Method for Evaluation of Placental Transfer in Embryofoetal Developmental Studies in Göttingen Minipigs at Scantox

Sisse Ellemann-Laursen, MSc Pharm, ERT
Nanna Grand, DVM, DipRCPath

scantox

For further information
and inquiries contact
clientservice@scantox.com

Background

For an embryo-foetal developmental study it may be of importance to determine the placental transfer ratio of a drug, ensuring that foetal exposure has taken place.

In embryofoetal developmental studies, the dosing period covers the period of organogenesis. In minipigs, this period ranges from gestation day 11 to 36, but due to the long pregnancy period of a minipig (114 days, or “3 months, 3 weeks and 3 days”), the dams are not terminated until gestation day 110, when an evaluation of the uterus and a foetal examination to investigate for test article induced abnormalities are performed. At this time, it is no longer possible to determine the exposure of the foetuses due to the elimination of the test item.

To determine the actual exposure of the foetuses, it is thus necessary to terminate dams on the last day of dosing (gestation day 36).

The Placenta

The placenta plays a vital role in the exchange between the maternal and the foetal blood circulation. It also acts as a potential barrier determining whether toxic compounds will reach the foetus at a concentration that will affect foetal development. Placentation is species-dependent and is classified according to the shape and histological structure of the placenta.

The placenta consists of foetal and maternal components and separates the maternal and foetal blood circulation. In the human discoid haemomonochorial placenta, all maternal components disappear during placentation, whereas in the diffuse epitheliochorial type in the minipig, all maternal components persist.

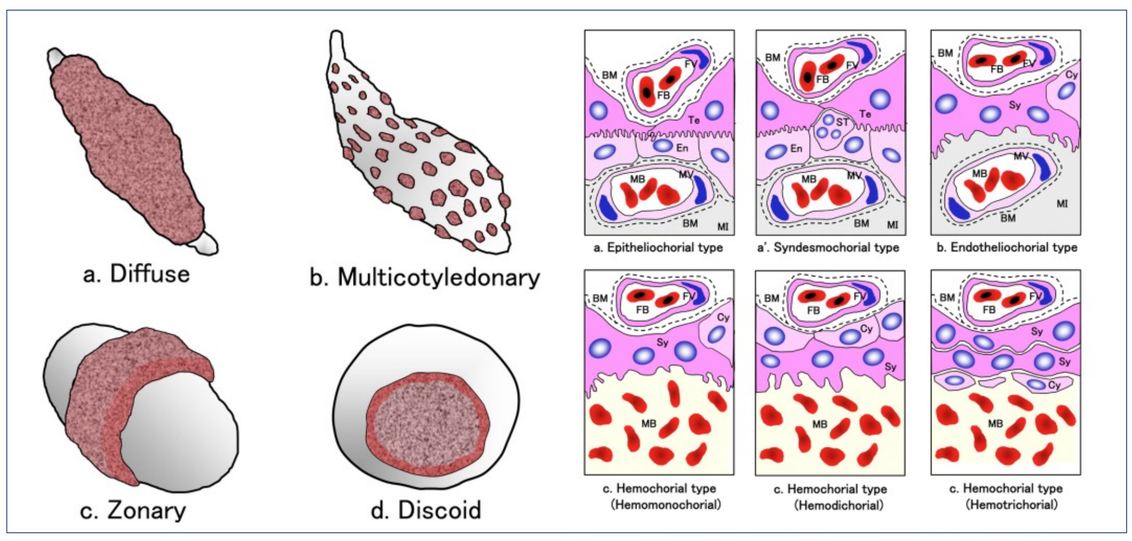

Figure 1: Types of placenta (Ref (2))

Molecules will either cross the placenta by passive diffusion or by active transport. For passive diffusion, the thickness of the barrier is rate-limiting, with a thin barrier allowing a faster transfer. The many-layered barrier in minipigs is known to be impermeable to macromolecules such as antibodies; however, the pig placenta is also known to be reduced in thickness in some areas, which can greatly affect the transfer of molecules across the placenta. It has been shown that the placental transfer of most drugs is determined mostly by the placental blood flow and thus not entirely dominated by the placenta type.

Method

To determine foetal exposure, two extra dams (satellite animals) were included in the groups selected for evaluation. Three possible ways of determining foetal exposure in minipigs were investigated: 1) sampling of blood from the umbilical cord; 2) sampling of foetal fluid (fluid from the allantoic sac and the amniotic sac); and 3) using a homogenate of foetuses.

1) Foetal blood: On the day of termination, a blood sample for bioanalysis was taken from each dam right before termination for calculation of the foetal/maternal exposure ratio. Blood from the foetus was drawn from the umbilical cord before separating the foetus from the umbilical cord and the placenta. The blood can be pooled (per litter or per group) to obtain the volume necessary for analysis.

