Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2021:83
Faculty of Veterinary Medicine and Animal Science

Nursing and anaesthesia care of growing pigs

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SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS
Uppsala 2021

Acta Universitatis Agriculturae Sueciae
2021:83

Cover: Blood sampling
(photo: Mari Wallbring)

ISSN 1652-6880
ISBN (print version) 978-91-7760-843-1
ISBN (electronic version) 978-91-7760-844-8

Uppsala
Print: SLU Service/Repro, Uppsala 2021

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Abstract

The overall aim of the present thesis was to improve the welfare of animals in research by refining the perioperative nursing and anaesthesia care of growing pigs in accordance with the 3Rs, replace, reduce and refine.

Forty-six pigs were trained during a 14-day acclimatisation period to accept blood and urine sampling and ultrasound examination. A polymer coated catheter for repeated blood sampling from the jugular vein was assessed. The effects of zolazepam-tiletamine and medetomidine (ZTMe) was compared with midazolam, ketamine and fentanyl (MiKF) regarding the quality of induction and physiological variables in a subsequent total intravenous anaesthesia (TIVA) with MiKF. A combination of zolazepam-tiletamine, dexmedetomidine and butorphanol (ZTDeB) intended for short-term anaesthesia was evaluated and physiological responses and drug plasma concentrations were examined. An artificial intelligence (AI) technology based on image vision was adapted for monitoring of the activity prior to anaesthesia and post-anaesthesia during treatment with transdermal fentanyl or buprenorphine injections. Facial expression was scored and plasma concentrations of the drugs were analysed.

The training enabled blood and urine sampling and ultrasound examination without restraints. It was possible to collect blood from the catheters for up to ten days. ZTMe had better results than MiKF in areas such as shorter induction time, better intubation scoring results and less adjustment and amount of TIVA required for up to six hours of TIVA. ZTDeB provided two hours of anaesthesia with stable physiological variables and spontaneous breathing. The plasma concentration profile of the drugs was in line with the duration of the effect. Measurement of activity in pigs with the AI technique was encouraging. Both opioids, at the doses used, resulted in plasma concentrations above the suggested therapeutic levels. Assessment of facial expressions was time consuming and several factors influenced the result.

In summary, the results showed that nursing interventions, adjustment of anaesthesia techniques and the use of AI technology for the measurement of activity can contribute to stress-free handling and improved animal welfare in growing pigs according to the 3Rs.

Keywords: 3Rs, animal welfare, acclimatisation, training, plasma concentration, opioid, artificial intelligence, facial expression, blood sampling, research animals

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Omvårdnad av växande grisar i samband med anestesi

Sammanfattning

Det övergripande syftet med denna avhandling var att förbättra djurens välbefinnande i forskning genom att förfina den perioperativa omvårdnaden och anestesivården för växande grisar i enlighet med 3R (ersätta, minska och förfina).

Fyrtiosex grisar tränades under en 14-dagars acklimatiseringsperiod för att acceptera blod- och urinprovstagning och ultraljudsundersökning. En polymerbelagd kateter för upprepad blodprovstagning från jugularvenen utvärderades. Effekterna av zolazepam-tiletamin och medetomidin (ZTMe) jämfördes med midazolam, ketamin och fentanyl (MiKF) avseende kvaliteten på induktion och fysiologiska variabler i en efterföljande totalintravenös anestesi (TIVA). En kombination av zolazepam-tiletamin, dexmedetomidin och butorfanol (ZTDeB) avsedd för kortvarig anestesi utvärderades avseende fysiologiska svar och koncentrationer av läkemedel i plasma. En teknik för artificiell intelligens (AI) baserad på bildigenkänning anpassades för att övervaka aktiviteten hos djuren före och efter anestesi och under behandling med buprenorfin eller transdermal fentanyl. Grisens ansiktsuttryck registrerades och plasmakoncentrationer av läkemedlen analyserades.

Träningen möjliggjorde blod- och urinprovstagning och ultraljudsundersökning då grisarna var lösa i boxen. Det var möjligt att samla blod från katetrarna i upp till tio dagar. ZTMe hade bättre resultat än MiKF avseende kortare induktionstid, lättare intubering, färre justeringar och lägre unerhållsdos av TIVA krävdes. ZTDeB gav två timmars anestesi med stabil andning och tillfredställande fysiologiska variabler. Plasmakoncentrationen för läkemedlen var i linje med effektens varaktighet. Mätning av aktivitet hos grisar med AI-tekniken var lovande. Båda opioiderna, i aktuella doser, resulterade i plasmakoncentrationer över de föreslagna terapeutiska nivåerna. Bedömning av grisarnas ansiktsuttryck var tidskrävande och flera faktorer påverkade resultatet.

Sammanfattningsvis visade resultaten att omvårdnadsinsatser, anpassning av anestesitekniker och användning av AI-teknik för mätning av aktivitet kan bidra till stressfri hantering och förbättrad djurvälfärd hos växande grisar enligt 3R.

Nyckelord: 3R, djurskydd, acklimatisering, träning, plasma koncentration, opioid, artificiell intelligens, ansiktsuttryck, blodprov, forskningsdjur

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Dedication

To my family

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Rydén A*, Manell E*, Biglarnia A, Hedenqvist P, Strandberg G, Ley C, Hansson K, Nyman G, Jensen-Waern M. (2020) Nursing and training of pigs used in renal transplantation studies. Laboratory Animals 54(5), pp 469-478.

II. Rydén A, Fisichella S, Perchiazzi G, Nyman G. (2021) Comparison of two injectable anaesthetic techniques on induction and subsequent anaesthesia in pigs. Laboratory Animals https://doi.org/-10.1177/00236772211029810

III. Rydén A, Jensen-Waern M, Nyman G, Olsén L. (2021) Physiological and clinical responses in pigs in relation to plasma concentrations during anesthesia with dexmedetomidine, tiletamine, zolazepam, and butorphanol. Animals 11(6):1482.

IV. Rydén A, Olsén L, Marntell S, Jensen-Waern M, Nyman G. Evaluation of an AI technique for objective assessment of activity in research pigs after two different analgesic regimens; a pilot study. (*manuscript*)

Papers I-III are reproduced with the permission of the publishers.

*Shared first author

The contribution of Anneli Rydén to the papers included in this thesis was as follows

I. Study design, data collection, analysis and interpretation, manuscript preparation, critical revision of the manuscript

II. Study design, data collection, analysis and interpretation, manuscript preparation, critical revision of the manuscript

III. Study design, data collection, analysis and interpretation, manuscript preparation, critical revision of the manuscript

IV. Study design, data collection, analysis and interpretation, manuscript preparation, critical revision of the manuscript

Abbreviations

3Rs	Replace, reduce, refine
AI	Artificial intelligence
ANOVA	Analysis of variance
AUC	Area under the plasma concentration
BID	Bis in die (twice a day)
CI	Cardiac index
C_{max}	Maximal concentration in plasma
C_{min}	Minimum concentration in plasma
C_{trough}	Trough concentration in plasma
CO	Cardiac output
CRI	Constant rate infusion
$ETCO_2$	End-tidal carbon dioxide concentration
FAU	Facial action units
F_IO_2	Inspired oxygen fraction
HR	Heart rate
IM	Intramuscular
IV	Intravenous
MAP	Mean arterial blood pressure
MiKF	Midazolam, ketamine and fentanyl

MPC	2-methacryloyloxyethyl phosphorylcholine
NC3Rs	National Centre for the Replacement Refinement & reduction of Animals in Research
MRI	Magnetic resonance imaging
NMB	Neuromuscular blocking
NRC	National Research Council
$PaCO_2$	Arterial carbon dioxide tension
PaO_2	Arterial oxygen tension
MPAP	Mean pulmonary artery blood pressure
PGS	Pig grimace scale
RR	Respiratory rate
SaO_2	Arterial oxygen saturation
SD	Standard deviation
SLU	Swedish University of Agricultural Sciences
SpO_2	Oxygen saturation measured by pulse oximetry
$T_{1/2}$	Terminal half-life
TIVA	Total intravenous anaesthesia
t_{max}	Time to reach C_{max}
TZ	Tiletamin and zolazepam
ZTDeB	Zolazepam, tiletamine, dexmedetomidine and butorphanol
ZTDe	Zolazepam, tiletamine and dexmedetomidine
ZTMe	Zolazepam, tiletamine and medetomidine

1. Introduction

Animals are used in a variety of scientific disciplines and continue to aid our understanding of various diseases and the development of new medicines and treatments in both humans and animals (Festing & Wilkinson 2007). The welfare of animals used in research is very important. There are good ethical, scientific, legal and economic reasons for making sure that animals are treated properly and used in minimum numbers. The principles of the 3Rs (Replacement, Reduction and Refinement) were developed 60 years ago to provide a framework for performing more humane animal research (Russell & Burch 1959). Since then, the 3Rs have been embedded in national and international legislation and regulations regarding the use of animals in scientific procedures. The EU directive 2010/63/EU, is the legislation designed for the protection of animals used for scientific purposes and is firmly based on the principle of the 3Rs. Animal studies, whether for new medicines, meat production, education or new food additives must be performed in compliance with EU legislation (EU, 2010).

Although animals cannot be completely replaced, it is important that researchers maximize methods for reduction and refinement when using animals in experimental studies (Festing & Wilkinson 2007). Good animal welfare and good science go hand in hand. If an animal is exposed to stress or pain it causes not only suffering for the animal, but it can also possibly affect the results of the research (Bailey 2018).

Veterinary nurses play an important role in the care of the animals and are obvious members of the research team. The duties of the professional veterinary nurse involved in research with animals are commonly adapted to certain areas such as husbandry, anaesthetics, technical advice, ethical principles as well as the practical handling of animals. To be able to develop the nursing care given the animals, whether it is in clinical practice or in

research, there is a need for evidence-based knowledge (Clarke 2012). If evidence-based guidelines are missing in either veterinary medicine or in veterinary nursing research, it could in the end lead to a lower quality of care for the animal. Therefore, the overall aim of the thesis was to refine perioperative nursing and handling of growing pigs in experimental studies requiring anaesthesia care.

2. Background

2.1 The pig as a research animal model

The pig has become an increasingly requested model in biomedical research, due in large part to its physiological and anatomical similarities to humans (Spurlock & Gabler 2008; Swindle *et al.* 2012; Dalgaard 2015; Huppertz *et al.* 2015). For example, the renal system of the pig is more similar to that of humans than most of the other animal species (Dyce *et al.* 2010). The size of the pigs makes them suitable for the development of surgical techniques requiring the dimensions of a human being (Fruhauf *et al.* 2004; Swindle 2007b). In addition, studies on imaging techniques using pigs can be readily used in humans (Alstrup & Winterdahl 2009). Pigs have a short reproduction cycle and produce large litters compared to many other large animals, which could decrease the interindividual variance between study objects. Despite all the potential benefits, many species specific concerns, both biological and ethical in nature, remain to be addressed (Gutierrez *et al.* 2015).

Purebred domestic pigs are exclusively found in breeding herds, consequently pigs for laboratories will most likely be of mixed breed, *e.g.* Landrace, Yorkshire and Hampshire (Bollen *et al.* 2010). Domestic pigs can have a growth rate of 0.5 kg day^{-1} at 12–22 weeks of age. Their rapid growth rate limits their usefulness in, *i.e.* survival and long-term studies (Smith & Swindle 2006). For that reason, minipigs with a growth rate of 0.5 kg $week^{-1}$ are more commonly used in such studies.

Experimental studies at our Faculty of Veterinary medicine and Animal science are carried out on pigs to study the functions and diseases of this species as well as the outcomes of surgical interventions. These studies are

conducted in collaboration with medical personnel, *e.g*. physicians and pharmacologists. In these studies, domestic growing pigs, 25–45 kg are used since their size makes them suitable to handle, and since they are prepuberal, the boars have not developed problematic sexual maturity behaviours (Fredriksen *et al.* 2009). In addition, the breeds and size of pigs we use are common in research laboratories. For these reasons, the animals included in the present thesis are exclusively domestic growing pigs. Even though there are scientific facts regarding the anatomy and physiology of growing pigs, important information is lacking regarding nursing measures that can improve and simplify the interactions with the animals and increase animal welfare during experimental situations, which is highlighted in this thesis.

2.2 Housing

It is desirable to house the pigs in small compatible groups in the laboratory; preferably from the same herd. However, pigs living in group housing may be contraindicated postoperatively for some protocols because of their propensity for increased aggression (Smith & Swindle 2006) and their tendency to bite at the incisions of cage mates (Swindle 2007a). Although not desirable, individual housing is common in survival experiments. When the pigs are housed individually, adequate socialisation can be met if they are allowed to see, hear and smell other pigs in the stable (Swindle 2007a). Laboratory pigs spend 70–80% of the day lying down or sleeping, except when it is feeding time or people are working in the stable (Smith & Swindle 2006). During the times they are active, the natural behaviours of pigs include almost uninterrupted grubbing, gnawing, rooting, chewing and foraging activities (Mkwanazi *et al.* 2019). Before puberty, the piglets also play with one another (Malavasi 2005).

2.3 Acclimatisation and training

To allow the animals to recover from the stress of transport and to acclimate to different husbandry conditions, an acclimatisation quarantine of two weeks is recommended before the start of a research project (Obernier & Baldwin 2006; Smith & Swindle 2006). During this period it is possible to examine the pigs for, *e.g*. infectious diseases (Clary *et al.* 2002), or as described in Study I, perform ultrasound examinations of organs. To

minimize fearful responses from the pigs, it is beneficial if all interactions and procedures with the animals are carried out by trained professionals (McGlone 2001; Damy *et al.* 2010). Pigs are intelligent animals with excellent memories of both bad and good experiences (Kaiser *et al.* 2006). Gentle handling procedures instead of forceful techniques will allow them to be petted and bond with the handlers (Smith & Swindle 2006).

It is an advantage if there is no need to restrain the animals. However if needed, pigs can be trained to voluntarily enter a restraining device if the method used does not involve pain or discomfort. Several methods to restrain pigs have been described and are used in numerous research laboratories. Small pigs can be handled in the staff member's arms, or placed in sling devices where the treatment and measuring can be performed (Panepinto *et al.* 1983; Lighty *et al.* 1992; Swindle 2007a) and larger animals can be herded against the cage with handheld panels (Swindle 2007a).

In a study by Nicholls *et al.* (2012), the effect of preoperative visits by project personnel on the compliance of 26 miniature pigs was examined. In the study, preoperative interaction variables and preoperative socialization measures were positively correlated with postoperative outcomes. The group of pigs that received more time with the personnel preoperatively had higher socialization scores, were associated with less stress and were easier to administer medication to postoperatively when compared to the control animals (Nicholls *et al.* 2012). Stress has been shown to affect immune responses (Dalin *et al.* 1993; Einarsson *et al.* 2008; Lee *et al.* 2016), and it can affect the outcomes of an experiment (Smith & Swindle 2006). Even though pigs are easily trained (Swindle 2007a), information concerning the best practices to prepare pigs for specific studies is limited.

2.4 Anaesthesia

Since pigs are easily stressed and not accustomed to being handled, it is often necessary to anaesthetise them for such things as diagnostic procedures, imaging, minor surgical procedures, catheterisation and during transport. Anaesthesia techniques that safeguard the animal's welfare in medical investigations are essential (Swindle 2007a; Geovanini *et al.* 2008). Consequently, it is desirable if the anaesthetic protocols are selected on the basis of the condition of the animal, the planned surgical procedure and the physiologic effects of the anaesthetic agents (McGlone 2001). Furthermore,

the anaesthetic protocol should provide fast and reliable immobilization, adequate analgesia and sufficient muscle relaxation without cardiovascular and respiratory depression (De Monte *et al.* 2015). The effects of anaesthesia and analgesic drugs differ between species (Lemke 2007) and can cause various physiological changes thus possibly affecting the outcome and results of the research (Alstrup & Smith 2013).

2.4.1 Induction

Pigs get stressed easily during preoperative handling, which can interfere with the onset of action of the anaesthetic drugs targeting the central nervous system (Grandin 1986). Therefore, the primary objective is to provide a quiet environment without anxiety and stress that is preferably in the pigs pens when anaesthesia is induced (Grandin 1986; Smith & Swindle 2006).
Since venous cannulation can be stressful for the animal, and the availability of superficial veins for drug injection is limited to the fragile ear veins, it is desirable to administer anaesthetic drugs by a single intramuscular (IM) injection in the muscles on the sides of the neck or behind the ears in small swine (Swindle 2007a). In addition, to reduce the discomfort for the animal, it is beneficial if the injection of the drug is rapid (National Research Council Committee on recognition & Alleviation of Distress in Laboratory 2009). A common induction protocol for pigs described in the literature, and used in our research laboratory, is a combination of ketamine and midazolam given IM followed by an intravenous (IV) injection of an opioid before intubation (Boschert *et al.* 1996; Swindle 2007a). In addition, it has been previously reported in a similar breed and age group of pigs that the induction of anaesthesia using an α_2-agonist in combination with zolazepam-tiletamine administered IM can produce a reliable and rapid induction (Henrikson *et al.* 1995; Malavasi 2005). However, the effects of the two different drug combinations on the induction and the physiological variables during subsequent anaesthesia are important to know.

2.4.2 Intubation

Endotracheal intubation is strongly recommended during general anaesthesia in pigs (Bollen *et al.* 2010) since it maintains a patent airway, permits assisted ventilation and protects the airways from aspirates (Ettrup *et al.* 2011). The pig may be intubated in dorsal, lateral or sternal recumbency

facilitated with a standard laryngoscope with straight blades (McGlone 2001; Kaiser *et al.* 2006; Swindle *et al.* 2012). Considering the laryngeal passage is narrow, and the swallowing reflex is strong, it is advantageous if salivation is reduced and the laryngeal reflexes are eliminated before the start of the procedure (Lemke 2007)**.** If the pig is not intubated on the first attempt, repeated attempts become more difficult since pigs are susceptible to laryngospasm and oedema of the larynx mucosa (Oshodi *et al.* 2011). Therefore, the animal should be at a sufficient anaesthetic level to facilitate an appropriate intubation (Swindle 2007a). Several complications associated with endotracheal intubation (Oshodi *et al.* 2011; Steinbacher *et al.* 2012; Tonge & Robson 2021) and extubation (Lin 2014) have been reported in pigs. However, few studies have examined if there is a possibility to maintain ventilation and oxygenation during spontaneous breathing in non-endotracheally intubated pigs.

2.4.3 General anaesthesia

To maximize the volume of the data collected from the pigs, maintenance of the anaesthesia is sometimes required for a long time. Maintaining a stable physiological state minimizes data variability and increases study power, which will contribute to the need for a lesser number of animals in a given project (Clutton *et al.* 2013). Maintaining physiological variables within appropriate limits is particularly important for recovery experiments in which deviations from normality may compromise the pig's convalescence rate and welfare (Smith & Swindle 1994; Swindle 2007a). Volatile anaesthetics are commonly used in research settings. They provide a better control of the anaesthesia depth and have a reduced recovery time over many of the injectable agents (Swindle 2007a).

Total intravenous anaesthesia (TIVA) with injectable anaesthetic agents is encouraged as an adjunct to volatile anaesthetics and may be required for some experimental procedures that do not allow the use of inhalants (Lundeen *et al.* 1983; Swindle 2007a).

Dissociative anaesthetic agents such as ketamine, are the most common injectable anaesthetic agents utilized in pigs. However, ketamine does not provide good visceral analgesia or muscle relaxation and is therefore usually used together with opioids (Benson & Thurmon 1979; Smith *et al.* 1997; Swindle 2007a). Benzodiazepines are also added to this anaesthetic combination to provide adequate muscle relaxation (Swindle 2007a).

Regardless of the need for maintenance anaesthesia, the anaesthesia may be induced with an injectable anaesthetic combination, after which the pigs are endotracheally intubated and maintained with gas anaesthesia or TIVA for prolonged periods. However, induction drugs can alter the physiological variables and the requirement for subsequent anaesthesia. This is important to consider because it can possibly affect the outcome and results of the research (Clutton *et al.* 1997; Alstrup & Smith 2013).

2.4.4 Short-term anaesthesia

In pigs, short-term anaesthesia is often required to avoid stress and the need to restrain them during short procedures such as transport, imaging, examinations, specimen sampling and minor surgical procedures (Heinonen *et al.* 2009). The challenge is to identify an anaesthetic technique that, without the use of sophisticated equipment, *e.g.* infusion pumps and ventilators ensures an acceptable depth and length of anaesthesia, a regular spontaneous breathing pattern, a stable hemodynamic condition for the animal and a satisfactory recovery phase. In addition, the possibility of prolonging anaesthesia without compromising the animal's safety must be considered (Albrecht *et al.* 2014). Anaesthesia with various combinations of α_2-agonists, dissociative anaesthetics, benzodiazepines and opioids have been suggested as induction agents prior to general anaesthesia or for use as short-term anaesthesia (Henrikson *et al.* 1995; Sakaguchi *et al.* 1996; Malavasi *et al.* 2008; Heinonen *et al.* 2009; De Monte *et al.* 2015). However, when drug combinations are administered, drug interactions may occur and repeated administrations of the anaesthetics may cause physiological changes, which can also influence the outcome of the results (Albrecht *et al.* 2014). For different experimental procedures in pigs, a short-term stable anaesthesia is valuable. Yet, there is limited information in the literature.

2.5 Nociceptive testing

Pain is a complex sensory experience normally generated by the activation of nociceptors (Craig 2003). Nociception represents the peripheral and central nervous systems' processing of information about the internal or external environment that is generated by nociceptor activation (National Research Council Committee on Recognition & Alleviation of Pain in Laboratory 2009). An evaluation of the severity of pain is particularly

important when making decisions during anaesthesia. Mechanical nocistimulation has been shown to be applicable to pigs (Jaber *et al.* 2015). In some studies, a method to ensure an appropriate depth of anaesthesia has been an observed depression of the somatic reflex responses after mechanically clamping the dewclaw (Haga *et al.* 2011; Jaber *et al.* 2015). That method has been compared with other methods such as electrical stimulations that are evaluated with bispectral index and electroencephalography (Jaber *et al.* 2015; Lervik *et al.* 2018). The conclusions in these studies suggest that clamping the dewclaw is acceptable for assessing nociception in anaesthetised pigs. Mechanically clamping the dewclaw is routinely used to evaluate nociception in pigs in our research laboratory, and therefore it was used in Study II and Study III.

2.6 Sampling

2.6.1 Blood

Intravascular access for injection and blood sampling is one of the most common experimental surgical procedures performed on laboratory animals (Swindle *et al.* 2005; Trim & Braun 2011). The process may be unnecessarily stressful due to the handling and the restraining and discomfort encountered during painful sampling techniques. The physiological changes and release of the endogenous hormones insulin, glucagon, catecholamines and cortisol associated with increased stress can even invalidate the research result (Brenner & Gurtler 1981; Rushen *et al.* 1993; Waern & Fossum 1993; Cooper 2007). If repeated blood samples need to be collected over a prolonged period of time, it is less stressful for both the animal and handler to use surgically implanted indwelling catheters or vascular access ports (Cooper 2007). There are some studies made with catheters surgically implanted in the external jugular vein, tunnelled through the subcutaneous tissue and then exteriorised on the back between the scapulae (Harris 1974; Lombardo *et al.* 2010; Manell *et al.* 2014). In those studies, the outcome of the technique has shown to be suitable for experimental animals even though some of the pigs had minor complications. The auricular vein is suitable for intravenous administration of drugs or collection of small volumes of blood. These veins are prone to form hematomas that complicate repeated punctures (Seldinger 2008). Nonsurgical techniques in which catheters have been

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 four hours (Shearer & Neal 1972; Zanella & Mendl 1992) and in other studies they remained for more than 48 hours (McGuill & Rowan 1989; Porter *et al.* 1992; Phillips *et al.* 2012; Pairis-Garcia *et al.* 2014). In those studies, the catheterisation was easy to perform and collection of blood samples worked adequately in pigs weighing 90–283 kg. However, in one study on 20 kg piglets, the catheterisation was smooth, but the lumen of the cardiac catheter used was too narrow to use for collecting blood samples (Niiyama *et al.* 1985).

The Seldinger technique is specifically designed to introduce catheters (Seldinger 2008). Under aseptic conditions the vessel is located and then punctured with a sharp hollow needle. A guidewire 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. In pigs thrombotic occasions are problematic, particularly when catheters are left *in situ* for a long period (Jacobson 1998).Therefore, as a general recommendation, the catheter is flushed with saline and then filled with 10% heparinized saline every time it is accessed or two times per week when not used (Jurewitsch & Jeejeebhoy 2005). Despite the treatment with heparin, a fibrin sleeve formation at the catheter tip can cause obstruction, and it is the most common cause of thrombotic obstruction in catheters placed in humans (Baskin *et al.* 2009).

In humans a 2-methacryloyloxyethyl phosphorylcholine (MPC) based polymer has been successfully used for coating medical devices and preventing plasma proteins and blood cells from interacting with surface material (Ishihara 2012). In addition, MPC coated catheters have been evaluated *in vitro* (Asif *et al.* 2019) and *in vivo* for 48 hours with promising results.

2.6.2 Urine

An accurate monitoring of the pigs' urine output is central during prolonged anaesthesia and during the postoperative period. It is especially valuable for experiments evaluating drug metabolism, nutritional protocols and urological surgeries (Holliman *et al.* 1982; Kurien *et al.* 2004). In male pigs, urethral catheterization of the urinary bladder through the penis is difficult as the tip of the penis is shaped like a cork-screw (Swindle *et al.* 2012). The female urethra can be catheterized conventionally as in other female

mammals (Swindle 1983). In a short report by Musk *et al*. (2015), a Foley catheter was inserted in 16 female pigs, and then 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 minutes in some pigs (Musk *et al.* 2015).

Insertion of an urinary catheter may carry urethral microorganisms into the bladder (Daifuku & Stamm 1984). In humans, nosocomial urinary tract infections are the most common infection acquired in hospitals and nursing homes, and are usually associated with urinary bladder catheterization (Warren 2001). Other systems used for the collection of urine in large animals are, *e.g.* metabolic pens (Moughan *et al.* 1987; Ivers & Veum 2012) suprapubic catheterization (Holliman *et al.* 1982) and an external apparatus affixed to the animals (Paulson & Cottrell 1984). Even though there are proper ways to measure urine output, it has been reported that these methods sometimes cause bladder infections and animal discomfort (Aschbacher 1970). Additionally, when the modified equipment fits poorly, and the animals move around, a failure to collect urine can occur (Aschbacher 1970).

To facilitate the recognition of urinary retention in humans, a 3D portable ultrasound device (BladderScan®) using automated technology to provide bladder volume has been developed and is part of routine care. The product has been evaluated and the results recommend the device as an alternative to catheterization (Al-Shaikh *et al.* 2009; Thanagumtorn 2016). Nevertheless, research regarding techniques for sampling and measuring urine in pigs postoperatively are rare.

2.7 Postoperative care

For recovery after anaesthesia, it is desirable to have an adequate place in a warm, quiet area that is distanced from other pigs. The recovery phase in large animals is slow, and the animals require support and continuous monitoring upon awakening (Swindle & Smith 2013). During the postoperative period, it has been recommended that personal on-site monitoring of such things as temperature, pulse, and respiration be performed until the pigs are fully awake. Thereafter, camera monitoring can be used whereby the animals can be viewed from a device outside the stable that will minimize disturbance from humans, yet allow continued monitoring of the pigs during the entire study period.

2.7.1 Postoperative analgesia

Both restraint and injections are potential acute stressors in pigs and may result in physiological and behavioural changes that, if possible, should be avoided (Bradbury & Clutton 2016). The most common analgesics used for postoperative pain relief in pigs are the opioids (Bradbury *et al.* 2016). Opioids have a short serum half-life in pigs, which leads to a need for repeated restraint and drug administration to achieve adequate analgesia (Harvey-Clark *et al.* 2000).

Fentanyl, a full μ-opioid receptor agonist, is frequently described in publications and can be administered from transdermal patches that are designed for slow release of the drug over several days. Accordingly, transdermal patches may be considered as an alternative method of drug administration in animals (Riviere & Papich 2001; Malavasi *et al.* 2005). The most often mentioned systemic analgesic used in pigs is buprenorphine (Bradbury & Clutton 2016). The drug has a relatively slow onset and is commonly administered preoperatively followed by repeated injections during the postoperative period. Malavasi (2005) reported in her thesis that treatment with transdermal fentanyl or buprenorphine IM after abdominal surgery in pigs, resulted in different behaviours postoperatively (Malavasi *et al.* 2005). Transdermal fentanyl alone in conscious pigs did not cause inactivity or sedation, but resulted in interindividual variations in the fentanyl serum concentrations. When 0.1 mg kg^{-1} buprenorphine was administered IM alone, 50% of the animals increased and 50% decreased their activity level postoperatively. However, there is a lack of information about the optimal dosing and the behavioural effects of transdermal fentanyl or buprenorphine given IV in pigs. Information about the analgesics' influence on the pigs' activity levels is *per se* important when assessing postoperative pain.

2.7.2 Postoperative assessment

Postoperative pain management is an important animal welfare issue. An accurate pain assessment is essential for animal welfare in order to estimate the consequences of painful interventions and develop effective pain control (Charlton 2005).

It is acknowledged that in pigs, the assessment of acute pain in the postoperative period is difficult (Bradbury & Clutton 2016). However, the use of a pain score can be helpful when evaluating the need for postoperative care and analgesic administration (Swindle 2007a). Recently, researchers

have examined specific nociceptive assessment methods during painful stimulation in conscious pigs utilizing nociceptive threshold testing and facial grimace scoring (Nalon *et al.* 2013; Di Giminiani *et al.* 2015; Di Giminiani *et al.* 2016; Luna *et al.* 2020). Still, behavioural observation remains subjective; it is time consuming, requires observer training and is prone to interobserver variation (Roughan & Flecknell 2003).

In recent years, there has been an increased focus on objective solutions that can determine and measure the behaviours and animal welfare in pig farming. Among other techniques, the application of artificial intelligence using machine learning has been used to study the behaviour of pigs, *e.g*. as an indication of climate conditions in slaughterhouse stables (Nilsson *et al.* 2015) and automatic warning of tail biting in slaughter pigs (D'Eath *et al.* 2018).

Recently, an AI device for the surveillance of mares that alerts caretakers to early signs of foaling was launched. The intelligent software learns to recognize the activity of the individual horse and the result provides the programme's reference baseline for that specific horse. Such intelligent software has not been developed yet for other animal species.

3. Aims of the thesis

The overall aim of the thesis was to refine perioperative nursing and handling of growing pigs in experimental studies requiring anaesthesia care

The specific aims were to:

Determine if pigs subjected to systematic training during the pre-operative acclimatisation period could tolerate postoperative sampling of blood and clinical examination (studies I, III and IV) as well as ultrasound examination and the collection of urine (study I) without restraint.

Assess if 2-methacryloyloxyethylphosphorylcholine (MPC) polymer-coated catheters inserted with the Seldinger technique enables blood sampling for a prolonged period (Studies I, III and IV).

Study the effects of two different injectable anaesthetic techniques on induction to general anaesthesia, the physiological changes, and total intravenous anaesthetic drug requirement during eight subsequent hours of anaesthesia (Study II).

Assess the physiological and clinical responses in relation to drug plasma concentrations after a single or repeated dose of a combination of zolazepam, tiletamine, dexmedetomidine and butorphanol (Study III).

Adapt an artificial intelligence technique for objective assessment of activity and evaluate a facial expression method in growing pigs treated with postanaesthetic transdermal fentanyl or intravenous buprenorphine injections (Study IV).

4. Materials and methods

4.1 Animals, housing and acclimatisation

The experimental protocols were approved by the Ethics Committee for Animal Experimentation, Uppsala, Sweden. The approval numbers are presented in each study. In the studies, clinically healthy domestic crossbreed pigs (Yorkshire x Swedish Landrace or Yorkshire x Hampshire) of both sexes, aged 7–9 weeks upon arrival were used. In Study II, the research was performed at the Hedenstierna laboratory, Department of Medical Sciences, Uppsala University. Since that laboratory does not have the possibility to house animals, the pigs arrived from the breeder the same day the study was performed.

Study I, Study III and Study IV were performed at the Department of Clinical Sciences at SLU. Upon arrival, the pigs were examined by a veterinarian to determine their health status and check for any clinical signs of disease. From day one, the animals underwent a 14-day acclimatization period. During this period, possible changes in appetite and thirst were monitored daily. Two times a week the pigs were weighed on an electronic scale and clinically examined by a veterinarian to check for any diseases and physiological abnormalities. The pigs were housed in individual pens measuring 3 m^2 where they could see and hear each other. Straw and wood shavings were provided as bedding. The ambient temperature inside the pens was 18 ± 2 °C, and a 10:14 hour light–dark schedule was used. An infrared lamp was provided in a corner of each pen in Study I and Study III. A lamp was not provided in Study IV because it affected the camera recording during the nights. The pigs were fed a commercial finisher diet (Solo 330 P SK, Lantmännen, Sweden) twice daily. The amount fed was according to body

weight and SLU's regimen for growing pigs. Water was provided *ad libitum*. Twice a day when the pens were cleaned, the animals were allowed to walk in the corridor where they could have physical contact with the other pigs. Study I was performed in four trials with 36 pigs included in the training programme. Eleven of these pigs were later used in other studies at SLU. The remaining 25 animals were used as recipients in a renal transplantation study. In Study II, 12 pigs were used. Additionally, in Study III, 12 pigs were used (six pigs from Study I that were involved in the training programme and six pigs from another study). In Study IV three pigs were used. At the end of that study the animals were also used in another study performed at SLU. In Study II, III and IV, pigs were randomly chosen for different protocols in each study. In Study IV, a crossover study was performed in which each pig was treated with transdermal fentanyl or buprenorphine, and after a wash-out period lasting a minimum of 72 hours, the pigs received the other analgesic. After the studies performed at SLU, all pigs underwent post-mortem examination at the Department of Pathology, SLU.

4.2 Acclimatisation and training

In Study I, III and IV, each pig was trained in a four-step training programme (Table 1). In Study I and III, five persons who were experienced in training pigs, took part in the training programme. In Study IV, three persons were involved in the training. Steps 1, 2 and 4 were similar for all animals, but step 3 differed due to the different study design. In step 1, the pigs were allowed to adapt to the new environment for three days. During this time no specific training took place. The staff only entered the housing area for feeding the pigs and cleaning the pens. In step 2, the trainer sat in the pen for 15 minutes each day so the pig could get accustomed to the person. Once the pig was close enough, the trainer started to gently touch and brush the animal, and offered it pieces of fruit from their hand. The pigs were also trained to accept touching and palpation of the ears as preparation for blood sampling from the auricular vein. In Study I, step 3, the training included touching the pigs on the abdomen with an ultrasound transducer dummy with lubricating gel to aid in their tolerance of an ultrasound examination of the urinary bladder and transplanted kidney post-operatively. In Study I, a trainer held a paper dish under the pigs belly so they would be accustomed to free-flow urine collection. In step 4, the training from step 2 and 3 continued. In Study

III and IV, steps 3 and 4 were devoted to touching and handling of the ears and clinical examination. In Study IV, the pigs were trained to accept handling from two trainers at same time to prepare them for the postoperative activities. In that study, the animals were also trained to become accustomed to wearing protective jackets. For each individual pig in all three studies, steps 3 and 4 were introduced once the pig had been completely accustomed to the procedures in the previous step.

Table 1. Training of pigs in Study I, III and IV in four steps during the two-week acclimatisation period.

	Step 1	Step 2	Step 3	Step 4
Study I	Pigs left to settle down	Trainer sits in the pen Touches and brushes Offers fruit Trainer talks	Ultrasound of abdomen Collection of free-flow urine	Training (step 2-3) continues Clinical examination
Study III	Pigs left to settle down	Trainer sits in the pen Touches and brushes Offers fruit Trainer talks	Touches and manipulates the ears	Training (step 2-3) continues Clinical examination
Study IV	Pigs left to settle down	Trainer sits in the pen Touches and brushes Offers fruit Trainer talks	Touches and manipulates the ears Two persons in the pen at specific times Pigs dressed with protective jackets	Training (step 2-3) continues Clinical examination

4.3 Anaesthesia

The day before the anaesthesia, the animals were weighed and a clinical examination was performed. Before induction, a light meal was given three to six hours before anaesthesia, and water was provided *ad libitum*. The induction combinations used in these studies were given IM in the brachiocephalic muscle using a butterfly needle (CHIRAFLEX Scalp vein set 21G x 3/4", 0.8 x 20 mm Luer-Lock, CHIRANA T. Injecta, Stara Tura Slovakia). One bottle of tiletamine and zolazepam (TZ) (Zoletil 100® vet.

tiletamine 250 mg + zolazepam 250 mg, Virbac, Carros, France) in powder form was reconstituted with 5 mL of either medetomidine (Domitor vet. 1 mg mL^{-1}, Orion Pharma AB Animal Health, Sweden) or dexmedetomidine (Dexdomitor®vet. 0.5 mg mL^{-1}, Orion Pharma AB Animal Health, Danderyd, Sweden). In Study III 10 mg of butorphanol (Dolorex®vet. 10 mg mL^{-1}, Intervet AB, Stockholm, Sweden) was added to the solution. The different anaesthetic and analgesic protocols were as follows:

Study I: Anaesthesia was induced with TZ 2.5 mg + 2.5 mg kg^{-1} in combination with Medetomidine 0.05 mg kg^{-1}. Ten minutes later buprenorphine 0.01 mg kg^{-1} (Vetergesic® vet, 0.3 mg mL^{-1}, Orion Pharma Animal Health, Solna, Sweden) was given IM. Epidural morphine 0.1–0.12 mg kg^{-1} (Morfin Epidural Meda 2 mg mL^{-1}, Meda AB, Sweden) was administered 40 minutes before the start of surgery. Anaesthesia was maintained with isoflurane (IsoFlo® vet. Orion Pharma Animal Health, Sweden). At the end of the anaesthesia and during the study period, additional buprenorphine (0.03 mg kg^{-1}) was given IV after assessment of the general condition and behaviour of each pig.

Study II: In group ZTMe, anaesthesia was induced with TZ 2.5 mg + 2.5 mg kg^{-1} in combination with Medetomidine 0.05 mg kg^{-1}. In group MiKF, anaesthesia was induced with midazolam 2 mg kg^{-1} (Midazolam Actavis 5 mg mL^{-1}, Actavis AB, Sweden) in combination with ketamine 10 mg kg^{-1} (Ketaminol® vet 100 mg mL^{-1}, Intervet AB, Sweden), which was followed by fentanyl 4 µg kg^{-1} (Fentanyl B. Braun 50 µg mL^{-1} B. Braun Medical AB, Sweden) IV before intubation. In both groups maintenance of anaesthesia was performed for eight hours with a TIVA mixture of midazolam 0.015 mg mL^{-1}, ketamine 4 mg mL^{-1} and fentanyl 0.5 µg mL^{-1} in 947 mL 0.9% Lactated Ringer's solution (Ringer-acetate, Fresenius Kabi AB, Sweden). The starting infusion rate was midazolam 0.105 mg kg^{-1} h^{-1}, ketamine 28 mg $kg^{-1}h^{-1}$ and fentanyl 3.5 µg $kg^{-1}h^{-1}$ respectively. At the end of anaesthesia, but while still anaesthetised, the animals were euthanised using a potassium chloride (2 mmol kg^{-1}) IV injection.

Study III: The pigs were randomly divided in two groups; single injection or repeated injections. Three days before the start of the study, anaesthesia was induced and maintained with sevoflurane (SevoFlo® Orion Pharma,

Danderyd, Sweden) in oxygen (F_IO_2 0.5) and air that was delivered using a non-rebreathing system with a face mask. During the anaesthesia, an internal jugular catheter was placed. When the study started, anaesthesia was induced with TZ 2.5 mg + 2.5 mg kg^{-1} in combination with Dexmedetomidine 0.025 mg kg^{-1} and butorphanol 0.1 mg kg^{-1} (0.06 mL kg^{-1} of the anaesthetic combination solution). A cannula (BD Venflon™ 20 G x 32 mm, BD Medical, Franklin Lakes, NJ, USA) was inserted in the auricular vein on the non-catheterized ear 30 minutes after induction in both groups. Sixty minutes after induction, only Group Repeated received a repeated injection containing one-third of the initial dose (TZ 0.83 mg + 0.83 mg kg^{-1} in combination with Dexmedetomidine 0.008 mg kg^{-1} and butorphanol 0.033 mg kg^{-1}) IV.

Study IV: Anaesthesia was induced with TZ 2.5 mg + 2.5 mg kg^{-1} in combination with Dexmedetomidine 0.025 mg kg^{-1}. Anaesthesia was maintained with sevoflurane (SevoFlo® Orion Pharma, Danderyd, Sweden). When treated with fentanyl, the animals received a bolus of fentanyl 0.025 mg kg^{-1} IV followed by constant rate infusion (CRI) of fentanyl 0.025 mg kg^{-1} h^{-1}. During anaesthesia, a fentanyl patch (Fentanyl ratiopharm 100 µg h^{-1}, Teva Sweden AB, Helsingborg, Sweden) was placed on the skin in the interscapular area and covered with tape. Before placement of the patch, the hair of the area was clipped and the skin was washed carefully so as not to cause bleeding or irritation. The skin was then dried before patch attachment. The patch was removed 72 hours after placement on the pigs. When treated with buprenorphine, the animals received an injection of buprenorphine (0.03 mg kg^{-1}) IM during anaesthesia followed by 0.03 mg kg^{-1} IV (pigs 1 and 2) or IM (pig 3) BID for three consecutive days.

4.3.1 Induction

During induction in Study II and Study III, the pigs were observed for any signs of discomfort from the injection and their position and level of consciousness were monitored continuously. The time to unconsciousness was noted; as evidenced by lateral recumbency, head down, lack of reaction when manipulating or moving their body, and absence of the palpebral reflex.

4.3.2 Intubation

Pigs in Study I, II and IV were intubated in their trachea. Before intubation, 100% O_2 (4 L min^{-1}) was delivered by use of a face mask to the pigs for five minutes. Pigs were placed on an operating table with a heating pad under them in sternal recumbency (Study II) or in dorsal recumbency (Study I and IV). The trachea was intubated with an endotracheal tube (6–8 mm ID) with the use of a laryngoscope (Standard handle with Miller blade 12″, Jorgensen Labs, CO, USA)**.** In Study II, when the intubation was performed, the ease of the intubation was evaluated by a laboratory technician who was unaware of the drug combination used. An intubation scoring sheet based on a system for humans (Sluga *et al.* 2005) and pigs (Duke-Novakovski *et al.* 2012) was used. Scoring was rated from one to three, where a higher score indicated a more difficult procedure. All criteria needed to be met for each level, and if they were not, the procedure was classified one level higher.

4.3.3 Anaesthesia equipment

The endotracheal tubes used in the animals in Study I and IV were connected to an anaesthesia circle with an integrated ventilator (FLOW-i® Anaesthesia Delivery System, Getinge AB, Sweden), and in Study II to a ventilator (SERVOi®Anesthesia delivery system, Getinge AB, Sweden). The pigs' lungs were ventilated with oxygen and air (F_IO_2 0.4 in Study II and IV and 0.3 in Study I). The minute ventilation (MV) was adjusted to maintain arterial partial pressure of carbon dioxide ($PaCO_2$) to 5.5–6 kPa.

4.3.4 Monitoring of physiological parameters

During anaesthesia in Study I-IV, clinical parameters were monitored continuously and recorded every 5–15 minutes. In Study I, II and IV, respiratory parameters included respiratory rate (RR), F_IO_2 and end-tidal carbon dioxide concentration ($ETCO_2$). The anaesthetic agents (Study I and IV) were also measured. The gas monitor was calibrated before each session by use of a commercially prepared calibration gas. The circulatory parameters measured were: heart rate (HR) based on a 3-lead electrocardiogram, and peripheral oxygen saturation (SpO_2) measured with the use of an ear probe placed on the pig's tongue, snout or tail. Arterial blood pressure was intermittently measured oscillometricly using an inflatable cuff placed around a forelimb. Temperature was measured with a

temperature probe placed in the oesophagus. In Study III palpation and monitoring of the HR and monitoring of RR, SpO_2 and rectal temperature began once the animals were in lateral recumbency and continued throughout the anaesthesia every 5–30 minutes until recovery.

In Study II one catheter was placed percutaneously into the femoral artery and two catheters were inserted from an incision into the jugular vein. One Swan-Ganz catheter was placed using pressure monitoring and by observing the pulse pressure contour change when the catheter passed through the heart and into the pulmonary artery. One pigtail catheter was also placed in the right atrium under pressure monitoring. This allowed for the measurement of the central venous pressure, pulmonary artery blood pressure, pulmonary artery occlusion pressure and cardiac output (CO). Thermodilution was used to determine CO. During the expiratory phase, the same person injected a 10 mL bolus of ice-cold saline through the pigtail catheter. A minimum of three measurements were made at each data collection point and the data were averaged at each time point. All pressure measurements were made with the use of a pressure transducer positioned at the level of the pig's right atrium. Before each pressure was measured and noted, the transducer was calibrated. Parameters measured in addition to those described and the monitoring systems used are described in detail in each Study.

4.3.5 Blood gas measurements

In Study II, arterial and mixed venous blood samples were collected simultaneously at each data collection point every 15 minutes during the first hour and every 30 minutes for the remaining seven hours. The blood was withdrawn from the femoral arterial and the Swan-Ganz catheters simultaneously into heparinised syringes and analysed immediately using a standard analysis instrument. Arterial pH, arterial oxygen tension, carbon dioxide tension, arterial oxygen saturation, haemoglobin concentration, mixed venous oxygen tension and oxygen saturation were analysed. Blood gases were corrected for atmospheric pressure. Results from the analyses are described in detail in Study II.

4.4 Nociceptive testing

In Study II and Study III, the response to noxious stimuli was elicited by mechanically clamping with a forceps the coronary band of the medial or

lateral dewclaw on either the right or left pelvic limb. The site of the stimulation was changed slightly each testing interval to prevent sensitisation to stimuli. When an obvious positive reaction, such as a reflex withdrawal of the leg occurred, it was interpreted as a response. In Study II the anaesthesia infusion rate was increased by 1 mL kg^{-1} h^{-1} if a response occurred, if no reaction was observed, the infusion rate was decreased by 1 mL kg^{-1} h^{-1}, but never below 7 mL kg^{-1} h^{-1}.

4.5 Sampling and examinations

4.5.1 Ultrasound examination

In Study I, a postoperative ultrasound of the kidneys was performed using linear and curvilinear (4 MHz) probes. A colour Doppler was used to evaluate the blood flow in the kidneys. The examination was performed in the pigs' individual home pens.

4.5.2 Blood

In Study I, III and IV, internal jugular catheters were placed under general anaesthesia (Study I had 27 catheters, Study III had 12 catheters and Study IV had 6 catheters) in the jugular vein to facilitate blood sampling and the injection of drugs IV postoperatively. A polyurethane catheter (BD Careflow™ 3Fr 200 mm, BD Medical, Franklin Lakes, NJ, USA) was introduced via the vena auricularis into the vena jugularis using an aseptic Seldinger technique. The catheter was sutured onto the ear with monofil-coated polyamide (Supramid 2-0, B Braun Medical, Danderyd, Sweden) and covered with tape (Tensoplast Sport 6 cm x 2.5 m, BSN Medical, Hamburg, Germany) and a bandage (Snøgg AS, Vennesla, Norway). In Study I, 12 catheters were coated with MPC-polymer (Asif *et al.* 2019) and sterilized in an ethylene gas autoclave, whereas 15 were uncoated. In Study III and IV, all catheters were coated with MPC-polymer. In Study I, III and IV, blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes via the central venous catheter according to predetermined protocols for each study (samplings occurred for 3–10 days). Before injecting or withdrawing samples through the catheter, the extended part of the catheter was wiped with aseptic solution. Three mL of blood were first withdrawn and discarded, and then the blood sample was withdrawn. Immediately after the sampling,

the uncoated catheters were flushed with saline and filled with heparinised saline, whereas the MPC coated catheters were flushed with saline only. During the handling of the catheters, all personnel wore gloves and followed aseptic routines. The caps and bandages were changed as needed. Plasma was separated by centrifugeation within 90 minutes from sampling for five minutes and stored at -80°C until analysis.

4.5.3 Urine

In Study I, the urinary bladders of the pigs that had received a kidney transplant were examined one or two times per day with a portable ultrasound machine (Imago 1401MG05, ECM, France). Additionally, a semi-automated ultrasound instrument (BladderScan® BVI 9400, Allytec AB, Sweden) intended for the measurement of urine volume in humans was evaluated for use in non-sedated pigs. Ten scans were performed at each measuring occasion, and the volume results from the scans were compared to results from the ultrasound examinations. Free-flow urine samples were collected from pigs with a paper dish once daily.

4.6 Postoperative care

Postoperatively, the pigs in Study I and IV were placed on a blanket under a heating lamp and were continuously monitored until extubation. The extubation occurred when they could breathe unassisted and the swallowing reflex had returned. During their recovery, the monitoring of RR and HR continued until the pigs were fully awake, after which they were offered food and water. During the remaining study period the animals were weighed at least every other day and health status, urination and defecation were controlled several times per day. Clinical examination was performed by a veterinarian once a day. All the nursing measures and physiological deviations were noted.

In Study I, the health status and signs of pain-related behaviour were monitored continuously by the staff during each pig's recovery (24 hours). During the postoperative period, if needed, the pigs were hand-fed fruit to stimulate their appetite, supported to drink water, and assisted to stand and walk. During the following days, in addition to the physical examination and inspection and care of the surgical wounds; RR, HR, temperature, appetite and estimates of faecal colour and consistency were monitored and recorded.

Additionally, the kidneys and blood flow in the transplanted graft were examined in the non-sedated animals. In Study III the animals were monitored during the entire time of their anaesthesia. The time of their first spontaneous movement, when the pig achieved a standing position, and the number of attempts to stand were observed and recorded. The quality of their recovery was assessed and recorded manually during the experiment, and at a later time reassessed by watching the video recordings. The assessment was made using a scoring system adapted and modified from an anaesthesia scoring system used in antelopes (Laricchiuta *et al.* 2012) 1 (excellent), 2 (good), 3 (poor).

4.6.1 Evaluation of activity with an AI technique

In Study IV, a 3D camera (Raspberry-pi-Camera (H), Waveshare Electronics, Shenzhen, China) with an infrared illuminator, was installed on the back wall of each pen. The 3D camera recorded continuously 24 hours a day during the entire study period (28 days) (Figure 1). Recordings were transmitted wirelessly to a router base unit (Teltonika RUT240, Kaunas, Lithuania) that enabled the data to be downloaded.

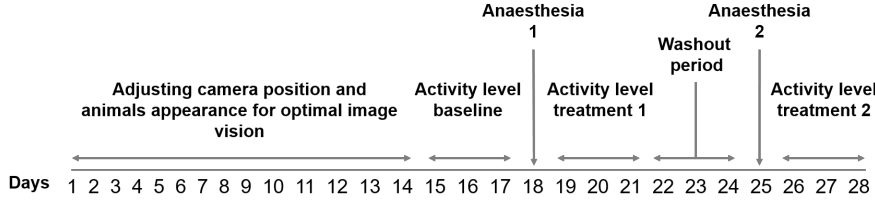

Figure 1. Study IV experimental timeline showing days and events during a four-week period.

A semantic segmentation model to detect the animal in the pen and a custom classification model to detect its behaviour and activity level were used. Deep neural networks to train computers proprietary algorithms were produced by the company (Videquus AB, Skövde, Sweden). Activity level data during the three days prior to the first anaesthesia trial was used as baseline and was compared with the activity level three days after each anaesthesia trial.

To improve the detection of the animals, the pigs were painted on their backs with a coloured marking spray (KRUUSE blue marking Spray, Kruuse A/S, Langeskov, Denmark) that is specially adapted for marking animals. In addition, the pigs wore a coloured protective jacket (Swine Jacket with Pocket, Ludomed Equipment Inc, QC, Canada) (Figure 2).

Figure 2. A 3D camera continuously recorded the pig during the entire study period (28 days). The pigs were painted on their back and equipped with a coloured jacket to improve detection and to protect the transdermal fentanyl patch (top). Identification of the pig in its pen with the AI image vision (bottom). The green masking shows what the computer recognizes as the pig. Activity levels are calculated based on changes in the position of the masking each second. Photo: Anneli Rydén

4.6.2 Pig Grimace Scale

In Study IV, front and profile photographs of each pig's face were taken in its home pen before anaesthesia and once a day (midday) during the next three consecutive days in both sessions. The photographer was the same

person and was familiar with the animals. A non-participating assistant selected an image set comprising three images of each pig every day. The images were organized and marked in a random order, which resulted in images from seven different days for each pig. The evaluation of the images included three Facial Action Units (FAU): cheek tightening/snout bulge, ear position and orbital tightening as used in earlier investigations (Viscardi *et al.* 2017; Vullo *et al.* 2020). Cheek tightening/snout bulge and ear position were set to a 3-point scale (0–2), while orbital tightening was set to a 2-point scale (0–1). If the FAU was impossible to score from the image, not accessible (N/A) was noted. A description of each FAU and scoring system was prepared and distributed together with the images to six assessors who were well experienced with laboratory pigs. Three assessors (A, B, C) were aware of the study protocol, but they did not know which opioid treatment the pig had received when the images were taken. The other group of three assessors (D, E, F) were completely unaware of the study protocol including any opioid treatment involved. The scores were also compared and analysed to assess if knowledge of study design influenced the scorings.

4.7 Analyses and calculations of drug plasma concentrations

For the analyses of drug plasma concentrations in Study III and IV, the samples were submitted for liquid chromatography-mass spectrometry analysis (Admescope Ltd, Oulu, Finland). For each animal and drug, the plasma drug concentrations vs. time were plotted. Different models and weighting factors were assessed by visual inspection of the curve fits and the residuals' scatter plots. The best options were then chosen after also considering the goodness of fit measures incorporated in the software (*e.g.* including the Akaike criteria). A non-compartmental model was used for all drugs. In Study III, for Group Single (IM), the maximal concentration of each drug in the plasma (C_{max}), time to reach C_{max} (t_{max}), and terminal half-life ($T_{1/2}$) were calculated using the PK Solver add-in for Microsoft Office Excel. For Group Repeated, after the IV bolus administration, the $T_{1/2}$, the volume of distribution and the clearance were obtained from the model. In Study IV, for fentanyl after a CRI of 60 minutes, the IV $T_{1/2}$ was calculated from the end of the CRI at one hour to three hours with a one phase decay using GraphPad Prism 9.1.0. The means and C_{max} concentration of each drug in

plasma, and the trough concentration (C_{trough}) just before the repeated administration of buprenorphine was read from the graph. Estimation of the area under the plasma concentration time curve (AUC) was made for buprenorphine for the first to the last sampling with GraphPad Prism 9.1.0.

4.8 Physiological calculations and statistics

From the measurements obtained in Study II, the following calculation was made and used as the standard equation:

$$Cardiac\ Index\ (CI) = \frac{Cardiac\ Output\ (CO)}{bodyweight\ in\ kg}$$

A repeated measures analysis using a mixed model method was used to analyse the physiological data. A mixed model approach that implemented the MIXED procedure of the SAS (SAS Institute Inc. (2017): Cary, NC, SAS Institute Inc.) system was used. The fixed part of the models included induction protocol, anaesthesia time and the interaction between them. The relationships between the time points within an animal were modelled using a spatial power covariance structure. Pairwise comparisons between treatments were made at all time points and evaluated after the application of the Bonferroni correction. The assumptions were checked using diagnostic plots. Time (minutes) data were analysed using a Mann-Whitney U test (NPAR1WAY procedure in SAS 2017). The intubation score data are ordinal with three values. We tested the effect of the treatments with the scores obtained during the intubation using a Mann-Whitney U test. The p-value was calculated in an exact way using a randomisation algorithm NPAR1WAY procedure of the SAS (ibid). In Study III, physiological and clinical data were compared among study times and groups using a one-way analysis of variance (ANOVA) for repeated measures. In Study IV, since the photos of the face included several observations for each experimental unit, mixed linear models for repeated-measures data (Littell 2006) were used for analysis. The Mixed procedure of the SAS (2018) package was used. The model included pig, judge, site and day, and all two-way interactions between these. Pig, and all interactions including these, were regarded as random and judge was regarded as fixed factor. The repeated measurements on the same site during several days were modelled using an autoregressive

AR (1) covariance structure. Post-hoc comparisons were adjusted for multiplicity using Tukey's method. Although we are aware that the measurements are on a discrete ordinal scale, the residual plots, in combination with the large sample size, suggest that the model is acceptable. Data are presented as median, mean ± SD, or as a range.

5. Results and discussion

5.1 General condition of the animals

The transport of the animals from the breeder took at most 30 minutes and was accomplished by the staff and nurses from the SLU research facilities in Study I, III and IV and by the breeder in Study II. The pigs' health status was good upon arrival, and during the acclimatisation period included in Study I, III and IV, the animals were eating and drinking adequately and adapting quickly to the new environment. The pigs' average daily weight gain during this period was 0.67 ± 0.03 kg, which is in line with the growing rate of pigs in conventional herds.

5.2 Preoperative training and handling intended for postoperative examination and sampling

All pigs fulfilled their specific training programme and accepted all procedures within 14 days, however there were differences in how fast the individual animals reached the different training steps (Table 1, Figure 3). During the first three days after arrival, the pigs were left to settle (step 1). This is based upon our previous experience and it is recommended that research animals are allowed to get accustomed to the new environment, individual housing and daily routines before they start training (Gieling *et al.* 2011). On the first day in step 1, most of the pigs tended to be anxious when the staff entered the stable, but became less afraid during the two following days and were calmer when the training in step 2 began. Time to adapt to the moments included in step 2 (touching and handling) ranged from 2–6 days. This corresponds to results from another study on the training of research

pigs (Nicholls *et al.* 2012) in which it was suggested that touching and handling is the most important, but also the most challenging part of the training. Two pigs in Study I needed up to three times as much time to adapt to step 2 then the others. However, after step 2, the two pigs quickly adapted to the remaining interventions, scans of the urinary bladder, clinical examinations and midstream catches of urine specimens. One pig in Study IV showed aggressive behaviour, and needed two more days than the others to accept being touched and brushed. The rest of the animals in Study I and IV and all of the pigs in Study III quickly adapted to the interventions. Even though the training of animals to perform various tasks has a long tradition and is popular, there are still few published standard protocols regarding the training of pigs for specific purposes in research trials. In general, pigs are friendly, curious, very adaptable and willing to cooperate if they are handled carefully without stress. Nevertheless, it has been described in studies that some individuals can be difficult to train (Kaiser *et al.* 2007; Nicholls *et al.* 2012; Swindle & Smith 2013), and some are more adaptable than others. This was also the case in Study I and IV with the three pigs that were difficult to train. By extending the training period in steps 2 and 3 for two pigs in Study I, and step 3 for one pig in Study IV, the same tameness was obtained for the difficult to train pigs within the two weeks that was obtained for the rest of the pigs.

In general, the recommendation is to start the acclimatization period 3–14 days before the experiment begins (Obernier & Baldwin 2006). In the present studies (I, III, IV), even though all of the pigs were adapted to the interventions before the studies began, the time needed for training could not have been less due to the number of interventions in the studies. The results from the present studies show that a training period of at least 14 days was necessary. This period also covers the incubation time of most infectious diseases encountered by pigs, and the recommended length of quarantine in Sweden, making it possible to detect diseases before the experiments began.

It has been described that pigs can recognize their handlers (Manteuffel *et al.* 2009) and are easier to handle by trainers that have spent more time with them (Nicholls *et al.* 2012). That result indicates that persons who will be handling the animals postoperatively should preferably be the ones who train the animals preoperatively. Based on that information, our training schedule was designed so that all persons who were supposed to be involved

in the samplings and examinations postoperatively were also involved in the training programmes before anaesthesia.

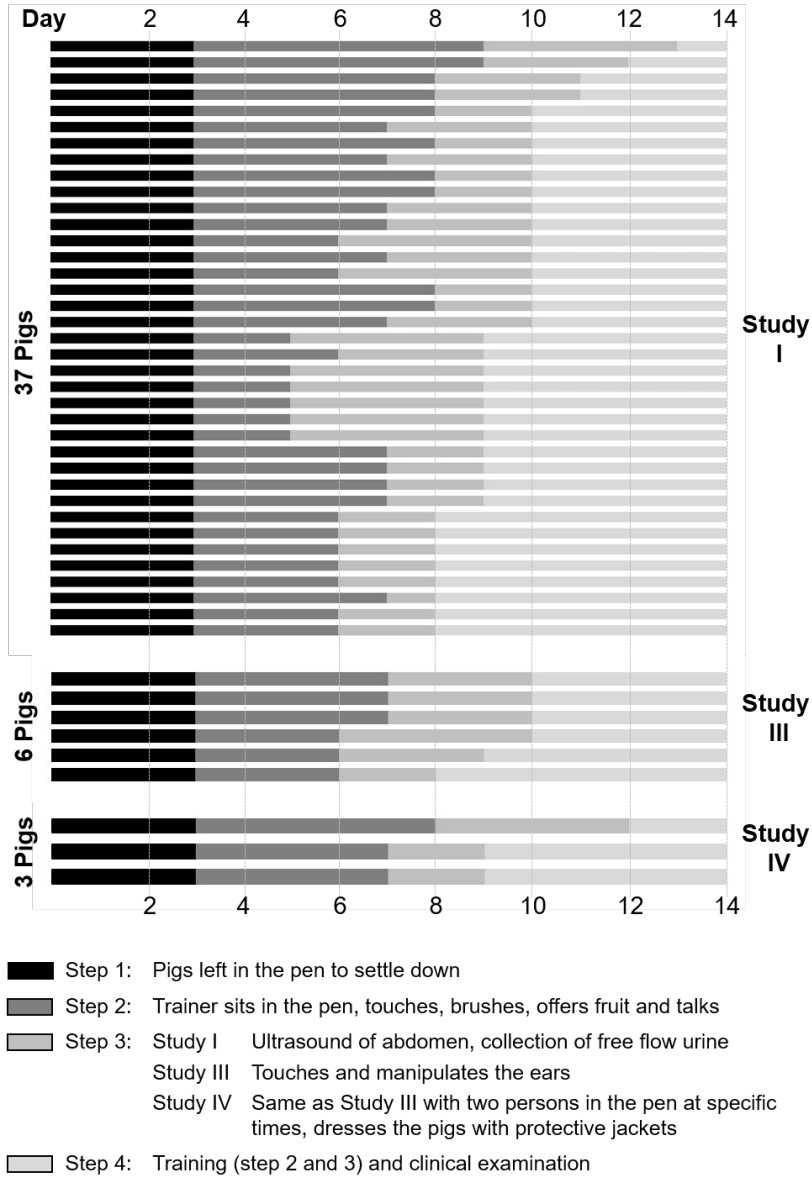

Figure 3. Length of time (days) to complete steps 1–4 included in the training programme for each pig. The horizontal lines indicate the length of time (days).

It has been reported that an indication of a positive effect from the training is when the staff receives a positive response from the pig in the form of increased contact seeking and less anxiety during the handling. The training of basic cooperative behaviours will make the daily routines safer and easier by decreasing the need to restrain the pigs and easing the ergonomic challenges such as lifting, holding and chasing the animals (Sørensen 2010). In addition, if the stress-induced effects on research data can be minimized, the physiological as well as haematological and serum chemical results will be more valid (Raad & Hanna 2002; Lambeth *et al.* 2006). Therefore, the overall goal of the training sessions preoperatively in the present studies were to improve the animals' welfare and refine the sampling procedures and examinations of the animals postoperatively.

5.3 Clinical examination

In Study I, the pigs were accustomed to an ultrasound examination of the kidney graft in the postoperative period. The pigs were standing or laying down voluntarily in a lateral position during the scanning, and were rewarded with fruit or being brushed during the procedure that lasted approximately 15 minutes (Figure 4). In studies with major surgery performed (as in Study I), a number of post-operative examinations to identify a healthy kidney transplant or detect complications are necessary. Ultrasonography, which is the most widely applied imaging modality for kidney transplant follow-ups was used in the present study (Kolofousi *et al.* 2013). Several protocols for the sedation of pigs in order to perform examinations postoperatively have been published (Swindle 2007a). However, administration of it may induce stress, and the drugs may affect the general recovery of the animals. In the present study, examinations of the kidney graft could be performed without the use of restraints or anaesthesia.

In Study I, III and IV, control of the health status and clinical examinations were performed once a day or more often if needed by veterinary nurses and veterinarians. The pigs accepted examination of surgical incisions (Study I), palpation of the body, and auscultation of the heart with a stethoscope (Study I, III and IV) without restraint.

5.4 Blood sampling

In Study I, III, IV, Careflow® catheters were successfully placed during anaesthesia in the auricular vein and advanced to an internal vein. The catheterisation according to the Seldinger technique was smooth and the collection of repeated blood samples functioned well during anaesthesia. Postoperatively, most animals were comfortable while the catheter in the auricular vein was manipulated. However, some pigs shook their heads when the blood samples were withdrawn or when an injection was infused. Even though blood withdrawal was possible, and these particular pigs did not show any signs of stress, the samplings were more time-consuming and the aseptic routines were more difficult to maintain than with the calmer pigs. Although there were no visible signs of infection at the catheter exit site, it is possible that inflammation was present. When blood samples were being collected, fruit rewards were given if needed. If two people were present, one person brushed the animal, which was the most effective way to get them to stand still during the sampling. When handling catheters intra- and postoperatively, and especially in survival procedures, meticulous aseptic techniques are essential to avoid infections (Swindle *et al.* 2005; Urzainqui-Laborda 2012). We therefore always use stringent aseptic measures during connection, disconnection and sampling in our studies.

For good science as well as for animal welfare, it states in the National Centre for the Replacement Refinement & Reduction of Animals in Research's (NC3Rs) guidelines that when taking blood samples, stress should be kept to a minimum, the sampling should be prepared in an appropriate manner and a correct aseptic technique should be used so that the most refined and current method is employed (https: //www.nc3rs.org.). Consequently, in the present study, even though some pigs shook their heads and the blood sampling procedure required extra time, the handling of the animals and the aseptic techniques, *e.g.* changing of gloves and disinfection of the connections and caps, were followed.

5.4.1 Evaluation of MPC-coated catheters

During the first trials in Study I, blood could only be drawn up to 24 hours after placement in four of the 15 uncoated catheters, but sampling was still possible for up to 96 hours in the remaining 11 uncoated catheters. In the remaining trials in Study I, Study III and Study IV, blood could be drawn throughout the study periods from the 30 MPC coated catheters for three and

up to ten days. Three catheters (two in Study I and one in Study IV) were dislodged. Even though the catheters were carefully sutured to the skin and fixed using surgical tape and bandaged, the catheters had slipped out while the tape and parts of the sutures remained attached to the earlobe. The main reason for the catheters slipping out was probably mechanical damage caused by the pigs, but insufficient fixation is always possible. In the future, we plan to try to develop a technique for attaching the catheters to the ear, so they do not dislodge.

At the post mortem examinations, none of the animals were found to have thrombophlebitis in the vessel where the catheters had been located. In addition, no blood clotting was evidenced at the surface of the MPC-coated catheters.

In experimental studies, central venous access is crucial, especially if discomfort is to be avoided when repeated blood sampling and/or the administration of IV medications are required. The catheterisation technique that included the use of coated catheters as described in the present study, is a minimally invasive surgical procedure that improves the welfare of experimental animals by reducing their suffering and number of possible confounders induced if surgery is needed (Fudge *et al.* 2002; Furbeyre & Labussiere 2020). In addition, with the catheter method we used, one person is usually enough to draw the samples from the pigs, but to perform venous punctures it usually requires two handlers. In Study I, four uncoated catheters were partially occluded, and even though they could be flushed, blood could not be withdrawn. Therefore, these catheters were removed. In humans, the most common cause of thrombotic obstruction is a fibrin sleeve formation at the catheter tip. Fibrin sleeves cause obstruction when they are pulled over the catheter tip, but flushing or infusion is still commonly easy (Baskin *et al.* 2009). Experimental studies in pigs have demonstrated that this species is hypercoagulable when compared to humans (Karges *et al.* 1994). Therefore, heparin is frequently used as an antithrombotic agent to maintain venous catheter patency. However, there are some studies that report no extra beneficial effect of heparin compared to saline, and there are no universal guidelines for the concentrations of the heparin solution or the frequency the catheters should be flushed (You *et al.* 2017). Even though the MPC coated catheters were flushed with only saline, all of them could be used to collect blood throughout the experimental periods. The possible benefit of coated catheters needs to be evaluated in a larger number of animals. Nevertheless,

MPC coated catheters are promising for prolonged implantation in pigs as they seem to eliminate the formation of fibrin sheaths.

From a 3R perspective, the goal is to use catheters for repeated blood sampling in pigs and to avoid occlusion of the catheter, which might require frequent replacements or venepunctures. In addition, it is vital that internal catheters are maintained properly to avoid the risk of contamination, and that the animals are closely monitored while the catheter is in place. The veterinary nurse plays an important role in ensuring that this takes place (Hurley 2012).

5.5 Urine monitoring and sampling

Accurate monitoring of the urine output in pigs is central for assessing the results during different experimental manipulations as well as biochemical, nutritional, urological and physiological studies (Holliman *et al.* 1982; Kurien *et al.* 2004). Additionally, in humans, the urine volume may correlate with favourable short- and long-term allograft survival after a kidney transplantation (Khosroshahi *et al.* 2007).

Non-invasive techniques to measure urine production in pigs are few. (Golriz *et al.* 2012). In our study, the ultrasound examination of the bladder was very valuable as urine was detected in the bladder from the first day after transplantation and every day until the end of the study in most of the pigs. The amount of urine was only estimated since the general aim was to evaluate the possibility of using ultrasound examinations on non-sedated pigs after renal transplantation.

In Study I, when the ultrasound examination of the urinary bladder was performed, the pigs remained calm during the scanning (Figure 4). Urine was detected in the bladders of 12 out of 21 scanned pigs by ultrasound on day one. Within three days postoperatively, urine was detected in the bladders of all of the pigs that had not urinated spontaneously. We decided to evaluate the BladderScan® as a possible alternative to the conventional ultrasound technique to measure urine in animals. In several pigs, the results from the BladderScan® showed divergent results, 0–120 mL, 5–270 mL, 0–90 mL etc. on the same measurement occasion. The urine was measured at the same time with the conventional ultrasound machine and the results were 200 mL, 50 mL and 170 mL respectively. The results were thus disappointing and showed that the BladderScan® did not have the ability to be more precise in

the determination of urine in the bladder. However, since the device was developed for use on humans (Al-Shaikh *et al.* 2009; Thanagumtorn 2016), it might be of interest to adapt a technique that is usable with animals and more useful in animal research laboratories.

Postoperatively, midstream urine specimens were sampled with a paper kidney shaped dish. The urine was collected whenever possible, and we managed to successfully retrieve urine from all the graft recipients. The first few times when we were attempting to collect free-flow urine, the pigs stopped urinating when the dish was put under them. However, it was noted during the training sessions that pressing the water nipple in the pen to create the sound of running water, was often an effective way of stimulating the pigs to produce free-flow urine.

Figure 4. Ultrasound examinations of urinary bladder (left) and renal graft (right). The pigs were brushed and offered fruit if needed. Photo: Anneli Rydén

5.6 Evaluation of anaesthesia techniques for different purposes

5.6.1 Induction

Induction of anaesthesia with zolazepam/tiletamine and medetomidine or dexmedetomidine (ZTMe and ZTDe, respectively) in Study I, II, III and IV was uncomplicated and reliable in all pigs. The animals were down in lateral recumbency within six minutes (range 2–6) and unconscious within 15 minutes (range 5–15). Induction of anaesthesia in pigs with the ZT combinations (ZT-xylazine, ZT-medetomidine) has been used at our

department for several years (Henrikson *et al.* 1995; Malavasi *et al.* 2005) and has been reported to rapidly immobilize pigs (Lee *et al.* 2010; Chang *et al.* 2021). The combination has been proven to be easy to administer since the volume of the mixture is small enough for a single IM injection and the sedation produced by this combination is relatively fast.

In some experimental laboratories, the choice of drugs in the induction protocol is based on tradition in the laboratory and not the specific purpose of the study. In addition, research laboratories have requested alternative and less stressful induction protocols intended for use in pigs. For that reason, we wanted to compare the induction time and ease of intubation after the ZTMe combination with a combination of midazolam and ketamine followed by fentanyl before intubation (MiKF) (Study II). The MiKF combination is routinely used in several research facilities, including ours. The results showed that the time from injection to unconsciousness was shorter in the ZTMe group than in the MiKF group (4.0 ± 1.0 min and 6.8 ± 2.8 min respectively) (p=0.030) (Figure 5). In Study II, the reaction of the animals receiving IM injections was not assessed. It is noteworthy that the maximum volume recommended for an IM injection at one site (0.25 mL kg^{-1}) (Diehl *et al.* 2001) is larger than the actual volume of the ZTMe injection (0.05 mL kg^{-1}), and the volume of the MiKF injection (0.50 mL kg^{-1}) is twice the recommended amount.

5.6.2 Intubation

The endotracheal intubation in Study I, II and IV was possible without increasing the depth of anaesthesia. Pigs were intubated 13–25 minutes after the start of the anaesthesia induction and the intubation procedure took less than two minutes. In Study II the intubation was easier to perform in the ZTMe group (p=0.041) when compared with MiKF group (Figure 5). The IM combination of ZTMe resulted in a loss of consciousness that lasted long enough to permit endotracheal intubation within 15 minutes from induction. Our results supported earlier published results regarding the efficacy of the induction protocol ZTMe (Malavasi *et al.* 2005; Lima-Rodríguez *et al.* 2008). Intubation in pigs can be challenging and it has been reported that laryngotracheal damage may occur due to the species-specific anatomical characteristics and the persistence of airway reflexes during the light plane of anaesthesia (Clutton *et al.* 1997). In Study III, when plasma concentrations were measured after an IM administration with a similar drug combination

(ZT-dexmedetomidine), the pigs laid down after three minutes (range 2–4 minutes). The concentrations increased further and the average t_{max} for all drugs was 12 minutes after the induction (range 10–13 minutes). The relationship between plasma concentrations and the effect on the airway reflexes or any lag time for this, has not been established in this drug combination. However, both the measured t_{max} in Study III and the smooth intubation without complications or need for additional drugs in Study I, II and IV suggests that an optimal time to perform endotracheal intubation after induction with ZTDe might be from 13 minutes to 25 minutes after the IM injection.

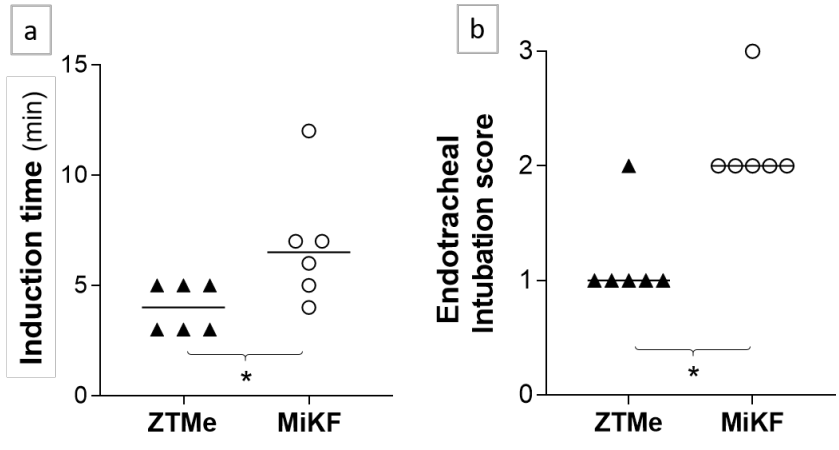

Figure 5. (a) Induction time (from injection to unconsciousness) (horizontal line indicates mean value) and (b) scoring of endotracheal intubation (scoring was rated from one to three, where a higher score indicated a more difficult procedure) (horizontal line indicates median value) after intramuscular injection with zolazepam–tiletamine 5 mg kg^{-1} (Z 2.5 + T 2.5 mg kg^{-1}) in combination with medetomidine 0.05 mg kg^{-1} (ZTMe) (▲) or midazolam 2 mg kg^{-1} , in combination with ketamine 10 mg kg^{-1} followed by fentanyl 4 µg kg^{-1} intravenously before intubation (MiKF) (○). Data considered significant (Mann–Whitney U test) at $p<0.05$ (*$p<0.05$).

5.6.3 General anaesthesia

An aim in Study II was to compare the requirement of anaesthetics in a subsequent eight hours of TIVA after induction with ZTMe or MiKF. The results showed differences in the required TIVA infusion rates in some of the time intervals between the two groups (p=0.040). Less adjustment of the rate, based on the response to the noxious stimuli, was required with ZTMe than with MiKF from 2–6 hours into the anaesthesia (Figure 6).

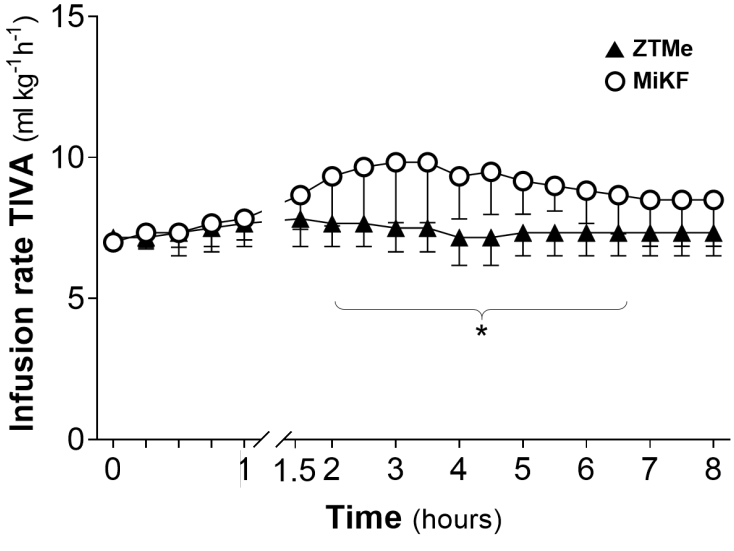

Figure 6. Infusion rate of total intravenous anaesthesia (TIVA) (mean ± SD) including 0.015 mg mL^{-1} midazolam, 4 mg mL^{-1} ketamine and 0.5 µg ml^{-1} of fentanyl. TIVA administration was based on a nociceptive stimulus response created by mechanically clamping the dewclaw. Data considered significant (mixed model) at $p<0.05$ (*$p<0.05$). ZTMe, zolazepam tiletamine in combination with medetomidine; MiKF, midazolam in combination with ketamine followed by fentanyl.

The combination of zolazepam-tiletamine has been previously reported to provide dissociative anaesthesia for 40 minutes (Lee & Kim 2012; Kumar *et al.* 2014) and midazolam ketamine for 2.5 hours (Boschert *et al.* 1996) in studies with a similar breed and age of pigs. Therefore, the induction combination probably provides satisfactory analgesia in both groups during the first hours of anaesthesia, which could explain the stable requirement of

the TIVA during the first hours. In a recent study, the antinociceptive effect in pigs of an α_2-agonist has been reported to provide superior analgesia when compared to fentanyl (Lervik *et al.* 2018), and a reduced minimal alveolar concentration of isoflurane anaesthetics in dogs (Pascoe *et al.* 2006; Lervik *et al.* 2012). Moreover, the response threshold to an electrical stimuli increased significantly in dogs and lasted up to six hours after an IM injection of medetomidine (Vainio *et al.* 1989) and up to 6.5 hours in horses (Ison *et al.* 2016). Additionally in Study III, plasma concentrations of dexmedetomidine were detected up to 19 hours after a single IM dos. Even though the therapeutic concentration might be less than the lower limit for detection, and medetomidine rather than dexmedetomidine was used, it is still possible that the α_2-agonist enhanced the antinociceptive effect for several hours (2–6) in the pigs induced with ZTMe compared to the pigs induced with MiKF (Study II).

Significant physiological differences were measured between the two drug combinations during the first two hours of TIVA (Figure 7). Some of the cardiovascular differences measured were probably an effect of the medetomidine included in the ZTMe induction drug combination. Therefore, the physiological responses in the animals during the first hours of anaesthesia, could be considered an effect of the anaesthetics included in the induction protocol. With that in mind, planned study interventions measuring physiological aspects might be influenced for at least two hours after induction if similar drugs and doses like those in Study II are used.

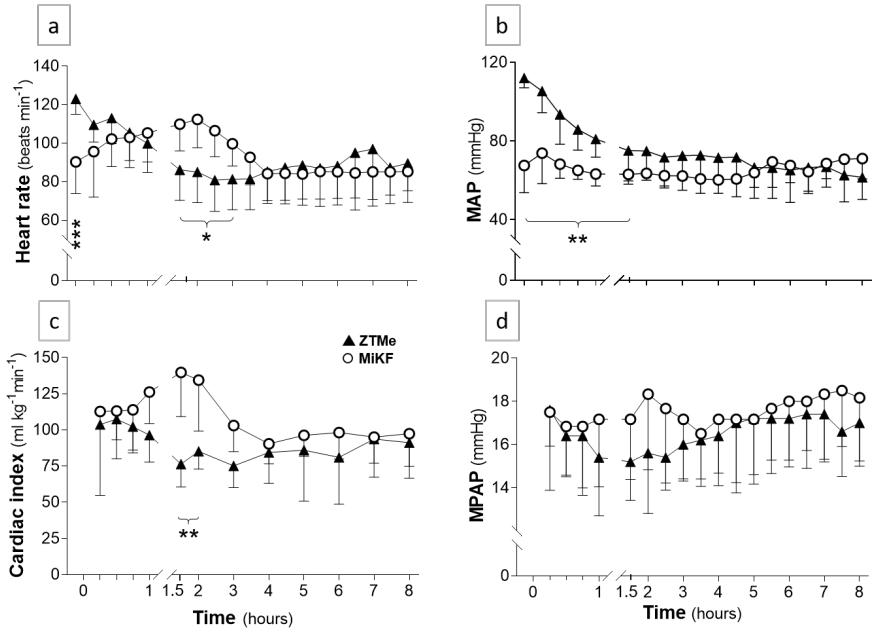

Figure 7. (a) Heart rate, (b) mean arterial blood pressure (MAP), (c) cardiac index and (d) mean pulmonary arterial pressure (MPAP) (mean SD) in pigs undergoing TIVA. Data considered significant (mixed model) at $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$). TIVA, total intravenous anaesthesia; ZTMe, zolazepam-tiletamine in combination with medetomidine; MiKF, midazolam in combination with ketamine followed by fentanyl.

