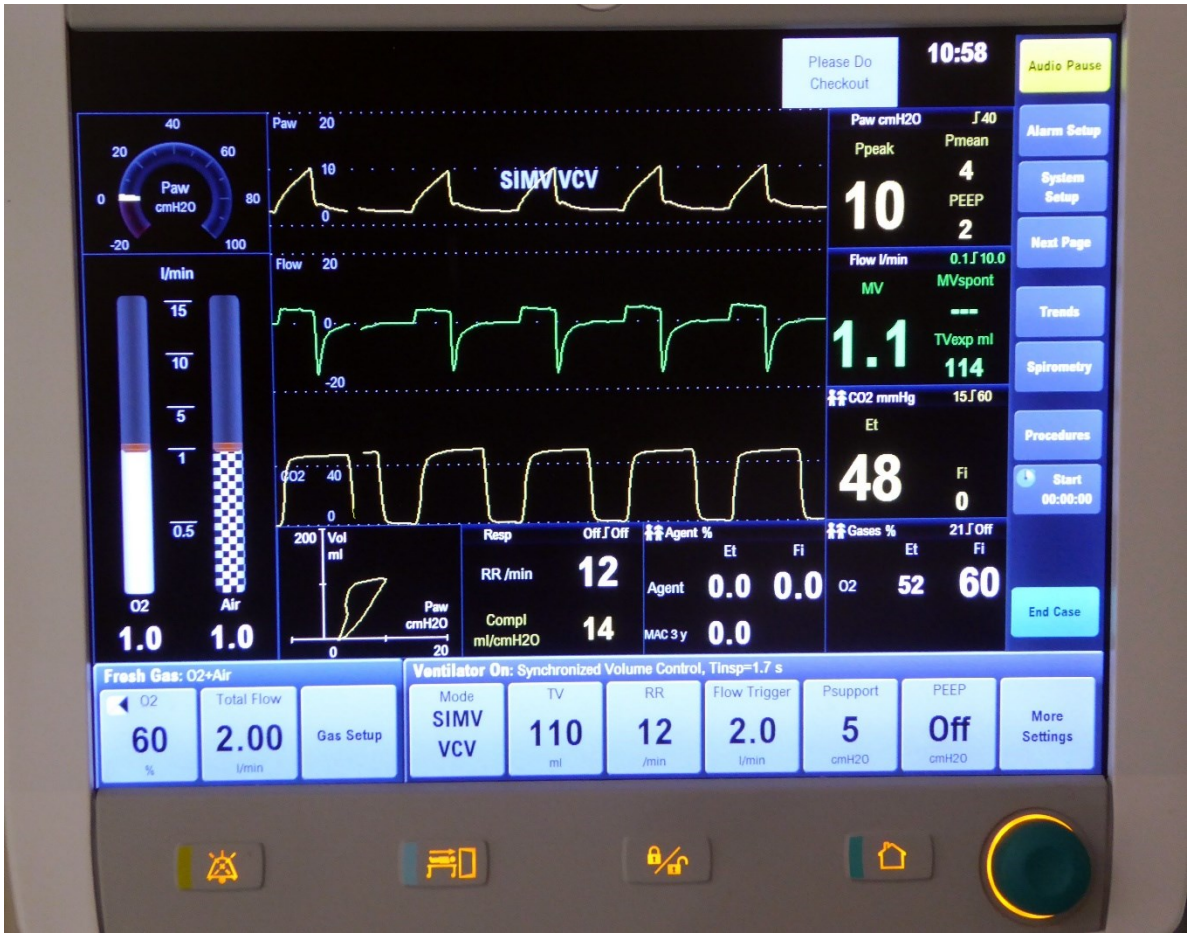
Technical Guide

Anaesthesia and Analgesia Göttingen Minipigs



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ELLEGAARD

GÖTTINGEN MINIPIGS

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Introduction

Ellegaard Göttingen Minipigs can easily be anaesthetised for several hours without a higher risk of complications. As they are microbiologically defined, with the absence of certain pathogens, the risk of respiratory arrest and cardiovascular failure is decreased. Furthermore, the absence of the halothane gene prevents the development of malignant hyperthermia.
However, any anaesthesia procedure should be carefully planned, and the protocol adjusted to the study design prior to carrying out the anaesthesia. It is of utmost importance that the physiologic effects of the anaesthetic protocol are considered in advance to avoid complications of the experiment.
The purpose of this paper is to provide information on the material and methods tested at Ellegaard Göttingen Minipigs and other Minipig users. The details are based on our in-house experience and feedback from users and are by no means exhaustive. Although we will provide a lot of practical information and ready to use protocols this document is not meant to act as a textbook on veterinary anaesthesia. Before attempting to anesthetize a Minipigs the investigator should acquire profound knowledge Anaesthesia, the pharmacology of the drugs used as well as the physiology of the respiratory and cardiovascular systems.

If you plan to anesthetize juvenile Minipigs you need to give special attention to the fact that they have an immature drug metabolism and transport. Therefore, it might not be able to metabolize certain drugs. [1]
Inhalation drugs might be more appropriate as they are eliminated by ventilation.

An important point is that Göttingen Minipigs are out-bread and interindividual differences should be expected. Therefore, every patient should be evaluated on its own merits.

Summary

Successful anaesthesia in general should provide sufficient analgesia, narcosis, and muscle relaxation. Göttingen minipigs are sedated by intramuscular injection, prior to anaesthesia to avoid physical restraint and stress. If the Göttingen Minipigs are well socialized, they can be induced with Sevoflurane in Oxygen via mask. Thereafter, an ear vein catheter is placed and used to deepen sedation or induce anaesthesia. After induction, establishing an airway is recommended, particularly for prolonged anaesthesia. Either by tracheal intubation or by placing a LMA.
The anaesthesia can be maintained by inhalation (e.g., isoflurane/sevoflurane) or by a continuous rate infusion CRI (e.g., propofol).
As Göttingen Minipigs have a poor thermoregulation system, it is important that measures to prevent hypothermia are in place. That starts from the time of sedation until homeostasis is reached after recovery.
Anaesthesia and analgesia are essential for the prevention of pain during surgery. A multi modal analgesic regime that suits the planned procedure is advised.
Careful postoperative observation is of importance following anaesthesia and surgery in Göttingen Minipigs.

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Basic concept of an Anaesthesia

1. Pre-Surgery
   - full clinical examination
   - obtain an accurate bodyweight
   - Withhold of food but not water, 12h prior to anesthesia
2. Sedation
   - Tranquilizer
3. Induction
   - Injectables/Mask
   - IV access
   - (Anticholinergic)
   - Intubation
4. Maintenance
   - Volatile gases
   - Intermitted IV doses
   - Continuous infusion
5. Recovery

What do we want in a balanced general anaesthesia?

No single drug can archive that. A fast acting, easy controllable system is desired.

Pre-Anaesthesia Considerations

Acclimation

Göttingen Minipigs should be well acclimatised in the experimental unit before the start of the experiment. Depending on circumstances that can be from one, up to three weeks.

A clinical examination should be carried out to assure the animal is fit for the procedures or surgery to be carried out. The extend of this should be decided by the veterinarian and based on the planned procedures. As a minimum, a brief examination should include observation of behaviour and respiration rate. (a high frequency may indicate lung disease or stress).

