Aus dem Institut für Veterinär-Anatomie
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Comparative cephalometric studies of the mandible in growing Göttingen Minipigs using 3D Computed Tomography: Refining experimental dental and orofacial research

Inaugural-Dissertation
zur Erlangung des Grades eines
Doctor of Philosophy (PhD)
in "Biomedical Sciences"
an der
Freien Universität Berlin

vorgelegt von
Giuliano Mario Corte
Tierarzt aus Pforzheim

Berlin 2019
Journal-Nr.: 4165

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Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Johanna Plendl
Zweiter Gutachter:	PD Dr. Stefan M. Niehues
Dritter Gutachter:	Univ.-Prof. Dr. Christa Thöne-Reineke

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To my beloved family

And

In loving memory of Salvatore Rametta

"l'angelo del ciclismo"

***** 21.03.1993 - **†** 09.07.2008

"*You go through life wondering what is it all about but at the end of the day it's all about family*" — Rod Stewart

Comparative cephalometric studies of the mandible in growing Göttingen Minipigs using 3D Computed Tomography:

Refining experimental dental and orofacial research

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List of abbreviations

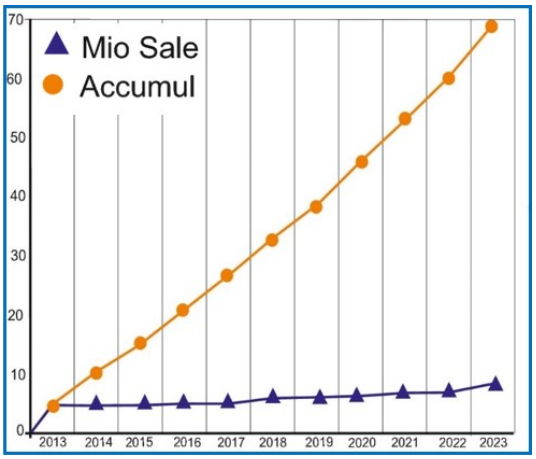
2D =	Two-Dimensional (**2**-**D**imensional)
3D =	Three-Dimensional (**3**-**D**imensional)
3R =	**R**eplace, **R**educe, **R**efine
AAW =	**A**verage **A**dult **W**eight
ABW =	**A**verage **B**irth **W**eight
ADO =	**A**lveolar **D**istraction **O**steogenesis
BB3R =	**B**erlin-**B**randenburg Research Platform **3R**
BMEL =	**B**undes**m**inisterium für **E**rnährung und **L**andwirtschaft
BMP =	**B**one **M**orphogenic **P**rotein
BW =	**B**ody **W**eight
CADD =	**C**omputer **A**ided **D**rug **D**esign
cm =	**c**enti**m**etre
CSD =	**C**ritical **S**ize **D**efect
CSIRO =	**C**ommonwealth **S**cientific and **I**ndustrial **R**esearch **O**rganisation
CT =	**C**omputed **T**omography
EC =	**E**uropean **C**ommission
ECVAM =	**E**uropean **C**entre for the **V**alidation of **A**lternative **M**ethods
EEC =	**E**uropean **E**conomic **C**ommunity
E.g. =	**E**xemplum **g**ratum (For example)
EN =	**E**uropean Standard (**N**orm)
EU =	**E**uropean **U**nion
EURL =	**E**uropean **U**nion **R**eference **L**aboratory
GDR =	**G**erman **D**emocratic **R**epublic
ISO =	**I**nternational Organization for Standardization
kg =	**k**ilo**g**ram
LD_{50} =	**L**ethal **D**ose (to kill **50% of test population)**
ml =	**m**illi**l**itre
mm =	**m**illi**m**etre
mm^3 =	cubic **m**illi**m**etre
OECD =	**O**rganisation for **E**conomic **C**o-operation and **D**evelopment
OP =	**O**steogenic **P**rotein
MDO =	**M**andibular **D**istraction **O**steogenesis
MRI =	**M**agnetic **R**esonance **I**maging
MPR =	**M**ultiplanar **R**econstruction
NA =	**N**orth **A**merica
PET =	**P**ositron **E**mission **T**omography
PLOS =	**P**ublic **L**ibrary **o**f **S**cience
SPECT =	**S**ingle-**P**hoton **E**mission **C**omputed **T**omography
U.S. =	**U**nited **S**tates (of America)
VR =	**V**olume **R**endering
ZEBET =	**Z**entralstelle zur **E**rfassung und **B**ewertung von **E**rsatz- und Ergänzungsmethoden zum **Ti**erversuch

The abbreviations and definitions of the cephalometric parameters and landmarks used in this thesis, are listed separately in Chapter 4.3.3 and Table 5.3.4-1 and 5.3.5-1.

1 Introduction

Bone and tooth loss due to trauma, infections or congenital abnormalities constitutes a therapeutic challenge in the daily life of dentists and oral-maxillofacial surgeons. For the oral restoration of both fully and partially edentulous patients, dental implants have been proven as an effective and successful tool [1]. The annual global market for dental implants is estimated between 12–18 million implants sold. Over the last decades, several hundred million patients have been provided with dental implants. In 2016, an estimated European market size of nearly 1.5 billion U.S. Dollar was reached and approximately 5-6 million implants were sold. This makes Europe the biggest market for dental implants worldwide (Figure 1.1).

Global dental implant market size
(Transparency Market Research)
https://transparencymarketresearch.com/images/global-dental-implants-market.jpg [2019-05-08]



Estimated figures of European annual sales (in millions and accumulated) of dental implants
Derived and modified from [2].

Figure 1-1: Global dental implant market size and European sales figures.

According to a study conducted by Transparency Market Research (www.transparencymarketresearch.com), during the next decade, the global annual market growth of dental implants is assumed to expand by nearly 7% (Figure 1-1). In 2019, the U.S. dental implant market size will presumably exceed the mark of 1 billion U.S. Dollar (Figure 1-2).

Besides prosthetic dentistry, implants are used for reconstructive surgical treatment of facial and mandibular defects. In order to increase the therapeutic success rates and reduce the duration for treatment and healing, new implants and biomaterials are being researched. Implants are considered as medicinal products. Therefore, every new modification to implants needs to be pre-clinically tested in order to proof its biocompatibility and safety for clinical tests in humans [3]. A major part of the pre-clinical evaluation involves *in vivo* testing in animals. Consequently, reliable animal models are demanded and needed to obtain valid and predictive

results. Due to anatomical and physiological considerations, pigs and especially minipigs such as the Göttingen Minipigs, are being extensively used as animal models in pre-clinical dental and orofacial research. Due to ethical concerns over the use of primates and dogs, the frequency of use of minipigs will continuingly increase in the future [4].

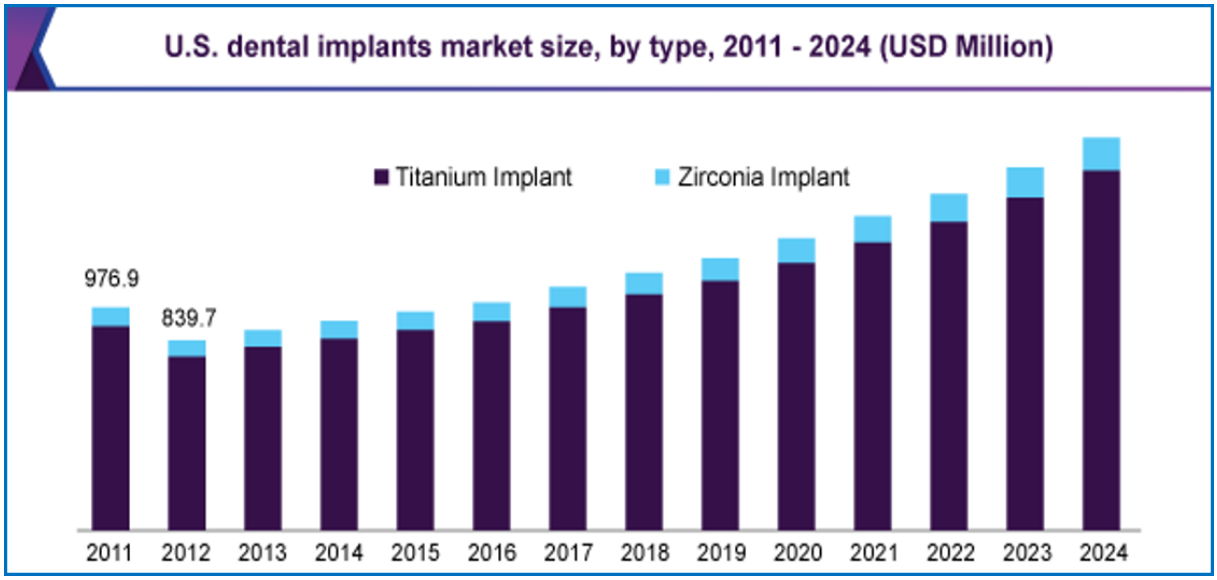


Figure 2.1.1-2: U.S. dental implant market size. https://www.grandviewresearch.com/static/img/research/ us-dental-implants-market.png [2019-05-10].

In recent literature, several authors have raised concerns over the use of Göttingen Minipigs in dental and orofacial research, observing problems such as implant failure or other post-operative complications. In some studies, implant success rates lower than 60% have been reported. The overall aim of this thesis is to provide relevant anatomical data of the mandible of the growing Göttingen Minipig to evaluate its suitability as animal model for dental and orofacial research and to provide additional knowledge in order to avoid complications during experimental procedures. This constitutes a methodological refinement in the sense of the 3Rs.

This thesis was designed as part of the Berlin-Brandenburg Research Platform **BB3R**, which aims to strengthen the 3R expertise of the region Berlin-Brandenburg and to provide substantial progress in 3R alternatives by intensive systematic research. The integrated graduate school "Innovations in the 3R Research - Genetic Engineering, Tissue Engineering and Bioinformatics" offers a comprehensive, well-structured training program in the wide field of 3R research and aims to prepare doctoral students for a subsequent career in the field of life sciences and science administration. **BB3R** was established in 2014 under the umbrella of the Dahlem Research School of Freie Universität Berlin.

2 Literature Review

2.1 Pigs

Pigs are even-toed ungulates and belong to the order *Artiodactyla,* the sub-order *Suina*, the family *Suidae* and its genus *Sus* [5]. From the varying number of species of the genus *Sus*, the Eurasian wild boar (*Sus scrofa scrofa*) is considered to be the wild ancestor of all domesticated pig breeds (*Sus scrofa domestica)*, with whom they share a close genetic affinity and the capability to hybridize [6, 7]. Besides the supply of meat, since the 20th century, the pig started to have an increasing importance as an animal model in biomedical research.

2.1.1 Minipigs - History and breeds

Already centuries ago, pigs were used in anatomical studies, due to the assumption of analogies of the porcine and human anatomy [8]. However, the size and weight of domestic pigs and the difficulties in handling and husbandry due to extensive space and food consumption, have always been considered to be big limitations in their experimental laboratory use [9]. Therefore, in 1949 at the Hormel Institute of the University of Minnesota, the first miniature pig breed, referred to as the Minnesota or Hormel Miniatures, was developed to overcome these problems. Miniature pigs are more closely scaled to the dimensions of the human body, possess a docile behaviour, have a decreased requirement for food and space, and need lower amount of administered compounds, such as anaesthesia (Table 2.1.1-1). When sexually mature, most miniature pigs weigh between 12-45kg. When domestic pigs reach their sexual maturity, it is not unusual that they exceed an adult average body weight (ABW) of 200kg (Figure 2.1.1-1) [4]. Micropigs are the even smaller versions of minipigs. As an example, with half a year of age, the male Yucatan weighs about 46kg, whilst male micro Yucatan miniatures weigh only around 20kg. Although the acquisition of domestic pig breeds is considerably less expensive compared to minipigs, the higher cost for feed and husbandry, coupled with higher personnel safety risks, clearly negate any perceived savings. In addition, their growth rate scientifically skews the results of long-term studies [10]. Today, a large variety of miniature pig strains and breeds are used in biomedical research worldwide (Table 2.1.1-1). Some are of native origin, occurring as feral animals; others were captured and imported from different countries and continents. However, the majority of existing miniature pig strains are a product of crossbreeding. In some breeds an introduction of normal-sized pig strains or wild boars was conducted [11, 12].

Table 2.1.1-1: Overview of the minipig breeds in biomedical research and their main biological characteristics. *Derived and modified from [12].*

Breed	Origin	ABW (g)	AAW (kg)	Colour	Year[a]
Yucatan	Native	500–900	70–83	Black, slate grey	1970
Micro-Yucatan	Native	600–700	55–70	Black, slate grey	1958
Westran	Native	930	80–93	Mainly white	1993
Sinclair	Crossbreed	590	55–70	Black, red, white, roan	1949
Clawn	Crossbreed	500	40	White	1978
Hanford	Crossbreed	730	80–95	White	1958
Munich	Crossbreed	600–900	60–100	White, black, red, brown	1993
Mini-LEWE	Crossbreed	550	60	White	1975
Göttingen	Crossbreed	450	45	White	1961

[a] Year of foundation; ABW= Average birth weight; AAW= Average adult weight.

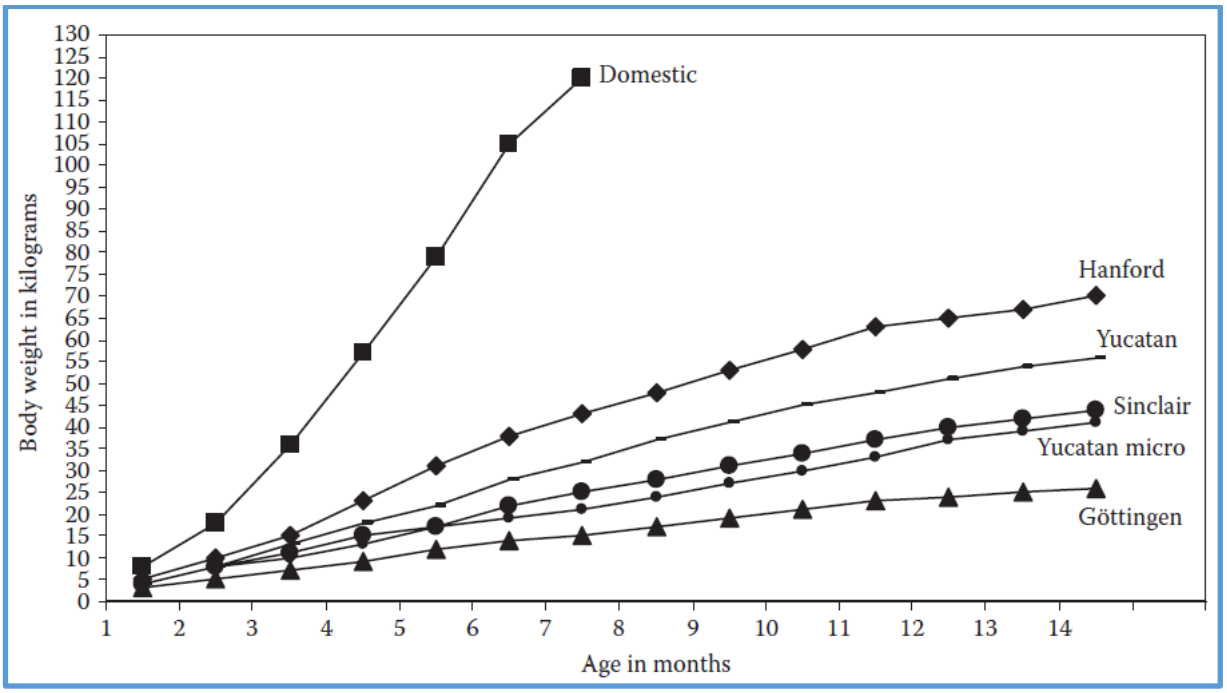


Figure 2.1.1-1: The developmental growth and body weight of domestic pigs in comparison with minipig and micro-minipig breeds. *Adapted from [10].*

2.1.2 Minipigs of native origin

2.1.2.1 Yucatan and Micro-Yucatan Miniature Swine

The Yucatan Miniature Swine is a naturally occurring breed of swine imported from the Mexican Yucatan peninsula. Today, five breeding and multiplier sites in the USA exit. Yucatan Miniature Swine are of black or grey colour, have minimal hair coat or are even hairless. They possess a docile temperament, which makes them amenable to handle. AAW of mature sows and mature non-obese boars ranges between 70 to 83kg. They reach puberty within 4-6 months and become sexually mature within 5-6 months [12, 13]. Amongst others, they are used in fields of cardiovascular research, diabetes- and dermal studies, metabolism, orthopaedic and ophthalmic research [14-17]. The Micro-Yucatan Miniature Swine is a Yucatan strain established in 1985 by the Charles River Laboratories. It primarily differs in size and a lower ABW and AAW [12, 13].

2.1.2.2 Westran

Westran pigs originated from an isolated feral pig population on Kangaroo Island in South Australia. These white or white and black-spotted pigs were originally domesticated for research purposes by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) of Australia [18]. AAW ranges from 80 to 93kg and maturity is reached within 6-7 months. While developing these highly inbred minipigs, the main intention was to establish a non-human tissue and organ donor for allo- and xeno-transplantation. Amongst other things, they are therefore used as a model for pancreatic islet transplantation, in order to cure patients suffering from diabetes type I. Due to the limited number of available Westrans for research, no international shipping is conducted [12].

2.1.3 Foreign crossbred minipig strains

2.1.3.1 Sinclair™S-1 Miniature Swine (Minnesota Miniature, Hormel Miniature)

The Minnesota Miniature Swine is a product of crossbreeding four American pig strains. Initially female Guinea hogs were mated to a wild boar, which originated from Santa Catalina Island, off the coast of California (USA). To subsequently reduce the size of the new breed, Piney Woods and Ras-n-lansa (Guam pigs) pigs were introduced [19]. Later in 1963, a Yorkshire boar was mated to a Minnesota sow to develop a white-coloured skin. Interestingly, the founder breeds of the Minnesota Minipig are strains originating from islands. The breeders made use

of the evolutionary phenomenon of “island dwarfism”, reflecting the observation that populations of mammalian species isolated on small islands, often develop dwarfism. In 1965, the Sinclair Comparative Medicine Research Farm of the University of Missouri in Columbia acquired part of the breeding stock. Since then, the name of the breed changed to Sinclair Miniature Swine [20, 21]. Their AAW ranges between 55 and 77kg, so they are smaller than the common Yucatan and slightly larger than the Micro-Yucatan Miniature Swine. They are used in a variety of fields such as cardiovascular, musculoskeletal, urogenital, gastroenterology and dermatology research. Some lineages have a significantly high incidence of melanoma, showing similar histopathology compared to humans [12, 22, 23].

2.1.3.2 Clawn Miniature Swine

The Clawn Miniature Swine was developed at Kagoshima University, Japan, by crossbreeding the Göttingen Minipig with the Ohmini miniature swine, which in turn was bred out of small pigs from Manchuria, China [24]. By introducing genetic materials from the large Landrace and Yorkshire pigs, white colour was established and their reproductive performance was improved [25, 26]. Nowadays, it is the predominant pig breed used in Japanese biomedical research. Their AAW is around 40kg and their main scientific use is in pharmacological and toxicological research, transplantation studies and regenerative medicine [12, 27, 28].

2.1.3.3 Hanford™ Miniature Swine

The Hanford Miniature Swine was developed at the Hanford Laboratory of the General Electric Company in 1958 in the U.S., and was initially intended for radiation biology studies. In the beginning of the radiation experiments, sheep were used as animal models. Soon they were exchanged for pigs, because researchers assumed that it would provide a better extrapolation to humans. However, the size of these adult domestic pigs made it questionable that accurate extrapolation was actually achieved. By crossbreeding Pitman-Moore pigs with the Palouse strain, a new white-skinned miniature pig breed was developed, in order to more precisely study the effects of radiation on the skin. Ultimately, a Mexican Labco pig was introduced to achieve smooth skin with very little hair. The Hanford Miniature Swine has an of 80-95kg [29]. Between 6-8 months of age, they possess organs and structures precisely corresponding to human organ sizes. Other breeds never develop equivalent organ and structure sizes [4]. Due to the dimensions of the heart of the Hanford Miniature Swine, they are considered as suitable animal models for cardiovascular studies. Other areas of use are in musculoskeletal and pharmacological research as also in dermal studies, which focus on toxicology [12, 30].

2.1.4 German crossbred minipig strains

2.1.4.1 Munich Miniature Swine

The Munich Miniature Swine is a small breed, which was developed in the 1990s by crossing Hanford and the Columbian Miniature Swine. It was especially bred for melanoma studies [31]. They possess an AAW of 60-100kg and besides of melanoma research are also used for cardiovascular, orthopaedic and implantation studies [32]. Nowadays, the only existing population is held at the University Hospital Düsseldorf, Germany [12].

2.1.4.2 Mini-Lewe (Berlin Miniature Pig)

The Mini-Lewe was developed in the former GDR as a counterpart to the already existing and frequently used Göttingen Minipig. The breeding goal was a small, fertile and precocious pig with a docile behaviour and calm temperament. The animals were bred by crossing a Vietnamese Potbelly Pig with a white-skinned boar, which in turn was a crossbreed of German Landrace and German Saddleback Pig. Adult animals reach an AAW of 45-60kg. At the time of its development, it was one of the smallest minipig breed worldwide [33].

2.1.4.3 Göttingen Minipig®

Inspired by the existence of the Minnesota Minipig, in 1961, Professor Fritz Haring from the Institute of Animal Breeding and Genetics of the University of Göttingen, Germany, developed the Göttingen Minipig (Figure 2.1.4.3-1) by crossbreeding the Minnesota Miniature and the Vietnamese Potbelly Pig. It was soon realized, that white skin would be desirable for many applications, e.g. for dermatological research. In addition, a leaner pig was demanded for studies on muscular growth. For this purpose, the white-skinned German Landrace pig was introduced by artificial insemination [21]. Glodek and Oldigs (1981) reported the genetic proportions of the three original strains as follows: 60% of Vietnamese potbelly pigs, 33% of Minnesota Minipigs and 7% of German Landrace [34]. Göttingen Minipigs reach sexual maturity within 3-5 months, and possess an AAW between 35 and 45kg [35]. The growth curve of Göttingen Minipigs (Figure 2.1.4.3-2) avoids a dramatic increase in adulthood as seen in domestic pig breeds [36]. Nowadays, four different breeding facilities exist worldwide. The University of Göttingen, Germany; Ellegaard Göttingen Minipigs A/S in Denmark; Marshall Bio Resources in the U.S. and the Oriental Yeast Company Ltd. in Japan [12]. Also due to their worldwide availability, Göttingen Minipigs have emerged to one of the most frequently used breeds in both North America and the EU [37]. They are mainly used for regulatory toxicity, diabetes and cardiovascular studies as well as in orthopaedic, surgical and dental research [30, 38-40].

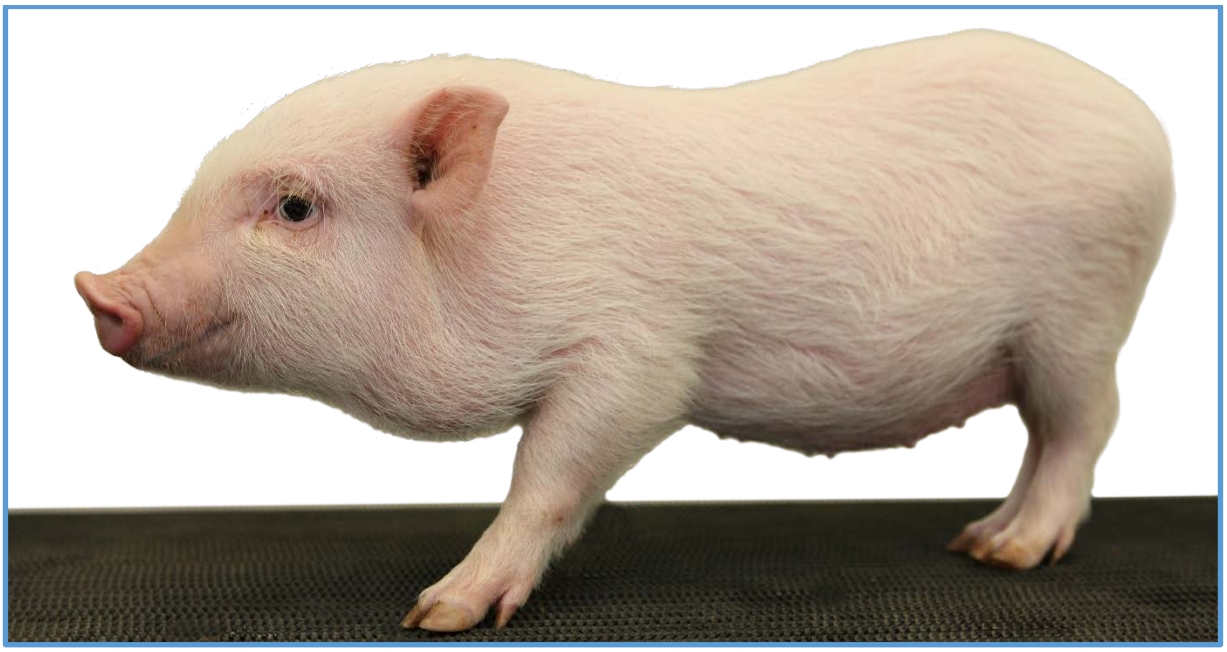


Figure 2.1.4.3-1: A pre-adult Göttingen Minipig. *Licensed under "CC BY-SA"; Minipigs; cropped; https://upload.wikimedia.org/wikipedia/commons/8/8a/G%C3%B6ttingen_Minipig.jpg;*

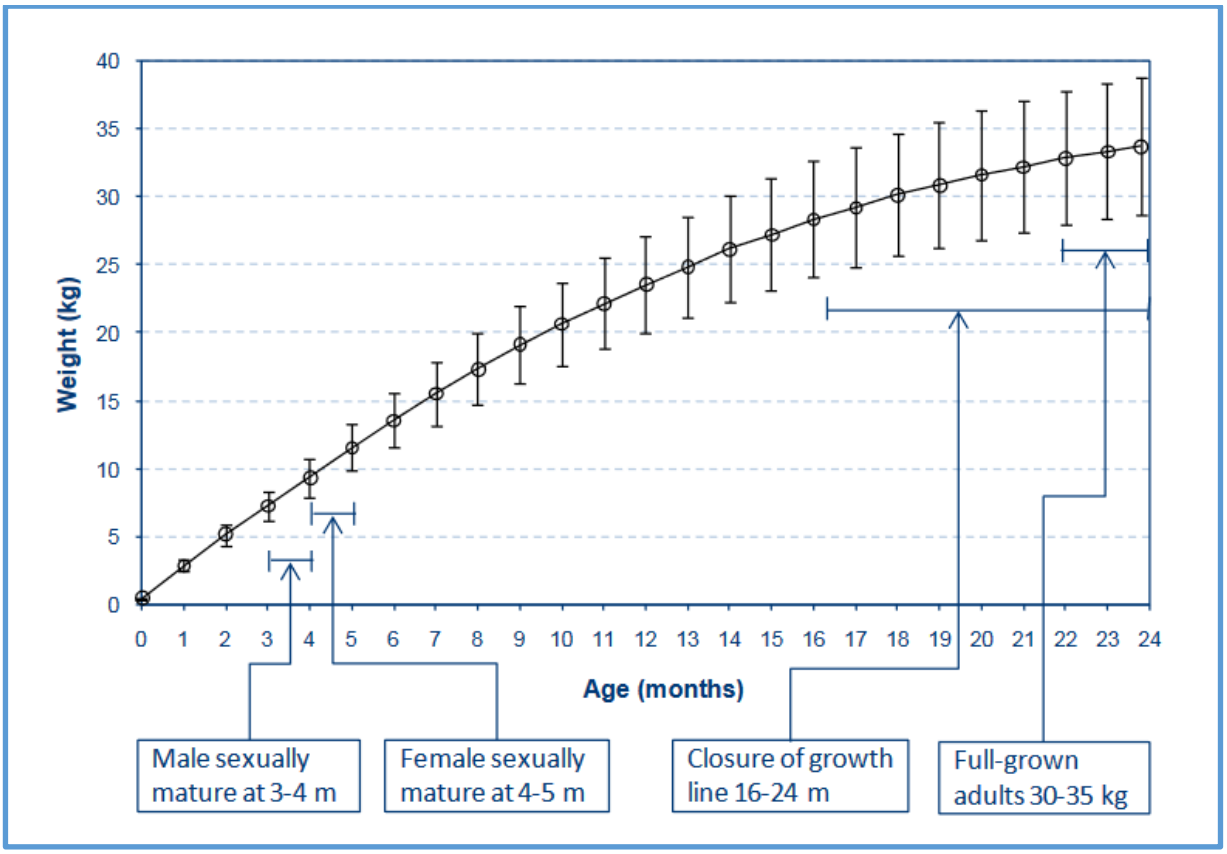


Figure 2.1.4.3-2: Growth curve of Göttingen Minipigs. *Adapted from "Taking good care of GöttingenMinipigs®".https://minipigs.dk/fileadmin/filer/pdf/Taking_good_care_of_Ellegaard_Goettingen_Minipigs_13.03.13.pdf; Ellegaard Göttingen Minipigs (Dalmose, Denmark).* m= months.

2.1.5 Animal research numbers

Animal research numbers of 2017, published by the BMEL, revealed, that in total 16130 pigs were used in animal testing in Germany. In addition, 1217 pigs were reused in experiments. Compared to cattle (5842), sheep (2832) and goats (195), the pig represented the most frequently used large animal model. In total 24999 even-toed ungulates (*Artiodactyla)* were used in research and approximately 65% of them were pigs (Figure 2.1.5-1). With 8817, over 50% of all pigs were used in “Translational and Applied Research” and more precisely 1654 of them were specifically used for “Human Gastrointestinal disorders”.

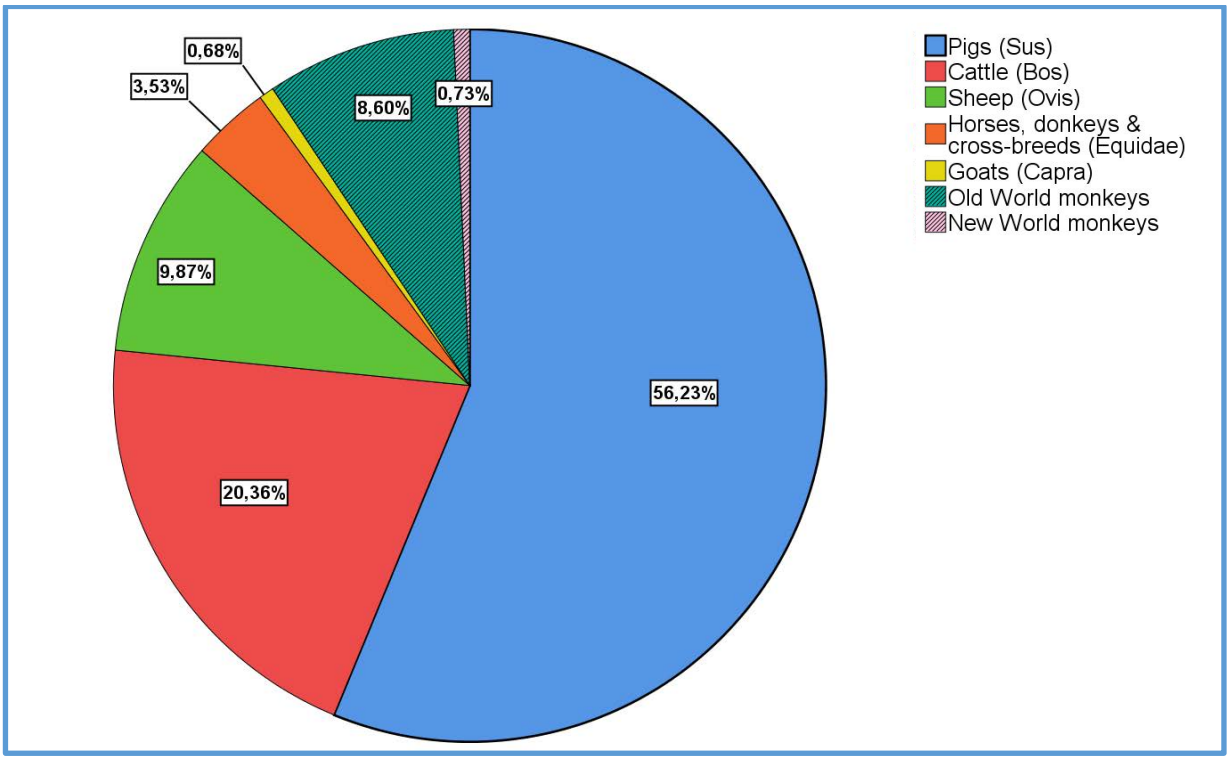


Figure 2.1.5-1: Percentages of large animals used in research in Germany in 2017. According to animal numbers issued by the BMEL (*https://www.bmel.de/DE/Tier/Tierschutz/_texte/TierschutzTierforschung.html?docId=11850874)* [2019-05-08].

Compared with 16995 pigs used in the year 2016, the German animal numbers remained quite stable and only a small decline of 5.1% could be registered. Over the years, there has been a clear fluctuation in the usage of pigs (Figure 2.1.5-2)**.** Unfortunately, the statistics of the BMEL do not distinguish between domestic pig breeds and minipigs. Therefore, exact numbers of involved minipigs, and especially Göttingen Minipigs, were not ascertainable. The EU animal numbers published in 2011 revealed that a total of 77280 pigs were used in research, 20806 for the “Research and development of products and devices for human medicine and dentistry and for veterinary medicine”. Thus, in total 2589 pigs were especially used for safety

evaluations of “Products/Substances or devices for human medicine and dentistry and for veterinary medicine” (https://eur-lex.europa.eu/resource.html?uri=cellar:e99d2a56-32fc-4f60-ad69-61ead7e377e8.0001.03/DOC_1&format=PDF) [2019-05-07].

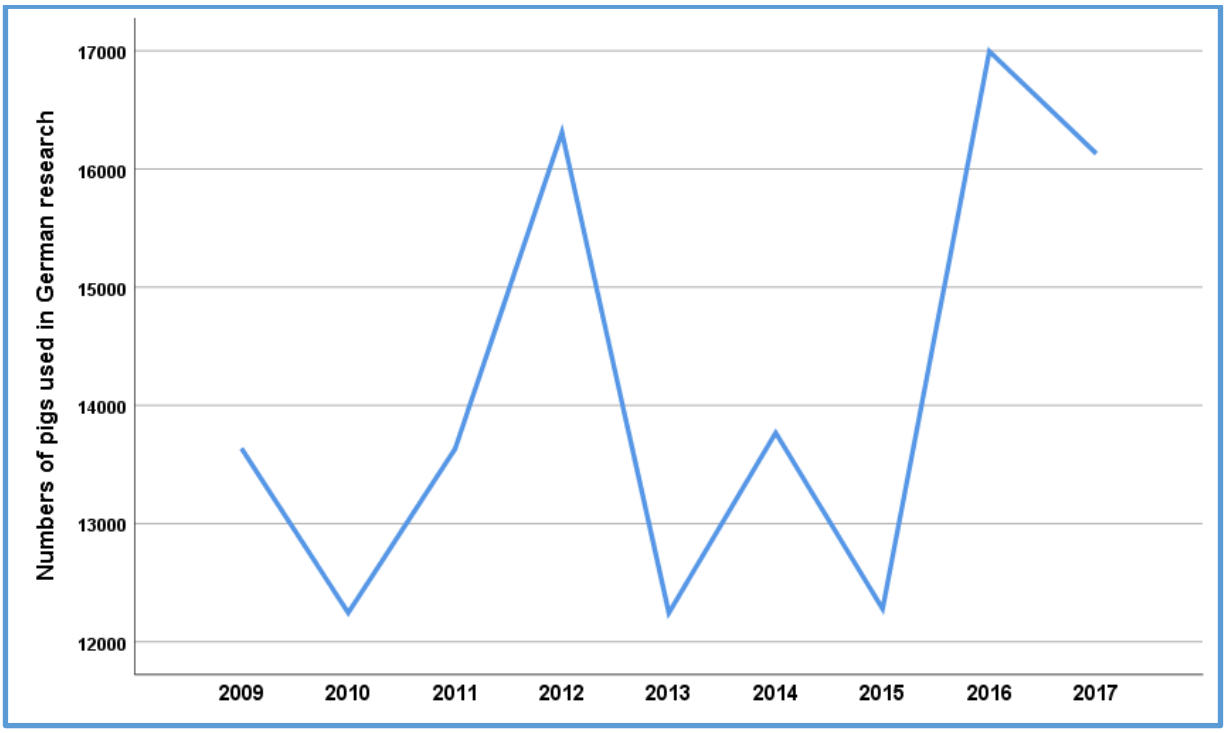


Figure 2.1.5-2: Annual development of the numbers of pigs used for scientific purposes. According to animal numbers issued by the BMEL (*https://www.bmel.de/DE/Tier/Tierschutz/_texte/Tierschutz_Tierforschung.html?docId=11850874*) [2019-05-08].

