NEWSLETTER 41

SPRING 2014

ELLEGAARD
GÖTTINGEN MINIPIGS

Clean pigs
for clear results

Dear Reader

The year 2013 already seems far behind us. But as this is the first newsletter of 2014, I would like to reflect on some of last year's achievements and highlights.

Shortly after the printing of our previous newsletter, I was appointed new CEO of Ellegaard Göttingen Minipigs. Thus, this is my first leading article for our newsletter.

As owner of and ambassador for the company, Jens Ellegaard now wants to focus more on visiting customers and partners to maintain and strengthen our good relationships and to find out how we can improve our services.

Even though we have experienced positive developments for our company and the Göttingen Minipig, we anticipate further growth in the future. We have a strong vision and are certain that the minipig will continuously prove to be more than an alternative model for many types of studies. Our vision is that the Göttingen Minipig should always be included as an option on equal terms with other non-rodent species in the selection of the most appropriate model for a study. Comparing the available animal models before a study and selecting the most suitable model will make the results clearer and reduce the risk of having to redo the study. Thus, meticulously selecting the proper species can contribute to the 3Rs, as well as to the corporate social responsibility of the company and the entire industry.

The Ellegaard team is very strong and comprises devoted and expert employees. Our barrier facilities are staffed with a good team of animal technicians who ensure excellent housing, handling and daily care of the minipigs. Our office is staffed with an excellent support team comprising a veterinarian, an animal technician and a new scientific specialist. The team is ready to assist our customers with any guidance or advice whenever needed, and it is pleasing for me to experience the rewarding collaboration we have with our customers.

I hope you will enjoy reading our newsletter which includes many interesting and useful articles.

Yours sincerely,

Michael Lehd, CEO

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Göttingen Minipigs in South Korea!

In January 2014, Ellegaard Göttingen Minipigs signed an agreement with Woojung BSC of South Korea.

Preclinical research is done worldwide, and one of the Göttingen Minipig's strengths is its availability in Europe, USA and Japan. This makes it possible to compare results and to utilize background data obtained around the world.

We have noted increasing interest and received several requests regarding the availability of Göttingen Minipigs in South Korea.

Woojung BSC has been selected as our partner in South Korea because the company has many of the same qualities as our other partners – Marshall BioResources and Oriental Yeast Co. – and primarily because Woojung BSC shares our focus on animal welfare. Woojung BSC will not breed Göttingen Minipigs but will from now on be responsible for distributing Göttingen Minipigs on the South Korean market.

We have high expectations for the South Korean market and we are looking forward to supporting South Korean customers in preclinical research as much as possible.

New Scientific Specialist

In March 2014, Anette Blak Grossi started at Ellegaard as a Scientific Specialist.

Anette is devoted to research and looks forward to providing scientific consultancy and facilitating knowledge-sharing between our customers.

Anette is a veterinarian and recently completed her PhD on neoplasia in cattle and pigs at the Section for Experimental Animal Models, University of Copenhagen. Anette has worked in a mixed animal practice in Sweden and at the veterinary pathology diagnostic laboratory at the University of Copenhagen where she performed necropsies and histopathological examinations.

Anette lives in Copenhagen with her husband and children: Giacomo, 5, and Gaia, 1. In her spare time she enjoys taking long bike rides or going on trips with her family.

We hope that you will have the opportunity to meet Anette and discuss your work with minipigs. You are welcome to contact her if you have any questions or challenges regarding minipigs in biomedical research. She can also help you with information relating to the selection of the Göttingen Minipig for your research.

Follow us on LinkedIn!

Now you can follow Ellegaard Göttingen Minipigs on LinkedIn! Our Company Page on LinkedIn will keep you updated with useful and interesting information regarding our company and the Göttingen Minipig!

Update from North America

We have experienced a cold and snowy winter in New York this year, but this has not stopped our excitement about Gottingen Minipigs. Marshall BioResources welcomes some big changes in 2014. This year marks the 75th anniversary of the company; and Marshall has now been the exclusive supplier of Gottingen Minipigs in North America for over a decade. The use of the Gottingen Minipig has grown considerably, with almost 20 percent more Gottingen Minipigs sold last year compared to the year before. We are very happy to see interest in the Gottingen Minipig continue to increase, as our new barrier housing facility will be complete later this year. This additional space will allow us to maximize our production capabilities within our current breeding facility. Complete barrier conditions will be maintained in the new building, and Gottingen Minipigs received from either facility will have the same clean health status and gentle temperament that customers have grown to expect.

Marshall also hosted our first ever Gottingen Minipig Symposium this past September in Rochester, New York. The symposium was three days long, and included attendees from all across North America. Adrian Zeltner of Ellegaard even came from Denmark to join us and share his experience and expertise with working with Gottingen Minipigs. The symposium covered a wide range of topics including a day of lectures and posters presented by attendees, two hands-on workshops, and tours of our animal facilities. Overall, the Gottingen Minipig Symposium allowed for the creation of a minipig community in North America, which will hopefully facilitate the opportunity for those working with Gottingen Minipigs to continue to share ideas with others doing similar work, much in the way that the Minipig Research Forum (MRF) has created such a community in Europe. The next Symposium is scheduled for 2015. Please contact infous@marshallbio.com for more information, or if you would like to provide any suggestions for the next meeting.

There has been growing interest in determining the exact timing of sexual maturity in the Gottingen Minipig, and we have also just completed collaboration with the University of Pennsylvania to histologically evaluate when the male Gottingen Minipig reaches sexual maturity. Testis tissue and epididymis tail samples were collected at Marshall BioResources and shipped fixed in Bouin solution to Dr. Gary Althouse at University of Pennsylvania School of Veterinary Medicine. A full spermatogenetic cycle was observed in some samples collected from animals as young as 5 weeks of age. Complete spermatogenesis was observed in all samples collected from animals at 8 weeks of age. This histological evaluation provides strong evidence that male Gottingen Minipigs reach sexual maturity by 8 weeks of age. This data will be presented in full later this year.

We also have several other projects going on: including some detailed work on the behavior of Gottingen Minipigs, and the investigation of new enrichment devices that help facilitate and promote rooting behaviors in a laboratory environment. Michelle Salerno, our production manager and minipig training facilitator here at Marshall, has also developed a procedure for the collection of Cerebral Spinal Fluid (CSF) that is outlined in this newsletter, and will also be presented later this year as a poster.

Cerebrospinal Fluid Collection from the Cerebellomedullary Cistern in a Gottingen Minipig

Michelle Salerno, Ben Grambo and Nicole Navratil

Marshall BioResources, North Rose, NY

Introduction:

As the Gottingen Minipig is increasingly used for neurological research, there is a growing need to obtain and test cerebrospinal fluid (CSF). Limited information is available on how to collect CSF from Gottingen Minipigs for experimental purposes. The following procedure was developed at Marshall BioResources for the collection of CSF from euthanized minipigs.

Supplies used for this procedure:

Clippers, scalpel, tissue forceps, 3 cc luer lock syringes, 20 guage X 1 ½ " needles, 20 gauge spinal collection needles, collection vials, guaze pads, anesthesia, euthanasia agent

CSF Collection in an Anesthetized or Euthanized Minipig

It is possible to perform the following collection procedure in a minipig under anesthesia and recover the minipig following collection. If the animal is to be recovered, is it very important sterile technique is used to avoid introducing infection. There is also a risk of trauma to the spinal cord. For the purposes of this paper, collections were performed in euthanized animals.

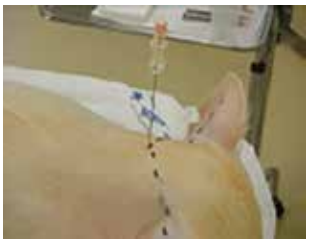
1. Once the animal has reached an adequate level of anesthesia, or has been completely euthanized confirmed by the absence of a heartbeat through auscultation, then place the minipig belly down on a table with the snout hanging off the table from the shoulders forward. Pull the front legs to the chest and secure the head in a perpendicular position (snout pulled in to the table and pointing toward to the floor) to create a flat midline behind the ears and cranial knuckle, along the spinal column. An assistant can be helpful to hold the snout in place or secure the snout with tape, ties or vet wrap. Shown here using vet wrap.

2. Shave the course hair from the ridge of the neck behind the ears to expose the skin layer.

3. Mark the location of the cerebellomedullary cistern by creating a line with a permanent marker. Pull the ears slightly forward and start from the middle of each ear following onto the neck line.

4. Use the intersection of the lines as a guide to pinpoint entry for the needle.

5. Prior to inserting the needle, prep the skin with an anesthetic wipe, alcohol pad or povidone iodine. The stick will be blind and fairly deep. Slowly insert the spinal collection needle straight in at the intersection point of the marked lines. As you insert past the fat layer and muscle layer, you will be begin to feel more resistance at the spinal column and may feel a pop as you penetrate the spinal column.



6. Pull the center wire from the spinal collection needle and attach the 3 cc luer lock syringe to the needle.

7. Pull the plunger up slightly to create a small vacuum. If no fluid is evident slowly continue inserting the needle with a vacuum and watch for the flash of fluid into the syringe.

8. Continue to draw the fluid slowly and monitor the color to ensure fluid remains clear and the sample is not tainted with blood. If it is necessary to change syringes, hold the base of the needle using caution not to interrupt the location of the needle as you proceed with the syringe change.

9. Open the collection vial, empty the spinal fluid from each syringe into a collection vial, replace the cap and label the vial.

Viewing the Cut Down

In euthanized animals, the tissues can be cut back to view where the collection is actually taking place. This technique can be useful for bulk collections or in practice before attempting collection in a recovery animal.

1. Make sure the animal is completely euthanized, confirmed by the absence of a heartbeat through auscultation. Position the animal as outlined in Step 1 above.

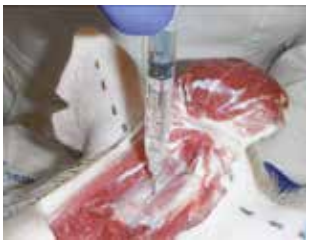
2. Make an incision across the neck just in front of the shoulders using a scalpel. Hold the tissue back with forceps and continue to cut through the fat layer and muscle layer but not too deep; avoid entering into the fascia layer above the vertebrae. A number 11 straight blade is recommended.