Reference values for clinically healthy Göttingen Minipigs:

Respiration rate:	11–29
Heart rate:	65–95
Body temperature [°C]	37–39

Fasting

Göttingen minipigs are not prone to vomit and it rarely occurs, however it is a good practice to deprive them of food for six to twelve hours before any anaesthesia procedure. For abdominal surgery up to 24 hours is appropriate, but even after that period the stomach will not be empty.
During the fasting period the bedding material must be removed as well, otherwise it will be ingested.

Most used drugs

Inhalable Drugs

- Isoflurane
- Sevoflurane
- Nitrous Oxide

Injectable Drugs

- Diazepines
  - Diazepam
  - Midazolam
  - Zolazepam
- Dissociative
  - Ketamine
  - Tiletamine
- α-2 Agonists
  - Xylazine
  - Medetomidine
- Opioids
  - Butorphanol
  - Buprenorphine
  - Fentanyl
- Propofol

Pharmacology of the most used drugs

Please note that the following data in the tables are collected from several textbooks and are indicative only.

Drug	Main effects	Duration	Route	Remarks
Diazepam	Minor tranquillizer Muscle relaxation	1.5 h	IM or IV	No analgesic effect, good moderate sedation
Midazolam		1.0 h		
Ketamine	Dissociative Pseudo narcosis	0.5 h	IM or IV	Weak analgesia
Zoletile		1.0 h		Contains Tilletamine and Zolazepam
Xylaxine	Sedative Analgesic	1.0 h	IM or IV	Not very effective in pigs, they should be associated with Ketamine and true analgesic
Medetomidine		1.0 h		
Propofol	Narcosis and muscle relaxation	0.25 h	IV	No analgesic effect Bolus or CRI
Fentanyl	Opioid analgesic	0.25 h	IV - CRI	Analgesia maintenance with continuous infusion.
Butorphanol		2-4 h	IM or IV	No strong sedative, could be associated with tranquillizers and or Ketamine.
Buprenorphine		8-12 h		
Isoflurane	Narcosis and muscle relaxation	0.2 h	Inhalation	For narcosis induction and maintenance
Sevoflurane		0.1 h		

Drug	Anxiolysis	Sedation	Hypnosis	Analgesia	Amnesia	Anesthesia
Diazepam	+	+	+	0	+	0
Midazolam	+	+	+	0	+	0
Ketamine	0	0	0	+	+	+ Dissosiative
Zoletile	+	+	+	+	+	+
Xylaxine		+	(+)	+		0
Medetomidine		+	(+)	+		(+ C)
Propofol	0	+	+	0	+	+
Fentanyl	0	+	+	+	0	0
Butorphanol	0	(+)		+	0	(+ C)
Buprenorphine	0	+		+	0	0
Isoflurane	0	+	+	0	+	+
Sevoflurane	0	+	+	0	+	+

Drug		HR	BP	Ventilation	Cerebral blood flow	Intracranial Pressure	PK/PD	
							t_{max}	½ time
Diazepam		0*	0	0*	0	0	30	180
Midazolam		↓	0/↓	↓	0/↓	0/↓	15	140
Ketamine		↑	↑	0/↑	↑	↑	15	120
Zoletile	Tiletamine	↑	↑	0/↑	↑	↑	30	180
	Zolazepam	↓	0/↓	↓	0/↓	0/↓	60	480
Xylaxine		↓	↑then↓	↓	↓	↓	15	35
Medetomidine		↓	↑then↓	↓	↓	↓	15	60
Propofol		0/↑	↓25 %	↓	↓	↓	1	45
Fentanyl		↓	↓	↓	↓	↓	5	15
Butorphanol		↓	↓	↓	↓	↓		360
Buprenorphine		↓	↓	↓	↓	↓	60	37h
Isoflurane		↑MAC .25	↓	Vt↓ RR↑	↑	↑	10*	<5
Sevoflurane		↑MAC1.5	↓	Vt↓ RR↑	↑	↑	6*	<5

Anticholinergic

Atropine is the most common agent used, but severe tachycardia is associated with atropine and could lead to cardiac collapse in hypovolemic pigs.
It is not recommended to prevent bradycardia for routine use, but if bradycardia occurs a dose of 0,04mg/kg IM or 0,02mg/kg IV is appropriate.
Bradycardia is defined by HR < pre-anaesthetic HR value /2
Pre-anaesthetic atropine can used to prevent Hypersalivation induced by Ketamine or Tiletamine, but this is rarely the case. If stimulation of the vagus nerve is expected the use of atropine might be advised.
Pharmacology: Cardiovascular effects 5 min. after IM injection, peak after ca. 15 min. HR up 30-40% for 30 min

Sources:
Lumb&Jones Veterinary Anesthesia and Analgesia Blackwell Publishing
Pharmacology for Anaesthesia and intensive care T.E.Peck and S.A. Hill
Veterinary Anaesthesia K.W. Clarke, C.M. Trim, L.W. Hall
Swine in the Laboratory M. Swindle

Protocols

General anaesthesia needs an anaesthetic protocol with specific techniques to obtain safety and research data reproducibility.
An anaesthetic protocol is always a combination of drugs to achieve both clinical and practical components of anaesthesia

- Adapted according to the surgery
- Adapted according to the animal
- Adapted according to the aim of the experimental study

All anaesthetic protocols are associated to:

- Hypoventilation inducing hypercapnia and hypoxemia
  - RR/Vt ↓ CO_2 ↑ blood O_2 ↓ blood
- Hypotension inducing hypoxia, hypoxemia, and hypothermia
  - BP↓ O_2 ↓tissue O_2 ↓ blood BT ↓

This is due to narcosis components, explaining the clinical relevance of:

- Narcosis depth monitoring
- Hypothermia prevention
- (pre) anaesthesia fluid administration
- Oxygenation before and during anaesthesia

Sedation-Induction

Sedation covers a progressive band of stages, ranging from relaxation to anaesthesia

	Minimal Sedation	Moderate Sedation	Deep Sedation	General Anesthesia
Consciousness	Awake; Relaxed coordination (-) Cognitive function (-)	Drowsy; Sleepy. Light sleep	Asleep	Unconscious
Responsiveness	Normal response to verbal and tactile stimuli	Purposeful; awakens When touched.	Unresponsive to verbal commands; purposeful movements to painful stimuli	Not arousable; Unresponsive to verbal or tactile stimuli; usually no movement except to painful stimuli
Airway; Protective Reflexes	Unaffected	Patent	Airway may be impaired. May require airway management	Airway often impaired, often requires airway support
Ventilation Status	Unaffected	Adequate	Possibly inadequate. supplemental oxygen indicated	Impaired; often requires support of Mechanical ventilation
Cardiovascular Function	Unaffected	Stable	Stable	May be impaired Requires fluids Vasopressor

Protocols for sedation by intramuscular injection

The following protocols have been used with Göttingen Minipigs successfully. Please note that time to LOR, deepness and duration of sedation is subject to great variation. This can be due to size and age as well as interindividual variation in metabolism.
Effects vary from light sedation, applicable for painless procedures like scanning, to anaesthesia that allows short minor procedures. Protocols used for induction might need an IV top up (once iv access is established) with propofol or other drugs to enable endotracheal intubation.
Please make sure, that you have a proper IM injection as even smaller Minipigs can have an unexpected thick fat layer. Do also consider the total Volume that is required for the injection. Ideally the cocktails are given mixed in one injection and not more than 5ml per site.
Please consult our Technical Guide: *Handling, Dosing and Training of the Göttingen Minipig* for more information regarding IM injection.
Some cocktails have analgesic drugs included, others have not. Please make sure that you provide appropriate analgesia for the proposed intervention.

