See where you can meet us in 2015

The 2015 Meeting of the Minipig Research Forum

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Clean pigs for clear results
Dear Reader

Spring is just around the corner and not only nature is changing, so is the Ellegaard organization. As most of you already know, I was recently appointed the new CEO of Ellegaard Göttingen Minipigs. This is for me a very exciting challenge and an excellent chance to use my experience, scientific knowledge and not least huge interest within the field of laboratory animal science. At the same time it is a great opportunity for me to meet, network and collaborate with both new and old colleagues and friends.

Looking back, 2014 was another good year for Ellegaard Göttingen Minipigs with growth in both the European, US and Japanese markets through a fruitful collaboration with our partners Marshall BioResources in the US and Oriental Yeast Company in Japan. Furthermore, we signed a contract with Woojung BSC in Korea and are now able to supply minipigs to the Korean research community. We also accomplished our AAALAC re-accreditation in 2014 and had continuous focus on animal welfare initiatives both for our own breeding colony, but also through exchange of knowledge with our customers and the arrangement of training courses.

We believe that 2015 will be another good and exciting year for the company. Jens Ellegaard will still be an important part of the team and will in the future focus on strategy initiatives and business development. We will focus much more on scientific collaborations and animal model development and will as always support all of you and do our utmost in living up to our company values - animal welfare, quality, respect and collaboration.

I already visited some of you, spent a week in Japan visiting Oriental Yeast Company and several Japanese customers and soon I will visit Marshall BioResources and meet more of you at the SOT meeting in San Diego. Another upcoming event is the Minipig Research Forum in Rome, 21-22 May 2015, which again is a great opportunity to gain and exchange knowledge at the scientific sessions and to network throughout the event.

Please enjoy reading the Newsletter.

Yours sincerely,
Lars Friis Mikkelsen, CEO

New Veterinarian at Ellegaard

In November 2014 Ann-Sofie Cæcilie Søndergaard started at Ellegaard Göttingen Minipigs as Head of Veterinary Services. Ann-Sofie has a MSc in Veterinary Medicine from the University of Copenhagen where she completed her Master’s Thesis on non-invasive stress evaluation in relation to animal welfare. After her graduation Ann-Sofie worked at a small animal practice before joining Ellegaard. Ann-Sofie is devoted to animal health and welfare and looks forward to providing veterinarian support and guidance to our customers as well as keeping Ellegaard at a high standard of both health and welfare of the Göttingen Minipigs.

Ann-Sofie lives in Copenhagen together with her two cats and in her spare time she very much enjoys to travel. We hope that you will have the opportunity to meet Ann-Sofie and discuss your work with minipigs. You are welcome to contact her (veterinary@minipigs.dk)

if you have any veterinary questions or challenges regarding the use of our minipigs.

New CEO

As of February 1st 2015 Lars Friis Mikkelsen was appointed as the new CEO of Ellegaard Göttingen Minipigs. In close collaboration with Jens Ellegaard and the Board of Directors, Lars will develop Ellegaard further in the minipig business based on the company values, long standing relationship with our business partners, and high quality minipigs, which Ellegaard’s business is built on.

Lars holds a veterinary degree and a master degree in laboratory animal science from Copenhagen University as well as an Executive MBA from the National University of Singapore.

During his career, Lars worked in general veterinary practice, before he in 2000 joined the pharmaceutical industry, first by working at Novo Nordisk in Copenhagen and later at Merck/MSD in Singapore with responsibilities including both scientific and business areas. At Novo Nordisk, he was globally responsible for external preclinical collaborations and lobbying in relation to the use of research animals and at Merck/MSD in Singapore, Lars was Director of In Vivo Pharmacology and established and headed the In Vivo Pharmacology Section with special focus on non-human primate animal models within diabetes, obesity, dyslipidemia and CNS related therapeutic areas.

Jens Ellegaard will continue as part of the Ellegaard organization as Director of Strategy & Business Development and will concentrate on the strategic development of the company including the continued good collaboration with our business partners and strategic customers.

We are looking forward to introducing you to Lars.
Update from North America

It has been yet another frigid, snowy winter in Upstate New York, however, that has not affected demand for Gottingen Minipigs. After a record breaking year in 2014, we are still experiencing increased demand, and the first quarter of 2015 is on track to be our best quarter ever.

With the opening of our new housing space this past November, we have already increased production over 40%, and our new building will allow us to eventually increase supply by up to 60%. Availability of minipigs from our facility is improving, and supply should grow over the next few months as we continue to see the results of our production increases.

This past September we hosted a Gottingen Minipig Training Course in Rochester, NY. This course reviewed normal swine behavior, enrichment and socialization, and offered hands on experience with handling, restraint and dosing methods. We also offered training on the placement of central venous catheters for blood collection. We are happy to offer customized training for our customers in North America. Please contact us at infous@marshallbio.com if you are interested in learning more about our training program and the services we can offer.

We also presented our data on sexual maturity development in the male Gottingen Minipig via a poster at the American College of Toxicology 35th Annual Meeting, held November 9-12th, 2014 in Orlando, FL. This work was completed in collaboration with the University of Pennsylvania. Histological preparation and analysis was performed at the Reference Andrology Laboratory at New Bolton Center (University of Pennsylvania, Kennett Square, PA, USA). Sexual maturity was defined by the presence of elongated spermatids in the seminiferous tubules along with the presence of epididymal spermatozoa. We found that male Gottingen Minipigs appear to reach sexual maturity at 8 weeks of age, and with some males mature as early as 6-7 weeks of age. A copy of the poster can be found on page 4. Our next step will be to evaluate the influence of the environment on the onset of puberty, especially in female Gottingen Minipigs.
Introduction

The use of the Göttingen Minipig for the toxicity testing of new drugs and chemicals has been increasing over the past few decades. Minipigs are a useful model for evaluating if a compound causes toxicity to the male reproductive organs or has an adverse effect on male fertility (Jørgensen et al., 1998). Fertility rate, percentage of morphologically abnormal sperm, percentage of progressively motile sperm, and incidence of cryptorchidism in the minipig all have close similarities to men (Svendsen, 2006). The stages of spermatogenesis in the minipig have been described, with a reported spermatogenic cycle of 35 days (Jørgensen et al., 1998). However, no precise data has been published comparing both temporal and histological changes in the minipig testes and epididymides in relation to sexual maturity. It is important to document the histological characterization of sexual maturity, and when maturity can be observed, as the use of immature minipigs can confound interpretation of testicular toxicity. The purpose of this study was to evaluate the testis tissue from young Göttingen Minipigs to determine the age of puberty, which was defined in this study as the majority presence of elongated spermatids in the seminiferous tubules/tubule lumen.

Methods

A total of 24 male Göttingen Minipigs were evaluated, ranging in age from 5-8 weeks (six boars/week of age). All minipigs were weaned at 4 weeks of age, and were subsequently housed with age matched males until the tissue collection. The animals were housed on a 12 hour light/dark schedule, and they had no contact with mature breeding boars, so as to avoid influence on their reproductive development.

Tissues were collected postmortem, and all procedures followed were in accordance with the policies of the Institutional Care and Use Committee at Marshall BioResources. Paired tissue samples were collected from both testes and epididymides, and were immediately fixed in Bouin’s solution for 24 hours. The samples were then sent to the Reference Andrology Laboratory at New Bolton Center (University of Pennsylvania, Kennett Square, PA, USA) for histological preparation and examination.

Tissue samples (3 areas/testis) were dehydrated in an ethanol series and embedded in paraffin wax, followed by histological sectioning (4-6 µm) and routine hematoxylin-eosin (H&E) staining (Kiernan, 2008). Randomly selected seminiferous tubules (n= 200 per testis) were assessed by a single researcher via light microscopy (x200 magnification). Cell types (e.g., primary spermatocytes, round and elongated spermatids, luminal sperm) were determined through observation of morphological characteristics (França et al., 2005), and each tubule was grouped based on the most advanced cell type present within the tubule.

Statistical analyses were performed using SPSS®, version 20 (IBM, SPSS Inc., Chicago, IL, USA). The descriptions of the most advanced cell types in the seminiferous tubules were considered as a percentage for each age group. Comparisons of cell type distribution by week (age) were performed using a Kruskal Wallis test, with the differences between weeks determined using a Mann Whitney comparisons test. To determine the age of sexual maturity, an analysis comparing maturity category by age was performed using Chi Square analysis. Significance was set to P<0.05 for all tests.

