The Reproductive Development of the Mini-Pig and its Impact on the Assessment of Reproductive Toxicity

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Abstract

The value of the mini-pig as a non-rodent model for non-clinical toxicity testing is becoming increasingly appreciated. Its use in general toxicology studies has been widely documented but use of minipigs for assessment of reproductive toxicity, including effects on fertility, and juvenile toxicity has received less attention. There is a need for an additional model for these studies particularly in the assessment of biotherapeutics where non-human primates may currently be the only realistic option. As the use of peripubertal animals can result in difficulties of interpretation, the timescale of reproductive development is a critical consideration in the planning of many toxicity studies. Work has been conducted at Sequani to establish suitable histological background data from Göttingen minipigs at various ages throughout development, in order to assess and compare the organ development and relevance to man. This poster provides background data in relation to development of reproductive organs, specifically testes and ovaries and provides important data about age at sexual maturity.

Objectives

The objective of the overall exercise was to provide background data to support the use of the minipig in toxicity studies. It has provided background data by microscopic examination of tissues from the minipig at the following ages 1, 7, 14, 21, 28 days of age and 3, 4, and 5 months of age, in order to show how organs develop as the animals get older. This poster aims to specifically highlight the key developmental changes identified by histology of the critical reproductive organs (testes and ovaries), with a view to further supporting the use of minipigs in reproductive and juvenile toxicity studies and in general toxicity studies where there is a focus on assessment of fertility.

Introduction

Assessment of many aspects of reproductive toxicity require sexually mature animals. Use of peripubertal animals can result in uninterpretable results or false positives.

There is now a heavy emphasis in pharmaceutical development towards biological agents, at the expense of small molecule R&D. Currently around a third of all new medical entities are biologics and a good model for reproductive toxicity does not currently exist for biological agents. In traditional models it is rare that the receptor for the biological entity that is to be tested exists in the model species. Even in the primate there is only a limited number of receptors that are fully developed. Hence, unless the clinical usage would specifically exclude young adults as a target group, this is the recommended age range at the start of most standard toxicity studies. Here, there is a substantial advantage in using the minipig because the difficulties of sourcing older dogs that will be sexually mature during short-term toxicity studies can be avoided.

In consideration of a suitable model for testing of biological agents there are several factors to be taken into consideration. There is the need for the relevant receptor to be present in the target species, an area in which both non-GM rodents and dogs fail almost completely. The dog is also inadequate in other aspects of reproductive biology.

The two remaining models are the primate and the minipig. The minipig has major advantages over the primate in terms of ethics and economy, with sexual maturity occurring between 3-6 months rather than 4-5 years for a cynomologus monkey. