Minipigs as a non-rodent species for embryofetal toxicology studies

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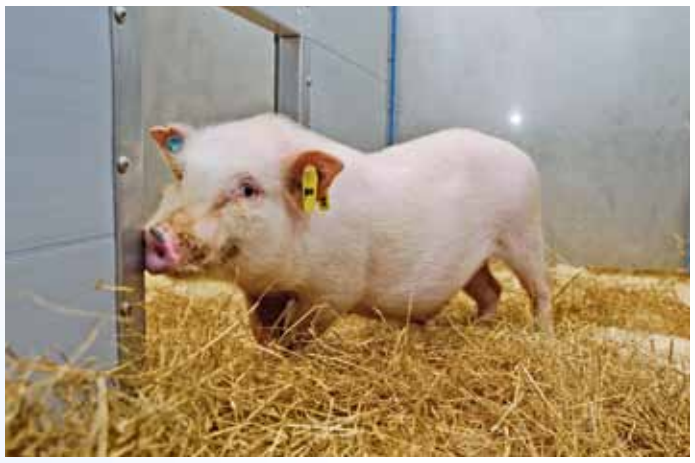
Introduction

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 mouse are inappropriate. Furthermore the susceptibility of minipigs to known human teratogens and their regulatory acceptance are documented in the literature (Jørgensen, 1998; McAnulty, et. al., 2012). 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.



The picture shows an Alizarin stained minipig fetus on GD 111.

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 11-35 of 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 us 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 tail 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.

Table 1: Litter data

	Mean
Pregnancy Rate %	94.7 n = 74
Fetuses/litter	5.41 (4.4-6.0)
Early resorptions/litter	0.66 (0.35-1.06)
Late resorptions/litter	0.11 (0.11-0.12)
Implantations/pregnant dam	6.18 (5.61-6.89)
Corpora Lutea/pregnant dam	6.71 (6.3-7.4)
Pre implantaion loss %	7.61 (6.4-10.4)
Post implanta-tionloss %	13.0 (8.7-21.3)
Uterus weight g	3873 (3380-4356)

Number of litters = 70; (Range of values in brackets)

Tables 2 to 5 illustrate some of the findings seen in the minipig. About one third (33.9%) of control fetuses have one or more external or visceral findings, although the number of these per affected fetus is normally small. Individual external findings are typically present at less than 2% but incidences of visceral findings can reach up to 10%.

Table 2: Fetal data

	Fetal weight g	Placenta weight g	Nose to tail head cm	Jaw length	Males/ Females
Mean	372 (344-385)	116 (103-130)	22.2 (21.7-22.6)	4.7 (4.5-4.9)	185/193

Number of fetuses = 378; (Range of values in brackets)

Certain visceral findings are seen regularly in the minipig, the most common being absent or short innominate artery; thereafter malpositioned testes, and septal defects in the heart. We see a wide range of skeletal findings, many of which are seen at a low incidence. Table 5 shows a selection of the findings that we have seen in these fetuses; we have excluded from the table all variations in ossification and any finding with an incidence of less than 1%. We do of course have access to these data in our database. Some skeletal variations have a very high incidence, for instance short supernumerary ribs are seen in 37% of all control fetuses examined. A large database allows a correct evaluation of study-to-study variability and low incidence findings. We have used this in our studies to provide context to findings such as cryptorchidism and supernumerary ribs, which could not be clarified using the control data from the study alone.

Table 3: External findings

(n=378)	Finding	Incidence	% Incidence
Head	Domed head	3	0.8
	Misshapen head	4	1.1
	Microtia (abnormal small ear)	1	0.3
	Open eye	6	1.6
	Small upper jaw	1	0.3
Body	Scoliosis	2	0.5
	Polydactyly	6	1.6
	Syndactyly	2	0.5
	Angulated tail	6	1.6
	Absent anus	1	0.3

Table 4: Visceral findings

(n=378)	Finding	Incidence	% Incidence
Body	Edema in abdominal and thoracic wall	1	0.3
	Heart – absence of valves, chordae tendinae	1	0.3
	Heart - atrial septal defect	9	2.4
	Heart - Ventricular septal defect	7	1.9
	Innominate artery absent	39	10.3
	Innominate artery short	24	6.4
	Small lung	5	1.3
	Diaphragmatic hernia	6	1.6
	Absent gall bladder	1	0.3
	Malpositioned gall bladder	1	0.3
	Small gall bladder	2	0.5
	Haemorrhagic kidney	1	0.3
	Fused kidney	3	0.8
	Fused adrenal	3	0.8
	Absent adrenal	1	0.3
	Malpositioned testis	22	5.8
	Large epididymis	1	0.3

Table 5: Selected skeletal findings

	Finding	Incidence	% Incidence
(n=308)			
Skull	Enlarged fontanel	24	11.5
	Misaligned palatine	8	3.8
	Parietal with additional suture(s)	2	1.0
	Occipital with extra suture(s)	3	1.4
	Small upper jaw	1	0.3
(n=378)			
Thoracic limb	Supernumerary carpal bone	4	1.1
	Supernumerary metacarpal	4	1.1
Sternebra	Fused sternebra	67	17.7
	Misaligned sternebra	10	2.6
	Small sternebra	5	1.3
	Misshapen sternebra	38	10.1
	Short sternebra	26	6.9
	Split sternebra	6	1.6
Ribs	Short supernumerary cervical rib	140	37.0
	Cervical rib fused with thoracic rib	79	20.9
	Rib fused with thoracic transverse process	11	2.9
	Detached rib	15	4.0
	Full supernumerary cervical rib	82	21.7
	Short rib	98	25.9
	Short supernumerary thoracolumbar rib	7	1.9
	Thickened rib	4	1.1
Vertebrae	Supernumerary thoracic vertebra	8	2.1
	Misshapen thoracic transverse process	12	3.2
	Supernumerary thoracic processus spinosus	4	1.1
	Misshapen lumbar transverse process	7	1.9
	Misshapen lateral process(es)	6	1.6
	Fused caudal centrum	4	1.1
Pelvic girdle	Fused ischium	17	4.5
	Partly fused ischium and pubis	5	1.3
	Pubis grown towards ischium	104	27.5
Pelvic limb	Supernumerary tarsal bone	14	3.7

Conclusion

We present here 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 embryo-fetal studies.

References

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