Results

As would be expected, the percentage of cell types observed changed based on tissue, collected at 5 weeks (85.8±4.4%). At 8 weeks of age, 77.8±4.7% of tubules significantly (P<0.05) higher than that observed for all other weeks. Figure 1 shows differences (p<0.05) were found between the right and left testes, as shown in Figure 2.

The samples were categorized based on the evaluation of the cells found in the tissue from the method previously described by Lervik et al. (2013). The categories are listed in Table 2 and shown in Figure 2. Our findings demonstrate that the categories are considered immature, and at 8 weeks all were mature.

<table>
<thead>
<tr>
<th>Category</th>
<th>Sexual stage</th>
<th>Criteria to quantify the level of spermatogenesis in seminiferous tubules/epididymides of male minipigs</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>Immature</td>
<td>Seminiferous tubules show predominantly spermatagonia and (&gt;70%) primary spermatocytes.</td>
</tr>
<tr>
<td>II</td>
<td>Transitional (Peri-pubertal)</td>
<td>Seminiferous tubules show spermatagonia, spermatocytes and (&gt;50%) round/elongated spermatids. Few, if any, spermatoza in lumen.</td>
</tr>
<tr>
<td>III</td>
<td>Sexually Mature</td>
<td>Seminiferous tubules show a full spermatogenetic cycle, including the presence of spermatoza in both the seminiferous tubule lumen and in the epididymides.</td>
</tr>
</tbody>
</table>

Representative histological images of testis and epididymal tissue from immature and mature samples are presented below in Figure 3.

Minipig: Assessing the Onset of Puberty

Jumbo B¹, Althouse G².
University of Pennsylvania, Kennett Square, PA, USA.

Discussion and Conclusion

The onset of puberty (e.g., sexual maturity) was defined histologically in this study based on the presence of elongated spermatids in the seminiferous tubules in addition to the presence of epididymal spermatozoa. Based upon these findings, male Göttingen Minipigs appear to have reached sexual maturity by 8 weeks of age, with some reaching maturity as early as 6-7 weeks of age. This is consistent with Smith & Roth (1970) who also described the detection of spermatozoa in the lumen of the seminiferous tubules by as young as 30 days of age in Göttingen Minipigs. In contrast, the onset of puberty in boars used for conventional commercial pork production is considered to be between 120-180 days of age (Allrich et al., 1983; Young et al., 1986; Trudade et al., 1992). Spermatozoa are absent at 100 days of age and first appear in the seminiferous tubule lumen at 130 days (Allrich et al., 1983). Göttingen Minipigs appear to reach maturity younger than conventional swine breeds, and our study demonstrates that Göttingen Minipigs mature even earlier than previously reported in the literature (Bode et al., 2010; Jørgensen et al., 1998).

This work serves to complement the work by Tortoreau et al. (2013) and De Rijk et al. (2014) which examined sexual maturity in female Göttingen Minipigs. In a production setting, it is well known that the onset of puberty can be influenced to some degree through manipulation of the environment. Infl uential factors can include contact with mature boars, light cycle, increased access to diet, and stressors such as introduction to new conspecífics (Agricultural and Horticultural Development Board, 2010; Andersson et al., 1998; Kemp et al., 2005; Patterson et al., 2002; Prunier & Mounier, 1991). Future research could further evaluate the extent of these external factors on the development of puberty in young Göttingen Minipigs.

References

Management of Pregnant Sows and Cross-fostering

By Anette Blak Grossi and Adrian Zeltner, Ellegaard Göttingen Minipigs A/S

There is increasing interest in using minipigs for reproductive and juvenile toxicity studies, which also prompts a need for information about the normal reproductive characteristics of the Göttingen Minipig. Correct handling of the sow before, during and after farrowing is crucial for the ease of handling, sow well-being and piglet survival rates. The purpose of this article is to share the lessons we have learned in our management of pregnant sows and the cross-fostering of piglets.

At Ellegaard Göttingen Minipigs A/S, breeding animals are selected based on a number of phenotypic and genetic criteria. Fertilization is by natural mating, and the female minipig is mated for the first time at the age of 7.0–7.5 months. Usually, Göttingen minipigs have two litters a year.

