Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science

Preoperative training of pigs used for kidney transplantation research

- Refinement of postoperative procedure

Anneli Rydén

Biomedicine and Veterinary Public Health
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Anneli Rydén

Supervisor:	Marianne Jensen-Waern, Swedish University of Agricultural Sciences, Department of Clinical Sciences
Assistant Supervisor:	Görel Nyman, Swedish University of Agricultural Sciences, Department of Clinical Sciences
Examiner:	Patricia Hedenqvist, Swedish University of Agricultural Sciences, Department of Clinical Sciences

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Summary

The use of pigs as a preclinical model has increased dramatically the last decades. The specie´s unique anatomy and physiological features make it an appropriate transplantation study model for humans. In human medicine, kidney transplants have been established as the best and most cost-effective treatment for people with end-stage renal failure. To improve the outcome of renal transplantation in humans, the graft survival rate needs to be improved and an animal model is essential for this research. However, information regarding preoperative training to allow for stress- and pain-free repeated blood sampling as well as blood sampling techniques and measurement of the urine volume postoperatively to kidney transplantation is limited.

Eight Swedish high-health domestic pigs were included in a training program in which touching and brushing the ears, ultrasound of the urinary bladder and physical examinations were performed for 15 minutes per pig every day for two weeks before transplantation surgery. Six of eight pigs underwent kidney transplantation and insertion of a catheter in the auricular vein with the Seldinger technique. After surgery, the pigs were kept for five days and blood sampling and ultrasound of the urinary bladder were performed daily. The effect of the preoperative training on postoperative examinations were evaluated.

The transplantation surgery and the anaesthesia were successful and the pigs recovered well after surgery. The training period of two weeks was sufficient to enable blood sampling and ultrasound examination of the urinary bladder without restrain. Furthermore, placement of a catheter with the Seldinger technique in the auricular vein was successful and withdrawal of blood was possible in four out of six pigs for five days post-surgery. In conclusion this study shows that the pig can be trained preoperatively to accept interventions and measurements postoperatively, which makes the pig a suitable animal model in transplantation studies.

Keywords: urine, blood sampling, stress-free, nursing, swine, catheterization

Sammanfattning

Nyttjande av grisen som preklinisk modell har ökat markant de senaste decennierna. Djurslagets unika anatomi och fysiologi gör den till en lämplig modell för människan i transplantationsstudier. Inom humanmedicinen har njurtransplantationer blivit den bästa och mest kostnadseffektiva behandlingen för människor som drabbats av njursvikt. För att resultatet efter transplantationen ska kunna förbättras, behöver överlevnaden av transplantatet bli bättre. I dessa studier är en djurmodell oumbärlig. Information gällande preoperativ träning för att kunna utföra stress- och smärtfri upprepad blodprovs tagning och mätning av urinvolymen postoperativt till njurtransplantation är begränsad.

Åtta svenska SPF grisar var inkluderade i ett träningsprogram där t.ex. klappa och borsta öronen, ultraljudsundersökning av urinblåsan och klinisk undersökning tränades i 15 minuter per gris, varje dag, i två veckor före transplantationskirurgi. Sex av åtta grisar genomgick njurtransplantation och kateterisering i auricularvenen med Seldingers teknik. Efter kirurgin utfördes blodprovstagning och ultraljudsundersökning av urinblåsan varje dag under fem dagar. Effekten av den preoperativa träningen på de postoperativa interventionerna utvärderades.

Transplantationskirurgin och anestesin förlöpte utmärkt och grisarna återhämtade sig bra efter kirurgin. Den två veckor preoperativa träningen var tillräcklig för att möjliggöra blodprovstagning och ultraljudsundersökning av urinblåsan postoperativt utan stress för djuren. Därutöver var kateteriseringen i auricularvenen med Seldingers teknik tillfredställande och blodprovstagning var möjlig i fyra av sex grisar i fem dagar efter kirurgin. Resultatet visar att grisar kan tränas preoperativt för att acceptera interventioner och mätningar postoperativt, vilket gör grisen till en lämplig djurmodell i transplantationsstudier

Nyckelord: urin, blodprov, stressfri, omvårdnad, svin, kateterisering

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1 Introduction

During the last three decades, the use of pigs as a preclinical model in research settings has increased dramatically. The pig has developed to be the major translational large animal model replacing the dog and nonhuman primates (Smith & Swindle 2006; Swindle 2007b).The species unique anatomy and physiological features makes it an appropriate model for human diseases. For example, the renal system of the pig is more similar to that of humans than most other animal species (Dyce, Sack & Wensing 2010). Furthermore, the size of the pigs is suitable for development of surgery techniques requiring the dimension of a human being (Fruhauf et al. 2004; Kaiser et al. 2007; Swindle 2007b).

The increased use of pigs in the laboratory settings would not have been possible if refinement of treatment, anaesthesia and husbandry had not been improved during the last decades (Swindle 2007b). However, to meet the demands of adequate handling of the pig during advanced surgery, improved nursing throughout the entire perioperative period is needed in order to reduce stress and pain. So far, some guidelines for handling, surgery, anaesthesia, and perioperative care of the pig have been described in the literature (Damy et al. 2010; Kaiser et al. 2006; Swindle 2007a), but information regarding preoperative training to ease stress and pain-free blood sampling and measurement of urine volume is limited.

This master degree essay is part of a study which aims to reduce tromboinflammation caused by ischemia/reperfusion injury due to kidney transplantation. Thereby rejection can be avoided and the success rate of implantation increased. The evaluation of the transplantation study depend on the possibility to measure and analyse physiological parameters in the early postoperative phase. Thus, the animals had to be trained to facilitate stress-free postoperative measurements, sampling of blood and examination of the urinary tract with ultrasound.

Hence, the present study focuses on the perioperative training and nursing of research pigs in kidney transplantation studies. The main aim is to study if preoperative training during the acclimatization period allows postoperative blood

sampling and ultrasonic urine bladder detection without restraint. A second objective is to evaluate if a central venous polyurethane catheter introduced percutaneously from an ear vein by the Seldinger technique would enable repeated blood sampling during five consecutive days postoperatively.

2 Literature review

2.1 The pig as an animal model in transplantation of kidneys

In general, pigs are appropriate as experimental animals because of their size, their physiological resemblance with humans and relatively short reproduction cycle. The pig is a valuable preclinical model for medical research on organs in experiments that require an organ size of human organ e.g. liver, kidney, heart, skin, blood and intestine. Moreover, there is a strong morphological and functional similarity of the organ systems between human and pigs (as reviewed by Kaiser et al., 2006). New surgical techniques have usually been evaluated and investigated in pigs before application on humans, for feasibility, safety, and efficiency. Also pathophysiological investigations like organ failure and sepsis studied in swine have high validity (Kaiser et al. 2006).

The porcine anatomy of the kidney and the urological system is more like that of primates than any other mammal (Nath et al. 2014; Sampaio, Pereira-Sampaio & Favorito 1998). The kidney of a 70-kg pig is approximately the size of that of an adult human kidney. Both kidneys are located dorsally with the left kidney cranial to the right. The adrenal glands are located near the cranial poles of both kidneys (Swindle & Smith 1998). Blood supply to the kidneys distributes transversely between the cranial and caudal poles like in humans, rather than longitudinally like most other species. The avascular plane of the kidney is transverse in pigs, and not longitudinal as in humans and dogs (McGlone 2001). The porcine anatomy of the collecting system and renal morphometry are compared in detail with that of humans in a publication by Sampio et al (1998). In that study, it was concluded that the pig kidney is a good model for training and urologic research in human medicine (Sampaio, Pereira-Sampaio & Favorito 1998).

Through the years, kidney transplants have been established as the best and most cost-effective treatment for people with end-stage renal failure (Prihodova et al. 2014). In May 2012, over 92 000 patients in the United States, were awaiting a solitary kidney transplant, and the need of kidney transplant is increasing by 7% per year (Heaphy et al. 2013). The graft survival rates in a 5- and 10-year period from 2005-2008 in Europe, has been reported to be 77 and 56% respectively (Gondos et al. 2013). The corresponding survival rates for the grafts were markedly lower in the United States (Heaphy et al. 2013). To improve the outcome of renal transplantation in humans, graft survival rate needs to be improved and an animal model is decisive for future research.

2.2 Housing, acclimatization and training of pigs in transplantation studies

Pigs housed in the laboratory should be kept in small groups and preferably obtained from the same herd. However group housing may be contraindicated postoperatively for some protocols because the propensity of increased aggression during that period (Smith & Swindle 2006) or due to the tendency to cannibalize incisions on cage mates (Swindle 2007b). For that reason, individual housing is common in a research setting. When the pigs are housed individually, adequate socialization can be met if they are allowed to see, hear, snout and smell other pigs in the stable (Smith & Swindle 2006).

Proper husbandry in the laboratory setting requires knowledge of the behaviour characters of pigs. In the wild, they live in large close groups with females and their young offspring in which a social dominance order among females is created. The males leave the group when mature and form bachelor herds until they are old enough to mate (as reviewed by Smith and Swindle, 2006). Laboratory pigs spend 70 to 80% of the day, lying down or sleeping, unless it is feeding time or people work in the stable. During the active periods of the day, natural behavioural of the pig includes almost uninterrupted grubbing, gnawing, rooting, chewing and foraging. Before puberty, the piglets also play with one another (Malavasi et al. 2005) Lack of abilities for these activities, can result in behavioural stereotypies (Kaiser et al. 2006).

The pigs have to be free from specified infectious diseases that may have an adverse effect on the research protocol (Clary et al. 2002). Upon arrival, animals of unknown health condition should be kept separately from other animals until their health condition can be defined properly (Kaiser et al. 2007). However, it is preferred to buy pigs with a good health from a SPF- (specific pathogen free) herd (Swindle 1996). A acclimatization quarantine of two weeks is recommended for

pigs before start of research (Clary et al. 2002; Fruhauf et al. 2004) to allow the animals to recover from the stress of shipping and to accommodate to different husbandry conditions (Smith & Swindle 2006).

It is essential to acclimate the animals to the facility and to handling before start of the study protocol. Due to ethical considerations, all procedures with animals should be carried out by trained professionals (Damy et al. 2010). All personnel working with the pigs should interact so that fearful responses are minimized (McGlone 2001). Pigs are intelligent animals with very good memory for both bad and good experiences. They easily become habituated to routines and unique practices in research settings. Pigs are relatively insensible to noise, even if extremely unexpected noises frighten them (Kaiser et al. 2006). Gentle handling procedures instead of forceful techniques will allow them to be petted and bond with the handlers (Kaiser et al. 2007; Smith & Swindle 2006). The impact of handlers on animal behaviour in a laboratory setting is significant, and the importance of bonding cannot be overemphasized (Smith & Swindle 2006).

Several designs for sling devices to restrain pigs, have been described and are used in numerous of research labs. The most commonly used is that described by Panepinto et al (1983). The pig is placed in sternal recumbency, in a hammock with holes for the limbs upon which the treatments and measuring can be performed (Lighty et al. 1992; Panepinto et al. 1983; Swindle 2007b). Small pigs can be handled in the staffs arms and larger animals can be herded against the cage with handheld panels (Swindle 2007b).

Pigs are easily trained (Swindle 2007b), but information concerning best practice to prepare pigs for surgical and transplantation studies is limited. In one study made by Nicholls et al (2012), the effect of preoperative visits by project personnel on compliance of 26 miniature pigs was examined. In the study, preoperative interaction variables and measures of preoperative socialization were positively correlated with postoperative outcome. The group that received more time with the personnel preoperatively had higher socialization scores, was associated with less stress and easier to give medication postoperatively compared to control animals (Nicholls et al. 2012).

2.3 Anaesthesia and analgesia

The anaesthetic protocols should be based upon the condition of the animal, the planned surgical procedure and the physiologic effects of the anaesthetic agents (McGlone 2001). Furthermore, as the use of pigs in experimental studies increases it is necessary to investigate new and effective anaesthetic protocols to preserve

animal welfare in extensive medical investigation and concurrently improve the quality of research (Geovanini et al. 2008; Kaiser et al. 2007; Usvald et al. 2008).

Pigs require careful perioperative care to avoid stress-related responses (Smith & Swindle 2006). Stress has been shown to affect immune responses (Dalin et al. 1993; Einarsson et al. 2008; Lee et al. 2016) and may affect outcome of the experiment (Smith & Swindle 2006). Anaesthesia is commonly induced in the stable, preferable in the pig`s pen. Intramuscular injection in the neck between the ear and the shoulder is the least stressful way to administer injectable agents (Smith & Swindle 2006). A butterfly catheter is usually used, and the needle should preferable be placed behind the ear in dorso-cranial aspect of the neck. When the pig is unconscious, a catheter can easily be placed in an auricular vein and intravenous anaesthesia can follow according to the intended protocol (Swindle 2007b). Continuous rate infusion of drugs is preferred over repeated bolus injections, when general intravenous anaesthesia will be used to maintain anaesthesia (McGlone 2001; Smith & Swindle 2008). All potent inhalation agents cause a decrease in the renal flow in proportion to the dose. The agents release fluoride as a by-product, but even if fluoride has been linked to renal failure, inhalation agents are only metabolised to a minor extent and unlikely to cause renal damage. Hence inhalation anaesthesia can be used in renal transplantation procedures (Martinez, Gasanova & Adesanya 2013).

Endotracheal intubation should be performed in all pigs undergoing general anaesthesia. When the specific anatomic considerations of this species are understood, the intubation is easier performed. The laryngeal passage is narrow, and the swallowing reflex is strong. It is useful to oxygenate the animal with 100% oxygen via an inhalation mask before the intubation if there should be a complication during the intubation procedure (Kaiser et al. 2006). The pig may be intubated in dorsal, lateral or sternal recumbency facilitated with a standard laryngoscope with straight blades (Kaiser et al. 2006; McGlone 2001; Swindle et al. 2012).

2.4 Measurements of urine

Accurate monitoring of the urine output of pigs is central for assessing the result after kidney transplantation but also during different experimental manipulations, biochemical, nutritional, urological and physiological studies (Holliman et al. 1982; Kurien, Everds & Scofield 2004). The urinary bladder in the pig is large and has a thin flimsy wall (Swindle et al. 2012). Maximum urine concentration in the pig is 1080 mOsm/kg, which is similar to human urine (Assimos et al. 1986; Dalmose et

al. 2000). Pigs produce 5–40 ml/kg urine per day depending upon the age and the water consumption (Swindle 2007b).

The procedure for insertion of a urinary catheter is seldom described in detail in scientific publications (Musk, Zwierzchoniewska & He 2015). In male pigs, urethral catheterization of the urinary bladder through the penis is difficult, even impossible, as the tip of the penis is shaped as cork-screw (Swindle et al. 2012). The female urethra can be catheterized conventionally as in other female animals (Swindle 1983). In a publication by Ettrup et al. (2011) the authors describe a stepwise procedure for placement of a urethral catheter in female pigs. Nevertheless, the description does not give any information of the difficulties the procedure may present. In a short report by Musk et al. (2015), a Foley catheter was inserted in 16 female pigs whereupon the placement was evaluated. The catheterization of the urethra was successful in 15 pigs, but the placement was unexpectedly challenging and took up to 60 min in some pigs.

Insertion of an urinary catheter may carry urethral microorganisms into the bladder (Daifuku & Stamm 1984), in humans, nosocomial urinary tract infection is the most common infection acquired in hospitals and nursing homes, and is usually associated with catheterization of the urinary bladder (Warren 2001). The external part of the catheter offers opportunity for bacteria entry directly into the bladder and this is the most common route of entry for bacteria (Daifuku & Stamm 1984). After bacteriuria develops, the ability to limit the complications is minimal. Once a catheter is placed, the clinician must keep the catheter system closed to postpone the onset of bacteriuria, and remove the catheter as soon as possible (Warren 2001).

Metabolic pens for the collection of urine are available, and use funnels, screens and gratings to separate urine and faeces. These stables have been used in pharmacokinetic and metabolic studies and are satisfactory for many purposes and easy to use. In some experiments with metabolic pens, female animals cannot be used because the separation of urine and faeces is not possible due to the proximity between vulva and anus (Ivers & Veum 2012; Moughan, Smith & Kies 1987; Schneider et al. 2014; Valle et al. 2012). In metabolic pens, the urinary collection system is not enclosed and the urine could be contaminated by feed or faeces (Moughan, Smith & Kies 1987). In one pharmacokinetic study made by Schneider et al. (2014) male pigs were fitted with a faeces collecting bag maintained by a nappy and the excreted urine could be collected from the metabolic cage.

Other systems for collection of urine in large animals, such as suprapubic catheterization (Holliman et al. 1982) and apparatus attached to animals (Paulson & Cottrell 1984) have been developed to minimize cross contamination of excreta. Even if there is a proper way to measure urine output, it has been reported that these

methods sometimes cause bladder infection, animal discomfort and badly adjusted apparatus, resulting in failure to collect of urine (Aschbacher 1970).