Interestingly in Study II, shivering was observed in four out of six pigs in the MiKF group, but none of the pigs in the ZTMe group shivered during the entire anaesthesia. Shivering has earlier been observed in pigs undergoing anaesthesia and is commonly associated with hypothermia (Thoresen *et al.* 2001; Noll *et al.* 2018). In Study II the body temperature of the pigs in the MiKF group was 39.1 ± 0.2°C when the shivering occurred. A body temperature above 36°C (normal 38–39.5°C) has been recommended for pigs undergoing anaesthesia (Swindle 2007a). Consequently, we did not consider hypothermia to be the reason for the shivering observed in the animals. Trembling/shivering might interfere with planned interventions during imaging and surgery. Neuromuscular blocking (NMB) is recommended as a possible treatment for the condition (Ringer *et al.* 2016). However, masking the reflexes induced by NMB agents can make it difficult

to assess the depth of anaesthesia (Bradbury & Clutton 2016). It has been reported, that shivering was observed in nine out of ten piglets after a 10 mg kg^{-1} IV fentanyl injection (Ringer *et al.* 2016). In that study, shivering was observed to begin as early as 20 seconds and up to 14 minutes after the fentanyl injection, and was no longer obvious 43 minutes after the injection. In Study II, shivering was observed as early as 7–15 minutes after fentanyl injection, and it continued for up to 50 minutes in two animals. In addition, shivering has recently been reported in pigs maintained with inhalation anaesthesia and fentanyl infusion 5 µg $kg^{-1}h^{-1}$ (Haga *et al.* 2021).
In Study II, an initial fentanyl injection of 4 µg kg^{-1} administered before intubation in the MiKF group was followed by 3.5 mg kg^{-1} h^{-1} IV during TIVA. More studies are needed to determine if the dose of fentanyl administered in the MiKF group is critical for the development of shivering and if the muscle relaxation produced by the α_2-agonist medetomidine prevents the development of shivering in pigs undergoing anaesthesia. Careful monitoring of physiological alterations as well as the registration and documentation of side effects like shivering is a cornerstone in the care of animals undergoing anaesthesia and is a vital task for the veterinary nurse to perform.

5.6.4 Short-term dissociative anaesthesia

There are situations when pigs need to be immobilised for short procedures such as for computer tomography or magnetic resonance imaging examinations, transportations or catheterisations, etc. One of the aims of this thesis was to investigate the pharmacokinetic and pharmacodynamic effects of ZT in combination with dexmedetomidine and butorphanol without intubation and subsequent general anaesthesia. Therefore, in Study III, the clinical responses were measured when a combination of zolazepam, tiletamine, dexmedetomidine and butorphanol (ZTDeB) was administered for short-term dissociative anaesthesia. The results of this study suggest that IM administration of ZTDeB provides up to two hours of anaesthesia with antinociception for up to 120 minutes (range 60–120), regular breathing patterns, and stable HR. In addition, the animals in this study exhibited good muscle relaxation with no spontaneous movements until recovery. These results are in line with previously published studies where an α_2 in combination with TZ, and either the addition or absence of an opioid was

evaluated (Chang *et al.* 2021). Muscle relaxation of the animals is crucial during transportation and diagnostic imaging procedures (Kaiser *et al.* 2007). In Study III these procedures were only imitated. Considering that after the successful placement of the catheters, sampling of blood and the ability to move the pigs in the pen without any reaction from the animals, it should be possible to accomplish short-term transports and examinations of pigs anaesthetised with the ZTDeB combination.

After one hour of anaesthesia, one-third of the initial dose of the combination was administered IV to the pigs in Group Repeated. The repeated dose extended the anaesthesia by approximately one-half hour. One pig exhibited a ten second episode of apnoea that occurred immediately after the IV administration of the repeated dose. It is known that respiratory effects such as apnoea can occur after injectable anaesthetic drugs are administered, especially if the anaesthetics are administered rapidly (Swindle 2007a). This side-effect should be considered whenever drugs are injected during ongoing anaesthesia. The episode was easily managed and the pig started to breathe spontaneously after the application of light pressure on its chest. In case of complications, skilled staff and equipment for prompt endotracheal intubation or placement of a laryngeal mask and equipment to ventilate the pigs with a self-inflating bag and supplemental oxygen were available. The repeated dose was based on a pilot study performed at our research laboratory and previous clinical experience with the drug combination. However, the plasma concentration, pharmacokinetics and pharmacodynamics of such agents may also aid in deciding the criteria for dose and when to administer a repeated injection to prolong anaesthesia (Lehmann *et al.* 2017). Since the pigs started to move at 70 minutes (range 70–140) see Figure 8 and Table 2 after the IM induction in the present study, that might be the ideal time for the repeated injection. However, further studies are needed to investigate the ideal dose and time for a repeated injection to safely prolong the anaesthesia if needed.

The pigs were not intubated in Study III because the aim was to relate respiratory function to the plasma concentration of the drugs used and not to examine the effects of a supramaximal stimulus, *i.e.* intubation. In addition, there are situations where intubation is usually avoided, such as when anaesthetizing free-ranging wild boars (Barasona *et al.* 2013; Morelli *et al.* 2021). In Study I, II and IV it was shown that intubation was possible 13–25 minutes after induction with the ZT combination, which was proven in Study

III to be shortly after the average t_{max} for IM administration of the drugs (Figure 8). In Study II, the induction was followed by maintenance inhalation anaesthesia and mechanical ventilation making intubation necessary. In any case, the intubation of pigs is challenging and the procedure can result in several complications, *e.g*. changes in the depth of anaesthesia, reflexes, and breathing pattern (Oshodi *et al.* 2011).

During anaesthesia in Study III, SpO_2 was, at its lowest 86% for one pig, and above 89% for the rest of the pigs (range 90–99%). One weakness in this study was the use of pulse oximetry when measuring oxygen saturation. Blood gas analysis is considered as gold standard when SaO_2 is assessed, however we considered that placement of an arterial catheter could affect the depth of the anaesthesia, which was one important variable during the trial. Furthermore, it has been reported that the accuracy of pulse oximetry is high when the SpO_2 is in the 80–100% (Jensen *et al.* 1998; Batchelder & Raley 2007).

There were no differences in HR (range 93–179 beats min^{-1}) or RR (range 20–68 breaths min^{-1}) over time in the pigs that were anaesthetised with one or two doses. Body temperatures decreased in both groups over time (range 37.3–40.1°C), but were still above the lowest recommended temperature (36°C) for pigs undergoing anaesthesia. No temperature differences were seen between the groups. The decrease in temperature occurring in both groups could probably have been avoided with the use of heating pads and blankets. Developing techniques and arranging heating measures for pigs during transport is another important nursing duty.

In Study III, the plasma concentrations of the drugs included in the combinations and their relationships to the clinical responses were assessed (Table 2). The drug concentration and pharmacokinetic analyses included data from seven pigs (Single=3 and Repeated=4) since samples from five pigs were lost. Physiological and clinical results included data from all 12 pigs (Single=6 and Repeated=6). The serum concentration of the different drugs varied between the pigs, which may be explained by the fact that the initial dose was given IM. When the pigs started to move and even stand up, the concentration of zolazepam and butorphanol still showed some variation during the awakening period, while both tiletamine and dexmedetomidine were lower compared to the initial levels after induction of anaesthesia. It is likely that these drug concentrations predict such events due to dexmedetomidine's sedative effect and tiletamine's dissociative anaesthetic

effect. In polypharmacy, the drugs may have an impact on each other's pharmacokinetic profiles, and a shorter t_{max} for zolazepam and tiletamine (72 ± 6 and 90 ± 12 minutes respectively) was observed in Study III when compared to other studies in pigs that only administered zolazepam and tiletamine (Lin *et al.* 1993; Kumar *et al.* 2006; Kumar *et al.* 2014). It was not possible to link each drug's pharmacokinetics to the pharmacodynamics, but rather, the combined effect of all drugs together for use in robust short-term anaesthesia have been described in Study III.

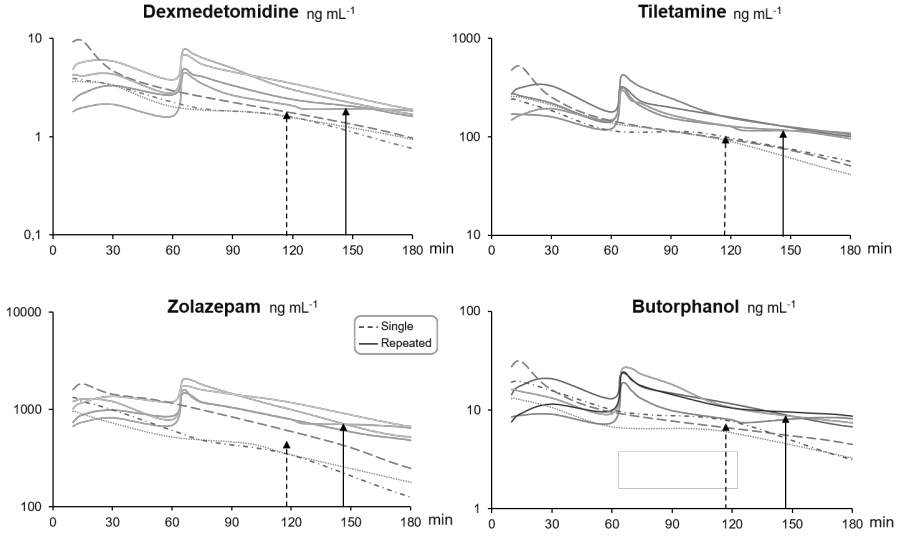

Figure 8. Profiles of log plasma concentrations over time for short-term anaesthesia induced by intramuscular administration of dexmedetomidine 0.025 mg kg^{-1}, zolazepam 2.5 mg kg^{-1}, tiletamine 2.5 mg kg^{-1} and butorphanol 0.1 mg kg^{-1} in pigs (n=7). Group Single (dotted lines n=3) received a single dose at time zero. Group Repeated (solid lines n=4) also received one-third of the initial dose intravenously at 60 min. Time to first spontaneous movement is indicated with a dotted arrow for Group Single and a solid arrow for Group Repeated

Table 2. Time range in minutes and the corresponding plasma concentration range for each drug and noted observation during the short-term anaesthesia induced by dexmedetomidine 0.025 mg kg^{-1}, tiletamine 2.5 mg kg^{-1}, zolazepam 2.5 mg kg^{-1}, and butorphanol 0.1 mg kg^{-1} after IM administration in growing pigs Single (n=6) and Repeated (n=6). All pigs received a single dose at time zero, and Group Repeated also received one-third of the initial dose intravenously at 60 minutes. All blood samples were taken relative to the noted observation except for lateral recumbency, which was taken at five minutes after induction. Plasma concentrations were available for seven pigs.

	Time (min) **from injection** (IM)		**Concentration range** (ng mL^{-1}) **Group Single & Repeated**			
Noted observations	**Single**	**Repeated**	**Dexmedetomidine**	**Tiletamine**	**Zolazepam**	**Butorphanol**
Lateral recumbency	2-4		<1.59–3.56	<126–268	<106–789	<5.22–15.60
Unconsciousness	5-15		1.99–6.00	169–337	737–1330	8.86–19.20
Last sample before response to noxious stimulus	60-120	120	2.01–3.48	115–158	520–1180	6.74–11.60
Palpebral reflexes	60-70	126-180	1.69–2.38	101–129	525–782	6.80–10.50
Response to noxious stimulus	70-140	126-180	1.69–2.38	101–129	525–782	6.80–10.50
First movement	70-140	126-180	1.69–1.97	106–123	444–713	6.47–9.42
Standing	120-180	175-240	1.14–2.01	73.7–109	250–678	4.74–8.70

5.7 Postoperative care

The pigs in Study III and IV recovered quickly after anaesthesia. The animals started to eat and drink as soon as they had access to food, and they gained weight at the same rate as before anaesthesia. The animals were then observed and filmed according to the different protocols in each study. In Study III the recovery was assessed through on-site observation of the animals and later from video recordings. The median recovery score was excellent, with the animals making one or two attempts to stand with very mild signs of excitement. Two animals, one in each group, scored as a good recovery, and none of the animals scored as a poor recovery. Overall, the drug combination ZTDeB was successful in producing a good to excellent recovery.

5.7.1 Observations in the early postoperative phase

During recovery from anaesthesia, the pigs were observed until they were fully awake. In Study I, the kidney transplanted animals were cared for and personally monitored by veterinarians and veterinary nurses during the first

24 hours after anaesthesia. During the remaining recovery period, they were observed personally on-site and via video cameras several times a day. One pig stopped breathing shortly after extubation. This side effect has been described previously in pigs (Smith & Swindle 2008) and reference was made to the species' tendency to develop partial airway obstruction during extubation. In addition, death due to laryngospasm after extubation has also been previously reported (He *et al.* 2013), making extubation a critical step. Thus, it is important to be prepared to treat complications in the early postoperative phase. Consequently, it is prudent that the pre- and postoperative unit is prepared with equipment for acute tracheal intubation and tracheostomy.

Three pigs out of six in the first trial (Study I) showed weakness in the left hind limb during recovery, to the degree that, the animals could not regain a standing position even when manually assisted. This can be explained by the fact that they were positioned in an unnatural position with their legs extended to facilitate the transplantation surgery. An additional cause of the complication, can be that they were placed on their 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 in humans, peripheral nerve damage is usually the result of the nerve being compressed or stretched, or an exaggerated positioning for prolonged periods of anaesthesia. In the present study, as soon as the paresis was seen, the staff began massaging the area and supporting the animals to help them stand and walk several times during the following 12 hours. All the affected pigs were recovered within 24 hours. In the following trials in Study I, the pigs were placed in a different position during surgery and recovery, and the staff turned the pigs from side-to side and massaged their musculus gluteus medius, biceps femoris and gluteus maximus several times during the first 24 hours. The pigs were also allowed to walk in the corridor to increase blood circulation. Despite the nursing measures, one pig out of 10, in the last trial (Study I) suffered a short, but transient weakness of its leg during the first two hours after surgery. Unfortunately, the reason for the paresis is only speculative, and therefore remains unknown. Veterinary nurses, surgeons and persons involved in the perioperative phase need to be aware of this potential complication (Borgeat 2005). In the future, investigations studying the cause would be beneficial so these complications in the postoperative period can be prevented. In Study I, the animals included

were a part of a transplantation study whose design was not within the scope of this thesis. However, some pigs underwent a nephrectomy and were moderately affected postoperatively. The weight gain in these pigs was 0.2 ± 0.4 kg the day after surgery. The reduced appetite postoperatively together with the use of energy for homeostasis can be the reason for the weight reduction in some animals. The animals started to eat and drink 24 hours after surgery, and increased their weight again. The animals that had not undergone a nephrectomy increased in weight postoperatively at the same rate as before anaesthesia. It has been reported that pigs receiving analgesia have shown an increased short (Malavasi *et al.* 2006) and long-term weight gain (Telles *et al.* 2016), when compared to pigs that have undergone abdominal surgery or castration without analgesia. In Study I during the first trial, the animals received 0.03 mg kg^{-1} day^{-1} buprenorphine postoperatively, which is admittedly within the recommended dose interval (Rodriguez *et al.* 2001). However, since the pigs' appetite and health status were affected negatively, they might have needed a higher dose or additional analgesia.

5.7.2 Adaptation of an AI technique intended for activity measurement

The collected recordings of three animals during nine days, led to the detection of the animals in approximately 50% of the sampled footage with the use of the semantic segmentation model. Based on the data, activity levels in the animals could be analysed. After treatment with transdermal fentanyl, the activity of the animals increased compared to baseline by 13% and 5% in pigs 1 and 2 respectively, but decreased 15% in pig 3. When pigs 1 and 2 were treated with buprenorphine IV, their activity levels based on the recorded data increased by 39% and 29%, whereas the pig treated with buprenorphine IM increased its activity with 9% compared to baseline (Figure 9).

During the first two weeks when the animals were in the training programme (Figure1), the semantic segmentation model was trained to detect the individual animals. During the first two days of this period, the programme failed to detect the animals. Since the programme is intended for horses, we thought that the low detection rate could be due to the pigs' light colour and small size. Therefore, we decided to improve the detection of the animals by painting the protective jackets and the pigs. This resulted in an increased detection rate each day during the remaining training period.

Based on personal on-site observations and later also from the videos, on some occasions two to five minutes after the administration of buprenorphine IV, pigs 1 and 2 showed increased signs of restlessness. Pig 2 additionally started to salivate, pant and climb up the sides of the pen wall several times during the first minutes. The behaviours continued for 10–15 minutes in pigs 1 and 2, but none of the behaviours were noted in the pig that received buprenorphine IM (pig 3). Side effects similar to those observed have been described following the administration of the opioid butorphanol to pigs (Hug *et al.* 2018; Pavlovsky *et al.* 2021) and following buprenorphine administration to goats (Ingvast-Larsson *et al.* 2007) and mice (Cowan *et al.* 1977). This side-effect should be addressed as it can cause stress for the animals, and also complicate blood sampling and examinations occurring shortly after IV administration. Reports of similar behaviours in pigs likely caused by buprenorphine are rare and need further investigation.

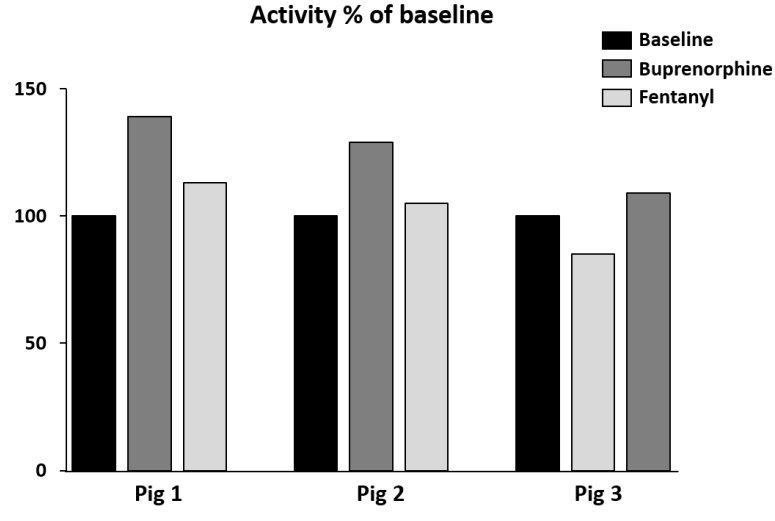

Figure 9. The activity of three individual pigs (1, 2, 3) measured with AI technique and presented as percent of baseline. Baseline measurements were collected during three consecutive days before the start of treatment. The pigs were randomly allocated to three days of treatment with either buprenorphine injections or transdermal fentanyl.

The assessment of acute pain in pigs is known to be difficult, and has recently been summarised in a review by Bradbury *et al* (2016). The National Research Council committee (2009) reported that signs of pain in animals can be depressed behaviour activity, *e.g.* the animals may remain immobile and be reluctant to stand or move. On the other hand, activities that can be interpreted as increased activity, *e.g.* lying down and getting up, shifting weight, circling, or pacing have also been associated with pain, which is described in the same report (National Research Council Committee on Recognition& Alleviation of Pain in Laboratory 2009). Even though specific nociceptive assessment methods utilising nociceptive threshold testing and facial grimace scoring have been published (Di Giminiani *et al.* 2015; Di Giminiani *et al.* 2016; Luna *et al.* 2020), extracting information from these pictures remains an expensive, time-consuming, manual task. In Study IV, we demonstrated that information of changed activity can be automatically extracted by deep learning, a cutting-edge type of artificial intelligence. These findings support an earlier study using a similar technique where pigs' behaviours were monitored to detect early signs of tail biting (D'Eath *et al.* 2018). This pilot study will be followed by similar trials and studies where pain can be expected, *e.g.* from surgery. In addition, the method could be validated against results from human observers and existing pain measuring instruments (Luna *et al.* 2020). The method can provide us with an objective efficient and automated means of monitoring animal welfare in research animals. It is still in its infancy and therefore more studies are needed to further develop this highly promising field.

The animals in the present study were not expected to change their behaviour activity due to pain. The observed changes in activity were probably due to the different opioid treatments. Specific assessment of any effects of the drugs *per se* might be useful and need to be investigated in future studies.

5.7.3 Plasma concentrations after the administration of opioids

The mean fentanyl plasma concentration 16–72 hours after the application of the transdermal patch was on average 0.25, 0.87 and 0.40 ng mL^{-1} for pigs 1, 2, 3 respectively. The fentanyl concentrations over time for each pig is presented in Figure 10.

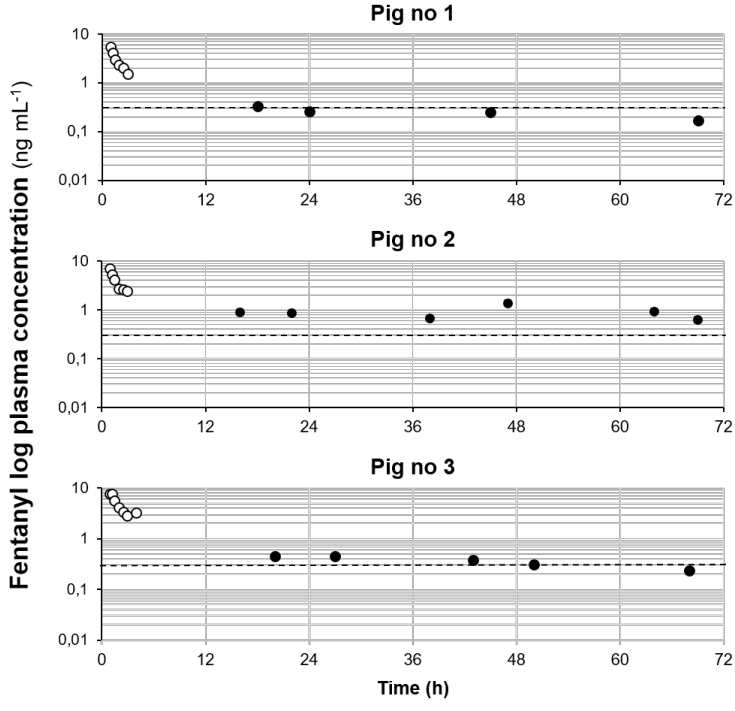

Figure 10. Profiles of plasma concentrations of fentanyl over time after an initial bolus of fentanyl 0.025 mg kg^{-1} IV at time 0 and followed by Constant Rate Infusion (CRI) of fentanyl 0.025 mg kg^{-1} h^{-1} for 60 minutes. A transdermal fentanyl patch (100 µg h^{-1}) was placed on the skin in the interscapular area at time 0. Blood samples were collected 5, 15, 30, 60, 120 minutes after the CRI was disconnected (○) and was followed by sampling twice a day for three consecutive days (●). The patch was removed at 72 hours. The dotted line indicates the suggested lowest therapeutic concentration for fentanyl (0.3 ng mL^{-1}) (Osorio Lujan *et al.* 2017).

As shown in Figure 11, the concentration declines rapidly after the CRI was discontinued and reached a steady state from the transdermal uptake from the fentanyl patch (100 µg h^{-1}) that was placed on the skin in the interscapular area at time 0. The concentration then stayed over or just below the suggested therapeutic concentration for fentanyl of 0.3 ng mL^{-1} (Harvey-Clark *et al.* 2000; Osorio Lujan *et al.* 2017) for all measured time points. As no sampling was performed for the time 4–16 hours, an extrapolated line was drawn for the elimination of fentanyl after the end of CRI indicating that the concentration will stay over 0.3 ng ml^{-1} until approximately 6-8 hours and that the onset of action from the patch applied at the start of the CRI was

uncertain in this study. The mean concentrations from other studies of fentanyl patches (50-100 µg h^{-1}) are included in Figure 11 for comparison.

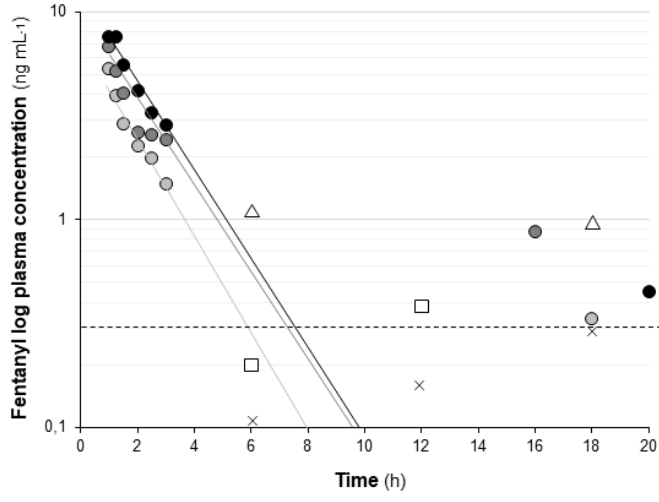

Figure 11. Individual values in 3 pigs from Study IV (●●●) of plasma fentanyl concentrations over time after an initial bolus of fentanyl 0.025 mg kg^{-1} IV that was followed by constant rate infusion of fentanyl 0.025 mg kg^{-1} h^{-1} over 60 minutes. A fentanyl patch (100 µg h^{-1}) was placed on the skin in the interscapular area at time 0. The solid lines represents the extrapolated decrease of fentanyl from the first six measurements after the CRI was discontinued. The dotted horisontal line indicates the suggested lowest therapeutic concentration for fentanyl (0.3 ng mL^{-1}). Mean concentration from other studies are included in the figure; 6 pigs Wilkinson *et al* (2001), 100 µg h^{-1} (x); 3 pigs Lujan *et al* (2017), 100 µg h^{-1} (Δ); 8 pigs Malavasi *et al* (2005), 50 µg h^{-1} (□).

After 72 hours, when the cover bandage and transdermal patch were removed from pig 1, a fold was noted on the fentanyl patch. The result of the fold was that only 70% of the patch had been in contact with the pig's skin. A similar case report has recently been published where the transdermal patch became dislodged and was probably ingested by the pig (Sredenšek *et al.* 2020). The incident in study IV might have been due to incorrect application of the patch or the pig managed to dislodge part of it during the study period. However, the patches were secured with thick elastic tape. The incident with the fold could be the reason for the wide variation in the plasma concentration of pig 1 when compared with pigs 2 and 3. However, a large

variation between pig 2 and 3 was also noted. Wide variations in plasma concentration in individual pigs have been previously reported (Harvey-Clark *et al.* 2000; Malavasi *et al.* 2005; Malavasi *et al.* 2006; Osorio Lujan *et al.* 2017). Nonetheless in those studies, the application or failure of contact with the skin is rarely discussed. Therefore, these important nursing measures need to be investigated and improved before fentanyl patches can be used in future studies in our research laboratory. The average fentanyl concentration was in the range of or just below the concentration level that other investigators have associated with adequate analgesia (0.3–0.6 ng mL^{-1}) (Osorio Lujan *et al.* 2017).

In pig 3, one of the jugular catheters dislodged. Since the remaining catheter was used for blood sampling only, the buprenorphine injections were given IM. After the IV administrations, the C_{max} for buprenorphine was 24.9 (± 8.3) for pig 1 and 24.0 ±17.8 ng mL^{-1} for pig 2 and the C_{trough} was 0.44 ± 0.26 and 0.67 ± 0.44 ng mL^{-1} respectively. For pig 3 that received IM administrations of buprenorphine, the C_{max} was 4.8 ± 2.3 and the C_{trough} was 0.39 (± 0.22) ng mL^{-1}.

For analyses of the buprenorphine concentrations, blood was sampled one minute before the buprenorphine was administered, five minutes after the IV administration, and 10 minutes after the IM administration. The results suggest that plasma concentrations in all three pigs were above the reported hypothesized therapeutic level of 0.1 ng mL^{-1} (Thiede *et al.* 2014) during the treatment period.

5.7.4 Pig grimace scale

During the training programme (Figure 1), the pigs were photographed in their pens once a day to discover a satisfactorily way to conduct the photography. Since the pigs were not restrained, they walked freely in the pen, which made it difficult to take photos from a correct angle and might have a negative impact on effective scoring (Dalla Costa *et al.* 2014; McLennan & Mahmoud 2019). Therefore, we decided to entice the animals with treats so that they would stand still. However, this could have altered the facial expression of the animals, and needs to be taken in to account. Restraining the animals or moving them to a specific observation arena has, however, been performed in similar studies (Di Giminiani *et al.* 2016; Viscardi *et al.* 2017)

Observation times for scoring the pictures were on average two hours (range 1–3 hours) for the six observers. A total of 131 of the 1044 assessments (13%) of the photos presented were judged as not assessable. Among the not assessable photos, the predominant reason was due to the assessment of the ears. There was a significant difference between scores over time and site of assessment. The median scores for facial expression of snout, ear and orbit were zero at baseline. During treatment, the range of scores varied between 0–2. The highest scores compared to baseline were seen among the evaluations of the snout expressions, and during the last day of the treatments with the buprenorphine injections ($p=0.042$) and the transdermal fentanyl patches ($p=0.021$). The scores during the last days in the treatments were higher compared to baseline. There was a significant difference ($p<0.03$) in the scoring between the assessors (A, B, C) who were aware of the study protocol, but unaware of which treatment was being used when the images were taken; and the assessors (D, E, F) who were unaware of protocol and treatments. There was also a difference in the scoring within the group where B scored significantly higher ($p<0.001$) compared to assessor A and C. No scoring differences were seen between assessors D, E and F.

The assessment of the ear was difficult as the catheter was secured with tape and these photos were often stated as not assessable. An interesting finding was that the snout assessments had the highest scores compared to baseline. The pig's snout is well developed to smell and root, and the presence as well as the taste of fruit could probably lead to changes in the expression of the snout, which in turn can be perceived as pain in a photograph. Nevertheless, the score was set to zero at baseline by all of the assessors, and at the end of each treatment the score was higher during both treatments.

Both the order of the treatments and the photographs were randomized, and although the pigs were treated with opioids, there can be a true sign of nociception from, *e.g*. the indwelling catheters in the ears. Besides injections administered in the buprenorphine group, the only invasive procedure performed in the present study was blood sampling from the catheter. We noticed that some pigs shook their head during the actual sampling, which can be a sign of nociception in the skin at the injection site. Other factors, such as the photographers improving their photography skills, or that the pigs learned that fruit is served when the camera is held up; makes the

interpretation of facial expression somewhat difficult in the present study. Another interesting result was that the assessors who had neither knowledge of the study design nor that the pigs were treated, graded the photos equally. The assessors who knew that the pigs were included in a research study gave both lower and higher scores, which can reflect the presence of bias.

6. Conclusions

The results from this thesis constitute refinements in perioperative nursing procedures and anaesthesia care of growing pigs in experimental studies. By applying refined nursing measures, improved animal welfare and better science can be achieved. Further, the number of animals used in research can be reduced.

In summary:
A two-week period of systematic training of pigs in research avoided stressful situations for the animals. Even if there are differences in how fast the individual animals' progress through the different training steps, a similar tameness was obtained within two weeks. Postoperatively, all animals accepted blood sampling, clinical examination, urine sampling, and ultrasound examination of their urinary bladder and the transplanted kidney without restraint.

It was possible to collect blood from MPC (2-methacryloylox-yethyl phosphoryl choline) catheters for up to 10 days during the experimental period. On post mortem examination, no blood clotting was seen on the surface of the MPC coated catheters even though the catheters were flushed with only saline.

Induction with the combination of ZTMe in the doses used provided a better quality of induction and endotracheal intubation was easier to perform compared to MiKF. Additionally based on an assessment of nociception, the required amount and adjustment of TIVA was less with ZTMe for up to six hours of anaesthesia. Some measured differences in HR and MAP occurred during the early phase of anaesthesia and were probably an effect of the medetomidine included in the ZTMe combination. The overall

cardiovascular stability was satisfactorily preserved with both drug combinations.

A combination of ZTDeB given intramuscularly provided a rapid induction of good quality, followed by up to two hours of anaesthesia with good muscle relaxation, no spontaneous movements and acceptable tolerability to nociceptive stimulus. The plasma concentration profile of the drugs was in line with the duration of the effect; showing a rapid uptake and distribution of the drugs. At one hour of anaesthesia, intravenous injection of one-third of the original dose extended the anaesthesia duration by another 30 minutes. In addition, the drug combination produced a good to excellent recovery. The technique used enabled an acceptable breathing pattern and physiological stability.

The pilot study showed that is seems to be possible to adapt an AI technology based on image vision for continuous perioperative monitoring and assessment of the activity in growing pigs. In the future, the technique might be of interest to use as a tool to evaluate activity among pigs expressing pain or other discomforts. Transdermal fentanyl or repeated buprenorphine injections at the current doses resulted in a serum concentration around the suggested therapeutic levels. Assessment of facial expressions was time consuming and many factors influenced the results.

References

Al-Shaikh, G., Larochelle, A., Campbell, C.E., Schachter, J., Baker, K. & Pascali, D. (2009). Accuracy of bladder scanning in the assessment of postvoid residual volume. *J Obstet Gynaecol Can,* 31(6), 526-532. https://doi.org/10.1016/s1701-2163(16)34215-3

Albrecht, M., Henke, J., Tacke, S., Markert, M. & Guth, B. (2014). Influence of repeated anaesthesia on physiological parameters in male Wistar rats: a telemetric study about isoflurane, ketamine-xylazine and a combination of medetomidine, midazolam and fentanyl. *BMC Vet Res,* 10, 310. https://doi.org/10.1186/s12917-014-0310-8

Alstrup, A. & Winterdahl, M. (2009). Imaging Techniques in Large Animals. *Scandinavian Journal of Laboratory Animal Science,* 36, 55-66.

Alstrup, A.K. & Smith, D.F. (2013). Anaesthesia for positron emission tomography scanning of animal brains. *Lab Anim,* 47(1), 12-8. https://doi.org/10.1258/la.2012.011173

Aschbacher, P.W. (1970). An adjustable metabolism stall for cattle. *J Anim Sci,* 31(4), 741-4.

Asif, S., Asawa, K., Inoue, Y., Ishihara, K., Lindell, B., Holmgren, R., Nilsson, B., Rydén, A., Jensen-Waern, M., Teramura, Y. & Ekdahl, K.N. (2019). Validation of an MPC Polymer Coating to Attenuate Surface-Induced Crosstalk between the Complement and Coagulation Systems in Whole Blood in In Vitro and In Vivo Models. *Macromol Biosci,* 19(5), e1800485. https://doi.org/10.1002/mabi.201800485

Bailey, J. (2018). Does the Stress of Laboratory Life and Experimentation on Animals Adversely Affect Research Data? A Critical Review. *Alternatives to laboratory animals: ATLA,* 46, 291-305. https://doi.org/10.1177/026119291804600501

Barasona, J.A., López-Olvera, J.R., Beltrán-Beck, B., Gortázar, C. & Vicente, J. (2013). Trap-effectiveness and response to tiletamine-zolazepam and medetomidine anaesthesia in Eurasian wild boar captured with cage and corral traps. *BMC Vet Res,* 9, 107. https://doi.org/10.1186/1746-6148-9-107

Baskin, J.L., Pui, C.H., Reiss, U., Wilimas, J.A., Metzger, M.L., Ribeiro, R.C. & Howard, S.C. (2009). Management of occlusion and thrombosis associated with long-term indwelling central venous catheters. *Lancet,* 374(9684), 159-69. https://doi.org/10.1016/s0140-6736(09)60220-8

Batchelder, P.B. & Raley, D.M. (2007). Maximizing the laboratory setting for testing devices and understanding statistical output in pulse oximetry.

Anesth Analg, 105(6 Suppl), S85-s94. https://doi.org/10.1213/01.ane.0000268495.35207.ab

Benson, G.J. & Thurmon, J.C. (1979). Anesthesia of swine under field conditions. *J Am Vet Med Assoc,* 174(6), 594-6.

Bollen, P., Kornerup Hansen, A. & Alstrup, A. (2010). *The laboratory swine, second edition*.

Borgeat, A. (2005). Neurologic deficit after peripheral nerve block: what to do? *Minerva Anestesiol,* 71(6), 353-5.

Boschert, K., Flecknell, P.A., Fosse, R.T., Framstad, T., Ganter, M., Sjøstrand, U., Stevens, J. & Thurman, J. (1996). Ketamine and its use in the pig. Recommendations of the Consensus meeting on Ketamine Anaesthesia in Pigs, Bergen 1994. Ketamine Consensus Working Group. *Lab Anim,* 30(3), 209-19. https://doi.org/10.1258/002367796780684863

Bradbury, A.G. & Clutton, R.E. (2016). Are neuromuscular blocking agents being misused in laboratory pigs? *Br J Anaesth,* 116(4), 476-85. https://doi.org/10.1093/bja/aew019

Bradbury, A.G., Eddleston, M. & Clutton, R.E. (2016). Pain management in pigs undergoing experimental surgery; a literature review (2012-4). *Br J Anaesth,* 116(1), 37-45. https://doi.org/10.1093/bja/aev301

Brenner, K.V. & Gurtler, H. (1981). [Further studies of swine reaction to fixation with a maxillary sling based on metabolic and hematologic parameters]. *Arch Exp Veterinarmed,* 35(3), 401-7.

Chang, L.J., Ko, J.C., Weil, A.B. & Weng, H.Y. (2021). Comparison of anesthetic and cardiorespiratory effects of tiletamine-zolazepam-detomidine-butorphanol, tiletamine-zolazepam-xylazine-butorphanol, and ketamine-detomidine-butorphanol in pigs. *J Am Vet Med Assoc,* 258(8), 883-891. https://doi.org/10.2460/javma.258.8.883

Charlton, J.E. (2005). A Report of the Task Force on Professional Education of the International Association for the Study of Pain. 4, 51.

Clarke, P. (2012). Veterinary nursing research: types, importance and dissemination. *The Veterinary Nurse,* 3(3), 142-146. https://doi.org/10.12968/vetn.2012.3.3.142

Clary, E.M., Bruch, S.M., Lau, C.L., Ali, A., Chekan, E.G., Garcia-Oria, M.J. & Eubanks, S. (2002). Effects of pneumoperitoneum on hemodynamic and systemic immunologic responses to peritonitis in pigs. *J Surg Res,* 108(1), 32-8.

Clutton, R.E., Blissitt, K.J., Bradley, A.A. & Camburn, M.A. (1997). Comparison of three injectable anaesthetic techniques in pigs. *Vet Rec,* 141(6), 140-6. https://doi.org/10.1136/vr.141.6.140

Clutton, R.E., Reed, F., Eddleston, M. & Hulse, E.J. (2013). Prolonged Anaesthesia in Minipigs. In.

Cooper, J.E. (2007). In: *Introduction to veterinary and comparative forensic medicine*. (Second Edition). Introductin Boca Raton, Fla, USA: CRP Press.

Cowan, A., Doxey, J.C. & Harry, E.J. (1977). The animal pharmacology of buprenorphine, an oripavine analgesic agent. *Br J Pharmacol,* 60(4), 547-54. https://doi.org/10.1111/j.1476-5381.1977.tb07533.x

Craig, A.D. (2003). Pain mechanisms: labeled lines versus convergence in central processing. *Annu Rev Neurosci,* 26, 1-30. https://doi.org/10.1146/annurev.neuro.26.041002.131022

D'Eath, R.B., Jack, M., Futro, A., Talbot, D., Zhu, Q., Barclay, D. & Baxter, E.M. (2018). Automatic early warning of tail biting in pigs: 3D cameras can detect lowered tail posture before an outbreak. *PLoS One,* 13(4), e0194524. https://doi.org/10.1371/journal.pone.0194524

Daifuku, R. & Stamm, W.E. (1984). Association of rectal and urethral colonization with urinary tract infection in patients with indwelling catheters. *Jama,* 252(15), 2028-30.

Dalgaard, L. (2015). Comparison of minipig, dog, monkey and human drug metabolism and disposition. *Journal of Pharmacological and Toxicological Methods,* 74, 80-92. https://doi.org/https://doi.org/10.1016/j.vascn.2014.12.005

Dalin, A.M., Magnusson, U., Haggendal, J. & Nyberg, L. (1993). The effect of transport stress on plasma levels of catecholamines, cortisol, corticosteroid-binding globulin, blood cell count, and lymphocyte proliferation in pigs. *Acta Vet Scand,* 34(1), 59-68.

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E. & Leach, M.C. (2014). Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS One,* 9(3), e92281. https://doi.org/10.1371/journal.pone.0092281

Damy, S.B., Camargo, R.S., Chammas, R. & Figueiredo, L.F. (2010). [Fundamental aspects on animal research as applied to experimental surgery]. *Rev Assoc Med Bras (1992),* 56(1), 103-11.

De Monte, V., Staffieri, F., Di Meo, A., Vannucci, J. & Bufalari, A. (2015). Comparison of ketamine-dexmedetomidine-methadone and tiletamine-zolazepam-methadone combinations for short-term anaesthesia in domestic pigs. *Vet J,* 205(3), 364-8. https://doi.org/10.1016/j.tvjl.2015.05.011

Di Giminiani, P., Brierley, V.L.M.H., Scollo, A., Gottardo, F., Malcolm, E.M., Edwards, S.A. & Leach, M.C. (2016). The Assessment of Facial Expressions in Piglets Undergoing Tail Docking and Castration: Toward the Development of the Piglet Grimace Scale. *Front Vet Sci,* 3(100). https://doi.org/10.3389/fvets.2016.00100

Di Giminiani, P., Stausholm, J.S., Viitasaari, E., Petersen, L.J. & Herskin, M.S. (2015). The effect of social isolation, gender and familiarity with the experimental procedure on tests of porcine nociceptive thresholds. *Vet Anaesth Analg,* 42(6), 648-56. https://doi.org/10.1111/vaa.12254

Diehl, K.H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vidal, J.M. & van de Vorstenbosch, C. (2001). A good practice guide to the

administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol,* 21(1), 15-23.

Duke-Novakovski, T., Ambros, B., Auckland, C.D. & Harding, J.C. (2012). The effects of succinylcholine or low-dose rocuronium to aid endotracheal intubation of adult sows. *Can J Vet Res,* 76(1), 57-61.

Dyce, K.M., Sack, W.O. & Wensing, C.J.G. (2010). *Textbook of Veterinary Anatomy*. 4th edition. (The urogenital apparatus 4th ed). Elsevier Health Sciences.

Einarsson, S., Brandt, Y., Lundeheim, N. & Madej, A. (2008). Stress and its influence on reproduction in pigs: a review. *Acta Vet Scand,* 50, 48. https://doi.org/10.1186/1751-0147-50-48

Ettrup, K., Glud, A., Orlowski, D., Fitting, L., Meier, K., Soerensen, J., Bjarkam, C. & Alstrup, A. (2011). Basic Surgical Techniques in the Göttingen Minipig: Intubation, Bladder Catheterization, Femoral Vessel Catheterization, and Transcardial Perfusion. *J Vis Exp*. https://doi.org/10.3791/2652

Festing, S. & Wilkinson, R. (2007). The ethics of animal research. Talking Point on the use of animals in scientific research. *EMBO Rep,* 8(6), 526-30. https://doi.org/10.1038/sj.embor.7400993

Fredriksen, B., Font, I.F.M., Lundström, K., Migdal, W., Prunier, A., Tuyttens, F.A. & Bonneau, M. (2009). Practice on castration of piglets in Europe. *Animal,* 3(11), 1480-7. https://doi.org/10.1017/s1751731109004674

Fruhauf, N.R., Oldhafer, K.J., Holtje, M., Kaiser, G.M., Fruhauf, J.H., Stavrou, G.A., Bader, A. & Broelsch, C.E. (2004). A bioartificial liver support system using primary hepatocytes: a preclinical study in a new porcine hepatectomy model. *Surgery,* 136(1), 47-56. https://doi.org/10.1016/j.surg.2003.12.017

Fudge, M., Coleman, R.E. & Parker, S.B. (2002). A minimally invasive percutaneous technique for jugular vein catheterization in pigs. *Contemp Top Lab Anim Sci,* 41(1), 38-42.

Furbeyre, H. & Labussiere, E. (2020). A minimally invasive catheterization of the external jugular vein in suckling piglets using ultrasound guidance. *PLoS One,* 15(10), e0241444. https://doi.org/10.1371/journal.pone.0241444

Geovanini, G.R., Pinna, F.R., Prado, F.A., Tamaki, W.T. & Marques, E. (2008). Standardization of anesthesia in swine for experimental cardiovascular surgeries. *Rev Bras Anestesiol,* 58(4), 363-70.

Gieling, E.T., Nordquist, R.E. & van der Staay, F.J. (2011). Assessing learning and memory in pigs. *Anim Cogn,* 14(2), 151-73. https://doi.org/10.1007/s10071-010-0364-3

Golriz, M., Fonouni, H., Nickkholgh, A., Hafezi, M., Garoussi, C. & Mehrabi, A. (2012). Pig kidney transplantation: an up-to-date guideline. *Eur Surg Res,* 49(3-4), 121-9. https://doi.org/10.1159/000343132

Grandin, T. (1986). Minimizing Stress in Pig Handling in the Research Lab. *Lab animal,* 15(3).

Gutierrez, K., Dicks, N., Glanzner, W.G., Agellon, L.B. & Bordignon, V. (2015). Efficacy of the porcine species in biomedical research. *Front Genet,* 6, 293. https://doi.org/10.3389/fgene.2015.00293

Haga, H.A., Lervik, A. & Nordgreen, J. (2021). Inhibition and facilitation of nociceptively evoked muscular activity by fentanyl or dexmedetomidine in isoflurane-anaesthetized pigs. *Vet Anaesth Analg,* 48(2), 230-238. https://doi.org/10.1016/j.vaa.2020.09.007

Haga, H.A., Ranheim, B. & Spadavecchia, C. (2011). Effects of isoflurane upon minimum alveolar concentration and cerebral cortex depression in pigs and goats: an interspecies comparison. *Vet J,* 187(2), 217-20. https://doi.org/10.1016/j.tvjl.2009.11.010

Harris, W.H. (1974). A technique for chronic venous cannulation in swine. *Lab Anim,* 8(2), 237-40.

Harvey-Clark, C.J., Gilespie, K. & Riggs, K.W. (2000). Transdermal fentanyl compared with parenteral buprenorphine in post-surgical pain in swine: a case study. *Lab Anim,* 34(4), 386-98. https://doi.org/10.1258/002367700780387750

He, B., Musk, G.C., Mou, L., De Boer, B., Delriviere, L. & Hamdorf, J. (2013). Laparoscopic surgery for orthotopic kidney transplant in the pig model. *J Surg Res,* 184(2), 1096-101. https://doi.org/10.1016/j.jss.2013.03.015

Heinonen, M.L., Raekallio, M.R., Oliviero, C., Ahokas, S. & Peltoniemi, O.A. (2009). Comparison of azaperone-detomidine-butorphanol-ketamine and azaperone-tiletamine-zolazepam for anaesthesia in piglets. *Vet Anaesth Analg,* 36(2), 151-7. https://doi.org/10.1111/j.1467-2995.2008.00443.x

Henrikson, H., Jensen-Waern, M. & Nyman, G. (1995). Anaesthetics for general anaesthesia in growing pigs. *Acta Vet Scand,* 36(4), 401-11.

Holliman, C.J., Kenfield, K., Nutter, E., Saffle, J.R. & Warden, G.D. (1982). Technique for acute suprapubic catheterization of urinary bladder in the pig. *Am J Vet Res,* 43(6), 1056-7.

Hug, P.J., Cap, V.H., Honegger, J., Schüpbach-Regula, G., Schwarz, A. & Bettschart-Wolfensberger, R. (2018). Optimization of analgesia for piglet castration under isoflurane anaesthesia with parenteral butorphanol, meloxicam or intratesticular lidocaine. *Schweiz Arch Tierheilkd,* 160(7-8), 461-467. https://doi.org/10.17236/sat00169

Huppertz, N.D., Tolba, R.H. & Grosse, J.O. (2015). Micturition in Göttingen minipigs: first reference in vivo data for urological research and review of literature. *Lab Anim,* 49(4), 336-44. https://doi.org/10.1177/0023677215570993

Hurley, J. (2012). How to place and maintain a jugular catheter. *The Veterinary Nurse,* 3(6), 374-379. https://doi.org/10.12968/vetn.2012.3.6.374

Ingvast-Larsson, C., Svartberg, K., Hydbring-Sandberg, E., Bondesson, U. & Olsson, K. (2007). Clinical pharmacology of buprenorphine in healthy, lactating goats. *J Vet Pharmacol Ther,* 30(3), 249-56. https://doi.org/10.1111/j.1365-2885.2007.00858.x

Ishihara, K. (2012). Successful Development of Biocompatible Polymers Designed by Natures Original Inspiration. *Procedia Chemistry,* 4, 34-38. https://doi.org/https://doi.org/10.1016/j.proche.2012.06.005

Ison, S.H., Clutton, R.E., Di Giminiani, P. & Rutherford, K.M. (2016). A Review of Pain Assessment in Pigs. *Front Vet Sci,* 3, 108. https://doi.org/10.3389/fvets.2016.00108

Ivers, D.J. & Veum, T.L. (2012). Effect of graded levels of niacin supplementation of a semipurified diet on energy and nitrogen balance, growth performance, diarrhea occurrence, and niacin metabolite excretion by growing swine. *J Anim Sci,* 90(1), 282-8. https://doi.org/10.2527/jas.2011-4035

Jaber, S.M., Sullivan, S., Hankenson, F.C., Kilbaugh, T.J. & Margulies, S.S. (2015). Comparison of Heart Rate and Blood Pressure with Toe Pinch and Bispectral Index for Monitoring the Depth of Anesthesia in Piglets. *J Am Assoc Lab Anim Sci,* 54(5), 536-44.

Jacobson, A. (1998). Continuous infusion and chronic catheter access *Lab Anim,* 27(7), 37-46.

Jensen, L.A., Onyskiw, J.E. & Prasad, N.G. (1998). Meta-analysis of arterial oxygen saturation monitoring by pulse oximetry in adults. *Heart Lung,* 27(6), 387-408. https://doi.org/10.1016/s0147-9563(98)90086-3

Jurewitsch, B. & Jeejeebhoy, K.N. (2005). Taurolidine lock: the key to prevention of recurrent catheter-related bloodstream infections. *Clin Nutr,* 24(3), 462-5. https://doi.org/10.1016/j.clnu.2005.02.001

Kaiser, G.M., Breuckmann, F., Aker, S., Eggebrecht, H., Kuehl, H., Erbel, R., Fruhauf, N.R., Broelsch, C.E. & Quick, H.H. (2007). Anesthesia for cardiovascular interventions and magnetic resonance imaging in pigs. *J Am Assoc Lab Anim Sci,* 46(2), 30-3.

Kaiser, G.M., Heuer, M.M., Fruhauf, N.R., Kuhne, C.A. & Broelsch, C.E. (2006). General handling and anesthesia for experimental surgery in pigs. *J Surg Res,* 130(1), 73-9. https://doi.org/10.1016/j.jss.2005.07.012

Karges, H.E., Funk, K.A. & Ronneberger, H. (1994). Activity of coagulation and fibrinolysis parameters in animals. *Arzneimittelforschung,* 44(6), 793-7.

Khosroshahi, H.T., Oskui, R., Shoja, M.M., Tubbs, R.S. & Ardalan, M.R. (2007). Time-dependent variations in urine output after renal transplantation. *Transplant Proc,* 39(4), 932-3. https://doi.org/10.1016/j.transproceed.2007.04.006

Kolofousi, C., Stefanidis, K., Cokkinos, D.D., Karakitsos, D., Antypa, E. & Piperopoulos, P. (2013). Ultrasonographic features of kidney transplants and their complications: an imaging review. *ISRN Radiol,* 2013, 480862. https://doi.org/10.5402/2013/480862

Kumar, A., Mann, H.J. & Remmel, R.P. (2006). Determination of constituents of Telazol--tiletamine and zolazepam by a gas chromatography/mass spectrometry-based method. *J Chromatogr B Analyt Technol Biomed Life Sci,* 842(2), 131-5. https://doi.org/10.1016/j.jchromb.2006.05.014

Kumar, A., Mann, H.J., Remmel, R.P., Beilman, G.J. & Kaila, N. (2014). Pharmacokinetic study in pigs and in vitro metabolic characterization in pig- and human-liver microsomes reveal marked differences in disposition and metabolism of tiletamine and zolazepam (Telazol). *Xenobiotica,* 44(4), 379-90. https://doi.org/10.3109/00498254.2013.833362

Kurien, B.T., Everds, N.E. & Scofield, R.H. (2004). Experimental animal urine collection: a review. *Lab Anim,* 38(4), 333-61.

Lambeth, S.P., Hau, J., Perlman, J.E., Martino, M. & Schapiro, S.J. (2006). Positive reinforcement training affects hematologic and serum chemistry values in captive chimpanzees (Pan troglodytes). *American Journal of Primatology,* 68(3), 245-256. https://doi.org/https://doi.org/10.1002/ajp.20148

Laricchiuta, P., De Monte, V., Campolo, M., Grano, F., Iarussi, F., Crovace, A. & Staffieri, F. (2012). Evaluation of a butorphanol, detomidine, and midazolam combination for immobilization of captive Nile lechwe antelopes (Kobus magaceros). *J Wildl Dis,* 48(3), 739-46. https://doi.org/10.7589/0090-3558-48.3.739

Lee, I.K., Kye, Y.C., Kim, G., Kim, H.W., Gu, M.J., Umboh, J., Maaruf, K., Kim, S.W. & Yun, C.H. (2016). Stress, Nutrition, and Intestinal Immune Responses in Pigs - A Review. *Asian-Australas J Anim Sci,* 29(8), 1075-82. https://doi.org/10.5713/ajas.16.0118

Lee, J.Y., Jee, H.C., Jeong, S.M., Park, C.S. & Kim, M.C. (2010). Comparison of anaesthetic and cardiorespiratory effects of xylazine or medetomidine in combination with tiletamine/zolazepam in pigs. *Vet Rec,* 167(7), 245-9. https://doi.org/10.1136/vr.c3611

Lee, J.Y. & Kim, M.C. (2012). Anesthesia of growing pigs with tiletamine-zolazepam and reversal with flumazenil. *J Vet Med Sci,* 74(3), 335-9.

Lehmann, H.S., Blache, D., Drynan, E., Tshewang, P., Blignaut, D.J.C. & Musk, G.C. (2017). Optimum Drug Combinations for the Sedation of Growing Boars Prior to Castration. *Animals (Basel),* 7(8). https://doi.org/10.3390/ani7080061

Lemke, K.A. (2007). Pharmacology In: Thurmon JC, T.W., Benson GJ (ed.) *Lumb & Jones Veterinary Anesthesia and Analesia*. (4th ed.). Ames, Iowa 50014, USA: Blackwell Publishing. 221-258.

Lervik, A., Haga, H.A., Ranheim, B. & Spadavecchia, C. (2012). The influence of a continuous rate infusion of dexmedetomidine on the nociceptive withdrawal reflex and temporal summation during isoflurane anaesthesia in dogs. *Vet Anaesth Analg,* 39(4), 414-25. https://doi.org/10.1111/j.1467-2995.2012.00713.x

Lervik, A., Raszplewicz, J., Ranheim, B., Solbak, S., Toverud, S.F. & Haga, H.A. (2018). Dexmedetomidine or fentanyl? Cardiovascular stability and analgesia during propofol-ketamine total intravenous anaesthesia in experimental pigs. *Vet Anaesth Analg,* 45(3), 295-308. https://doi.org/10.1016/j.vaa.2017.08.012

Lighty, G.W., Jr., Spear, R.S., Karatay, M.C., Hare, C.L. & Carlson, R.J. (1992). Swine models for cardiovascular research: a low stress transport and restraint system for large swine. *Cornell Vet,* 82(2), 131-40.

Lima-Rodríguez, J.R., García-Gil, F.A., García-García, J.J., Rocha-Camarero, G., Martín-Cancho, M.F., Luis-Fernández, L., Crisóstomo, V., Usón-Gargallo, J. & Carrasco-Jiménez, M.S. (2008). Effects of premedication with tiletamine/zolazepam/medetomidine during general anesthesia using sevoflurane/fentanyl in swine undergoing pancreas transplantation. *Transplant Proc,* 40(9), 3001-6. https://doi.org/10.1016/j.transproceed.2008.09.042

Lin, H. (2014). *Perioperative monitoring and management of complications. Farm animal anesthesia : cattle, small ruminants, camelids, and pigs*. Ames, Iowa: Wiley-Blackwell,.

Lin, H.C., Thurmon, J.C., Benson, G.J. & Tranquilli, W.J. (1993). Telazol--a review of its pharmacology and use in veterinary medicine. *J Vet Pharmacol Ther,* 16(4), 383-418. https://doi.org/10.1111/j.1365-2885.1993.tb00206.x

Littell, R., Milliken, G., Stroup, W. Wolfinger, R. and Schabenberger O. (2006). *SAS for mixed models*. second ed. edition. SAS Institute Inc.

Lombardo, C., Damiano, G., Cassata, G., Palumbo, V.D., Cacciabaudo, F., Spinelii, G., Calvagna, C., Gioviale, M.C., Maione, C.L. & Lo Monte, A.I. (2010). Surgical vascular access in the porcine model for long-term repeated blood sampling. *Acta Biomed,* 81(2), 101-3.

Luna, S.P.L., de Araújo, A.L., da Nóbrega Neto, P.I., Brondani, J.T., de Oliveira, F.A., Azerêdo, L., Telles, F.G. & Trindade, P.H.E. (2020). Validation of the UNESP-Botucatu pig composite acute pain scale (UPAPS). *PLoS One,* 15(6), e0233552. https://doi.org/10.1371/journal.pone.0233552

Lundeen, G., Manohar, M. & Parks, C. (1983). Systemic distribution of blood flow in swine while awake and during 1.0 and 1.5 MAC isoflurane anesthesia with or without 50% nitrous oxide. *Anesth Analg,* 62(5), 499-512.

Malavasi, L.M. (2005). *Physiological and behavioral effects of opioids in pigs subjected to abdominal surgery.* Doctoral thesis, Department of clinical sciences Swedish university of agricultural sciences

Malavasi, L.M., Augustsson, H., Jensen-Waern, M. & Nyman, G. (2005). The effect of transdermal delivery of fentanyl on activity in growing pigs. *Acta Vet Scand,* 46(3), 149-57.

Malavasi, L.M., Jensen-Waern, M., Augustsson, H. & Nyman, G. (2008). Changes in minimal alveolar concentration of isoflurane following treatment with medetomidine and tiletamine/zolazepam, epidural morphine or systemic buprenorphine in pigs. *Lab Anim,* 42(1), 62-70. https://doi.org/10.1258/la.2007.006048

Malavasi, L.M., Jensen-Waern, M., Jacobson, M., Ryden, A., Ohagen, P. & Nyman, G. (2006). Effects of extradural morphine on end-tidal isoflurane concentration and physiological variables in pigs undergoing abdominal

surgery: a clinical study. *Vet Anaesth Analg,* 33(5), 307-12. https://doi.org/10.1111/j.1467-2995.2005.00260.x

Manell, E.A., Ryden, A., Hedenqvist, P., Jacobson, M. & Jensen-Waern, M. (2014). Insulin treatment of streptozotocin-induced diabetes re-establishes the patterns in carbohydrate, fat and amino acid metabolisms in growing pigs. *Lab Anim,* 48(3), 261-269. https://doi.org/10.1177/0023677213517683

Manteuffel, G., Langbein, J. & Puppe, B. (2009). Increasing farm animal welfare by positively motivated instrumental behaviour. *Appl Anim Behav Sci*(118), 191-198.

McGlone, J.J., Curtis, S.E, Houpt, T.R. (2001). Husbandry, anesthesia, and surgery. In: Wilson G. Pond, H.J.M. (ed.) *Biology of the Domestic Pig*. 2 edition. Ithaca, United States: Cornell University Press.

McGuill, M.W. & Rowan, A.N. (1989). Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques. *Ilar j,* 31(4), 5-20. https://doi.org/10.1093

McLennan, K. & Mahmoud, M. (2019). Development of an Automated Pain Facial Expression Detection System for Sheep (Ovis Aries). *Animals (Basel),* 9(4). https://doi.org/10.3390/ani9040196

Mkwanazi, M.V., Ncobela, C.N., Kanengoni, A.T. & Chimonyo, M. (2019). Effects of environmental enrichment on behaviour, physiology and performance of pigs - A review. *Asian-Australas J Anim Sci,* 32(1), 1-13. https://doi.org/10.5713/ajas.17.0138

Morelli, J., Rossi, S., Fuchs, B., Richard, E., Barros, D.S.B., Küker, S., Arnemo, J.M. & Evans, A.L. (2021). Evaluation of Three Medetomidine-Based Anesthetic Protocols in Free-Ranging Wild Boars (Sus scrofa). *Front Vet Sci,* 8, 655345. https://doi.org/10.3389/fvets.2021.655345

Moughan, P.J., Smith, W.C. & Kies, A.K. (1987). Endogenous urinary metabolite excretion in the growing pig. *New Zealand Journal of Agricultural Research,* 30(2), 183-187.

Musk, G.C., Zwierzchoniewska, M. & He, B. (2015). Catheterization of the urethra in female pigs. *Lab Anim,* 49(4), 345-8. https://doi.org/10.1177/0023677215587637

Nalon, E., Maes, D., Piepers, S., van Riet, M.M., Janssens, G.P., Millet, S. & Tuyttens, F.A. (2013). Mechanical nociception thresholds in lame sows: evidence of hyperalgesia as measured by two different methods. *Vet J,* 198(2), 386-90. https://doi.org/10.1016/j.tvjl.2013.08.016

National Research Council Committee on, Recognition. & Alleviation of Pain in Laboratory, A. (2009). The National Academies Collection: Reports funded by National Institutes of Health. In: *Recognition and Alleviation of Pain in Laboratory Animals*. Washington (DC): National Academies Press (US)

Copyright © 2009, National Academy of Sciences. https://doi.org/10.17226/12526

Nicholls, S.M., Mitchard, L.K., Murrell, J.C., Dick, A.D. & Bailey, M. (2012). Perioperative socialization, care and monitoring of National Institutes of Health miniature swine undergoing ocular surgery and sampling of peripheral blood. *Lab Anim,* 46(1), 59-64. https://doi.org/10.1258/la.2011.011026

Niiyama, M., Yonemichi, H., Harada, E., Syuto, B. & Kitagawa, H. (1985). A simple catheterization from the ear vein into the jugular vein for sequential blood sampling from unrestrained pigs. *Jpn J Vet Res,* 33(1-2), 1-9.

Nilsson, M., Herlin, A.H., Ardö, H., Guzhva, O., Åström, K. & Bergsten, C. (2015). Development of automatic surveillance of animal behaviour and welfare using image analysis and machine learned segmentation technique. *Animal,* 9(11), 1859-65. https://doi.org/10.1017/s1751731115001342

Noll, E., Diemunsch, S., Pottecher, J., Rameaux, J.P., Diana, M., Sauleau, E., Ruetzler, K. & Diemunsch, P. (2018). Prevention of laparoscopic surgery induced hypothermia with warmed humidified insufflation: Is the experimental combination of a warming blanket synergistic? *PLoS One,* 13(7), e0199369. https://doi.org/10.1371/journal.pone.0199369

Obernier, J.A. & Baldwin, R.L. (2006). Establishing an appropriate period of acclimatization following transportation of laboratory animals. *Ilar j,* 47(4), 364-9. https://doi.org/10.1093/ilar.47.4.364

Oshodi, A., Dysart, K., Cook, A., Rodriguez, E., Zhu, Y., Shaffer, T.H. & Miller, T.L. (2011). Airway injury resulting from repeated endotracheal intubation: Possible prevention strategies. *Pediatr Crit Care Med,* 12(1), e34-9. https://doi.org/10.1097/PCC.0b013e3181dbeb28

Osorio Lujan, S., Habre, W., Daali, Y., Pan, Z. & Kronen, P.W. (2017). Plasma concentrations of transdermal fentanyl and buprenorphine in pigs (Sus scrofa domesticus). *Vet Anaesth Analg,* 44(3), 665-675. https://doi.org/10.1016/j.vaa.2016.09.002

Pairis-Garcia, M.D., Johnson, A.K., Bates, J.L., Stock, M., Barth, L.A., Brommel, A.S., Stalder, K.J. & Karriker, L.A. (2014). Development and refinement of a technique for short-term intravascular auricular vein catheter placement in mature sows. *Lab Anim,* 48(1), 78-81. https://doi.org/10.1177/0023677213514044

Panepinto, L.M., Phillips, R.W., Norden, S., Pryor, P.C. & Cox, R. (1983). A comfortable, minimum stress method of restraint for Yucatan miniature swine. *Lab Anim Sci,* 33(1), 95-7.

Pascoe, P.J., Raekallio, M., Kuusela, E., McKusick, B. & Granholm, M. (2006). Changes in the minimum alveolar concentration of isoflurane and some cardiopulmonary measurements during three continuous infusion rates of dexmedetomidine in dogs. *Vet Anaesth Analg,* 33(2), 97-103. https://doi.org/10.1111/j.1467-2995.2005.00236.x

Paulson, G.D. & Cottrell, D.J. (1984). An apparatus for quantitative collection of urine from male cattle, sheep, and swine. *Am J Vet Res,* 45(10), 2150-1.

Pavlovsky, V.H., Corona, D., Hug, P.J., Kümmerlen, D., Graage, R. & Bettschart-Wolfensberger, R. (2021). Butorphanol induces anxiety-like -behaviour and distress in piglets. *Schweiz Arch Tierheilkd,* 163(7), 485-491. https://doi.org/10.17236/sat00309

Phillips, C.E., Li, Y.Z., Johnston, L.J., Goplen, A.E. & Shurson, G.C. (2012). Technical note: a technique for ear vein catheterization in group-housed sows. *J Anim Sci,* 90(2), 501-4. https://doi.org/10.2527/jas.2011-4390

Porter, D.G., Ryan, P.L. & Norman, L. (1992). Lack of effect of relaxin on oxytocin output from the porcine neural lobe in vitro or in lactating sows in vivo. *J Reprod Fertil,* 96(1), 251-60.

Raad, II & Hanna, H.A. (2002). Intravascular catheter-related infections: new horizons and recent advances. *Arch Intern Med,* 162(8), 871-8. https://doi.org/10.1001/archinte.162.8.871

Ringer, S.K., Spielmann, N., Weiss, M. & Mauch, J.Y. (2016). Fentanyl bolus induces muscle tremors in sevoflurane-anaesthetized piglets. *Lab Anim,* 50(4), 312-4. https://doi.org/10.1177/0023677215623896

Riviere, J.E. & Papich, M.G. (2001). Potential and problems of developing transdermal patches for veterinary applications. *Adv Drug Deliv Rev,* 50(3), 175-203. https://doi.org/10.1016/s0169-409x(01)00157-0

Rodriguez, N.A., Cooper, D.M. & Risdahl, J.M. (2001). Antinociceptive activity of and clinical experience with buprenorphine in swine. *Contemp Top Lab Anim Sci,* 40(3), 17-20.

Roughan, J.V. & Flecknell, P.A. (2003). Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *Eur J Pain,* 7(5), 397-406. https://doi.org/10.1016/s1090-3801(02)00140-4

Rushen, J., Schwarze, N., Ladewig, J. & Foxcroft, G. (1993). Opioid modulation of the effects of repeated stress on ACTH, cortisol, prolactin, and growth hormone in pigs. *Physiol Behav,* 53(5), 923-8.

Russell, W.M.S. & Burch, R.L. (1959). *The principles of humane experimental technique*. Wheathampstead (UK) Universities Federation for Animal Welfare.

Sakaguchi, M., Nishimura, R., Sasaki, N., Ishiguro, T., Tamura, H. & Takeuchi, A. (1996). Anesthesia induced in pigs by use of a combination of medetomidine, butorphanol, and ketamine and its reversal by administration of atipamezole. *Am J Vet Res,* 57(4), 529-34.

Seldinger, S.I. (2008). Catheter replacement of the needle in percutaneous arteriography. A new technique. *Acta Radiol Suppl (Stockholm),* 434, 47-52. https://doi.org/10.1080/02841850802133386

Shearer, I.J. & Neal, G.D. (1972). The establishment and maintenance of indwelling venous catheters in adult female pigs. *N Z Vet J,* 20(5), 73-5. https://doi.org/10.1080/00480169.1972.34015

Sluga, M., Ummenhofer, W., Studer, W., Siegemund, M. & Marsch, S.C. (2005). Rocuronium versus succinylcholine for rapid sequence induction of anesthesia and endotracheal intubation: a prospective, randomized

trial in emergent cases. *Anesth Analg,* 101(5), 1356-61. https://doi.org/10.1213/01.Ane.0000180196.58567.Fe

Smith, A.C., Ehler, W. & Swindle, M.M. (1997). *Anesthesia and analgesia in swine in* (Anesthesia and Analgesia in Laboratory Animals). New Yourk: Academic Press.

Smith, A.C. & Swindle, M.M. (1994). *Research Animal Anesthesia, Analgesia and Surgery*. Scientists Center for Animal Welfare.

Smith, A.C. & Swindle, M.M. (2006). Preparation of swine for the laboratory. *Ilar j,* 47(4), 358-63.

Smith, A.C. & Swindle, M.M. (2008). *Anesthesia and analgesia in swine,* 2nd edition. (Anesthesia and Analgesia in Laboratory Animals). New Yourk: Academic Press (Elsevier).

Spurlock, M.E. & Gabler, N.K. (2008). The development of porcine models of obesity and the metabolic syndrome. *J Nutr,* 138(2), 397-402. https://doi.org/10.1093/jn/138.2.397

Sredenšek, J., Bošnjak, M., Lampreht Tratar, U., Kosjek, T., Cemazar, M., Kržan, M. & Seliškar, A. (2020). Case Report: Intoxication in a Pig (Sus Scrofa Domesticus) After Transdermal Fentanyl Patch Ingestion. *Front Vet Sci,* 7(995). https://doi.org/10.3389/fvets.2020.611097

Steinbacher, R., von Ritgen, S. & Moens, Y. (2012). Laryngeal perforation during a standard intubation procedure in a pig. *laboratory animals,* 46, 261-3. https://doi.org/10.1258/la.2012.012032

Swindle, M.M. (1983). *Basic Surgical Exercises Using Swine*. Philadelphia: Praeger Press.

Swindle, M.M. (2007a). Swine in the laboratory: Anesthesia, Analgesia, and Perioperative Care. In: Swindle, M.M. (ed.) (Second Edition). Boca Raton, Fla, USA: CRP Press. 35-75.

Swindle, M.M. (2007b). Pancreas and spleen. In: Swindle, M.M. (ed.) *Swine in the laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques*. (Second Edition). Boca Raton, Fla, USA: CRP Press.

Swindle, M.M., Makin, A., Herron, A.J., Clubb, F.J., Jr. & Frazier, K.S. (2012). Swine as models in biomedical research and toxicology testing. *Vet Pathol,* 49(2), 344-56. https://doi.org/10.1177/0300985811402846

Swindle, M.M., Nolan, T., Jacobson, A., Wolf, P., Dalton, M.J. & Smith, A.C. (2005). Vascular access port (VAP) usage in large animal species. *Contemp Top Lab Anim Sci,* 44(3), 7-17.

Swindle, M.M. & Smith, A.C. (2013). Best practices for performing experimental surgery in swine. *J Invest Surg,* 26(2), 63-71. https://doi.org/10.3109/08941939.2012.693149

Sørensen, D.B. (2010). Never wrestle with a pig …. *laboratory animals,* 44(2), 159-161. https://doi.org/10.1258/la.2010.009100

Telles, F.G., Luna, S.P.L., Teixeira, G. & Berto, D.A. (2016). Long-term weight gain and economic impact in pigs castrated under local anaesthesia.

Veterinary and Animal Science, 1-2, 36-39. https://doi.org/https://doi.org/10.1016/j.vas.2016.11.003

Thanagumtorn, K. (2016). Accuracy of Post-Void Residual Urine Volume Measurement Using an Ultrasound Bladder Scanner among Postoperative Radical Hysterectomy Patients. *J Med Assoc Thai,* 99(10), 1061-6.

Thiede, A.J., Garcia, K.D., Stolarik, D.F., Ma, J., Jenkins, G.J. & Nunamaker, E.A. (2014). Pharmacokinetics of sustained-release and transdermal buprenorphine in Gottingen minipigs (Sus scrofa domestica). *J Am Assoc Lab Anim Sci,* 53(6), 692-9.

Thoresen, M., Satas, S., Løberg, E.M., Whitelaw, A., Acolet, D., Lindgren, C., Penrice, J., Robertson, N., Haug, E. & Steen, P.A. (2001). Twenty-four hours of mild hypothermia in unsedated newborn pigs starting after a severe global hypoxic-ischemic insult is not neuroprotective. *Pediatr Res,* 50(3), 405-11. https://doi.org/10.1203/00006450-200109000-00017

Tonge, M. & Robson, K. (2021). Hypoxaemia following suspected intubation of the tracheal bronchus of a pig. *Veterinary Record Case Reports,* 9(1), e19. https://doi.org/https://doi.org/10.1002/vrc2.19

Trim, C.M. & Braun, C. (2011). Anesthetic agents and complications in Vietnamese potbellied pigs: 27 cases (1999-2006). *J Am Vet Med Assoc,* 239(1), 114-21. https://doi.org/10.2460/javma.239.1.114

Urzainqui-Laborda, P. (2012). Venous catheters: Problems and measures in nursing care. *Diálisis y Trasplante,* 33, 134–141. https://doi.org/10.1016/j.dialis.2012.06.011

Waern, M.J. & Fossum, C. (1993). Effects of acute physical stress on immune competence in pigs. *Am J Vet Res,* 54(4), 596-601.

Vainio, O., Vähä-Vahe, T. & Palmu, L. (1989). Sedative and analgesic effects of medetomidine in dogs. *J Vet Pharmacol Ther,* 12(2), 225-31. https://doi.org/10.1111/j.1365-2885.1989.tb00664.x

Warren, J.W. (2001). Catheter-associated urinary tract infections. *Int J Antimicrob Agents,* 17(4), 299-303.

Viscardi, A.V., Hunniford, M., Lawlis, P., Leach, M. & Turner, P.V. (2017). Development of a Piglet Grimace Scale to Evaluate Piglet Pain Using Facial Expressions Following Castration and Tail Docking: A Pilot Study. *Front Vet Sci,* 4, 51. https://doi.org/10.3389/fvets.2017.00051

Vullo, C., Barbieri, S., Catone, G., Graïc, J.M., Magaletti, M., Di Rosa, A., Motta, A., Tremolada, C., Canali, E. & Dalla Costa, E. (2020). Is the Piglet Grimace Scale (PGS) a Useful Welfare Indicator to Assess Pain after Cryptorchidectomy in Growing Pigs? *Animals (Basel),* 10(3). https://doi.org/10.3390/ani10030412

You, T., Jiang, J., Chen, J., Xu, W., Xiang, L. & Jiao, Y. (2017). Necessity of heparin for maintaining peripheral venous catheters: A systematic review and meta-analysis. *Exp Ther Med,* 14(2), 1675-1684. https://doi.org/10.3892/etm.2017.4706

Zanella, A.J. & Mendl, M.T. (1992). A fast and simple technique for jugular catheterization in adult sows. *Lab Anim,* 26(3), 211-3.

Popular science summary

Animals are used in a variety of scientific disciplines and continue to aid our understanding of various diseases and the development of new medicines and treatments in both humans and animals. The pig has become an increasingly requested model in biomedical research, due in large part to its physiological and anatomical similarities to humans. Important information is lacking regarding nursing measures that can improve and simplify the interactions with the animals and increase animal welfare during experimental situations, which is highlighted in this thesis. The welfare of animals used in research is very important. There are good ethical, scientific, legal and economic reasons for making sure that animals are treated properly and used in minimum numbers in research.

In this thesis, we have shown that a 14-day structured training of the animals during the acclimatisation period enabled blood and urine sampling and ultrasound examination without the need for restraints postoperatively. When a coated catheter was assessed, the results showed that it was possible to collect blood from the catheters for up to ten days. When two different induction combinations were compared, differences in the quality of induction and the requirement for maintenance anaesthesia were seen between the two induction techniques. In addition, a drug combination for short-term anaesthesia was evaluated. The drug combination provided two hours of anaesthesia with stable physiological variables and spontaneous breathing. The plasma concentration profile of the drugs was in line with the duration of the effect. The use of an artificial intelligence technology for an objective assessment of activity in pigs seems to be a promising tool in experimental studies. In the future, the technology might be useful to evaluate the activity of pigs expressing pain or other discomfort.

In summary, the results show that nursing interventions, adjustment of anaesthesia techniques and the use of artificial technology to measure activity can contribute to stress-free handling and improved animal welfare in growing pigs according to the 3Rs.

Populärvetenskaplig sammanfattning

Djur används inom en mängd olika vetenskapliga discipliner och bidrar till förståelse för olika sjukdomar, utvecklingen av nya läkemedel och behandlingar hos både människor och djur. Grisen har blivit en allt mer efterfrågad modell inom biomedicinsk forskning, till stor del beroende på dess fysiologiska och anatomiska likheter med människans Viktig information saknas om omvårdnadsåtgärder som kan förbättra och förenkla samspelet med djuren och öka djurens välbefinnande då grisen ingår i studier, vilket lyfts fram i denna avhandling. Välfärden för djur som används i forskning är mycket viktig. Det finns goda etiska, vetenskapliga, juridiska och ekonomiska skäl för att se till att djuren behandlas korrekt och att det används så få djur som möjligt i forskningsstudier.

I denna avhandling har vi visat att en 14-dagars strukturerad träning av grisarna under acklimatiseringsperioden möjliggjorde blod- och urin-provstagning och ultraljudsundersökning med grisarna lösa i boxen postoperativt. När en ytbehandlad kateter utvärderades visade resultaten att det var möjligt att samla blod från katetrarna i upp till tio dagar. Olika läkemedels-kombinationer jämfördes och utvärderades. Resultatet visade att skillnader kunde ses gällande induktionskvalitet och krav på underhåll av anestesi mellan de två induktionsteknikerna. Utvärdering av en läkemedels-kombination för kortvarig anestesi resulterade i två timmars anestesi med stabila fysiologiska mätningar och tillfredställande spontanandning. Koncentrationen av läkemedlet i blodplasman var i linje med effektens varaktighet. Applicering av en teknik med artificiell intelligens för bedömning av aktivitet hos grisar visade sig vara ett lovande i experimentella studier. I framtiden skulle tekniken kunna vara av intresse för att utvärdera aktivitet på grisar som uttrycker smärta eller andra obehag.

Acknowledgements

The present studies were carried out at Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science at the Swedish University of Clinical Sciences (SLU) and at Hedenstierna Laboratory, Department of Surgical Sciences, Uppsala University (UU). I wish to thank the heads of these departments.

The work in this thesis was generously supported by funding from the European Community's Seventh Framework Programme for project DIREKT, from Torvald and Britta Gahlins stiftelse, the Swedish Research Council (VR) and the Swedish Research Council for the Environment, Agricultural Sciences and Spatial Planning (FORMAS).

This thesis and the studies could not have been accomplished without all the amazing people who have supported me these past years.

First, I would like to pay particular tribute to the former dean
Kristina Dahlborn, who pushed through a PhD position in veterinary nursing science. Thank You!

In particular I would like to express my sincere gratitude to my group of supervisors:

Görel Nyman, my main supervisor, mentor, and friend; thank you for sharing your vast knowledge in anaesthesia care and for your never-failing support *e.g.* answering e-mails late at night, early in the morning and all times in between, and thanks for all the laughs when the world around us tends to get too serious.

Marianne Jensen Waern, co-supervisor, thank you for your patience, guidance, and support and for always taking time to answer my questions. I am extremely grateful that you encouraged me to do a PhD and introduced me to this highly important research area….and to the pigs of course ☺

Lena Olsén, co-supervisor, thank you for hours and hours of guidance and brainstorming about half-lifes, $C_{min,max,trough}$ and "spagettifigures", but most of all for being my friend and invaluable nurse colleague.

Gaetano Perchiazzi, co-supervisor, thank you for your many supporting "bravissimas", and excellent feed-back during the writing process.

I would also like to express my gratitude to:

Mari and **Carola**, my nurse colleagues at the animal facilities. This thesis is yours as much as it is mine since it describes your daily work in this important area. I am extremely grateful for all your help with the sampling (urine, blood, pens, fruits from the lunchroom to the pigs…black books…) training, painting, filming and all the other nursing measures. Thanks for your patience when I was constantly changing the protocols, and thanks for all the laughs and discussions. All the other staff at hus 3 and at KFUS and **Malin** and **Fia** for help and preparation when it time for research and for reminding me of everything.

Elin, my pig research colleague, for sharing so many hours in the pig pens with me, and for doing your PhD before me so I could benefit your experiences and expertise. **Patricia**, for always taking time to answer my questions, and for the many hours with the pigs before, during and after anaesthesia. **Leo** and **Pålle**, thank you for your help and professional contributions with training the pigs.

Hedenstierna laboratory, **Karin** and **Agneta** and other colleagues, for your professional help with the animals and everything concerning the studies, and **Kerstin** thank you for your patience with all my questions.

Bo, **Kristina**, **Yuji** and Sana at the Department of immunology, genetics and pathology, UU, and Alireza at the department of transplantation, Skåne University hospital, for collaborations and to Yuji for the magic coating of the MPC catheters.

The anaesthesia dream team; **Dessie, Maja** and **Vanessa**, thanks for all the in-depth anaesthesia discussions (which I mostly just pretended to understand), and for always volunteering when it was time for pig anaesthesia. **Emma** and **Jonna** thanks for teaching the veterinary nurse students when I was crawling around among the pigs. **Stina** thanks for proofreading the manuscript and for the interesting discussions (sometimes about pigs…). **Adam** thank you for making the dream team kind of wake up when you visit Sweden ☺.**Tammy**, my friend, thank you for your support and for all discussions and for always taking my questions so seriously, you are so important for me and I can`t wait until you visit us again. **Lais** we miss you, you are still a member of the dream team, and the pigs are waiting ☺. My co-author **Sheila** thanks for all the hours with pig anaesthesia at UU, and for inviting us to Sicily, we will never forget. **Gunilla and Peter** thanks for just being friends and for proofreading the kappa.

Eva-Maria thanks for making the figures and tables over and over again, and for your support with the thesis until the last minute (or actually second…) before submission. But also for listening when I can't stop talking and for making me laugh when I needed it. Thanks Evis!

Sussie thanks for your help with administrative things that always fail when I try to do it. Everyone should have a Sussie. **Elinora** thanks for always taking time for discussions and for fixing new computers or cell phones before I knew I needed one. To **Ulf** for statistical support. **Sabina** at SLU library, for support when the ghost took over the thesis template (or is it still here..).

Ylva for help with administrative parts and for answering all FUN questions, and for support.

Robin My “language hero” for proofreading and excellent English language editing and for endless language, nursing and cat discussions via Skype.

All colleagues and friends at KV and UDS, thank you for your support and “hejarop”, and for showing interest (even if you were probably pretending…except **Ann**) when I continuously talking about and showing pictures of pigs, pigs and more pigs.

Brian my mentor, friend and technical support, you were the first person encourage me to do a PhD, and here we are! You are definitely part of this thesis. Thank you Brian! **Marieann** thanks for sharing your expertise in the area of nursing research (inter alia), for always proofreading, editing and answering questions whenever I need help and where ever you are (*e.g.* in the hunting tower), and also thank you for being my friend.