The average length of gestation is 115 days and depends on factors such as the number of piglets, where farrowing in sows with only 1 or 2 piglets may be delayed up to ten days (Figure 1). Reproductive failure in Göttingen minipigs is generally low, with a pregnancy success rate following the first mating attempt of about 91% and a farrowing percentage of 86%. The average litter size is 7.2 piglets, but varies between inexperienced and experienced sows (see Figure 2).

Socialization:

Pregnant sows should be transferred to the farrowing pen 7–10 days prior to the due date. (For details on pen requirements, please contact us: handling@minipigs.dk). To calm the sows and make them easier to work with during farrowing and nursing, caretakers should enter the pen several times a day and take time to rub the sow’s back and udder. This will also make it easier to get a sense of how close to farrowing the sow is. The udder will be harder, and the teats will have milk in them as the sow nears parturition.

Feeding and water supply:

It is essential for sows to get the correct amount of feed up to farrowing, so that they have enough energy to farrow, but also to avoid problems with constipation and prolonged farrowing, as well as with reduced feed intake and milk production after farrowing. The following is based on Standard Minipig Diet (SMP) diet supplied by SDS. (http://www.sdsdiets.com/) and is only a guide: The eating habits and general condition of the sow must be observed closely, and the amount of diet should be decreased or increased accordingly.

Feed twice a day with 370 grams each time. During parturition, the sow might not want to eat, but food should be offered when parturition has concluded.

After farrowing, increase the amount of diet by 50% (2 x 550g). Depending on the sow’s appetite and condition, add around 90 g per feeding every day and observe whether the sow eats up. Some days after farrowing, the feeding regime is essentially ad libitum. Any diet left in the feeding trough one hour after feeding must be removed, and the trough should be cleaned. If a sow has not emptied her feeding trough within one hour after feeding, reduce her diet until her appetite is back. Sows with problems keeping their weight may be fed three times a day. The sows must have access to plenty of water, either from a water nipple supplying a minimum of 2–4 litres per minute or from a well-attached bowl.

Figure 1:

The length of gestation in minipigs varies, but most sows farrowed on days 114–116.

<table>
<thead>
<tr>
<th>Gestation length in days</th>
<th>No. of farrowings</th>
</tr>
</thead>
<tbody>
<tr>
<td>108</td>
<td>5</td>
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<tr>
<td>110</td>
<td>10</td>
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<td>112</td>
<td>15</td>
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<td>124</td>
<td>5</td>
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<td>126</td>
<td>5</td>
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Parturition and care for new-born piglets:
Sows usually become more restless and start nest-building behaviour a day or two before farrowing. For this purpose, it is important to provide adequate bedding material in the pen. (Link to video with nest-building sow: www.minipigs.dk/). Vaginal discharges are sometimes seen within 3–4 days of farrowing when a thick viscous and odourless material may be excreted. When the sow lies down in the nesting area and starts to go into labour, it is imperative not to disturb her. Sows often farrow during the night, but most do very well at parturition and no
help is needed, nor can much be given. If the sow has been in labour for more than two hours, and no piglets are born, take her out of the pen and walk her for 5–10 minutes in the aisle. It is good if the sow urinates while she is up and walking. In our experience, the time it takes from the first piglet is born until the last piglet and the placenta are expelled varies greatly. (You may not find the placenta if the sow has eaten it.). After farrowing, record the rectal temperature and check that the sow is alert and interested in the piglets. If the rectal temperature is above 39.5°C, treat her with NSAIDs and antibiotics, IM, in the neck muscle.

From 6 to 48 hours after birth, the piglets need to receive an iron supplement to prevent anaemia. The ambient temperature at sow height should be 18–20°C. In the piglet area, the temperature must be at least 34–37°C for the first week. Thereafter, reduce the temperature in the piglet area gradually.

If a piglet is found cold with low body temperature, the best way to bring the body temperature back to normal is to hold the piglet in a bucket of 38°C warm water for 10-15 minutes with only its head above water.

**Aggressive Sows and Agalactia**

It sometimes happens that a sow is confused and aggressive after farrowing, especially if it is her first time. If she is aggressive towards the piglets (attempts to bite or kill them), you can remove them if you have another sow that can take them on. It might also help if you take the sow out of the pen and let her have a walk-about. Alternatively, you can lightly sedate her with Azaperone (ca. 2 mg/kg) or other mild sedative. The piglets should be satisfied and alert with a rounded rather than sunken belly – in the latter case, this could indicate insufficient intake ofcolostrum. If, for example, the sow does not lie down to offer milk, it may help to rub the udder, and when the sow lies down, position the piglets at the teats. Oxytocin 20 IE IM (1–2 times) can also be administered to stimulate lactation.