M	Moderate sedation for ca. 30min. Useful for US - scanning and mask inhalation studies.		
	concentration mg/ml	Dose mg/kg	volume ml/kg
Medazolam	5	0,3 - 0,5	0,1
Total injection volume			0,1

MK	Deep sedation 30-60 min, for induction or CT scanning		
	concentration mg/ml	Dose mg/kg	volume ml/kg
Medazolam	5	1	0,2
Ketamine	100	10	0,1
Total injection volume			0,3

MKX	Deep sedation, light anaesthesia ca.60 min, good recovery		
	concentration mg/ml	Dose mg/kg	volume ml/kg
Medazolam	5	0,5	0,1
Ketamine	100	6	0,06
Xylazine	20	0,5	0,025
Total injection volume			0,185

MMA	Moderate to deep sedation, for induction		
	concentration mg/ml	Dose mg/kg	volume ml/kg
Medazolam	5	0,5	0,1
Medetomidine	1	0,05	0,05
Atropine	1	0,02	0,02
Total injection volume			0,17

MMB	Moderate to deep sedation, for induction or short procedures		
	concentration mg/ml	Dose mg/kg	volume ml/kg
Medazolam	5	0,2	0,4
Medetomidine	1	0,05	0,05
Butorphanol	10	0,2	0,02
Total injection volume			0,11

MDKM	Deep sedation, as induction for thoracic surgery		
	concentration mg/ml	Dose mg/kg	volume ml/kg
Medazolam	5	0,5	0,1
Dexmedetomidine	0,1	0,005	0,005
Ketamine	100	5	0,05
Methadone	10	0,2	0,02
Total injection volume			0,22

XK	Deep sedation, for induction or Seldinger catheter placement. 60-90 min. Reversable after 45 min. with alpha2 antagonists		
	concentration mg/ml	Dose mg/kg	volume ml/kg
Xylazine	20	2	0,1
Ketamine	100	20	0,2
Total injection volume			0,3

MBK	Deep sedation, for induction or short procedures. 60-90min. Reversable after 30min with alpha2 antagonists		
	concentration mg/ml	Dose mg/kg	volume ml/kg
Medetomidine	1	0,08	0,1
Butorphanol	10	0,22	0,022
Ketamine	100	10	0,1
Total injection volume			0,222

The protocols below are based on Zoletil(Telazol) Zoletil 50 is supplied in a vial that contains dry matter of 125mg Tiletamine and 125mg Zolazepam adding up to 250mg. An accompanying vial contains a mixing liquid. Only the dry matter is used in the protocols and the other products are added to it.

Zoletil is associated with prolonged recovery, where the Minipig tries to rise but falls again with every attempt and tumbles around in the pen. This might be due to the very log halve-life of Zolazepam. A well-padded recovery box is recommended

ZXKB	Deep sedation to anaesthesia, for induction or short procedures. 60-90min. The most widely used protocol at Ellegaard. The Minipigs are stable, minimally cardiovascular compromised and are breathing spontaneously also at higher doses			
	Concentration mg/ml	Volume added ml	Conc. in mix mg/ml	Dose mg/kg
Tiletamine/Zolazepam	Dry/250mg	0	25	2,5
Xylazine	20	6,25	12,5	1,25
Ketamine	100	1,25	12,5	1,25
Butorphanol	10	2,5	2,5	0,25
Total injection volume		10		0,1 ml/kg

ZXKM	Deep sedation to anaesthesia, for induction or short procedures. 60-90min. This protocol is similar to ZXKB but has with Methadone a full μ-agonist for more effective analgesia. It is especially helpful when other μ-agonist are used during surgery.			
	Concentration mg/ml	Volume added ml	Conc. in mix mg/ml	Dose mg/kg
Tiletamine/Zolazepam	Dry/250mg	0	25	2,5
Xylazine	20	6,25	12,5	1,25
Ketamine	100	1,25	12,5	1,25
Methadone	10	2,5	2,5	0,25
Total injection volume		10		0,1 ml/kg

ZM	This protocol can be used for induction or short procedures, but for the later analgesia needs to be administered. The advantage is a very low injection volume that's beneficial for large Minipigs			
	Concentration mg/ml	Volume added ml	Conc. in mix mg/ml	Dose mg/kg
Tiletamine/Zolazepam	Dry/250mg	0	125	5
Medetomidine	1	2	1	0,04
Total injection volume		10		0,04 ml/kg

Induction by volatile gases

The most used volatile gases to anesthetize Göttingen Minipigs are Isoflurane and Sevoflurane. Sometimes supported by Nitrous oxide. Minimal Alveolar Concertation is a measure for their potency. For humans it is the concentration where 50% of the patients do not react to a surgical stimulus. There are several publications for pigs, but each has used a different method to establish a value. Therefore, the following data for MAC are indicative only:

Isoflurane: 1.7%
Sevoflurane: 3,0%
Nitrous oxide 277%

Only a very small part of Isoflurane and sevoflurane is metabolized, elimination is mainly by ventilation.

For induction by mask, Sevoflurane should be used as it has the following advantages compared with isoflurane.

Shorter time to brain equilibrium
More pleasant odour
Less irritating to the airways

Socialized Göttingen Minipigs will accept a mask and can be induced without any sedatives. However, some prior habituation will be beneficial. Standard masks are available in various sizes and work ok, but as they are never absolutely tight. It is therefore advised to use some form of air extraction to have a save working environment.

The Minipigs can be placed in a sling or held on the arm of a sitting technician. The mask should have an appropriate flow of 100% oxygen (2-3x minute volume) and is placed over the snout. Once the Minipigs has accepted this and is relaxed, the vaporizer is set to 7% and within a short time the Minipig will fall asleep. The vaporiser setting can then be reduced to maintain the required plane of anaesthesia.

For faster induction up to 60% of nitrous oxide in 40% oxygen can be used to take advantage of the second gas effect.

Other anaesthetics used together with these volatile gases have an additive effect: MAC is reduced, and a smaller concentration is needed to reach the same plane of anaesthesia.

Ventilation

With most of the sedation protocols mentioned earlier the Göttingen Minipig will breathe spontaneously. Respiration however is compromised and as a minimum oxygen should be supplied.

For longer procedures, surgery where a deep plane of anaesthesia is required and when μ-agonist are used, endotracheal intubation and mechanical ventilation is recommended. Other indicators to use mechanical ventilation are:

PaO2 < 55-60 mm Hg with O_2 supplied

EtCO2 > 55mm Hg

Laborious breathing: RR >35/min, Vt <5ml/kg

Depending on the available ventilator, a pressure support setting with spontaneous breathing can be adequate.

General settings for ventilation

Mode:	Volume- or pressure-controlled modus depending on type of surgery and preference.
Fresh gas:	30-100% oxygen : 70-0% medical air. 40-60% oxygen : 60-40% nitrous oxide.
Fresh gas flow:	0,5-1,5 times minute volume. (70- 210 ml/kg)
Tidal volume:	8-10 ml/kg
Frequency	10-20 /min
PIP:	10-15 cm H_2O
PEEP:	max 5 cm H_2O* * most ventilator have a natural PEEP of ca. 2 which is ideal.
I:E ratio:	1 : 2

Adjust these initial settings to have normocapnia (35-45 cm H2O $EtCO_2$) an a SpO_2 > 95%

Anaesthesia by volatile gases

As mentioned in the induction section, Isoflurane and Sevoflurane are the most common gases used for anaesthesia. How these gases are delivered depends on available equipment, but with all systems F_iO_2, FGF (fresh gas flow) and anaesthetic concentration can be set by the clinician.

