

# Enrichment of Göttingen Minipigs: Models of human hepatocellular carcinoma

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The pig has long been a meaningful model in biomedical research. It provides hope for successful xenotransplantation and the development of induced pluripotent stem cell technology in the field of regenerative medicine. Insulin obtained from pigs is used to treat diabetes. Pig heart valves are being surgically placed into humans.

We are using Göttingen minipigs to achieve our goal of developing an animal model of human hepatocellular carcinoma (HCC), including background cirrhosis that would facilitate the development of diagnostic and prognostic therapies or markers. HCC is prevalent all over the world. Sorafenib is the only approved systemic therapy for patients with advanced disease, but its effect on overall patient survival is modest and the time to develop drug resistance is short. Studies with laboratory mice have failed to produce similar effects in clinical trials with humans. Despite thousands of publications on HCC studies with mice and other small-animal models, we are still in dire need of a model that mimics all the phases of HCC. Ultimately, large-animal models are needed to determine the diagnostic and prognostic relevance of the heterogeneous attributes of HCC. For our study, the cancer-causing agent diethylnitrosoamine (DENa) is being used to develop this model. The effects of the carcinogen DENa on the liver of pigs was initially described in 1977 and DENa is now known to cause liver cirrhosis. Because in most persons

liver cirrhosis preceded HCC, the swine model mimics patient conditions. Swine have continued to serve as a laboratory model to study a variety of human treatments since 1980. We expect our findings to reinforce the applicability of the swine model in the search for new therapies that can be translated into human clinical trials, reveal new areas of application of pig biology that would benefit humans, and demonstrate that the pig is good model for molecular mechanistic studies of human cancer. Our study is the first use of the Göttingen Minipig in cancer research. The findings will be highly relevant to patients with cirrhosis and a high risk of HCC.

It is our goal to characterize the DENa-induced HCC model in the pigs and identify its applicability in the search for new therapies that can be translated into human clinical trials and hopefully demonstrate that the pig is a good model for HCC in humans.

I would like to share our experiences with enrichment of Göttingen minipigs. We welcomed four animals from Marshall BioResources to our facility in January 2013. Each weighed about 10 kg upon arrival and currently weighs 14–15 kg. The animals eat a minipig grower diet, which consists of an average of 200 g (based on animal weight) daily.

Social or pair-housing is our main form of swine enrichment. The minipigs are group-housed in stalls that have sliding divider doors/panel which allow separation of animals for feeding and study procedures, as needed. The objective of the group housing is to meet the social needs of the minipigs and improves their pro-social behaviours.

Ideally, enrichment is designed to draw out behaviours that would be exhibited in nature (i.e., rooting for pigs). Not only do pigs display rooting while feeding, but larger “wobble” toys are great for drawing out this specific action: when nudged, these toys nudge back. The interaction is stimulating. Toys that can be pushed around, chewed on, or dug into appear to be used frequently and occupy the time of the minipigs, and appear to facilitate rooting and other natural behaviours. However, with continued exposure, a loss of interest in toys has been noticed. Manipulanda should be rotated regularly to maintain the animal’s interest and natural behaviour. We switch the toys every couple of days to prevent the minipigs from becoming bored.

In addition to enriching toys and manipulanda, we also frequently use novel treats for enrichment. Candy corn is used as a reward for weighing, receiving an injection, or being picked up or restrained. Medication in powder or pill form is drizzled with honey or hidden in yoghurt to encourage intake. The minipigs are also occasionally treated to raisins, fruits, popsicles, or Dum-Dum suckers. While eating these sweets is certainly not indigenous behaviour, their sugary attraction can be very useful





when a pig is required to perform an “unnatural” behaviour. Exposure to these tasty treats, typically more enticing than toys to the Göttingen minipigs, should be limited to small quantities to avoid distracting them from their normal diet or causing an undesirable increase in weight.

Enrichment is critical in laboratory animal medicine, especially for intelligent animals such as pigs, that are not in their natural environment. The goal of our animal team is to create a positive environment for the Göttingen minipigs while they are in our facility. Our small group is easily maintained with a variety of enrichment activities. Husbandry and veterinary interactions with the animals have been improved with the use of these toys and food items.

Listed are some of the toys we used for the enrichment of our minipigs:

- Big Red Apple: polyethylene, apple scented, can be hung (Bio-Serv)
- Kong Genius: rubber, designed to be filled with treats (Bio-Serv)
- Busy Buddy Football: rubber, has slots for hiding food treats (Bio-Serv)
- Jingle Ball: polyethylene, noisemakers inside, has holes that a chain can be attached to for hanging (Bio-Serv)
- Blue Kong: rubber, can be filled with treats (Bio-Serv)
- Precious Gem: polyethylene, can be filled with treats (Bio-Serv)
- Bobbin: rolls easily, can be pushed around the run (Otto-environmental)
- Sauras Egg: polyethylene, large oval toy with a weighted bottom that pops back up when it is pushed over (Otto-environmental)

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