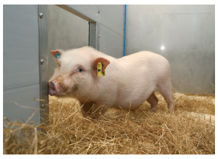
The minipig in embryofetal toxicity studies.

Thuri S. Kledal and Andrew Makin
LAB Scantox, Hestehavevej 36A, 4623 Ll-Skensved, Denmark

Excellence in Research

Introduction

Traditionally, rats and rabbits are the species of choice for developmental toxicity (embryofetal) studies. However, if these species are found unsuitable the choice of an alternative can be difficult. One alternative is the minipig, and the use of the minipig as a non-rodent in developmental toxicity testing is increasing. Several studies have now been performed in minipigs, and at LAB Scantox, we have a database that comprises data from 70 control Göttingen SPF minipig dams from 6 different studies and in all 308 control fetuses regarding incidences of variations and anomalies. This database is essential for interpretation of the results from an embryofetal/ teralogy study. Experiments have shown that the minipig is susceptible to teratogenic effects of tretinoin (Jørgensen, K.D. 1998).



Litter data

	Pregnancy Rate %	Fetuses/ litter	Early resorptions/ litter	Late resorptions/ litter	Implantations/ pregnant dam	Corpora Lutea/ pregnant dam	Pre implantaion loss %	Post implantation loss %	Uterus Weight g
Mean	81 n=70	5.3 n=61 (2)	0.9 n=61 (1.3)	0.3 n=61 (0.7)	6.3 n=61 (2.1)	7.3 n=61 (2.6)	12.2 n=61 (19.7)	16.1 n=61 (21.5)	3806 n=61 (1627)

n=number of litters, (sd=standard deviation in brackets)

Fetal data

Fetuses	Fetal weight g	Placenta weight g	Nose to tip of tail cm	Nose to tail head cm	Jaw length cm
Mean	379.6 n=308 (83)	105.5 n=308 (30.2)	26.7 n=202 (1.8)	21.8 n=308 (1.7)	4.7 n=250 (0.5)

n=number of fetuses, (sd=standard deviation in brackets)

External anomalies

(n=308)	Finding	Incidence	% Incidence
Head	Doomed head	5	1.62
	Flattened head	2	0.65
	Hole in head under the skin	1	0.32
	Cranioschisis (split cranium)	1	0.32
	Cheilognathopalatoschisis (Split jaw, lip and palate)	1	0.32
	Cleft palate	1	0.32
	Ankyloglossia (malformed tongue)	1	0.32
	Resistance in mouth opening	1	0.32
	Microtia (abnormal small ear)	1	0.32
	Open/ slightly open eye	7	2.27
	Exophthalmos (eye)	4	1.30
	Malpositioned eye	2	0.65
	Enlarged orbital (eye)	4	1.30
Body	Scoliosis	1	0.32
	Front legs, hyperflexion	6	1.95
	Bowed limb (limb)	1	0.32
	Polydactyly	18	5.84
	Syndactyly	1	0.32

Visceral anomalies

(n=308)	Finding	Incidence	% Incidence
Body	Cardiac ventricles - coagulated blood	1	0.32
	liver lobe(s) haemorrhagic	5	1.62
	Gall bladder agenesis	3	0.97
	Liver lobe(s) dark discolouration	1	0.32
	Discoloured liver	2	0.65
	Pale liver	1	0.32
	Gall bladder hypoplastic	2	0.65
	Enlarged gall bladder	1	0.32
(n=127)	Malpositioned testes /Cryptorchism	16	12.40
(n=127)	Testis/testes hypoplastic	1	0.78
(n=127)	Enlarged scrotum	1	0.78
	Hydronephrosis	1	0.32
	Kidney haemorrhagic	1	0.32
	Dilated renal pelvis	3	0.97
	Gnathoschisis (Split Jaw)	3	0.97
	Mild umbilical hernia	1	0.32
	Cardiac ventricles - coagulated blood	1	0.32

Material and Methods

- Group size (embryofetal studies): 14-18 Göttingen SPF minipigs.
- Primiparous mothers: age 6 to 8 months.
- Exposure: Day 11-35 of gestation (organogenesis).
- Body weight GD 10-11 = 20 kg (SD +/- 3.4), GD 35 = 23 kg (SD +/- 3.9).
- Route of administration: via oral route, subcutaneous injection, or intravenous injection via implanted vascular access port (VAP).
- Fetuses: Collected by caesarean section GD 109-111.
- 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 L.D. Wise et at., 1997

Skeletal anomalies

	Finding	Incidence	% incidence
(n=124)			
Skull	Enlarged fontanelle	12	9.7
	Unossified central hyoid	9	7.3
(n=308)			
Vertebrae	Incomplete oss of cervical arch	36	11.7
	Incomplete oss of cervical centrum	13	4.2
	Incomplete oss of cervical processus spinosus	30	9.7
	Incomplete oss of thoracic arch	38	12.3
	Incomplete oss of thoracic centrum	24	7.8
	Total incomplete oss of thoraic processus spinosus	34	11.0
	Incomplete oss of lumbar centrum	18	5.8
Sternebra	Two or more sternebrae fused	41	13.3
	Asymmetrical sternebrae	15	4.9
Ribs	Fused rib(s)	15	4.9
	Shortened rib(s)	33	10.7
	Rudimentary 14th rib	23	7.5
	Cervical rib	13	4.2
	Rudimentary cervical rib	27	8.8
	Cervical rib fused with thoracic rib	22	7.1
	15 ribs or rudimentary 15th rib	7	2.3
Extremities	Unossified oss center distal of fibula	23	7.5
	Incomplete oss. Center proximal of humerus	21	6.8
	Unossified oss. Center proximal of humerus	18	5.8
	Unossified oss center distal of ulna	28	9.1
	Ulna and radius fused	1	0.3
	Pentadactyly (forelimbs)	13	4.2
	Hexadactyly	2	0.6
	Unossfied oss. Site proximal of proximal(s) both front legs	44	14.3
	Unossified oss site(s) distal of metacarpal(s)	61	19.8
	More than one tarsal bone unossified on both legs	13	4.2
	Absent ossification site cranial of proximal(s)	13	4.2
	Extra proximal(s)	4	1.3
	Extra medial	3	1.0
	Extra distal	4	1.3

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

Head anomalies

Finding (n=123)	Incidence	% incidence
Slight dilation of ventricle(s)	6	4.9
Dilation of ventricle(s)	6	4.9
Nasal cavity slightly narrowed	2	1.6
Thalamic cyst	2	1.6
Cavity(ies) in cerebral tissue	1	0.8
Cavity in cerebrum	1	0.8
Cavity in cerebrum	1	0.81
Brain, hemisphere compressed	1	0.8

Results and Discussion

The database contains information on 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.

The data presented in the tables illustrate some of the variations and anomalies found in the minipig, as well as variations in the reproductive parameters used in embryofetal studies.

A range of external variations and anomalies are observed in the minipig. Some of these appear in 1 to approx. 6% of the fetuses. The most common ones are fetuses with doomed head, exophthalmos (protruding eye), enlarged orbital, open/ slightly open eye, hyperflexion and polydactyly. More severe anomalies are seen more seldom.

A few visceral variations and anomalies are seen in the minipig. The most common finding is malpositioned testes, which is seen in approx. 12 % of the male fetuses and dilatation of the ventricles of the brain in approx. 5 % of all fetuses.

In the minipig fetuses we have observed a number of the congenital skeletal anomalities. There are asymmetrical sternebrae, misshapen sternebrae, fused ribs, shortened ribs, a rudimentary 14th rib, cervical ribs, rudimentary cervical rib, cervical rib fused with thoracic rib, fused ulna and radius and polydactyly and absent ossification site cranial of proximal(s). There is a broad range of variations in the degree of ossification in a number of bones and more than 4% of the minipig fetuses display unossified or incomplete ossification of the following bones: central hyoid, cervical arches, cervical centrum, cervical processus spinosus, thoracic arches, thoracic centrum, thoracic processus spinosus, lumbar centrum, ossification center distal of fibula, ossification center proximal of humerus, ossification site proximal of proximal(s), ossification site(s) distal of metacarpal(s), and tarsal bones.

A gestation period of 113 days and 5.3 fetuses/litter, make the minipig a more suitable species to use compared to primates as an alternative species in embryofetal studies.

The picture shows an opened minipig uterus with fetuses on GD 111.

Conclusion

The LAB Scantox database provides us with highly useful information and makes it more feasible to judge possible teratogenic effects caused by a test item from background incidences of variations and malformations. Through these studies the minipig has shown to be a highly valuable alternative non-rodent species for use in developmental toxicity studies.

References

Wise, L.D et al.(1997). Terminology of developmental abnormalities in common laboratory mammals (version I). Teratology 55 (4), pp. 249-292.
Jørgensen, K.D. (1998). Teratogeneic activity of tretinoin in the Göttingen minipig. Scandinavian Journal of Animal Science Suppl. 1 (25), pp.235-243.