2.1.6 The Göttingen Minipig in dental and orofacial research

Because of their heterodont and diphyodont dentition with incisors and molars only slightly larger than in humans, the Göttingen Minipig has always been considered as a suitable and frequently used animal model in dental research [41]. Additionally, they possess quite similar tooth eruption patterns compared to humans [42]. Pigs are born with eight teeth, whilst the erupted deciduous dentition consists of twenty-eight, the permanent dentition of forty-four teeth.

In 1952 the Swedish researcher Per-Ingvar Brånemark discovered, that when placing titanium chambers in a rabbits’ femur, the chambers became firmly affixed to the bone over time and could not be removed without fracture. Brånemark coined the term “Osseointegration”, defined as "a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant". An implant is therefore considered as osseointegrated, when no progressive relative movement between the implant and the adjacent bone is present [43, 44]. The development of threaded implants made of pure titanium increased the popularity of

implants to new levels [45]. In the 1980s, the use of titanium implants was increasingly gaining acceptance in dentistry for the restoration of partially or completely edentulous jaws. Finally in 1988, titanium dental implant acquired the medical approval of the U.S. Food and Drug Agency [46].

Since then, the biggest endeavour of dentistry is to decrease the healing time for osseointegration, allowing earlier implant loading and thus enable shorter treatment periods for the patients. One possible way to achieve this goal is to modify the dental implant surface. The surface is the only part that is directly in contact with the surrounding tissue and the properties of the surface affect the mechanical strength of the implant-tissue interface [47]. Surface modifications can be chemical treatments such as acid etching, mechanical treatments such as sand blasting, electrochemical treatments, for instance anodic oxidation, thermal and laser treatments. All endosseous dental implants with different modifications and coatings are endosseously tested in *in vivo* experiments using animal models, such as the Göttingen Minipig [48-52].

Also several growth and differentiation factors have been tested as biocoatings of conventional implants to determine their contribution to acceleration and enhancement of bone ingrowth and implant fixation [44]. These factors were bone morphogenetic proteins (BMPs), in particular BMP-2 and BMP-7 [53] or osteogenic protein-1 (OP-1) [54]. In addition, collagen and other extracellular matrix proteins such as fibronectin and vitronectin were used as biological coating to improve the osseointegration of titanium implants [55]. Nowadays, a vast variety of dental implants are commercially available (Figure 2.1.6-1). The most common implant designs used in animal models are either cylindrical (rod-shaped) or screw type (threaded) implants. Other forms such as disc, plate or coin implants are less often used. In order to quantify the success of osseointegration, pull-out and torque removal tests are performed. A good osseointegration is indicated by the amount of force needed to pull the implant out of the bone [56].

Another field of applications of minipigs is the research on critical size defects (CSD). CSD are regarded as defects that will not spontaneously heal despite surgical stabilization and which will always require further surgical interventions [57]. In animal models, CSD are intentionally caused to serve as experimental control defects for the efficacy and efficiency of bone repair materials such as stem cells or bone graft materials [2, 53, 58-62].
Further, a variety of surgical techniques and instruments have been developed, improved and modified in the Göttingen Minipig, such as Mandibular Distraction Osteogenesis (MDO), used for the correction of mandibular abnormalities or Alveolar Distraction Osteogenesis (ADO), used for the reconstruction in patients with mandibular atrophy to facilitate dental implant placement by onlay block grafting [63].

Figure 2.1.6-1: Overview of different dental implant designs. *Adapted from [1].*

2.1.6.1 Procedures of experimental interventions

The procedures of testing endosseous dental implants and associated materials start with the extraction of the minipigs' teeth at the desired implantation side. Some authors even extracted all maxillary and all mandibular premolars and molars [64]. Following the extraction, a mucoperiosteal flap is retracted with an elevator and is, subsequently after tooth extraction, repositioned and closed using single sutures [65]. After a specific healing period of up to eight and on average three months, a crestal incision is made and again a mucoperiosteal flap is reflected [66]. Then holes are drilled and implants are inserted (Figure 2.1.6.1-1) and another healing period ranging between two weeks and four months, depending on the granted time for the implant osseointegration, is scheduled [48]. Another possibility is the so-called "immediate loading" procedure. There, the dental implants are directly placed after the tooth extraction. At the end of the experimental periods, the animals are euthanised and the mandibular segments containing the implants are resected en bloc. The segments either are prepared for the examination with micro-CT to evaluate bone changes [67-69], or embedded for further histological examination [70].

In interventions investigating bone healing abilities of biomaterials in CSD models, it is often reported that large parts of the mandible are resected [50].

The osteotomies in experimental MDO are usually performed from the superior junction of the mandibular body and ramus to the inferior border of the mandible near the mandibular angle [71].

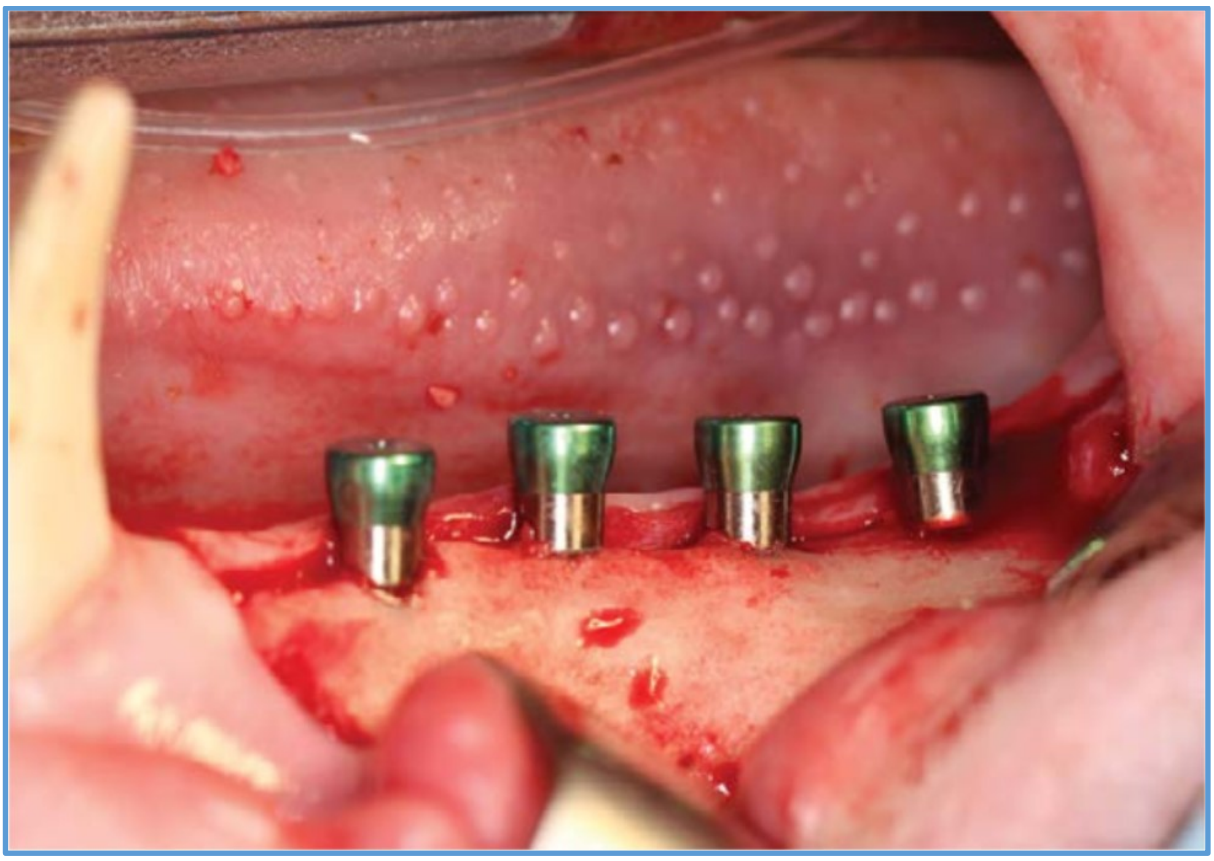


Figure 2.1.6.1-1*:* Intraoperative view. Four dental implants placed after tooth extraction in the mandible of a male Göttingen Minipig. *Adapted from [65].*

2.1.6.2 Complications

When performing surgery for dental and orofacial research in minipigs, knowledge of the specific anatomy of the mandible and the surrounding tissue is very important. Despite the fact that most of the failed studies and occurred complications remain unpublished, different incidences concerning implant stability [72, 73], implant loss [64], loosening of screws [50] and peri-implantitis with mucosal dehiscence have been reported. Peri-implantitis and mucosal dehiscence lead to infections, reduced bone regeneration, distorted results and failed experiments [74]. In some studies, the rate of successfully implanted teeth was reported only to be about 60% [66].

When similar anatomic dimensions as in human mandibles are assumed, the large mandibular canal will be penetrated through its superior wall, potentially harming the containing nerve and vessels [75]. In addition, the dimension and the root course of the large canine teeth in pigs, which represent a substantial part of the mandibular body, have to be taken into account.

Extracting the canine teeth without the destruction of a major part of the mandible, is very challenging [66, 76].

2.2 European laws on the protection of animals used for scientific purposes

In the year 1986, the EEC released its first legislation (86/609/EEC), which covered the protection and use of animals for experimental and scientific purposes [77]. Since then it is implemented that animal experiments are no longer allowed to be performed when an alternative method exists. Thereby, it concentrated on reducing the numbers of animals used for experiments and on encouraging the scientific community to develop and validate alternative methods. In addition, it also focused to improve the minimum standards for housing and the requirements in training of responsible persons working with laboratory animals and monitoring experiments. The need to revise the Directive 86/609/EEC resulted from the fact that the parameters specified therein neither adequately took account of the changed legal requirements of animal research nor of the increased importance of animal welfare at the EU level. In addition, a harmonization of the member states' law was urgently needed. In 2008, the European Commission proposed a revision of the Directive to the European Council and the Parliament [78-80]. Finally, in September 2010, the EU issued the new Directive 2010/63/EU to replace the Directive 86/609/EEC from 1986 [81]. While the 3Rs (Replace, Reduce, Refine) were not explicitly mentioned in in the Directive 86/609/EEC, one of the new Directives' major aim was to implement the principles of the 3Rs into EU legislation and the ultimate goal is the replacement of all animal experiments in the future (Directive 2010/63/EU, Article 4).

Other main elements of change between the former (86/609/EEC) and current Directive 2010/63/EU [78]:

- Inclusion of the protection of animals used for educational purposes.
- Rules on "the origin, breeding, marking, care and accommodation and killing of animals".
- Extension to cephalopods (e.g. squid and octopus) and foetal organisms.
- Avoidance of death as an endpoint.

In 2013, the legal requirements of the Directive 2010/63/EU were implemented by amending the German Animal Welfare Act (Tierschutzgesetz – TierSchG) and by the enactment of national regulations on the welfare of animals used for experiments or for other scientific purposes (Tierschutz-Versuchstierverordnung – TierSchVersV) [82, 83].

2.3 The 3R principle by Russel and Burch

The 3R principle was developed by William Russell and Rex Burch and described in their book "The Principles of Humane Experimental Techniques" of 1959 [84]. In the book they proposed, that if animals were to be used in experiments, every action should be taken to **r**eplace them with alternatives, to **r**educe the number of used animals to a minimum, and to **r**efine these experiments to cause the lowest possible level of distress and pain [85]. Initially the publication of the 3Rs only attracted little attention. Nowadays, they are regarded as important guiding principles, which influenced new legislation for the regulation of the use of animals for scientific purposes. Finally in 2010, they were formally incorporated into the EU Directive 2010/63/EU [81, 85].

David Smyth later gave the 3Rs the definition of „alternatives". In his book "Alternatives to Animal Experiments" from 1978, he stated that alternatives are "all procedures which can completely replace the need for animal experiments, reduce the number of animals required, or diminish the amount of pain or distress suffered by animals in meeting the essential needs of man and other animals" [86]. This provided more than a simple restatement of the 3Rs, since it forces all the people working in the field of animal testing, to name convincing arguments that their work is justifiable and necessary for good purposes [87].

In 2002, Flecknell pointed out, that: "Many of those who oppose animal experimentation, would also agree that until animal experimentation is stopped, Russell and Burch's 3Rs provide a means to improve animal welfare." He also stated that the adoption of the 3Rs is capable to improve the scientific quality. Because good and reliable data can only be obtained by appropriately designed experiments, that minimise variation and by standardised ideal animal care, which minimises stress and pain [85].

2.4 Definitions of the 3Rs and their applications

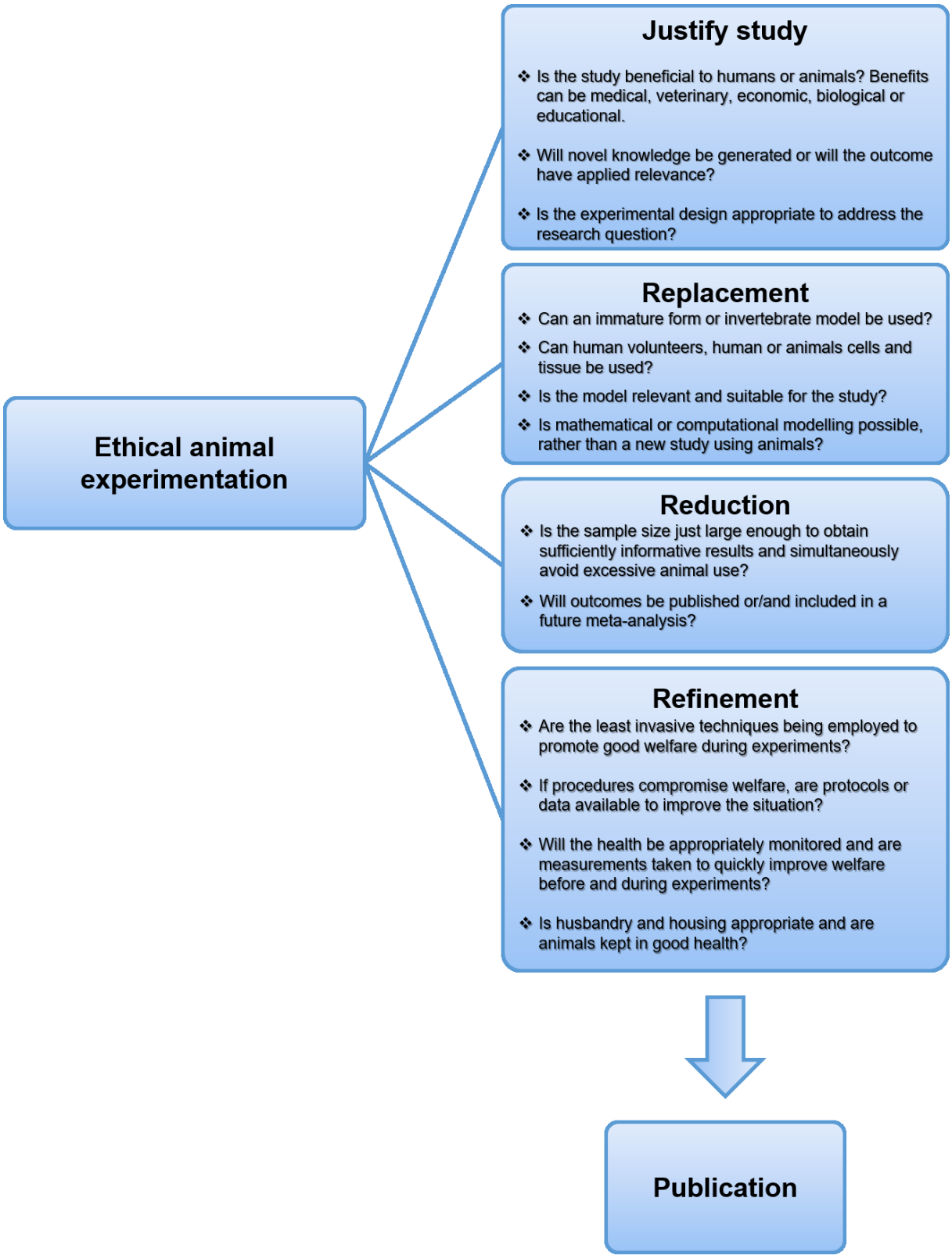


Figure 2.1.64-1: 3R ethical key questions. According to the 3Rs, this figure shows some of the major ethical key questions and concepts that scientists must address from the planning of the animal study to its publication *Derived and modified from [88].*

2.4.1 Replacement

Russell and Burch defined replacement in the sense of the 3Rs as “any scientific method employing non sentient material which may in the history of animal experimentation replace methods which use conscious living vertebrates”. They furthermore distinguished between two different forms of replacement. The relative replacement, in which animals would still be involved but not exposed to any distress in the actual experiment. The absolute replacement was described as a study in which animals are, nevertheless which state of experimentation, not needed at all. A relative replacement was for instance considered when animals were humanely killed in order to collect cells, tissues or organs for further assessment [84, 87]. Replacing animal tests by a non-killing test is the ultimate achievable goal. In the following, three different replacement methods are discussed more in detail:

- *In vitro* tests
- *In silico* tests
- The use of lower vertebrates, invertebrates and microorganisms

2.4.1.1 *In vitro* tests

The Latin term *in vitro* means “in the glass” and describes a method where, for instance cells of animals or humans are kept in Petri dishes, tubes or flasks outside their normal biological context in order to simulate the *in vivo* situation [89]. *In vitro* methods are often used in pre-clinical drug testing of chemicals or other pharmacological compounds to assess their biological safety. Traditionally pre-clinical drug testing involved a high number of animals, mostly mammals. The EU’s decision to ban animal testing for cosmetics ingredients (Regulation EC 1223/2009) can be regarded as additional driving force to develop alternative mammalian *in vitro* models [88, 90, 91]. Due to the increasing numbers of these *in vitro* models validated and developed over the years, it became possible to sort out molecules with a high potential of toxicity and to identify promising drug candidates at an early stage of the drug development. Thereby a substantial proportion of *in vivo* tests could be replaced. The replacement of *in vivo* tests has been empowered by the establishment of internationally acting organizations that support the regulatory validation of alternative methods, such as the European Union Reference Laboratory for Alternative to Animal Testing (EURL ECVAM), which was formally founded in 2011 [92]. After the successful validation process, several *in vitro* methods became an integral part of international regulatory OECD guidelines [93]. In Germany, the ZEBET, as a part of the Federal Institute for Risk Assessment (BfR), is the equivalent institution to the EURL ECVAM and also occupied with the development, validation and registration of alternative methods and all facets of the 3R research [94].

Relatively new are microsystemic modules, often called “body on a chip” or “organ on a chip”. These systems aim to combine several organ equivalents within a human-like metabolizing environment to evaluate *in-vivo*-like pharmacokinetics and pharmacodynamics. The modules consist of an external pump, microchannels with flowing media, and a reservoir of miniaturized cell culture compartments [95, 96].

Cell culture assays traditionally are comprised of two-dimensional (2D) monolayer cells, which are cultured on flat and rigid substrates, such as petri dishes. Although 2D cell culture has proven to be a valuable tool in cell-based studies, its biggest disadvantage is that is does not adequately consider the natural three-dimensional environment of cells *in vivo* [97]. In contrary, 3D cell culture systems, more accurately mimic *in vivo* conditions and results from 3D cell culture studies demonstrated a positive impact on cell proliferation, differentiation and cell survival compared to 2D cell culture [98]. Cell culture assays can thus either be mono-culture systems, consisting of only one cell type, or co-culture compositions of two or more different cell types. Using co-culture assays, it becomes possible to evaluate interactions between cell populations and are fundamental to cell–cell interaction studies of any kind [99, 100].

Several *in vitro* human cell culture tests of the skin have reached OECD Guideline status. In these co-culture tests (EpiSkin® and EpiDerm®), the substance to be tested is poured onto a static transwell system with a 3D reconstructed human primary epidermis layer [96, 101].

The Institute of Veterinary Anatomy has been active in the field of 3R *in vitro* research for many years. Already in 2004, Bahramsoltani and Plendl established an *in vitro* model for the quantification of angiogenesis [102]. The institutes’ expertise on the microscopic and ultrastructural level is used to establish cell cultures for a large variety of scientific questions, e.g. on blood vessel research [103, 104]. The group was one of only a few in the world to achieve the cultivation of equine endothelial cells in order to investigate the cause of arterial and venous thromboses, which are frequently occurring clinical complications in horses [105]. In order to avoid *in vivo* testing, the effects of iodinated contrast medium on the microcirculation has been studied using an *in vitro* three-dimensional soft-tissue co-culture assay [106]. The assay contained fibroblasts and angiogenic endothelial cells which were embedded in a fibroblast-derived extracellular matrix. The major advantage of this model was provided by its superiority over monocellular angiogenesis models in mimicking the *in vivo* angiogenesis and microvessel development. The study showed that all tested contrast media had a negative effect on the parameters of *in vitro* blood vessel development [106]. A current focus is the establishment of three-dimensional vascularized *in vitro* models of the skin [107].

2.4.1.2 In silico

Nowadays, using specialized software programs, computer models help to design new medicaments. The simulations predict possible toxic or biological effects of potential drug candidates or chemicals. This significantly reduces tests that would have been done in animals. Therefore, after primary *in silico*-screening only the most promising molecules are used for *in vivo* testing [108, 109].

The software known as Computer Aided Drug Design (CADD) is used to predict the receptor-binding site for a potential drug molecule and helps to avoid that unwanted chemicals with no biological activity are being tested. CADD is capable of identifying active drug candidates, called “hits”, and to select most likely candidates used for further evaluation, called “leads”. Leads can then be optimized into suitable drugs by improving their pharmaceutical, physicochemical or pharmacokinetic properties. Virtual screening is used to discover new drug candidates from different chemical scaffolds by searching 3D chemical structure databases [110].

Pre-clinical safety studies using animals are lengthy, expensive and frequently of limited predictability for human outcomes. This engages chemical and pharmaceutical companies to develop alternative *in silico* strategies for the predictive evaluation of drug toxicity in order to minimise animal testing. The expectation is that predictive toxicology will thus help to avoid resource waste, reduce regulatory review burden, and will increase accuracy, sensitivity, and specificity [110].

2.4.1.3 The use of lower vertebrates, invertebrates or microorganisms

Using mammals as animal models in research such as toxicity testing is still the current “gold standard” because they may share similar developmental pathways and most organs with humans. However, even human trials do often not predict outcomes in the wide population, showing that a perfect experimental model does not exist [111]. Thus, the use of mammals in toxicity testing is time consuming and expensive [112] and recent publications indicate the unreliability of rodent models to predict specific toxic effects in humans [113, 114]. Therefore, research disciplines, such as predictive toxicology, are trying to employ alternative methods and models to improve prediction of human outcomes and simultaneously reduce the cost, time and use of mammals [111]. These alternative species can be lower vertebrates, invertebrates or microorganisms. They have a positive influence on the drug development time, are economic and easy to house and handle. In addition, it is possible to perform high-throughput screening for various compounds to determine their toxicity, efficacy and selectivity [115].

The zebra fish (*Danio rerio)* is a small freshwater fish. Because of the availability of its whole genome sequence, it is often used in toxicological studies of chemicals and pharmaceuticals. According to Directive 2010/63/EU, fish become protected animals when they are capable of feeding independently. Therefore, embryos and larvae of the zebra fish are suitable for the testing in cell culture plates and Petri dishes [108, 116], since their use is not defined as animal testing [117].

The fruit fly *(Drosophila melanogaster)*, is the most commonly used invertebrate species in research. Based on genomic sequence similarity, it is the closest invertebrate model organism to humans [115, 118]. The species possesses a short life cycle and can be easily manipulated and cultured. These features make the Drosophila an ideal model for studies on development and genetics [119].

The second most used invertebrate species is the roundworm (Caenorhabditis elegans). Since its first characterization as experimental model in the 1960s, it helped to understand several basic aspects of biology. Its small size of a bit over 1mm length allows, that thousands of animals can be maintained in nutrient media in multi-well plates. Similar to *Drosophila melanogaster* they possess a very short life cycle of approximately 3 days and therefore most experiments can be easily completed within a week or less [111]. Among many other experiments *C. elegans* is involved in studies designed to rank toxicity. There they had consistently shown good correlation with oral LD_{50} rankings of rodents, at one-tenth of financial costs [120].

Even microorganism hold the potential to contribute to the replacement of mammalian animal testing. As an example, the unicellular budding yeast, *Saccharomyces cerevisiae*, normally used for baking, brewing and winemaking, has been used as eukaryotic model organism in studies of gerontology [121] and helped to understand fundamental aspects of cellular biology in Parkinson's and other neurodegenerative diseases [108, 122].

Unfortunately, it is often the case, that animals cannot be replaced easily, so that reduction and refinement are more realistic ethical strategies [88].

2.4.2 Reduction

Reduction concerns minimising the number of animals used to effectively achieve the goals of an experiment. Following the definition by Russell and Burch it is achieved by "reduction in the numbers of animals used to obtain information of given amount and precision" [84]. In practice, it is accomplished by experimental designs that improve the signal-to-noise ratio of the data analysis and therefore ultimately enable to reduce the sample size. The statistically clearer and cleaner the experiments are, the fewer experimental animals are required for the analysis

to be robust [88]. In 2005, de Boo and Hendriksen introduced three different levels to the concept of reduction [123].

1) “Intra-experimental Reduction”, which focuses on the reduction of animal numbers used within individual experiments by:

- Improving the experimental design (multifactorial designs, randomised block designs, precision, variation, statistical analysis) [124-126].
- Pre-screening with *in silico* or *in vitro* methods [123].
- Conducting pilot studies which’s data can be used to determine the number of animals needed in the main study [123].
- Retrospective analyses, which give information about the test variance in order to calculate the right number of animals [123, 127].

2) “Supra-experimental Reduction” which aims to reduce the number of animals by changing the settings in which a series of experiments takes place, and which is independent of the individual scientific procedure. It is achieved by:

- Reducing breeding surplus [123].
- Training scientists in experimental design, statistics and literature research [128].
- The employment of an experienced statistician in all animal ethics committees, in order to check whether experiments are statistically sound [129].
- Sharing tissues and organs of killed animals with other laboratories, for instance via AniMatch (www.animatch.eu).
- Reusing animals in procedures.

3) “Extra-experimental Reduction”, which concentrates to reduce developments not directly related to animal procedures [123], and is achieved by:

- Good Laboratory Practice (GLP), which guarantees consistency in testing. Protocols should be specified in standard operating procedures (SOPs).
- Good Manufacturing Practice (GMP), which ensures quality, safety and efficacy of produced medicines or biologicals.
- Harmonising international guidelines.
- Publishing negative data and results [130].

2.4.3 Refinement

In the publication “The increase of humanity in experimentation: replacement, reduction and refinement” by William Russel (1957), the definition of refinement was given as “any decrease

in the incidence or severity of inhumane procedures applied to those animals which still have to be used" [131]. In theory, refinement starts when replacement techniques are not available and when every practice or device of theory has been used to reduce the number of animals to a minimum [132]. In their book from 1959, Russell and Burch stated a different definition of refinement described as "simply to reduce to an absolute minimum the amount of stress imposed on those animals that are still used" [84]. This was done mainly to remove the ambiguity of what was meant by the word "procedures" and to point out that refinement also includes what happens before and after the scientific procedures. They furthermore referred to two distinct refinement areas. The "Contingent inhumanity", which was described as distress and pain, caused by housing animals in a research facility, and the "Direct inhumanity", which was defined as distress and pain caused directly by the applied research procedure [84, 85]. Here are examples for the practical implementation of refinement methods:

- Improving the use of anaesthesia and analgetics by testing their severity level in different species [133, 134].
- Improve husbandry
- Improve housing with environmental enrichment methods for laboratory animals [135].
- Defining new humane endpoints / Refining the method of humane killing [136, 137].
- Using less invasive methods or techniques.
- Using non-invasive imaging techniques such as CT, MRT, PET, SPECT or ultrasound.
- Apply visual methods to assess pain, such as the Mouse Grimace Scale [138].

2.4.4 Other Rs

Over recent years, several authors postulated new Rs that should extent the existing 3R-principle. The Indian scientists Pereira and Tettamanti (2005) proposed "**R**ehabilitation" as "India´s fourth R". Rehabilitation is defined as the after-care rendered to animals involved in experimentation, with the lone purpose of alleviating any pain or suffering the animals have been exposed to. Their life should be prolonged until the point of natural death and it is mandatory that during the period of rehabilitation, the animals should not be exposed to any kind of unnatural activity to their natural needs or behaviour. The expenses for the rehabilitation of animals are intended to be a part of the research budget and should be scaled in accordance with the level of sentience of the animals [139].

Sneddon et al. (2017) reminded that it is crucial, that the species chosen is relevant to the question being addressed. This would positively influence the scientific outcome of any study. Therefore, "**R**elevance" could be considered as a fourth R [88].

In a review article it was calculated, that 85% of basic and clinical research was wasted due to inadequate or inappropriate design, unpublished negative results and poor reporting [130]. As a consequence, Aske and Waugh (2017) proposed, that some of these issues could be improved by expanding the 3R to the 5R by including "**Ri**gour" and "**R**eproducibility". Animal studies that adhere to scientific rigour, in having a robust and unbiased experimental design and by providing full transparency, would consequently lead to more "reproducibility" and transparency in research [140].

"**R**ehoming" animals is also often considered as an extension to the 3R-principle. Article 19 of the European Directive 2010/63/EU allows the Member States to rehome or return their laboratory animals in "a suitable habitat or husbandry system appropriate to the species", if the following conditions are fulfilled: The health status of the animals must allow the process of rehoming; appropriate measures have been taken to ensure the wellbeing of the animal in its new surroundings. In addition, it is mandatory, that there is no danger to the public, environmental and animal health [81].

Russell and Burch reminded all researchers to act with responsibility towards the quality of their scientific work as also on the ethical treatment of the involved animals. Many regard this "**R**esponsibility" as fourth R**,** as it affects all participants directly or indirectly involved in the use laboratory animals. It concentrates on training involved persons in planning and communicating their research [84, 141-143].

Susan Iliff, who described "**R**emembering" as an additional R, reminds that researchers should recognize the contributions laboratory animals give for advancing biomedical research. Iliff furthermore proposes to organize acknowledgement events such as memorial ceremonies, as a formal sign of gratitude towards all involved animals. This might not only allow individuals to openly share their feelings and break the barrier of silence, but also can be a satisfying and meaningful experience for participating research groups [144].

2.4.5 Alternatives to experimental dental and orofacial animal testing

Law defines implants as medical devices. According to Annex 7 of the Council Directive 90/385/EEC, a medical device needs to be pre-clinically tested rigorously under both *in vitro* and *in vivo* conditions, in order to be accepted for clinical tests in humans [3]. These pre-clinical tests assess the biocompatibility, tissue response and mechanical stability of new implant materials. *In vitro* methods can be used as first stage tests for acute toxicity and

cytocompatibility, in order to avoid using inappropriate materials in animal testing. However, one major limitation is that no *in vitro* cell culture system is capable to produce implant loading, as present in the *in vivo* situation [56]. Therefore, a complete replacement of *in vivo* tests for dental and orofacial research is still not possible. Only animal models allow the long-term evaluation of materials under unloaded and loaded situations and in different ages and tissue qualities. In addition, also the influence on other tissues in remote locations can be studied [56]. The necessity of *in vivo* testing emphasizes the importance of developing and using refinement strategies. For the use of animals in the testing of endosseous dental implants, the standard EN ISO 7405:2018 “Dentistry – Evaluation of biocompatibility of medical devices used in dentistry” was issued. In contrast to laws, standards and norms are of voluntary nature and not necessarily legally binding. Nevertheless, they can be understood as an advisory guide. Instead of giving a recommendation for particular suitable animals models, the ISO 7405:2018 proposes different criteria for the selection process of the animal species [12, 145].

2.4.6 Reusing CT data sets

Instead of performing new experiments, older CT scans, which originated from a research study of 2007 and 2008, were reused. Despite this thesis, the CT data also served studies on the vascular growth [146] and the morphometry of the spinal column [147]. The process of reusing CT data contributed to the 3Rs by reducing the number of animals. The data sets, with their excellent quality, will remain an everlasting opportunity to conduct additional future studies on the Göttingen Minipigs’ characteristic anatomy, without any further animal use.

2.5 Methodologies

2.5.1 Computed Tomography as a non-invasive method

Computed Tomography uses a combination of many X-ray measurements, which are taken from various different angles and are processed by a computer. The production of cross-sectional images or so called “virtual slices” allows the operator to see inside of the scanned object, without cutting it [148].

The absorption of X-rays within the object is proportional to the linear attenuation coefficient and the thickness of the materials or tissues the beam passes. Bone possesses a higher linear absorption compared to low electron density tissue such as fat or fluids. The obtained attenuation values from the over 1000 different projections angles are re-calculated, and produce a matrix of the average relative X-ray absorptions in each volume element (short voxel) of the examined slice. The different materials or tissues are displayed as a 2D picture

consisting of pixel in shades of grey, which are chosen according to their mean attenuation values. A pixel therefore represents a two-dimensional image of a three-dimensional voxel within the scanned object [149, 150].

Although, the images are generated in the transverse plane (also called “axial plane”), perpendicular to the long axis of the object or body, todays CT scanners are reformatting the volume of data to different planes (coronal, sagittal or oblique) and even as 3D (volumetric) objects.

With regards to the 3Rs, the technology of CT is not only deployed as a diagnostic and preoperative tool, it can also be used to follow certain biological effects or anatomical changes in the same animal over a defined period. Thereby, the number of animals can be reduced as each animal serves as its own control. Although the negative effect of repeated anaesthesia required for the examination cannot be negated, CT can be performed in real-time. Invasive and potentially painful procedures as well as euthanasia after the examination, are therefore not necessary [151, 152]. By contrast-enhanced CT, vessels, vascularised organs and tissues as well as anatomical and pathological changes can be accurately assessed [153, 154].

2.5.2 2D reconstruction

Two-dimensional reconstructions such as Multiplanar Reconstruction (MPR) can be processed without digital loss, when image data with isotropic resolution is acquired. MPR uses the available 3D data to show other planes, reconstructed from the transverse images that were not acquired directly during the CT scan, such as coronal and sagittal cross-sections. As the volume data is fully available, it is possible to visualize any required planes for instance a curved plane to enable the presentation and measurement of irregular or oblique anatomical structures. This can be used for the analysis of vessels, through acquiring a plane cut parallel and showing its real anatomical dimensions. MPR is the most widely used post-processing 2D method (Figure 2.5.3-1 A-C) [153, 155-157].

2.5.3 3D reconstruction

The creation of 3D reconstructions is a very important and expanding use of CT in research and teaching (Figure 2.5.3-1 D). These 3D reconstructions can fundamentally assist surgeons during preoperative planning and during the recovery period. They can also be an invaluable and reusable tool in academic teaching in order to demonstrate complex anatomical structures, visualize complicated fractures or the required position of implants. 3D reconstructions can be

even used in virtual museums providing virtual dissection experience or as 3D printed solid objects as a preoperative practice device for experimental surgeons [152, 153].

