by Peter Singer and Ursula Wolf, understands the ability to avoid suffering as a property that distinguishes a morally significant existence from a morally indifferent one. As a preference utilitarian, Singer demands that living beings with a comparable ability to dispose of interests as humans should also be treated comparably. Each action is therefore morally required if it leads to the result of the maximum satisfaction of interests (Singer 1992, p.728-32). In the case of "Baby Fae" the following train of thought follows: Thus, if a baboon's capacity for interest is similar to that of an infant suffering from anencephaly, a condition in which the neonate's brain is not fully developed and whose life expectancy does not exceed a few days, then the removal of both mammals' organs is permitted or not (Wissenschaftlicher Beirat der Bundesärztekammer 1999, p.1924-1925). Ursula Wolf also represents a pathocentric position. However, she criticizes Singer's approach as too narrow. Living beings, apable of felling deep pain and suffering, e.g. animals in the context of organ removal, must not be exposed to any suffering deliberately. The ability to

suffer is not merely a sensation of pain, but rather a general term whose positive counterpart is well-being (Niess 2017, p.47-50).

6.2.5 Concluding remark to the case of “Baby Fae”

The animal and human ethical assessment of the case "Baby Fae" presents itself as a complex vignette of xenotransplantation. Bailey firmly believed that killing the baboon, extracting his heart and xenografting are medically indicated and morally justified. The death of Baby Fae, like that of every human being, was deplorable and a great loss to her parents. If the surgery had been successful not only technically, but also physiologically, Baby Fae could have survived longer than was possible because of her congenitally damaged heart - and the preservation of life is in principle a bioethically relevant good. Nonetheless, a baboon, a healthy primate with recognized homologies in human neurology and physiology, was killed for xenograft extraction. The case vignette of "Baby Fae" clearly shows that Bailey's actions were strongly anthropocentric. Since the death of Baby Fae, no xenogenic heart transplantations have been performed to date as far as clinical human medicine is concerned. Bailey only used more allografts for the treatment of critically ill children and achieved good, scientifically relevant, success. In a 1990 essay that reflected the events surrounding the "Baby Fae" case, Bailey said he did not regret his decisions. Rather, a baboon whose blood type and blood markers had been more compatible with those of Baby Fae could has better guaranteed that his patient would has survived (Devettere 2010, p.411-413).

The ethics of xenotransplantation in the human area is not clearly defined. Perhaps the use of xenografts is beneficial and allows mankind to compensate for the lack of human donor organs. Critical voices, however, claim that the research and use of xenotransplantation is an overzealous undertaking that distracts from other, more realistic, medical intentions. Without advanced knowledge in human medicine, human and veterinary immunology and genetics, research talents and appropriate resources, clinical xenotransplantation lies in the far distant future (Gericke 2014, p.1).

7 Summary

A potential long-term solution to counteract worldwide organ donation deficiency is the availability of animal donor organs. The lack of long-term, complete and permanent artificial replacement of organs makes the advancement of xenotransplantation research more necessary and urgent. However, xenotransplantation is still far from clinical implementation. The main problem here is the control of the xenogeneic rejection reaction. Although the hyperacute rejection reaction by genetically modified donor pigs can now be mastered relatively well, this is not yet the case in acute vascular (humoral) rejection. The pathophysiological processes during the xenogeneic rejection reaction are not fully understood. Present data, however, indicate a pronounced coagulopathy with a disturbance of microcirculation and thrombosis in the hyperacute and acute humoral rejection reaction. It is absolutely necessary to know the mechanisms of the xenogeneic rejection reaction in order to be able to intervene in a targeted manner. The immunological rejection of porcine xenografts by primates may be dominated by the production of multiple transgenic pigs - e.g. the "perfect pig" presented in this diploma thesis

The physiological compatibility of porcine and human organs, that is, the ability of the xenograft to maintain life-sustaining functions in humans, cannot be presumed safe without further investigation. In this diploma thesis molecular incompatibilities for the diverse organs were differentiated: xenogeneic heart transplantation seems to be the most promising, while already in the kidney transplantation model problems in the interaction of the enzyme and hormone balance are to be expected. The liver, on the other hand, is unlikely to be suitable for xenotransplantation because of its many complex metabolic and synthetic functions. In the lungs, there are doubts about the potency of the porcine lung. Beyond these organ-specific difficulties, basic problems of microcirculation or different electrolyte concentrations affect all organs equally.

In addition, the risk of infection for patients, their relatives and the public by pathogens that can be transmitted with the xenograft must be further characterized. On the one hand, relevant pig pathogens must be identified - especially those that are latent or not yet sufficiently well-known. On the other hand, the importance of porcine endogenous retroviruses needs to be analysed more thoroughly.

With the aim of assessing the developmental potential and possible consequences of xenotransplantation as well as the prerequisites for human use, this diploma thesis examined the scientific and medical problems as well as selected historical and ethical aspects of the procedure with the help of literature analyses. In this diploma thesis it could be shown that a number of obstacles are still in the way of a clinical trial of xenotransplantation. Only when the necessary evidence for successful and save xenotransplantations from long-term studies in vivo is available and, in addition, the above-mentioned open questions regarding the ethical-social, but also - not explicitly mentioned in this diploma thesis – the health-economic and legal frameworks have been clarified, it seems justified to test human xenotransplantation in clinical trials.

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