3. Cut back tissues along both sides of the neck and continue to dissect the fat and muscle layers only away from the fascia layer protecting the spinal column until you reach the cranial knuckle.

4. If there is any blood left on the fascia layer remove it with a gauze pad prior to proceeding.

5. Attach a 20 guage X 1 1/2'" needle to a 3 cc luer lock syringe. Insert the needle straight going just through the fascia layer.

6. Pull up slightly on the plunger of the syringe to create a small vacuum and continue inserting the needle until you see a flash of spinal fluid.



7. Continue to draw the fluid slowly and monitor the color to ensure the fluid remains clear and is not tainted with blood. Follow the collection instruction detailed above in Step 8. Following the collection, the CSF can be transferred into a collection vial as outlined above in Step 9.

Thermal Burn Modeling in Göttingen Minipigs

Carol Meschter, DVM, PhD, DACVP, CEO

Comparative BioSciences, Inc.

At CBI we have been using pigs and dogs from Marshall BioResources at our facilities for over 15 years for a wide variety of toxicology, pharmacokinetic and pharmacology studies. A burn is a tissue injury resulting from exposure to various noxious agents. For purposes of this article, we will limit the discussion to burns of the skin resulting from heat. Burn therapy has become a research priority, in part due to the recent combat experience and concern over the possibility of massive terrorist attacks. Consequently, our clients have become interested in investigating various treatments and methods in this therapeutic area. At CBI we have developed some excellent models of dermal wounds and burns using Göttingen Minipigs. We adapted models published by Singer et al. (2011) and Branski et al. (2008) to our own expertise and requirements as well as to meet the needs of our clients. As a contract research house, our mission is not so much to explore the nature of burn tissue injury or mechanisms of healing, but to quickly and thoroughly evaluate the safety and efficacy of new treatments, in a way that would meet regulatory standards. Our models are effective, reproducible and humane.

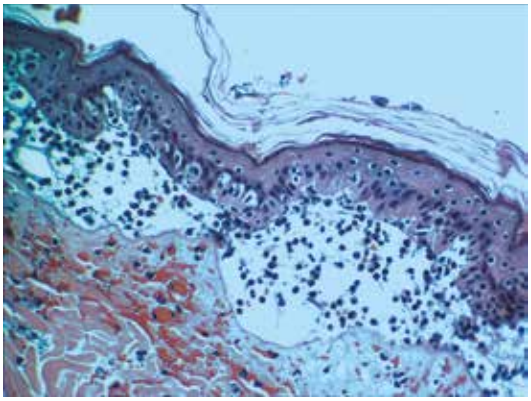


Figure 1. Histologic section of a superficial, partial-thickness dermal burn in a minipig demonstrating blister formation with inflammation and subjacent, thermal collagen damage. HE 200x.

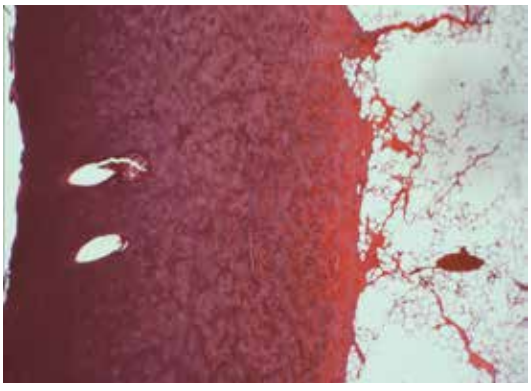


Figure 2. Histologic section of a deep, full-thickness dermal burn in a minipig demonstrating total epithelial loss, deep and severe collagen damage and damage to the underlying adipose tissue. HE 40x.

Types of burns

There are 3 major types of dermal thermal burns:

- first degree or superficial: superficial epidermis with hyperemia and no blistering;
- second or partial thickness: affects epidermis and dermis-superficial (epidermis and papillary dermis) or deep (reticular dermis);) *(see Fig. 1)*
- third or full thickness: affects epidermis and dermis and extends to the subcutaneous tissue; *(see Fig. 2)*

Some authorities also describe fourth degree burns, but these typically require extensive surgical treatment, including amputation, and are beyond the scope of this article.

Pain management

Pain is always a concern in wound studies and must be managed carefully. We use intraoperative pre-emptive pain relief followed by a fentanyl patch with supplemental buprenorphine or butorphanol. Pigs are carefully monitored throughout the study for signs of pain or distress. CBI had an unannounced USDA inspection while we were performing one of these models and they found no deficiencies. It is worth noting that in humans, first- and superficial second-degree burns are reported to be more painful than deep second- and third-degree burns.

Anesthesia and Surgery

For anesthesia, we use a combination of pre-emptive pain relief, fluids, sedation induction, intubation and isoflurane maintenance. EKG, body temperature, blood pressure, respiratory rate, hemoglobin saturation are monitored. Animals are on heating pads. Strict asepsis is critical to all the surgical procedures.

Burn induction

We have developed a method to consistently induce a uniform burn that is either a first-, second-(superficial or deep) or third degree burn *(See Fig. 3)*.

Bandage Changing and Dressings

Burn and wound management generally requires some sort of wound covering. *(see Fig. 4)*. The individual study protocol generally specifies the type of bandage changing and dressings. We are careful to minimize stress, excitement and pain during this sort of handling. The preconditioning of the animal is helpful in this regard.

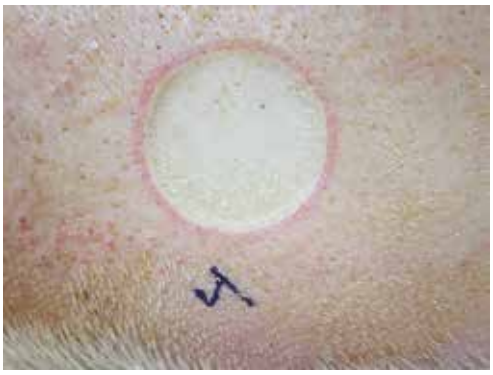
Burn treatment modalities

There is a variety of treatment modalities that are of current interest including:

- Topical or systemic administration
- Escharotomy and debridement
- Stem cell therapies
- Autologous cells
- Small molecules
- Biologics
- Bandaging or wound dressings
- Sealants
- Minced preps, split and full thickness grafting

Each modality may require variations on the model. Each study protocol is custom tailored to the needs of the client.

Figure 3.
Second degree burn at time of induction



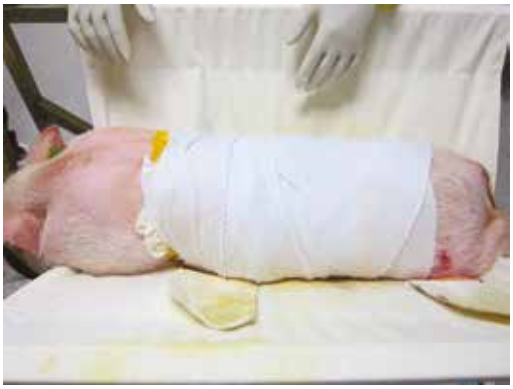


Figure 4.
Gottingen Minipig post-surgical bandage

Additional Surgical Procedures and Biopsies

Protocols often call for additional procedures such as escharectomy, skin grafts or biopsies at intervals following the original burn procedure. With these procedures, pigs are susceptible to problems with anesthesia and recovery. Consequently, we have developed some techniques and methods to optimize these secondary procedures.

Thermal Burn Modeling Endpoints

As part of a program addressing the three Rs, we feel it is very important to obtain as much information from each animal as possible. At minimum we assess the following:

- wound-healing progression with digital image analysis of wound size and closure
- draize scoring
- transepidermal water loss
- body temperature and body weights, food consumption
- hematology and clinical chemistry
- serum and tissue markers of inflammation
- serial biopsies
- histopathology and photomicroscopy
- special stains and immunohistochemistry
- Histomorphometry

Preconditioning

For preconditioning, pigs receive a physical exam and routine blood work. Pigs are acclimated to the facility and enrichment program. They are also handled and get to practice being carried and put into a sling with positive re-enforcement. This helps to facilitate bandage changing, scoring of wounds, biopsies and photography. In burn and wound studies it is important to keep the pigs in the most stress-free environment possible to minimize interference with the healing process.

Nutrition

High-quality nutrition and good food consumption are important to the success of the study. We provide an optimized diet plus supplements such as fruit and alfalfa.

Environmental Enrichment

Environmental enrichment is an important aspect of managing pigs on a wound study. They need to be kept comfortable and occupied to reduce fretting and keep them from damaging their bandages. Radios, television, toys, fruit, hay, alfalfa cubes, exercise pens, petting and scratching are helpful.

Why we like Göttingen Minipigs at CBI

As we optimized this model, we worked with several strains of pigs and settled on the Marshall BioResources Göttingen Minipig. The pigs are physically uniform, free of disease, gentle and easy to handle and carry and they have good appetites. Further, they handle multiple anesthetics, multiple sedations and restraint well. Subclinical conditions, particularly respiratory infections, can lead to poor surgical and anesthesia outcomes in swine, but the Göttingen Minipigs are free of pulmonary pathogens. Another important feature of these pigs is their uniformity in size and shape and their growth curves. This is important in following wound progression and healing over time. We can also conduct modeling in disease states such as hypertension, diabetes and immune suppression in burn- and wound-healing studies to mimic human disorders

For our burn studies, we design our studies to optimize the success of the study. This means that we have well-designed studies with adequate numbers of animals and study endpoints to ensure the relevance of the data. Initially, preconditioning, acclimation and environmental enrichment are key parts of the study design. This is followed by the procedure and treatment, as well as recovery and healing prior to necropsy.

Summary

In summary, we at CBI have developed and optimized a 1st, 2nd and 3rd-degree thermal dermal burn model using the Göttingen Minipig. For our burn studies, we design our studies to optimize the success of the study. This leads to well-designed studies with adequate numbers of animals and study endpoints to ensure the relevance of the data. Initially, preconditioning, acclimation and environmental enrichment are key parts of the study design. This is followed by the procedure and treatment, as well as recovery and healing prior to necropsy. Our methods allow us to evaluate various treatment modalities to assess healing in a manner that is both useful and humane.

Thermal Burn Modeling References

Singer AJ, Hirth D, McClain SA, Crawford L, Lin F, Clark RA. Validation of a vertical progression porcine burn model. J Burn Care Res. 2011 Nov-Dec; 32(6):638-46.

Branski LK, Mittermayr R, Herndon DN, Norbury WB, Masters OE, Hofmann M, Traber DL, Redl H, Jeschke MG. A porcine model of full-thickness burn, excision and skin autografting. Burns. 2008 Dec; 34(8):1119-27.

Genomic Considerations When Selecting a Model Organism

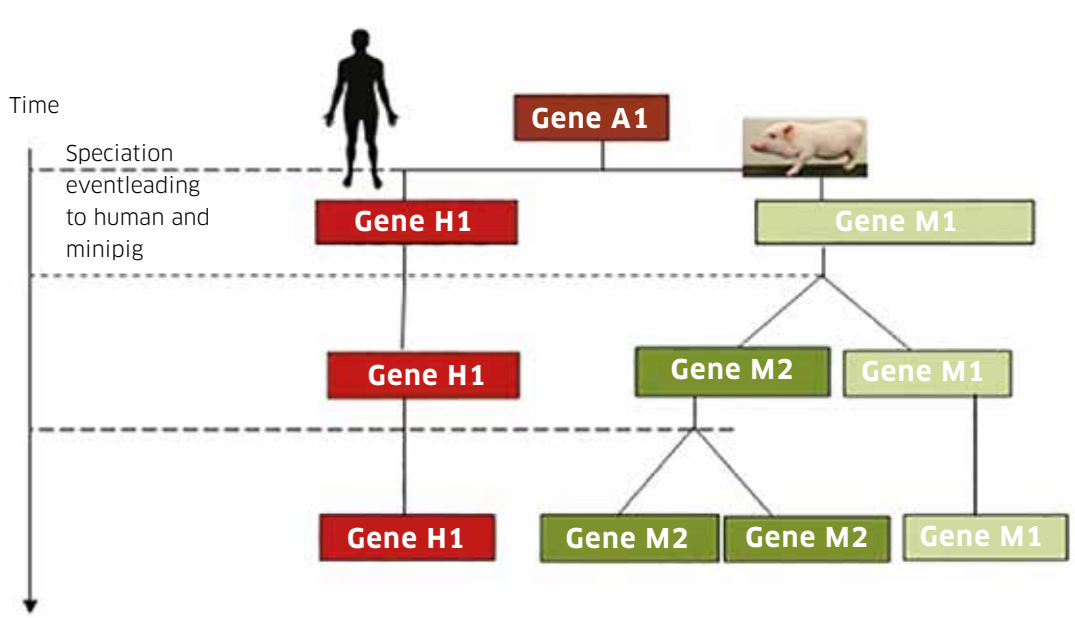
Peter Woollard,

Computational Biology, GlaxoSmithKline.

Introduction

Safety concerns are a major factor in the high attrition rate in drug discovery and development. Regulatory authorities mandate pre-clinical safety assessment in at least one non-rodent animal model. Ideally we want to choose appropriate animal models for a particular drug that are likely to demonstrate similar safety signals to what would be expected in humans. This will help us reduce the unnecessary use of animals http://www.nc3rs.org.uk/, as well as help to provide society with better drugs to treat unmet medical needs. For example, if a safety signal for a drug is seen in a beagle for a reason that is specific to beagles, but that was not known, further animal trials may be needed to understand this before terminating a potentially useful human drug unnecessarily. Knowing the genomic sequence of organisms is a key piece of knowledge along with biochemical, phenotypic and other evidence in selecting potentially appropriate species. Even a basic molecular knowledge of the genes in the genomes of model organisms helps *(Figure 1)*.

Figure 1:
Many changes may have occurred to individual genes since the last ancestor that minipigs and humans had in common. Genes in either species may now be non-functional (pseudogenisation), duplicated, undergone a change of function, etc.



The pig sequence exists, so why sequence minipig too?

A plethora of academically and agriculturally important animals have been sequenced in recent years, e.g. human, mouse, rat, fugu, pig [1], cow, domestic dog (boxer), etc. Many breeds/strains of domesticated animals are surprisingly different [2,3,4]. In 2011, we realised that we needed to know more about the key breeds of non-rodent species used in our studies, including the Ellegaard Minipig breed, to make a more informed species choice.

Accessing Minipig Sequence Data

Beijing Genomics Institute(BGI) sequenced and assembled the minipig sequence into contigs and then analysed the genome [4]. The minipig contig data is now publicly available (e.g. GenBank and EMBL), so it can be searched for and freely downloaded. Internally, gene predictions and other annotation were performed to allow easier analysis. At least one other company has sequenced the minipig and also has useful transcriptomics (RNASeq) information, but this is not publicly available at present. In the long term, it would be fantastic if integrated minipig genomic data was maintained in the public domain, in the same way as rat data in Ensembl.

Choosing the Right Species

The genes of particular interest to pharmaceutical companies are those to which drugs can typically be designed (e.g. by small molecules and biologicals), genes that metabolise drugs(ADMET) and other genes which have historically been implicated in adverse events. If a project has a promising therapeutic modulator, then there are some questions about the protein target in helping to determine which species is best for use in tests *(Table 1)*. Obviously, many factors are involved in determining the model's organism choice.

Question	Comment
Is my gene present in the minipig?	Can be found by a sequence search.
What is the sequence of my gene in the minipig? Is the sequence well conserved?	Best to look at peptide level. Are the key active and structural domains conserved?
Is my human gene a pseudogene in the minipig?	E.g. is the protein-coding sequence full-length compared to human?
How many copies of my gene are there in the minipig? 1:1 or duplicated?	A phylogenetic sequence tree with other placental mammals helps.
Has my gene undergone positive selection/ functional divergence in the minipig?	N.B. Quite complex phylogenetics and other analysis are needed to answer that.
Is the ADMET profile for a compound likely to be similar in minipigs and humans?	ADME Sarfari may help here.

Table 1: Sample target-validation questions that can be answered now that the minipig genome is known.

ADME Sarfari integrates genomic, pharmacokinetics (PK) and transcriptomics data

Abundant pharmacokinetics (PK) and genomic sequence data exist in the public domain for human and model organisms. Tissue-specific expression data are increasingly available too. The need to answer certain fundamental ADME/translational science questions motivated the development of ADME Sarfari https://www.ebi.ac.uk/chembl/admesarfari at the European Bioinformatics Institute(EBI). Questions such as this are becoming easier to answer: a lead molecule has been identified for a certain disease indication; is it possible to predict from available molecular data, if there is a specific animal model that might be best suited to model human ADME?

Summary

Having the minipig genome is already informing species choice for safety tests, this will improve as more information is integrated. Logically this helps to reveal obvious genomic reasons for choosing certain non-rodent species over others, reduces the unnecessary use of animals, helps to correctly fail drugs earlier and reduces the wrongful attrition of drugs.

Acknowledgements: Samiul Hasan and Jessica Vamathevan for material and advice.

In GSK all animal studies need to be ethically reviewed and carried out in accordance with the 1986 Animals (Scientific Procedures) Act and the GSK Policy on the Care, Welfare and Treatment of Animals.

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What You Can See In the Eye of a Minipig

(The ophthalmic examination in the Ellegaard Goettingen Minipig)

Helmut Ehall, Named Veterinary Surgeon, Department of Veterinary Services

Huntingdon Life Sciences

1. Introduction:

The minipig is today a commonly used non-rodent species in the safety assessment of new therapeutic drugs and other chemicals. Sometimes the endogenous or exogenous exposure to the test compounds will lead to structural and functional alterations within the visual system potentially affecting the ability to see. In many instances, ocular changes are the first and sometimes only clinical sign of toxicity. The ophthalmic examination is therefore an important integral part of most safety toxicology studies.

However, the majority of ocular lesions in animals are breed-specific or have at least a hereditary component to their pathogenesis. The examiner has to be able to recognise such lesions and differentiate them from a potential toxicological effect induced by the test material. It is therefore essential that the most common congenital and hereditary ocular lesions are known for each laboratory species. In comparison to other laboratory species, ocular lesions are relatively uncommon in minipigs, but incidental background findings will still be detected during routine examinations.

There are many ways to assess visual function in animals; however, in toxicology the routine ophthalmic examination will be limited to the use of a binocular indirect ophthalmoscope. By using a handheld converging lens as a magnifying lens (Panretinal 2.2) it is possible to view the anterior segments in appropriate detail and by using the same lens as a condensing lens a wide field of the posterior segments of the eye (i.e.: vitreal body, fundus) can be viewed. Some examiners may prefer to also use a slit lamp biomicroscope to examine the anterior segments of the eye in more detail. This may be of particular benefit if lesions are expected in the cornea or the lens.

To perform the examination, Tropicamide 1% (e.g.Mydriacyl®) is applied to the eyes prior to the examination. This will usually provide satisfactory dilatation of the pupils within 20 minutes after application. Mydriasis will be maintained for approximately an hour.

Minipigs up to an age of 6 months are best held in the arms of an assistant, when examined. Animals above 6 months of age are usually too heavy to be held by a person and are better restrained either in a sling or sat between the legs of the assistant with the pig's front legs lifted off the ground.

As with any observation of clinical signs, it is necessary to develop a routine for the ophthalmic examination. This will allow the examiner to carry it out quickly and to identify any relevant abnormalities.

Both eyes should be first examined from a distance, assessing the orbital and periocular conformation and the size and position of the globe. As the eyes are positioned in a quite lateral position within the skull in the pig, any asymmetrical abnormalities are best detected by looking at the orbitae and globes from the front and the top of the head.

By using the viewing lens s a magnifying lens, the eyelids and the conjunctiva should be examined. Due to the deep seated eyes and tight eyelids in the minipig, the examination will mainly involve the assessment of the lid margin and the marginally visible conjunctiva. The cornea should be assessed for irregularities and opacities, pigmentations and vascularisation. The limited area of cornea visible in the minipig may be increased by moving the head up and down, to the left and the right and by encouraging the minipig to look in different directions. The examination should continue with the anterior chamber, noting its depth and any abnormal haziness. Despite the induced mydriasis before examination, the iris should be included in the examination together with the pupil margin. The examination of the lens usually requires some experience. By moving the lens back and forwards all planes of the lens can be carefully searched for opacities/cataracts. The posterior suture line and the origin of the hyaloid remnant are useful landmarks to identify the focus on the posterior aspect and the anterior suture line and iris on the anterior aspect of the lens.

The ophthalmic examination will usually be concluded by the fundoscopy. This should include the assessment of the vitreous for any opacities or abnormal contents and the retina, optic nerve head and retinal blood vessels for any abnormalities.

2. Globe / Periorbita

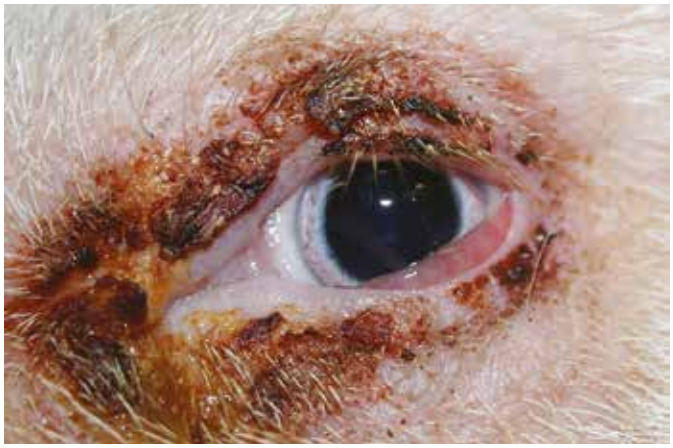


Figure 1: reddish brown mucoid and crusty discharge.

A reddish brown and crusty discharge is commonly observed in older minipigs. The discharge occurs usually bilateral and is neither associated with conjunctivitis nor seems the drainage of the tears through the nasolacrimal system compromised. The aetiology of the condition is not known, but as the first signs of discharge usually appear when the animals become sexually mature and due to its prevalence in boars, it may be considered a courtship signal. Bacteriological examinations are from personal experience usually negative. The discharge is best left in situ as the animals do not seem to be affected by it and removal and cleaning of the crusts may only lead to irritation of the underlying skin.

3.Eyelids

a. Entropion

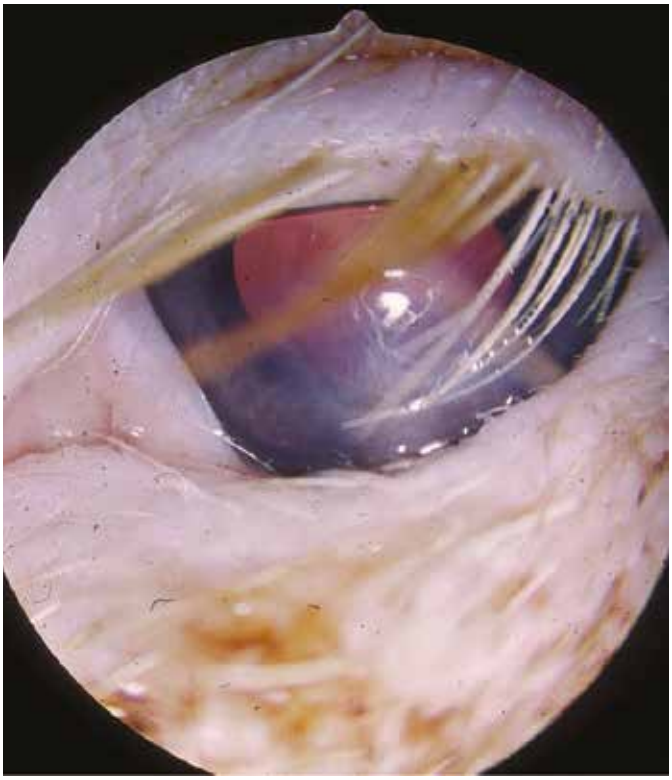


Figure 2: Entropion of the lower eyelid.

An entropion occurs when all or part of an eyelid is rotated towards the oculus, so that its hair-bearing margin touches the corneal surface causing permanent or intermittent corneal irritation. In regards to its treatment it is important to differentiate between primary entropions, which are due to poor conformation between eyelid and globe and secondary entropion, which is due to spasms of the orbicularis and /or malaris muscle or due to scar formation. It may also be induced by severe ocular pain and the associated endophthalmos. Most primary entropions will also be exacerbated by a secondary spastic component due to the corneal irritation [(1)]. It will be important to consider this when preforming a surgical repair. In the minipig, a large amount of subcutaneous fat in the periocular region and a disposition to entropion as part of its heritage from the Vietnamese pot-bellied pig may contribute to the occasional observation of entropion. Entropions are best repaired by a modified Hotz-Celsus procedure [(2)]. It has also been suggested that post-operative control of the animal's weight is beneficial for long-term success of the procedure.

4. Conjunctiva

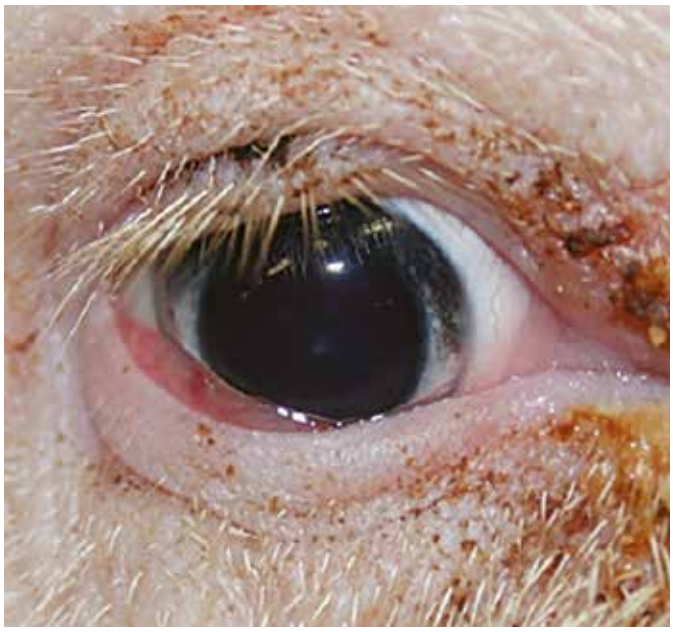


Figure 3: conjunctiva visible in the lateral half of the lower eyelid

In some minipigs, part of the conjunctiva is exposed in the area of the lateral part of the lower eyelid. It does not appear to be associated with conjunctivitis as hyperaemia is only very slight if present at all. Usually it is observed bilaterally and must not be mistaken as chemosis or conjunctival oedema. The conjunctiva may be mechanically exposed by a large amount of periorbital fat.

5. Cornea

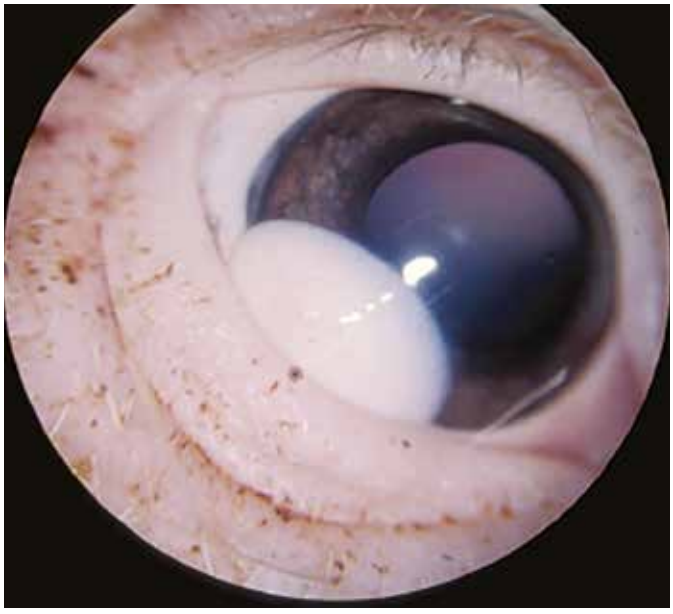


Figure 4: Dermoid on the tempero-ventral aspect of the cornea.

A Dermoid is a choristoma, which is a benign tissue element found on an abnormal location. An ocular dermoid is typically a skin-like, congenital mass of tissue, which can be found on

various ocular structures. It usually consists of keratinised epithelium, hair, blood vessels, connective tissue and smooth muscle, nerves, fat and glands [3]. They are frequently observed on the anterior surface of the globe near the temporo-ventral limbus [4]. In pigs, dermoids have only been described once as an approximately 7.5cm long tubular mass consisting of unorganised tissue derived form multiple embryonic germ layers, including bone and cartilage [3].

Metaplasia of the corneal epithelium is considered to be the most likely cause of dermoids, because it is the underlying mesodermal tissue, which determines whether surface ectoderm forms a non-keratinised stratified squamous epithelium and a Bowman's membrane as present in the cornea or keratinised epidermal adnexa as found in haired skin [4]. So dermoids are believed to either result form a primary aberration of invading corneogenic mesoderm or from abnormal inductive influences from underlying vestiges of the embryonic eye or from sequestration of dermal tissues destined to form keratinised skin.

Small dermoids may be left alone, in particular if they are unlikely to be irritant due to a lack of hair follicles. Larger dermoids or those, which are irritant to the eye, require surgical removal. On the cornea, the procedure of choice is a superficial keratectomy.

6.Iris

a. Heterochromia of the iris

Figure 5: Heterochromia iridis.

Figure 6: Heterochromia iridum

Both, Heterochromia irides, a different colouring of the two irides of an animal and Heterochromia iridum, a multicoloured iris, are very commonly seen in the Goettingen minipig. While both may also be acquired, usually as a result of previous inflammation, in the Goettingen minipig they are congenital and are most certainly remains of the pigmented Vietnamese pot bellied pig which forms part of its ancestry. While the introduction of the Large White Landrace into the breeding programme successfully eliminated the occurrence of skin pigmentation, it seemed to only have partially lead to colour dilution in the eye. Apart from the variation in appearance, heterochromia of the iris is of no clinical significance.

b. Iris coloboma:

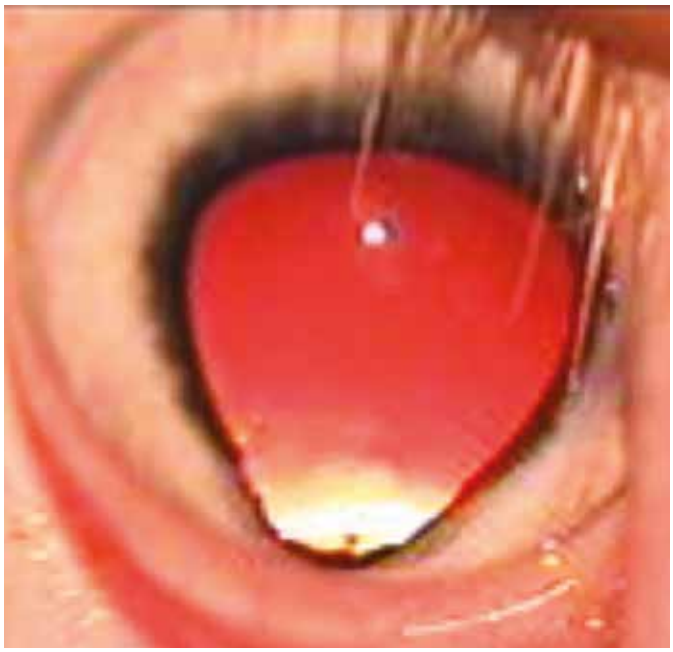
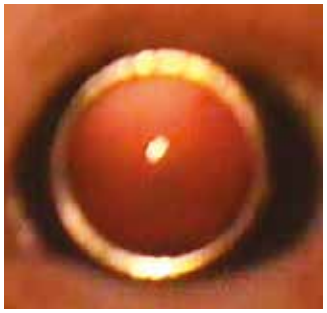


Figure 7: Iris coloboma at a typical position at 6 o'clock.

Ocular colobomas are embryological maldevelopments leading to fissure-like lesions of any ocular tissue formed by the optic cup. In their typical form, these appear at a 6 o'clock position and are considered a failed fusion or closure of the embryonic ventral fissure of the optic stalk or cup [5]. A coloboma appearing at any other location than the 6 o'clock position is a so-called atypical coloboma and are caused by primary abnormalities in the outer layer of the optic cup [6].

7.Lens

a. Microphakia:



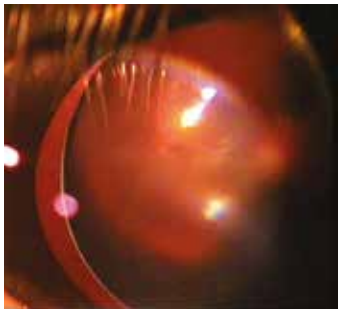


Figure 8 & 9: Microphakia

Congential microphakia is an abnormally small lens, which was observed on a breeding sow and one of its off-spring. A hereditary component can therefore not be excluded in the minipig. The area of contact between the optic vesicle and the surface ectoderm during embryological development will determine the ultimate size of the lens. Congenitally displaced lenses have also been described to be small and sphaerophakic, possibly due to an abnormal embryonic lens-zonule relationship [6,7]. Microphakia may also occur as a consequence of acquired lens zonular discorder and manifests in humans when the ciliary processes cause insufficient tractional forces on the lens [8].

b. Cataracts:

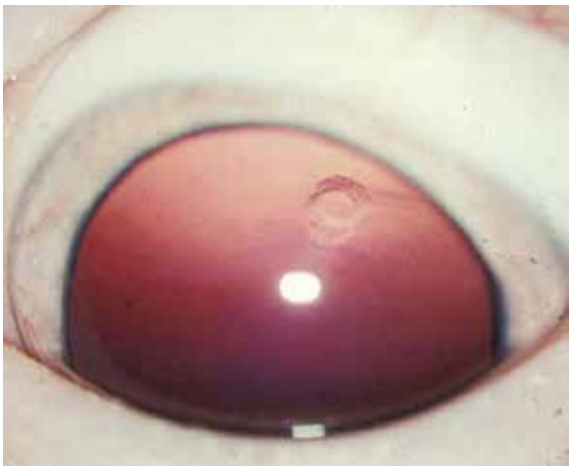


Figure 10: Anterior subcapsular opacity with peripheral extension.

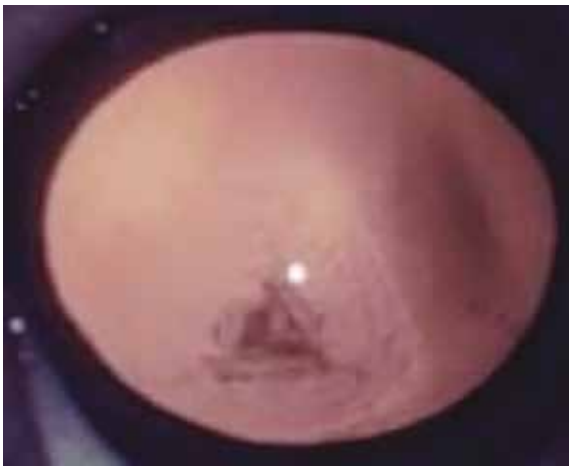


Figure 11: Posterior polar subcapsular cataract

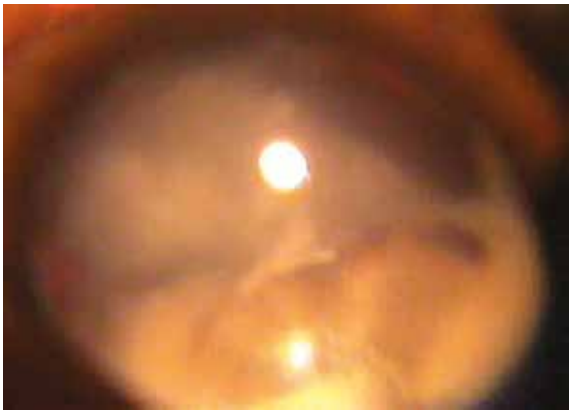


Figure 12: Total cataract

The lens is a refractive structure within the globe with the prime function to focus sharp images on the retina for accurate vision. Transperancy is therefore an essential property for the lens to fulfil its task. Due to the rather simple design, the lens depends on correct functioning of its biochemical processes. If disturbed, the lenticular fibres will react in a similar simplistic way and will loose transparency. Cataracts are therefore one of the most common test compound related ocular change in toxicology.
In contrast to other laboratory animals, congenital cataracts (which may only become apparent at a later age), are relatively rare in the minipig and a total cataract has so far only been observed once by the author. On occasions and appearing to be restricted to offspring from animals of one barrier, a faint to slight posterior subcapsular cataract can be observed. These seem to be similar to the cataracts described in Golden and Labrador Retriever breeds in dogs. The opacity appears at the confluence of the posterior suture line and in typically triangular, pyramidal or of an inverted Y shape *(Picture 11).* These are also, as observed in the minipig, non progressive and do not interfere with vision.

8. Vitreous

a. Hyaloid remnant:

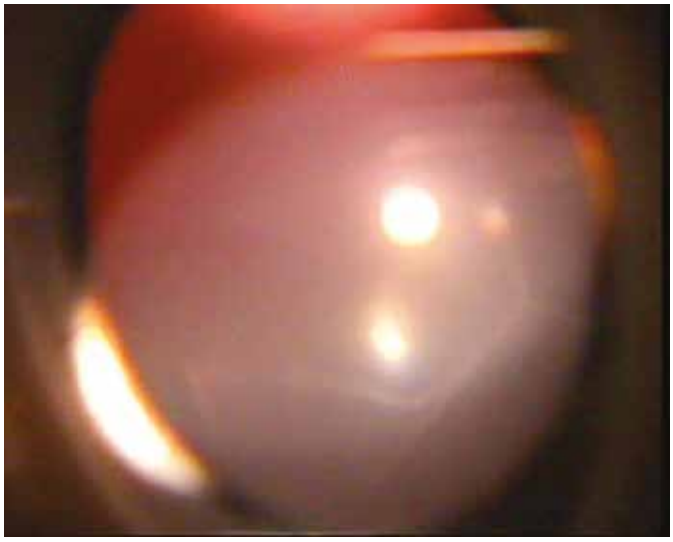


Figure 13: Hyaloid remnant

Figure 14: Hyperplastic hyaloid remnant

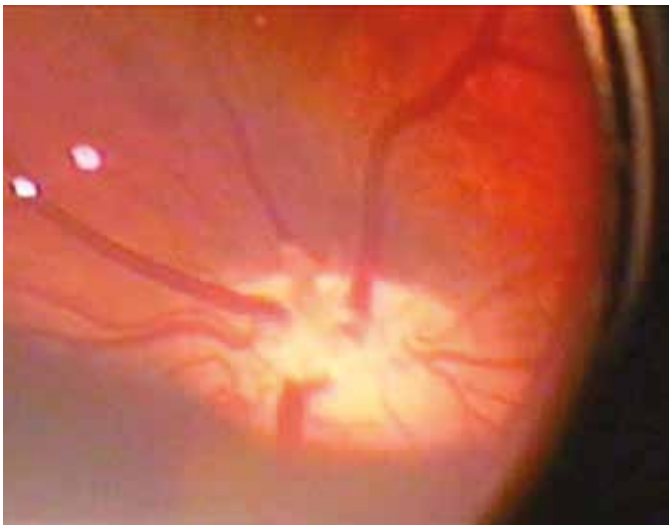
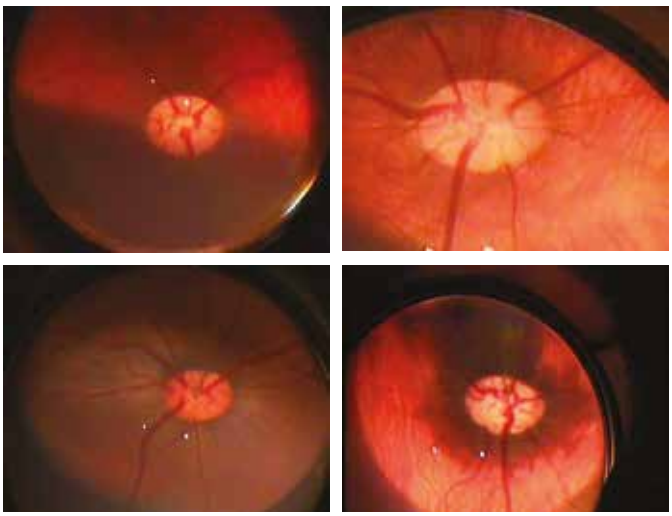


Figure 15: persistent hyaloid artery (white arrow)

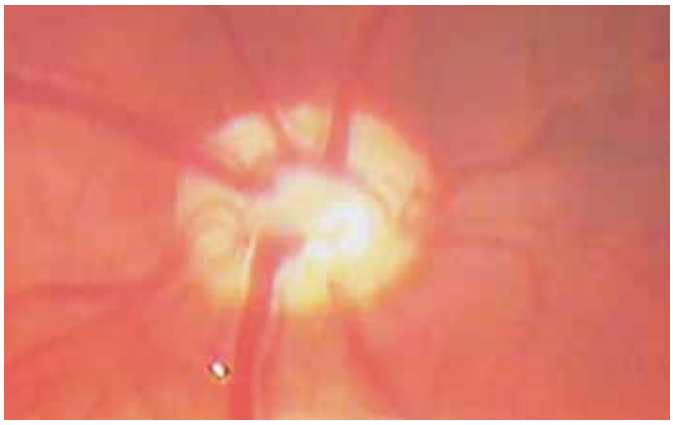
The hyaloid artery is the termination of the primitive ophthalmic artery and branches around the posterior lens capsule and continues anteriorly to anastomose with the network of vessels in the papillary membrane forming part of the tunica vasculosa lentis. The hyaloid artery and its associated vascular network provide the necessary nutrition to the developing lens in the foetus. Once aqueous humour is produced by the ciliary body and takes over nourishing the lens, the hyaloid system is no longer required and regresses. In most animals a remnant originating at the polar posterior lens capsule will continue to be evident. A small area of fibrosis on the posterior lens capsule, which represents the attachment of the hyaloids artery has been described as the Mittendorf's dot. In contrast to the dog, where it is seen as a small round opaque dot, in the minipig it features as a narrow line running along one of the Y-shaped posterior suture lines *(Picture 13 - arrow)*.

9. Fundus

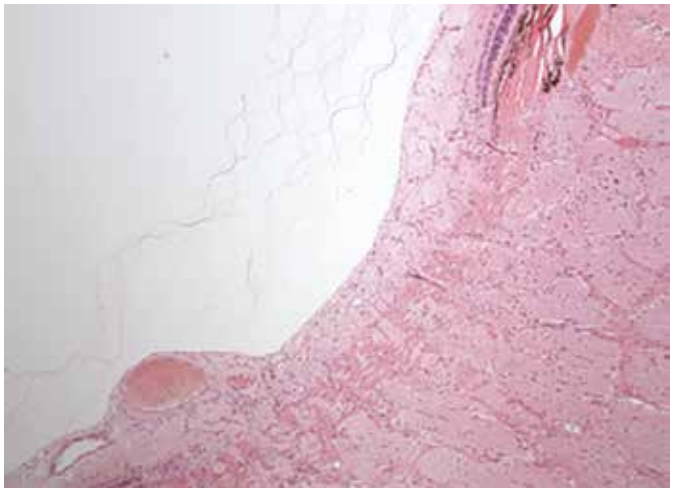


Picture 16 – 19: Normal variations in pigmentations of the retina

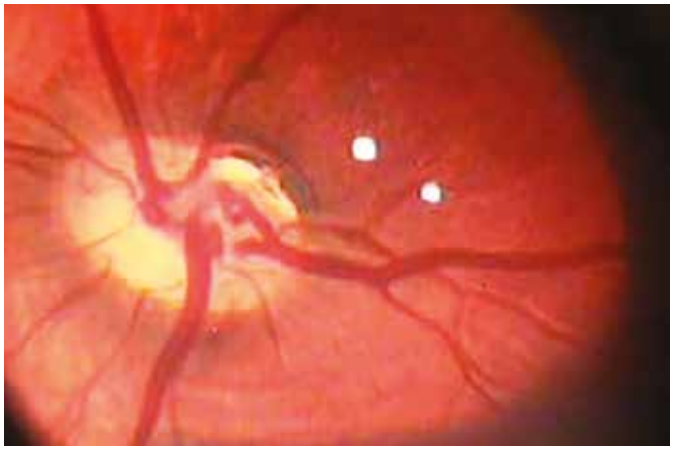
The fundus of the pig lacks a tapetum as seen in most other species and also does not have a visible macula like Primates. As mentioned with the iris, the retina has also got variable pigmentation due to the minipig's pigmented ancestors. The optic disc is horizontally shaped and sharply demarcated against the surrounding retina. The minipig possesses a holangiotic retina with direct blood supply to the inner neurosensory retina. Up to 10 arterioles branch dichotomously from the optic disc into the periphery, of which three to four are more prominent. Characteristic for the minipig is the commonly observed deep cup of the central part of the optic nerve head *(Picture 20 & 21)*. The deep cup of the optic disc can be a normal feature in the minipig and seems not to be associated with a compromise of vision or other ocular abnormalities. It must not be mistaken for cupping of the optic disc as is often observed in animals with glaucoma. In glaucoma, the raised intraocular pressure on the relatively weak lamina cribrosa will lead to optic nerve fibres exiting the globe and allowing the optic nerve head to bow outwards.



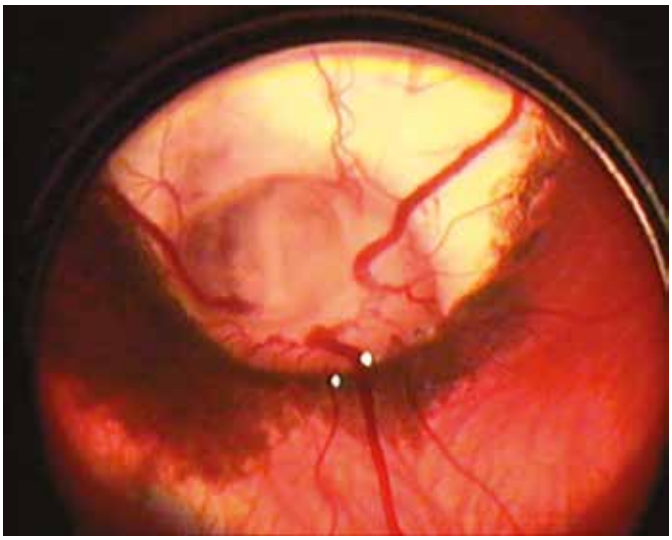
Picture 20: optic disc showing the deep cup often observed in the minipig



Picture 21: cross section disc showing the deep cup of the optic disc



Picture 22: Optic disc coloboma



Picture 23: Retinal coloboma involving the optic disc at a typical 6 o'clock position.

As mentioned earlier, ocular colobomas occur when the embryonic ventral fissure of the optic stalk and cup fails to fuse. If the most proximal portion of the optic stalk fails to close, this will lead to a coloboma of the optic disc. Optic disc or retinal colobomas have also been described in miniature swine [9] and the Yucatan micropig [10].

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Catheterization of the genital tract in female minipigs

- when using the minipig as a model of human genital infections

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The reproductive system of the minipig resembles the human reproductive system more closely than that of any other animal species. In addition, large parts of the porcine immune system are well described and very similar to the human system. These factors, together with practical advantages, such as easy handling and sufficient sample material, make the minipig an optimal model for studying human genital infections.

Chlamydia vaccine research

In our research group, we use sexually mature Göttingen Minipigs from Ellegaard as models of human genital *Chlamydia trachomatis* infection in the development of a chlamydia vaccine for human use. In our previous vaccine trial studies, we found that minipigs raise a clear antibody and cell-mediated immune response against the vaccine candidates. However, our challenge has been to establish a persistent chlamydia infection in the genital tract. It is important to establish an infection in order to evaluate the protective capacity of a vaccine candidate. One of the challenges in the establishment of an infection in the minipig's genital tract is to guide the bacteria through the cervix. The purpose of this article is to share our knowledge on how an inoculation of the (upper) genital tract can be done non-invasively in the minipig.

Catheters for the minipig genital tract

We tried different catheters for the inoculation of the genital tract in the minipigs – e.g. insemination catheters for conventional pigs, an insemination catheter for dogs and catheters for embryo transfer in pigs – but without success. The porcine cervix is long and complex with winding pulvini, which makes it difficult to insert a catheter through the cervical region. We finally found a successful non-invasive method that could convey bacteria through the cervical region *(Figure 3)* by combining two catheters: the Osiris dog catheter (E-vet, Denmark) and a urinary catheter (Buster, 2 x 500 mm, Kruuse, Denmark) *(Figure 1).*

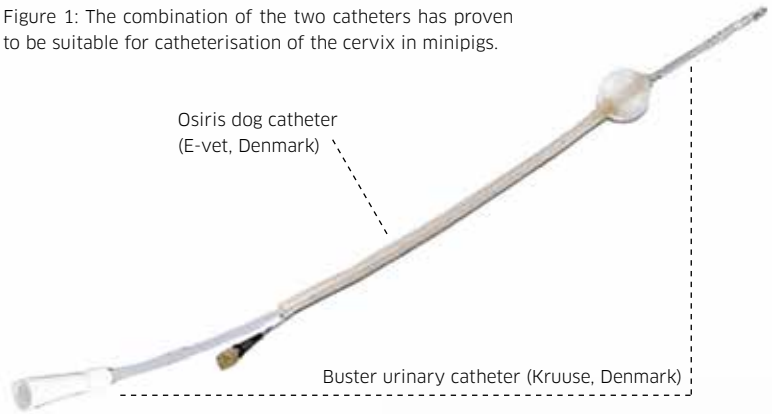
Initially, the inner catheter pipette is removed from the Osiris catheter. The labia are cleaned thoroughly with water and ethanol disinfectant before inserting the insemination catheter. The outer Osiris catheter tube is guided through the vagina (following the dorsal surface) to fit into the caudal end of cervix and to be used as a kind of trocar *(Figure 2).* Once the tube is fixated in the cervix, the balloon is inflated very carefully and with only a small amount of air (1 mL, depending on the flexibility of the genital wall), and the Buster urinary catheter is guided through the Osiris catheter as far as possible through the cervix. Slight pressure and, if necessary, winding counter-clockwise, are needed to pass the pulvini in the cervical region, however, one has to be very careful to avoid perforating the genital tract wall, which can easily happen, especially in anoestrus. Once the catheter has been placed properly *(Figure 2),* approximately 15 mL can be injected without direct reflux. We recommend a 10 mL bacterial suspension (or other test substance) and 2 mL of pure, sterile SPG (or something similar) to flush the catheter. After inoculation, we let the pigs lie for 20 minutes with elevated hindquarters to avoid reflux.

We inoculate the minipigs in oestrus when the cervix is softer, which makes it easier to properly position the insemination catheter. Furthermore, studies have shown that the endometrium is more susceptible to *Chlamydia* during oestrus. When working with risk-group 2 microorganisms such as C. *trachomatis,* we prefer to do the inoculation during anaesthesia. However, when working with other microorganisms or substances, it is also possible to inoculate the genital tract during standing oestrus, in cooperation with a teaser boar.

In conclusion, this short article describes how inoculation of the genital tract can be done non-invasively in the minipig. It will hopefully add useful knowledge to the use of the minipig as a model of genital tract infections and save others some initial work.

We would like to thank Ellegaard Göttingen Minipigs, and especially Helle Lorentsen, for their great help and professional and kind service at all times.

Figure 1: The combination of the two catheters has proven to be suitable for catheterisation of the cervix in minipigs.



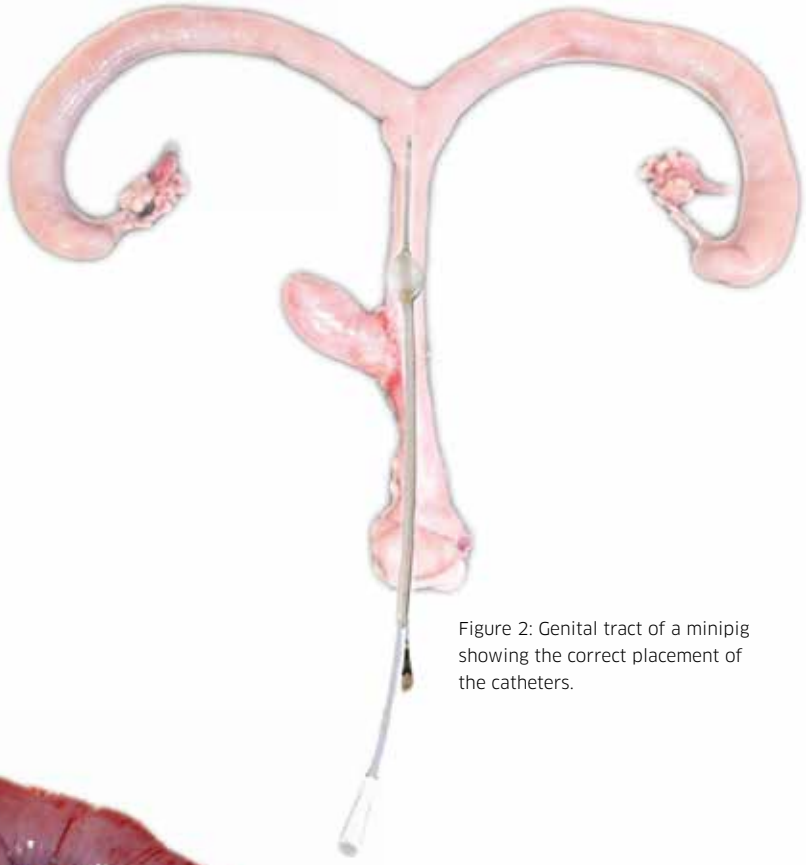


Figure 2: Genital tract of a minipig showing the correct placement of the catheters.

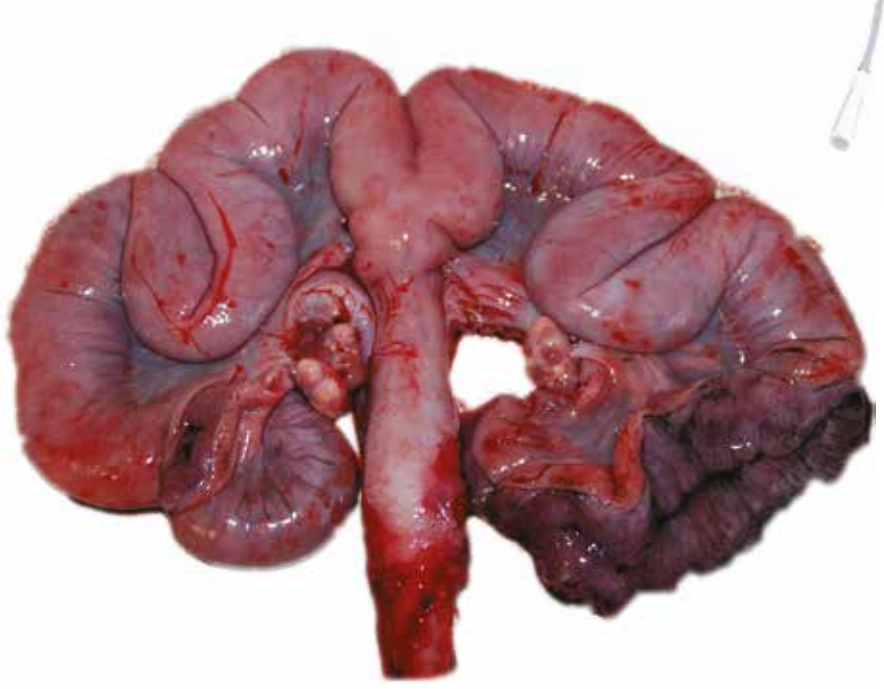


Figure 3: Following the injection of black ink using the described method of cervical catheterisation, this figure shows successful distribution to the outermost part of the uterine horns.

Central Venous Catheterisation of the Göttingen Minipig by Jugular Vein Placement

An Investigation of Potential Utility in Regulatory Safety-Evaluation Studies

Scott Hackford, BSc (Hons), MSc, Study Director

Sequani Limited

Introduction

In recent years, the use of the minipig for non-clinical toxicology studies has increased due to the model's anatomical, physiological and biochemical dispositions that allow extrapolation to humans. This has resulted in the minipig becoming a viable non-rodent alternative that is accepted by regulatory authorities. Furthermore, as a wealth of background data accumulates and ethical concerns continue to be raised by the public over the use of other non-rodent species such as the dog, there seem to be few factors that could limit the minipig's potential to become the primary non-rodent option.

One drawback of minipig use for non-clinical toxicology studies relates to the obtaining of serial blood samples. Usually, blood samples are obtained from the cranial vena cava due to the vein's easy access and the fact that large blood samples can be taken on a single occasion. However, with the increasing complexity of non-clinical studies, the need for more extensive blood sampling is apparent.

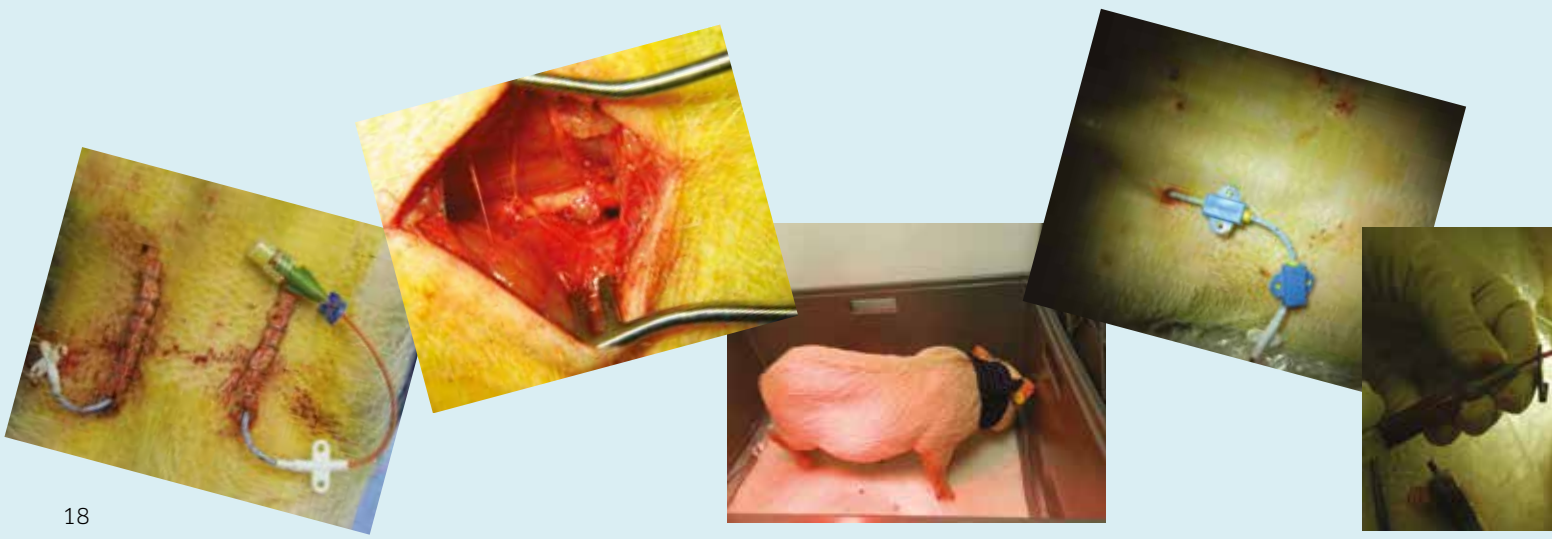
Serial sampling from the vena cava over a short period of time can cause stress in the minipig, leading to animal welfare concerns and potential non-test-item-related mortality. This has required the development of surgically-based blood-sampling approaches, such as the installation of vascular access ports and, whilst this approach is certainly appropriate for some study types, it is not ideal for use in routine regulatory toxicity studies. A positive advancement in this area appears to be the previously described method for the minimally invasive insertion of a central venous catheter into the external jugular vein, allowing the facilitation of multiple blood samples.

At Sequani, we have investigated the placement of a catheter in the external jugular vein. The study design is briefly discussed in detail, yet with the extensive technical details available in Ellegaard's catheter implantation educational package, the aim of this article is to share our first-hand experience with the technique and its potential use in future regulatory studies.

Study Design

The primary objective was to investigate the practicality of central venous catheterisation of the jugular vein, for potential use in regulatory non-clinical studies. Six minipigs were divided into three groups with the intention to study the insertion of a catheter for a period of time that would be useful in obtaining the toxicokinetic samples required in a one-month regulatory study.

On each occasion, a catheter (Arrow Gauge 14) was inserted using the Seldinger technique with the animal anaesthetised. The catheter was removed the next day following serial blood sampling for two groups, and the catheter remained in-situ for



an additional group to assess patency. Patency was assessed by visible signs of infection at the insertion site and by means leucocyte parameters analysed from the blood samples obtained. During the final catheterisation, a cut-down technique was employed to visualise the veins during the procedure. Blood-sample quality and the ability to reinsert the catheter were also assessed.

Discussion

Catheterisation Practicability and Success

Insertion of a catheter into the jugular vein became increasingly successful as the technicians gained first-hand experience and became accustomed to the procedure.

A total of 17 catheterisations were attempted (not including occasions where a cut-down method was applied); 11 were successful resulting in a catheterisation rate of 65%. The low success rate was a result of the first three catheterisations where none was successful. After these occasions where procedural experience was low, the catheterisation rate improved to 79%. The burden of the remaining unsuccessful catheterisations involved two animals where the first catheterisation attempt was unsuccessful and future attempts were made within a seven-day period. Surrounding vessels had been punctured causing swelling at the injection site, and insufficient recovery time was the likely cause of these unsuccessful attempts. It was found that animals could be successfully catheterised following a 14-day recovery period.

Experience in catheter insertion was associated with a clear reduction in the time taken to conduct each procedure, with later catheterisations being around 60% faster than the initial ones.
Patency and Re-insertion of Catheters

Patency was demonstrated for a period up to 22 days with no changes in related blood parameters or signs of infection. Longer patency is possible but could not be further demonstrated in this study due to one catheter becoming dislodged and another being damaged and having to be replaced.

Self-inflicted removal of the catheter by one minipig resulted in no injury to the animal and future removal was prevented by the adaptation of a neck collar to secure the catheter. This unintended removal allowed us to demonstrate the ability to reinsert the catheter on the same day or the day after removal. Reinsertion potential was also demonstrated on fortnightly occasions over a one-month period.

Blood Sample Quality

Blood sample quality was unaffected, with haematology and blood chemistry parameters comparable to background data collated from other collection routes. A number of dubious values was seen for standard coagulation parameters; however, this was attributed to heparin contamination from incomplete flushing of lines. This was resolved by more thorough flushing prior to blood sample-taking and resulted in coagulation parameters within the expected range.

Cut-down Method

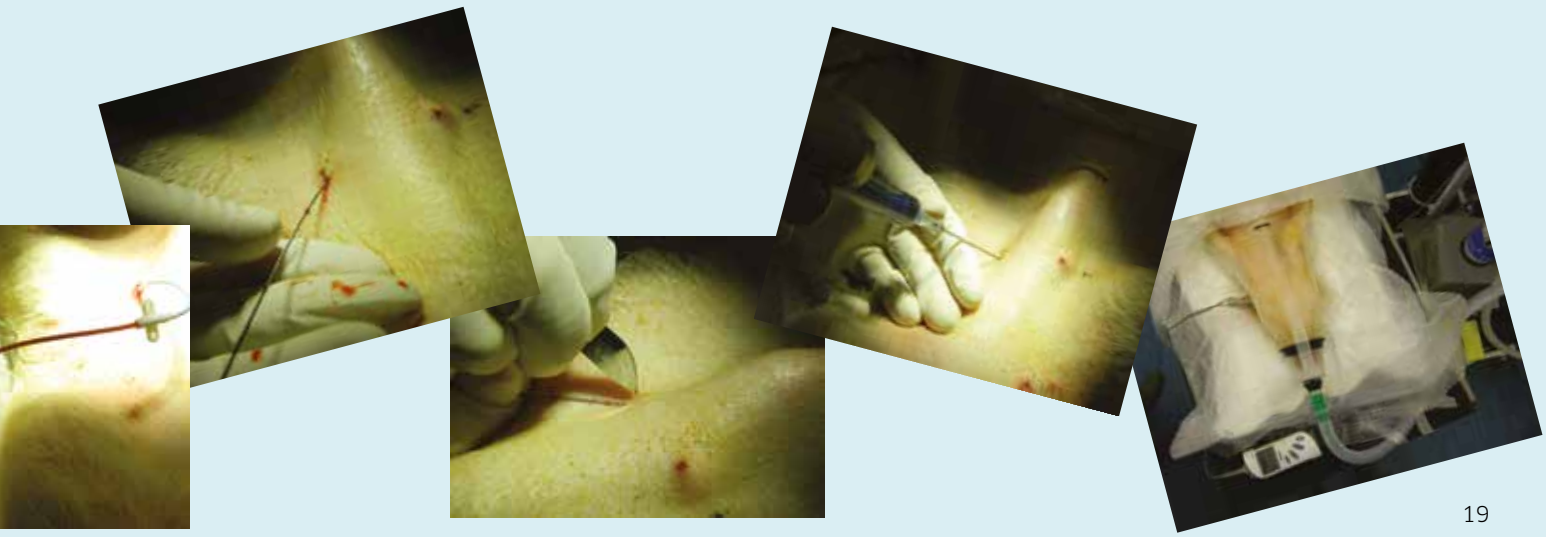
On all occasions where the cut-down method was used, the insertion of the catheter was successful. Procedural times were longer than those where only the Seldinger technique was used; however, visual access to the vein resulted in easier catheterisation and allowed the insertion of a catheter into both the left and right veins simultaneously.

Anaesthetic Regime

Minipigs were anaesthetised using a regime of injectable anaesthetics/analgesics comprising ketamine, medetomidine hydrochloride and butorphanol, as well as isoflurane through a nose cone. The amounts of injectables were reduced for each catheterisation to investigate the minimum required for a regulatory non-clinical study without compromising the state of anaesthesia. Initially, vocalisation and agitation were observed in one animal for the administration of ketamine; this was subsequently removed from the regime with no adverse effects on the state of anaesthesia. Reducing the remaining injectable anaesthetics shortened the recovery time following the procedure and subsequently reduced the level of agitation in the animals as they regained consciousness faster.

Conclusion

Central venous catheterisation of the jugular vein appears to be a promising alternative to serial blood sampling in toxicology studies involving the minipig. With experience, the insertion of a catheter becomes relatively quick and subsequently reduces



the manpower required for blood sampling. More importantly, a large reduction in stress was apparent which improved the welfare of the animal. Blood samples were obtained with ease on all occasions with the offering of a food treat eliminating the need for a sling or excessive handling.

These preliminary investigations suggest that the technique could be used in regulatory toxicology studies incorporating toxicokinetic bleeds that require serial blood samples. There are some issues concerning the amount of time needed to conduct the procedure in a study that may include 32 animals or more. Nevertheless, with well established procedures in place, this could easily offset the work involved in repeated blood sampling using the traditional method. At present it would seem appropriate to insert catheters for short periods to cover each serial blood-sampling session only (usually at the beginning and end of the study). However, further investigative work into line patency could open up the option of keeping catheters in place for a full one-month regulatory study.

The appeal of this technique would be further enhanced by adapting the anaesthetic regime to solely involve the administration of isoflurane through a nose cone, i.e. without any injectable anaesthetics. This requires further investigation but could substantially accelerate the recovery time.

In conclusion, we intend to further develop and enhance the procedure before introducing it into a regulatory study. However the progress made in this initial investigation will enable us to conduct additional small-scale studies using the technique and thereby develop proficiency and process efficiency at a suitable level. It should be noted that where less extensive blood sampling is required, bleeding from the cranial vena cava using the traditional method would still be considered the most appropriate.

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The 2013 meeting of the Minipig Research Forum

In November 2013, the Minipig Research Forum (MRF) broke the record by welcoming 90 attendees to the annual meeting in Rome.

17 speakers presented valuable information from their work and experience with minipigs. The presentations covered the three topics:

- Safety assessment of large molecules/biopharmaceuticals in minipigs
- CNS active compounds in minipigs
- GI-Tract

The programme also included workshops about these three topics where attendees could share their knowledge and experience with each other.

As always, the 2013 MRF meeting provided interesting presentations, useful information, knowledge sharing and unique networking opportunities.

The presentations from all MRF meetings are available on the website www.minipigresearchforum.org.

Meeting calendar

NAME	DATE	LOCATION
SOT ToxEXPO	23-27 March	Phoenix, Arizona
IAT	8-10 April	United Kingdom
Scand-LAS	24-27 April	Stockholm, Sweden
AFSTAL	4-6 June	Toulouse-Labége, France
EuroTox	7-10 September	Edinburgh, United Kingdom
GV-SOLAS/IGTp	10-12 September	Frankfurt, Germany

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