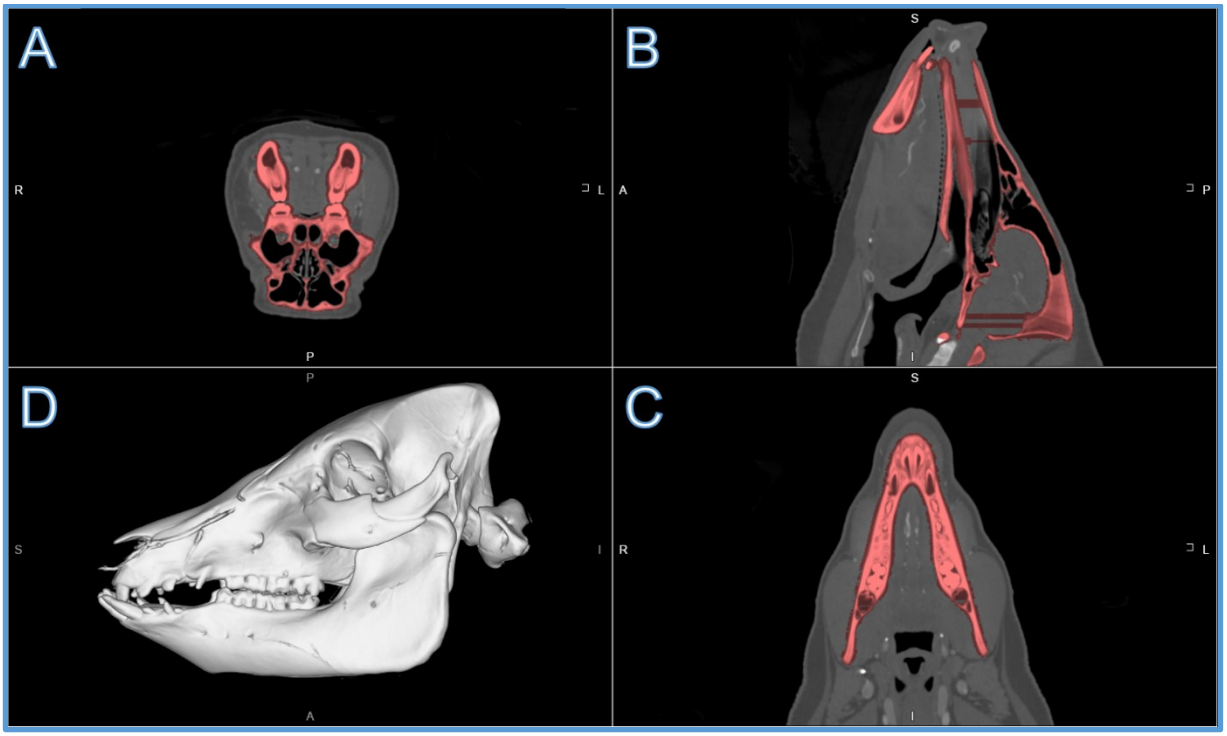


Figure 2.5.3-1: MPR and 3D reconstruction of a Göttingen Minipig skull. Transversal (axial) view (A) with MPR of (B) sagittal plane and (C) coronal plane; (D) 3D reconstruction created by VR.

3D reconstructions are mostly created by the technique of Volume rendering (VR) which takes the entire volume of the data and calculates each voxels' contribution through the data set. VR then displays the resulting composite for each pixel of the display. Volume rendering is widely used in CT and MRI, and in various applications such as cardiac imaging and orthopaedic applications. Depending on the opacity levels, the 3D reconstructions can be displayed as absolutely opaque or entirely transparent objects, which itself can be used to see the interior of the data volume. Using shading techniques, the appearance of surfaces can be improved [157, 158].

2.5.4 Cephalometry

Cephalometry is defined as the scientific measurement of the skull usually performed on radiographic x-rays. To diagnose skeletal malformations, malocclusions and plan the according treatments, these measurements are performed by orthodontists and oral-maxillofacial surgeons based on the location of different well-defined anatomical points, known as craniofacial landmarks. Once the landmarks are located, several linear and angular measurements can be performed for instance to compare the measurements with norms of

populations [159, 160]. Despite of data collections, which are dating back to the 19th century, much of the cephalometric data on human mandibles has been published in studies comparing the accuracy of different radiographic techniques and different cephalometric methods [161]. Also in recent times, forensic and anthropologic studies assessing mandibular anatomical indicators for the sex determination, have risen to prominence [162, 163]. Morphometrics or morphometry is defined as the quantitative studies of biological shape and size, as the variation of shape can be a valuable tool for interspecific discrimination [164, 165].

The software used for the measurements in this thesis was Vitrea Advanced® 6.6 (Vital Images Inc., Minnetonka, MN, USA), which is a multi-modality visualization system providing advanced imaging tools, such as in-suite 3D viewing and clinical applications. Clinical data from multiple modalities, such as CT, MRI or SPECT can be processed and analysed.

3 Aims and Objectives of the thesis

Though detailed anatomical data of the mandible is important to the success of dental and orofacial surgical experiments and the ethical use of animal models, no data of the Göttingen Minipigs' mandible is published. The knowledge of interspecies differences and of intraspecies anatomical variability in breeding lines contributes to a better understanding of the animal model and has the potential to improve animal welfare by reducing complications and misleading and meaningless results with limited transferability.

Therefore, the overarching objective of this anatomical thesis was to establish a data collection with detailed and age-related anatomical data of the mandible and the mandibular canal of growing Göttingen Minipigs and to compare this data with human mandibular anatomy. To avoid intra- and postoperative pain and distress for animals, this data collection shall prospectively be used in dental and orofacial surgical experiments as a refinement in the sense of the 3Rs.

Within this thesis, the following points have been addressed:

- Assess overall mandibular dimensions and mandibular growth of the Göttingen Minipig.
- Compare the anatomy of the mandibles of Göttingen Minipigs and humans.
- Define the time points of skeletal maturity.
- Assess whether significant anatomical differences between both hemimandibles exist.
- Perform 3D renderings of the course and dimensions of the masticatory muscles to reduce the invasiveness of surgical.
- Assess the 3D configuration of the blood vessels of the mandibular ramus to avoid bleeding during MDO or CSD tests.
- Visualize and measure the size and shape of the mandibular canal and the changes with higher age.
- Assess the 3D configuration and course of the inferior alveolar neurovascular bundle within the mandibular canal.

- Measure the anatomic dimensions of the alveolar ridge and alveolar bone height to determine the available space for dental implant placement, without harming the nerves and vessels of the mandibular canal.

- Create a data collection in order to design customized implants and for pre-surgical planning.

- Raise awareness for the different size and shape of the mandibular canal in Göttingen Minipigs compared to humans.

- Identify whether the frequently occurring post-operative complications in mandibular surgical experiments performed on Göttingen Minipigs are caused by their specific anatomical mandibular characteristics.

- Find out whether the Göttingen Minipig is an anatomically suitable animal model for dental and orofacial surgery.

- Identify further advantages and disadvantages of the Göttingen Minipig as an animal model for dental and orofacial research.

4 Publication I:

RESEARCH ARTICLE

Refining experimental dental implant testing in the Göttingen Minipig using 3D computed tomography – A morphometric study of the mandibular canal.

OPENACCESS

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Refining experimental dental implant testing in the Göttingen Minipig using 3D computed tomography – A morphometric study of the mandibular canal

Giuliano M. Corte[1]*, Johanna Plendl[1], Hana Hünigen[1], Kenneth C. Richardson[2], Ole Gemeinhardt[3], Stefan M. Niehues[3]

1 Institute of Veterinary Anatomy, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany, **2** College of Veterinary Medicine, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, **3** Department of Radiology, Charité – Universitätsmedizin Berlin, Berlin, Germany
* Giuliano.Corte@fu-berlin.de

4.1 Abstract

This study reports morphometric and age-related data of the mandibular canal and the alveolar ridge of the Göttingen Minipig to avoid complications during *in vivo* testing of endosseous dental implants and to compare these data with the human anatomy. Using 3D computed tomography, six parameters of the mandibular canal as well as the alveolar bone height and the alveolar ridge width were measured in Göttingen Minipigs aged 12, 17 and 21 months. Our null hypothesis assumes that the age and the body mass have an influence on the parameters measured. The study found that the volume, length and depth of the mandibular canal all increase with age. The width of the canal does not change significantly with age. The body mass does not have an influence on any of the measured parameters. The increase in canal volume appears to be due to loss of deep spongy bone in the posterior premolar and molar regions. This reduces the available space for dental implantations, negatively affecting implant stability and potentially the integrity of the inferior alveolar neurovascular bundle. Dynamic anatomical changes occur until 21 months. On ethical grounds, using minipigs younger than 21 months in experimental implant dentistry is inadvisable. Paradoxically the measurements of the 12 months old pigs indicate a closer alignment of their mandibular anatomy to that of humans suggesting that they may be better models for implant studies. Given the variability in mandibular canal dimensions in similar age cohorts, the use of imaging techniques is essential for the selection of individual minipigs for dental prosthetic interventions and thus higher success rates.

4.2 Introduction

Because of their similar anatomy and physiology to that of humans, the Göttingen Minipig is often used as a large animal model [1] in areas of research such as toxicology [2], neuroscience [3] diabetes [4] and obesity studies [5, 6]. Their small size, rapid growth and early sexual maturity allow easier handling and more economic housing, features that make them preferable to normal-sized pigs or other large animal species for long-term studies [7, 8]. Miniature pigs are used frequently as an animal model in dental research [9] because of their heterodont dentition with incisors and molars only slightly larger than in humans. Their being diphyodont and having similar eruption patterns validate their suitability as an animal model for dentistry [10–12].

Over recent decades, public concern about animal welfare has evolved gradually resulting in 2010 with the European Union issuing Directive 2010/63/EU for the implementation of the 3R concept i.e., replace, reduce, refine [13] in research [14]. The aim of the refine-principle is to modify animal testing to minimise distress, pain and suffering using improved experimental techniques [15].

This is particularly so when testing new dental implants and biomaterials in animal models. Here it is important to have detailed knowledge of the animals' facial anatomy. The knowledge of interspecific differences and of intraspecific anatomical variability in breeding lines, improves the outcomes of these surgical interventions and lessens the possibility of failure or of potentially misleading and meaningless results with limited transferability [16].

The mandibular canal (Canalis alveolaris inferior) originates at the foramen mandibulae and runs within the substance of the mandibular body to terminate immediately rostral to the first premolar tooth [17]. The canal conveys the inferior alveolar neurovascular bundle, which consist of the inferior alveolar artery and vein and the inferior alveolar nerve [18]. In recent times, dental research has often involved in vivo testing of endosseous dental implants in 12–24 months old Göttingen Minipigs [19–21]. In some cases, these interventions have failed entirely or have had less than satisfactory results. Often this has been due to implant instability. An additional procedural problem has been the penetration of the mandibular canal during experimental procedures resulting in injury to the inferior alveolar neurovascular bundle causing bleeding, swelling, neurosensory alterations like paraesthesia, hyperaesthesia or dysaesthesia and pain [22–25].

Although detailed morphometric data is important to the success of oral implant surgeries, only a few studies of miniature pigs' mandibular morphometry exist. Consequently, this study was designed to provide detailed morphometric and age-related in vivo data of the mandibular canal and the alveolar process of the Göttingen Minipig using 3D computed tomography. We

measured the volume, length, depth, width and inferior bone thickness of the mandibular canal as well as the alveolar bone height and the alveolar ridge width. We also focused on the configuration and course of the inferior alveolar neurovascular bundle within the mandibular canal.

Our null hypothesis is, that there are no significant differences between the left and right hemimandibles (mandibular halves) and that the age and body mass have an influence on the parameters measured.

4.3 Material and methods

The CT data sets were created in 2007 and 2008 in the course of another research study, that was approved by the Regional Office for Health and Social Affairs Berlin (permit IC113-G 0281/12) and was conducted at the medical faculty (certified by ISO 9001) of the Charité Campus Virchow-Klinikum, Berlin [26]. The reuse of the data is in accordance with the 3Rs, but precluded an optimal study design, however it was an opportunity to further our knowledge base on the little known mandibular morphology of the Göttingen Minipig.

4.3.1 Animal groups and husbandry

A total of 18 healthy female Göttingen Minipigs consisting of six animals examined at the age of 12 months (12m; n = 6; 357±31d) and 12 animals examined at an age of 17 months (17m; n = 12; 511±24d) and again at the age of 21 months (21m; n = 11; 620±37d). The animals' weight ranged from 23 to 44 kg. In the 21-month group (adult animals), one animal was excluded due to the loss of some of its data.

The minipigs were obtained from Ellegaard, Göttingen Minipigs® (Dalmose, Denmark) where they had been habituated to routine handling by humans to lessen the effects of humans as stressors in their daily life. Subsequently, at the research facility in Berlin, animals were held according to the Guidelines of the European Societies of Laboratory Animal Science. The pigs were grouped into pens of six animals, with a light/dark rhythm of 12/12 hours, a relative humidity of 55 ± 10% and temperatures between 15 and 24˚C. The animals were fed a restricted diet designed for minipigs (Ssnif Spezialdiäten GmbH, Soest, Germany) to prevent obesity [27]. Their body mass was measured weekly.

4.3.2 Computed tomography

4.3.2.1 Anaesthesia and drug administration

Prior to tomography, animals were fasted for 24 hours with water *ad libitum*. Then the animals were premedicated by intramuscular injection of 0.5 mg atropine (Atropinum sulfuricum, 1

mg/ml, Eifelfango, Bad Neuenahr-Ahrweiler, Germany). Anaesthesia was induced by intramuscular injection of ketamine (27 mg/kg, Ursotamin®, 100 mg/ml, Serumwerk Bernburg, Germany), xylazine (3.5 mg/kg, Rompun® TS, 20 mg/ml, Bayer Vital GmbH, Leverkusen, Germany) and 3 ml azaperone (Stresnil®, 40 mg/ml, Janssen Animal Health, Neuss, Germany). An electrolyte solution was infused intravenously throughout the entire procedure (Ionosteril®, Fresenius, Bad Homburg v.d.H., Germany) [28]. At the end of the experiment all animals were euthanised in deep anaesthesia by intravenous injection of 15 ml T 61 (Intervet Deutschland GmbH, Unterschleißheim, Germany) for separate studies of the vascular distribution of the whole body [26] and histologic experiments.

4.3.2.2 Equipment and software

Data acquisition was performed on a 64-slice scanner (Lightspeed 64®; GE Medical Systems, Milwaukee, USA). For contrast enhancement, 80 ml of a nonionic iodinated contrast medium (XenetiX® 350, Guerbet GmbH, Sulzbach, Germany 350 mg iodine /ml) with automatic intravenous injection of 4 ml/sec was injected through the lateral ear vein of every pig. The examination timing was multiphasic, bolus arterial triggered and venous (with an 80 sec delay). Scan parameters were standardised (voltage of 120 kV, maximal 500 mA with automatic mA-optimization at a noise index of 15, mean 490 mA; collimated slice thickness of 64×0.625 mm; total detector width of 55 mm; rotation speed of 0.4 sec; table feed per rotation: 55 mm) [26]. The scan field of view (SFOV) was 50 cm and the display field of view was 39.7 cm. The physical detector width covers 40 mm in z-axis and the used pitch factor was 1.375. The positioning and the following computed tomographic examination required only a few minutes per animal. The 12m pigs were imaged twice at an interval of 27 days, whilst the 17m and 21m pigs were imaged five times over 111 days. Then the data was transferred to an independent workstation using the software Vitrea Advanced® 6.6 (Vital Images Inc., Minnetonka, MN, USA). Volumetric assessment was reconstructed without overlap of images with a slice thickness of 1.25 mm.

4.3.3 Parameters measured

To identify landmarks and to ensure high reproducibility, multiplanar (sagittal, coronal, axial) views, reconstructed from the original axial slices were used [29]. In addition, bone reconstruction kernels were used (Bone plus, GE Medical Systems, Milwaukee, USA). All parameters were measured on both left and right hemimandibles. The volumes are given in millilitres (ml), all other parameters in millimetres (mm).

To measure the mandibular canal volume (VCM) the “vessel grow” function of Vitrea Advanced® was utilized to segment the mandibular canal and to calculate its’ volume. The short canals branching off the main canal and forming the numerous mental foramina in pigs were excluded from the volume calculations (Fig 1).

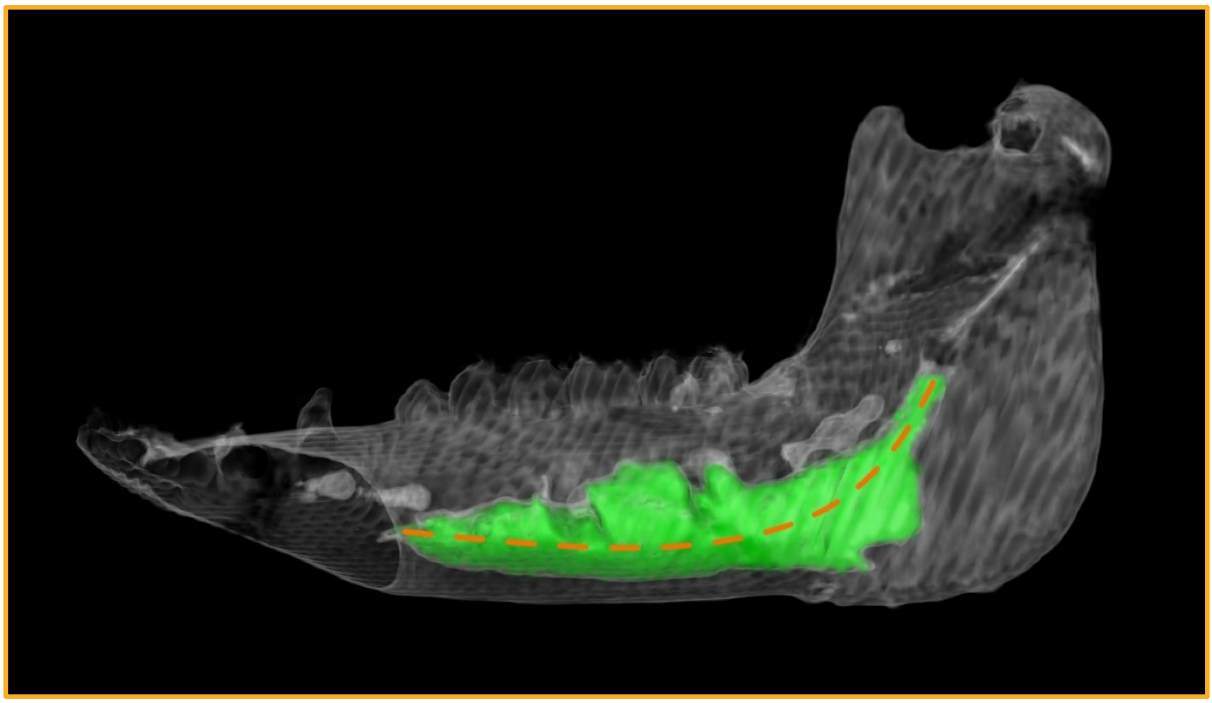

Figure 4.3.3-1: **Fig 1. The mandible of a 17m old pig showing the volume of the mandibular canal.** The volume of mandibular canal (VCM) appears in green and the length of mandibular canal (LCM) as the dashed red line.

The length of the mandibular canal (LCM) in each hemimandible was measured using images of the axial plane where the software connected the midpoints at each segmentation level along the entire length of the canal, forming a continuum (Fig 1).

All subsequent parameters (Fig 2B) were measured at the level of the most posteriorly located mental foramen (Fig 2A). This consistent anatomical landmark facilitated the comparison between the age groups.

The maximal vertical depth of the mandibular canal (MVD) was determined by drawing a vertical line from the midpoint of the superior aspect of the mandibular canal opposite to the inner surface of the canal.

The maximal oblique depth of the mandibular canal (MOD) was determined by drawing an oblique from the same superior midpoint to the most distal inferior point. If the MVD was less than MOD, the shape approaches an oval. If the MVD equalled the MOD, the canal was assumed to be circular.

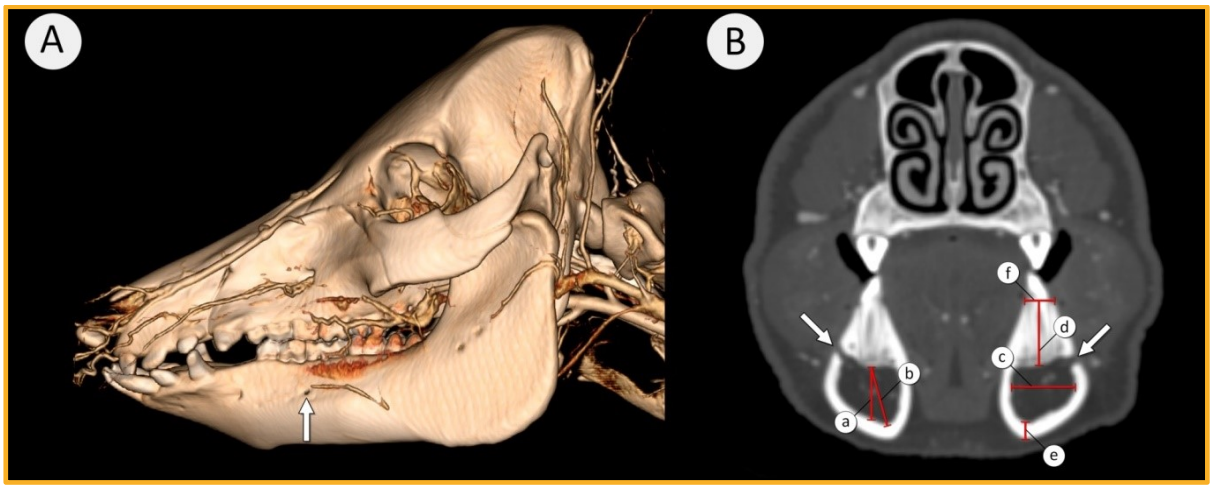

Figure 4.3.3-2: ***Fig 2. 3D rendering of the head of a minipig (A) and a transverse section view at the level of the posterior mental foramen (B).*** (A) Arrowed is the prominent posterior mental foramen. Image (B) shows the measured parameters with (a) Maximal vertical depth, (b) Maximal oblique depth, (c) Maximal width of mandibular canal, (d) Alveolar bone height, (e) Inferior bone thickness and (f) Alveolar ridge width. The white arrows indicate the posterior mental foramen.

The width of the mandibular canal (WCM) was defined by the largest horizontal measurement between the buccal and lingual aspects of the canal.

The alveolar bone height (ABH) was determined by drawing a vertical line from the midpoint of the superior aspect of the canal to a horizontal line connecting the buccal and lingual alveolar crests.

The inferior bone thickness (IBT) was measured by drawing a line from the most distal inferior point of the canal across the broadest bony dimension to the most inferior point of the mandibular body.

The alveolar ridge width (ARW) was measured by the length of a line connecting the buccal and lingual alveolar crests.

4.3.4 Dissection

To confirm that the inferior alveolar nerve runs in close proximity with the inferior alveolar vessels and to determine its' position within the mandibular canal, two hemimandibles of an adult Göttingen minipig were dissected. The mandible was a donation to the Institute of Veterinary Anatomy, Freie Universität Berlin. The mandible was transected by a saw cut through the mandibular symphysis into its' hemimandibles. All the external soft tissues were dissected from the hemimandibles. Subsequently one hemimandible was sawn transversally at 1 cm intervals along its entire length. In the other hemimandible, the mandibular canal was exposed from the medial aspect using a micro milling tool (Micromot®, Proxxon GmbH, Föhren,

Germany) from the mandibular foramen to the first molar tooth. Any fatty tissue within the canal was removed to clearly visualise the inferior alveolar neurovascular bundle.

4.3.5 Statistics

For statistical analysis we used IBM SPSS Statistics 23 (IBM Deutschland GmbH, Kassel, Germany). Every parameter was checked for normality. If normal distribution could be assumed, we used the student's t-Test and for non-normal data the Mann-Whitney-U, Wilcoxon and Kruskall-Wallis Test. When comparing 12m animals with animals of 17m and 21m, the Independent T-test was used. This was because the animals in the 12m group differ to those of 17m and 21m group. However, animals in 17m and 21m group were the same individuals and therefore paired samples. For this statistical comparison the Paired-student's t-Test was used. The correlations between parameters were analyzed with a bivariate Pearson-Test and Spearman-Rho-Test, depending on the distribution of the data. The values are given as mean values with the associated standard error. A p value of less than 0.05 was considered significant. A correlation coefficient (r) between 0.45 to 0.59 was considered to be a moderate correlation, whereas a correlation coefficient between 0.60 to 0.79 was perceived to be a strong and from 0.80 to 1.0 to be a very strong correlation. All measurements were conducted by the same trained examiner and under the supervision of an experienced radiologist. To estimate the observers' reproducibility of the measured values, several blind tests were conducted. A mean percentage standard deviation of 2.2% proved that the measurements were executed precisely and were therefore reliable.

4.4 Results

Table 1 shows the mean values and standard errors of all measured parameters. The correlation coefficient between left and right side, correlation with age and with body are in Table 2.

Table 4.4-1: *Table 1. Mean values and standard errors of all measured parameters.* Because data from all measure of the left and right hemimandibles were statistically similar, data from the left and right hemimandibles were pooled for this table.

Age group (months)		Volume of the mandibular canal [ml]	Length of the mandibular canal [mm]	Maximal vertical depth of the mandibular canal [mm]	Maximal oblique depth of the mandibular canal [mm]
12 months (n = 6)	Mean	**3.71**	**94.29**	**7.60**	**7.98**
	Std. Error	0.79	4.82	2.00	1.65
17 months (n = 12)	Mean	**6.86**	**104.13**	**9.78**	**11.37**
	Std. Error	2.26	3.65	1.63	2.18
21 months (n = 11)	Mean	**8.27**	**108.58**	**11.74**	**12.44**
	Std. Error	2.58	3.93	1.46	1.91
Age group (months)		**Width of the mandibular canal [mm]**	**Alveolar bone height [mm]**	**Inferior bone thickness [mm]**	**Alveolar ridge width [mm]**
12 months (n = 6)	Mean	**10.32**	**17.74**	**4.65**	**7.20**
	Std. Error	0.60	2.73	0.88	1.10
17 months (n = 12)	Mean	**11.21**	**13.99**	**3.65**	**8.26**
	Std. Error	1.17	1.20	1.20	0.86
21 months (n = 11)	Mean	**11.59**	**14.33**	**3.95**	**7.85**
	Std. Error	1.65	1.16	1.14	0.82

Table 4.4-2: *Table 2. Overview of the correlation between left and right hemimandibles, correlation with age and with body weight.* A correlation coefficient (r) between 0.45 to 0.59 was considered to be a moderate correlation, whereas a correlation coefficient between 0.60 to 0.79 was considered to be a strong and from 0.80 to 1.0 to be a very strong correlation. The significance levels are reported as *p<0.05; **p<0.01; ***p<0.001; ^{ns} = p>0.05

Parameter	Correlation between left and right hemimandible	Correlation with age (days), left/right hemimandible	Correlation with body mass (kg), left/right hemimandible
VCM	r= 0.994***	r= 0.616**/0.579**	r= 0.174^{ns}/0.145^{ns}
LCM	r= 0.883***	r= 0.783**/0.701**	r= 0.309^{ns}/0.293^{ns}
MVD	r= 0.892***	r= 0.613**/0.755**	r= 0.302^{ns}/0.394^{ns}
MOD	r= 0.913***	r= 0.618**/0.689**	r= 0.112^{ns}/0.249^{ns}
WCM	r= 0.511**	r= 0.282^{ns}/0.170^{ns}	r= 0.022^{ns}/-0.126^{ns}
ABH	r= 0.866***	r= -0.536**/-0.451*	r= -0.075^{ns}/-0.031^{ns}
IBT	r= 0.908***	r= -0.128^{ns}/-0.063^{ns}	r= -0.108^{ns}/-0.010^{ns}
ARW	r= 0.737***	r= 0.058^{ns}/0.072^{ns}	r= -0.010^{ns}/0.184^{ns}

4.4.1 Volume of the mandibular canal (VCM)

The volume of both mandibular canals increases with age. All age groups differ significantly from each other (Fig 3A). The values range from 2.5 ml in 12m animals to 13.4 ml in 21m animals. Left and right canal volumes do not differ significantly from each other. Between 17 and 21 months of age, the mean increase of the canal volume was 1.2 ml for the left and 1.1 ml for the right hemimandible. Highly significant differences within the same age groups could be observed (Fig 4). VCM correlates with age but not with body mass (Table 2).

4.4.2 Length of mandibular canal (LCM)

Mandibular canal length increased significantly with age (Fig 3B). Length ranges from 85.6 mm in 12m animals to 114.9 mm in 21m animals. The length of left and right canals do not differ significantly. LCM correlates with age but not with body weight (Table 2).

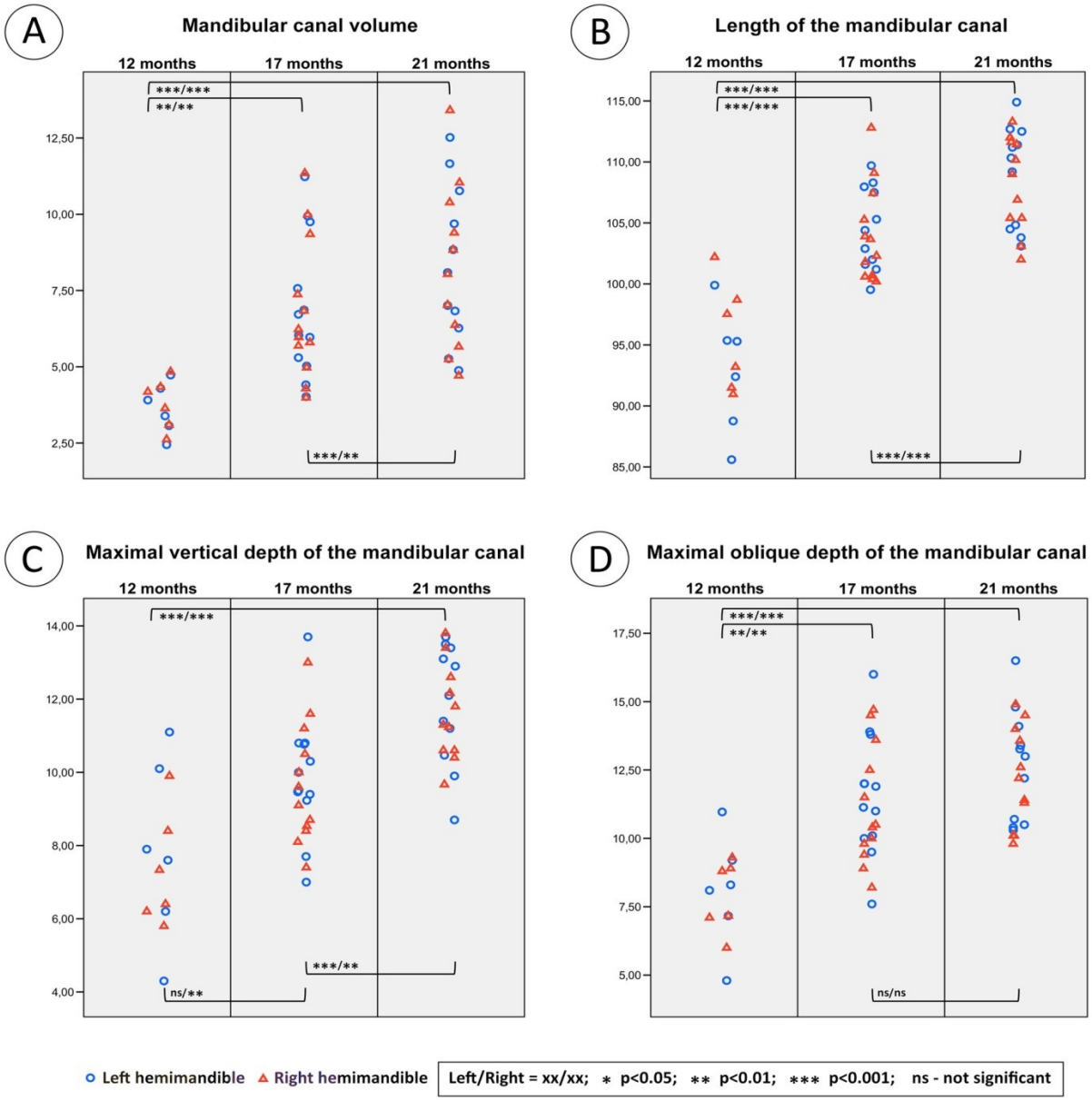

Figure 4.4.2-1: **Fig 3. Dot plots of the measured parameters VCM (A), LCM (B), MVD (C) and MOD (D).** The blue circles are for the left and the red squares for the right hemimandibles. VCM is given in millilitres, all others in millimetres.

4.4.3 Maximal vertical depth of mandibular canal (MVD)

The left canal depths of 12m and 17m animals do not differ significantly from each other. All other group comparisons were significant (Fig 3C). The values range from 4.3 mm at 12m to 13.8 mm at 21m. Left and right canal depths were similar. MVD correlates with age but not with body weight (Table 2).

4.4.4 Maximal oblique depth of mandibular canal (MOD)

The maximal oblique depth of the mandibular canal increases until the age of 17 months but then does not change significantly (Fig 3D). The lowest oblique depth was found at 12m at 4.8 mm, the highest at 16.5 mm in 21m animals. Left and right oblique canal depths do not differ significantly. MOD correlates with age but not with body weight (Table 2).

4.4.5 Width of mandibular canal (WCM)

The mean width of the mandibular canal does not change over time (Fig 5A). The only significant difference was between the left hemimandibles of 12m and 21m animals. The canal width ranged between 9.0 mm at 17m and 14.8 mm at 21m. The left and right canal widths do not differ significantly. WCM does not correlate with age or body weight (Table 2).

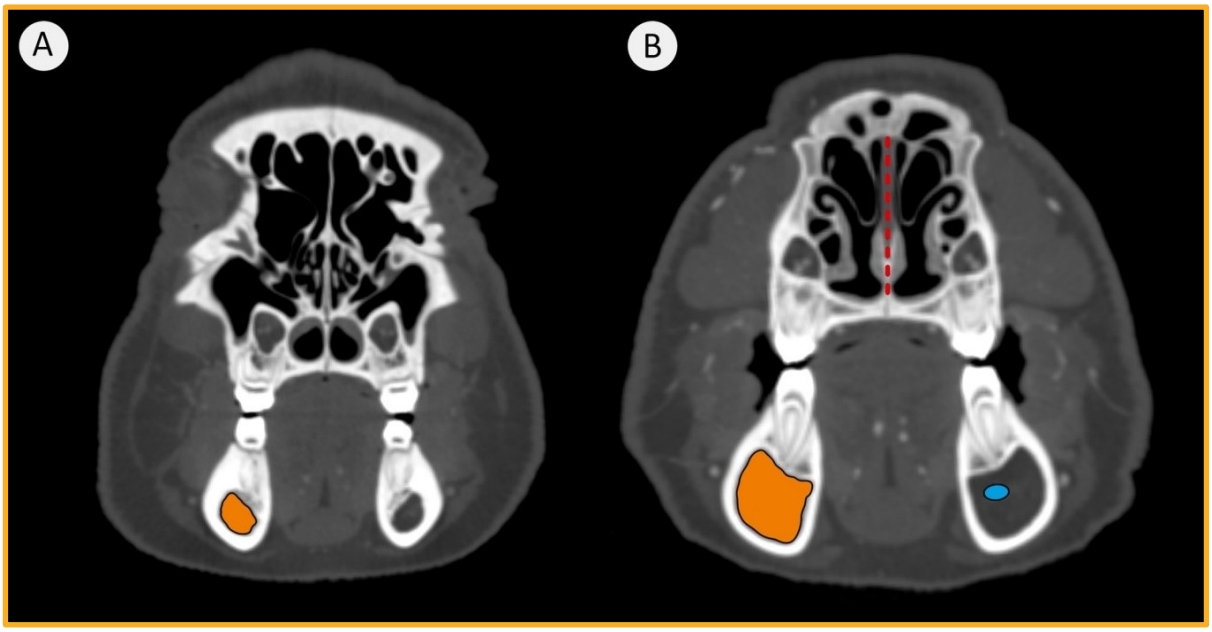

Figure 4.4.5-1: **Fig 4. Transverse plane view at the level of the first molar tooth (M1) of two different minipigs.** Where (A) is a minipig with a VCM of 4.88 mm (left) and 4.71 mm (right); (B) is a minipig with VCM of 12.52 mm (left) and 13.41 mm (right). The broken red line in (B) is the nasal septum, the orange areas are the lumina of the mandibular canals and the blue ellipse indicates the position of the inferior alveolar artery that lies beneath the inferior alveolar nerve.

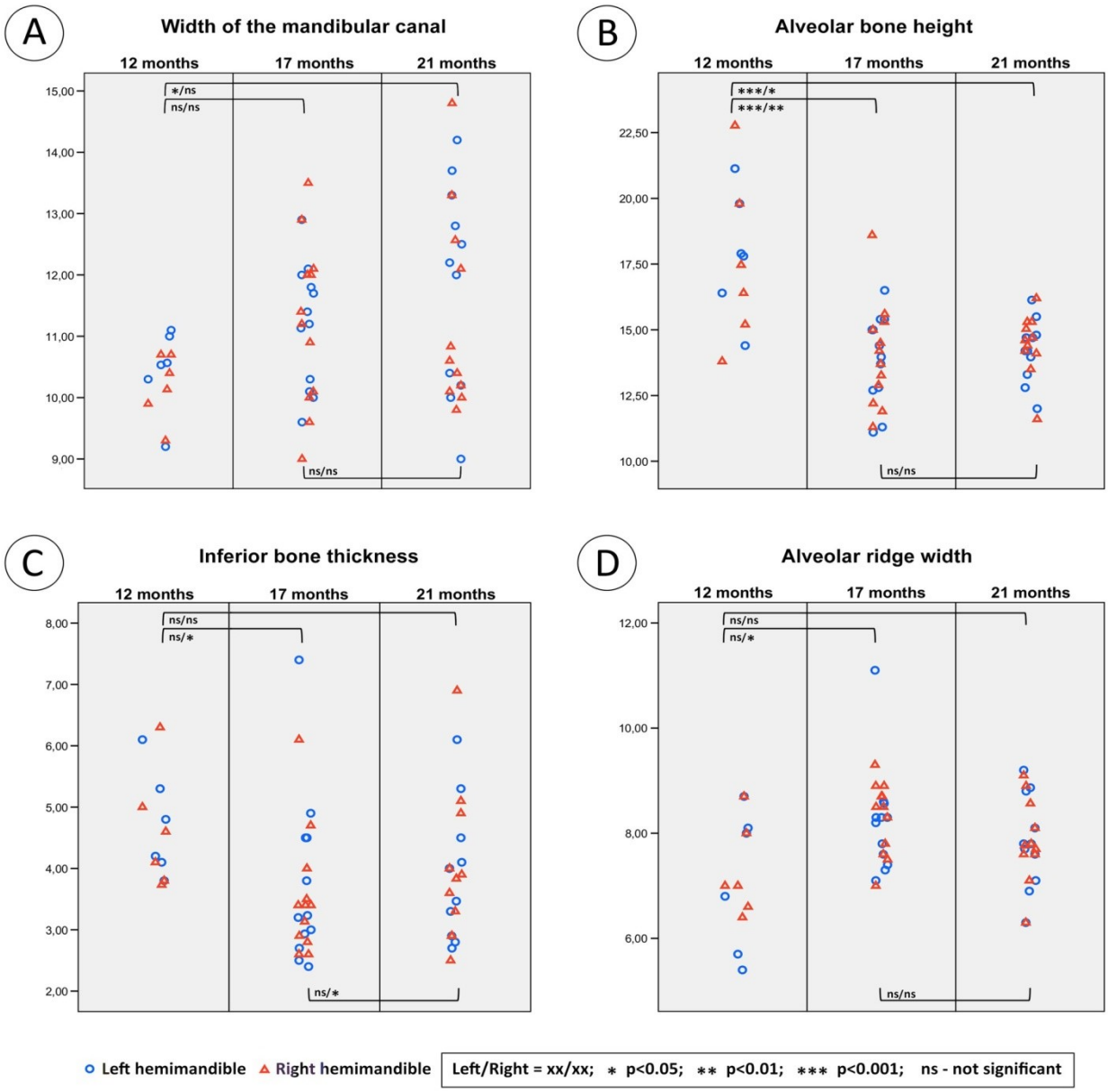

Figure 4.4.5-2: *Fig 5. Dot plots of the measured parameters WCM (A), ABH (B), IBT (C) and ARW (D).* The blue circles are for the left hemimandible and the red squares for the right. All parameters are given in millimetres.

4.4.6 Alveolar bone height (ABH)

Over the course of this study, ongoing anatomical changes in the vicinity of the tooth roots were observed. The spongy bone, normally located between and beneath the tooth roots, slowly resorbs. This was particularly obvious in the molar region (Fig 6A). After this gradual loss, the residual spongy bone could be seen as a lighter grey region (red oval) directly adjacent to the tooth necks. As a result, the inferior alveolar neurovascular bundle may come into close proximity with the tooth roots. ABH decreases between 12 and 17 months. After

17m, the height does not change significantly (Fig 5B). The distance ranges from 11.1 mm in 17m to 22.8 mm in 12m animals. There was no significant difference between left and right hemimandibles. ABH correlates negatively with age but does not correlate with body weight (Table 2).

4.4.7 Inferior bone thickness (IBT)

The IBT of the left hemimandible does not differ significantly between the age groups. However, the right hemimandible has significant differences between 12m and 17m and between 17m and 21m respectively (Fig 5C). Variability can be great e.g., in 17m animals the range was 2.4 mm to 7.4 mm. Left and right hemimandibles do not differ significantly. IBT does not correlate with age or body weight (Table 2).

4.4.8 Alveolar ridge width (ARW)

The alveolar ridge width does not change over time (Fig 5D). The only significant difference was seen when comparing the right hemimandibles of the 12m and 17m animals. The alveolar ridge width ranged between 5.4 mm in 12m and 11.1 mm in 17m animals. The left and right alveolar process widths do not differ significantly from each other. ARW does not correlate with age or body weight (Table 2).

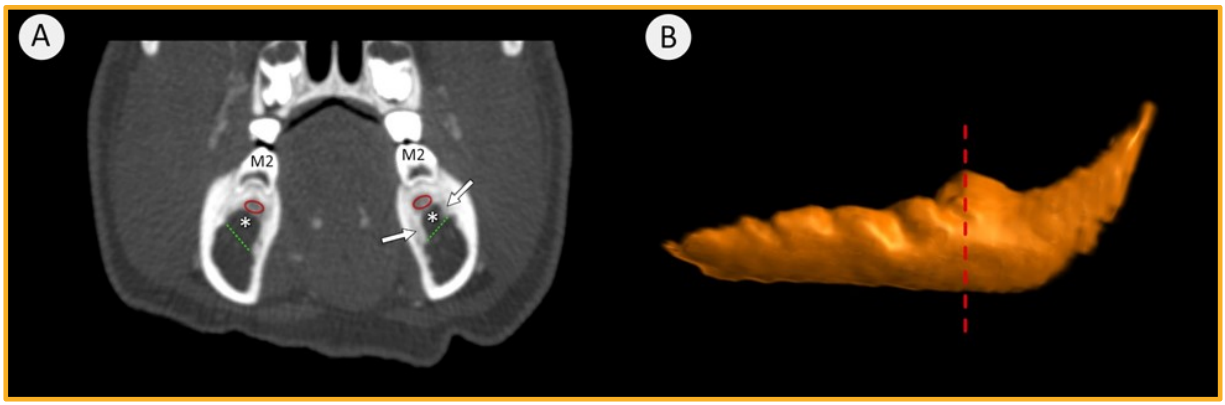

Figure 4.4.8-1: **Fig 6. 3D transverse section view and mandibular canal reconstruction.** Where (A) is a transverse image at M2. The loss of spongy bone within the space around the tooth roots white arrows is shown by white stars. The residual spongy bone is demonstrated by a red oval. The green dashed lined indicate the normal superior extent of the mandibular canal. Image (B) shows the segmented mandibular canal where the dashed red line indicates the level of the second molar where image (A) was taken.

4.4.9 Shapes of segmented mandibular canals

Segmentation composite (Fig 7A) shows a sickle-shaped mandibular canal, typical for animals of 12m of age. Segmentation composite (Fig 7B) is typical for animals of 17 and 21 months of

age. It shows an obvious increase of canal depth along the whole length of the canal. The posterior border of the canal rises sharply at the level of the mandibular foramen.

The mandibular canal volume (Fig 8) of the same canal in the same pig, measured at 17 and 21 months of age differs. The merged volumes clearly show that the increase in volume between 17 and 21 months of age was caused mainly by a vertical superior extension in the premolar and molar regions. The ascending posterior portion of the canal near the mandibular foramen does not change in size and shape.

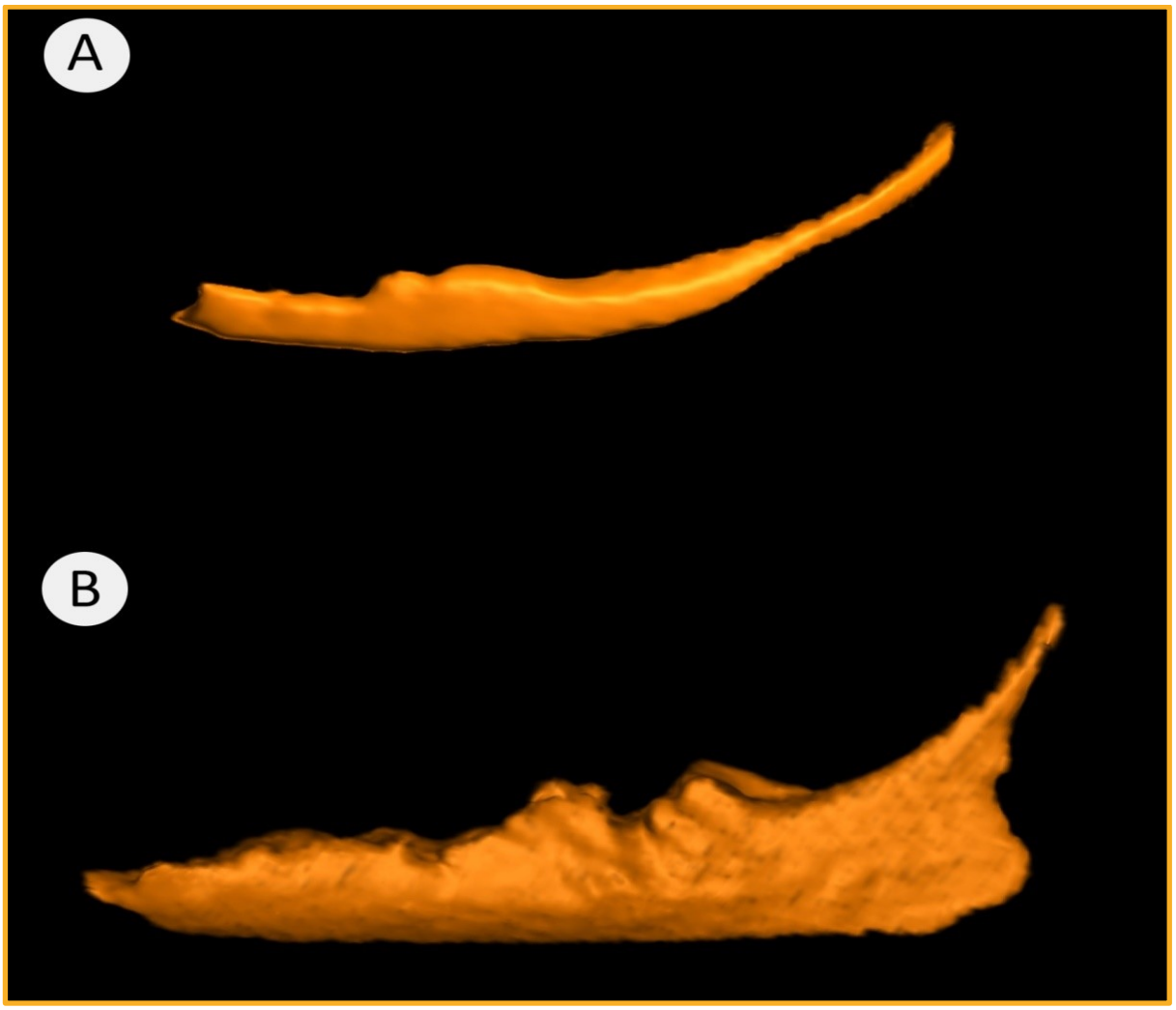

Figure 4.4.9-1: ***Fig 7. A visual comparison of mandibular canals from animals of two different age groups.*** Where (A) is of a 12m old minipig with a canal volume of 2.4 ml and (B) is of a 17m old animal with a canal volume of 9.9 ml. The images are scaled to size.

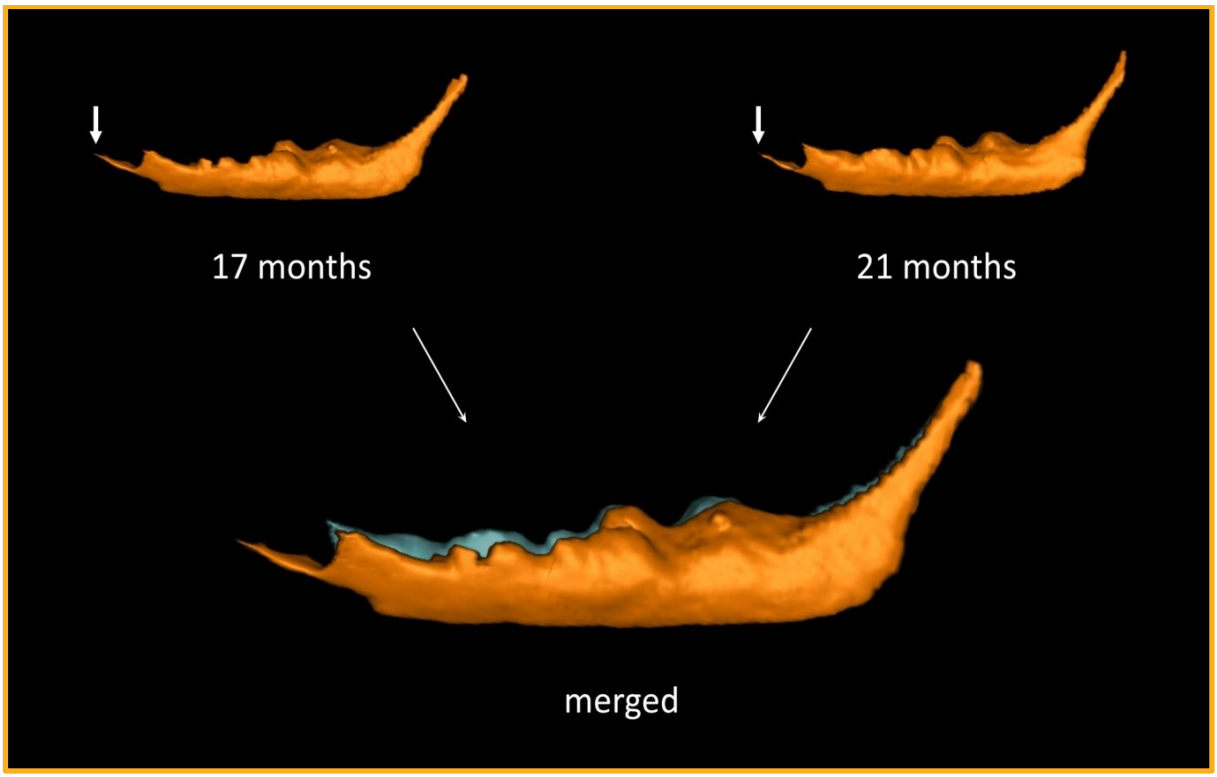

Figure 4.4.9-2: **Fig 8. Visualization of the changes in mandibular canal volume over time.** The white arrow shows the incisive canal, which is the anterior prolongation of the mandibular canal. The lower image shows the merged segmentations to enable a better visual comparison. The orange segmentation is at 17m and the bright blue at 21m.

4.4.10 Inferior alveolar neurovascular bundle

The CT segmentations show that inferior alveolar vessels lie in the superior aspect of the mandibular canals. They may have either an undulating or a straight course (Fig 9B and 9C).

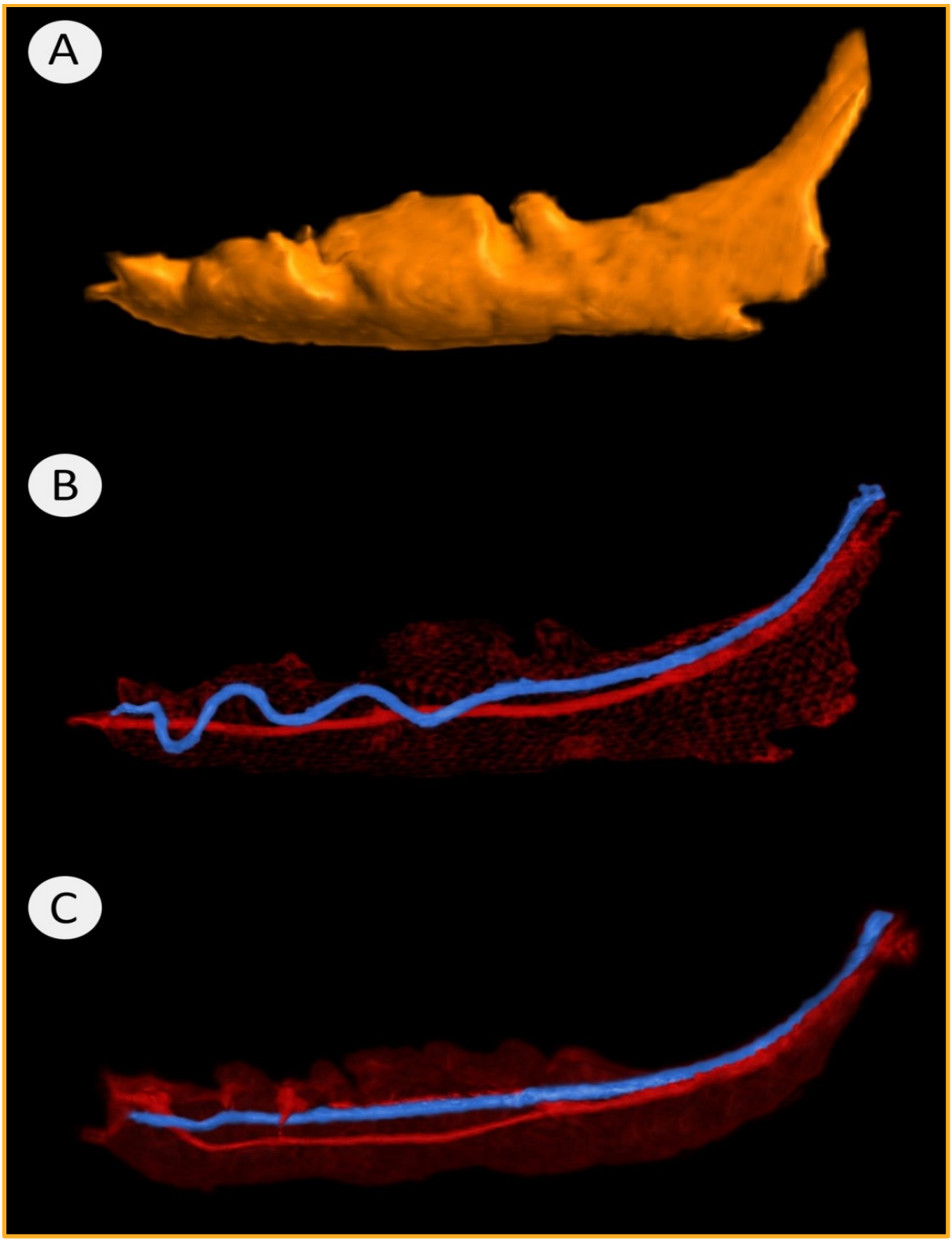

Figure 4.4.10-1*: Fig 9. Mandibular canals and inferior alveolar vessels.* Where (A) shows the typical mandibular canal outline (12.5 ml) of a 21-month-old animal. Image (B) shows the same canal

but with the inferior alveolar artery (red) having a straight route and the inferior alveolar vein (blue) having an undulating route. In Image C, the inferior alveolar vessels anteriorly have a straight course.

4.4.11 Dissection

Both the transverse serial interrupted sections and the longitudinal excavation of the hemimandibles revealed that the inferior alveolar nerve ran in close association with the inferior alveolar blood vessels within the mandibular canal. The inferior alveolar neurovascular bundle lay adjacent the superior border of the mandibular canal in close proximity to the tooth roots. Between the mandibular foramen and the second molar, the inferior neurovascular bundle had a pronounced sheath of connective tissue. Posteriorly the neurovascular bundle lay superiorly in the midline of the mandibular canal however as it ran anteriorly it became more and more lateral (buccal).

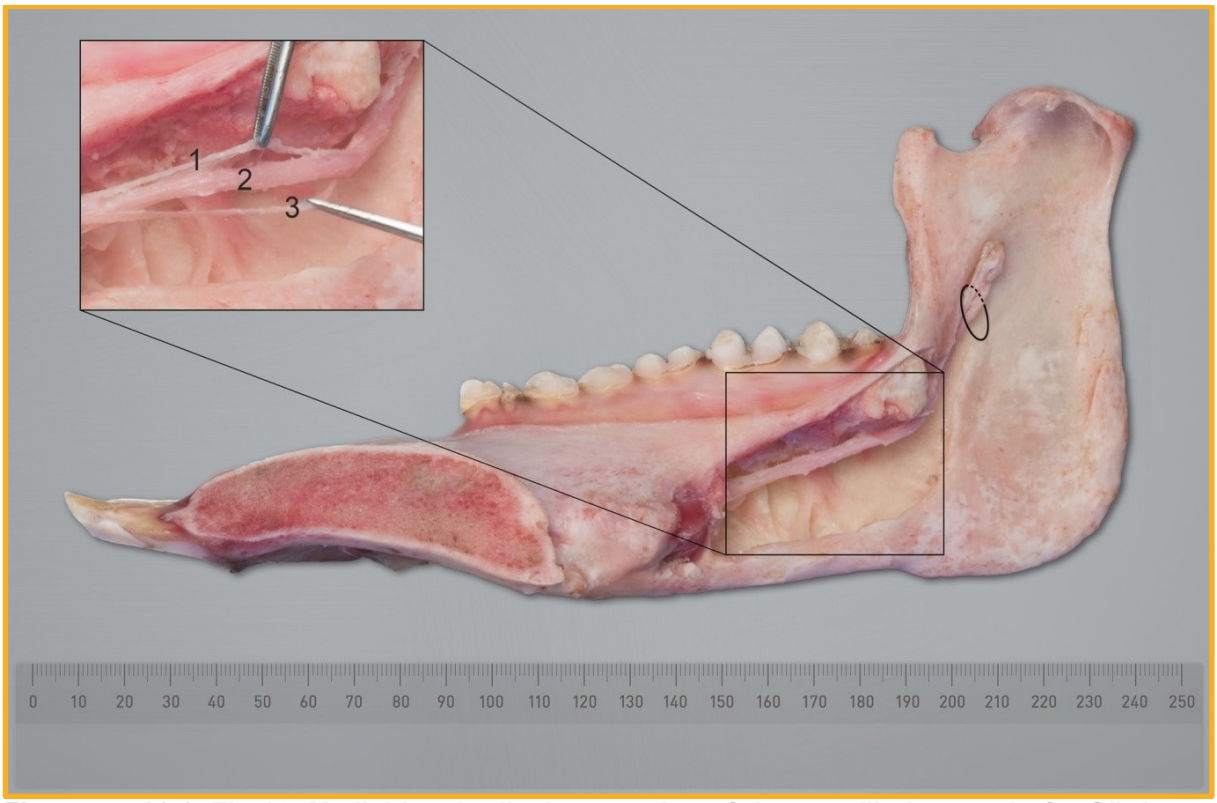

Figure 4.4.11-1: **Fig 10. Medial longitudinal excavation of the mandibular canal of a Göttingen minipigs' right hemimandible showing the inferior alveolar neurovascular bundle.** The inset shows the inferior alveolar nerve (2) and the inferior alveolar vein (1) and artery (3). The mandibular foramen is indicated by a black ellipse.

4.5 Discussion

This CT study of the mandibular canal of live Göttingen Minipigs aged between 12 months and 21 months found that the volume, length and depth of the canal increased over time. The width

remained constant. However, as one may expect the superior region of the canal showed clear dynamic changes over the experimental period. This was particularly evident with the slow increase in volume of the mandibular canal notably associated with a gradual loss of deeper spongy bone adjacent the distal tooth roots (Fig 5). This resulted in the inferior alveolar neurovascular bundle coming into close proximity with these tooth roots. All measures of the mandibular canal within the left hemimandible were statistically similar to those of the right hemimandible. Likewise, body mass did not have an influence on any of the eight parameters reported here.

To date, the only previous study of the dimensions of the minipigs' mandibular canal were reported by Koppe, et al in 1994 and 1997. Their study sampled 103 dried left hemimandibles of female MINI-LEWE miniature pigs. These were of 11 groups ranging from 3 days to 24 months. They found, that in contrast to the uniform diameter of the human mandibular canal that that of the minipig was enlarged in the molar and premolar regions [30, 31]. Because the MINI-LEWE pigs are considerably larger than Göttingen Minipigs their morphological dimension cannot be compared readily with each other [32].

The present study found that the volume of the mandibular canal increased between 12 and 21 months of age with a mean value of 6.7 ± 2.7 ml. There were large individual differences within each age group, for example within the 21 months old group the animal with the lowest canal volume of 4.7 ml was 14 kg heavier than the animal that had a significantly higher volume of 13.4 ml, a variation of 285% (Fig 4). These differences are not due to the length of the canal because they were identical.

As one would expect, in minipigs the length of the mandibular canal increased at a steady rate with age. The values range between 85.6 mm and 114.9 mm with a mean value of 103.8 ± 6.6 mm. Liu et al. (2009) in a study of 200 two dimensional panoramic radiographs of male and female human patients with an average age of 31.6 years reported a length of 62.46 ± 4.32 mm [33].

In this study we found that the minipigs had a mean vertical mandibular canal depth of 10.1 ± 2.2 mm with a range of 4.3 mm to 13.8 mm. Lindh et al. (1995) in a study of 71 radiographs of humans with a mean age of 76 years reported a value of 3.0 ± 0.7 mm [34]. The elderly age of the corpses in the study suggests that there may have been some mandibular atrophy present. Another study of adult Japanese cadavers reported the depth to be around 5 mm [35]. Dogs, which are also a potential animal model for dental implant testing, have a higher mean canal depth of 6.1 ± 2.1 mm than humans [36].

In the present study, all of the minipigs had an oval-shaped canal except for one individual that had a circular-shaped canal, where the mean vertical depth equals the oblique depth of the canal.

The width of the mandibular canal in the minipigs ranged from 9.0 to 14.8 mm and had a mean value of 11.2 ± 1.4 mm. However, the t-Tests show that the canal width in the minipigs does not change significantly with the age of the pigs. Humans have much narrower mandibular canal widths. Rajchel et al. (1985) reported a width of 2.0 to 2.4 mm proximal to the third molar in 45 Asian adults [37]. Ikeda et al. (1996) reported from a cadaver study a width of approximately 3.4 ± 0.5 mm midway along the length of the mandibular canal and 4.1 mm near the mandibular foramen [38]. Similar values are provided by Miller et al. (1990) in their study on 22 human mandibles. Here the mean horizontal diameter was 2.2 ± 0.5 mm with a range from 1.1 to 3.6 mm [39].

The alveolar bone height is important in the assessment of the available space for dental implant placement [40]. In the 12-month-old minipigs, it had a mean value of 17.7 ± 2.8 mm, whilst the 17 and 21 months old animals had a mean value of 13.9 ± 1.8 mm and 14.3 ± 1.2 mm respectively. Levine et al. (2007), in a morphological study of 50 adult human patients with a mean age of 42.1 years, reported a mean height of 17.4 ± 3.0 mm and a range from 8.7–23.0 mm. However, some of their subjects were partially edentulous [41]. This could result in an underestimation of the alveolar bone height due to the commencement of, or presence of, alveolar ridge atrophy [42, 43]. Liu et al. (2009) reported similar mean values with the largest height of 17.8 ± 2.2 mm [33]. Likewise, another study reported a height of 17.4 ± 3.1 mm using CT imaging on 79 Japanese patients [44]. These comparative studies show that older minipigs have a shorter alveolar bone height compared to humans. This should be taken into account when positioning a dental implant without risking the penetration of the mandibular canal [45–47], especially in the older minipigs. Dogs have an even lower mean alveolar bone height of 8.5 ± 2.6 mm [36].

In the present study of Göttingen Minipigs the mean inferior bone thickness was 4.0 ± 1.2 mm and had a range between 2.4 to 7.4 mm. Watanabe et al. reported that humans have a mean bone thickness of 9.4 ± 2.0 mm in the molar region and 15.0 ± 1.5 mm at the level of the mental foramen [44]. Another study reported a mean inferior bone thickness of 12.6 ± 1.7 mm in humans [48].

The mean alveolar ridge width of the minipigs was 7.9 ± 1.0 mm ranging between 5.4 mm in 12m and 11.1 mm in 17m animals. A classification of the alveolar ridge width published by Tolstunov (2014) states that the physiologic width in humans should be above 10 mm. Alveolar ridge widths between 6 and 8 mm are considered to indicate a mild alveolar ridge deficiency

represented by a loss of buccal cortical bone and therefore an indication for alveolar distraction osteogenesis surgery [49]. When applying Tolstunov's classification to Göttingen Minipig, their narrower alveolar ridge compared with that of healthy humans, suggests that they may be unsuitable for some experimental procedures such as dental implantation.

The morphology of the mandibular canal presented in this study aimed to visually evaluate its position, course and shape (Fig 7). In humans, the high variability in the course and shape of the mandibular canal has been described elegantly by Liu, et al. (2009), who have classified these into four distinct patterns [33]. Young Göttingen Minipigs (12m) have a rather simple, slightly curved mandibular canal (Fig 7) that closely resembles that described by Liu et al (2009) as being the most common found in humans. However, in older animals the canal becomes larger and much more complex than any seen in humans (Fig 9A). Consequently, at this stage, a similar classification is not possible for Göttingen Minipigs but is a potential subject of further studies. In the older groups over the period 17m to 21m the horizontal part of the mandibular canal i.e., within the mandibular body, enlarges vertically and has a highly convoluted superior surface. These dynamic changes are clearly seen in the merged image (Fig 8).

Over most of its course, the inferior alveolar neurovascular bundle lies superiorly in the canal adjacent the distal part of the tooth roots. When one images the inferior alveolar vein, two basic patterns occur as it traverse the canal. One being a straight traverse, the other being a variable undulating route where it rises and falls as it traverses the length of the canal (Fig 9). In contrast, the inferior alveolar artery lies below the inferior alveolar vein and has a simple straight route. Dissection of the hemimandibles confirmed that the inferior alveolar nerve runs in close proximity with the blood vessels (Fig 10). Because the inferior alveolar vein lies above the inferior alveolar nerve, it is quite probable that in surgical procedures such as the implantation of dental prostheses, that damage to the vein and the nerve can occur steadily. This conclusion is supported by Pogrel et al. who described the position of the vein in humans to be in a 12 o'clock position superior to the nerve [18].

4.6 Conclusions

This study showed that the volume of the mandibular canal in Göttingen Minipigs increases with age. This increase appears to be caused primarily by loss of deep spongy bone of the canal roof notably in the posterior premolar and in the molar regions. This results in the closer approximation of inferior alveolar neurovascular bundle to the distal tooth roots. In practice, this reduces the available space for dental implantation and could negatively affect implant stability or jeopardize the integrity of the inferior alveolar neurovascular bundle. Contrary to the

increase in volume as well as its' length and depth, the width of the mandibular canal does not change significantly over time. Despite the uniform canal width, other parameters clearly indicate progressive ongoing anatomical changes. This strongly suggests, on ethical grounds, not using minipigs younger than 21 months in experimental implant dentistry. However, paradoxically the measurements of the 12 months old pig indicate a closer alignment of the anatomy of their mandibular canal to that of humans. As such, these younger animals may be better models for implant studies.

This study also showed that the body mass of the minipigs does not have an influence on the dimensions of the mandibular canal. Consequently, choosing animals for implant surgery based on physical appearance and body dimensions is inappropriate. This, linked in with the high variability in mandibular canal dimensions in similar age cohorts, indicates that the use of CT imaging and other diagnostic imaging techniques is essential for the best selection of animals for experimental surgery as well as for probable higher success rates and thus the use of fewer animals in the long run.

4.7 Supporting information

S1 Dataset. Data of the morphometric studies of the mandibula in Göttingen Minipigs.

4.8 Acknowledgments

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4.9 Author Contributions

Conceptualization: Giuliano M. Corte, Johanna Plendl, Hana Hünigen, Stefan M. Niehues.

Data curation: Giuliano M. Corte.

Formal analysis: Giuliano M. Corte.

Funding acquisition: Johanna Plendl.

Investigation: Giuliano M. Corte, Stefan M. Niehues.

Methodology: Giuliano M. Corte, Ole Gemeinhardt, Stefan M. Niehues.

Project administration: Johanna Plendl.

Resources: Johanna Plendl, Stefan M. Niehues.

Software: Giuliano M. Corte, Stefan M. Niehues.

Supervision: Johanna Plendl, Hana Hünigen, Stefan M. Niehues.

Validation: Hana Hünigen.

Visualization: Giuliano M. Corte.

Writing – original draft: Giuliano M. Corte, Kenneth C. Richardson.

Writing – review & editing: Giuliano M. Corte, Johanna Plendl, Hana Hünigen, Kenneth C. Richardson, Ole Gemeinhardt, Stefan M. Niehues.

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5 Publication II

RESEARCH ARTICLE

Cephalometric studies of the mandible, its masticatory muscles and vasculature of growing Göttingen Minipigs – A comparative anatomical study to refine experimental mandibular surgery.

OPENACCESS

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Cephalometric studies of the mandible, its masticatory muscles and vasculature of growing Göttingen Minipigs – A comparative anatomical study to refine experimental mandibular surgery

Giuliano Mario Corte[1]*, Hana Hünigen[1], Kenneth C. Richardson[2], Stefan M. Niehues[3], Johanna Plendl[1]

1 Institute of Veterinary Anatomy, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany, **2** College of Veterinary Medicine, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, **3** Department of Radiology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
* Giuliano.Corte@fu-berlin.de

5.1 Abstract

Over many decades, the Göttingen Minipig has been used as a large animal model in experimental surgical research of the mandible. Recently several authors have raised concerns over the use of the Göttingen Minipig in this research area, observing problems with postoperative wound healing and loosening implants. To reduce these complications during and after surgery and to improve animal welfare in mandibular surgery research, the present study elucidated how comparable the mandible of minipigs is to that of humans and whether these complications could be caused by specific anatomical characteristics of the minipigs' mandible, its masticatory muscles and associated vasculature. Twenty-two mandibular cephalometric parameters were measured on CT scans of Göttingen Minipigs aged between 12 and 21 months. Ultimately, we compared this data with human data reported in the scientific literature. In addition, image segmentation was used to determine the masticatory muscle morphology and the configuration of the mandibular blood vessels. Compared to data of humans, significant differences in the mandibular anatomy of minipigs were found. Of the 22 parameters measured only four were found to be highly comparable, whilst the others were not. The 3D examinations of the minipigs vasculature showed a very prominent deep facial vein directly medial to the mandibular ramus and potentially interfering with the sectional plane of mandibular distraction osteogenesis. Damage to this vessel could result in inaccessible bleeding. The findings of this study suggest that Göttingen Minipigs are not ideal animal models for experimental mandibular surgery research. Nevertheless if these minipigs are used the authors recommend that radiographic techniques, such as computed tomography, be used in the specific planning procedures for the mandibular surgical experiments. In addition, it is

advisable to choose suitable age groups and customize implants based on the mandibular dimensions reported in this study.

5.2 Introduction

In experimental surgery, the use of the most common experimental animals worldwide i.e. mice, rats and hamsters, is limited due to their small body size. Consequently, large animal models that have closer comparability to human dimensions are needed [1]. Over recent decades, the use of primates and dogs in research, has met with increasing societal resistance, mostly on ethical grounds. However, the pig has emerged as an acceptable alternative species because it is regarded by society as a production animal [2]. Furthermore, many aspects of a pig's physiology are similar to that of humans, making them especially suitable as large animal models for biomedical research [3–5]. Domestic pig breeds have a high adult body weight and large size that is frequently coupled with aggressive behaviour that have proven to be challenging in their husbandry [6, 7]. In 1949, the first miniature pigs namely, Minnesota minipigs, were bred to overcome these problems [8]. Subsequently since its development in the 1960s, the Göttingen Minipig has become the most widely used pig breed and one of the smallest available for research [9]. Its small size, low average adult body weight of around 35 kg and rapid growth allows easier handling and more economic housing than conventional domestic pig breeds. Furthermore, its early sexual maturity makes it more convenient for long-term studies than normal-sized pigs or other large animal models [10–13]. Because of that, the Göttingen Minipig has been used frequently in mandibular surgical research over recent decades [14, 15].