**Cross-fostering**

Cross-fostering can be done to equalize litter size and for research purposes. It is crucial that all the piglets get colostrum before cross-fostering takes place. At Ellegaard, we do not remove the piglets until their umbilical cord is dry. If a small piglet is moved to a sow that is still farrowing or has just finished farrowing, the piglet will get colostrum here. To be certain that all piglets get a functioning teat, cross-fostering allocation must be completed no more than 48 hours after farrowing. If you cross-foster later than this, you risk that there are inactive teats which no longer provide milk. The piglets that are moved must be strong enough to handle the move, and it is advisable to remove the largest piglets from the large litter and mix them in a small warm area (34–37°C) with the piglets from the smaller litter that they are allocated to. The mixed piglets should remain together for about 30 minutes. To stimulate milk production and facilitate the acceptance of the new piglets, the foster sow can be given ½ ml oxytocin IM in the neck muscle. It is imperative to observe the situation for a while to make sure the piglets are accepted.

A. New-born Göttingen minipig with a fresh umbilical cord, B. The Umbilical cord is now dry, the belly is rounded as a sign that the piglet has received sufficient colostrum and the new-born piglet is now ready to be placed with a foster sow.
First-pass metabolism and absorption model in minipigs – a pilot study

Contact: Lars Siim Madsen, COO, Msc Biology, PhD, lsm@bioadvice.dk, Tel: +45 3030 2315

BioAdvice A/S, Lyshøjvej 21, DK-3650 Ølstykke

The limitations of current in vitro models in the accurate prediction of first-pass metabolism described in this section clearly highlight the need for an integrated in vivo model that takes full account of the complexity of drug absorption and metabolism. To our knowledge, such a first-pass metabolism model, based on the serial blood sampling in double permanent catheterized animals, has not yet been described in the literature.

This R&D project aims to meet the need for assessing first-pass metabolism early on in drug discovery and development in order to be able to anticipate potential high presystemic metabolism affecting drug bioavailability. Göttingen minipigs implanted with intraportal VAPs for studying first-pass metabolism of test substances were used as model since a substantial body of literature has shown the similarities between Göttingen minipigs and humans, thus placing the minipig as a promising animal in drug development. Diazepam was used as test substance. Diazepam is widely used as a muscle relaxant, sedative and anticonvulsant. After oral administration, its serum values are fairly low as Diazepam is subject to significant first-pass metabolism. It has been reported to undergo oxidative metabolism by demethylation (CYP 2C9, 2C19, 2B6, 3A4, and 3A5), hydroxylation (CYP 3A4 and 2C19) and glucuronidation in the liver as part of the cytochrome P450 enzyme system. It has several pharmacologically active metabolites, including primarily desmethyldiazepam (also known as nordazepam or nordiazepam).

In the first-pass metabolism model in Göttingen minipigs presented here, two venous catheters are implanted in the same minipig. The first catheter is implanted in the portal vein for the sampling of blood coming from the stomach and intestine before entering the liver, and the second catheter is implanted in the jugular vein for the collection of blood from the systemic circulation after passing through the liver. Diazepam was administered at 2 mg/kg by oral route and blood samples were collected simultaneously from the jugular and the portal veins at 5, 15, 30 minutes, and 1, 1½, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours after dosing.

Serum sample analysis showed that Diazepam could be detected in serum up to 120 hours after dosing. The peak concentration in the portal vein was 123 ng/ml and occurred after 30 minutes, while the peak concentration in the jugular vein was at 71.1 ng/ml and occurred after 60 minutes. The AUC values were 27527 and 22602 ng/ml×min respectively in the portal vein and the jugular vein. This corresponds to 18% of Diazepam having been metabolized in the liver before reaching the systemic circulation.

