Minipigs as a non-rodent species for embryofetal toxicology studies

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INTRODUCTION

There is increasing current interest in the minipig as an animal model for embryofetal development (EFD) studies. The large litter size and short gestation period of the minipig make it a convenient species for these studies when traditional choices such as the rat, rabbit or monkey are inappropriate. Furthermore the suitability of minipigs to known human teratogens and their regulatory acceptance are documented in the literature (Jørgensen, 1998; McNalty, et. al., 2002). In our facility we have more than 20 years experience performing EFD studies in the Göttingen minipig, permitting us to refine study designs and ensure the robustness of our data. In this poster we present control data from 4 full studies performed between 2010 and 2012 and intended for regulatory submission. Our database is sufficient to allow us to judge possible teratogenic effects based on the background incidence of variations and malformations. On the basis of these data we conclude that the minipig is a valuable alternative non-rodent species for use in regulatory embryofetal studies.

MATERIALS AND METHODS

Group size (embryofetal studies): 18 Göttingen SPF minipigs.

Primiparous mothers: age 6 to 8 months. Boars of proven fertility are used for the mating and we use sufficient to ensure that there is a good genetic variation.

Exposure: Day 9-13 gestation (organogenesis).

Fetuses: Collected by caesarean section (GD 110).

Examination of fetuses: external and visceral examination of fresh tissue at necropsy and skeletal examination of Alizarin stained bones. Heads from half of the fetuses were fixed in Bouin’s fixative, sectioned and examined for abnormalities and described mostly according to the terminology published by S.L. Makris et. al. 2009. Because some of the heads are sectioned, the total number of heads for skeletal examination shown in Table 4 (n=308) is less than the total number of fetuses (n=378).

DISCUSSION

The study design resembles that of an embryofetal study in other species. The guidelines indicate that there should be sufficient animals to be able to interpret low incidence findings and to be able to separate these from the background. Fetuses from around 16 litters per group at the time of cesarian sectioning is generally considered to be the minimum. Our study design and our mating success give confidence that we can get a sufficient number of fetuses for optimal study data interpretation. We present here data from control animals from 4 embryofetal studies, in total 378 fetuses from 70 litters. The studies were conducted between 2010 and 2012. Data collected routinely includes bodyweight gain, abortion rate, pregnancy rate, number of fetuses, number of early and late resorptions, number of implantation sites, total number of corpora lutea, uterine weight (including fetal and placental weight), preimplantation loss, postimplantation loss, fetal weight, placenta weight, nose to tip of tail length, nose to head length, and external, visceral and skeletal anomalies. Table 1 presents the litter data. We have a very high mating success, the pregnancy rate is 94.7%. Losses are small and the average litter size is 5.4 fetuses/litter. This provides substantial data to enable interpretation of findings. This litter size is slightly better than that of the breeder, Ellegaard Göttingen Minipigs, (with litter sizes of 5.1 (total) and 4.8 (viable) piglets from primiparous females), thus validating our husbandry procedures.

MaterIals and Methods

RESULTS

Table 1: Litter data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestation stage (%)</td>
<td>94.7%</td>
<td></td>
</tr>
<tr>
<td>Fetuses/litter (%)</td>
<td>5.4 (4.4-6.0)</td>
<td></td>
</tr>
<tr>
<td>Early resorptions/fetus (%)</td>
<td>0.66 (0.35-1.06)</td>
<td></td>
</tr>
<tr>
<td>Early resorptions/litter (%)</td>
<td>0.11 (0.01-0.12)</td>
<td></td>
</tr>
<tr>
<td>Corpora Lutea/pregnant dam</td>
<td>6.17 (6.2-7.4)</td>
<td></td>
</tr>
<tr>
<td>Post-implantation losses (%)</td>
<td>13.0 (7.8-21.3)</td>
<td></td>
</tr>
<tr>
<td>Placenta weight g</td>
<td>3873 (1350-4550)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Fetal data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight</td>
<td>972 (544-985)</td>
<td></td>
</tr>
<tr>
<td>Placenta weight g</td>
<td>102 (135)</td>
<td></td>
</tr>
<tr>
<td>Nose to tail head cm</td>
<td>22.2 (19-22.6)</td>
<td></td>
</tr>
<tr>
<td>Jaw length</td>
<td>4.7 (4.5-5.9)</td>
<td></td>
</tr>
<tr>
<td>Males/ Females</td>
<td>185/93</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: External findings