2.5 Vascular catheterization for long-term use

Intravascular access for injection and blood sampling is one of the most common experimental surgical procedures in laboratory animals (Swindle et al. 2005; Trim & Braun 2011). Scientists should be aware that the process may well be unnecessarily stressful due to the handling, including restraint, the anaesthetics used, but also discomfort during painful sampling techniques. The physiological changes and release of endogenous hormones associated with increased stress may even invalidate the research result (Cooper 2007; Rushen et al. 1993). If repeated blood samples need to be collected over a prolonged period of time, it is less stressful for both animal and handler to use previously surgically implanted indwelling catheters or vascular access ports (Cooper 2007). The ideal vascular access device is accessed without stress in the animal and is free of infectious and thrombotic complications. The route for blood sampling may also be used for administration of substances and for treatments of disease (Trim & Braun 2011). In a report from the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement ("Removal of blood from laboratory mammals and birds. First report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement" 1993), the authors compared blood obtained through chronic indwelling cannula in unrestrained animals with blood obtained from restrained animals, a significant difference in blood counts was observed. As part of stress reaction, catecholamine's are produced, which cause the spleen to contract and the red blood cell count, packed cell volume and haemoglobin will be artificially high. In one study by Brenner et al (1981), the effects of five-minute fixation by means of maxillary sling on the levels of various blood components were recorded. Blood was sampled through an indwelling catheter from the jugular vein up to 60 minutes after start of restraint. The fixation led to a maximum rise of lactate (0.53 g/l) and increase in glucose (0.37 g/l). The values returned to their initial levels within 60 minutes from the end of the restraint (Brenner & Gurtler 1981). It is well known that the number of granulocytes increase when the cortisol levels are high. Thus, both the number of red and white blood cells are altered (Waern & Fossum 1993).

Skin and hair are a reservoir of bacteria and the catheter site should be prepared aseptically before start of the catheterization (Madan, Alexander & McMahon 1992). Clipping of the hair provides good visualization of the vessels. Contradictory there is a proven association between wound infection and pre-operative shaving (Mishriki, Law & Jeffery 1990). In a study from 1997, made on horses, the authors

reported that aseptic preparation of the skin over the midcarpal and distal interphalangeal joints can be accomplished without hair removal (Hague et al. 1997). However if the hair is shaved or clipped, sharp blades should be used to minimize skin trauma and infections (Mishriki, Law & Jeffery 1990). Standard methods of aseptic preparation with chlorhexidine-alcohol solution should be used (Rupp et al. 2012) and 3 to 7 applications is recommended. The procedure should last for at least two minutes (Coolman et al. 1998; Feldman, Zinkl & Jain 2000).

The vascular access sites in pigs are relatively deep in the tissues (Bobbie & Swindle 1986) and covered with well-developed subcutaneous fat (Niiyama et al. 1985). In addition, vessels are prone to vasospasm on puncture and also relatively easy to rupture during catheterization techniques (Swindle 2007b). When performing surgical vascular access, the division of muscular tissues should be performed bluntly between muscle bodies instead of sharp dissection of muscle fibres. Blood vessels should be handled gently and suture material used around the blood vessels should not be abrasive. Pigs tend to show exaggerated inflammatory reaction to silk and surgical gut sutures, thus arteries, veins and operation wound may be repaired using synthetics and monofilament non-absorbable suture material in this species (Swindle et al. 2005). Catheter access should be tunnelled through subcutaneous tissue and externalized away from the surgical site, because of the stress on the suture line and the high probability of contamination (Cooper 2007).

There are some studies made with catheters surgically implanted in the external jugular vein and tunnelled through the subcutaneous tissue, externalized to the posterior region of the auricle (Harris 1974; Lombardo et al. 2010; Manell et al. 2014). In these studies, the outcome of the technique has shown to be suitable for experimental animals even if some of the pigs got minor complications.

Superficial access to a vein makes it possible to cannulate the central venous system either with a small incision in the skin or percutaneously. Possible veins to cannulate include the auricular vein, the cephalic vein close to the thoracic inlet, and the cranial epigastric vein on the ventral abdomen (Cooper 2007). Advantages of percutaneous techniques include minimal damage to the catheterized vessels, a shorter healing time than surgical procedures, and a possibility to perform serial catheterizations in the same animal (Swindle 2007b).

Superficial percutaneous cannulation methods have been described in which authors used a through the needle catheterization technique with commercial introducer kit for access to the femoral vessels (Gaymes et al. 1995; Smith et al. 1989) and to the external jugular vein (Carroll et al. 1999; Damm et al. 2000; Fudge, Coleman & Parker 2002). In additional studies, percutaneous cannulation on pigs have been performed with introducers and tubes made in their own laboratories (Damm et al. 2000; Ford & Maurer 1978; Smith & Ficken 1991). The technique of locating and accessing blood vessels for percutaneous catheterization can be guided

with fluoroscopy (Larsson et al. 2015) or by ultrasound guidance (Wallace, Ahrar & Wright 2003).

Ear veins are suitable for intravenous administration of drugs or collection of small-volume blood samples. Branching and size of ear veins differ from animal to animal and between breeds. Moreover, these veins are prone to form hematomas that complicate repeated puncture of the vessel (Seldinger 2008). In the dorsal area of the visible veins of the auricle, the middle or lateral vein is to prefer for cannulation, since the inner vein run under thick skin and is situated in an unfavourable area for insertion (McGuill & Rowan 1989). Nonsurgical techniques in which catheters were placed in the jugular vein via an auricular vein has been published. In two of these studies the catheters remained in place for less than 4 hours (Shearer & Neal 1972; Zanella & Mendl 1992) and in other studies for more than 48 hours (McGuill & Rowan 1989; Pairis-Garcia et al. 2014; Phillips et al. 2012; Porter, Ryan & Norman 1992). In these studies the catheterization were easy to perform and collection of blood samples worked adequate in pigs weighing 90-283 kg. However, in one study made by Niiyama et al. (1985) in 20 kg piglets, the catheterization (Argyl´s Sentinel Line Cardiac Catheter 14 gauge 1.35mm x 400mm, Nippon Sherwood Medical Industries, Japan) was smooth, but the canal of the catheter was too narrow to use for collection of blood samples. Additionally, the length of each vein was measured in the piglets. The caudal auricular vein (ear bas to junction with maxillary vein) measured 5-6 cm, the maxillary vein (distance between the caudal auricular vein and the external jugular vein) was further 3-4 cm. The length of the external jugular vein was 8-10 cm (Niiyama et al. 1985) fig. 1.

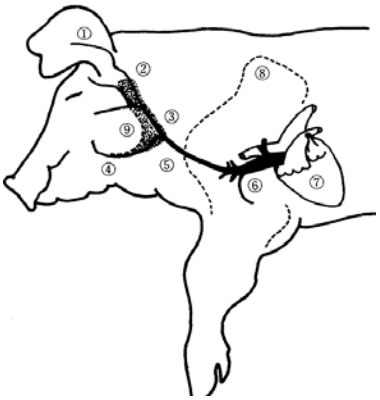

Figure 1. A schematic presentation of the cranial venous system in a 20-kg piglet, according to Niiyama (1985).
1. The auricular vein
2. The caudal auricular vein measures 5-6 cm
3. The maxillary vein measured 3-4 cm
5. The external jugular vein measured 8-10 cm

The Seldinger technique is specifically designed to introduce catheters into vessels via needle puncture (Seldinger 2008). The vessel is located and then punctured with a specially shaped needle. A guide wire is inserted into the vessel through the needle and the needle is removed. The catheter is inserted over the guidewire and guided into the vessel. The wire is then removed from the catheter.

The vascular access port was developed as an alternative to externalization of catheters (Swindle et al. 2005).The access port is implanted subcutaneously, firmly sutured in place, and with an attached catheter that is tunnelled in the subcutaneous tissue to a blood vessel. The port is accessible through the skin, punctured with a Huber needle (Moroni et al. 2011; Raad & Bodey 1992). As port catheters do not exit the skin, the risk of infection, tissue damage and, morbidity are reduced (Chuang et al. 2005) and there are few concerns with the animal disturbing the implanted device (Swindle et al. 2005).

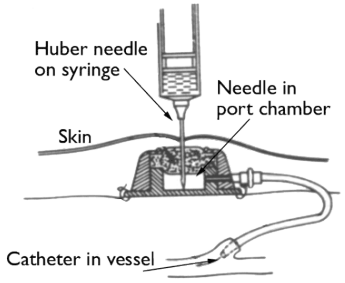

Figure 2. The subcutaneous vascular access port (with permission from Norfolk Vet Products Illinois, USA).

In a study by Chuang et al. (2005), the outcomes of surgically placed externalized catheters and subcutaneous vascular access ports implanted in a pig model, were compared. After placement of the devices the pigs underwent serial blood sampling for 30 days. In the group with externalized catheters, 72% of the animals developed infectious complications and samples from the blood and wounds were frequently marked by the presence of several species of microorganisms. In addition 17% of the pigs with externalized catheter developed thromboembolic complications, and one animal was euthanatized secondary to sepsis. In the group with subcutaneous vascular access port, one animal developed transient fever. No other complications were noted in the rest of the pigs with subcutaneous vascular access port. Anaesthesia was not needed during blood draws. However, there are some disadvantages to consider when using the subcutaneous vascular access port including higher costs than external catheters, a needle puncture to access the port, dead volume within the port, required meticulous aseptic skin prep, risk of necrosis

or port erosion through the skin, and the potential for the needle to dislodge during flushing (Swindle et al. 2005).

2.6 Aseptic considerations

For survival procedures, meticulous aseptic techniques are obligatory intra- and postoperatively when handling chronic catheters (Swindle et al. 2005). Infection is one of the leading complications of indwelling vascular catheters, and catheter-related septicaemia represents the most frequent life-threatening complication of vascular catheters (Raad & Bodey 1992). Thrombotic occasions are also problematic, particularly when catheters are left *in situ* for a long period (Jacobson 1998). Anticoagulant therapy is necessary to prevent blood from clotting within the catheter. As a general recommendation, the catheter is flushed with 10% heparinized saline every time it is accessed or twice per week when not used (Jurewitsch & Jeejeebhoy 2005). Before injecting or withdrawing samples through the catheter, the extended part of the catheter should be washed with aseptic solution. The old heparinized saline is withdrawn until blood is visualized and the blood sample is withdrawn. Immediately after the sampling, the catheter is flushed with large volumes of saline after which an amount of heparinized saline required to fill the catheter is injected. All personnel should wear gloves and follow aseptically routines during the procedure (Swindle et al. 2005).

2.7 Blood sampling

The volume of blood sampled from the animal should be stated in the experimental protocol. However it is desirable that analyzing techniques that allow sampling of small blood volumes (Morton et al. 1993) are used. The maximum blood volume that can be drawn is calculated from data on the circulating blood volume. There is considerable variation in these values, relating to the techniques used and the strain and gender of animal (Diehl et al. 2001). The blood volume of the pig commonly described in safety evaluation studies in pigs consists of an overall blood volume of 65 mL/kg and 6.5% of the body weight (Feldman, Zinkl & Jain 2000; Kaiser et al. 2006; McGuill & Rowan 1989; Morton et al. 1993). As a rough guide, up to 10% of the circulating blood volume can be withdrawn on a single occasion every two weeks from healthy animals on adequate nutrition with minimal adverse effect. For repeat bleeds at shorter intervals a maximum of 1 % of an animals circulating blood volume can be removed every 24 hour. (Morton et al. 1993; Smith & Ficken 1991). In case of frequent blood sampling, the weekly quantity of blood should not exceed 7.5% (Smith & Ficken 1991).

3 Material and methods

All procedures were approved by Ethics Committee for Animal Experimentation, Uppsala, Sweden. The total duration of the experiment was three weeks including training and the postoperative period.

3.1 Animals and housing

Eleven Swedish high-health-certified domestic pigs (Yorkshire x Hampshire) were obtained from the University herd (Lövsta, SLU, Sweden). Three female pigs from one litter were used as donors. Six pigs, of both sexes, from another litter unrelated to the donors were used as recipients, and two pigs, from the recipient litter were used as controls (no surgery). Eight pigs (six recipients and two controls) were used in the present investigation.

The pigs arrived 14 days before the start of the surgical procedure and were housed at the Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden. Upon arrival, the seven-week-old recipients and controls were separated and moved to individual pens measuring 3 m². The six-week-old donors were kept together in a bigger pen measuring 4.3 m². Both groups were within sight and sound of one another. The pigs were provided straw and wood shavings as bedding before surgery, whereas the floors were covered with blankets (Vetbed® Petlife International Ltd, Suffolk, UK) after surgery. The temperature in the stable was 18 ± 2 °C, and a light/dark schedule (10:14h) was used and an infrared lamp was placed in a corner of each pen. The pigs were fed a commercial finisher diet (Solo 330 P SK, Lantmännen, Sweden) every day at 7 a.m. and 4 p.m. and water was provided *ad libitum.* The pens were cleaned twice daily throughout the study.

3.2 Acclimatization and social training

Five persons, with experience of training pigs, were involved in the social training program of the six recipient pigs and controls. The training was divided in four different steps (see Table 1), and the training was performed for 15 minutes per pig daily. In step 1 (day 1-3), the pigs were left without any handling for adaption to the new environment. The staff only entered the stable for feeding of the pigs and cleaning of the pens. In step 2 (day 4-5), the trainer sat in the pen allowing the pig to get accustomed to the individual. Once the pig was close enough, the trainer started to gently touch and brush the animal and treated it with pieces of apples, pears and bananas from the hand. The pigs were also adapted to accept touching, brushing and handling of the ears as preparation for blood sampling from a catheter in the auricular vein. The training in step 2 also included talking to the animals. In step 3 (day 6-9), the training from step 2 continued and also included touching with an ultrasound transducer over the pigs abdomen to tolerate ultrasound evaluation of the urinary bladder and the kidneys postoperatively, and sampling of a midstream urine specimen. In step 4 (day 10-14) the training from step 2 and 3 continued. They were also trained to undergo a physical examination including auscultation of the heart and lungs, rectal measurement of body temperature. Extra time was spent on the pigs that not fully adapted the different procedures. Additionally to the training program, the pigs, were trained to step on an electronic spring scale (Ecco 101, Farmer Tronic Industries A/S, Vamdrup, Denmark) for recording of body weight (b.w.) throughout the study.

3.3 Anaesthesia and catheterization

Twelve hours before surgery, food was withheld but water provided *ad libitum.* The anaesthesia was induced in the home pen, with a mixture of 5 mg/kg b.w. tiletamine-zolazepam (Zoletil Forte® vet. 250 mg/mL, Virbac, Carros, France) and 2.2 mg/kg b.w. xylazin (Rompun® vet. 20 mg/mL, Bayer), followed by 0.01 mg/kg b.w. buprenorphine (Vetergesic® vet. 0.3 mg/mL, Orion Pharma Animal Health, Sollentuna, Sweden) intramuscularly (IM) in the semispinalis capitis muscle. After administration of the drugs, the pigs were left for five to ten minutes, without manipulation, to achieve an adequate depth of anaesthesia. After the induction, the principals were administered 20 mg/kg b.w. bensylpenicillinprokain (Penovet® vet. 300 mg/mL, Boehringer Ingelheim) to prevent infection. The animals were covered with a blanket and transported on a transport cart to the preparation room, where they were placed in sternal recumbency on a surgical table and measurement of oxygen saturation was started. To prevent heat loss, socks were placed on feet and legs. An intravenous (IV) catheter (BD Venflon™ 20G x 32mm, BD Medical,

Sweden) was placed in an auricular vein and the trachea was intubated with an endotracheal tube (6-8 mm internal diameter and cuffed) with the use of a laryngoscope. Five minutes before and during intubation the pigs were administered oxygen (4 L/min). Anaesthesia was maintained with Isoflurane (IsoFlo® vet Orion Pharma Animal Health, Sollentuna, Sweden) in 30% oxygen in a rebreathing circuit, mechanically ventilated (Pressure control) with a Flow-I ventilator (FLOW-i ® Anesthesia delivery system, MAQUET Medical Systems, NJ, USA).

To allow for blood sampling intra- and postoperatively, an uncoated catheter (BD Careflow™ 3Fr 200mm BD Medical Sweden) was introduced via *Vena auricularis* into an internal vein using the Seldinger technique. The hair on the ears was removed with a clipper if needed and the skin was cleaned with chlorhexidine gluconate 4% (Hibiscrub 40 mg/mL) followed by 0.5% chlorhexidine gluconate in 70% isopropyl alcohol (Klorhexidinsprit Fresenius Kabi 5 mg/mL, Fresenius Kabi, Uppsala, Sweden). All personnel performing or assisting with catheter insertion, washed their hands with an antimicrobial scrub (chlorhexidine-based) and wore caps and sterile gloves. An assistant placed the thumb on the vein at the base of the ear, providing pressure for occlusion. The catheterization was made by first inserting a sterile steel needle introducer (20G OD 0.9mm x 40mm, BD Medical, Sweden) at 30°- angle through the skin caudally along the expected path of the vein. Blood backflow showed successful puncture of the vessel. A guidewire (OD 0.46mm x 450mm, BD Medical, Sweden) was passed through the needle and advanced into the vessel. The needle was withdrawn over the guidewire which was left in the vessel. A small stab incision was made at the puncture site and a catheter (3Fr OD 1.10mm x 200mm, BD Medical, Sweden) filled with heparinized saline (100 IU/mL) (Heparin LEO, 5000 IU/Ml, Leo Pharma) was advanced over the guidewire and into the vessel. The catheter was capped with a threaded injection cap and sutured in place with monofil coated polyamide (Supramid 2-0, B Braun Medical, Danderyd, Sweden) and covered with a polster (Snögg, Animal Polster, Snøgg AS, Norway). The catheter was filled with heparinized saline (100 IU/mL) when not used.

All recipients were administered an epidural injection of morphine (Morfin Epidural Meda 2 mg/mL, Meda AB, Solna, Sweden) diluted to 0.1 mg/ml in saline. The technique of the epidural injection was performed according to Strande (1968). The volume of drug administration was related to the length of the vertebral column, measured from the external occipital protuberance to the first coccygeal vertebra. One mL of the solution was given for the first 40 cm length of vertebrae and for every 10 cm of vertebral length an extra 1.5 mL was added. An epidural needle (8 cm long and 1.3 mm diameter) was introduced in the large interarcuate space in an angle of 90° during aseptic conditions. Before surgery, the incision area was shaved with an electric clipper, and cleaned with chlorhexidine gluconate 4% (Hibiscrub

40 mg/mL) followed by 0.5% chlorhexidine gluconate in 70% isopropyl alcohol (Klorhexidinsprit Fresenius Kabi 5 mg/mL, Fresenius Kabi, Uppsala, Sweden). The cleaning procedure was performed for at least five minutes. The pigs were moved to the operation theatre and placed in sternal recumbency on a heating pad on the operation table. Bland ophthalmic ointment (Viscotears®, Laboratoires Théa Clermont-Ferrand, France) was administered into the eyes. The cleaning procedure with chlorhexidine in alcohol was repeated and the entire surgery was performed under aseptic conditions.

Throughout anaesthesia the pigs received IV infusion of lactated Ringer solution (Ringer-acetat, Fresenius Kabi AB, Uppsala, Sweden) 10-15 ml//kg/h and succinylated gelatine (Gelofusine®, B. 40 mg/mL, Braun Melsungen AG, Melsungen, Germany) as a bolus of 3 mL/kg during 10 min., followed by 3 mL/kg/h. To achieve desired blood flow, dobutamine (DOBUTAMIN Carino® 250 mg/50ml, Carinopharm, Elze, Germany) was given as an infusion dose of 5 µg/kg/min stepwise increased to 15 µg/kg/min if necessary.

Oxygen saturation of hemoglobin (SpO_2), Heart Rate (HR), Arterial Blood Pressure (SAP, MAP, DAP), Electrocardiogram (ECG), Respiratory Rate (RR), End-tidal carbon dioxide (Et CO_2), End-tidal isoflurane (Et AA), End-tidal oxygen (Et O_2), spirometry and Body Temperature (BT) were monitored (AS/3 Compact Anesthesia Monitor, Datex-Ohmeda, Finland) continuously and recorded every five minutes during the anaesthesia.

3.4 Transplantation surgical procedure

A vertical midline incision was made in the *linea alba* through which both kidneys and ureters were removed from the donors. The total duration of the surgery was 60-75 min. Subsequently the kidneys were flushed with cold HTK (Custodiol® Kaliumklorid 2 mmol/mL, Fresenius Kabi AB) and stored at 4°C (static cold storage) for 24 hours. After nephrectomy, the donors were euthanized with 12 mL pentobarbitalnatrium (Euthasol Vet. 400 mg/mL, Virbac, Kolding, Danmark) IV. Before recipient surgery, a random kidney from the donor was manually perfused with 20 mL of HTK solution and the other kidney was prepared with polyethylene glycol lipid 2 mg/mL. After perfusion, both kidneys were placed in boxes filled with perfusion solution and stored at 4°C for 40 minutes. In the recipient pig, one kidney was transplanted according to Zakariasson (2016). Nephrectomy of the recipients own kidneys were made after transplantation was completed. Thus, the recipient pig had one transplanted kidney (Zakariasson 2016).

The abdominal wall was closed with absorbable suture (Vicryl 2-0, Ethicon, Sollentuna, Sweden) and the skin was closed with a surgical stapler (Appose™

Single Use Skin Stapler, Medtronic, Sweden). Thereafter a band aid was placed over the surgical wound. The surgery in the recipients lasted for 1¾ – 3¼ hours. The surgery procedure is described in details in a published master thesis by Zakariasson (2016).

3.5 Postoperative care

Towards the end of surgery isoflurane administration was discontinued, and the concentration of inspired oxygen was increased to 100%. Once the abdominal wall was closed, the pigs were weaned off the mechanical ventilator and spontaneous breathing was restored. After the animals were disconnected from the breathing system they were supported with oxygen and placed in an intensive care cage where they could easily be continuously monitored by the staff. The monitoring of RR, HR, SpO2 and BT continued until extubation. The pigs were extubated when they could breathe unassisted and the swallowing reflex had returned. The animals were transferred back to their pens and placed under a heating lamp and the monitoring of RR and HR continued until the pigs were fully awake after which they were monitored at least hourly for 12 hours.

After surgery, all recipients were given 0.01 mg/kg b.w. buprenorphine (Vetergesic® 0.3 mg/mL, Orion Pharma) IV, when needed, up to four times a day, enrofloxacin (Baytril® vet. 100 mg/mL, Bayer) 2.6 mg/kg b.w. IV, once daily and bensylpenicillinprokain (Penovet® vet. 300 mg/mL, Boehringer Ingelheim) 21 mg/kg b.w. IM, daily for two days.

Pain evaluation was performed by clinical examination, registration of changes in the normal posture (sitting position or lying) and behavioural characteristics (restlessness, isolation, appearance and gnashing) several times every day and the body weight was recorded three times a week.

3.6 Urine sampling and analyses

Midstream urine specimen was sampled if possible post-surgery with a paper kidney dish. Urine samples were analysed for *e.g.* urine specific gravity (USG), a biomarker of hydration, with a refractometer. Protein, blood and pH were measured using urine test strips (Chemstrip® Test Strips Roche Diagnostics, North America).

3.7 Ultrasound examination

To assess renal perfusion and urinary production, the urinary bladder was examined by ultrasound scan (Imago 1401MG05, ECM, France) once on the day before surgery and at 9 a.m. and 6 p.m. every day postoperatively. The ultrasound probe was placed over the skin and moved over the abdomen. To ensure the continuous contact between the probe and the skin and allow the probe to move smoothly, a lubricating gel was put onto the skin before examination. To succeed with the examination, the pigs were preferably lying down in a lateral position.

Two days after surgery the transplanted kidneys were examined by ultrasound (Logiq e R6, GE Healthcare, Wauwatosa, U.S.A.). The length, the echogenicity and the cortico-medullary definition were estimated and the blood supply in the kidney was evaluated. Additionally, the renal pelvic region was measured.

3.8 Blood sampling

Blood samples were collected from the venous catheter during surgery and once daily during the five postoperative days. Before sampling, the heparinized saline and 1 mL blood were withdrawn. Ten mL blood was withdrawn for analyses and the catheter was then flushed with 10 mL of saline followed by 1 mL of heparinized saline. If needed, the cap and the bandaid were changed. During the sampling procedure, all personnel used gloves and followed aseptically routines.

3.9 Euthanasia

The pigs were euthanized five days after surgery in the pens with an intravenous injection of 1.2 grams of pentobarbital sodium (Pentobarbital® vet. 100 mg/ml, Apoteksbolaget, Sweden) via the venous catheter. All pigs underwent a full *post mortem* examination including histopathology.

3.10 Statistics

Data is expressed as mean ± SD, or min - max. Groups were compared with nonparametric Mann-Whitney U test and p values ≤ 0.05 were considered statistically significant.

4 Results

4.1 Training during the acclimatization period

The general condition of the pigs was good during the acclimatization period. They were all eating and drinking and quickly adapted to the new environment. The first three days, when the pigs were left to settle, they tended to be nervous and afraid when the staff entered the stable. All moments in the four different training steps worked adequate in all pigs. After 14 days of social training, all pigs tolerated the moments included in the training (tab. 1). After surgery five out of six pigs were comfortable during manipulation with the catheter in the auricular vein. One pig continuously shook the head when blood samples were withdrawn from the catheter or if an injection was made. All six pigs were accustomed to ultrasound examination of the urinary bladder in the postoperative period.

Table 1. *Intervention and result of training in four steps of eight pigs during the acclimatization period before kidney transplantation*

	Step 1	Step 2	Step 3	Step 4
	Day 1-3	Day 4-5	Day 6-9	Day 10-14
Intervention by the trainer	-Cleaning of stables -Feeding the animals -Pigs left to settle down	-Trainer sits in the pen -Touch and brush pigs -Offer pieces of fruits -Trainer talks	-Training continuing according to step 2 -Ultrasound of abdomen -Sampling of urine	-Training continuing according to step 3 - Clinical examination -Extra training in one of the pigs
Result	All the pigs tended to be nervous and stressed when the staff enters the stable	On day 5 the trainer could touch six out of eight pigs	On day 9, seven pigs accept manipulation with the ears and use of ultrasound probe and sampling of urine	On day 14, all eight pigs were accustomed to all moments included in the training

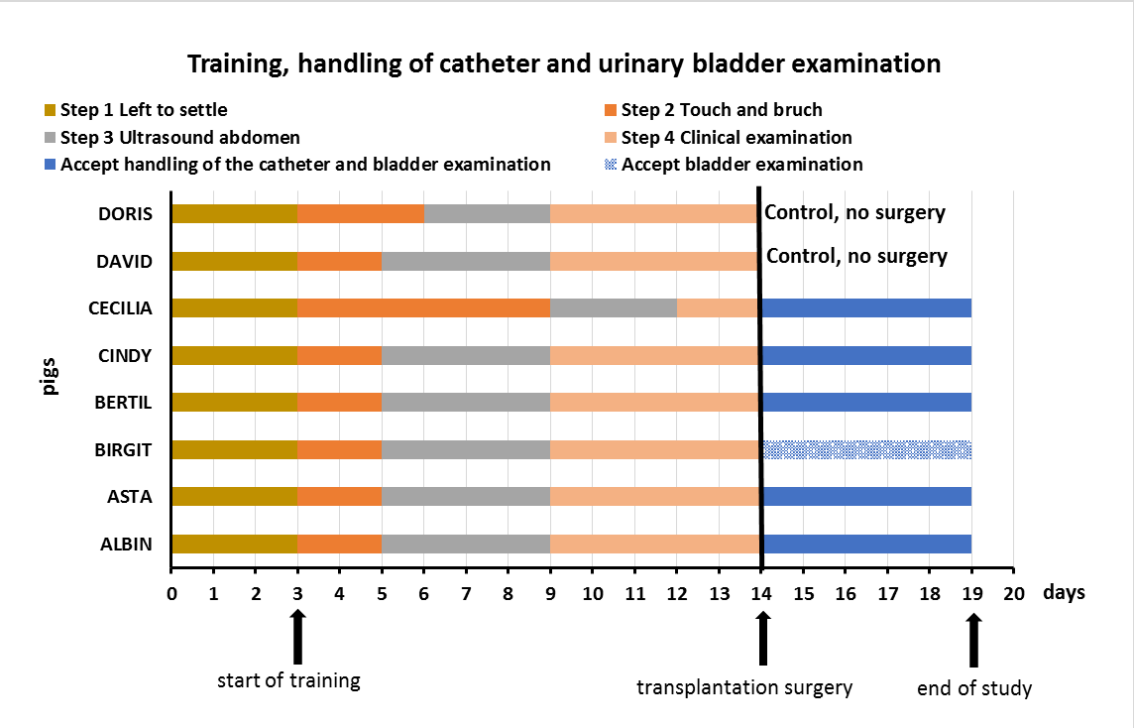

Figure 3. Individual result of training of eight pigs in four steps before kidney transplantation. For explanations see table 1.

4.2 Surgery and Anaesthesia

The chosen anaesthetic protocol was adequate for both donors and recipients. Fifteen minutes after reperfusion, the HR had increased from 119 ± 24 bpm (before perfusion) to 153 ± 19 bpm (p=0.01) (fig. 4). The blood pressure was kept within acceptable physiological limits and the other measured physiological parameters were stable throughout the surgery. After the transplantation was completed, urine could be collected from the bladder by cystocentesis, showing that all the transplanted kidneys had started to produce urine soon after reperfusion.

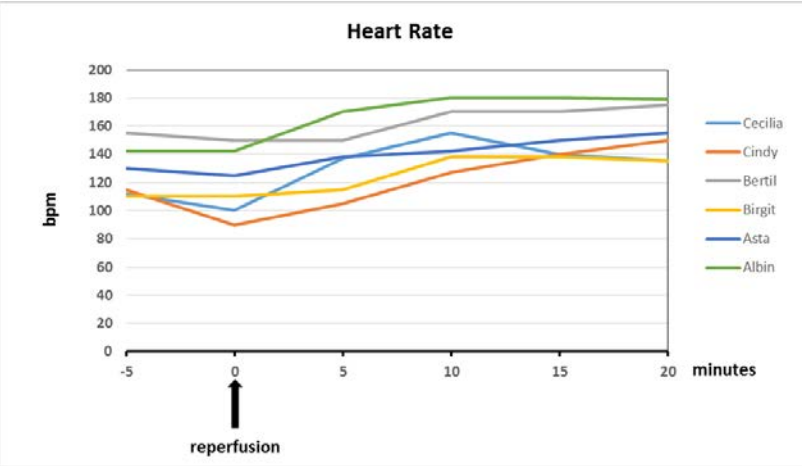

Figure 4. Individual heart rate before and after reperfusion of the graft in six pigs

4.3 Results postoperative period

The pigs were extubated 10 to 30 minutes after the end of surgery. One pig stopped breathing shortly after extubation and was intubated and ventilated manually until spontaneous breathing returned. This pig could later be successfully extubated. All the pigs were fully awake within 1 to 3 hours after extubation. The animals showed signs of pain the first 24 hours after surgery and analgesia was administered intravenously when needed. The day after surgery, paresis of the left hind leg was obvious in three pigs and the animals could not regain a standing position even when assisted by the staff. The pigs received massage over gluteus medius, biceps femoris and gluteus maximus and were supported to stand and walk several times during the day. They all recovered within 24 hours. There daily weight gain before surgery was (1.1 ± 0.1 kg) for recipients (n=6) and (1.0 ± 0.1 kg) for the controls (n=6). After surgery (5 days), the daily weight gain was (- 0.2 ± 0.4 kg) for recipients and (1.0 ± 0.0 kg) for the controls (n=2).

4.4 Ultrasound of the bladder

The ultrasound examination of the bladder and kidneys was successfully performed in all pigs. The pigs were quickly adapted to the procedure during training, and accepted the scanning after surgery without restrain or stress. Urine was detected in the bladder in four out of six pigs on the first and second day after surgery. Urine was detected in the bladder of all pigs during the remaining postoperative days (fig. 5).

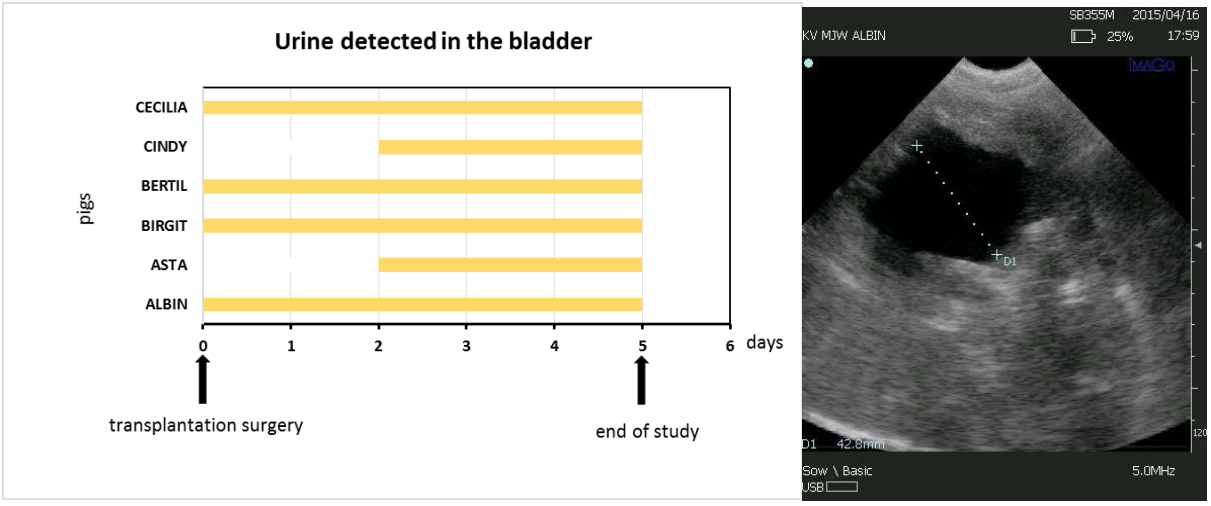

Figure 5. Individual result of ultrasound examination of the urinary bladder in six pigs. To the right, ultrasound scan of urinary bladder (Albin three days post-surgery)

4.5 Catheterization

In all six pigs, the Careflow catheter was successfully placed in Vena auricularis and advanced to an internal vein. The catheterization with the Seldinger technique was smooth and collection of blood samples functioned satisfactory during surgery. Postoperatively blood sampling and injections via catheter post-surgery were possible without restrain or stress. Injection of fluid through the Careflow catheter was possible during the whole study period in all pigs. In four out of six pigs it was possible to draw blood samples from the catheter until termination (5 days post-surgery). In the two remaining pigs, the catheter admitted blood sampling for 24 h but were only suitable for injections the following 4 days (fig. 6). None of the animals were found to have thrombophlebitis in the vessel on the post mortem examination

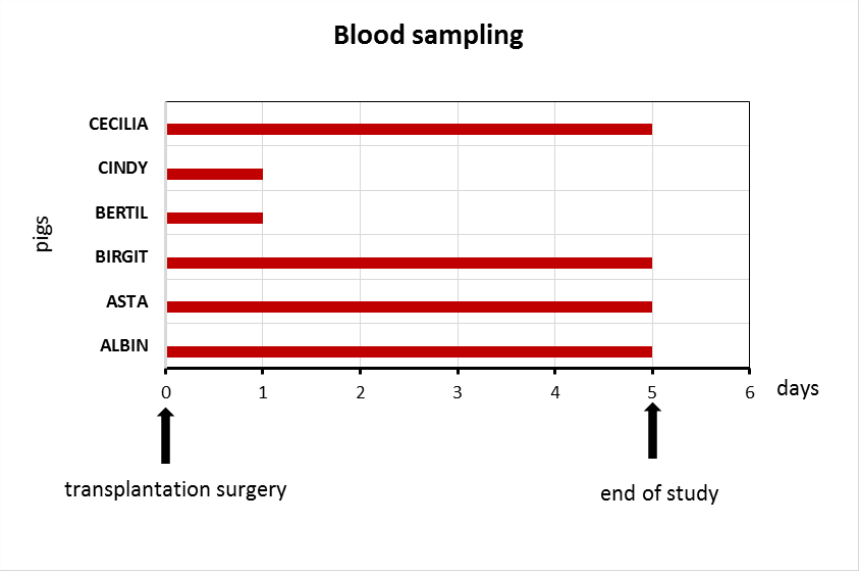

Figure 6. Individual result of possibility to withdraw blood from the Careflow catheter in six pigs

5 Discussion

The aim of the present study was to evaluate if preoperative training of pigs during an acclimatization period, will enable postoperative blood sampling and ultrasonic urine bladder examination without pain or restraint. The results show that a four-step training schedule for 14 days before surgery was sufficient for this purpose. Postoperatively, five out of six pigs were comfortable with all moments i.e. blood sampling, ultrasound examination and clinical examination, included in the training program. One pig was uncomfortable during blood sampling, but accepted all other interventions. Another objective was to evaluate if a central venous polyurethane catheter, introduced percutaneously from an ear vein by the Seldinger technique would make repeated blood sampling possible during five consecutive days postoperatively. The placement of the catheters was successful, and in four out of six pigs withdrawal of blood was possible throughout the whole study period. Even if collection was not possible in two pigs for more than two days, the catheters were suitable for injections in all pigs.

5.1 Training program and interventions in the postoperative period

The primary aim of the training was to make the pigs as comfortable at handling as possible and gradually accustom them to the necessary interventions during the postoperative period. The moments included in the four step training program were suggested in earlier published training studies on pigs (Gieling, Nordquist & van der Staay 2011). During the first three days after arrival the pigs were left to settle (step 1). This is based upon our previous experience that research animals need to get used to the new environment, individual housing and adaption to daily routines before start of training. The pigs were stressed and afraid the first days, but had become calmer when the training step 2 begun, indicating that they had adapted to the new settings. The training was successful in all pigs but two. These two pigs

needed more time to adapt to the moments included in step 2. This is in line with other studies on training of research pigs (Nicholls et al. 2012), in which it was suggested that touching and handling is the most important but also challenging part of the training. Although training has been popular when working with animals as pets, there are still few published standard protocol for training of pigs as research animals. In general, pigs are friendly and very adaptable and willing to cooperate if they are handled carefully without stress impact. Despite that, it has been described in studies that individual pigs differ (Kaiser et al. 2007; Nicholls et al. 2012; Swindle & Smith 2013), and some are more adaptable than others. However, in the present study, with prolonged training up to four days in two animals, these pigs became as tame as the rest of the group. After step 2, the pigs quickly adapted to the remaining interventions, scanning of the urinary bladder, clinical examination and sampling of midstream catch urine specimen.

It is known that pigs can recognize their handlers, have preferences, and benefit from environmental enrichment (Manteuffel, Langbein & Puppe 2009). Based on that information, our training schedule was allocated so that the five trainers had the same amount of time for socializing and training of the pigs. In addition, it is described by Nicholls et al (2012) that pigs were easier to handle by trainers that had spent more time with the animals. That result indicates that animals should preferably be socialized and trained by the researchers who will handle them in long-term survival studies.

In general the recommendation is to start the acclimatization period 3-14 days before the experiment (Obernier & Baldwin 2006). Even if all eight pigs in our study were adapted to all interventions before the transplantation surgery started, the time for training with the amount of interventions and number of animals, could not have been less. The result from the present study shows that a 14 day training period was necessary. This time also covers the incubation period of most infectious diseases in pigs in Sweden, which thereby can be detected before the experiments begin.

Several methods how to restrain pigs postoperatively are described in the literature. The most common method is the Panepinto sling (Lighty et al. 1992), or herd the animals against the cage walls (Swindle 2007b). Even if these techniques are presented as easy to perform and quickly accepted by the animals, the methods still need to be trained before the experimental procedure starts and is limited for pigs up to 50 kg. In the future, it would be interesting to compare the handling of pigs in a sling with the training protocol described in this study.

5.2 Interventions in the postoperative period

After surgery, the handling of the pigs was smooth. The examinations and handling were performed by the same staff that was involved in the training. There were no problems to do the clinical examinations, to measure the physiological parameters or inspect the operation wounds. Additionally, the scanning with the ultrasound probe for presence of urine in the bladder was easy to perform and none of the pigs seemed to be stressed during the procedures. If needed, the animals were offered pieces of fruit to keep them still during the procedures. One pig continuously shook the head when blood samples were withdrawn from the catheter or when saline was injected through the catheter. Even if the withdrawal of blood was possible and the pig did not show any symptoms of stress, the sampling was more time-consuming and the aseptic routines more challenging to comply with than in the other pigs. However in this particular pig, there was no signs of infections around the catheter site. Moreover, the preoperative training had been successful according to the protocol, and the other interventions included in the protocol were performed without problem postoperatively.

5.3 Anaesthesia

The chosen anaesthetic protocol was appropriate for the transplantation surgery. Isoflurane is a suitable choice as only 0.2% is metabolised in the body and may also have less effects on cardiac output and renal blood flow than other agents e.g. enflurane (Rabey 2001). The pigs received analgesics before the operation and opioids were administered if needed during the postoperative period. The increase of the pulse during the reperfusion of the graft is a known side effect of the actual surgical intervention (Rabey 2001). Hypotension and hypovolaemia should be avoided due to the risk of acute tubular necrosis of the grafted kidney (Rabey 2001). However in our study, the physiological changes could be stabilized with infusions of fluids, dobutamine and by adjustments of the inhalation agent concentration. The blood pressure was measured by an oscillometric non-invasive technique since invasive blood pressure measurements may have increased the risk of postoperative complications due to the cut-down techniques, manipulation and perforation of the vessel (Scheer, Perel & Pfeiffer 2002). It has been described in several studies that oscillometric measurements offer reliable prediction in comparison with invasive techniques in anaesthetized pigs (Chow et al. 1999; Ypsilantis, Didilis & Simopoulos 2013). Additionally it would have been beneficial to measure the central venous pressure, which is a suitable predictor of the hydration status. Hence the catheterization of the vena cava in pigs, is often performed during surgical procedures. In the current study additional surgery was avoided because of the

increased risk of pain and infections that could interfere with the outcome of the actual study. In other similar transplantation studies in pigs, it is common that muscle relaxants are used. Infusion with rocuronium and atracurium is reported to give an adequate muscle relaxation during surgery (Pehbock et al. 2015), and the excretion of the relaxant is independent of the kidney (Rabey 2001). However, no muscle relaxants agents were needed during the surgery performed in the present study.

5.4 Postoperative nursing

All the recipients survived general anaesthesia, transplantation surgery and the postoperative period and were euthanazied five days post-surgery. During recovery from anaesthesia, the pigs were observed and supervised until fully awake. One of the pigs stopped breathing shortly after extubation. This side effect has been described in pigs by Smith and Swindle (2008) and referred to the species tendency to suffer from partial airway obstruction during extubation. Fortunately, in our study, the postoperative unit was equipped with monitors, oxygen, tracheal tubes and laryngoscope so action could be taken immediately and the pig was re-intubated, ventilated with oxygen and monitored until spontaneous breathing returned. Due to the unique laryngeal anatomy in the pig, the endotracheal intubation can be challenging (Takala et al. 2004). Consequently, it is beneficial that the pre- and postoperative unit is prepared with equipment for acute tracheal intubation and tracheostomy. Interestingly, three out of six pigs showed paresis of the left hind leg the day after surgery. The pigs had problem to stand and even more difficulties to walk. This can be explained by the fact that they were laying in an unnatural position with the legs extended to facilitate the transplantation surgery. Another reason for the complication, can be that they were placed on the left side in the pen after surgery and the intra-compartmental pressure in the muscle might have been increased during the recovery period. Aitkenhead (2005) reported that peripheral nerve damage is usually the result of compression or stretching of the nerve, or exaggerated positioning for prolonged periods of anaesthesia time in humans. Both the anaesthetist and the surgeon should be aware of this potential complication and the patient should be moved on a regular basis if possible (Borgeat 2005). In our study, directly when the paresis was noticed, the staff started to massage the area and give support to standing and walking several times during the first 12 hours and the pigs recovered within 24 hours. Still, the reason for the paresis is only speculative and therefore remains unknown.

Before surgery all pigs increased in weight in line with pigs in conventional herds (Swindle 2007b). The controls continued to increase in weight at the same rate as

before, but the recipients decreased in weight post-surgery. Even if the recipients started to eat and drink 24 hours after surgery, the pre-surgical fasting and reduced appetite postoperatively together with the acquisition of energy for homeostasis could be the reason for the weight reduction. The present results indicate that nutrition and hydration maintained with fluids, electrolytes and glucose might be beneficial in the early postoperative period. The latency to begin to eat and drink, may be an indicator of postsurgical pain (Malavasi et al. 2005). However a quick return to homeostasis is crucial for recovery after transplantation surgery, because of the initiation of wound-healing (Short 1999).

5.5 Analgesia

Pain evaluation was based on physical examinations and behavioural indicators. Perceived pain in non-verbal patients cannot be directly measured, only inferred (Dobromylskyj et al. 2000) but a wide range of behavioral indicators, pain scales have been described in studies and in the literature. The use of pain scales could have been used in the present study to evaluate the pain in a more structured and objective way. The animals were given morphine epidurally before surgery and buprenorphine 0.01 mg/kg every three to six hours during the first 24 hours post-surgery, based on the subjective assessment of pain by the staff. Buprenorphine, a partial μ–agonist, is frequently used for perioperative analgesia in pigs because of its relatively long half-life. In one study, Hermansen et al. (1986) investigate the assessment of the analgesic action on thermal and mechanical noxious stimuli on pigs treated with buprenorphine. The result of their study showed that the duration of action was between seven and 24 h (Hermansen, Pedersen & Olesen 1986). However, in our study pain assessment of in the pigs resulted in more frequent injections than that. Today, buprenorphine is available in two long-acting formulations, one subcutaneous injectable sustained-release buprenorphine and another as a transdermal buprenorphine patch. Pharmacokinetic studies on Göttingen minipigs with these two formulations have been performed. The authors found that the therapeutic concentration lasted for 264 and 72 h respectively (Thiede et al. 2014). Nevertheless, in another study from 2005 on domestic pigs, the authors declare that transdermal delivery of buprenorphine, 50 μg/h, caused a large variation in serum fentanyl concentration of the animals, indicating that drug absorption from transdermal patches is unpredictable and sometimes lacking in pigs (Malavasi et al. 2005). Additionally, the maximal effect of morphine administered epidurally is expected to last for 12–24 h (Nolan 2001). However, pigs differ in their response, and administration of systemic analgesics without

continuous evaluation of the individual pain score is not recommended. In future transplantation studies, refinement of the analgesic treatment needs to be addressed.

5.6 Detection of urine in the bladder

In humans who have undergone kidney transplantation surgery, diuresis usually begins soon after renal transplantation. Although the urine volume shows considerable variation early after renal transplantation, the urine volume may correlate with favourable short- and long-term allograft survival (Khosroshahi et al. 2007). The information confirms the importance to detect and measure the urine volume in experimental studies on kidney transplantation. None-invasive techniques to measure urine production in pigs are limited. Even if catheterization of the bladder is possible in female pigs, it is impossible in male pigs as they have many folds in their urethra (Golriz et al. 2012). There is also an increased risk of infections in the urinary tract and problems to fixate the catheters and the urine collection bags to the animal postoperatively. Thus, we chose to not catheterize the pigs in the present study. Metabolic pens and special methods have been described (Kurien, Everds & Scofield 2004), but none of these methods were suitable for measuring the amount of urine in this transplantation study. There are no studies published describing the use of ultrasound examination of the urinary bladder after renal transplantation surgery in pigs.

In our study, the ultrasound examination of the bladder was very valuable. In four out of six pigs, urine was detected in the bladder from the first day postoperatively and every day until the end of the study. In two pigs, there was no detectable urine in the bladder during the first two days after transplantation surgery which could be due either to lack of detection or the fact that no urine was produced or that the urine had just been voided. The last option was highly impossible, because urine could not be detected in the pen. In our study, the amount of urine was not estimated and the aim of the study was only to evaluate if the ultrasound examinations was possible on pigs after renal transplantation. Still it would be interesting in future studies to measure the volume of urine in the bladder with ultrasound technique.

5.7 Catheterization in the auricular vein

Repeated blood samples for measurement of biomarkers such as urea nitrogen and serum creatinine is essential for evaluation of how the kidneys are functioning following renal transplantation (Liu et al. 2006). In our study, the placement of the polyurethan vascular catheters with the Seldinger technique in the auricular vein

was successful in all six pigs (Seldinger 2008). The procedure has been reported previously, is easy to perform, no surgery is needed, and is therefore associated with a lower risk of infection, bleeding and placement complications (McBride et al. 1997). In all six pigs, the identification ear mark was placed on the right earflap closed to the auricular vein, which made it challenging to place the catheter in that ear. Pigs often have permanent identification in the ear for husbandry, management and research purposes (Widowski and Torrey 2002). Several methods are available such as ear tattoos or smaller ear tags placed in a more proper way to make catheterization possible. Another method for marking which does not interfere with possibilities to venous catheterization will be considered in future studies.

In two out of four pigs, the catheter could not be used for withdrawal of blood for more than one day post-surgery. In a study by Niiyama et al (1985), catheters were placed in the auricular vein in 20 kg piglets. These catheters were 1.35 mm OD, they claimed to be too narrow for blood sampling. The size of the catheter in our study was even smaller (3 Fr, 1 mm OD) and yet we could withdraw blood in four out of six pigs. However, if possible in future studies we will use a catheter with a larger diameter if blood sampling is necessary in the postoperative period. In the remaining four pigs, the blood sampling was smooth and injection in the catheters was possible in all six pigs during the whole study period. The technique and polyurethane catheter used proved to be suitable since no signs of thrombophlebitis was seen in the pigs on necropsy. Experimental studies have demonstrated that pigs are hypercoagulable compared to humans (Karges, Funk & Ronneberger 1994). In a study from 2002, the outcome of heparin coated and uncoated catheters placed in a rat model for 30 days were compared (Foley, Barthel & Brausa 2002). In that study the presence of e.g. intraluminal fibrous sheaths and organized thrombi were highly associated with uncoated catheters. Further it has been found that catheters with sharp or rough edges can damage the endothelium and increase the thrombogenicity (Hecker 1981). With that in mind we can show that the anticoagulant therapy, the catheter with round tip shape and the aseptic maintenance of the catheters were appropriate in the present study. Another practical implication was that the blood sampling was more efficiently carried out by two persons. When the procedure with blood sampling was made by a single person, it turned out to be necessary to have all equipment prepared and accessible, so that once the animal was still, the sampling of blood could be performed in an aseptic course of action.

In conclusion, a training period of two weeks was sufficient to establish a protocol which enables blood sampling and ultrasound examination of the urinary bladder on pigs without stress for neither the pigs nor trainers. The result also show that placement of a catheter with the Seldinger technique in the auricular vein was

successful and withdrawal of blood was possible in four out of six pigs for five days post-surgery

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