<table>
<thead>
<tr>
<th></th>
<th>T_{\text{max}} (hours)</th>
<th>C_{\text{max}} (ng/ml)</th>
<th>AUC (min×ng/ml)</th>
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<tbody>
<tr>
<td>Portal vein</td>
<td>0.5</td>
<td>123</td>
<td>27527</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>1</td>
<td>71.1</td>
<td>22602</td>
</tr>
</tbody>
</table>
Background literature:


Bode, G., P. Clasung, F. Gervais et al., 2011. “The utility of the minipig as an animal model in regulatory toxicology.” J.Pharmaco-


Ellegaard Göttingen Minipigs kindly sponsored minipigs for this pilot study and for a follow-up study (under preparation).

In conclusion, double catheterization was carried out successfully, and the simultaneous pharmacokinetic profile of Diazepam in portal and peripheral blood demonstrated that Diazepam is subjected to first-pass metabolism in the liver, demonstrating proof-of-concept of the model offering the following applications:

- a first pass metabolism model both addressing absorption and metabolism as outlined below;
- the investigation of intestinal absorption by collecting plasma samples from the portal vein after oral drug administration;
- the investigation of hepatic metabolism by collecting plasma samples from both the portal and jugular veins after oral drug administration;
- the investigation of intestinal/hepatic metabolism by collecting plasma samples from both the portal and jugular veins after drug administration in the portal vein.


Van der Laan JW., Brightwell J., McNulty P., Ratky J., Stark C., Steering Group of the RETHINK Project, 2010. Regulatory acceptability of the minipig in the development of pharmaceuti-
cals, chemicals and other products. Nov-Dec.62(3) :184-95

Webster J., Bollen P., Grimm H., Jennings M., 2010. Ethical impli-

# Meeting calendar

<table>
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<tr>
<th>NAME</th>
<th>DATE</th>
<th>LOCATION</th>
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<tbody>
<tr>
<td>IAT</td>
<td>17-20 March</td>
<td>Scotland</td>
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<tr>
<td>SOT ToxExpo</td>
<td>22-26 March</td>
<td>San Diego, CA</td>
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<tr>
<td>The Minipig Research Forum</td>
<td>21-22 May</td>
<td>Rome, Italy</td>
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<tr>
<td>AFSTAL</td>
<td>3-5 June</td>
<td>Lille, France</td>
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<td>Scand-LAS</td>
<td>9-12 June</td>
<td>Turku, Finland</td>
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<tr>
<td>EAVPT – European Association for Veterinary Pharmacology and Toxicology</td>
<td>19-22 July</td>
<td>Nantes, Frankrig</td>
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<td>ETS</td>
<td>30 August-2 September</td>
<td>Amsterdam, The Netherlands</td>
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<td>EuroTox</td>
<td>13-16 September</td>
<td>Porto, Portugal</td>
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<td>GV-SOLAS</td>
<td>14-16 September</td>
<td>Hannover, Germany</td>
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<td>Juvenile Tox. Symp.</td>
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<td>Beerse, Belgium</td>
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<td>BSTP/ESTP</td>
<td>22-25 September</td>
<td>Surrey, UK</td>
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<tr>
<td>BSTP/ACCP/MRF Meeting</td>
<td>13-14 November</td>
<td>Alderley Park, Cheshire, UK</td>
</tr>
</tbody>
</table>

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Now you can follow Ellegaard Göttingen Minipigs on LinkedIn! Our Company Page on LinkedIn will keep you updated with useful and interesting information regarding our company and the Göttingen Minipig!
The 2015 meeting of the Minipig Research Forum

The annual meeting of the Minipig Research Forum will take place in Rome, Italy on 21-22 May 2015.

The topics will be:
- The use of minipigs in development of anti-cancer products: relevance and species comparison in efficacy and safety testing
- Models of metabolic characterisation of test compounds in the minipig
- Efficacy testing of medical devices in minipigs

There will also be a practical/technical session with different cases, e.g. blood sampling in the sling and serial microsampling. Furthermore there will be workshops and time for networking with other minipig users.

The preliminary programme is available and will be updated soon.

Registration for the meeting is open and the €300 registration fee covers full attendance in the meeting, lunch on both days, refreshments and a meeting dinner on the first day.

The venue for this year’s meeting will be the NH Roma Collection Vittorio Veneto. Further information is available on the MRF website www.minipigresearchforum.org

Please contact the MRF if you have any questions (info@minipigresearchforum.org). Deadline for registration is the 1st of May and hopefully we will see you at this meeting.