The mandible consists of two hemimandibles joined anteriorly by a symphysis that in the pig is usually ossified by 12 months of age [16]. Each hemimandible consists of a horizontal tooth-bearing mandibular body and a perpendicular mandibular ramus. The mandibular body has an anterior incisive part that contains three incisor teeth and a single canine tooth. Further posteriorly the molar part of the mandibular body houses three to four premolar and three molar teeth. A short diastema separates the incisive and molar parts of the mandible. Within the substance of the mandibular body runs the mandibular canal. This originates posteriorly at the mandibular foramen and runs anteriorly within the mandibular body to terminate immediately rostral to the mandibular molar part. The canal conveys the inferior alveolar neurovascular bundle that consists of the inferior alveolar artery, vein and nerve [17–19].

Posteriorly the mandibular ramus rises superiorly from the mandibular body. Its lateral aspect is slightly recessed forming the masseteric fossa housing a large masseteric muscle. When both left and right masseter muscles contract together, they elevate the mandible and when they contract separately they move the mandible laterally [20, 21]. The medial aspect of the

ramus has a shallow recess where the medial and lateral pterygoid muscles both insert. The larger medial pterygoid muscle acts synergistically with the masseter muscle to elevate the mandible, whilst the lateral pterygoid muscle is occupied mainly with lateral movements of the mandible [21].

The posteroinferior transition of the mandibular body into the mandibular ramus forms the gonial angle. From here, the posterior border of the mandibular ramus runs nearly vertically to its free superior aspect. Here a posteriorly located condylar process connects anteriorly via a sigmoid notch, also called mandibular notch, to a much smaller coronoid process. The coronoid process is the insertion point for the temporal muscle that is partly responsible for raising the mandible. The condylar process articulates with the temporal bone, forming the temporomandibular joint [20, 21].

In many mandibular research studies, the principle of distraction osteogenesis (DO) is used in skeletal reconstruction to exploit the body's innate capacity for bone formation in response to tensile forces. Here a distractor is fixed to the aligned bone segments to keep them in the desired plane and to separate them gradually over time at a controlled rate [22, 23]. This performed in three stages; a latency period of several days after osteotomy which allows haematoma formation and local bridging of the gap by soft callus formation, then a slow gradual distraction to stimulate ossification during elongation, followed by a period of stable fixation allowing hard callus maturation and bone remodeling [24]. Distraction osteogenesis is a lengthy and risky procedure that can result in post-operative non-union, infection, bleeding and device failure. Any of these complications ultimately prolong the period of treatment [25].

Mandibular distraction osteogenesis (MDO) and alveolar distraction osteogenesis are among many surgical techniques that have been studied using Göttingen Minipigs [23, 26– 32]. Even more important has been the search for methodologies to enhance the process of distraction by accelerating the rates of activation and bone healing or to promote the Osseointegration of bony implants utilizing novel biomaterials, implant coatings, growth factors such as morphogenetic proteins, angiogenic factors and autologous mesenchymal stem cells [22, 23, 25, 33, 34].

In experimental MDO in minipigs, the osteotomy is usually performed from the superior junction of the mandibular body and ramus and extends to the inferior border of the mandible in close proximity to the mandibular angle [23]. Alveolar distraction osteogenesis is used often for the reconstruction of the alveolar bone and surrounding soft tissues to enable dental implant placement [35].

Recently several authors have raised concerns over the use of the Göttingen Minipig in dental and orofacial surgery research, observing problems with post-operative wound healing as well as loosening of implanted plates and screws [36–38]. Some authors report that the success rate of implant studies is below 60 percent [39, 40].

These situations are problematic and it is important to refine procedures to reduce these complications during and after surgery to improve animal welfare in orofacial surgery research by minimizing pain, distress and discomfort for the animals. This is in accordance to the principles of the 3Rs by Russell and Burch [41]. To fulfill these goals, it is necessary to answer the following questions [42–44]. The first being, how comparable is the mandible of minipigs to that of humans in general, and the second being, could these post-operative complications be caused by specific anatomical characteristics of the minipigs' mandible, its masticatory muscles and associated vasculature? To address these questions we measured 22 mandibular cephalometric parameters that are measured routinely in most presurgical planning of human mandibular surgery and reconstruction. We then measured these on computed tomographic (CT) scans of Göttingen Minipigs aged between 12 and 21 months [45–48]. Ultimately, we compared our data with human data reported in the scientific literature. The parameters were chosen to evaluate the overall changes of the mandibular dimensions of subadult and adult Göttingen Minipigs. Measurements between the same landmarks on the left and right hemimandibles evaluated laterolateral growth, whilst distances between anterior and posterior landmarks served to evaluate longitudinal growth. Measurements between vertically located landmarks assessed the vertical growth of the mandibular ramus, whilst vertical parameters between the mental foramen and the alveolar ridge or the inferior border determined the posterior mental foramen's vertical position. Manual segmentation of the coronoid and mandibular condyle was conducted to evaluate changes in their morphology and dimensions. In addition, image segmentation was used to determine the masticatory muscle morphology and the configuration of the mandibular blood vessels.

5.3 Materials and methods

A computed tomographic study of Göttingen Minipigs approved by the Regional Office for Health and Social Affairs Berlin (permit IC113-G 0281/12) was conducted in 2007 and 2008 at the research facility for experimental surgery of the medical faculty (certified by ISO 9001) at Charité – Universitätsmedizin Berlin, Campus Virchow-Klinikum [49]. These CT scans were reused for the cephalometric measurements of the present study. Whilst this precluded an optimal study design, it promoted the 3Rs by eliminating additional animal experiments.

5.3.1 Animal groups and husbandry

The animals in this study consisted of 18 healthy female Göttingen Minipigs. Six animals were examined at the age of 12 months (12m; n= 6; 357 ± 31d) and another 12 animals were examined twice, once at 17 months (17m; n= 12; 511 ± 24d) and again at 21 months (21m; n=

11; 620 ± 37d). Their body mass ranged from 23 to 44 kg. Due to the loss of some of its data, one animal in the 21-month group was excluded from the study.

The minipigs were obtained from Ellegaard, Göttingen Minipigs (Dalmose, Denmark). To lessen the effects of humans as stressors, the animals had been habituated to routine handling and basic techniques such as blood sampling.

At the research facility in Berlin, the animals were housed according to the Guidelines of the European Societies of Laboratory Animal Science. The pigs were grouped into pens of six animals, with a relative humidity of 55 ± 10%, a light/dark rhythm of 12/12 hours and temperatures between 15 and 24˚C. The animals were fed a specific diet formulated for minipigs to prevent obesity (Ssnif Spezialdiäten GmbH, Soest, Germany) [50]. Their body mass was measured weekly using a decimal scale.

5.3.2 Adult human mandible

The image of a human mandible shown in the results, originated from a free anonymous CT sample provided by the software company (Vital Images Inc., Minnetonka, MN, USA). The gender and exact age of the sample is unknown, however the overall mandibular dimensions indicate that it is from an adult person.

5.3.3 Computed Tomography

Anaesthesia and drug administration.

Prior to tomography, animals were fasted for 24 hours with water ad libitum. Premedication consisted of an intramuscular injection of 0.5 mg atropine (Atropinum sulfuricum, 1 mg/ml, Eifelfango, Bad Neuenahr-Ahrweiler, Germany). For the induction of anaesthesia, an intramuscular injection of ketamine (27 mg/kg, Ursotamin, 100 mg/ml, Serumwerk Bernburg, Germany), xylazine (3.5 mg/kg, Rompun TS, 20 mg/ ml, Bayer Vital GmbH, Leverkusen, Germany) and 3 ml azaperone (Stresnil, 40 mg/ml, Janssen Animal Health, Neuss, Germany) was administered. Throughout the entire procedure, an isotonic electrolyte solution was infused intravenously (Ionosteril, Fresenius, Bad Homburg v. d. H., Germany) [49]. For separate studies on the vascular distribution of the whole body [49] and further histologic examination, all animals were euthanised when in deep anaesthesia by a 15 ml intravenous injection of T61 (Intervet Deutschland GmbH, Unterschleißheim, Germany).

Equipment and Software.

The data acquisition was performed using a 64-slice scanner (Lightspeed 64, GE Medical Systems, Milwaukee, USA). For contrast enhancement, an automated intravenous injection of

80 ml nonionic iodinated contrast medium (XenetiX 350, Guerbet GmbH, Sulzbach, Germany 350 mg iodine /ml) was used in every pig. Scanning parameters were standardised (voltage of 120 kV, an amperage of 500 mA with automatic mA-optimization at a noise index of 15, mean 490 mA; collimated slice thickness of 64×0.625 mm, total detector width of 55 mm, rotation speed of 0.4 sec and table feed per rotation of 55 mm) [52]. The positioning and the following computed tomographic examination required only a few minutes per animal. The 12m minipigs were imaged twice over 27 days, and the 17m and 21m minipigs were imaged five times over 111 days. Then the data was transferred to an independent workstation and the software Vitrea Advanced 6.6 (Vital Images Inc., Minnetonka, MN, USA) was used for measurements, segmentation and 3D rendering. Without overlap of images, the volumetric assessment was reconstructed with a slice thickness of 1.25 mm.

5.3.4 Anatomical landmarks

Table 5.3.4-1: ***Table 1. Cephalometric landmarks and their definitions.*** List of the anatomical landmarks that were used in this study and their definition, listed in anterior to posterior order.

Landmark	Definition
Infradentale (Id)	The apex of the septum between the mandibular central incisors [55].
Menton (Me)	Lowest midsagittal point of the intermandibular symphysis [55].
Diastema (Dia)	Prominent toothless gap of each hemimandible, located between the canine and the premolar teeth.
Midpoint of the diastema (mDia)	Midtransversal point of the diastema.
Mental foramen (Mf)	Posterior prominent mental foramen.
Alveolar crest (Ac)	Point on the buccal alveolar crest at the level of the posterior mental foramen (Mf).
Inferior border (Ib)	Most inferior point of the mandibular body at the level of the posterior mental foramen (Mf).
Dental ridge length (Ld)	Length of the premolar and molar dental arch.
Coronion (Cor)	Most superior point of the coronoid process.
Condylion (Con)	Most superior point of the mandibular condyle.
Lowest point of the sigmoid notch (Sn)	Most inferior point of the sigmoid notch, located between the coronoid and mandibular process.
Gonion (Go)	Most posterior, inferior and lateral point on the external angle of the mandible [56].

The definitions of the cephalometric landmarks used in this study are presented in Table 1. These landmarks are derived primarily from anthropometric landmarks that have been defined and modified by different authors over many decades [53, 54].

5.3.5 Parameters measured

Table 5.3.5-1: *Table 2: List of the cephalometric parameters, their abbreviations and definitions.* Parameters are described by distances between two distinct anatomical landmarks, which are defined in Table 1

Abbreviation	Parameters	Definition	Figure
MRH	Mandibular ramus height	Con—Go	1
oMRH	Oblique mandibular ramus height	Cor—Go	1
iMBL	Inferior mandibular body length	Go—Me	2
MBL	Mandibular body length	Go—Id	2
DL	Diastemal length	Dia	1
DAL	Premolar and molar dental arch length	Ld	1
IB	Interdiastemal breadth	mDia—mDia	3
LIB	Lingual intercrestal breadth	Ac—Ac	3
MIB	Mental foramen to inferior mandibular border height	Mf—Ib	1
MAC	Mental foramen to alveolar crest height	Mf—Ac	1
MGO	Mental foramen to gonion length	Mf—Go	1
IFB	Interforaminal breadth	Mf—Mf	3
GA	Gonial angle	Ga	1
MRL	Mandibular ramus length	aCol—pCol	1
SRL	Superior ramus length	Cor—Con	1
CPV	Coronoid process volume	Cpv	1
MCV	Mandibular condyle volume	Mcv	1
AMH	Anterior mentum height	Me—Id	2
ICOB	Intercoronoidal breadth	Cor—Cor	3
SNB	Breadth between sigmoid notches	Sn—Sn	3
ICB	Intercondylar breadth	Con—Con	3
IGB	Intergonial breadth	Go—Go	2

Except for the coronoid process volume (CPV) and the mandibular condyle volume (MCV), all parameters measured are distances between two defined landmarks (Table 1). For the segmentation and calculation of CPV and MVC, as well as for the segmentation of the mandibular condyles, the masticatory muscles and the whole mandible, the “sculpt” function of Vitrea Advanced was used. To evaluate the different morphologies of mandibles of humans and minipigs, two segmentations were scaled to the same size and superimposed upon each other. To ensure high reproducibility and for the correct identification of landmarks, multiplanar (sagittal, coronal, axial) views that were automatically reconstructed from the original axial slices, were used. In addition, bone reconstruction kernels were applied (Bone plus, GE Medical Systems, Milwaukee, USA) [17]. Table 2 lists all measured parameters, their abbreviations and definitions. All parameters were measured on both left and right hemimandibles. All parameters are given in millimeters (mm) except for CPV, MCV and GA

that are given in cubic millimeters (mm^3), millilitres (ml) and degrees. In Figs 1–3, a segmented mandible of a 17 months-old Göttingen Minipigs is pictured with all landmarks and measured parameters.

The diastemal length (DL) is the length of the toothless gap from the distal aspect of the canine tooth to the mesial aspect of the premolar tooth (Fig 1).

The premolar and molar dental arch length (DAL) was measured from the mesial aspect of the premolar to the posterior surface of the last molar tooth (Fig 1).

The interdiastemal breadth (IB) is the distance between both midpoints (mDia) of the diastemal length (Fig 3).

The lingual intercrestal breadth (LIB) is the distance connecting the lingual alveolar crests (Ac) of the canine teeth (Fig 3).

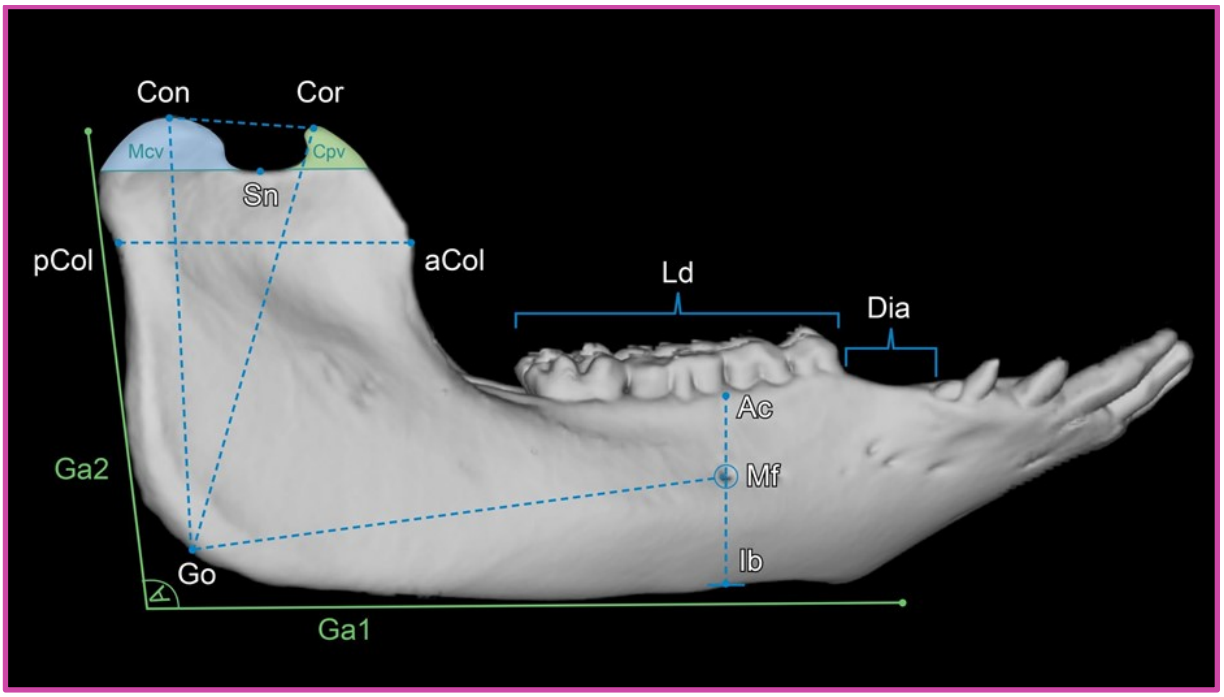

Figure 5.3.5-1: **Fig 1. Lateral view of a 3D rendered mandible of a 17 months-old Göttingen Minipig with landmarks and measured parameters.** Where: Con = condylion, Cor = coronion, Sn = lowest point of the sigmoid notch, pCol = posterior point of the mandibular collum, aCol = anterior point of the mandibular collum, Ga1 = horizontal tangent alongside the inferior border of the mandibular body, Ga2 = near vertical tangent alongside the posterior border of the mandibular ramus, Ac = point on the buccal alveolar crest at the vertical level of the posterior mental foramen, Mf = posterior prominent mental foramen, Ib = most inferior point of the mandibular body at the vertical level of the posterior mental foramen, Go = gonion. The parameters measured were: Con–Go = mandibular ramus height (MRH), Cor–Go = oblique mandibular ramus height (oMRH), Dia = diastemal length (DL), Ld = premolar and molar dental arch length (DAL), Mf–Ib = mental foramen to inferior border (MIB), Mf–Ac = mental

foramen to alveolar crest (MAC), Mf–Go = mental foramen to gonion (MGO), Ga1-Ga2 = gonial angle (GA), aCol–pCol = mandibular ramus length (MRL), Cor–Con = superior ramus length (SRL), Cpv = coronoid process volume (CPV), Mcv = mandibular condyle volume (MCV).

The parameters MIB, MAC and MGO describe the position of the mental foramen (Mf).

The gonial angle (GA) is the angle measured between two intersecting tangents. Tangent 1 runs horizontally alongside the inferior border of the mandibular body (Ga1), and tangent 2 runs vertically alongside the posterior border of the mandibular ramus (Ga2) (Fig 1).

The lines for calculating the coronoid process volume (CPV) and the mandibular condyle volume (MCV) were drawn manually in the coronal plane, starting from the coronion and the condylion to a horizontal plane through the inferior point of the sigmoid notch (Sn) (Fig 1).

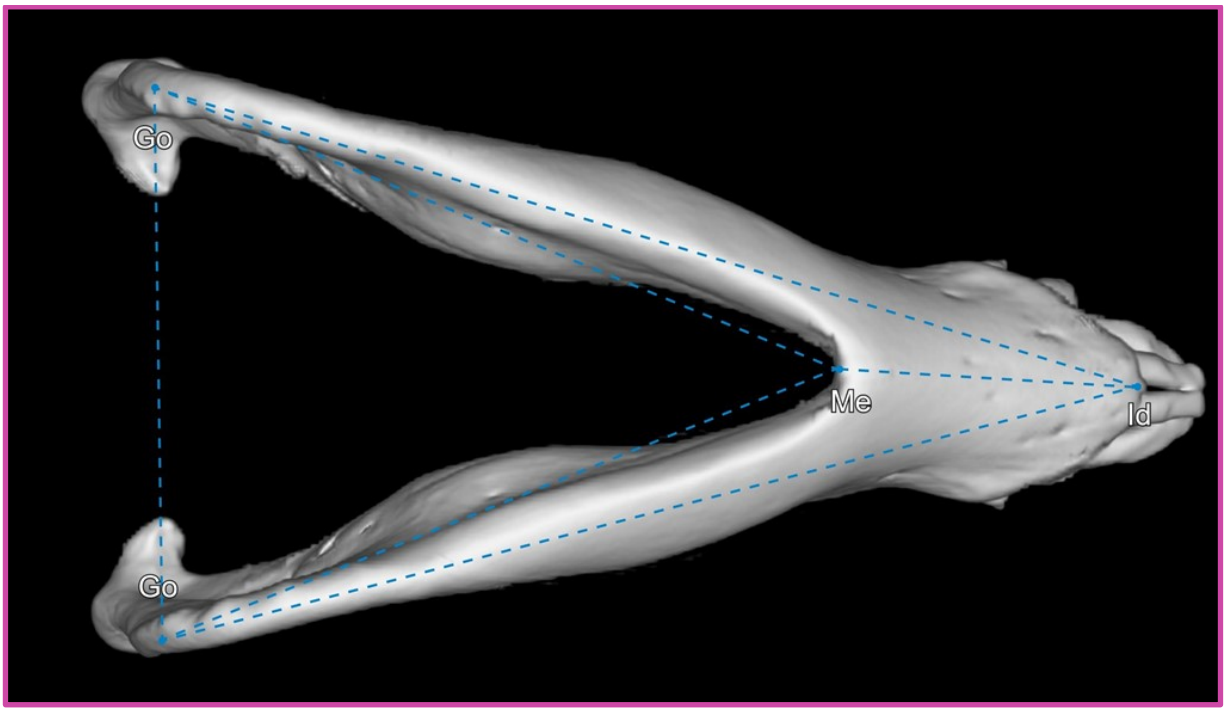

Figure 5.3.5-2: **Fig 2. Inferior view of a 3D rendered mandible of a 17 months-old Göttingen Minipig with landmarks and measured parameters.** Where: Go = gonion, Me = menton, Id = infradentale. The parameters measured were: Go–Go = intergonial breadth (IGB), Go–Me = inferior mandibular body length (iMBL), Go–Id = mandibular body length (MBL), Me–Id = anterior mentum height (AMH).

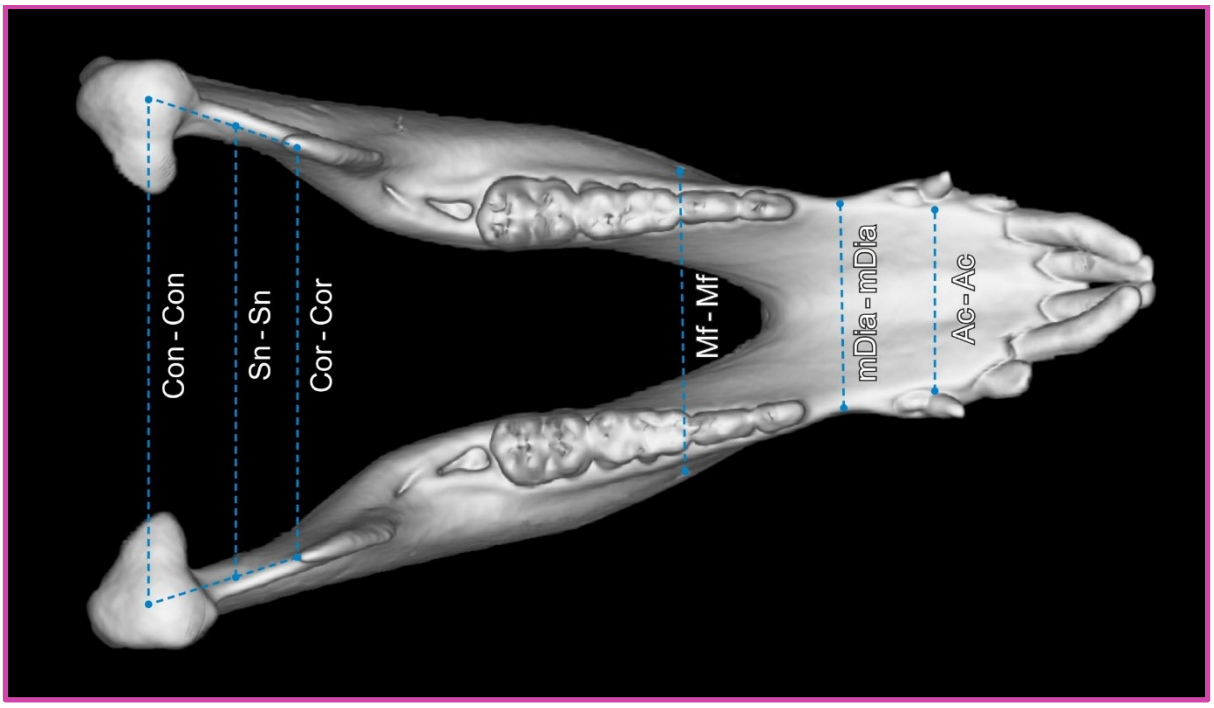

Figure 5.3.5-3: **Fig 3. Superior view of a 3D rendered mandible of a 17 months-old Göttingen Minipig with landmarks and measured parameters.** Where: Con = condylion, Cor = coronion, Sn = lowest point of the sigmoid notch, Mf = posterior prominent mental foramen, mDia = midpoint of the diastema, Ac = point on the buccal alveolar crest at the vertical level of the posterior mental foramen. The parameters measured were: mDia–mDia = interdiastemal breadth (IB), Ac–Ac = lingual intercrestal breadth (LIB), Mf–Mf = interforaminal breadth (IFB), Cor–Cor = intercoronoidal breadth (ICOB), Sn– Sn = breadth between sigmoid notches (SNB) and Con–Con = intercondylar breadth (ICB).

5.3.6 Statistics

IBM SPSS Statistics 23 was used for statistical analysis (IBM Deutschland GmbH, Kassel, Germany). All parameters were checked for normal distribution. If normal distribution was revealed, the student's *t*-Test was used. For non-normal distributed data, the Mann-Whitney-U-, Wilcoxon- and Kruskall-Wallis Tests were utilised. For the comparison of 12m animals with animals of 17m and 21m, the Independent T-test was used because the animals in the 12m group differ from those of 17m and 21m. Because the animals in 17m and 21m groups were the same individuals measured at different time points, they were treated statistically as paired samples and the Paired-student's *t*-Test was used. Correlations between parameters were analyzed with the bivariate Pearson-Test or Spearman-Rho-Test, depending whether normal or non-normal distributed data was present. Values are given as mean values with the associated standard deviations. A *p* value of less than 0.05 was considered significant. Correlation coefficients (r) between 0.45 to 0.59 were considered to be moderate correlations, whereas correlation coefficients between 0.60 to 0.79 were considered as strong and from 0.80 to 1.0 to be very strong correlations. All measurements were executed by the same

trained examiner (GMC) and under the supervision of an experienced radiologist (SMN). For the estimation of the observer's reproducibility of the measured values, several blind tests were conducted and it was proven that the measurements were precise and reliable.

Comparison with human data from literature. The relationship of age specific values of minipigs and human data, averaged over all available published means, was expressed as a minipig-human ratio (MP:H). Ratios lower than 0.85 and higher than 1.15 were defined as substantial anatomical deviations between both species. Parameters with ratios within the range of 0.85 and 1.15 were considered to have a moderate (>0.85 and <1.15) or high (>0.9 and <1.1) comparability.

5.4 Results

The mean values, standard deviations and p-values of all parameters measured are presented in Table 3. The p-values are the results of the statistical hypothesis tests conducted to determine if the parameter data of the three minipig age groups differ significantly from each other. Depending whether normal or non-normal distribution was present, student's t-(Independent and Paired), Mann-Whitney-U-, Wilcoxon- or Kruskall-Wallis Test was utilized. The data of left and right hemimandibles did not show any significant differences and were therefore pooled. All parameters (Table 3) showed significant correlations between the left and right hemimandibles and therefore no significant asymmetries were observable.

Table 4 presents an overview of all parameters measured indicating significant changes, lowest and highest individual values as well as correlations between the left and right hemimandible, with age and with body mass. The Figs 4–6 are boxplots of all measured parameters.

Table 5.4-1: *Table 3. Mean values, standard deviations and p-values of all measured parameters.*The data of left and right hemimandibles were statistically similar and were therefore pooled. The p-values presented are the results of the statistical hypothesis tests conducted to determine if the parameter data of the three minipig age groups differ significantly from each other.

Parameter	12 months (n = 6)	17 months (n = 12)	21 months (n = 11)	p-values 1) 12m-17m 2) 12m-21m 3) 17m-21m
Mandibular ramus height [mm] (MRH)	73.43 ± 3.44	78.08 ± 3.88	81.58 ± 4.00	0.001
				0.000
				0.012
Oblique mandibular ramus height [mm] (oMRH)	74.51 ± 3.69	77.15 ± 3.58	81.46 ± 4.98	0.047
				0.015
				0.026
Inferior mandibular body length [mm] (iMBL)	105.70 ± 2.68	112.49 ± 3.32	120.33 ± 2.90	0.000
				0.000
				0.000
Mandibular body length [mm] (MBL)	144.30 ± 5.23	152.14 ± 3.19	160.40 ± 4.06	0.000
				0.000
				0.000
Diastemal length [mm] (DL)	14.57 ± 2.40	14.54 ± 1.47	15.30 ± 1.70	0.970
				0.316
				0.245
Premolar and molar dental arch length [mm] (DAL)	57.19 ± 7.8	61.27 ± 1.60	63.15 ± 5.62	0.753
				0.444
	1			0.807
Interdiastemal breadth [mm] (IB)	33.38 ± 1.42	35.48 ± 1.28	37.57 ± 1.24	0.006
				0.000
				0.001
Lingual intercrestal breadth [mm] (LIB)	28.30 ± 1.24	29.19 ± 1.76	30.37 ± 2.06	0.289
				0.041
				0.152
Mental foramen to inferior mandible border height [mm] (MIB)	20.59 ± 1.62	22.90 ± 2.08	23.87 ± 2.12	0.002
				0.000
				0.144

Mental foramen to alveolar crest height [mm] (MAC)	13.40 ± 1.82	10.62 ± 1.50	11.40 ± 1.63	0.000
				0.003
				0.140
Mental foramen to gonion length [mm] (MGO)	81.57 ± 2.56	85.71 ± 3.15	90.92 ± 3.70	0.000
				0.000
				0.000
Interforaminal breadth [mm] (IFB)	51.59 ± 2.93	55.79 ± 2.86	57.91 ± 2.75	0.010
				0.000
				0.108
Gonial angle [degree] (GA)	97.53 ± 4.43	99.36 ± 3.80	97.32 ± 4.42	0.205
				0.898
				0.118
Mandibular ramus length [mm] (MRL)	42.27 ± 2.12	42.27 ± 1.48	44.46 ± 1.13	0.998
				0.001
				0.000
Superior ramus length [mm] (SRL)	25.42 ± 2.22	27.42 ± 1.56	27.43 ± 1.73	0.004
				0.006
				0.988
Coronoid process volume [mm3] (CPV)	193.42 ± 82.29	108.19 ± 56.39	187.75 ± 95.02	0.009
				0.719
				0.016
Mandibular condyle volume [ml] (MCV)	1.94 ± 0.52	2.68 ± 0.39	2.94 ± 0.65	0.000
				0.000
				0.045
Anterior mentum height [mm] (AMH)	45.92 ± 4.46	46.00 ± 2.76	48.03 ± 3.36	0.963
				0.286
				0.066
Intercoronoidal breadth [mm] (ICOB)	68.01 ± 2.36	71.44 ± 1.75	73.51 ± 1.59	0.003
				0.000
				0.015
Breadth between sigmoid notches [mm] (SNB)	75.29 ± 4.27	77.44 ± 3.66	80.06 ± 2.86	0.283
				0.014
				0.107
Intercondylar breadth [mm] (ICB)	88.08 ± 4.08	88.84 ± 2.85	91.61 ± 3.54	0.648
				0.082
				0.052

Intergonial breadth [mm] (IGB)	96.95 ± 7.72	106.62 ± 3.58	111.83 ± 4.79	0.002 0.000 0.002

Table 5.4-2: *Table 4. Overview of significant changes, lowest and highest individual values and correlations.* Significant correlations are pictured in green, negative correlation in yellow and non-significant values in red. Correlations were considered moderate (0.45 to 0.59), strong (0.60 to 0.79) and very strong (0.80 to 1.0). Significance levels are reported as p<0.05, p<0.01, p<0.001.

Param.	Significant changes with age	Lowest individual value (Group)	Highest individual value (Group)	Correlation between left and right hemi-mandible	Correlation with age	Correlation with body mass
MRH [mm]	Increase	68.3 (12m)	88.1 (21m)	r = 0.977	r = 0.685	r = 0.508
oMRH [mm]	Increase	70.8 (12m)	89.3 (21m)	r = 0.974	r = 0.686	r = 0.327
iMBL [mm]	Increase	102.6 (12m)	125.4 (21m)	r = 0.968	r = 0.835	r = 0.671
MBL [mm]	Increase	138.6 (12m)	167.4 (21m)	r = 0.959	r = 0.832	r = 0.511
DL [mm]	No changes	10.1 (12m)	18.1 (21m)	r = 0.719	r = 0.132	r = 0.103
DAL [mm]	No changes	44.5 (12m)	80.2 (21m)	r = 0.481	r = 0.188	r = 0.059
IB [mm]	Increase	31.1 (12m)	39.3 (21m)	---	r = 0.799	r = 0.487
LIB [mm]	Increase when comparing 12 and 21m	26.2 (17m)	34.6 (21m)	---	r = 0.440	r = 0.041
MIB [mm]	Increases between 12 and 17 m. No change after 17m	17.7 (12m)	27.1 (21m)	r = 0.918	r = 0.490	r = 0.110
MAC [mm]	Decreases between 12 and 17m. No change after 17m	7.7 (17m)	16.8 (12m)	r = 0.593	r = -0.194	r = -0.209
MGO [mm]	Increase	78.0 (12m)	99.2 (21m)	r = 0.932	r = 0.782	r = 0.610
IFB [mm]	Increases between 12 and 17 m. No change after 17m	46.7 (12m)	63.9 (21m)	---	r = 0.563	r = 0.080
GA [degree]	No change	91.5˚ (21m)	107.9˚ (17m)	r = 0.951	r = -0.130	r = -0.367
MRL [mm]	Change after 17m.	40.0 (17m)	46.9 (12m)	r = 0.946	r = 0.373	r = 0.392
SRL [mm]	Increase between 12 and 17 m. No change after 17m.	22. (12m)	30.8 (21m)	r = 0.932	r = 0.348	r = 0.448
CPV [mm^3]	Only when directly comparing 12-17m and 17-21m	44.5 (17m)	399.2 (21m)	r = 0.958	r = 0.013	r = -0.130

MCV [ml]	Increase	1.2 (12m)	3.8 (21m)	r = 0.907	r = 0.581	r = 0.623
AMH [mm]	No change	41.7 (12m)	54.7 (21m)	---	r = 0.220	r = 0.135
ICOB [mm]	Increase	65.5 (12m)	75.3 (21m)	---	r = 0.761	r = 0.451
SNB [mm]	Significant when directly comparing 12m and 21m	70.6 (12m)	86.3 (21m)	---	r = 0.473	r = 0.137
ICB [mm]	No change	82.2 (17m)	96.2 (21m)	---	r = 0.349	r = 0.638
IGB [mm]	Increase	83.7 (12m)	120.8 (21m)	---	r = 0.781	r = 0.621

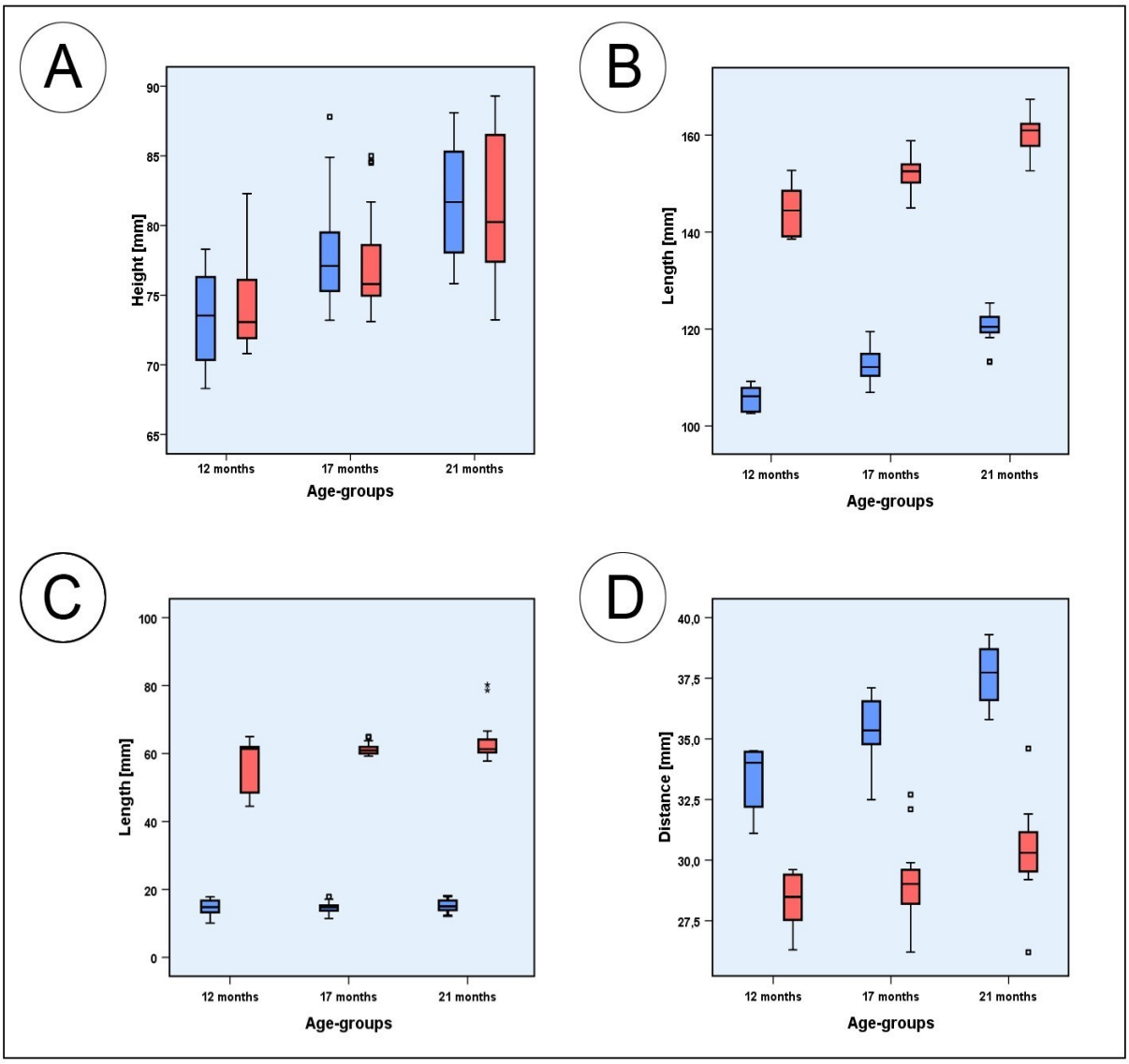

Figure 5.4-1: *Fig 4. Box plots of measured parameters.* (A) Box plots of the mandibular ramus height (blue) and oblique mandibular ramus height (red); (B) Box plots of the inferior mandibular body length (blue) and mandibular body length (red); (C) Box plots of the diastemal length (blue) and the premolar

and molar dental arch length (red); (D) Box plots of the interdiastemal breadth (blue) and lingual intercrestal breadth (red). Squares associated with the box plots are individual outliers. Outliers marked with asterisks are values that exceed the triple interquartile range.

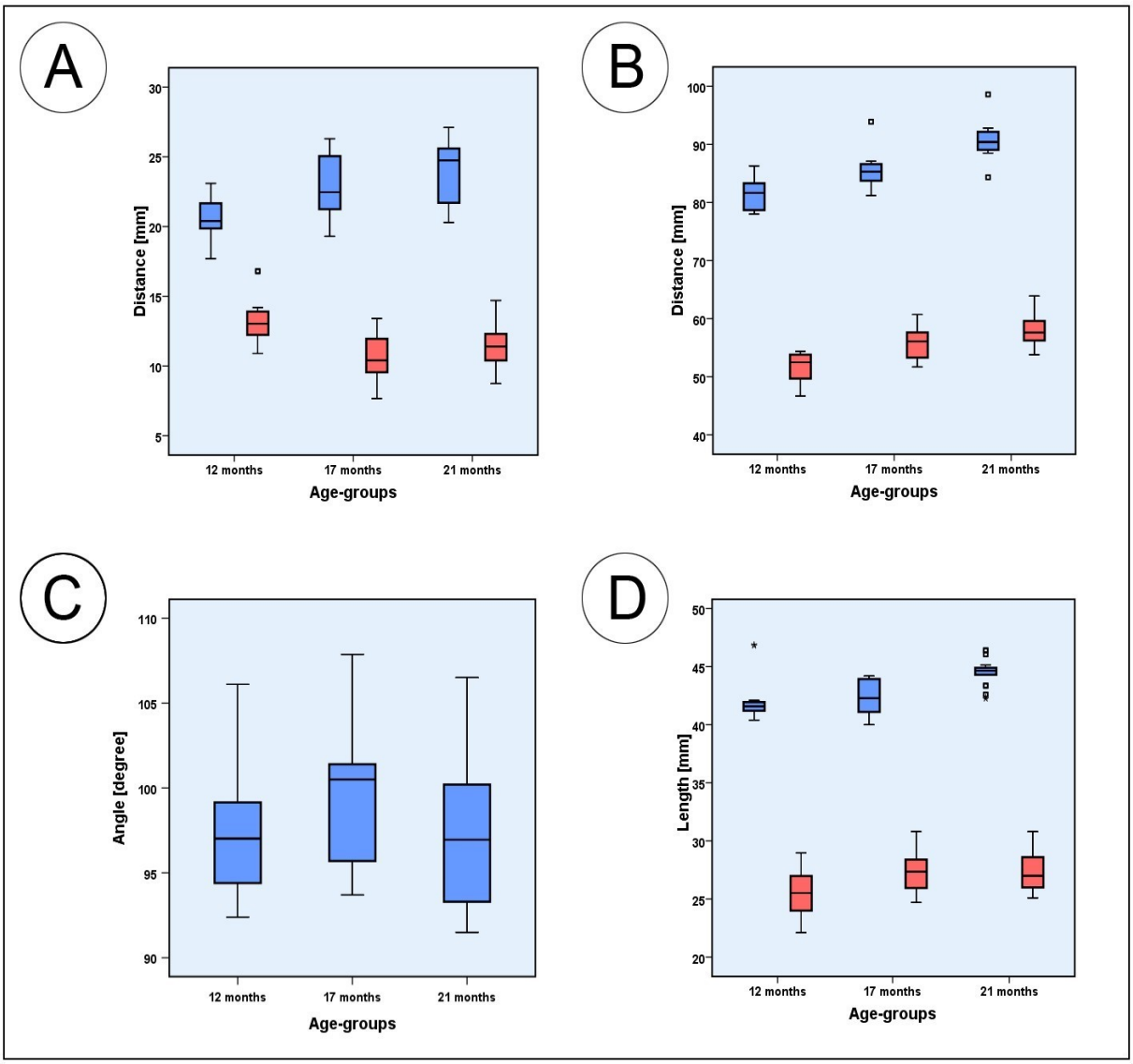

Figure 5.4-2: ***Fig 5. Box plots of measured parameters.*** (A) Box plots of mental foramen to inferior mandible border height (blue), and mental foramen to mandibular alveolar crest height (red); (B) mental foramen to gonion length (blue) and interforaminal breadth (red). (C) Box plots of the gonial angle measurements (blue); (D) Box plots of the mandibular ramus length (blue) and superior ramus length (red). Squares associated with the box plots are individual outliers. Outliers that are marked with asterisks are values that exceed the triple interquartile range.

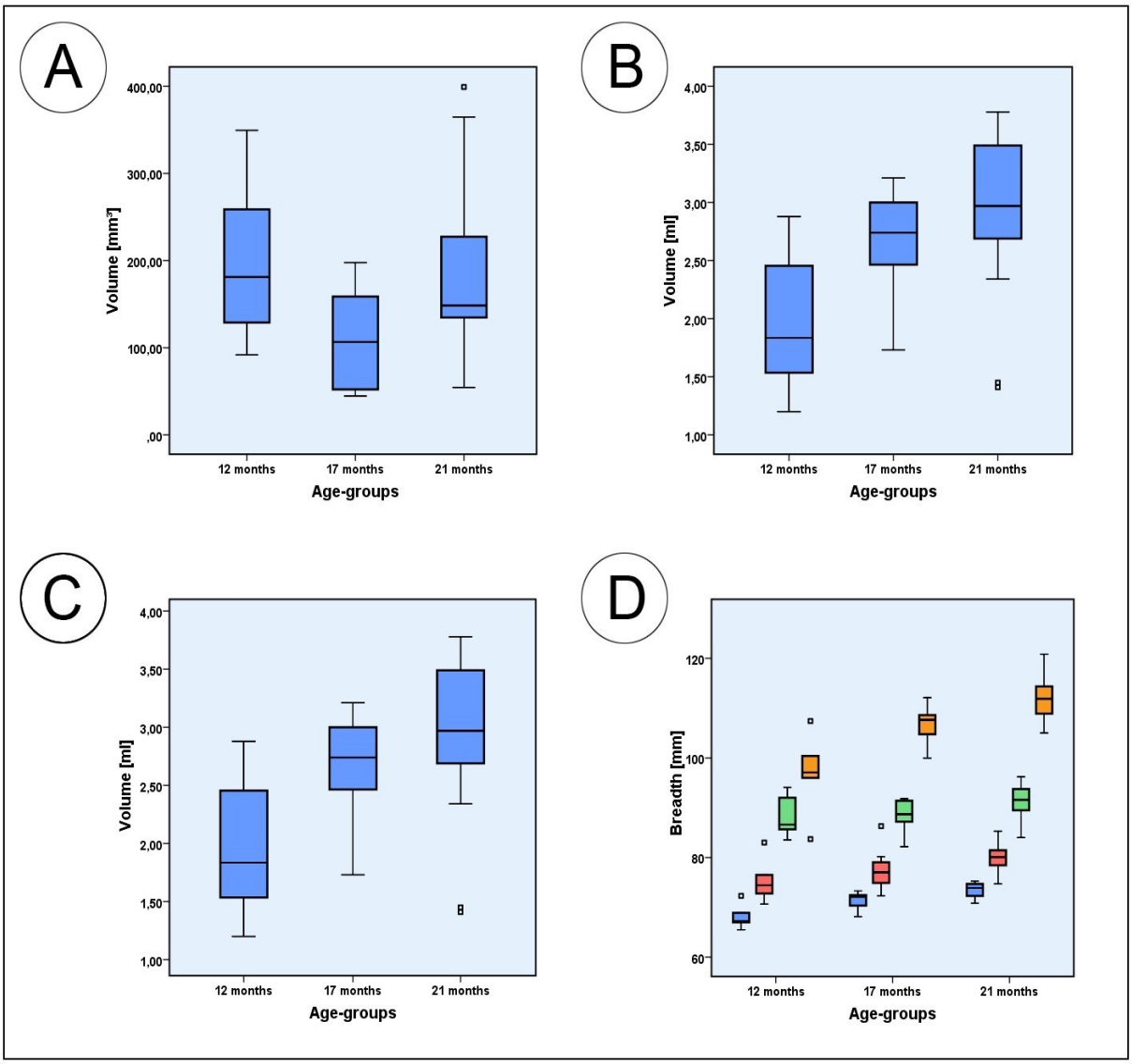

Figure 5.4-3: **Fig 6. Box plots of measured parameters.** (A) Box plots of the coronoid process volume; (B) Box plots of the mandibular condyle volume; (C) Box plots of the anterior mentum height; (D) Box plots of the intercoronoidal breadth (blue); intercondylar breadth (green); intergonial breadth (orange) and breath between sigmoid notches (red). Squares associated with the box plots are individual outliers.

5.4.1 Comparison to human data

The comparison to human data (Table 5) shows that 4 parameters, namely the MAC, MGO, MCV and IGB are highly comparable between the two species. Three other parameters have moderate comparability. All others are either not comparable or could not be compared due to insufficient data in the literature.

Table 5.4.1-1: *Table 5. Comparison to human data.* The total mean values of all parameters measured in each of the three minipig age groups and the corresponding data of humans from the scientific literature. The three different colors identify the differences between the age-group values of the minipigs and the mean values of human data, presented as a minipig/human ratio (MP:H). Ratios lower than 0.85 and higher than 1.15 were defined as substantial anatomical deviations (in red) between the two species where no comparability is present. Parameters with ratios within the range of 0.85 and 1.15 were considered to have a moderate (>0.85 and <1.15 in yellow) or a high (>0.9 and <1.1 in green) comparability.

Param.	Age (m)	Minipigs ($\bar{x}$ ±SD)	Humans ($\bar{x}$± SD)	Authors	MP/H ratio
MRH	12	73.4 ± 3.4 mm	53.1 ± 5.3 mm 56.5 ± 5.1 mm 57.6 ± 5.8 mm 59.3 mm	Lopez et al. [57] Moshiri et al. [58] Bayome et al. [59] Ozturk et al. [60]	0.771
	17	78.1 ± 3.9 mm			0.725
	21	81.6 ± 4.0 mm			0.694
MRH	12	74.5 ± 3.7 mm	53.2 ± 3.6 mm 64.5 ± 4.2 mm	Franklin et al. [61] Kim et al. [62]	0.789
	17	77.2 ± 3.6 mm			0.762
	21	81.5 ± 5.0 mm			0.721
iMBL	12	105.7 ± 2.7 mm	72.7 ± 5.3 mm 79.4 ± 5.6 mm 79.4 ± 5.6 mm; 88.0 ± 5.0 mm and 93.0 ± 5.0 mm	Steyn and Iscan [63] Moshiri et al. [58] Bayome et al. [59] Weijs and Hillen [64]	0.787
	17	112.5 ± 3.3 mm			0.740
	21	120.3 ± 2.9 mm			0.692
MBL	12	144.3 ± 5.2 mm	**No comparison possible**		
	17	152.1 ± 3.2 mm			
	21	160.4 ± 4.1 mm			
DL	12	14.6 ± 2.4 mm	**No comparison possible**		
	17	14.5 ± 1.5 mm			
	21	15.3 ± 1.7 mm			

DAL	12	57.2 ± 7.8 mm	38.4 ± 2.7 mm	Al-Zubair et al. [65]	0.726
	17	61.3 ± 1.6 mm	41.5 and 44.7 mm	Braun et al. [66]	0.678
	21	63.2 ± 5.6 mm			0.657
IB	12	33.4 ± 1.4 mm	**No comparison possible**		
	17	35.5 ± 1.3 mm			
	21	37.6 ± 1.2 mm			
LIB	12	28.3 ± 1.2 mm	24.4 ± 1.4 mm 25.4 ± 1.8 mm 25.3 ± 0.9 mm and 26.4 ± 2.9 mm	Bishara et al. [67] Singh et al. [68] Tamewar et al. [69]	0.897
	17	29.2 ± 1.8 mm			0.869
	21	30.4 ± 2.1 mm			0.802
MIB	12	20.6 ± 1.6 mm	11.5 mm	Ozturk et al. [60]	0.648
	17	22.9 ± 2.1 mm	15.2 mm	Tebo and Telford [70]	0.583
	21	23.9 ± 2.1 mm			0.559
MAC	12	13.4 ± 1.8 mm	11.4 mm	Ozturk et al. [60]	0.866
	17	10.6 ± 1.5 mm	11.8 ± 3.0 mm	Lorenzo et al. [71]	0.906
	21	11.4 ± 1.6 mm			1.017
MGO	12	81.6 ± 2.6 mm	74.6 mm	Tebo and Telford [70]	0.914
	17	85.7 ± 3.2 mm			0.870
	21	90.9 ± 3.7 mm			0.820
IFB	12	51.6 ± 2.9 mm	44.6 ± 2.5 mm 43.2 ± 2.8 mm 47.2 ± 2.8 mm and 49.9 ± 3.0 mm	Lopez et al. [57] Kumar et al. [72] Dong et al. [73]	0.853
	17	55.8 ± 2.9 mm			0.806
	21	57.9 ± 2.8 mm			0.781
GA	12	97.5 ± 4.4˚	115.5 ± 4.0˚ 118.6 ± 5.2˚ 123.9 ± 7.3˚ 125.7 ± 5.6˚	Bayome et al. [59] Weijs and Hillen [64] Lopez et al. [57] Dong et al. [73]	1.194
	17	99.4 ± 3.8˚			1.178
	21	97.3 ± 4.4˚			1.195
MRL	12	42.3 ± 2.1 mm	32.7 ± 2.8 mm 37.8 ± 2.9 mm and 39.8 ± 3.7 mm	Kim et al. [62] Giles [74]	0.869
	17	42.3 ± 1.5 mm			0.869
	21	44.5 ± 1.1 mm			0.826

SRL	12	25.4 ± 2.2 mm	31.3 ± 2.9 mm	Lopez et al. [57]	1.216
	17	27.4 ± 1.6 mm	33.5 ± 3.6 mm	Kim et al. [75]	1.154
	21	27.4 ± 1.7 mm			1.154
CPV	12	193.4 ± 82.3 mm^3	250.0 ± 9.0 mm^3	Gomes et al. [76]	1.226
	17	108.2 ± 56.4 mm^3			1.567
	21	187.8 ± 95.0 mm^3			1.249
MCV	12	1.9 ± 0.5 ml	2.3 ml and 2.4 ml	Safi et al. [77]	1.230
	17	2.7 ± 0.4 ml	2.7 ± 0.4 ml	Saccucci et al. [78]	0.914
	21	2.9 ± 0.7 ml			0.851
AMH	12	45.9 ± 4.5 mm	24.6 mm	Ozturk et al. [60]	0.600
	17	46.0 ± 2.8 mm	28.5 ± 3.0 mm	Giles [74]	0.599
	21	48.0 ± 3.4 mm	29.6 ± 3.5 mm	Kumar et al. [72]	0.574
ICOB	12	68.0 ± 2.4 mm	90.8 ± 5.7 mm	Lopez et al. [57]	1.251
	17	71.4 ± 1.8 mm	92.0 ± 5.7 mm	Kumar et al. [72]	1.219
	21	73.5 ± 1.6 mm			1.244
SNB	12	75.3 ± 4.3 mm	**No comparison possible**		
	17	77.4 ± 3.7 mm			
	21	80.1 ± 2.9 mm			
ICB	12	88.1 ± 4.1 mm	111.2 ± 6.2 mm and 117.0 ± 5.3 mm	Steyn and Iscan [63]	1.226
	17	88.9 ± 2.9 mm	110.5 ± 6.2 mm and 116.4 ± 7.0 mm	Lopez et al. [57]	1.219
	21	91.6 ± 3.5 mm			1.195
IGB	12	97.0 ± 7.7 mm	85.9 ± 5.0 mm	Ozturk et al. [60]	0.935
	17	106.6 ± 3.6 mm	91.5 ± 5.0 mm 91.8 ± 5.9 mm	Steyn and Iscan [63] Lopez et al. [57]	0.851
	21	111.8 ± 4.8 mm	93.7 ± 6.8 mm	Carvalho et al. [79]	0.811

5.4.2 Visualization of the growth changes

Between 17 and 21 months of age, there is an obvious increase in mandibular body length, mandibular ramus height and oblique mandibular ramus height. The gonial angle does not change visually (Fig 7).

Between 17 and 21 months, the mandibular condyle (Fig 8A) has an increase in horizontal width, with greater growth at its medial aspect. Beneath the condyle, the upper mandibular ramus increases in thickness over time. In addition, there is a slight increase in mandibular ramus length (Fig 8B). The superior mandibular ramus length does not change.

Fig 9 shows the elongate mandible of minipig and its anteriorly directed mentum. Humans have a much shorter mandible and a more vertical mentum, with an anteriorly located menton. Minipigs have a longer and steeper mandibular ramus with a longer and larger mandibular condyle. Their coronoid process and mandibular condyle are approximately located at the same height. Humans have a more elongate and deeper sigmoid notch as well as an inferiorly located mandibular condyle in relation to the coronoid process.

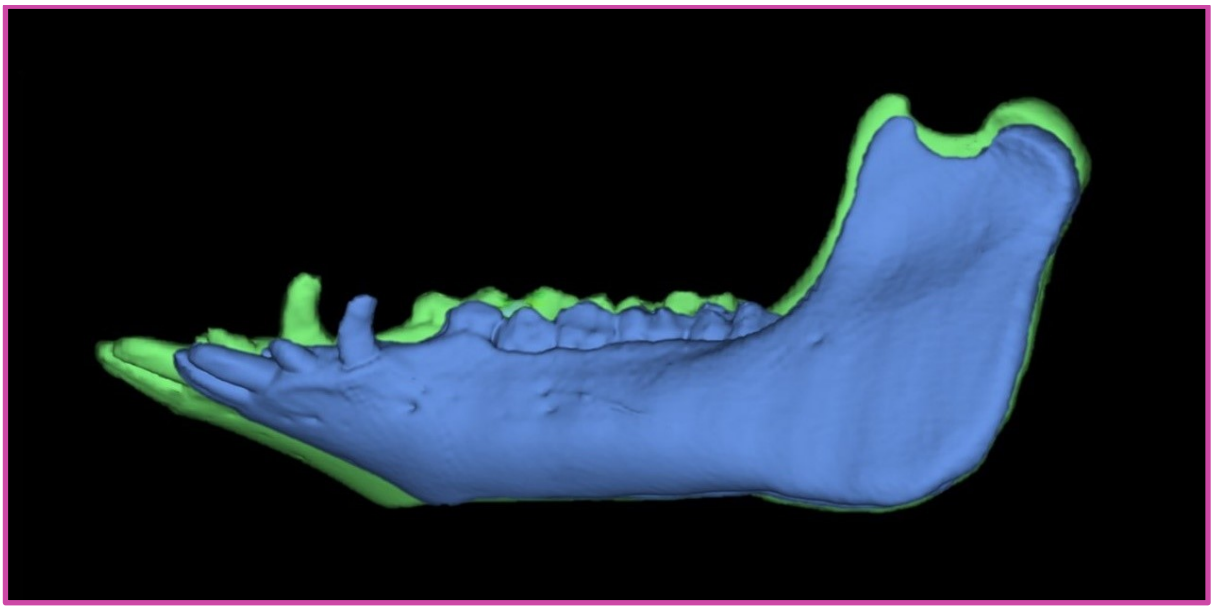

Figure 5.4.2-1: **Fig 7. Lateral view of the same segmented mandible showing growth changes.** The segmentations show the mandibular volume at 17 (blue) and 21 (green) months of age, merged and presented at the same scale.

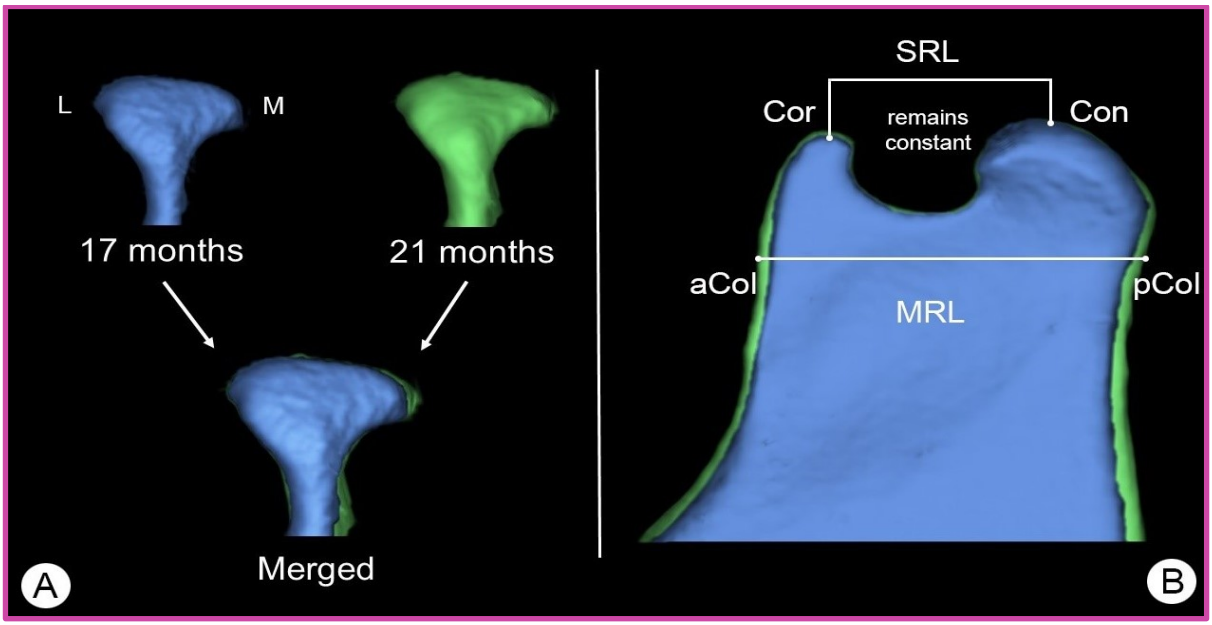

Figure 5.4.2-2: **Fig 8. Growth changes of the mandibular condyle and superior ramus.** (A) Posterior view of a mandibular condyle of the same individual animal at 17 (blue) and 21 months (green) of age scaled to same size, showing changes in mandibular condyle volume (MCV) over time. Here: L = lateral aspect of the mandibular condyle and M = medial aspect of the mandibular condyle. (B) Lateral view of the superior area of the mandibular ramus, showing growth changes of the mandibular ramus. Here: Cor = coronion, Con = condylion, aCol = anterior point of the mandibular collum, pCol = posterior point of the mandibular collum. Parameters were: SRL = Cor-Con, MRL = aCol-pCol. Segmentations are presented at the same scale.

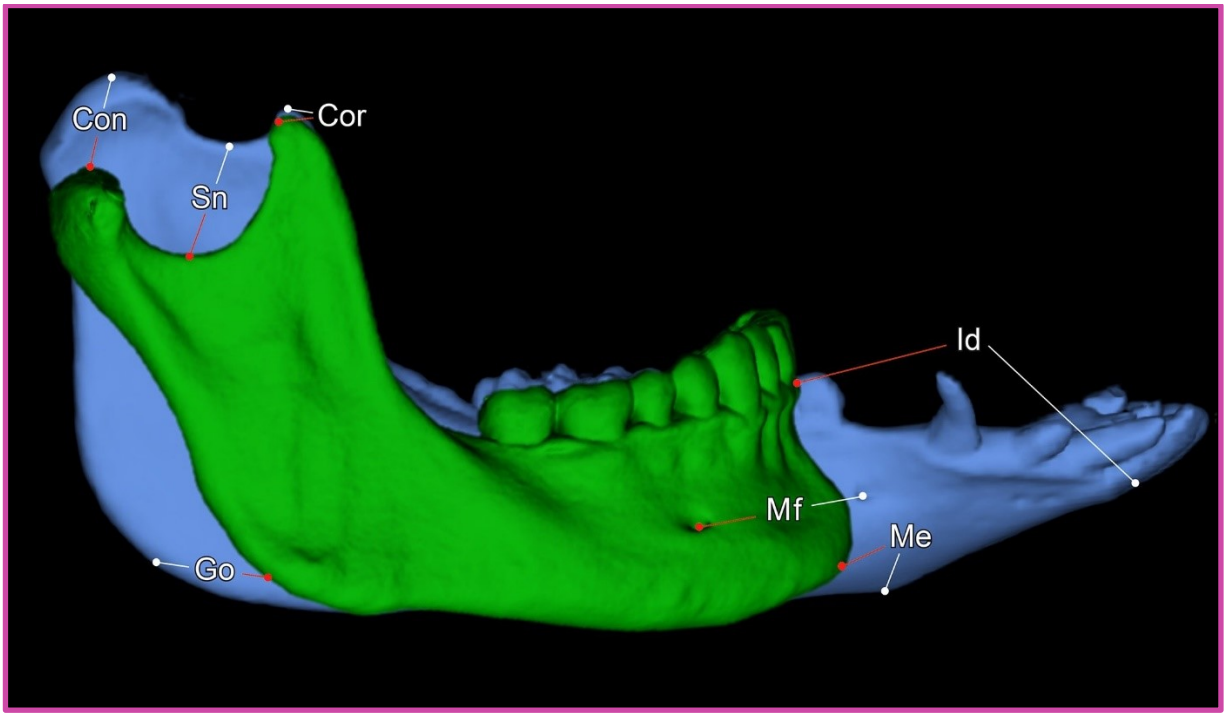

Figure 5.4.2-3: **Fig 9. The different morphology of the minipig and human mandible.** 3D renderings of an adult human mandible (green) and a mandible of a 21-months old Göttingen Minipig. Both segmentations are presented at the same scale. Where: Con = condylion, Cor = coronion, Sn = lowest

point of the sigmoid notch, Go = gonion, Mf = posterior prominent mental foramen, Me = menton, Id = infradentale.

5.4.3 The position and dimensions of the masticatory muscles

In the minipigs, the masseter muscle (Fig 10A and 10B) is a nearly square shaped, thick muscle that originates from the inferior aspect of the facial crest, the complete inferior aspect of the zygomatic arch and the lateral aspect of the mandibular process, directly inferior to the mandibular condyle. Its insertion is the mandibular body extending from the vertical at the level of the distal aspect of the second molar tooth (M2) through to the posterior border of the mandibular ramus.

The temporal muscle (Fig 10A and 10B) is much thinner than the masseter muscle. It originates from the temporal fossa, terminating anteriorly at the level of the zygomatic process of the frontal bone and posteriorly adjacent to the nuchal line and supramastoid crest. The temporal muscle also originates from the superior aspect of the zygomatic process of the temporal bone. The temporal muscle inserts on the coronoid process and the anterior aspect of the mandibular ramus, in close proximity with both pterygoid muscles.

The pterygoid muscles consist of a large medial muscle block and a smaller lateral muscle block. The inferior alveolar nerve passes between these to traverse the mandibular foramen into the mandibular canal.

The medial pterygoid muscle (Fig 10B and 10C) originates from the inferolateral aspect of the pterygoid bone, the pterygoid hamulus and the sphenoidal process of the palatal bone. It travels in close proximity to the tympanic bulla to its insertion at the lateral and posterior borders of the mandibular ramus. An inferior portion extends across the medial aspect of the mandibular body as far anteriorly as the second premolar tooth.

The lateral pterygoid muscle (Fig 10B and 10C) originates from the dorsolateral aspect of the pterygoid bone and the dorsal aspect of the pterygoid hamulus. Its insertion is directly beneath the medial aspect of the mandibular condyle.

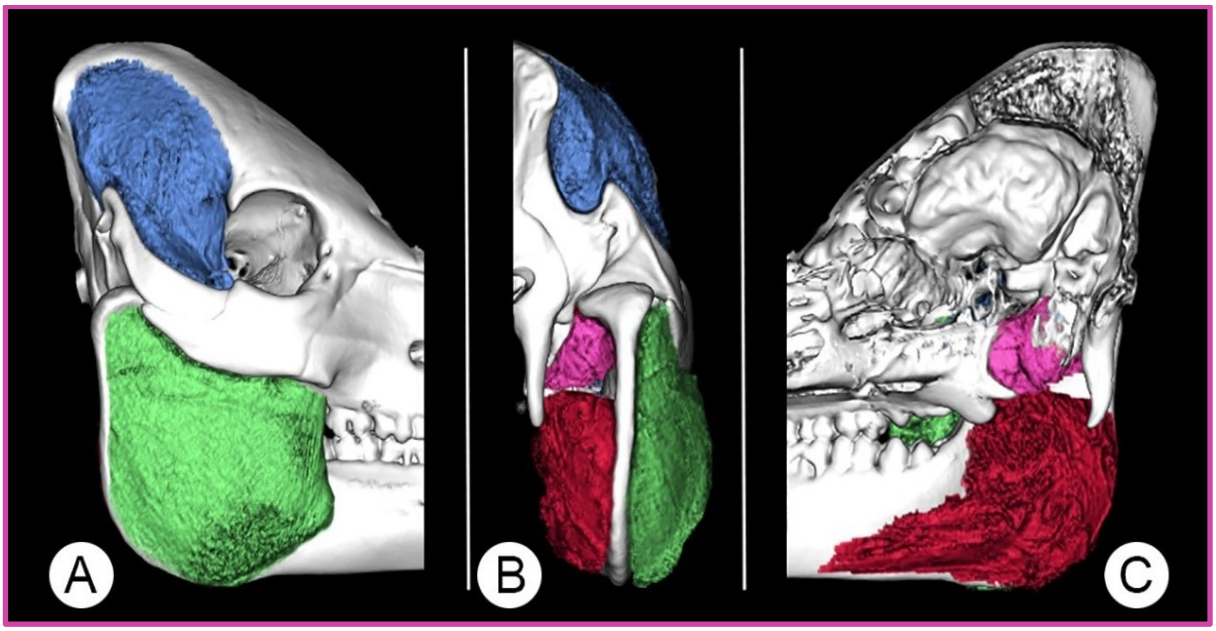

Figure 5.4.3-1: **Fig 10. A 3D-rendered skull of a 12 months-old Göttingen Minipig showing the segmented masticatory muscles.** Where (A) is a lateral, (B) a posterior and (C) a medial view. Pictured are the masseter (green), temporal (blue), medial pterygoid (red) and lateral pterygoid (pink) muscles.

5.4.4 Blood vessel architecture adjacent the mandibular ramus

The 3D rendering of the blood vessel architecture shows that both the maxillary artery and the deep facial vein (V. faciei profunda) lie in close proximity to the medial aspect of the mandibular ramus. The deep facial vein originates from numerous slender superficial facial veins immediately anterior to the mandibular ramus. From here it dives around the anterior edge of the mandibular ramus, to run posteriorly immediately adjacent the mandibular ramus. At this level, it has a diameter of approximately 6 mm. It then drains posteriorly into the maxillary vein. The deep facial vein is accompanied by the maxillary artery as it traverses medial to the mandibular ramus. Inferior to the maxillary artery and the deep facial vein runs the lingual artery along its arcuate course (Fig 11A).

The two-dimensional coronal plane image (Fig 11B) shows the horizontally running deep facial vein and its mediolateral course around the anterior aspect of the mandibular ramus. The portion of the vein medial to the ramus has a diameter of approximately 6 mm.

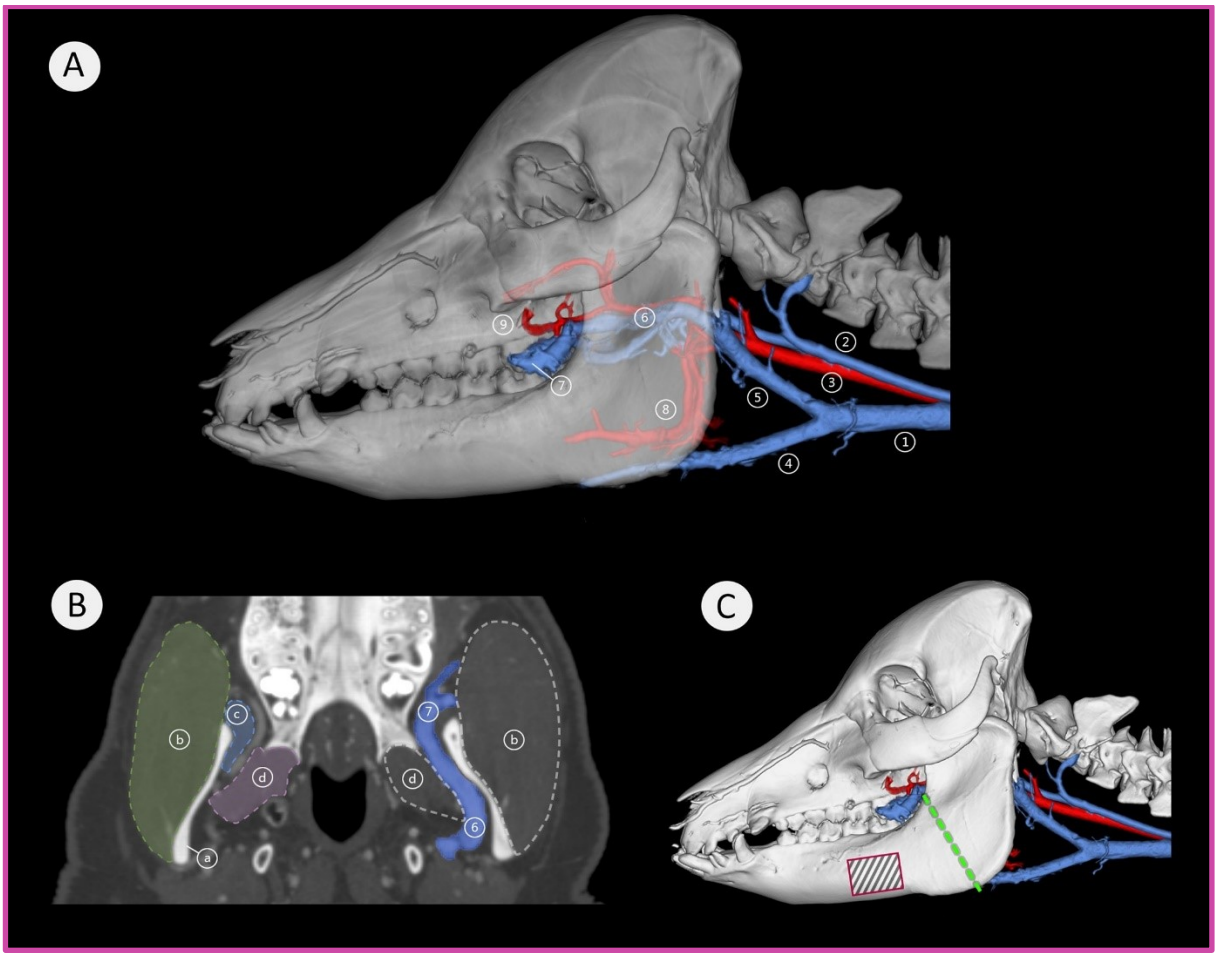

Figure 5.4.4-1: **Fig 11. Vascular architecture medial to the mandibular ramus.** Image (A) is a lateral view of a semitransparent segmentation of a 21 months-old minipig head with associated major blood vessels of the neck and the mandibular region. Arteries are pictured in red and veins in blue. Here: (1) external jugular vein, (2) internal jugular vein, (3) common carotid artery, (4) linguofacial vein, (5) maxillary vein, (6) deep facial vein with maxillary artery, (7) deep facial vein traversing from medial to lateral, (8) lingual artery, (9) buccal artery. Image (B) shows a coronal view with the prominent deep facial vein (6) (in blue), adjacent to the medial aspect of the mandibular ramus (a). The vein has a diameter of approximately 6 mm and traverses from medial to lateral across the anterior aspect of the mandible (7). Here; (a) mandibular ramus, (b) masseter muscle, (c) temporal muscle insertion, (d) lateral pterygoid muscle, (6) deep facial vein, (7) deep facial vein traversing from medial to lateral. Image (C) is a lateral view of a 21 months-old minipig skull with associated large blood vessels of the neck and the mandibular region. Arteries are pictured in red and veins in blue. The green dashed line indicates the most common sectional plane used in experimental mandibular distraction osteogenesis procedures, the black-striped red rectangle indicates a common site for fixation plate placement in some experimental surgery (Fig 12B and 12C).

5.4.5 Theoretical space available for mono- and bicortical screw insertion

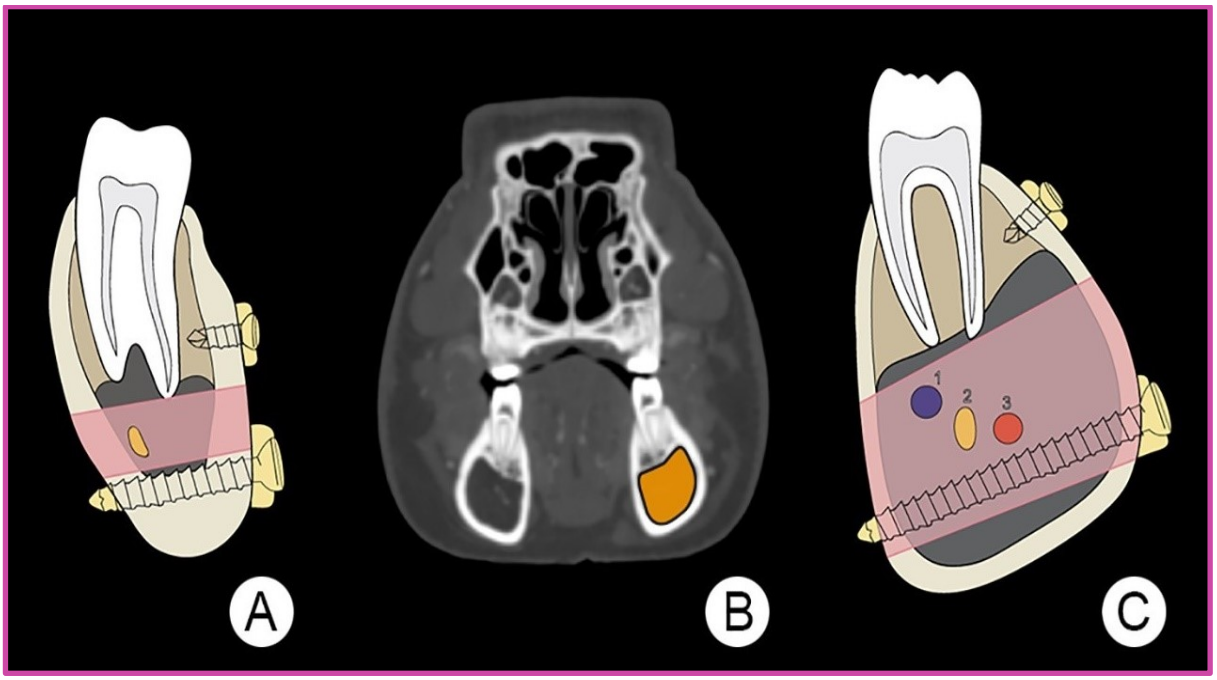

Figure 5.4.5-1: **Fig 12. Illustration of theoretical space available for mono- and bicortical screw insertion.** Here (A) is an illustration of the human mandibular body (after the AO Foundation, Switzerland), showing the potential space for positioning both mono- and bicortical screws. The pink area indicates a zone, which extends from the tooth roots to the inferior aspect of the mandibular canal that conveys the inferior alveolar nerve and its associated blood vessels. The yellow oval indicates the inferior alveolar nerve. Image (B) is a transverse plane CT image of a 21 months-old minipig head at the level of the first premolar tooth. The area coloured in orange indicates the dimensions of the mandibular canal. Illustration (C) depicts the right mandibular body of the minipig seen in (B), showing one extreme of the highly variable mandibular canal dimensions and the potential space for positioning both mono- and bicortical screws. The pink area indicates a zone where the inferior alveolar nerves and blood vessels are located. Portrayed is the inferior alveolar neurovascular bundle consisting of the inferior alveolar vein (1), the inferior alveolar nerve (2) and the inferior alveolar artery (3).

The illustration of the human mandibular body (Fig 12A) demonstrates the correct positioning of mono- and bicortical screws in order to avoid damage to the tooth roots and the inferior alveolar neurovascular bundle. The minipig shown in Fig 12B has a large mandibular canal volume [17]. Compared to the human (Fig 12A), the inferior mandibular bone thickness of the minipig is notably thinner as are the buccal and lingual cortices of the mandibular body (Fig 12C). In addition, the shape of the mandibular body in both species clearly differs greatly with each other. Whilst the mandibular body cross section of humans is usually ovoid in shape (Fig 12A), that of minipigs is highly variable, ranging from ovoid to pear-shaped (Fig 12C). In some minipigs, the most inferior point of the mandibular body can be located at the lingual side of

the body, whereas the most buccal point is more or less located on a horizontal midline through the center of the mandibular canal.

5.5 Discussion

In presurgical planning of human mandibular surgery and reconstruction, numerous cephalometric parameters are measured routinely. Because experimental approaches for these procedures are often developed in Göttingen Minipigs, we selected 22 of these parameters and measured them using CT scans of subadult and adult Göttingen Minipigs. By doing so, we evaluated the dimensions and the overall anatomical growth changes and ultimately compared these with human data from the literature. Of the 22 parameters measured, only four were found to be highly comparable, whilst the others were not.

These four parameters were the distance from the mental foramen vertically to the mandibular alveolar crest (MAC), the distance from mental foramen to the gonion (MGO), the mandibular condyle volume (MCV) and the intergonial breadth (IGB). They all had a MP:H between 0.9 and 1.1 in at least one age group (Table 5).

In the present study, the MAC in minipigs decreased from 13.4 mm at 12m to 10.6 mm at 17m but not thereafter. Comparably in humans, Ozturk et al. (2013) reported a MAC of 11.4 mm [60]. In another study on 307 human patients, a mean MAC of 11.84 ± 3.02 mm was reported [71]. Compared to humans, especially older minipigs of the 17m and 21m group, showed a high comparability, indicating that in these age groups, the position of the mental foramen in relation to the alveolar crest is very similar.

In the minipigs of the present study, the second highly comparable feature, i.e. the distance from the mental foramen to the gonion, increased significantly with age. At 12m it was 81.6 mm and by 21 m it was 90.9 mm. Tebo and Telford (1950) reported a MGO in humans of 74.6 mm, which is highly comparable to values found in 12m minipigs [70].

The third highly comparable parameter, the mandibular condyle volume, in the minipigs ranged from 1.9 ml at 12m to 2.7 ml at 17 m and 2.9 ml at 21m. However, there were large individual differences within each age group. As an example, the 21 months group showed values ranging from 1.4 ml to 3.8 ml. In a volumetric assessment of 700 human mandibular condyles, Safi et al. (2017) reported a mean MCV of 2.44 ml in the right and 2.27 ml in the left condyle [77]. Similarly Saccucci et al. (2012) reported a mean MCV of 2.7 ± 0.5 ml for the right and 2.7 ± 0.4 ml for the left condyle in 65 adolescent human patients [78]. This indicates that the MCV of 17m old minipigs is highly comparable to that of humans.

The fourth highly comparable parameter was the intergonial breadth that in minipigs ranged from 97.0 mm at 12m to 111.8 mm at 21 m. In the present study, only the 12m old animals' parameters were highly comparable with humans. In humans, Weijs and Hillen (1984) reported an IGB of 107.0 ± 5.0mm [64]. Steyn and Iscan (1998) presented an IGB of 99.6 ± 5.5 mm in males and 91.5 ± 5.0 mm for females [63]. Similarly a Brazilian study from 2013 reported that the IGB ranged from 93.7 ± 6.8 mm to 94.5 ± 9.1 mm [79]. More recently Lopez et al. (2017) reported an IGB of 91.8 ± 5.9 mm for males and 84.5 ± 5.0 mm for females [57]. Ozturk et al. (2013) published an IGB of 85.86 mm [60]. Thus whilst the IGB of the 12m minipigs has a high comparability with humans that of older minipigs has not.

The lingual intercrestal breadth (LIB), the interforaminal breadth (IFB) and the mandibular ramus length (MRL) showed moderate comparability in at least one minipig age group with published data for humans (MP:H between 0.85 and 1.15) (Table 5).

The LIB, a rough indicator of the intercanine width, in minipigs ranged from 28.3 mm at 12m to 30.4 mm at 21m. Bishara et al. (1997) reported the intercanine width to be 25.4 ± 1.8 mm in 13 year old and 24.4 ± 1.4 mm in 26 year old human females [67] whilst Tamewar and Parakh (2018) reported it to be 26.4 ± 2.9 mm in adolescents [69] and Singh et al. (2017) found it to be 25.3 ± 0.9 mm in 209 females [68]. As seen in MGO and IGB, especially the younger groups of minipigs had comparable values and therefore similar dimensions in this region.

In minipigs, the IFB ranged between 51.6 mm at 12m to 57.9 mm at 21m. The closest comparability was between 12m old minipigs and humans. The older minipigs showed no comparability. In humans, Lopez et al. reported (2017) an IFB of 46.5 ± 3.7 mm for males and 44.6 ± 2.5 for females, Kumar and Lokanadham (2017) reported an IFB of 43.2 ± 2.8 mm [72] and Dong et al. (2015) an IFB of 49.93 ± 3.01 mm in males and 47.23 ± 2.80 mm in female individuals [73]. Hence, the IFB of 12m old minipigs is comparable to that of humans; the older age groups are not comparable.

The mandibular ramus length remained at 42.3 mm in 12 and 17 months old minipigs but then increased to 44.5 mm by 21m. In humans, Kim et al. (1997) reported a mean MRL of 32.7 ± 2.8 mm [62], whilst Giles (1964) reported an MRL of 39.84 ± 3.69 mm in males and 37.83 ± 2.93 mm in females [74]. When compared to 12m and 17m minipigs the MRL of humans had a moderate level of comparability.

The 11 remaining parameters showed no comparability between Göttingen Minipigs and humans, as they had a MP/H-ratio <0.85 and >1.15in in all three age groups.

1. MRH: Compared to the published values of humans, Göttingen Minipigs have a significantly higher mandibular ramus [57–60].

2. oMRH: The oblique mandibular ramus height is significantly higher in Göttingen Minipigs than in humans [61].

3. iMBL: In minipigs, the infradentale is the most anteriorly located mandibular point and contributes to the overall length of the mandible. Minipigs possess a significantly longer mandible than humans [58, 59, 63, 64].

4. DAL: The presence of a diastema in minipigs prevents a reasonable comparison with humans but served as an anteroposterior growth indicator. Our data show, that the premolar and molar dental arch length is longer in minipigs than the overall dental arch length in humans, measured from central incisors to the last molar tooth [65, 66].

5. MIB: In minipigs the MIB is nearly twice that of humans [60]. The mental foramen in humans is in the vertical center of the mandibular body, whilst in minipigs, the mental foramen has a more superior position, relative to the mandibular body height.

6. GA: Measurements of the gonial angle revealed that minipigs have a much more oblique mandibular angle compared to humans [57, 59, 64, 73]. In humans, the most posterior point of the mandible is the mandibular condyle whilst in minipigs it is the posterior ramus edge above the gonion (Fig 9).

7. SRL: Minipigs have a shorter superior ramus length than humans [57, 75].

8. CPV: The coronoid process volume of minipigs is not comparable to that of humans who have a significantly higher volume [76]. Noteworthy is the high individual variation in minipigs of the same age. For example in the 21m group, the lowest volume was 53.8 mm^3 whilst the highest was 399.2 mm^3.

9. AMH: Minipigs have a higher anterior mentum height compared to human values reported in the scientific literature [60, 72, 74]. This is because minipigs do not have a posteriorly located infradentale as found in humans.

10. ICB: Because of the smaller area between their superior mandibular ramus, minipigs have a lower intercondylar breadth (ICB) than humans [57, 63, 72].

11. ICOB: Of the three parameters (ICOB, ICB and SNB) which assess laterolateral growth between the superior ramus of both hemimandibles, only the ICOB had statistically significant ongoing changes with age. This indicates that the superior ramus width does change in the anterior region between the coronions and remains constant in the posterior region.

Four parameters, namely the mandibular body length (MBL), the diastemal length (DL), the intercrestal breadth (IB) and the breadth between sigmoid notches (SNB), could not be compared, because to the best of our knowledge, there is no data reported on humans (Table 5). Mandibular body length of minipigs increased steadily with age. Whilst the presence of a

diastema in the minipigs prevents a direct comparison with humans, increases in minipig diastemal length indicate longitudinal growth. However, in our study there were no significant changes in DL over time. This suggests that the major part of anteroposterior mandibular growth occurs in the posterior ramus area. Studies conducted on mandibular growth in humans and pigs confirm this observation [80–82].

In this study, the 3D segmentations show that the growth changes of the whole mandible, the mandibular condyles and the superior mandibular ramus between minipigs of 17 and 21m, corresponded to the cephalometric measurements undertaken in this study.

The quadrupedal mode of life has a significant influence on the architecture and distribution of the vasculature of the head and neck when compared to that of bipedal humans. In a quadruped at the transition of the neck to the head the vasculature courses in a horizontal, posteroanterior manner, whilst that of bipeds is vertically directed [20, 21]. The 3D examinations of the minipig vasculature showed an extensive, large, tortuous network of veins and to a lesser extent arteries immediately medial to the mandibular ramus (Fig 11). The very prominent, deep facial vein and maxillary artery form a deep facial vascular complex that has not been reported previously and is potentially important to experimental MDO procedures in Göttingen Minipigs. Commonly the principal sectional plane for MDO procedures extends from the inferior border anterior to the mandibular angle to the retromolar region [83, 84]. In Göttingen Minipigs, the presence of the deep facial vascular complex adjacent to where the mandible is sectioned, constitutes a major risk factor. Any accidental transection of these blood vessels could result in uncontrollable inaccessible bleeding. Whilst the lingual artery and linguofacial vein could potentially interfere with the MDO sectional plane their more medial location makes them less vulnerable.

As illustrated in Fig 12 the morphology and dimensions of the mandibular body in humans and minipigs are very different. Whilst humans have a mandibular body with an ovoid cross-section (Fig 12A), that of minipigs can be pear-shaped (Fig 12C). In a previous study we showed large individual differences in the dimension of the mandibular canal of Göttingen Minipigs of the same age [17]. Minipigs also have a significantly thinner inferior mandibular body bone thickness (4.7 mm at 12m and 4.0 mm at 21m) than humans (9.4 mm to 12.6 mm) [17, 85, 86]. Consequently, bicortical screws that are positioned in the inferior part of the mandibular body routinely in humans could, when placed in a similar way in a Göttingen Minipig, cause trauma to the inferior alveolar nerves and vessels. This could be compounded by the erratic highly variable position of the inferior alveolar nerves and vessels with their possible undulating course, often resembling a corkscrew [17]. Bicortical screws implanted in the inferior cortex would probably, due to the thin inferior bone thickness, have impaired stability.

The segmentation of the masticatory muscles of the minipigs revealed similar findings to that reported in the literature on larger domestic pig breeds. However, we found that the masseter muscle of Göttingen Minipigs extended more anteriorly than previously described [21]. When compared with humans, minipigs have a larger masseter but smaller temporal muscle. Whilst the lateral pterygoid muscle of the minipig has a comparable anatomical position and dimension to that of humans, the medial pterygoid muscle is larger and has a similar origin, but its insertion is located far more anteriorly. It extends to the height of the first molar tooth [84, 87–89]. Herring et al. observed that the dynamics of mastication in pigs and in humans differ greatly. Under natural conditions, pigs have a rapid rate of mastication and each side of the dental arcade is used independently. Contrary to this, humans have a slower and unilateral mastication. In addition, pigs have a higher crushing force and closing velocity than humans, that could potentially impair wound healing and implant stability [20]. An additional negative influence potentially promoting these post-operative complications often observed by surgeons undertaking mandibular surgical procedures, is that post-operatively pigs grind their teeth extensively as well as bite hard objects such as their cages [20, 37, 90, 91].

In 2002, Swennen et al. stated, “that appropriate animal models would be those that exhibit similar regional growth vectors and patterns to humans. Because it is obvious that a single animal model cannot be appropriate for all craniofacial regions, fitting appropriate animal models should be based on comparative data of anatomical characteristics and growth patterns of the craniofacial region of interest and the expected level of extrapolation to the human clinical condition, rather than on the phylogenetic affinity” [92]. Our study corroborates Swennen’s observations. We found significant differences in the mandibular anatomy of minipigs compared to data of humans. This raises concerns, that extrapolating acquired scientific results of Göttingen Minipigs to humans could be misleading or incorrect. This in turn suggests that Göttingen Minipigs are not ideal for experimental mandibular surgery research. Due to the lack of alternative large animal models, the authors recommend to precisely plan mandibular surgical experiments based on radiographic techniques, such as Computed Tomography, and to choose suitable age groups and use customized implants based on the mandibular dimensions as reported in this study.

5.6 Conclusions

Based on the results of this study, the authors consider the Göttingen Minipig not to be an anatomically ideal animal model for experimental mandibular surgery research. The minipig mandible not only differs greatly from that of humans but also is highly variable in its morphology within animals of the same age group. This in fact requires carefully conducted

presurgical planning using radiographic techniques, such as Computed Tomography. The minipig mandibular anatomy of younger animals (12m) is aligned more closely to that of humans. However, because of ongoing growth changes until the age of 21 months, only older Göttingen Minipigs should be used. The anatomical properties of mandible of the minipigs, i.e. the blood vessels medial to the ramus interfering with the sectional plane for MDO, can result in complications that are relevant to animal welfare and may additionally contribute negatively to their suitability.

5.7 Supporting information

S1 Dataset. Table with results of all measured parameters of the different age groups of Göttingen Minipigs.
(XLSX)

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5.8 Author Contributions

Conceptualization: Giuliano Mario Corte, Hana Hünigen, Stefan M. Niehues, Johanna Plendl.

Data curation: Giuliano Mario Corte.

Formal analysis: Giuliano Mario Corte.

Funding acquisition: Johanna Plendl.

Investigation: Giuliano Mario Corte.

Methodology: Giuliano Mario Corte, Hana Hünigen, Kenneth C. Richardson, Stefan M. Niehues.

Project administration: Johanna Plendl.

Resources: Stefan M. Niehues, Johanna Plendl.

Software: Giuliano Mario Corte, Stefan M. Niehues.

Supervision: Hana Hünigen, Stefan M. Niehues, Johanna Plendl.

Validation: Giuliano Mario Corte, Hana Hünigen, Kenneth C. Richardson.

Visualization: Giuliano Mario Corte.

Writing – original draft: Giuliano Mario Corte.

Writing – review & editing: Giuliano Mario Corte, Hana Hünigen, Kenneth C. Richardson, Stefan M. Niehues, Johanna Plendl.

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6 Discussion

6.1 Suitability of the Göttingen Minipig for dental and orofacial research

Because of the results of this thesis, the Göttingen Minipig cannot be considered an anatomically ideal animal model for dental and orofacial research. Comparative measurements showed that its overall mandibular morphology differs significantly from that of humans.

The superior region of the mandibular canal clearly showed dynamic changes over the experimental period, notably associated with a gradual loss of deeper spongy bone adjacent the distal tooth roots. When testing endosseous dental implants, the reduced alveolar bone height and variable morphology of the extensive mandibular canal may cause procedural problems. These include, for instance, the penetration of the mandibular canal during experimental procedures resulting in reduced implant stability, impaired osseointegration and damages to the inferior alveolar neurovascular bundle, causing bleeding, neurosensory alterations and pain. The dissection of the mandible revealed that the inferior alveolar nerve runs in close association with the inferior alveolar blood vessels and in close proximity to the tooth roots. Additional challenges for experimental implantation poses the narrow alveolar ridge of the minipigs. In human dentistry, these physiological dimensions would be considered as a mild alveolar ridge deficiency. An additional pitfall is the variable course of the inferior alveolar vein, having two basic patterns [75].

In the planning process of animal studies involving Göttingen Minipigs, several obstacles have been identified. The body mass has proven to be an unreliable indicator to judge skeletal development. Therefore, choosing animals for implant surgery based on physical appearance and body dimensions seems to be inappropriate. This problem is intensified by the high variability in mandibular dimensions of animals of the same age groups.

The large canine teeth constitute a high risk for complications, especially in male minipigs. Removing them would most certainly damage the outer cortices, reduce the alveolar bone to a minimum and would impede proper implant placement. When ignoring them, unintentional implant placement into the canine tooth itself is possible, resulting in poor implant stability and scientific outcome [66]. Inserting implants in the diastema is also not recommendable, due to missing agonists and missing load. In long-term studies, this can lead to atrophic conditions, which negatively influence osseointegration. Thus, the anatomical dimension of the ridge dimensions are much smaller than in the premolar and molar region.

In interventions such as MDO, care has to be taken not to harm the extensive, large, tortuous network of veins and to lesser extent arteries, immediately medial to the mandibular ramus.

These vessels are commonly interfering with the principal sectional plane for MDO procedures and damages would lead to massive and inaccessible bleeding [166].

For long-term studies on the osseointegration of endosseous dental implants, skeletally mature animals are necessary (ISO 7405:2018). However, the results of this thesis clearly demonstrated ongoing significant mandibular growth until the age of 21 months. It was often observed in literature that pre-adult, skeletally immature animals of 12 to 17 months of age were used [64, 167-169]. Interestingly, the mandibular anatomy of younger minipigs is more comparable to that of humans. Because of the described ongoing growth, only older Göttingen Minipigs should be used.

The dynamics of mastication in pigs and in humans is far from comparable. Compared to humans, pigs show a rapid rate of mastication, a dissimilar occlusion and use both sides of the dentition for crushing and grinding. The powerful transverse jaw movements [170], combined with unintentional post-operative loading by food or cage biting are difficult to avoid and disturb the process of bone formation [66]. Extensive tooth grinding, which could be repeatedly observed in pigs, could also lead to the reported complications after surgery [171]. Martinez-Gonzales et al. performed alveolar distraction osteogenesis in minipigs and noticed many complications such as dehiscence, contamination, inflammation and ultimately the instability of the distractor. The authors considered that the constant rooting and biting movements of minipigs, compromise the surgical maintenance and hygiene of the soft tissues protecting the distraction [172]. It has been reported, that gingivitis, periodontitis and accumulations of calculus are common in minipigs older than 6 months, correlating with local resorption of alveolar bone and ultimately resulting in impaired implant stability [12, 41].

With 44, the pig has the most permanent teeth of all large animal models. Pigs thus possess a denser, thicker trabecular bone, higher bone mass and lamellar bone structure similar to that of humans [56]. Differences have also been recorded in the remodelling of cortical bone. In pigs, the bone remodelling rate at tissue level, defined as the volume of bone turned over per unit volume of tissue per day, was 10% per month, compared to only 3% per month in humans [2, 173]. Bone mineral concentration, volumetric bone mineral density and fracture stress are significantly higher than in humans; whilst bone ash is lower (Figure 6.2.3-1).

When studies using miniature pigs shall be compared with each other, the differences in anatomy, physiology and in maturity at a given time point need to be carefully considered. Therefore, this breed-specific data as reported in this thesis, is very important [10].

6.2 Alternative animal models to Göttingen Minipigs

Considering the Göttingen Minipig as an unideal animal model for dental and orofacial research, what other alternative species are available and what are their advantages and disadvantages? Instead of giving a recommendation for particular suitable animal models, the ISO 7405:2018 proposes five different criteria for the selection process of the animal species [145].

- The jaws of the animals should be of sufficient size to allow normal surgical access and to accommodate the dental implant system in its form intended for use in humans.
- The used animals should be skeletally mature if appropriate for its intended use.
- The site into which the dental implant system is to be placed should have opposing teeth.
- Animals possessing a nonherbivorous pattern of masticatory jaw movements are preferable.
- In suitable animal models, oral hygiene can be maintained, either naturally or artificially.

Using animal models, the ultimate goal is to obtain transferable results, which are usable in clinical practice. Therefore, not only the anatomic properties and dimensions, masticatory patterns and oral hygiene of animals has to be considered, but also their direct and indirect responses in bone metabolism [174]. For the investigations and extrapolation of bone-implant interactions, species-specific bone characteristics such as bone composition and microstructure as well as the bone remodelling is of major importance [56]. For the following discussion on suitability, the five criteria of the ISO 7405:2018 shall now serve as basis to determine the suitability of other species for the use in dental and orofacial research. In addition, the focus is put on biological bone parameters of the different species, rather than comparing cephalometric data.

6.2.1 Small rodents

Small rodents, and especially mice and rats, represent the most commonly used species in bone research. Although they have advantages such as the ease of care and accommodation or the low costs and ethical acceptance, their small size clearly prohibits their use in endosseous dental implant testing. As ISO 7405:2018 suggests, only implant dimensions as applied in humans should be utilized. Rodents possess an inflexum pellitum, infolds of the lips, which have separated gnawing teeth from the remaining tooth row [175]. This could complicate

the surgical oral access. Their tooth morphology with two pairs of long, thin and mostly rootless incisors also as their herbivorous pattern of mastication are not comparable to the ones of human anatomy and physiology. Between the incisors and the three molars of each quadrant, a very large diastema is located. Canine and premolar teeth are mostly missing. Therefore, it is assumable that rodents would not be frequently considered as animal models for endosseous dental implants, even if they would possess comparable size ratios to humans. In contrast to larger animals, rodents possess very fragile cortices and do not show Harversian-type remodelling in the cortex [176]. Aerssens et al. studied differences in bone mineral density, mechanical competence and biochemical composition in bone specimens from different species, including human samples. They concluded, that among all examined species, the bone of rats differ the most in comparison with humans. The authors state that "the large biochemical differences in bone composition in the rat and human indicate that bone research data derived from this most frequently used animal model should be transferred to the clinical situation with utmost care" [174]. Nevertheless, a large number of studies tested size-adapted endosseous dental implants in rats and guinea pigs by using either an intraoral approach [177] or the implantation in long bones, favourably the tibia [178, 179]. Due to their even smaller size, the use of mice and hamsters is even more inappropriate. To the best of the author's knowledge there are no studies in mice, testing dental implants intra-orally for tooth replacement. However, studies with extra-oral approach in bones, such as the tibia, femur and calvaria are numerously published. Extraorally, dental implants of reduced size are inserted (lengths approaching 2 mm and a diameter around 1 mm) [180]. Hamsters are very rarely used. Only a few studies on periodontal diseases have been conducted [181].
Using rodents as osseous defect models, no CSD can be induced due to their small body dimensions [182].

6.2.2 Rabbits

Because rabbits are considered to be initial pre-clinical animal models, used for the screening of implant designs or materials prior to the use in larger animals, they are one of the most commonly used animal model in musculoskeletal research [180, 183, 184]. Rabbits are docile animals, are easy to house and handle, economic to purchase and sustain, and are reaching skeletal maturity within 7-8 months [185]. Rabbits own an inflexum pellitum and a large diastema, such as rodents do. Different to rodents, the rabbit possesses four maxillary incisive teeth and in total six maxillary and four mandibular teeth. All teeth grow continuously throughout life. Because of this continuous growth of all teeth, dental care and corrections are very important and need to be regularly done. For the testing of endosseous dental implants, its small size and herbivorous pattern of mastication is a major disadvantage. For an intraoral

approach, the dental implants would need a substantial reduction in size. Therefore, the dental implants need to have much smaller dimensions as intended for the use in humans. This is conflicting with the criteria proposed by ISO 7405:2018. Another infringement would constitute the implantation in the diastema, as no opposing teeth are developed in this region. Compared to the secondary osteal bone structure in humans, rabbits own a very different microstructure of bone, comprising of a primary vascular longitudinal tissue structure (Table 6.2.2-1) [56]. Only little data is published about the bone composition of rabbits and the only reported similarities are described for bone mineral density and fracture stress [186]. Compared to other species, rabbits possess faster bone turnover and skeletal change. This might complicate the extrapolation of results obtained from rabbits [56, 187]. Munhoz et al. tested a socket defect model in the anterior rabbit mandible. Therefore, the lower incisors were surgically extracted and the sockets remained either untreated or were filled with biomaterial. After a healing period of eight weeks, the former sockets were reopened for implant placement. Regular placements of dental implants into the mandible were possible, but the method is ethically not justifiable, as the lower incisors are inevitably important for the animals. Extraction and long-term evaluation causes major feeding issues and distress. Due to the staggered arrangement of the upper and lower canines, no direct loading situation can be simulated [188, 189]. Freilich et al. described a procedure for implant placement into the rabbit mandible using an extra-oral approach from the submandibular region and custom-made implants [190]. Other extra-oral anatomical locations for implant placements were the calvaria, fermur and knee [180].

Table 6.2.2-1: List of different key attributes for the comparison of animal and human bone quality. *Derived and modified from [56].*

Key attributes	Pig	Rabbit	Canine	Sheep/Goat
Macrostructure	++	+	++	+++
Microstructure	++	+	++	+
Bone Composition	+++	++	+++	++
Bone Remodelling	+++	+	++	++
+ least similar; **++** moderately similar; **+++** most similar				

6.2.3 Dogs

Since the 1960s, the dog has been extensively used in musculoskeletal and dental research, especially as a model for periodontal surgery. This research aims at understanding the etiopathology of periodontal diseases and to find a treatment by using growth factors and other signalling molecules [180, 183]. As demanded by the ISO 7405:2018, dogs have a nonherbivorous dentition, which is adapted to the carnivorous lifestyle. In addition, dogs have a diphyodont dentition with 28 deciduous and 42 permanent teeth. The first premolar has no deciduous precursor and at the age of five months it erupts as single tooth. Dog teeth have thin enamel, probably because carnivores eat nonabrasive food and chew little [191]. Differences to humans exist in the dogs' lack of lateral mandibular movements and missing occlusal contact for all premolar teeth. Because of the variety of different breeds, the morphologies and lengths of the dogs' skull, snout and mandible have developed very heterogeneously [192]. Therefore, only normo- and dolichocephalic dog breeds can be considered for endosseous dental implant testing. The medium-sized Beagle is the most frequently used dog breed in research. Disadvantageously, are observations of spontaneous periodontitis in this dog breed [193]. This makes them unsuitable for studies focusing on oral bone regeneration [180]. Even though Beagles are much larger than small rodents and rabbits, their mandible is significantly smaller than of any other large animal model. Therefore larger dog breeds such as mongrels, Labradors and greyhounds are more widely used in dental research [180]. Canine trabecular bone can withstand significantly higher compressive strains compared to humans bone [194]. Concerning bone composition, density and quality, Aerssens et al. point out, that based on a combination of all the parameters examined, the characteristics of human bone are best approximated by the properties of dog bone (Figure 6.2.3-1) [174]. The dog thus fulfills all five criteria of the ISO 7405:2018. However, complications in connection with the loss of the alveolar ridge after a tooth extraction and implant placement have been reported. Immediately after extraction, the bone remodelling process initiated and interfered with the successful osseointegration of new dental implants [180, 195-197]. Compared to minipigs, dogs own an even lower average alveolar bone height of 8.5 ± 2.6 mm, which means very limited space for the placement of a dental implant. In contrast to the secondary osteonal microstructure of humans, canine long bones consist of a mixture of secondary osteonal bone located in the cortical bone centre and plexiform bone, in the areas near the periosteum [56, 186]. The biggest disadvantage using dogs is their status as companion animals, as in recent times, ethical resistance is constantly increasing [56].

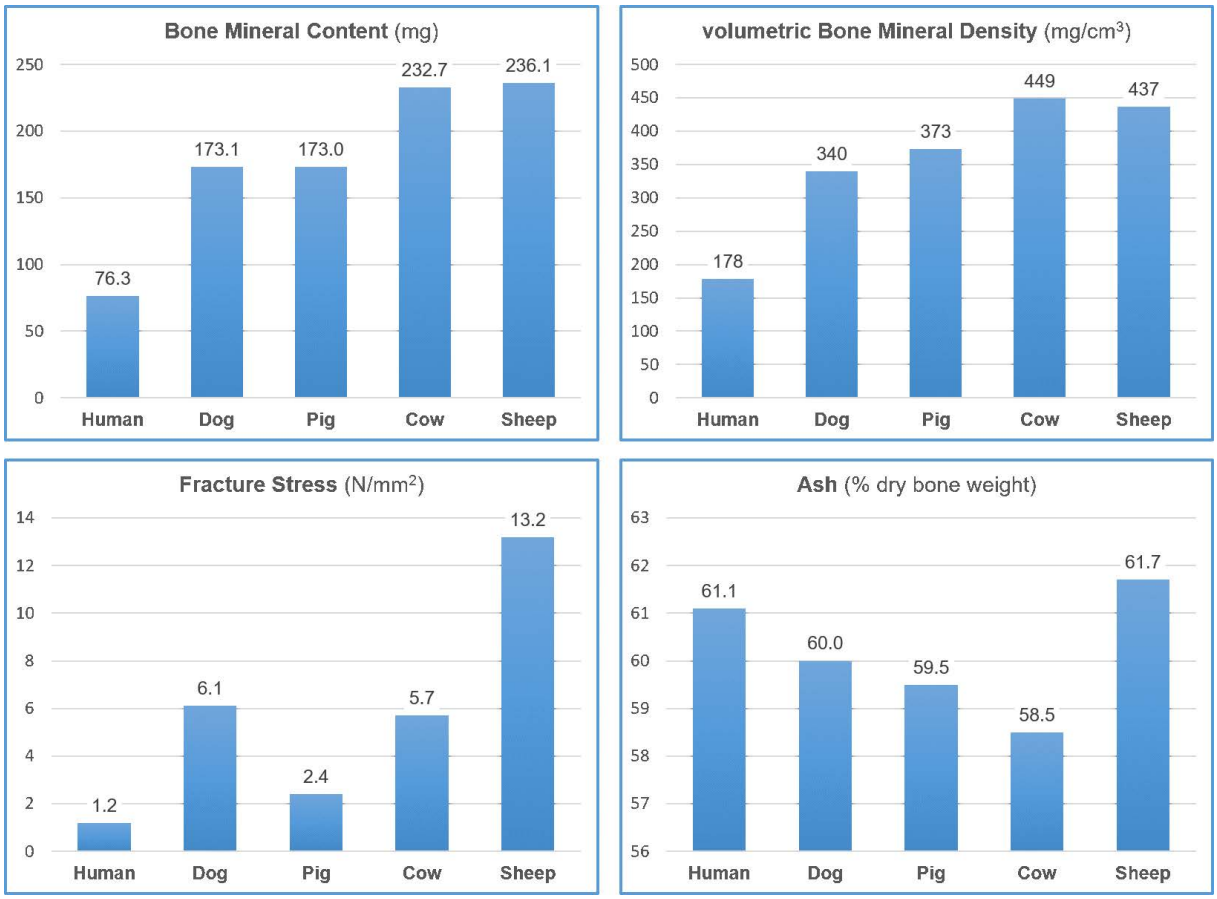

Figure 6.2.3-1: Comparison of bone parameters from five different species. *Derived and modified from [174].*

6.2.4 Sheep/Goats

Sheep and goats are considered food animals. Therefore, the emotional attachment is not the same as for companion animals such as dogs or cats. Concerning husbandry, the flock animals prefer to be housed in groups of two or more [198]. Their mandibular size is more suitable than that of small rodents and rabbits. However, their herbivorous pattern of masticatory jaw movements are contradicting the ISO 7405:2018. They only possess 32 permanent teeth, a large diastema and in the maxilla, the incisive and canine teeth are missing. The criteria of maintaining good oral hygiene was reported as very difficult to achieve and gingival recession could be observed [199]. After surgery most animals in experimental dental implant studies are fed a soft diet in order to avoid excessive loading. It is doubtful that this will be in accordance with species-appropriate nutrition of ruminants. Sheep and goat bone is very different from human bone, since it predominantly consists of primary bone structure, compared to the largely secondary bone structure in humans [176]. Equally to pigs, the sheep has a higher bone strength through a significantly stronger trabecular bone. The bone mineral content in sheep trabecular bone is three times as high as in humans and ranks first in comparison with all other large animal models. It also possesses the highest fracture stress

that is 13 times higher than in humans. The young ovine bone is plexiform, which is a combination of woven and lamellar bone. Plexiform bone is rapidly deposited but capable to provide better mechanical properties for rapidly growing, large animals such as the artiodactyls, elephants, and larger dog breeds. Plexiform bone in humans is found exclusively in the medial side of the mandibular ramus. Haversian remodelling in sheep has been seen between the age of 7 to 9 years [176, 200]. As all ruminants, sheep and goats own specific oral biomechanics. In the rare cases where they were utilized in mandibular dental implant testing, a high frequency of implant failures, due to poor implant osseointegration occurred [180, 201]. Extra-oral anatomical locations for implant placements were the tibia, femur and iliac bone.

6.2.5 Non-human primates

Of all large animal models, non-human primates are the phylogenetically most similar ones to humans [202]. Monkeys and apes, such as macaques, cynomolgus macaques, marmorsets or baboons, differ significantly in weight and size, ranging from 350 grams for certain marmorsets to baboons [180]. Compared to humans, they possess a diphyodont dentition with great similarities in tooth size, anatomy, masticatory movements and healing characteristics, making them an adequate animal model in experimental implant dentistry [182, 202, 203]. In most non-human primates, the canine teeth are prehensile and elongate. Macaques and baboons have the same dental formula as humans, whilst marmosets have one additional premolar tooth. *Macaca fascicularis* seems to be the most frequently used strain. Because all the teeth are usable, a considerable number of test sites per animal are available [202]. In general, implant loading is likely to be the most important area for using non-human primate models in implant dentistry. In contrast, studies addressing the loss of the alveolar ridge are rare. This suggests, that for the assessment of alveolar ridge pathologies, other animal models seem to have a superior suitability to monkeys. Though non-human primates are forming calculus and plaque, they rarely exhibit progressing gingival inflammation or periodontal diseases [180, 182]. Wang et al. (1998) constitute, that from a material science perspective, the bones of baboons are the most similar to humans concerning microstructural and compositional properties. Also, no significant differences in fracture stress between both species was found [186]. Dard (2012) accurately describes that “The non-human primate instinctively represents the most appropriate animal model because of its anatomical, morphological and physiological features resembling those of humans. However, clear ethical considerations arise in using these species for surgical research purposes.” [180]. Additional problems are handling difficulties, high costs and the risk of zoonotic diseases [56]. Moreover,

for studies involving non-human primates, it remains very challenging to obtain enough animals to reach statistical power [180].

6.2.6 Other species

Due to their body size, bovines and horses are not being used in dental and orofacial experimentation. They possess a herbivorous dentition with substantially bigger and morphologically different teeth. In addition, their bones are partly composed of plexiform bone structure, which is very different to humans [186].

Despite one study about ankyloses during periodontal regeneration, the cat is surprisingly not used at all [204]. This could be due to ethical reasons concerning their status as companion animals or their short mandible and small oral cavity. In addition, their difficult character can make examining and handling them quite challenging.

In the past, the ferret was used as a model to test the biocompatibility of retrograde filling materials, such as amalgam [205]. The ISO 7405:2018 even mentions ferrets as alternative animal models, however the size and number of usable teeth is very limited. In addition, some authors reported, that even in adult ferrets only the canine teeth qualify for the use in biocompatibility tests [12, 180].

6.3 Limitations of the thesis

This thesis has several limitations. The animals of the 12 months-old group were different individuals to those of the 17 and 21 months-old groups, which themselves were the same animals, measured at different time points. Therefore, the 12 months-old minipigs had to be treated as independent sample, thus preventing a more realistic comparison. This limitation was caused by reusing the CT datasets from a previously conducted study. Also because of the reuse, the animal groups only consisted of female minipigs. Sex differences could therefore not be determined. Nevertheless, it can be assumed that similar conditions as in humans are present, where in most cases, male individuals had bigger mandibular dimensions than females [206].

6.4 Conclusions

It can be confidentially stated, that no perfect animal model for dental and orofacial research exists. Each of the discussed species has its own specific advantages and disadvantages. If an animal model for dental and orofacial research would be simply chosen by its anatomical

and biologic characteristics, non-human primates surely constitute the most appropriate and suitable model. The dog, which possesses the most similar bone structure to humans, would probably be the second most suitable.

However, the stricter legal requirements regarding the use of monkeys and the societal resistance against the use of companion animals, such as dogs and cats, have become the major key deciding factor [56]. In contrast, some species such as the minipig and the sheep are considered as farm and meat animals and their use for scientific purposes might therefore be more accepted. Despite all unsuitable considerations and disadvantages of minipigs and sheep revealed in this thesis, the omnivorous minipig might be the more appropriate choice, tough it possesses a nonherbivorous dentition.

Smaller species such as the rabbit or the rat constitute the least anatomically and physiologically convenient animals, compared to the human situation. Some pre-clinical studies involving rats have failed to predict accurately the biocompatibility of dental material for human trials [182, 207]. The ISO 7405:2018 even proposes, that no rodents should be used for the biocompatibility tests, which have to be conducted anterior to the actual animal experiments. Instead, they suggest monkeys, dogs, ferrets and minipigs as suitable genera. The animals used, should have an intact and completed permanent dentition with completed root growth. Therefore, monkeys, dogs and minipigs are usable when all permanent teeth, except the third molar have erupted. Ferrets only when all four permanent canines have erupted, as only these teeth are suitable for testing. The ISO further explains that the chosen model should be the lowest animal capable of achieving the scientific purposes and causing the least possible infliction with animal welfare [145].

In contrary to ISO 7405:2018, some authors proposed that the initial screening for material biocompatibility should be entirely done using rats and mice. This would have the advantage of potentially using genetically altered disease models of mice and rats, to predict implant use in humans with osteoporosis or diabetes. Monkeys or dogs, should be restricted to the following and more advanced pre-clinical validation studies where bone loading is needed [202].

An important aspect, which urgently needs improvement, is the choice of the implantation site. When testing dental implants, the intra-oral approach probably produces the most comparable and transferable results. The use of dental implants in extra-oral approaches, such as in the tibia, the femur or the iliac crest has been observed extensively in literature [2]. These extra-oral approaches should be regarded with concerns, as the insertion of a dental implant in cortical bone, does not properly simulate its use in the mandible. It is very well known, that the mechanical properties of cortical bone are generally superior to those of trabecular bone [208]. Therefore, it can be expected that the interactions between the tissue and the material would

significantly differ between cortical and trabecular bone, especially regarding remodelling timeframes [56]. It has been scientifically proven that the simultaneous implantation in cortico-trabecular bone and pure cortical bone, for instance in the compacta of long bones, causes very different time-depending responses to the same implant material. The quadrupedal mode of life in most mammals has also to be considered. The different loading situation in long bones can act as a confounder and often ignored influence on implant stability.

In addition, as there are major differences in the vascular distribution of osteonal bone in humans and plexiform bone in large animal models, it is assumable that the responses to surgical trauma, as provoked by implantation, are not comparable. The healing and remodelling characteristics in plexiform bone can be expected to be more rapid than in osteonal bone [56].

Whatever animal model is selected, well-founded reasons against its use exist. No species is entirely fulfilling all requirements of an ideal animal model. This makes refinement methods even more important. Researchers are obliged to deepen the knowledge on different animal model species. All interspecies differences should be understood and carefully considered in order to further improve the choice of animal species and the interpretation and translation of obtained results [56]. With the ongoing technical and ethical progress in research and society, the promotion and implementation of the 3Rs should always have the highest priority.

6.5 Outlook

The results of this dissertation are the basis for an online database for cephalometric data, in order to act against data crisis and to support transferability discussions and the further refinement of different surgical purposes and presurgical planning. The database shall include published cephalometric data from other authors and on all different species used for dental and orofacial research. It is also intended to include 3D models of bones, such as the mandible, so that experimental surgeons are able to print them with 3D printers. This would allow to precisely plan and practice surgical interventions prior to *in vivo* experiments.

7 Summary / Zusammenfassung

7.1 Summary of the PhD-Thesis

Title of the PhD: **Comparative cephalometric studies of the mandible in growing Göttingen Minipigs using 3D Computed Tomography: Refining experimental dental and orofacial research.**

This thesis was created as a subproject of the Berlin-Brandenburg Research Platform, with the integrated graduate school "Innovations in the 3R Research - Genetic Engineering, Tissue Engineering and Bioinformatics". BB3R was established under the umbrella of the Dahlem Research School (DRS) of Freie Universität Berlin and aims to provide substantial progress in 3R alternatives by intensive systematic research. The overarching objective of this thesis was to establish an anatomical data collection with detailed and age-related data of the mandible and the mandibular canal of growing Göttingen Minipigs using 3D Computed Tomography and to compare this data with human mandibular anatomy. Therefore, it could be determined, whether the Göttingen Minipig represents an anatomically suitable animal model for orofacial surgery. In order to refine and avoid intra- and postoperative complications in the sense of the 3Rs, this data collection should be prospectively used in dental and orofacial experiments.

Chapter 1 generally introduces the market size, sales figures and the utilization of dental implants in dentistry and orofacial reconstructive surgery. It also focusses on the required pre-clinical testing procedures to proof the implants' biocompatibility and safety in order to be authorized for clinical testing in humans. This involves *in vivo* testing in large animal models. Due to stricter legal requirements over the use of primates for scientific purposes, the Göttingen Minipig is nowadays extensively used as animal model in pre-clinical dental and orofacial research. Nevertheless, several authors have raised concerns over the use of the Göttingen Minipig in this research area, observing complications and success rates below 60%.

Chapter 2 gives a literature review about the development of different minipig breeds, their general scientific use and the corresponding German and European animal research numbers. Then the reader is introduced to the role of the Göttingen Minipig in dental and orofacial research, how the *in vivo* procedures are performed and what complications can occur. In addition, the European law, the definitions of the 3Rs and their implementation in research are elucidated. At the end of the chapter, the technical methodologies (CT, 2D and 3D reconstruction, Cephalometry and visualization software) used in this study, are explained.

Chapter 3 lists the detailed objectives of the thesis.

Chapter 4 covers the publication “Refining experimental dental implant testing in the Göttingen Minipig using 3D computed tomography – A morphometric study of the mandibular canal”. In this study, morphometric and age-related data of the mandibular canal and the alveolar ridge of the Göttingen Minipig was reported and compared with the human anatomy, in order to avoid complications during *in vivo* testing of endosseous dental implants. Using 3D computed tomography, six parameters of the mandibular canal as well as the alveolar bone height and the alveolar ridge width were measured in Göttingen Minipigs aged 12, 17 and 21 months. The study found that the volume, length and depth of the mandibular canal all increased with age. The width of the canal did not change significantly with age. There were high individual differences within each age group, for example within the 21 months old group the animal with the lowest canal volume of 4.7ml was 14kg heavier than the animal with the significantly highest volume of 13.4ml. In contrast to humans, minipigs possess a significantly larger mandibular canal. With higher age and increasing canal volume, loss of deep spongy bone in the posterior premolar and molar regions could be observed, which potentially results in the inferior alveolar neurovascular bundle coming into close proximity with the tooth roots. This reduced the available space for dental implants and could negatively affect implant stability and the integrity of the inferior alveolar neurovascular bundle. This problem is aggravated by the minipigs’ narrower alveolar ridge width in comparison with healthy humans. Surprisingly, the body mass does not have an influence on any of the measured parameters. When one images the inferior alveolar vein, two basic patterns occur. One being a straight traverse, the other being a variable undulating route where it rises and falls as it travels through the length of the canal. Dynamic anatomical changes could be proven until the age of 21 months. Due to ongoing growth it is inadvisable to use minipigs younger than 21 months in experimental implant dentistry. Paradoxically, the measurements of the 12 months old pigs indicate a closer alignment of their mandibular anatomy to that of humans, suggesting that they may be better models for implant studies. Given the variability in mandibular canal dimensions in similar age cohorts, the use of imaging techniques is essential for the selection of individual minipigs for the interventions and thus higher success rates [75].

Chapter 5 covers the publication “Cephalometric studies of the mandible, its masticatory muscles and vasculature of growing Göttingen Minipigs – A comparative anatomical study to refine experimental mandibular surgery”. This study elucidated how comparable the mandible of Göttingen Minipigs and humans are, and whether the frequently reported complications could be caused by specific anatomical characteristics of the minipigs’ mandible, its masticatory muscles and associated vasculature. Therefore, twenty-two mandibular cephalometric parameters were measured on CT scans of Göttingen Minipigs aged between 12 and 21 months. Of the 22 parameters measured, only four were found to be highly

comparable, whilst the others were not. Again, especially younger minipigs showed a closer alignment to human anatomy. Minipigs generally showed a higher mandibular ramus, anterior mentum height and a significantly longer mandible with a much steeper mandibular angle. They in contrast possess a shorter superior ramus length and a lower coronoid process volume. The 3D examinations of the minipigs' vasculature pictured a large, tortuous network medial to the mandibular ramus, mainly consisting of the very prominent deep facial vein and maxillary artery. This vascular complex interferes with the principal sectional plane for MDO that could cause strong and inaccessible bleeding. The morphology and dimensions of the mandibular body in humans and minipigs are very different. Whilst humans have a mandibular body with an ovoid cross-section, that of minipigs can be pear-shaped. Consequently, bicortical screws that are positioned in the inferior part of the mandibular body as routinely performed in humans, could, when placed in a similar way in a Göttingen Minipig, cause trauma to the inferior alveolar nerves and vessels. Bicortical screws implanted in the inferior cortex would probably have impaired stability, due to the thin inferior bone thickness. Literature revealed that the dynamics of mastication in pigs and in humans differ greatly. Pigs have a higher crushing force and closing velocity than humans. In addition, they postoperatively grind their teeth extensively as well as bite on hard objects such as their cages that could potentially impair wound healing and implant stability. Based on the results of this study, the authors consider the Göttingen Minipig not to be an anatomically ideal animal model for experimental mandibular surgery research. The minipig mandible not only differs greatly from that of humans but also is highly variable in its morphology within animals of the same age group. This raises concerns, that extrapolating acquired scientific results of Göttingen Minipigs to humans could be misleading or incorrect. Due to the lack of alternative large animal models, the authors recommend to precisely plan mandibular surgical experiments based on radiographic techniques, such as Computed Tomography, and to choose suitable age groups and use customized implants based on the mandibular dimensions as reported in this study [166].

Chapter 6 more precisely discusses the suitability of Göttingen Minipigs as an animal model for dental and orofacial research. In addition, alternative animal models for the Göttingen Minipigs, with their anatomical and physiological advantages and disadvantages are, in regards to the recommendations of the ISO 7405:2018, discussed. The chapter ends with conclusive remarks, while giving an outlook on a database planned in the future.

7.2 Zusammenfassung der Dissertation

Titel der Dissertation: Vergleichende zephalometrische Untersuchungen zum Unterkiefer des heranwachsenden Göttinger Minischweins mittels 3D-Computertomographie: Ein Beitrag zum Refinement experimenteller dentaler und orofazialer Forschung.

Die vorliegende Doktorarbeit entstand als Teilprojekt der Berlin-Brandenburger Forschungsplattform BB3R mit integriertem Graduiertenkolleg „Innovationen in der 3R-Forschung - Gentechnik, Tissue Engineering und Bioinformatik", welche unter dem Dach der Dahlem Research School (DRS) der Freien Universität Berlin gegründet wurde. Das Ziel von BB3R ist die breite Implementierung von Alternativmethoden und tierschonenden Verfahren in der Forschung. Übergeordnetes Ziel dieser Dissertation war es, eine anatomische Datensammlung mit detaillierten und altersbezogenen Daten des Unterkiefers und des Unterkieferkanals von heranwachsenden Göttinger Minischweinen mittels 3D-Computertomographie zu erstellen und diese Daten mit solchen der menschlichen Unterkieferanatomie zu vergleichen. Dadurch konnte festgestellt werden, ob das Göttinger Minischwein ein anatomisch geeignetes Tiermodell für die orofaziale Chirurgie darstellt. Um ein Refinement im Sinne der 3R zu erzielen und intra- und postoperative Komplikationen zu vermeiden, sollte diese Datensammlung prospektiv in der experimentellen dentalen und orofazialen Chirurgie an Göttinger Minischweinen eingesetzt werden.

Kapitel 1 stellt das Marktvolumen, die Verkaufszahlen und die Verwendung von Implantaten in der Zahnheilkunde und der orofazialen rekonstruktiven Chirurgie vor. Ebenso werden die erforderlichen präklinischen Testverfahren zum Nachweis der Biokompatibilität und Sicherheit von Implantaten erläutert, um für die klinische Erprobung am Menschen zugelassen zu werden. Hierfür werden häufig *in-vivo*-Tests an Großtiermodellen durchgeführt. Aufgrund strengerer gesetzlicher Vorgaben an Tierversuche mit Primaten, wird das Göttinger Minischwein heute sehr häufig als Tiermodell in der präklinischen zahnärztlichen und orofazialen Forschung eingesetzt. Trotz allem haben sich mehrere Autoren hinsichtlich der Verwendung des Göttinger Minischweins in diesem Forschungsbereich kritisch geäußert, da sehr viele Komplikationen und ein geringer Prozentsatz erfolgreicher Setzungen von Implantaten, teilweise unter 60%, beobachtet wurden.

Kapitel 2 gibt eine Literaturübersicht über die Entwicklung der verschiedenen Minipig-Rassen, ihre allgemeine wissenschaftliche Verwendung und die entsprechenden deutschen und europäischen Tierversuchszahlen. Anschließend wird der Leser in die Rolle des Göttinger Minipigs in der dentalen und orofazialen Forschung eingeführt und mit den Abläufen von *in-vivo*-Eingriffen und deren Komplikationen vertraut gemacht. Darüber hinaus werden das

europäische Recht, die Definitionen der 3Rs und deren Umsetzung in der Forschung erläutert. Am Ende des Kapitels werden die technischen Methoden (CT, 2D- und 3D-Rekonstruktion, Kephalometrie und Visualisierungssoftware) erklärt, die in dieser Studie verwendet wurden.

Kapitel 3 listet die detaillierten Ziele der Arbeit auf.

Kapitel 4 beinhaltet die Publikation „Refining experimental dental implant testing in the Göttingen Minipig using 3D computed tomography – A morphometric study of the mandibular canal“. In dieser Studie wurden morphometrische und altersbezogene Daten des Unterkieferkanals und des Alveolarkamms des Göttinger Minischweins gemessen und mit der menschlichen Anatomie verglichen, um Komplikationen bei *in-vivo*-Tests enossaler Zahnimplantate zu vermeiden. Mittels 3D-Computertomographie wurden an Göttinger Minischweine im Alter von 12, 17 und 21 Monaten sechs Parameter des Unterkieferkanals, sowie die Höhe des Alveolarknochens und die Breite des Alveolarkamms gemessen. Die Studie ergab, dass Volumen, Länge und Tiefe des Unterkieferkanals mit dem Alter zunahmen. Die Breite des Kanals änderte sich mit dem Alter nicht signifikant. In jeder Altersgruppe konnten große individuelle Unterschiede festgestellt werden. Aus der Gruppe der 21 Monate alten Minischweine war das Tier mit dem niedrigsten Kanalvolumen von 4,7 ml 14 kg schwerer als das Tier mit dem höchsten gemessenen Volumen von 13,4 ml. Minischweine besitzen im Gegensatz zum Menschen einen deutlich größeren Unterkieferkanal. Mit zunehmendem Alter und zunehmendem Kanalvolumen konnte ein Verlust von tiefem spongiösem Knochen im hinteren Prämolar- und Molarbereich beobachtet werden, was möglicherweise dazu führt, dass sich die Gefäße und der dazugehörige Nerv des Unterkieferkanals unmittelbar an die Zahnwurzel annähert. Dies führt zur Reduktion von verfügbarem Knochen für die Setzung von Zahnimplantaten und zu verringerter Stabilität und Integrität und kann die Verletzungsgefahr der Leitungsstrukturen im Unterkieferkanal erhöhen. Dieses Problem wird durch einen, im Vergleich zum gesunden Menschen schmaleren Alveolarkamm noch einmal verschärft. Überraschenderweise hat das Körpergewicht keinen Einfluss auf die gemessenen Parameter. Bei der Visualisierung der Vena alveolaris inferior, treten zwei Konfigurationsmuster auf. Einerseits ein gerader, andererseits ein variabler Verlauf mit korkenzieherartigen Windungen, die sich während der Route durch den Kanal auf und ab bewegen. Kieferwachstum konnte bis zum Alter von 21 Monaten nachgewiesen werden. Aufgrund des anhaltenden Wachstums ist es daher nicht ratsam, Minischweine die jünger als 21 Monate sind, in Studien zu Zahnimplantaten einzusetzen. Paradoxerweise zeigen die Messungen an 12 Monate alten Minischweinen eine bessere anatomische Vergleichbarkeit, was diese Altersgruppe für Zahnimplantatsstudien potenziell geeigneter macht. Angesichts der hohen Variabilität der Unterkieferkanaldimensionen in Tieren gleicher Altersklassen, ist der Einsatz bildgebender

Verfahren zur Auswahl einzelner Minischweine und zur Erhöhung der Erfolgsaussichten von chirurgischen Interventionen von entscheidender Bedeutung [152].

Kapitel 5 befasst sich mit der Veröffentlichung „Cephalometric studies of the mandible, its masticatory muscles and vasculature of growing Göttingen Minipigs – A comparative anatomical study to refine experimental mandibular surgery“. In dieser Studie wurde untersucht, wie vergleichbar der Unterkiefer von Göttinger Minischweinen und Menschen ist und ob die häufig auftretenden Komplikationen bei Göttingen Minipigs durch spezifische anatomische Merkmale des Unterkiefers, seiner Kaumuskulatur und Gefäße verursacht werden. Daher wurden 22 zephalometrische Parameter an CT-Aufnahmen des Unterkiefers Göttinger Minischweine im Alter zwischen 12 und 21 Monaten gemessen. Von diesen 22 Parametern erwiesen sich nur vier als gut vergleichbar, die anderen dagegen nicht. Wiederum zeigten insbesondere jüngere Minischweine eine bessere Übereinstimmung zur menschlichen Anatomie. Minipigs hatten im Allgemeinen einen höheren Kieferast, ein höheres Kinn und einen signifikant längeren Unterkiefer mit deutlich steilerem Unterkieferwinkel. Sie besitzen im Gegensatz zum Menschen einen kürzeren oberen Kieferastbereich und ein geringeres Volumen des Processus coronoideus. Die 3D-Visualisierung des Gefäßsystems der Minischweine zeigten ein ausgedehntes, gewundenes Netzwerk medial des Unterkieferastes, das hauptsächlich aus der sehr ausgeprägten, tiefen Gesichtsvene und der Oberkieferarterie bestand. Dieser Gefäßkomplex interferiert mit der Schnittführung von Unterkiefer-Distraktionsosteogenesen, was starke und unzugängliche Blutungen zur Folge haben kann. Die Morphologie und Dimensionen des Unterkieferkörpers von Mensch und Minischwein sind sehr unterschiedlich. Während Menschen einen Unterkieferkörper mit hochovalem Querschnitt besitzen, kann der von Minischweinen Birnenform annehmen. Infolgedessen könnten bikortikale Schrauben, die beim Menschen im unteren Teil des Unterkiefers routinemäßig eingesetzt werden, bei vergleichbarer Positionierung im Minischwein, zu Verletzungen des Unterkiefernerv und der Gefäße führen. Bikortikale Schrauben, die in die untere Kortikalis implantiert werden, würden zudem höchstwahrscheinlich aufgrund der dünnen Wandstruktur eine beeinträchtigte Stabilität besitzen. Der Fachliteratur war zu entnehmen, dass die Dynamik des Kauvorgangs von Schweinen und Menschen sehr unterschiedlich ist. Schweine haben eine höhere Mahlkraft und Schließgeschwindigkeit. Außerdem knirschen sie postoperativ ausgiebig mit den Zähnen und kauen auf harten Gegenständen wie Käfigstangen, was möglicherweise die Wundheilung und die Stabilität des Implantats beeinträchtigen könnte. Basierend auf den Ergebnissen dieser Studie bewerten die Autoren das Göttinger Minischwein als ein anatomisch nur bedingt geeignetes Tiermodell für die experimentelle dentale und orofaziale Forschung. Der Unterkiefer des Minipigs unterscheidet sich nicht nur stark von dem des Menschen, sondern ist in seiner Morphologie auch bei Tieren gleicher Altersgruppen sehr unterschiedlich. Dies gibt Anlass zur Sorge, dass

die Übertragbarkeit der von Göttingen Minischweinen erworbenen wissenschaftlichen Ergebnisse auf den Menschen, irreführend oder falsch sein könnten. Aufgrund des Fehlens alternativer Großtiermodelle, empfehlen die Autoren die sorgfältige Planung chirurgischer Eingriffe am Unterkiefer mittels bildgebenden Verfahren wie der Computertomographie, um auf Grundlage der in dieser Dissertation angegebenen Dimensionen maßgeschneiderte Implantate und geeignete Altersklassen zu entwerfen und auszuwählen [153].

In Kapitel 6 wird die Eignung des Göttinger Minischweins als Tiermodell für die dentale und orofaziale Forschung diskutiert. Darüber hinaus werden alternative Tiermodelle mit ihren anatomischen und physiologischen Vor- und Nachteilen aufgeführt und mit den Empfehlungen der ISO 7405:2018 diskutiert. Das Kapitel endet mit abschließenden Bemerkungen und Denkanstößen und gibt einen Ausblick auf eine geplante Datenbank.

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9 List of Publications

Original articles (peer-reviewed)

Corte, G. M.; Hünigen, H.; Richardson, K. C.; Niehues, S. M.; Plendl, J. (2019):
Cephalometric studies of the mandible, its masticatory muscles and vasculature of growing Göttingen Minipigs - A comparative anatomical study to refine experimental mandibular surgery.
PLoS ONE; 14(4): e0215875; doi: 10.1371/journal.pone.0215875

Corte, G. M.; Plendl, J.; Hünigen, H.; Richardson, K. C.; Gemeinhardt, O.; Niehues, S. M. (2017):
Refining experimental dental implant testing in the Göttingen Minipig using 3D computed tomography - A morphometric study of the mandibular canal.
PLoS ONE; 12(9): e0184889; doi: 10.1371/journal.pone.0184889

Gemeinhardt, O.; Poch, F. G.; Hiebl, B.; Kunz-Zurbuchen, U.; Corte, G. M.; Thieme, S. F.; Vahldiek, J. L.; Niehues, S. M.; Kreis, M. E.; Klopfleisch, R.; Lehmann, K. S. (2016):
Comparison of bipolar radiofrequency ablation zones in an in vivo porcine model: Correlation of histology and gross pathological findings.
Clinical hemorheology and microcirculation; 64(3): 491-499; doi: 10.3233/CH-168123

Demeler, J., Knapp, F., Corte, G. M., Katzschke, O., Steininger, K., von Samson-Himmelstjerna, G. (2012):
Recovery of strongylid third-stage larvae from herbage samples: standardisation of a laboratory method and its application in the field.
Parasitology research; 110(3): 1159-1164; doi: 10.1007/s00436-011-2606-y

Abstracts in proceedings and conference participations

Corte, G.M.; Humpenöder, M.; Pfützner, M.; Merle, R.; Ladwig-Wiegard, M.; Thöne-Reineke, C.; Plendl, J. (2018):
SimulRATor – Manufacturing a 3D-printed simulator of the rat for laboratory animal training courses.
18th Annual Congress of EUSAAT. Linz, Austria – 23.09.-26.09.2018.
In: Altex Proceedings, 7(2), p. 37. Talk
Awarded with the Young Scientist Travel Award 2018

Humpenöder, M.; Corte, G. M.; Pfützner, M.; Merle, R.; Ladwig-Wiegard, M.; Plendl, J.; Thöne-Reineke, C. (2018):
3Rs in education – systematical evaluation of simulators for rats and mice.
18th Annual Congress of EUSAAT, Linz, Austria. 23.09.-26.09.2018.
In: Altex Proceedings, 7(2), p. 100. Talk
Awarded with the Young Scientist Travel Award 2018

Humpenöder, M.; Corte, G. M.; Pfützner, M.; Ladwig-Wiegard, M.; Merle, R.; Plendl, J.; Thöne-Reineke, C. (2018):
Training with simulators for rats and mice as Refinement in education.
11. Doktorandensymposium & DRS Präsentationsseminar “Biomedical Sciences”. Berlin, Germany – 21.09.2018. Talk
In: 11. Doktorandensymposium & DRS Präsentationsseminar "Biomedical Sciences" Mensch und Buch-Verlag Berlin, p. 18

Corte, G. M.; Hünigen, H.; Niehues, S. M.; Richardson, K.; Plendl, J. (2018):
Refining experimental dental implant testing in Göttingen minipigs using 3D computed tomography.
The 32nd Congress of the European Association of Veterinary Anatomists. Hannover, Germany – 25.07.-28.07.2018. Poster
In: Anatomia, histologia, embryologia; 47(S1), p. 15
Awarded as the best poster of the session “Musculoskeletal System”

Corte, G. M.; Pfützner, M.: Humpenöder, M.; Thöne-Reineke, C.; Plendl, J. (2018):
SimulRator - Manufacturing a 3D-printed simulator of the rat for medical experimental training.
The 32nd Congress of the European Association of Veterinary Anatomists. Hannover, Germany - 25.07.-28.07.2018. Poster
In: Anatomia, histologia, embryologia; 47(S1), p. 15

Bahramsoltani, M.; Corte, G. M.; Rieger, J.; Lübbe, K. (2018):
Constructive alignment in teaching anatomy.
The 32nd Congress of the European Association of Veterinary Anatomists. Hannover, Germany - 25.07.-28.07.2018. Poster
In: Anatomia, histologia, embryologia; 47(S1), p. 9

Corte, G. M.; Humpenöder, M.; Pfützner, M.; Thöne-Reineke, C.; Plendl, J. (2018): **Simulatoren der Ratte und Maus als Alternativ- und Ergänzungsmethode zum Tierversuch.** 37. Jahrestagung der Deutschen Gesellschaft für Klinische Mikrozirkulation und Hämorheologie e.V. Neubrandenburg, Germany – 08.06.-09.06.2018. Talk

Corte, G. M.; Humpenöder, M.; Pfützner, M.; Merle, R.; Ladwig-Wiegard, M.; Thöne-Reineke, C.; Plendl, J. (2018):
Simulatoren der Ratte und Maus – Tierschutz oder Spielzeug?
PhD Symposium of the 4th BB3R Spring School. Berlin, Germany 09.04.2018. Talk
Awarded with the 1st prize for best Speed Talk.

Corte, G. M.; Hünigen, H.; Niehues, S. M.; Plendl, J. (2017):
Anatomy for 3R-relevant refinement of animal experiments: Dental implant testing in Göttingen Minipigs™.
10. Doktorandensymposium & DRS Präsentationsseminar "Biomedical Sciences". Berlin, Germany – 22.09.2017. Poster
In: 10. Doktorandensymposium & DRS Präsentationsseminar "Biomedical Sciences" Mensch und Buch-Verlag Berlin, p. 33
Awarded with the 3rd prize for best poster.

Corte, G. M.; Hünigen, H.; Niehues, S.; Plendl, J. (2017):
Refining experimental dental implant testing using 3D computed tomography – A morphometric study of the mandibular canal in Göttingen Minipigs.
9th Young Generation of Veterinary Anatomists Meeting. Brno, Czech Republic – 12.07.-14.07.2017. Poster
In: YGVA Proceedings of the 9th Meeting of the Young Generation of Veterinary Anatomists – M. Kyllar, P. Čížek (Publishers), pp. 58–59

Corte, G. M.; Plendl, J.; Hünigen, H.; Richardson, K. C.; Niehues, S. M. (2017):
Refining experimental dental implant testing using 3D Computed Tomography - A morphometric study of the mandibular canal in Göttingen Minipigs.
JRC Summer School on Alternative Approaches to Risk Assessment. Ispra, Italy – 16.05.-19.05.2017. Poster
In: Abstract book, p. 41

Corte, G. M.; Hünigen, H.; Niehues, S. M.; Plendl, J. (2017):
Refining Experimental Dental Implant Testing in the Göttingen Minipig Using 3D Computed Tomography – A Morphometric Study of the Mandibular Canal
9th BB3R PhD Symposium, Berlin, Germany – 15.03.2017. Talk

Gemeinhardt, O.; Poch, F.; Zurbuchen, U.; Buhlmann, A.; Corte, G.; Vahldiek, J.; Thieme, S.; Niehues, S.; Kreis, M.; Lehmann, K. (2016):
Korrelation von Makroskopie und Histologie bipolarer Radiofrequenzablationszonen der Leber am in vivo-Schweinemodell
71. Jahrestagung der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten. Hamburg, Germany – 21.09. – 24.09.2016 Talk
In: Zeitschrift für Gastroenterologie 08/16

Corte, G. M.; Plendl, J.; Niehues, S. M.; Hünigen, H. (2016):
Assessing mandibular morphometric changes in the growing Göttingen Minipig using 3D Computed Tomography.
31st Conference of the European Association of Veterinary Anatomists. Vienna, Austria – 27.07.-30.07.2016. Poster
In: Anatomia, histologia, embryologia; 45 (Suppl. 1), p. 18

Corte, G. M.; Plendl, J.; Hünigen, H.; Niehues, S. (2015):
Refinement of orofacial surgical experiments in the Göttingen minipig™: Morphometry of the mandibula by 3D-computed tomography.
16th Annual Congress of EUSAAT. Linz, Austria – 20.09.-23.09.2015. Poster
In: Altex Proceedings; 4(2), p. 50
Awarded with the Young Scientist Travel Award 2015

Corte, G. M.; Plendl, J.; Hünigen, H.; Niehues, S. (2015):
3D-CT morphometric studies of the mandibula in Göttingen Minipigs.
8th Meeting of the Young Generation of Veterinary Anatomists. Poznan, Poland – 15.07.-17.07.2015. Talk
In: 8th Meeting of the Young Generation of Veterinary Anatomists (YGVA) – Faculty of Veterinary Medicine and Animal Science (Publisher), p. 3.

Corte, G. M.; Plendl, J.; Hünigen, H.; Niehues, S. M. (2015):
Morphometric 3D-CT studies in pigs and minipigs.
BB3R Seminar, Berlin, Germany – 07.07.2015. Tal

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11 Declaration of Authorship

I hereby declare that the thesis submitted is my own unaided work. All direct or indirect sources used are acknowledged as references.

This thesis was not previously presented to another examination board and has not been published.

Berlin, den 12.11.2019

Giuliano Mario Corte

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