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The incidence of congenital malformations and variations in Göttingen minipigs

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ABSTRACT

Knowledge of the incidence of spontaneous congenital abnormalities is critical for the accurate interpretation of findings in teratogenicity studies in any species. In this paper, results of the examination of 1739 neonatal Göttingen Minipigs are presented. Over the 2-year period under consideration, the incidence of external and visceral malformations was less than 0.2 and 0.1%, respectively. The most common external malformations were syndactyly, limb hyperflexion, domed head and scoliosis. The most common internal malformations were undescended testes, ventricular septal defect, diaphragmatic hernia and atrial septal defects. Pentadactyly and variation in the aortic arch's bifurcation (absent truncus bicaroticus) were the most common variations. These data will help support the use of the Göttingen Minipig as a non-rodent species in embryofetal development studies where concerns persist about the availability of background data.

1. Introduction

The small size, microbiologically defined health status and the many similarities to humans make the Göttingen minipig an ideal animal model in many types of biomedical studies. Early onset of sexual maturity compared with other large animal species, duration of gestation, and large litter size, as well as the susceptibility of the minipig to known human teratogens, make the minipig the logical economical and scientific alternative to other species for developmental and reproductive toxicology studies.

Traditionally, rats and rabbits are the species of choice for developmental toxicity studies (ICH Guideline S5R2 (2005)) [1]; however, when these species are found unsuitable e.g. because the metabolites in the animals are not relevant to those in humans, the minipig may be an appropriate alternative [22].

The minipig has several benefits over other non-rodent species e.g. sexual maturity is reached at approximately 4–8 months of age, the average gestation length is 115 days, and the litter size is typically 4–6 piglets in primiparous, and 5–9 piglets in multiparous, minipigs [3,13,18]. By comparison, the cynomolgus monkey attains puberty/sexual maturity much later (age 2.5–4 years), gestation length is about 160 days and, in general, has a single offspring only [23]. There are also significant ethical issues involved in using large numbers of non-human primates for reproductive and juvenile toxicity studies. Whilst the dog (beagle) may also appear a good alternative for several reasons such as body weight (approximately 10 kg), gestation length (average of 63 days) and a litter size comparable with that of the minipig, animal supply in the numbers required for regulatory embryofetal development studies is unrealistic due to seasonal breeding.

Compared with the non-human primate, it is easy to synchronize the ovarian cycles of minipigs and the fertility rate is high (89%) (www.minipigs.dk). This facilitates the planning and management of a study and reduces the number of sexual mature animals needed in reproductive toxicology studies.

In contrast to humans, the placenta of the pig has a six layered epitheliochorial structure and maternal-derived antibodies are transferred to the offspring postpartum via colostrum [24]. However, minipigs exhibit sensitivity to small molecule teratogens that are known to be teratogenic in man. For example, pyrimethamine administered in the diet during early organogenesis can lead to cleft palate, club-foot and micrognathia in minipig fetuses [6,15,17], and

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Fig. 1. Pentadactyly.

Fig. 2. Meningocele.

tretinoin given by oral gavage from day 11 up to day 37 of gestation results in multiple malformations resembling retinoic acid teratogenicity in humans [9].

Knowledge of the incidence of spontaneous congenital abnormalities in the minipig is critical for the accurate interpretation of findings in teratogenicity studies. In this paper, we present results of the examination of a large number of neonatal Göttingen Minipigs (1739) that were stillborn, died shortly after birth or were euthanized within 72 h because of abnormalities or failure to thrive. This material may therefore be used as a reference to add context to the aetiology of morphological changes to support evaluation of study-to-study variability of low incidence findings.

2. Materials and methods

At Ellegaard Göttingen Minipigs A/S, all live-born piglets are examined shortly after birth. Piglets that are weak or have external abnormalities are euthanized. Selected findings are recorded in an internal database at Ellegaard. During a 2-year period (2010–2011), data from these piglets, and piglets that were stillborn or found dead within 72 h of birth were collected. For each piglet, the following were recorded: gender, identification number of the parents, mating date, date of birth (postnatal day) and litter size. In addition, the reason for euthanasia or, when possible, the cause of death, were recorded. The piglets were stored frozen (−20 °C) until shipment and examination at CiToxLAB Scantox A/S, WIL Research Europe, Envigo or Sequani.

Fig. 3. Frontal meningo-encephalocele.

Before necropsy, the piglets were thawed and body weight was recorded. The external abnormalities of the piglet, and, for a proportion, the nose to rump (head to tail) and the jaw length measured in cm were recorded.

External and visceral examinations were performed on 1739 piglets to identify congenital abnormalities according to the following definitions:

Malformation: Permanent structural change likely to adversely affect the survival or health of the species under investigation [4].

Variation: Change that occurs within the normal population under investigation studied and is unlikely to adversely affect survival or health [4].

Each finding was identified by a code according to Makris et al., "Terminology of Developmental Abnormalities in Common Laboratory Mammals (Version 2)" [12]. The approximate time from death until placement in the freezer was recorded for each piglet and varied considerably. Therefore, findings with an inconclusive description or that were considered probable artefacts caused by post-mortem changes, physical trauma and/or the freeze/thaw procedure are listed in Table 6.

3. Results

3.1. Farrowing and litter data

During the 2-year period (2010–2011), 16,898 minipigs were born in the barriers at Ellegaard Göttingen Minipigs A/S (female: 8334 male: 8564) of which 621 (3.7%) were stillborn. i.e. born at term (after GD 106) without any sign of life. The average litter size was 5.0 and 7.4 piglets for primiparous and multiparous sows, respectively. The average gestation length was 114.1 days (range 107–127 days) and 114.7 (range 108–133) in primiparous and multiparous sows, respectively. A proportion (355) of the piglets died postnatally because they were accidentally crushed by the sow. Table 1 summarizes the clinical condition and/or the cause of death or euthanasia of the piglets during the period. At Ellegaard, the clinical condition was recorded at birth and results are summarized in Table 1. Of the 1739 piglets subsequently examined at the participating laboratories, the clinical condition at birth was recorded for 1205 piglets and results are summarized in Table 1. Abnormalities were present in 492 of the 897 (54.85%) examined piglets born alive and in 149 of the 308 (48.38%) stillborn piglets.

3.2. Piglet data

Piglet data are presented in Table 2 summarising weight, nose-rump length and jaw length.