Anaesthetics are not given in fixed doses but are administered to effect.

Potency, MAC

There are no standardised methods to determine the MAC values for pigs/minipigs but based on available literature the following values can be used as indication:

Isoflurane:	1.7%
Sevoflurane:	3,0%

The standard deviation of MAC is 10%, thus 95% of patients will not respond to 1.2 MAC, and 99% will not respond to 1.3 MAC.

In most cases the minipigs have received other sedative drugs that reduce MAC

The Mac is influenced by many factors like e.g.:

- Temperature: ↓ 2%-5% for 1 °C ↓ temp.
- Age: ↓ by age
- Benzodiazepine, opiates, alpha-2 agonists: ↓ MAC
- Inhaled anaesthetics: additive effect, 1% N_2O decreases MAC by 1%
- Pregnancy: ↓ MAC
- Metabolic acidosis, hypoxia, hypotension: ↓ MAC

Pulmonary Effects

The following effects are associated with anaesthetic gases:

- Respiratory depression
  - V_t↓ RR↑ , MV is often not changed but $EtCO_2$ rises because of a constant dead space
- Depressed ventilatory responses to hypercarbia and hypoxia in a dose dependent manner
- Respiratory Irritation
  - 50% experience irritation at 2 MAC Isoflurane, but none at 2 MAC Sevoflurane
  - No irritation experienced at 1 MAC of either
  - Sevoflurane is the agent of choice for inhalation induction
- Potent bronchodilators
- Apnoea at 2-3 MAC

Cardiovascular Effects

Isoflurane leads to a more significant dosage-dependent cardiovascular depression.

- MAP ↓ Systemic Vascular Resistance (SVR) ↓
- HR ↑
  - Sevoflurane at MAC 1.5
  - Isoflurane at MAC 0.25
- CO ↓ NC
- QT interval ↑
- Cardiac arrest at 3-5 MAC

Uptake and distribution

There is a concentration gradient associated with the uptake of inhaled anaesthetics.

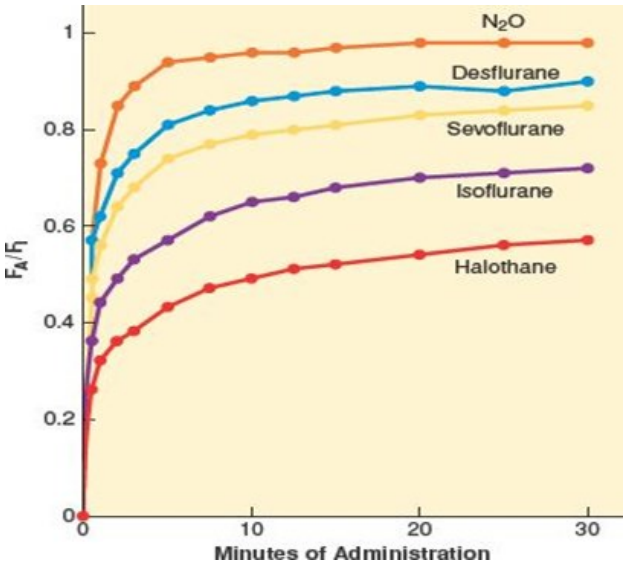
Delivered>Inspired>Alveolar>Arterial>Brain

Brain with its high perfusion per gram rapidly equilibrates with anaesthetic partial pressure in blood. Complete equilibration with any tissue takes 3 time constants, time constant for isoflurane is 3-4 minutes, for sevoflurane, 2 minutes. For all inhaled anaesthetics, the inhaled concentration should therefore be decreased after 6-12 minutes.

	Time constant	Brain Equilibration time
Isoflurane	3-4 mins	10-15 mins
Sevoflurane	2 mins	6 mins

The balance between the delivery of anesthetic and its removal by uptake or metabolism determines F_A/F_I ratio at any given time after administration of inhaled anesthetic. The rate of rise of alveolar concentration (F_A) toward inspired concentration (F_I) or F_A/F_I ratio determines speed of induction of anesthesia and can be influenced by:

- Alveolar ventilation
- The inspired concentration
- Second gas effect

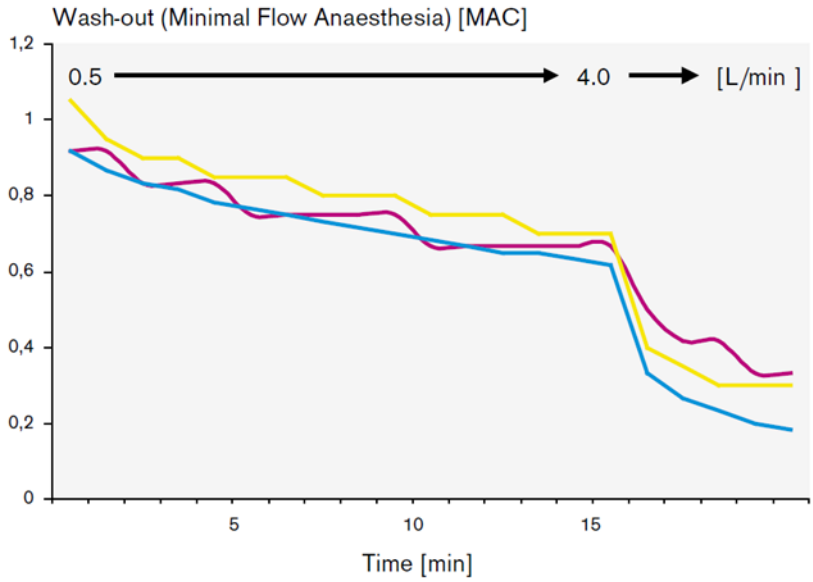


Wash out

Recovery correlates with fall in alveolar concentration and is influenced by the following factors.

- Solubility: Low solubility = Rapid recovery.
    - Solubility Sevoflurane>Isoflurane
- Duration of anesthesia.
- MAC awake: Varies with different anesthetics.

Ventilation is the most important factor affecting the decrease of the anesthetics in the system.



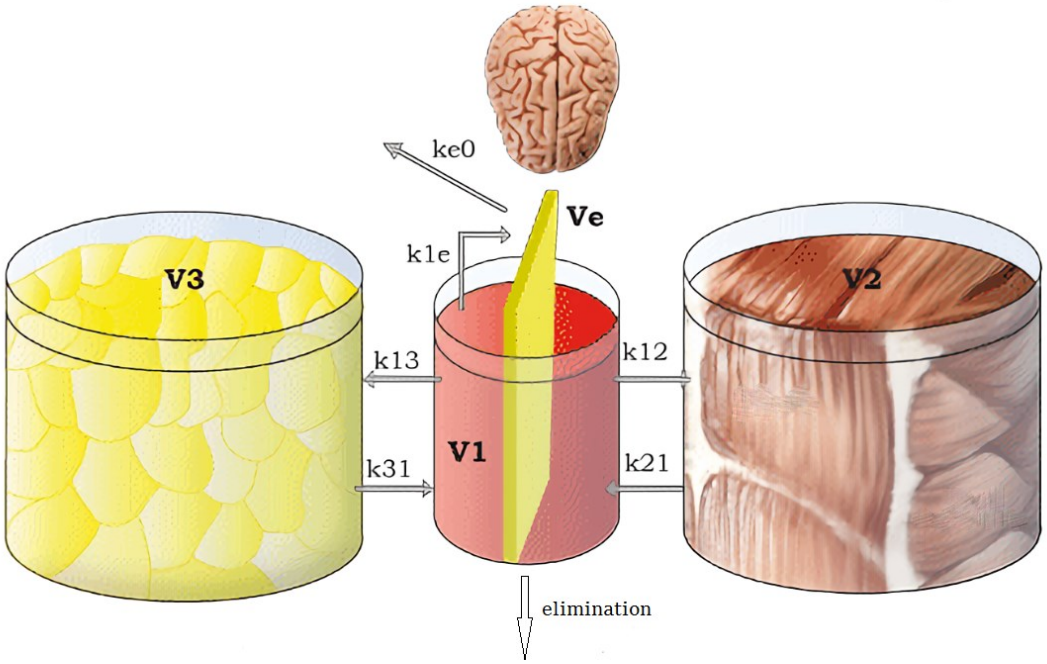
Effect of different FGF settings on alveolar concentration (y-axis) against wash-out time of Sevoflurane, Isoflurane and Desflurane.

TIVA

Propofol is the most used agent for total intravenous anesthesia; barbiturates are being phased out and not used at Ellegaard Göttingen Minipigs.
Strictly speaking, in practice it is not TIVA that is applied as the patients are often heavily sedated previously with other drugs.

The pharmacokinetics of propofol can be well described by a three-compartment linear model, with the compartments representing plasma (V1), rapidly (muscle V2) and slowly (fat V3) equilibrating tissues.

Propofol is highly lipophilic and distributes extensively in the body. Propofol is extensively metabolized by the liver prior to its elimination by the kidney. During induction, propofol decreases the systolic and diastolic blood pressure by approximately 20–30 percent with minimal change in heart rate; apnea is also common. [3]



Adaptado de Mani V, Morton NS. Overview of total intravenous anesthesia in children. Paediatr Anaesth. 2010;20(3):211-22.

The blood concentration-time profile of propofol after an iv bolus injection follows the three-compartment model with half-lives of 2–4 min, 30–45 min, and 3–63 h, respectively. The elimination half-life of Propofol is context sensitive. As Propofol has a large volume of distribution the Morgan et al. [4] conclude, that despite the very long elimination half-life, blood propofol concentrations appeared to approach steady state within 20 min rather than the 4-5 half-lives normally expected. This is because for this drug, which displays multicompartment pharmacokinetics, the rate of initial rise of blood concentrations is governed primarily by the very short distribution half-life of the drug. Therefore, the long elimination half-life of propofol is probably of little significance in designing infusions regimens, but the lower systemic clearance should be taken into account to avoid unwanted accumulation.

There will be accumulation and a rise of blood plasma level at a fixed rate infusion, therefore the rate needs the be reduced during the duration of the anesthesia to maintain constant plasma levels.

There are several well described models in the humane sector (https://www.bjaed.org/article/S2058-5349(17)30085-9/pdf), but not many published data for pigs and Minipigs can be found.

At Ellegaard we use propofol as anesthetic agent in a CRI routinely and our protocols are based on modified human models. We assume the following.

- rapid distributed into peripheral tissues.
- Onset of action 20-40 s
- Time to peak 90-120 s
- Duration: 5-10 min
- Propofol has no analgesic effect

Induction

If there is an IV access, we use a bolus induction dose of:

1-6mg/kg – to effect.

As there is a lag to peak effect, it is advised to dose slow and observe the patient carful and pay particular attention to apnea.

This might be enough for short painless procedures. Anesthesia can be prolonged with small, intermitted doses.

In most cases, there is no established venous access, so the Göttingen Minipigs are sedated to a various degree with one of the protocols mentioned under Protocols. This allows to place an over the needle catheter and do another preparation for surgery. Anesthesia might be induced now, but the drugs used for sedation must be considered and only a small dose of Propofol might be necessary. Monitor carefully.

Continuous Rate Infusion CRI

The aim is a Bolus, Elimination, Transfer (BET) regimen:

1. a bolus dose calculated to fill the central (blood) compartment,
2. a constant-rate infusion equal to the elimination rate,
3. an infusion that compensates for transfer to the peripheral tissues

As a rule of thumb, the following protocol is appropriate for a manual infusion scheme. Infusion pumps might have programmed TIVA settings. The settings are based humane models, regularly accompanied by opioids. They might not be appropriate and have not been tested with Göttingen minipigs.

Loading dose	1-2mg/kg	then
	10mg/kg/h for 10 min	then
	8mg/kg/h for 10 min	then
	6mg/kg/h thereafter	

But please make allowances for the other drugs the patient may have received during prep. They are often accumulative so less propofol is needed.

At Ellegaard, we often use a CRI of propofol with large Minipigs that have been sedated with the ZXKB cocktail initially. Knife time of these surgeries is between 1 and 3 h. For these adult Minipigs, usually around 30kg or more the following Protocoll is applied.

1. Im injection of ZXKB, IV access, intubation and other prep
    a. Connect to ventilator, spontaneous breathing mode
2. Propofol loading dose 0,5 mg/kg over 2 min
    a. Spontaneous breathing often ceases and SIMV takes over
3. After 3 min CRI is started at:
    a. 6mg/kg for ca. 20min, then
    b. 5mg/kg for ca. 20min, then
    c. 4mg/kg for the remaining time.

We observe a great variation of anaesthesia between different patients. Reflexes are continuously checked, and infusion rates adjusted. The patients can have different stages of obesity and as V3 has the largest volume of distribution and the longest half-life, corpulence should be considered.

Monitoring

Anaesthetic depth

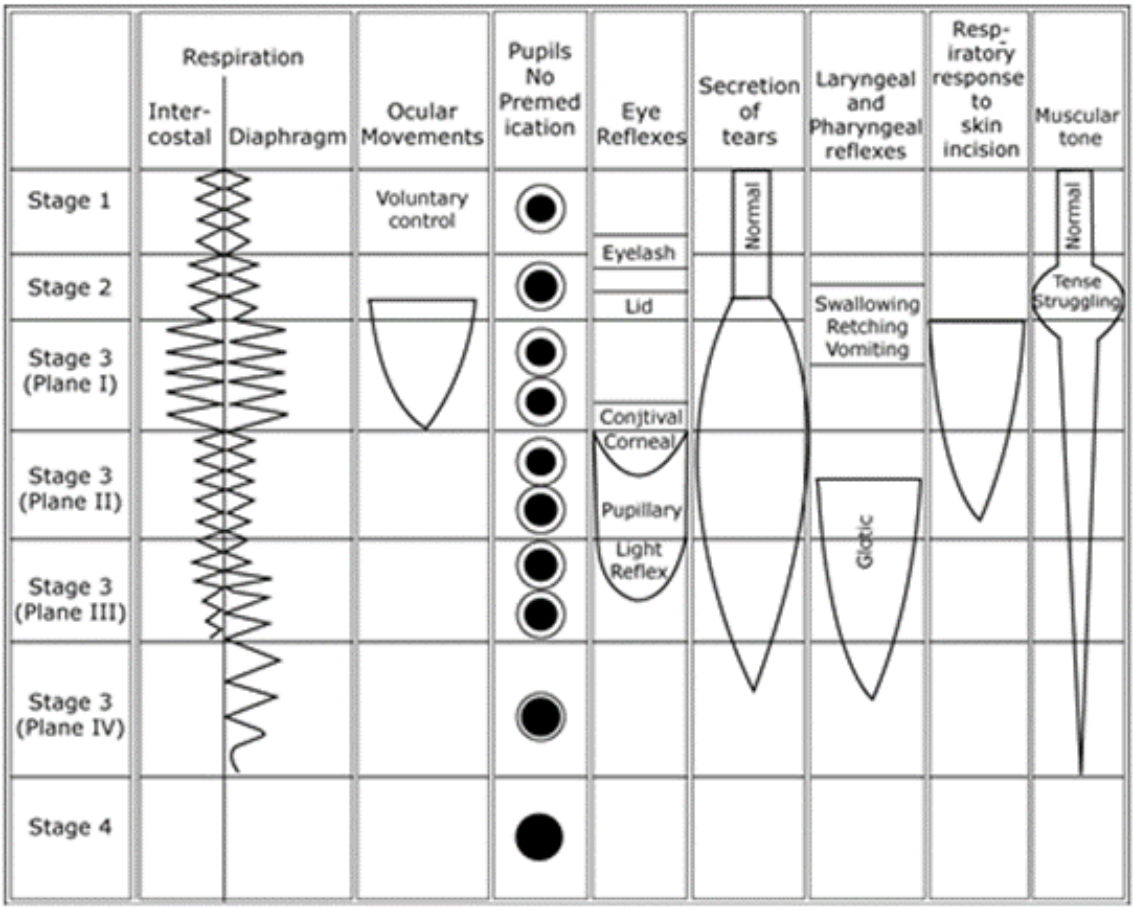
Aesthetic depth is the degree to which the central nervous system (CNS) is depressed by a general anaesthetic agent, depending on the potency of the anaesthetic agent and the concentration in which it is administered.

Determination of anaesthetic depth

Anaesthetic depth is mainly monitored by existence and degree, or absence of reflexes, but jaw tone can be useful as well. Eye position and pupils are less reliable, but HR and respiration change can be helpful.

Reflexes can be tested by touching the eyelid or corner of the eye. Applying a clamp under the tail or interdigital is an alternative. The response is not the same at the different places, it depend also on the drugs used and on the individual. Try to establish a standardised procedure for the type of anaesthesia you use.

Touching the cornea should be avoided to reduce the risk of injury.



After Gillepsie 1940

Adjustment

Depth of anaesthesia is controlled by increasing or decreasing the narcotics. Depending on type of protocol this can be easy and fast or slower and more complicated.

Type	Depth adjustment	Action	Remarks
IM Injectable:	↓	Redosing	not recommended for cocktails
	↑	Antagonists	Not possible for all drugs
IV Injectable	↓	Re-dose	CRI: give bolus, then increase dose
	↑	Antagonists, stop CRI	stop CRI
Inhalation	↓	Dial a higher dose	increase FGF temporarily
	↑	Dial a lower dose	increase FGF, increase RR

Normal vital parameters

- Body Temperature BT 38 +/-1
- Hearth Rate HR 80 +/-15
- Electrocardiography ECG no arrhythmias
- Blood Pressure NIBP/IBP 130/80(90) +/-15%
- Respiration Rate RR 12 +/-4
- O_2 Saturation SpO_2 >95%
- End Tidal CO_2 $ETCO_2$ 35-45 mmHg

Body Temperature

If not managed, body temperature will sink gradually during anaesthesia. The reason is lack of movement, effect of drugs (Vasodilation, Xylazine suppresses CNS thermoregulatory mechanisms.) and sedation. This affects metabolism, immune system, oxygen saturation, recovery, and the healing process. Hypothermia also results in higher use of analgesics and anaesthetics. Lost temperature is very hard to regain. To prevent hypothermia the following measures should be applied.

- Sedate/induce on insulated floors, pick the patient up asap
- Heat pillows, warm air blankets
- Blankets, socks
- Warm infusion liquids

Hearth Rate

HR should be regular and without arrhythmias. HR is influenced by various anaesthetic compounds. Isoflurane can cause tachycardia already at low doses, and propofol at high doses, in Göttingen Minipigs.

Telemetric measurements have shown that the resting HR can be as low as 60 and an active as high as 110 in unstressed Göttingen Minipigs.

Blood Pressure

NIBP can be obtained by a cuff on the foreleg, hindleg or tail. It is not always easy to get a reliable measurement. It is important that the size is appropriate. The width of the cuff should be 40% of the circumference.

In comparison to IBP, the systolic value corresponds very well but the diastolic is around 20% lower when measured with cuff.

End Tidal CO_2

The $EtCO_2$ in an anesthetised, spontaneous breathing Minipig is typically 50 cm H_20 or more. If the value is considerably above, ventilation should be considered.

If a patient is ventilated it is essential that $EtCO_2$ is monitored as it is too easy to over-ventilate. In addition, a capnogram gives important information about the general state of the patient.

Fluids

Fluids should be administered during anaesthesia to maintain the fluid balance. Replacement of fluids should be ml for ml. Deficit in the balance occurs trough:

- Blood loss
- Trauma
- Respiration – dry gases/Nose bypassed
- Renal activity
- GI activity
- (Sweating)

Crystalloids are cheap and easily available. Saline associated with acidosis. More balanced fluids, like Ringer lactate, that represent the electrolyte composition of plasma closer are recommended.

Appropriate fluid infusion is 5 – 10 mL/kg/h

Maintaining Fluid balance under normal condition, can also be calculated with the 4 – 2 – 1 formula:

First 10 kg	4 ml/kg/h
Second 10 kg	2 ml/kg/h
After that	1 ml/kg/h

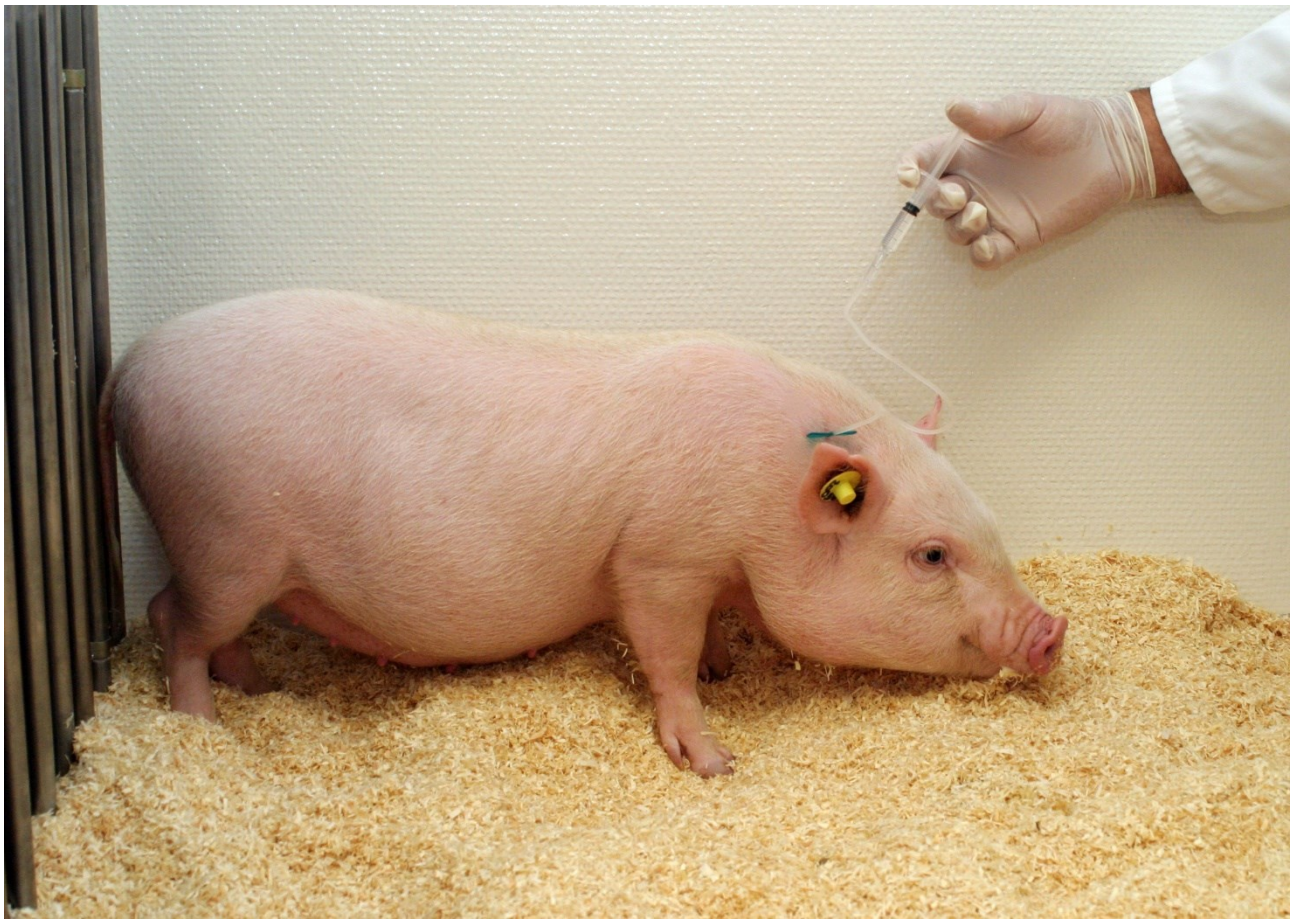
Peri-anaesthetic procedures

Ellegaard Göttingen minipigs has a series of instruction videos the cover the following procedures. Please contact us if you would like to have access to them.

Intramuscular injection

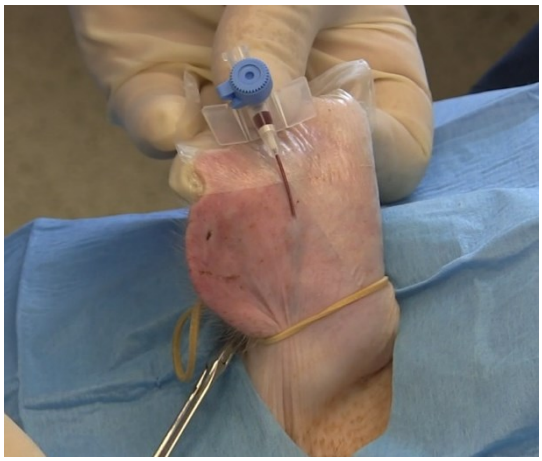
Intramuscular injections for initial sedation are preferably given in the neck muscle with a butterfly needle or flexible extension to the needle. This allows minimal restraining of the Göttingen minipig.

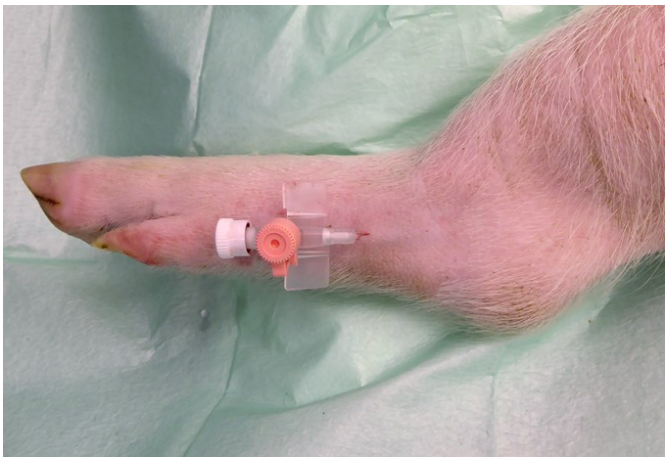
Depending on the age 23 or 21 ga needles with a length of 19- 25mm are appropriate.



Intravenous access

There are not many superficial veins in a Göttingen Minipig. The easiest one to use is the auricular vein, where a 22ga over the needle catheter can be placed in most cases.
As an alternative the saphenous vein can be cannulated.





Endotracheal intubation

The preferred position for endotracheal intubation is sternal recumbence. The mouth can be held open by an assistant, by a needle cover (or similar) or by a hanging rope. Pre-oxygenation via nasal tube is advised.

The patient must be deeply sedated for this procedure, any swallowing or laryngeal reflexes are not acceptable. You can deepen sedation by administration of propofol if necessary but watch out for apnoea. Should that happen, proceed with intubation as fast as possible and ventilate.

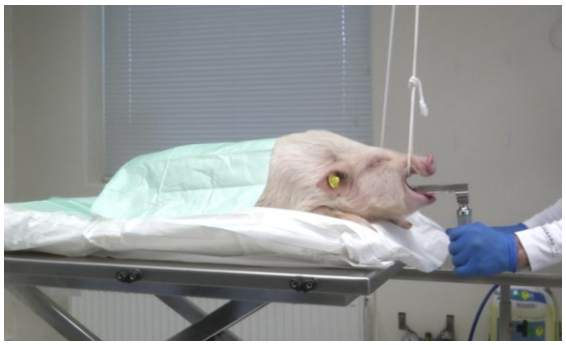
There is no hypersalivation as it is observed with landrace pigs after administration of Ketamine.

The epiglottis needs to be disengaged from the soft palette. The larynx is sprayed with lidocaine and intubation can take place. The path of intubation is angled. The tube needs to be turned as it is advanced, from the downwards position of the initial approach past the larynx to pointing upwards at the end.

The cranial lobe of the pig is ventilated trough and opening in the trachea a bit further cranial of the bronchi. Please make sure that the cuff of the tube is proximal and is not blocking that branch. Do also avoid touching the carina with the tip of the tube, it is a very sensitive area.

Once proper placement has been confirmed the cuff is inflated to a pressure between 20 and 30 cm H_2O. Avoid over pressuring the cuff, it can damage the trachea.

A



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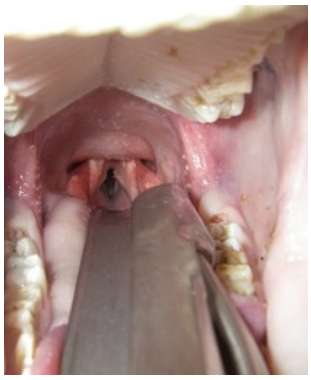

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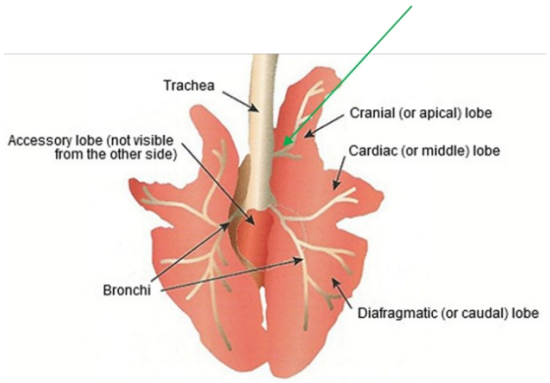

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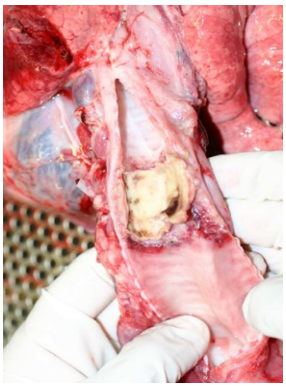


E

A: mouth held open by a needle cap and by a hanging rope B.
C: View to the larynx after the epiglottis has been disengaged from the soft palate.
D: Independent ventilation of the cranial lobe.
E: Necrosis caused by an overinflated cuff.

The following table is a guide for choosing an appropriate ETT

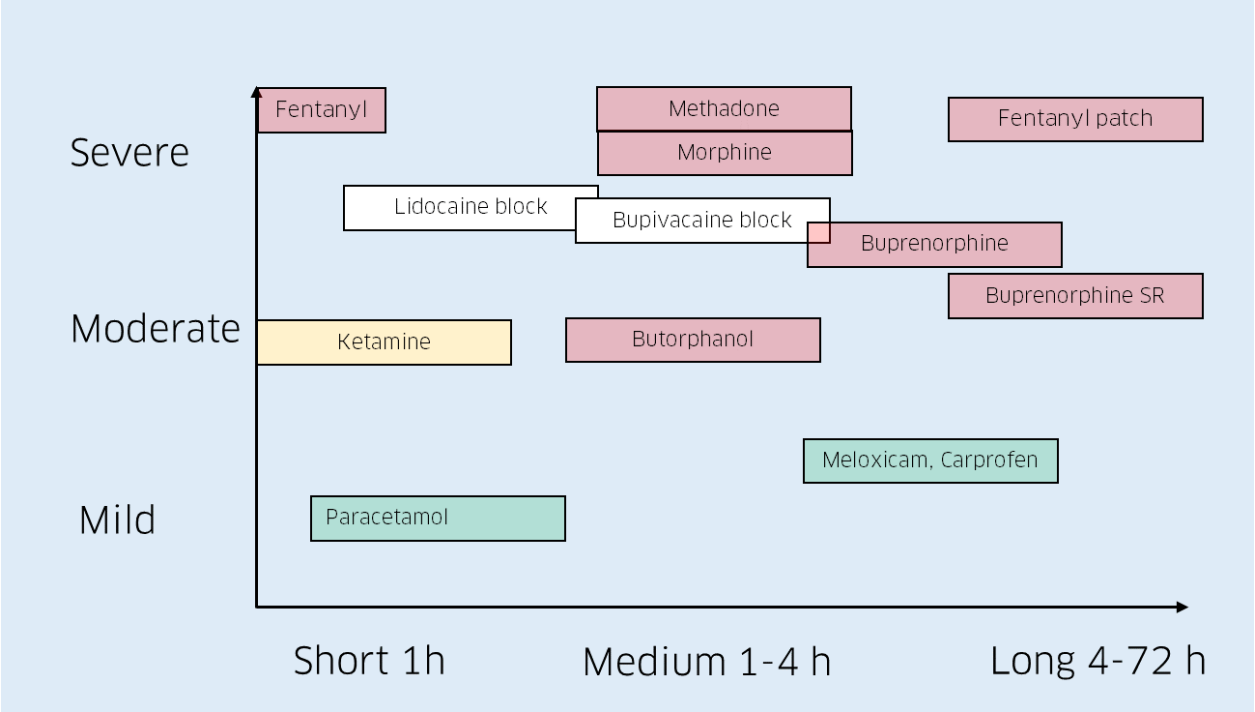
Weight	Age Months	Trachea ø	Suggested Tube size
4	2	6	3 – 3,5
12	6	9	4,5 – 5,5
18	9	12	5,5 – 6,5
25	12	17	6,5 – 7,5
30	12	No data	7- 8

Analgesia

It is essential that analgesia is provided before any intervention and that it is adequate in all the following stages. To give a complete guide to analgesia is outside the possibility of this document, but we would like to give some examples of products and doses that have been used with Göttingen Minipigs.

Each procedure must be carefully evaluated, and analgesia requirements determined. A multi modal approach is then recommended to establish an analgesic protocol. Pre-emptive analgesia is advised; some analgesia might already be included in the sedation cocktails, but they can always be supplemented by other products. NSAID' and opioids in combination have synergetic effect.

Overview commonly used products



We use three classes of drugs for peri- and postoperative analgesia.

Local anaesthetics

Lidocaine and Bupivacaine are applied peri- and intraoperatively as required. Weras Lidocaine has a relative fast onset and a shorter duration of action it takes longer for Bupivacaine to be effective, but it lasts longer. In some cases, the two are mixed in one syringe to a concentration of 2mg/ml each before application. Typically, we use them to infiltrate the intended site of incision when preparing the patients for surgery.

NSAID's

Both Carprofen and Meloxicam are frequently mentioned in literature, at Ellegaard we use Meloxicam exclusively.
During preparation we dose Meloxicam at 0.4mg/kg IM or IV. This should provide moderate analgesia for up to 24h. Postoperatively we give Meloxicam daily in form of an oral suspension (P.O.) at the same dose. This can be up to 10 days depending on duration of the expected pain.

Opioids

Fentanyl is a very potent, short acting analgesia. We use it during painful surgery by CRI at a dose of 10µ/kg/h after a loading dose of 4µg/kg.
For postoperative analgesia Fentanyl patches can be used. For Minipigs between 20 and 30 kg we use patches with a strength of 25µg/h. Please note that with patches, it takes up to 12 h before a therapeutic level of the drug is reached in the plasma. Provdes analgesia for three days
Methadone is a full µ-agonist, potent and medium long lasting (ca. 4h) It can be used as for acute analgesia or as part of the sedation cocktail. We use when the patient receives other µ-agonists at a dose of 0.2-0.3 mg/kg
Buprenorphine a partial µ-agonist, has a ceiling for effect and is less potent than Fentanyl and Methadone. It has a slow onset, time to peak is 30-45 min, but it lasts up to 10h. Some sources[5] claim the duration of analgesia is dose dependent, from 0.01 mg/kg and 4h to 0.04mg/kg 10h duration. We use it at doses between 0.05 and 0.07 mg/kg. Buprenorphine has a strong affinity to the receptor and will replace Fentanyl or Methadone. To avoid injections every 8 h we also use patches at strengths between 10 and 50 µg/h for postoperative analgesia. Provides analgesia for 7 days.
Butorphanol is k-agonist and a µ-antagonist, which makes it a bad candidate for mixing with µ-agonist. On the other hand, I can be used to reverse the effects µ-agonists and still providing analgesia. It has a relative high potency initially but that drops quickly to a lower level. It provides analgesia for around 4h. We use it at doses between 0.2 and 0.3 mg/kg, but mainly as part of sedation cocktails.

Example Protocols

Minipigs are scored for pain at every stage and if analgesia seems not to be sufficient, Buprenorphine or Methadone is dosed.

For procedures with light to moderate pain

1. Sedation with a cocktail containing Butorphanol
2. Meloxicam during preparation
3. (Local anaesthetic at site of incision)
4. (Buprenorphine IM 4h after sedation)
5. Meloxicam post-op, up to three days

For procedures with moderate pain

1. Sedation with a cocktail containing Butorphanol
2. Meloxicam and Buprenorphine patch applied during preparation
3. Local anaesthetic at site of incision
4. Buprenorphine (IM, IV) 4h after sedation
5. Meloxicam post-op, up to 5 days

For procedures with severe pain

1. Sedation with a cocktail containing Methadone
2. Meloxicam and Fentanyl applied patch during preparation
3. Local anaesthetic at site of incision
4. (Local anaesthetic block during surgery)
5. CRI of Fentanyl and low dose Ketamine during surgery
6. Methadone IV 4h after sedation
7. Meloxicam post-op, up to 10 days

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