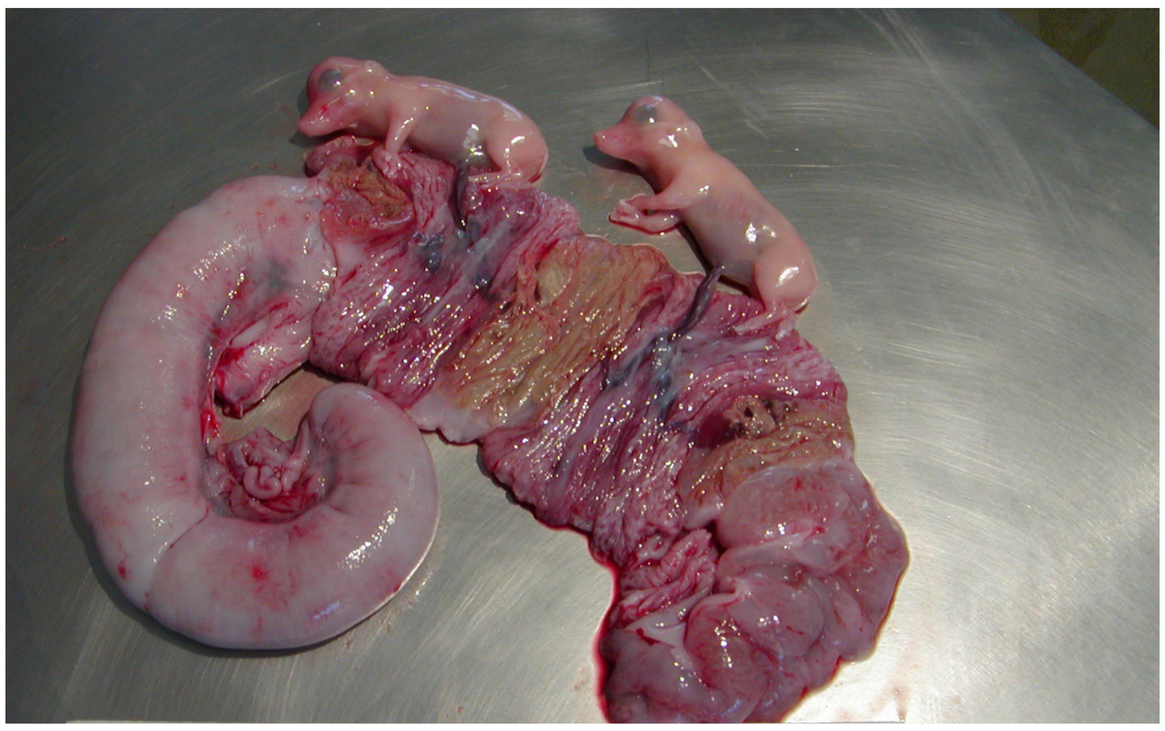

Figure 2: Minipig uterus with foetuses (Photo source: Scantox)

2) Foetal fluid: Foetal fluid was sampled right after opening the uterus, with the foetuses still within the uterus, and with the amniotic sac still intact. The allantoic and amniotic fluid can be collected separately, when possible, to differentiate between the two compartments. Otherwise, samples can be pooled.

3) Foetal homogenate: Foetuses were collected without the umbilical cord and were homogenised in methanol/water 50/50 v/v in a ratio of 1:4.

It was possible to analyse all three types of matrices using standard bioanalytical approaches.

Conclusion

It is possible to determine the exposure of minipig foetuses by including, e.g. two extra (satellite) dams per group for termination on the last day of dosing.

It is possible to sample umbilical cord blood and to sample allantoic and amniotic fluid from minipig foetuses to perform bioanalysis and get valid results. It is also possible to use a foetal homogenate for bioanalysis to determine foetal exposure.

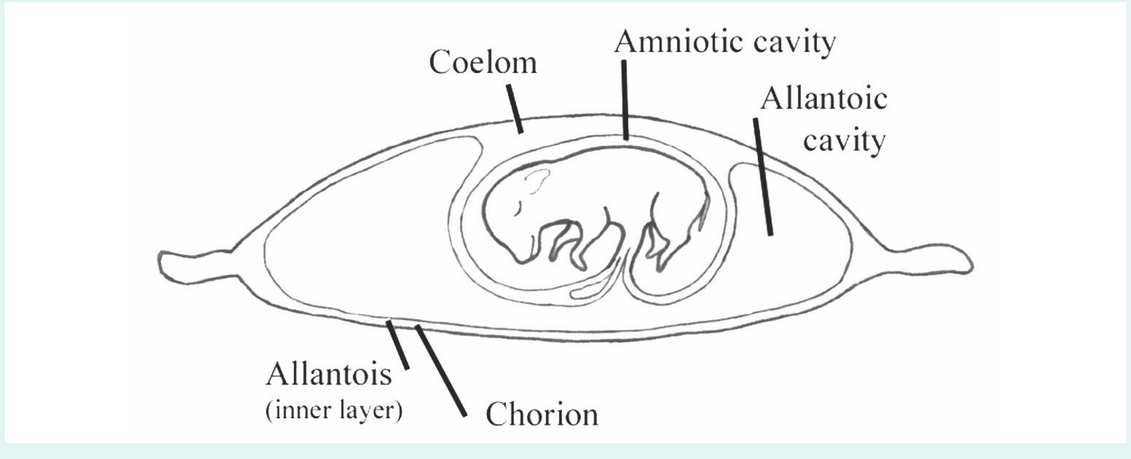

Figure 3: Allantoic and amniotic sacs (Animal biosciences: LEC3)

Allantoic and Amniotic Fluid

The allantoic sac is developed from the primitive gut and acts as a waste reservoir for the foetal metabolism. The allantoic sac is very large in the minipig epitheliochorial type of placenta. The amniotic sac provides a mechanical protection of the foetus during its development.

Practically, it can be challenging to differentiate between the fluid-filled compartments of the allantoic sac and the amniotic sac.

References: (1) The Minipig in Toxicology, Ove Svendsen, 1998. (2) Furukawa S et al: A Comparison of the Histological Structure of the Placenta in Experimental Animals. J Toxicol Pathol 2014; 27: 11-18.