My former nurse colleagues and friends from the horse anaesthesia clinic; **Ylva**, **Andrea**, **Jennie** and **Lotta**, thanks for listening and pepping and just being my friends. It’s beer time!! Also thanks to you all and to Mälaren Hästklinik for running the MPC catheters in the ETO autoclave, even if I always forgot to bring fika.

My girlfriends in “the real world” **Lotta** thanks for being my friend for so many years, for accepting my last minute cancelations for my sometimes-stupid ideas, and for lifting me up and sitting me down when I needed it, now It’s time for the Finland road trip. **Ywonne** thank you for your friendship and for our discussions about everything one can think of, except pigs, anaesthesia care and SLU. **Anna** the neighbour, I am so grateful for our friendship and for all the discussions, our travels, and the “just pass trough’s” Thank you all! I long to see you more often

To my parents **Maud** and **Aimo**, I know you would be proud of me, because you always were. Thank you for the “do not give up” genes.

To my brother **Bengt**, for always being there for me, even if we get together far too seldom.

Lastly, my beloved family deserves endless gratitude:

Amanda and **Isac** thank you for reminding me what's important in life
Bill thank you for your patience, support and never ending love

III

animals

MDPI

Article

Physiological and Clinical Responses in Pigs in Relation to Plasma Concentrations during Anesthesia with Dexmedetomidine, Tiletamine, Zolazepam, and Butorphanol

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Simple Summary: Reliable protocols are needed for short-term anesthesia in pigs. The study's aim is to identify an anesthetic procedure that, without the use of sophisticated equipment, ensures an acceptable depth and length of anesthesia, a regular spontaneous breathing pattern, and a stable hemodynamic condition for the animal. A total of 12 pigs were given a single intramuscular injection of dexmedetomidine, tiletamine, zolazepam, and butorphanol. To investigate the possibility of prolonging the anesthesia, six of the pigs also received an intravenous dose of the drug combination after one hour. Physiological and clinical responses and drug plasma concentrations were examined. The main results suggest that intramuscular administration of the drug combination provides up to two hours of anesthesia with stable physiological parameters and an acceptable level of analgesia. An intravenous administration of one-third of the original dosage prolonged the anesthesia for another 30 min. Since the pigs were able to breathe spontaneously, none of them were intubated. The study also provides new information about each drug's plasma concentrations and the impact of the drug combination in pigs. This technique can be used to perform nonsurgical operations or transports when short-term anesthesia is required.

Abstract: Reliable protocols for short-term anesthetics are essential to safeguard animal welfare during medical investigations. The aim of the study was to assess the adequacy and reliability of an anesthetic protocol and to evaluate physiological and clinical responses, in relation to the drug plasma concentrations, for pigs undergoing short-term anesthesia. A second aim was to see whether an intravenous dosage could prolong the anesthesia. The anesthesia was induced by an intramuscular injection of dexmedetomidine, tiletamine zolazepam, and butorphanol in 12 pigs. In six of the pigs, a repeated injection intravenously of one-third of the initial dose was given after one hour. The physiological and clinical effects from induction to recovery were examined. Plasma concentrations of the drugs were analyzed and pharmacokinetic parameters were calculated. Each drug's absorption and time to maximal concentration were rapid. All pigs were able to maintain spontaneous respiration. The route of administration did not alter the half-life of the drug. The results suggest that intramuscular administration of the four-drug combination provides up to two hours of anesthesia with stable physiological parameters and an acceptable level of analgesia while maintaining spontaneous respiration. A repeated intravenous injection may be used to extend the time of anesthesia by 30 min.

Keywords: animal welfare; pharmacokinetic; swine; transportation; short term

Citation: Rydén, A.; Jensen-Waern, M.; Nyman, G.; Olsén, L. Physiological and Clinical Responses in Pigs in Relation to Plasma Concentrations during Anesthesia with Dexmedetomidine, Tiletamine, Zolazepam, and Butorphanol. *Animals* **2021**, *11*, 1482. https://doi.org/10.3390/ani11061482

Academic Editor: Nélida Fernández

Received: 31 March 2021
Accepted: 17 May 2021
Published: 21 May 2021

1. Introduction

Pigs have genetic, anatomical, and physiological similarities to humans, which makes them one of the most useful and versatile animal models in biomedical research [1]. Anesthetic protocols for safeguarding the animal's welfare and refinement in medical investigations are essential [2,3]. To avoid stressing and restraining the pig during short procedures

such as transport, imaging, and sampling, short-term anesthesia is often required in this species [4]. The challenge is to identify an anesthetic treatment that, without the use of sophisticated equipment, ensures an acceptable depth and length of anesthesia, a regular spontaneous breathing pattern, and a stable hemodynamic condition for the animal. Desirably, the anesthetic protocol should provide fast and reliable immobilization, adequate analgesia, and muscle relaxation without cardiovascular and respiratory depression [5]. Furthermore, prolongation of anesthesia must be possible without compromising the safety of the animal [6]. In terms of safety and efficacy in pigs, it has been reported that the combination of two or more drugs that target specific clinical effects is currently what constitutes the best standard for injectable intramuscular (IM) anesthesia [7]. Anesthesia with various combinations of α_2-agonists, dissociative anesthetics, benzodiazepines, and opioids have been suggested as induction agents prior to general anesthesia or for use as short-term anesthesia [4,5,8,9]. In those previous studies, physiological parameters and time to unconsciousness and recovery have been presented without correlation to actual drug concentrations in the body. Drug concentration from a single injection of a certain drug and pharmacokinetic data have also been published [10–12]. However, when drug combinations are administered, drug interactions may occur and repeated anesthetics may further alter the physiology of the animal, which can impact the outcome of the results [6,13]. In a previous study, we have shown that the induction of anesthesia using an α_2-agonist in combination with tiletamine-zolazepam and butorphanol administered IM produces a reliable and rapid anesthesia induction in pigs [14]. In that study, the anesthesia induction was followed by endotracheal intubation and general anesthesia; however, none of the factors such as the anesthesia length, the standard of recovery, or the drug plasma concentrations of the drugs were assessed. To the authors' knowledge, the possible pharmacokinetic interactions between the drugs or the plasma concentrations and effects of these drug combinations used for induction of anesthesia have not yet been evaluated.

The aim of the study was to assess the adequacy and reliability of an anesthetic protocol and to evaluate physiological and clinical responses in relation to the drug plasma concentrations for growing pigs undergoing short-term anesthesia. A second aim was to see whether an intravenous (IV) dosage could be used to prolong the anesthetic time.

We hypothesized that a single IM and repeated IV injection of the drug combination in pigs will result in a rapid uptake and distribution of the drug mixture, as well as the maintenance of physiological parameters within acceptable ranges.

2. Material and Methods

2.1. Animals and Housing

The experimental protocol was approved by the Ethics Committee for Animal Experimentation, Uppsala, Sweden (Approval No. C123/14, C2/16). The study design and reporting are in accordance with the ARRIVE guidelines. The study was performed at the Department of Clinical Sciences at the Swedish University of Agricultural Sciences, Sweden. The animals were handled according to the guidelines set out by the Swedish Board of Agriculture and the European Convention of Animal Care. A total of 12 pigs Yorkshire $\times$ Hampshire, of both sexes, were included in the study. Upon arrival from the university farm, they were 7–8 weeks old and weighed 25 $\pm$ 2 kg. The pigs were clinically examined before the start of the study and found to be healthy according to the American Society of Anesthesiologists (ASA 1). The animals were video recorded from the start of the acclimatization period until the end of the study. The animals underwent a 14-day acclimatization period at the Department of Clinical Sciences. They were housed in individual pens measuring 3 m^2, where they could see and hear each other. Straw and wood shavings were provided as bedding. The ambient temperature inside the pens was 18 $\pm$ 2 °C, and a 10:14 h light–dark schedule was used. An infrared lamp was provided in a corner of each pen. The pigs were fed a commercial finisher diet (Solo 330 P SK, Lantmännen, Sweden) twice daily. The amount fed was according to body weight and the Swedish University of Agricultural Sciences (SLU) regimen for growing pigs. Water

was provided ad libitum, and once a day, their general condition was examined. All of the pigs were included in a previously published study during the acclimatization period in which the animals were trained to accept touching and palpation of the ears in preparation for blood sampling from the auricular vein [14]. The pigs were randomly assigned into two groups using the R statistical software version 2.3.1 (GNU operation systems, Free Software Foundation, Boston, MA, USA): those who received a single IM injection (Group S) and those who received an additional injection IV (Group R), with $n = 6$ in each group.

2.2. Central Vein Catheterization

Three days before the start of the study, an internal jugular catheter was placed under general anesthesia in all pigs to facilitate blood sampling. A light meal was given to the animals six hours before anesthesia, but water was provided ad libitum. Topical anesthesia (2.5 g) with lidocaine and prilocaine (EMLA 25 mg/g + 25 mg/g AstraZeneca, Södertälje, Sweden) was applied on the pigs' ear flaps two hours before induction. Anesthesia was induced with sevoflurane (SevoFlo® Orion Pharma, Danderyd, Sweden) in oxygen and air (FIO_2 0.5) that was delivered using a nonrebreathing system with a face mask. Initially, the fresh gas flow (FGF) was set at 300 mL/kg/min and the vaporizer at 8%. When the animal assumed a lateral recumbent position, FGF and the concentration of sevoflurane were decreased to 100 mL/kg/min and 4%, respectively. A polyurethane catheter (BD Careflow™ 3Fr 200 mm, BD Medical, Franklin Lakes, NJ, USA) coated with MPC [14] was introduced via *V. auricularis* into *V. jugularis* using an aseptic Seldinger technique. The catheter was sutured onto the ear with monofil-coated polyamide (Supramid 2-0, B Braun Medical, Danderyd, Sweden) and covered with a bandage (Snøgg AS, Vennesla, Norway). Sevoflurane administration was discontinued at the end of the catheter placement, and the pigs were returned to their home pens to recover. The catheters were flushed with saline once daily until the start of the study.

2.3. Anesthesia

A light meal was given to the animals six hours before anesthesia, and water was provided ad libitum. The anesthesia was induced IM while the pigs were in their home pen using a butterfly needle (CHIRAFLEX Scalp vein set 21G × 3/4", 0.8 × 20 mm Luer-Lock, CHIRANA T. Injecta, Stara Tura Slovakia). The pigs were given an IM injection in the brachiocephalic muscle on the side opposite the catheterized ear. One bottle of tiletamine and zolazepam (Zoletil 100® vet. tiletamine 250 mg + zolazepam 250 mg, Virbac, Carros, France) in powder form was reconstituted with 5 mL of dexmedetomidine (Dexdomitor®vet. 0.5 mg/mL, Orion Pharma AB Animal Health, Danderyd, Sweden) and 1 mL of butorphanol (Dolorex®vet. 10 mg/mL, Intervet AB, Stockholm, Sweden). Thus, each milliliter contained 0.42 mg/mL dexmedetomidine, 83.3 mg/mL of tiletamine–zolazepam, and 1.67 mg/mL of butorphanol. The anesthesia dose for each pig was dexmedetomidine 0.025 mg/kg, tiletamine 2.5 mg/kg, zolazepam 2.5 mg/kg, and butorphanol 0.1 mg/kg (0.06 mL/kg of the anesthetic combination solution). The pigs were observed for any signs of discomfort during the injection, and their position and level of consciousness were monitored continuously. The time to unconsciousness, evidenced by lateral recumbency, head down, lack of reaction when manipulating or moving their body, and absence of the palpebral reflex, was noted. The trachea of the animals was not intubated and pigs breathed room air over the anesthetic period. Equipment for endotracheal intubation, laryngeal masks, self-inflating bag, and supplementary oxygen was prepared and available if cyanosis occurred or if the hemoglobin oxygen saturation (SpO_2) decreased below 85%. After induction, the animals were positioned and supported on straw bedding to avoid discomfort or pressure injuries. During anesthesia, the animals' bodies were checked for discomfort and manipulated or moved in the same position to another location in the pen every hour to mimic transport. The palpebral reflex, jaw tone, the occurrence of spontaneous movements, and response to stimuli, such as cannulation and blood sampling, were all used to determine the depth of anesthesia. The response to

the nociceptive stimulus was elicited by mechanically clamping the coronary band of the medial or lateral dewclaw using forceps. The site of the stimulation was changed slightly each test to prevent sensitization to stimuli [15]. A cannula (BD Venflon™ 20 G × 32 mm, BD Medical, Franklin Lakes, NJ, USA) was inserted in the auricular vein on the noncatheterized ear 30 min after induction in both groups. Through this IV access, only Group R was given a further dose containing one-third of the initial dose, (0.02 mL/kg of the anesthetic combination solution) that was administered 60 min after induction. The time to first spontaneous movement, standing position, and the number of attempts to stand were observed and recorded. The quality of their recovery was assessed and recorded manually during the experiment, and at a later time reassessed by watching the video recordings. The assessment was made using a scoring system adapted and modified from an anesthesia scoring system used in antelopes that ranged from 1 (excellent) to 3 (poor) [16] (Table 1.) Any side effects observed during the procedure and the subsequent 72 h were recorded.

Table 1. Recovery Scoring System.

Recovery Score	Description
1 Excellent	Animal transitions from lateral to sternal recumbency with minimal ataxic movements. Stands in one or two attempts. Walks with only slight ataxia.
2 Good	Animal in transition from lateral to sternal recumbency displays moderate ataxic movements and may take one or two attempts. Some imbalance in sternal recumbency and requires more than two attempts to stand. Walks with moderate ataxia.
3 Poor	Animal makes frequent attempts with severe ataxic movements to transition from lateral to sternal recumbency before being successful. Severe imbalance in sternal recumbency. Makes numerous attempts to stand but falls before being successful and displays marked ataxia when walking.

Scoring system (1–3) used to categorize the quality of recovery in pigs.

2.4. Physiological Measurements and Blood Sampling

Palpation and monitoring of the pulse (HR) and monitoring of respiratory rate (RR), SpO_2 and rectal temperature (temp) (GE B40 Patient Monitor, GE Healthcare, Danderyd, Sweden) began once the animals were in lateral recumbency and continued throughout the anesthesia at the following intervals: 5, 10, 15, 30 and every 30 min until recovery.

Ethylenediaminetetraacetic acid (EDTA) tubes were used for the blood samples that were obtained via the central venous catheter before induction and repeated after at the following intervals: 5, 10, 15, 30, 60 min; 2, 3, 4, 5, 6, 7, 10 h; and finally, once daily, for up to two days after drug administration. Additional sampling was made when the pigs became unconscious, had a first spontaneous movement, and assumed a standing position. Plasma was separated by centrifugation in 5 min within 90 min and stored at −80 °C until analysis.

2.5. Drug Analyses

The samples were prepared by mixing 50 µL of plasma with 100 µL of the standard internal solution used at the lab (50 ng/mL of midazolam and phenacetin in Acetonitrile), which were vortexed and centrifuged (Thermo SL16, 20 min, 4000 rpm). The samples were then transferred to a Waters 96-well plate and submitted for liquid chromatography–mass spectrometry analysis. Samples for making the standard curves were prepared into pig plasma by spiking the matrix into concentrations of 0.05–10,000 ng/mL of the tiletamine, 0.05–10,000 ng/mL of zolazepam, 0.005–1000 ng/mL of dexmedetomidine, and 0.005–1000 ng/mL butorphanol, and were otherwise treated as the samples.

Quality control (QC) samples were prepared into pig plasma by spiking the matrix into concentrations of 2, 20, 200, and 2000 ng/mL of tiletamine and zolazepam, but the high concentration QC samples were not used in the primary analysis since the calibration range

extended only up to 1000 ng/mL. Samples were otherwise treated as samples. Quality control samples in pig plasma for dexmedetomidine and butorphanol were prepared in 0.2, 2, 20, and 200 ng/mL concentrations and were otherwise treated as samples. Samples with zolazepam concentrations higher than 1000 ng/mL were diluted and reanalyzed. Midazolam was used as an internal standard for the quantification of dexmedetomidine, tiletamine, and butorphanol. Zolazepam was quantified successfully without the use of an internal standard.

2.6. Pharmacokinetic Analyses

For each animal and drug, the plasma drug concentrations vs. time were plotted. Different models and weighting factors were assessed by visual inspection of the curve fits and the residuals' scatter plots, together with the accuracy of fit measures incorporated in the software, e.g., the Akaike information criterion. A noncompartmental model was used for all drugs. For Group S (IM), the maximal concentration of each drug in plasma (C_{max}), time to reach C_{max} (t_{max}), and terminal half-life ($t_{1/2}$) were calculated with a noncompartmental model using the PK Solver add-in for Microsoft Office Excel. For Group R, after the IV bolus administration, the $t_{1/2}$, the volume of distribution (Vd) and the clearance (cl) were obtained from the model.

2.7. Statistical Analysis

Physiological and clinical data were compared among study times and groups using a one-way analysis of variance (ANOVA) for repeated measures. Data were presented as median, mean ± SD, or as a range. A *p*-value of <0.05 was considered significant in all tests.

3. Results

The study was completed with a minor incident occurring in only one of 12 pigs. The drug concentration and pharmacokinetic analyses include data from a total of seven pigs (S = 3 and R = 4) since samples from five pigs were lost. Scoring and duration of anesthesia, as well as physiological and clinical results, include data from all 12 pigs (S = 6 and R = 6).

3.1. Anesthesia

Induction of anesthesia was possible without the need to restrain any of the animals. The mean time from injection to lateral recumbency was 2.6 (±0.7) min, and from injection to unconsciousness, 10.0 (±3.7) min. During the anesthesia, excellent muscle relaxation was sustained until the first movement, and there were no reactions to cannulation, manipulation/movement of their body, and blood sampling in any of the animals. The withdrawal reflex was absent in 10 animals within 10 min after the induction, and it was present in two pigs for up to 60 min after the induction. The palpebral reflex was absent in all pigs 10 min after the induction. After the repeated injection IV, none of the pigs showed withdrawal reflexes when their dewclaw was mechanically clamped. The mean time from induction to the first spontaneous movement was 117 (±27) and 147 (±25) min, and from induction to standing position, 158 (±22) and 199 (±25) min, in Group S and R, respectively. In Group R, the time from the repeated injection to the first movement was 98 (±22) min, and to a standing position, 139 (±25) min (Table 2). The median recovery score was 1 (range 1–2) in both groups.

Table 2. Observed clinical responses in relation to plasma concentrations.

Noted Observations	Time (min) from Injection (IM)		Concentration Range (ng/mL) Group S and R			
	S	R	Dexmedetomidine	Tiletamine	Zolazepam	Butorphanol
Lateral recumbency	2–4		<1.59–3.56	<126–268	<106–789	<5.22–15.60
Unconsciousness	5–15		1.99–6.00	169–337	737–1330	8.86–19.20
Last sample before response to noxious stimulus	60–120	120	2.01–3.48	115–158	520–1180	6.74–11.60
Palpebral reflexes	60–70	126–180	1.69–2.38	101–129	525–782	6.80–10.50
Response to noxious stimulus	70–140	126–180	1.69–2.38	101–129	525–782	6.80–10.50
First movement	70–140	126–180	1.69–1.97	106–123	444–713	6.47–9.42
Standing	120–180	175–240	1.14–2.01	73.7–109	250–678	4.74–8.70

3.2. Physiological Data

The mean values for HR and RR (n = 12) are shown in Figure 1. There were no differences in HR (p = 0.72) or RR (p = 0.073) over time in either group. During anesthesia, SpO_2 was never below 86% (99–86%) in any of the pigs. One pig in Group R showed initial but transient apnea (about 10 s) immediately after the repeated injection IV. Rectal temperature decreased over time in both groups (range 40.1–37.3 °C), but no differences were seen between the groups.

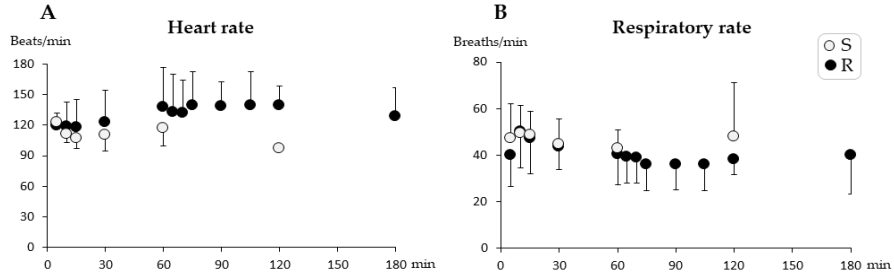

Figure 1. Heart rate (**A**) and respiratory rate (**B**) (mean ± SD) during short-term anesthesia recorded at 5, 10, 15, 30, and every 30 min from injection until recovery. Pigs from Group Single (S) (n = 6) received a single dose intramuscularly of dexmedetomidine 0.025 mg/kg, tiletamine 2.5 mg/kg, zolazepam 2.5 mg/kg, and butorphanol 0.1 mg/kg at time zero. Pigs from Group R (n = 6) also received one-third of the initial dose intravenously at 60 min. No significant differences were found between groups or over time.

3.3. Concentration and Pharmacokinetic Data

None of the catheters became obstructed or dislodged. No infection was observed at any of the catheter sites. None of the animals showed signs of local pain during palpation or blood sampling. Quality data for the analyses are presented in Table 3. Individual data on plasma concentration over time for each drug are shown in Figure 2. The main pharmacokinetic parameters are shown in Table 4, and observed clinical responses in relation to plasma concentrations are shown in Table 2. In all pigs, the average C_{max} for all drugs was 12 (range 10–13) after administration of the IM induction drug combination. The highest concentrations in Group R were measured 5 min after the repeated injection IV (Table 2). In Group R, the drug concentrations were 1.4–1.6 times higher 5 min after

the repeated injection IV, compared to C_{max} after the IM induction (Figure 2). Within 15 min after the repeated injection IV, the mean plasma concentrations of the drugs in Group R were similar (tiletamine 336 ng/mL, dexmedetomidine 5.1 ng/mL, butorphanol 19.8 ng/mL, and zolazepam 1387 ng/mL) (Figure 2), compared to the C_{max} after IM administration. It was possible to detect the drugs up to 19 and 22 h for dexmedetomidine, 10 and 24 h for tiletamine, 29 and 31 h for zolazepam, and 23 and 31 h for butorphanol after administration of the induction dose in Group S and R, respectively.

Table 3. Pharmacokinetic parameters.

Compound	Dexmedetomidine	Tiletamine	Zolazepam		Butorphanol
Detection limit (ng/mL)	<0.02	0.2	0.05	0.05	0.02
Quantitation limit (ng/mL)	0.02	0.5	0.1	0.1	0.05
Range (ng/mL)	0.02–200	0.5–1000	0.1–1000	0.1–500	0.05–500
R^2	>0.999	>0.998	>0.997	>0.996	>0.999

Detection limit (LoD), quantitation limit (LoQ), range of standard curve, and coefficient of determination R-squared value (R^2) of each drug.

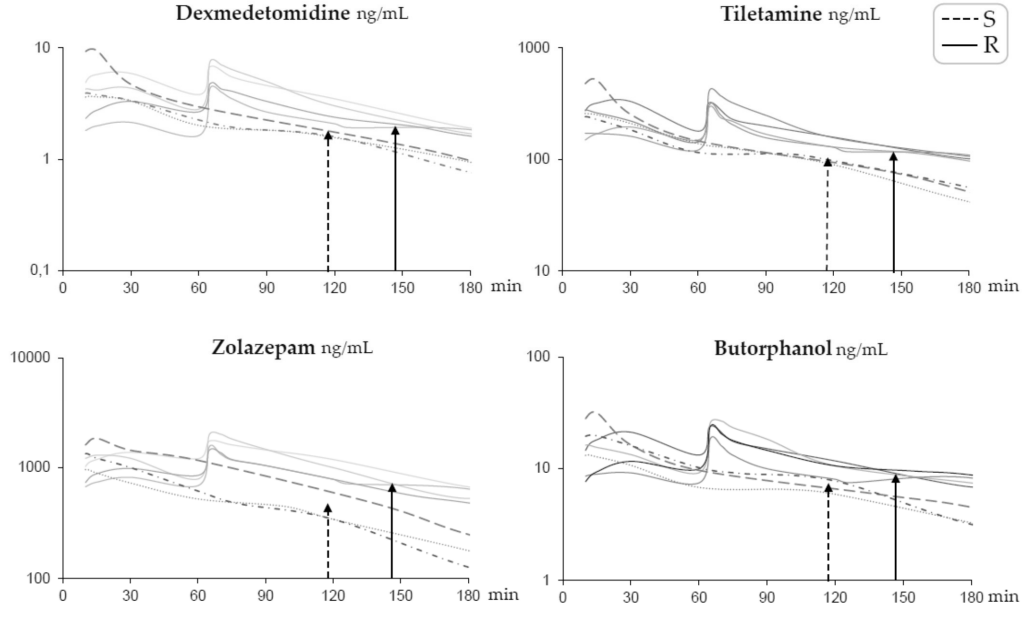

Figure 2. Profiles of plasma concentrations for each drug over time for short-term anesthesia induced by intramuscular administration of dexmedetomidine 0.025 mg/kg, tiletamine 2.5 mg/kg, zolazepam 2.5 mg/kg, and butorphanol 0.1 mg/kg in pigs (n = 7). Group S (dotted lines n = 3) received a single dose at time zero. Group R (solid lines n = 4) also received one-third of the initial dose intravenously at 60 min. Time to first spontaneous movement is indicated with a dotted arrow for Group S and a solid arrow for Group R.

Table 4. Pharmacokinetic parameters.

	IM Group S (*n* = 3)			IV Group R (*n* = 4)		
	$t_{1/2}$ (min)	t_{max} (min)	C_{max} (ng/L)	$t_{1/2}$ (min)	cI (L/kg/min)	Vd (L/kg)
Dexmedetomidine	125 ± 37	13 ± 3	5.6 ± 3.2	117 ± 24	0.012 ± 0.004	1.7 ± 0.5
Tiletamine	90 ± 12	10 ± 5	342 ± 152	80 ± 13	0.050 ± 0.010	5.8 ± 0.9
Zolazepam	72 ± 6	12 ± 3	1372 ± 438	76 ± 13	0.009 ± 0.002	1.0 ± 0.2
Butorphanol	97 ± 11	13 ± 3	21 ± 9	101 ± 16	0.012 ± 0.002	1.6 ± 0.4

Time range in min and the corresponding plasma concentration range for each drug and noted observation during the short-term anesthesia induced by dexmedetomidine 0.025 mg/kg, tiletamine 2.5 mg/kg, zolazepam 2.5 mg/kg, and butorphanol 0.1 mg/kg after intramuscular (IM) administration in growing pigs single (S) (*n* = 6) and repeated (R) (*n* = 6). All pigs received a single dose at time zero, and group Ralso received one-third of the initial dose intravenously at 60 min. All blood samples were taken in relation to the noted observation except for lateral recumbency, which was taken at 5 min after induction. Plasma concentrations were available for seven pigs.

4. Discussion

The results of this study suggest that intramuscular administration of the drug combination dexmedetomidine, tiletamine, zolazepam, and butorphanol provided up to two hours of anesthesia with an acceptable level of analgesia, regular breathing pattern, and stable physiological parameters. Furthermore, at one hour of anesthesia, IV administration of one-third of the initial dose extended the anesthesia duration by another 30 min.

4.1. Anesthesia

In the present study, all of the pigs were recumbent within 4 min and were unconscious within 15 min after the IM injection. This demonstrates a rapid uptake from the injection site resulting in a rapid onset of action. In addition, the observed C_{max} time of 10–13 min for all drugs after administration is in good agreement with the manually assessed time of unconsciousness. The time from drug administration to unconsciousness plays a critical role regarding the quality of the anesthesia [17]. Pigs are easily stressed and the time between injection and onset can cause ataxia during the induction phase [17,18]. Assessing the depth of anesthesia in pigs in the absence of surgical stimuli is challenging [19]. Clamping the dewclaw has been described in previous studies as an intense stimulus that persists until a high degree of central nervous system depression is reached, i.e., a supramaximal stimulus [20,21]. In those studies, response to dewclaw clamping was not consistent between the animals. Despite this, the authors stated that the method is a reliable indicator of anesthetic depth. In the present study, none of the 12 pigs reacted to cannulation, blood sampling, or being moved in the same position to another location in the pen, but there was variability in the withdrawal reflex, which was absent for up to 60 min in two of the animals. Hence, the levels of anesthesia and analgesia seem to be adequate for short procedures intended in this study.

Muscle relaxation of the animals is crucial during transportation and diagnostic imaging procedures [22]. The animals in this study were characterized by complete muscle relaxation with no spontaneous movements until the recovery time. $Alfa_2$ agonist sedatives are used in both veterinary medicine and biomedicine, and the drug has been described to provide central sedative effects, accompanied by muscle relaxation and analgesia when combined with tiletamine–zolazepam [3,23,24].

The pigs were not endotracheally intubated in the present study because the aim was to relate respiratory function to the plasma concentration of the drugs used and not to the effect of a supramaximal stimulus, i.e., intubation. Previous publications state that intubation is possible shortly after induction with a drug combination similar to the combination used in the present study [14,24,25]. In those reports, the induction was followed by maintenance with inhalation anesthesia and mechanical ventilation making intubation necessary. However, since intubation is a potent stimulus in the pig, the procedure may cause complications, e.g., changes in depth of anesthesia, reflexes, and breathing pattern. Additional anesthesia, such as fentanyl or propofol, may be needed in pigs induced

with the combination of drugs used to suppress laryngeal reflexes and provide adequate relaxation for endotracheal intubation [26,27]. Under very deep sedation or anesthesia, complications such as hypoxemia, vomiting, and aspiration pneumonia may occur; therefore, endotracheal intubation is always recommended for pigs undergoing procedures under the described combination of drugs [28]. In case of complications, skilled staff and advanced equipment for prompt endotracheal intubation or placement of a laryngeal mask for the possibility to ventilate the pigs with a self-inflating bag and supplemental oxygen were prepared.

Moreover, 15 min after the repeated injection IV, the plasma concentrations of all drugs were similar to the C_{max} after the initial IM administration. One-third of the initial dose prolonged the anesthesia by approximately 26% when given IV to the animals. The plasma concentration ranges of each drug from the last blood sample that was taken while the pigs were still unconscious are overlapping for zolazepam and butorphanol, but not for dexmedetomidine and tiletamine (Table 4). There is no doubt that these drug concentrations predict such events due to dexmedetomidine's sedative effect and tiletamine's dissociative anesthetic effect. Moreover, since the four drugs were given in combination, the effects of concentrations could not be calculated in this study. Additionally, the t_{max} results for all drugs in this combination were rather similar, and as a result, they will decline in a similar matter. In polypharmacy, the drugs may have an impact on each other's pharmacokinetic profiles, and a shorter t_{max} for zolazepam and tiletamine was observed in this study, compared to other studies in pigs when only zolazepam and tiletamine were given [10,11,29]. In this study, it is not possible to link each drug's pharmacokinetics to pharmacodynamics, but rather, the combined effect of all drugs together for use in robust short-term anesthesia has been discovered and described. The sole adverse event observed was a short episode of apnea (about 10 s) that occurred immediately after giving the repeated injection IV containing one-third of the initial dose. It was easily managed, and the pig started to breathe spontaneously after the application of light pressure on its chest. Therefore, it was decided to include the pig in the analyses.

Over the course of the anesthesia and even after the repeated injection, the physiological measures remained within normal limits. This was unexpected because α_2-agonists commonly produce bradycardia [30]. However, tiletamine that was included in the induction combination increases HR by stimulating the sympathetic nervous system [31].

Since the observer was aware of the animals' treatments, the scoring system was based on what was written in the notes, as well as the number of attempts to stand and the degree of ataxia seen in the video recordings. The median recovery score was excellent (score 1), with the animals making one or two attempts to stand with very mild signs of excitement. Two animals, one in each group, had a good recovery (score 2), but none of the animals was scored as poor recovery (score 3) (Table 1). Overall, the drug combination was successful in producing a good to excellent recovery.

Hypothermia is a common anesthesia complication, and it occurs due to altered thermoregulatory control as well as evaporative and conductive heat loss [32]. The decrease in temperature occurring in both groups could have probably been avoided with the use of heating pads and blankets. However, the body temperature did not fall below 37.3 °C in any animal, which is within the normal range for pigs undergoing anesthesia [33].

The initial IM dose was based on previous studies conducted on pigs at our research laboratory. The repeated dose used was based on a procedure in a similar population of animals where catheters were placed via subcutaneous tunneling. In that procedure, an IV injection after one hour of anesthesia containing one-half the initial dose of the combination resulted in apnea (>10 s) in approximately 50% of the pigs. When one-third of the initial dose was used, sufficient spontaneous respiration was maintained, and reflexes from catheterization stimuli were absent.

4.2. Pharmacokinetics

There were no $t_{1/2}$ differences in regard to route of administration for any of the drugs. After an extravascular drug administration, such as IM in this case, the $t_{1/2}$ can be more prolonged than after an IV administration. Thus, the route of administration for a drug can be one reason for the prolongation. This (flip-flop phenomenon) occurs when the rate of absorption is the rate-limiting step in the sequential processes of drug absorption and elimination. The drug cannot be eliminated before it has been absorbed, and the $t_{1/2}$ of the absorption depends on the disappearance of the drug from the site of administration, which, in turn, depends on the physiological absorption process and how much of the drug is bioavailable.

Since the process of absorption does not seem to be a limiting factor for this drug combination, the $t_{1/2}$ expresses the overall rate of the actual drug elimination process during the terminal phase. This overall rate of elimination depends on drug clearance and on the extent of drug distribution.

We were unable to compare pharmacokinetic studies for this drug combination. The pharmacokinetic parameters for dexmedetomidine (or medetomidine and detomidine) and butorphanol in pigs seem to be unpublished, and only a few reports for tiletamine and zolazepam are available. There are similarities between the results for butorphanol in a goat study [34], in which the Vd was 1.27 L/kg, cl 0.0096 L/kg/min and $t_{1/2}$ 1.87 h with IV administration, and for the pigs in the present study1.6 L/kg, 0.012 L/kg/min and 1.68 h. After IM administration, the time to C_{max} was comparable (t_{max} 16 min), but the $t_{1/2}$ was longer (2.75 h in the goat), suggesting a flip-flop phenomenon in the goat that was not observed in the pigs in the present study. The goats received only butorphanol and became hyperactive within the first 5 min after administration, which was not the case for the pigs since they received butorphanol in a combination of drugs meant for short-term anesthesia.

Polypharmacy, as well as other variables including species and age, may affect a drug's pharmacokinetics, making comparisons difficult. In another study [35], the IV pharmacokinetics of butorphanol, detomidine, and a combination of both administered to horses revealed that butorphanol given alone showed about a twofold larger clearance but similar $t_{1/2}$, when compared with the combination. When detomidine was administered alone and compared with the combination, there was a similar clearance but a slightly shorter $t_{1/2}$. The $t_{1/2}$ for butorphanol in the horse (mean 5.2 h as a single drug and 5.4 h in the combination) is considerably longer than in the pig. There is a similar clearance (0.01 L/kg/min) when it is given alone but noticeably lower (0.006 L/kg/min), when compared with the combination of both.

A clearance of about 0.01 L/kg/min for butorphanol was also found in a study involving horses [36]. It has been suggested that the $t_{\frac{1}{2}}$ is influenced by a physiological compartment in the horse that can be saturated and that a lower dose of butorphanol is sufficient to be efficacious when used in combination with the α_2-agonist detomidine [35]. In pigs, the $t_{1/2}$ for dexmedetomidine was in our study almost two hours, which is longer than the $t_{1/2}$ (less than one hours) in horses, dogs, and cats [36–40] In the present study, only the pharmacokinetics of the combination with the α_2-agonist dexmedetomidine was explored. It is yet to be investigated if a similar influence by the combination also exists in pigs.

An equal-dose combination of tiletamine and zolazepam is often used for the induction of short-term anesthesia in various species of animals, but information regarding its pharmacokinetics and metabolism is scarce. The anesthetic effects of the drugs may differ from species to species depending on the elimination and metabolic clearance. The plasma concentrations in the present study are consistent with the data obtained in a previous study [11] that showed higher concentrations of tiletamine–zolazepam than in pig plasma from 16 Yorkshire-crossbred pigs given a single dose of 3 mg/kg tiletamine and zolazepam IM. The zolazepam had a lower $t_{1/2}$ (zolazepam 2.76 h versus tiletamine 1.97 h) and clearance, compared to the tiletamine, which demonstrates the major pharmacokinetic

and metabolic differences between the two drugs. However, the findings of the current study did not appear to have this difference in $t_{1/2}$ (zolazepam 1.2 h versus tiletamine 1.5 h) after administration IM. Our findings may be a result of the combination of drugs that were similar to those found in a study of pregnant pigs [41], where the C_{max} for both tiletamine and zolazepam was 50–60 min (compared to 10–12 min in the present study), and where the elimination of zolazepam was slow and tiletamine rapid. The study also showed that zolazepam, but not tiletamine, was detected in the uterus and umbilical cord and thereby probably having an effect on the fetus; this finding must be taken into consideration when used in pregnant sows.

Three metabolites of zolazepam and one metabolite of tiletamine in plasma, urine, and microsomal incubations were analyzed in a study [11]. Unfortunately, the muscle-relaxant, antianxiety, and sedating properties of the metabolites of zolazepam were not reported. The metabolism and plasma clearance of zolazepam was reported to be slower than tiletamine, thus leading to prolonged sedative and muscle-relaxant effects. For the welfare of the pig, and as long as good analgesia/anesthesia during the intervention is provided, this must be considered to be a better scenario than vice versa. Since the effect of tiletamine alone as a dissociative anesthetic does not provide muscle relaxation, it can cause a cataleptic state. The $t_{1/2}$ of tiletamine has been reported to be shorter than that of zolazepam in other animal species. In a polar bear study, an average $t_{1/2}$ of 1.8 h and 1.2 h, for tiletamine and zolazepam, respectively, was reported [42].

It has been rereported in a review [29] that tiletamine has a $t_{1/2}$of 2–4 h in cats, 1.2 h in dogs, 1–1.5 h in monkeys, and 30–40 min in rats. For zolazepam, the reported $t_{1/2}$ results are 4.5 h in cats, 4–5 h in dogs, 1 h in monkeys, and 3 h in rats. Comparable pharmacokinetic properties of fixed-dose combination drugs may be desirable in order to permit similar dosing intervals. For anesthetic/muscle-relaxant combinations, the pharmacodynamics/anesthetic effects such as dissociative anesthesia and muscle relaxation should also be considered. According to the same review [29], the explanation for using a 1:1 dose ratio was based on pharmacodynamic considerations from studies performed on cats, dogs, and monkeys, despite differences in the $t_{1/2}$ of tiletamine and zolazepam in these species.

5. Limitations

The number of animals included was based on a previous sample size calculation. However, the sample size was decreased by the lost plasma samples. In addition, relatively high individual variability limited the power to detect significant differences that might have been found if all samples had been included. Before the start of the present study, the pigs had been involved in a training program for two weeks. The pig training, which lowered patient stress levels, may have contributed to the short period between injection and unconsciousness, as well as the excellent recovery rates. Different findings can be obtained in laboratories that do not have this stress-reduction program.

It should be emphasized that arterial blood gas analysis remains ideal for the assessment of oxygenation and to address the presence of hypoxemia [43,44]. In the present study, arterial blood gas analysis was not performed. Arterial catheterization usually requires surgical exposure of the deeply located vessels that would likely produce a potent stimulus in the pigs. Due to the small sample of animals used and the use of pulse oximetry instead of blood gas analysis, further studies are warranted to investigate the presence of hypoxemia.

6. Conclusions

In conclusion, the combination of dexmedetomidine, tiletamine, zolazepam, and butorphanol, given intramuscularly, provides a rapid induction of good quality, followed by up to two hours of anesthesia with acceptable tolerability to nociceptive stimulus in growing pigs. The plasma concentration profile of the drugs was in line with the duration of the effect, showing a rapid uptake and distribution of the drugs. Since the technique

used ensures a satisfactory breathing pattern and physiological stability, this procedure is suitable for the care and health of pigs. In addition, at one hour of anesthesia, intravenous injection of one-third of the original dose extended the anesthesia duration by another 30 min.

Author Contributions: Conceptualization and methodology, A.R., M.J.-W., G.N., and L.O.; formal analysis, L.O.; data curation, A.R.; writing—original draft preparation, A.R. and L.O.; writing—review and editing, M.J.-W. and G.N.; visualization, A.R.; project administration, G.N.; funding acquisition, M.J.-W. and G.N. All authors have read and agreed to the published version of the manuscript.

Funding: The research has received funding from the European Community's Seventh Framework Programme in the project DIREKT (No. 602699).

Institutional Review Board Statement: The study was conducted according to the guidelines set out by the Swedish Board of Agriculture and the European Convention of Animal Care, and approved by the Ethics Committee of Animal Experimentation, Uppsala, Sweden (Protocol code C123/14, C2/16, 2017-08-20).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

Acknowledgments: We are grateful to the staff at the Department of Clinical Sciences SLU, Uppsala for the precious technical support, to Eva-Maria Hedin for her invaluable help creating and editing the tables and figures, to Robin Quell for the proofreading and English language editing, and to Stina Marntell for her critical reading of the manuscript.

Conflicts of Interest: The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

References

1. Schook, L.B.; Collares, T.V.; Darfour-Oduro, K.A.; De, A.K.; Rund, L.A.; Schachtschneider, K.M.; Seixas, F.K. Unraveling the swine genome: Implications for human health. *Annu. Rev. Anim. Biosci.* **2015**, *3*, 219–244. [CrossRef] [PubMed]
2. Geovanini, G.R.; Pinna, F.R.; Prado, F.A.; Tamaki, W.T.; Marques, E. Standardization of anesthesia in swine for experimental cardiovascular surgeries. *Rev. Bras. Anestesiol.* **2008**, *58*, 363–370. [CrossRef] [PubMed]
3. Swindle, M.M. *Swine in the Laboratory: Anesthesia, Analgesia, and Perioperative Care*, 2nd ed.; Swindle, M.M., Ed.; CRP Press: Boca Raton, FL, USA, 2007; pp. 35–75.
4. Heinonen, M.L.; Raekallio, M.R.; Oliviero, C.; Ahokas, S.; Peltoniemi, O.A. Comparison of azaperone-detomidine-butorphanol-ketamine and azaperone-tiletamine-zolazepam for anaesthesia in piglets. *Vet. Anaesth. Analg.* **2009**, *36*, 151–157. [CrossRef] [PubMed]
5. De Monte, V.; Staffieri, F.; Di Meo, A.; Vannucci, J.; Bufalari, A. Comparison of ketamine-dexmedetomidine-methadone and tiletamine-zolazepam-methadone combinations for short-term anaesthesia in domestic pigs. *Vet. J.* **2015**, *205*, 364–368. [CrossRef]
6. Albrecht, M.; Henke, J.; Tacke, S.; Markert, M.; Guth, B. Influence of repeated anaesthesia on physiological parameters in male Wistar rats: A telemetric study about isoflurane, ketamine-xylazine and a combination of medetomidine, midazolam and fentanyl. *BMC Vet. Res.* **2014**, *10*, 310. [CrossRef] [PubMed]
7. Nishimura, R.; Sakaguchi, M.; Mochizuki, M.; Sasaki, N.; Takahashi, H.; Tamura, H.; Takeuchi, A. A balanced anesthesia with a combination of xylazine, ketamine and butorphanol and its antagonism by yohimbine in pigs. *J. Vet. Med. Sci.* **1992**, *54*, 615–620. [CrossRef]
8. Malavasi, L.M.; Jensen-Waern, M.; Augustsson, H.; Nyman, G. Changes in minimal alveolar concentration of isoflurane following treatment with medetomidine and tiletamine/zolazepam, epidural morphine or systemic buprenorphine in pigs. *Lab. Anim.* **2008**, *42*, 62–70. [CrossRef]
9. Sakaguchi, M.; Nishimura, R.; Sasaki, N.; Ishiguro, T.; Tamura, H.; Takeuchi, A. Anesthesia induced in pigs by use of a combination of medetomidine, butorphanol, and ketamine and its reversal by administration of atipamezole. *Am. J. Vet. Res.* **1996**, *57*, 529–534.
10. Kumar, A.; Mann, H.J.; Remmel, R.P. Determination of constituents of Telazol®—Tiletamine and zolazepam by a gas chromatography/mass spectrometry-based method. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2006**, *842*, 131–135. [CrossRef] [PubMed]
11. Kumar, A.; Mann, H.J.; Remmel, R.P.; Beilman, G.J.; Kaila, N. Pharmacokinetic study in pigs and in vitro metabolic characterization in pig- and human-liver microsomes reveal marked differences in disposition and metabolism of tiletamine and zolazepam (Telazol). *Xenobiotica* **2014**, *44*, 379–390. [CrossRef] [PubMed]

12. Ezzati, M.; Broad, K.; Kawano, G.; Faulkner, S.; Hassell, J.; Fleiss, B.; Gressens, P.; Fierens, I.; Rostami, J.; Maze, M.; et al. Pharmacokinetics of dexmedetomidine combined with therapeutic hypothermia in a piglet asphyxia model. *Acta Anaesthesiol. Scand.* **2014**, *58*, 733–742. [CrossRef] [PubMed]
13. Glass, P.S. The art of reasonable combining drugs in anesthesia. *Cah. D'Anesthesiol.* **1994**, *42*, 635–640.
14. Rydén, A.; Manell, E.; Biglarnia, A.; Hedenqvist, P.; Strandberg, G.; Ley, C.; Hansson, K.; Nyman, G.; Jensen-Waern, M. Nursing and training of pigs used in renal transplantation studies. *Lab. Anim.* **2020**, *54*, 469–478. [CrossRef]
15. Re, M.; Canfrán, S.; Largo, C.; de Segura, I.A.G. Effect of Lidocaine-Ketamine Infusions Combined with Morphine or Fentanyl in Sevoflurane-Anesthetized Pigs. *J. Am. Assoc. Lab. Anim. Sci. JAALAS* **2016**, *55*, 317–320.
16. Laricchiuta, P.; De Monte, V.; Campolo, M.; Grano, F.; Iarussi, F.; Crovace, A.; Staffieri, F. Evaluation of a butorphanol, detomidine, and midazolam combination for immobilization of captive Nile lechwe antelopes (Kobus magaceros). *J. Wildl. Dis.* **2012**, *48*, 739–746. [CrossRef]
17. Henrikson, H.; Jensen-Waern, M.; Nyman, G. Anaesthetics for general anaesthesia in growing pigs. *Acta Vet. Scand.* **1995**, *36*, 401–411. [CrossRef] [PubMed]
18. Grandin, T. Minimizing Stress in Pig Handling in the Research Lab. *Lab. Anim.* **1986**, *15*, 15–20.
19. Whelan, G.; Flecknell, P.A. The assessment of depth of anaesthesia in animals and man. *Lab. Anim.* **1992**, *26*, 153–162. [CrossRef] [PubMed]
20. Eger, E.I., 2nd; Johnson, B.H.; Weiskopf, R.B.; Holmes, M.A.; Yasuda, N.; Targ, A.; Rampil, I.J. Minimum alveolar concentration of I-653 and isoflurane in pigs: Definition of a supramaximal stimulus. *Anesth. Analg.* **1988**, *67*, 1174–1176. [CrossRef] [PubMed]
21. Haga, H.A.; Tevik, A.; Moerch, H. Motor responses to stimulation during isoflurane anaesthesia in pigs. *Vet. Anaesth. Analg.* **2002**, *29*, 69–75. [CrossRef] [PubMed]
22. Kaiser, G.M.; Breuckmann, F.; Aker, S.; Eggebrecht, H.; Kuehl, H.; Erbel, R.; Fruhauf, N.R.; Broelsch, C.E.; Quick, H.H. Anesthesia for cardiovascular interventions and magnetic resonance imaging in pigs. *J. Am. Assoc. Lab. Anim. Sci. JAALAS* **2007**, *46*, 30–33.
23. Lu, D.Z.; Fan, H.G.; Wang, H.B.; Hu, K.; Zhang, J.T.; Yu, S.M. Effect of the addition of tramadol to a combination of tiletamine-zolazepam and xylazine for anaesthesia of miniature pigs. *Vet. Rec.* **2010**, *167*, 489–492. [CrossRef]
24. Lima-Rodriguez, J.R.; Garcia-Gil, F.A.; Garcia-Garcia, J.J.; Rocha-Camarero, G.; Martin-Cancho, M.F.; Luis-Fernandez, L.; Crisostomo, V.; Uson-Gargallo, J.; Carrasco-Jimenez, M.S. Effects of premedication with tiletamine/zolazepam/medetomidine during general anesthesia using sevoflurane/fentanyl in swine undergoing pancreas transplantation. *Transplant. Proc.* **2008**, *40*, 3001–3006. [CrossRef]
25. Rydén, A.; Nyman, G.; Nalin, L.; Andreasson, S.; Velikyan, I.; Korsgren, O.; Eriksson, O.; Jensen-Waern, M. Cardiovascular side-effects and insulin secretion after intravenous administration of radiolabeled Exendin-4 in pigs. *Nucl. Med. Biol.* **2016**, *43*, 397–402. [CrossRef] [PubMed]
26. Clutton, R.E.; Blissitt, K.J.; Bradley, A.A.; Camburn, M.A. Comparison of three injectable anaesthetic techniques in pigs. *Vet. Rec.* **1997**, *141*, 140–146. [CrossRef] [PubMed]
27. Morgaz, J. Swine model in transplant research: Review of anaesthesia and perioperative management. *World J. Anesthesiol.* **2015**, *4*, 73. [CrossRef]
28. Pehbock, D.; Dietrich, H.; Klima, G.; Paal, P.; Lindner, K.H.; Wenzel, V. Anesthesia in swine: Optimizing a laboratory model to optimize translational research. *Der Anaesthesist* **2015**, *64*, 65–70. [CrossRef]
29. Lin, H.C.; Thurmon, J.C.; Benson, G.J.; Tranquilli, W.J. Telazol—A review of its pharmacology and use in veterinary medicine. *J. Vet. Pharmacol. Ther.* **1993**, *16*, 383–418. [CrossRef]
30. Santos González, M.; Bertrán de Lis, B.T.; Tendillo Cortijo, F.J. Effects of intramuscular alfaxalone alone or in combination with diazepam in swine. *Vet. Anaesth. Analg.* **2013**, *40*, 399–402. [CrossRef]
31. Pawson, P.; Forsyth, S. Anesthetic agents. In *Small Animal Clinical Pharmacology*, 2nd ed.; Maddison, J.E., Page, S.W., Church, D.B., Eds.; W.B. Saunders: Edinburgh, UK, 2008; pp. 83–112. [CrossRef]
32. Musk, G.C.; Costa, R.S.; Tuke, J. Body temperature measurements in pigs during general anaesthesia. *Lab. Anim.* **2015**, *50*, 119–124. [CrossRef]
33. Alstrup, A.K. Blood Lactate Concentrations in Göttingen Minipigs Compared with Domestic Pigs. *J. Am. Assoc. Lab. Anim. Sci. JAALAS* **2016**, *55*, 18–20.
34. Carroll, G.L.; Boothe, D.M.; Hartsfield, S.M.; Waller, M.K.; Geller, S.C. Pharmacokinetics and selected behavioral responses to butorphanol and its metabolites in goats following intravenous and intramuscular administration. *Vet. Anaesth. Analg.* **2001**, *28*, 188–195. [CrossRef] [PubMed]
35. Paine, S.W.; Bright, J.; Scarth, J.P.; Hincks, P.R.; Pearce, C.M.; Hannan, C.; Machnik, M.; Hillyer, L. The intravenous pharmacokinetics of butorphanol and detomidine dosed in combination compared with individual dose administrations to exercised horses. *J. Vet. Pharmacol. Ther.* **2020**, *43*, 162–170. [CrossRef]
36. Knych, H.K.; Casbeer, H.C.; McKemie, D.S.; Arthur, R.M. Pharmacokinetics and pharmacodynamics of butorphanol following intravenous administration to the horse. *J. Vet. Pharmacol. Ther.* **2013**, *36*, 21–30. [CrossRef] [PubMed]
37. Honkavaara, J.; Restitutti, F.; Raekallio, M.; Salla, K.; Kuusela, E.; Ranta-Panula, V.; Rinne, V.; Vainio, O.; Scheinin, M. Influence of MK-467, a peripherally acting α2-adrenoceptor antagonist on the disposition of intravenous dexmedetomidine in dogs. *Drug Metab. Dispos.* **2012**, *40*, 445–449. [CrossRef] [PubMed]

38. Pypendop, B.H.; Ilkiw, J.E. Pharmacokinetics of dexmedetomidine after intravenous administration of a bolus to cats. *Am. J. Vet. Res.* **2014**, *75*, 441–445. [CrossRef] [PubMed]
39. Ranheim, B.; Risberg, Å.I.; Spadavecchia, C.; Landsem, R.; Haga, H.A. The pharmacokinetics of dexmedetomidine administered as a constant rate infusion in horses. *J. Vet. Pharmacol. Ther.* **2015**, *38*, 93–96. [CrossRef]
40. Grimsrud, K.N.; Ait-Oudhia, S.; Durbin-Johnson, B.P.; Rocke, D.M.; Mama, K.R.; Rezende, M.L.; Stanley, S.D.; Jusko, W.J. Pharmacokinetic and pharmacodynamic analysis comparing diverse effects of detomidine, medetomidine, and dexmedetomidine in the horse: A population analysis. *J. Vet. Pharmacol. Ther.* **2015**, *38*, 24–34. [CrossRef]
41. Li, W.; Li, G.; Zhong, Z.; Xie, B.; Zhou, Z.; Gu, W.; Shi, X.; Tang, T.; Ai, S.; Fu, H.; et al. Validation of an HPLC assay for determination of Telazol in pregnant pigs: Application to placental transfer study. *J. Vet. Med. Sci.* **2017**, *79*, 801–806. [CrossRef]
42. Semple, H.A.; Gorecki, D.K.; Farley, S.D.; Ramsay, M.A. Pharmacokinetics and tissue residues of Telazol in free-ranging polar bears. *J. Wildl. Dis.* **2000**, *36*, 653–662. [CrossRef]
43. Farrell, K.S.; Hopper, K.; Cagle, L.A.; Epstein, S.E. Evaluation of pulse oximetry as a surrogate for PaO(2) in awake dogs breathing room air and anesthetized dogs on mechanical ventilation. *J. Vet. Emerg. Crit. Care* **2019**, *29*, 622–629. [CrossRef] [PubMed]
44. Rigg, J.R.; Jones, N.L. Clinical assessment of respiratory function. *Br. J. Anaesth.* **1978**, *50*, 3–13. [CrossRef] [PubMed]

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2021:83

The aim of this thesis was to improve the welfare of growing pigs in research by refining the perioperative nursing and anaesthesia care. Pigs underwent a training program which enabled interventions postoperatively without restraint. Furthermore, a refined technique for blood sampling was evaluated. In summary, the results showed that nursing interventions, adjustment of anaesthesia techniques and the use of AI technology for activity measurement can contribute to stress-free handling and improved animal welfare in growing pigs.

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Online publication of thesis summary: http://pub.epsilon.slu.se/

ISSN 1652-6880
ISBN (print version) 978-91-7760-843-1
ISBN (electronic version) 978-91-7760-844-8