<table>
<thead>
<tr>
<th>Finding</th>
<th>Incidence</th>
<th>% Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domed head</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Misshapen head</td>
<td>11</td>
<td>3.0</td>
</tr>
<tr>
<td>Meningeal abnormalities</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Open eye</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Smaller jaw</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Polydactyly</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Syndactyly</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Angulated tail</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Absent anus</td>
<td>1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 4: Visceral findings

<table>
<thead>
<tr>
<th>Finding</th>
<th>Incidence</th>
<th>% Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema in abdominal and thoracic wall</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Heart - absence of valves, chordae tendines</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Heart - atrial septal defect</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Heart - Ventricular septal defect</td>
<td>7</td>
<td>1.9</td>
</tr>
<tr>
<td>Immotile artery absent</td>
<td>10</td>
<td>10.3</td>
</tr>
<tr>
<td>Immotile artery short</td>
<td>24</td>
<td>6.4</td>
</tr>
<tr>
<td>Small lung</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>Diaphragm hernia</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Absent gall bladder</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Malpositioned gall bladder</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Small spil bladder</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Haemorrhagic kidney</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Fused kidney</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Fused sacrum</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Absent adrenal</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Malpositioned testis</td>
<td>22</td>
<td>5.8</td>
</tr>
<tr>
<td>Large epididymis</td>
<td>1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 5: Selected skeletal findings

<table>
<thead>
<tr>
<th>Finding</th>
<th>Incidence</th>
<th>% Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic limb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernumeray vertebral bone</td>
<td>41</td>
<td>11.1</td>
</tr>
<tr>
<td>Supernumeray meralcal bone</td>
<td>12</td>
<td>17.9</td>
</tr>
<tr>
<td>Pelvic girdle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelvic girdle</td>
<td>142</td>
<td>37.7</td>
</tr>
<tr>
<td>Pelvic limb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernumeray femoral rib</td>
<td>76</td>
<td>20.9</td>
</tr>
<tr>
<td>Rib fused with thoracic transverse process</td>
<td>61</td>
<td>2.9</td>
</tr>
<tr>
<td>Full supernumeray rib</td>
<td>35</td>
<td>4.0</td>
</tr>
<tr>
<td>Short rib</td>
<td>98</td>
<td>25.9</td>
</tr>
<tr>
<td>Short supernumeray clavicular rib</td>
<td>17</td>
<td>1.9</td>
</tr>
<tr>
<td>Chloride rib</td>
<td>11</td>
<td>1.1</td>
</tr>
<tr>
<td>Vertebral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernumeray scapular rib</td>
<td>11</td>
<td>1.1</td>
</tr>
<tr>
<td>Supernumeray thoracic vertebrae</td>
<td>10</td>
<td>2.1</td>
</tr>
<tr>
<td>Supernumeray thoracic processus spinous</td>
<td>4</td>
<td>1.1</td>
</tr>
<tr>
<td>Supernumeray lumbar transverse process</td>
<td>7</td>
<td>1.9</td>
</tr>
<tr>
<td>Supernumeray processus spinous</td>
<td>6</td>
<td>1.1</td>
</tr>
<tr>
<td>Fused caudal segment</td>
<td>14</td>
<td>4.5</td>
</tr>
<tr>
<td>Fused ischium</td>
<td>17</td>
<td>4.5</td>
</tr>
<tr>
<td>Partly fused ischium and pubis</td>
<td>15</td>
<td>1.3</td>
</tr>
<tr>
<td>Pubis grown towards ischium</td>
<td>204</td>
<td>27.5</td>
</tr>
</tbody>
</table>

CONCLUSION

The use of minipigs as embryofetal study models is increasing due to the availability of more background data on fetal findings in our facility, which provide an invaluable interpretative tool for the evaluation of studies performed using the same in-life procedures and fetal processing and diagnostic criteria. The availability of this data allows us to characterise the experimental model and distinguish treatment related effects from background incidences of variations and malformations. This data base supports the use of the minipig as a valuable alternative non-rodent species for embryofetal studies.

REFERENCES

Makris, et. al., Terminology of Developmental Abnormalities in Common Laboratory Animals (Version 2). Birth Defects Research (Part B) 86 200-201 2008

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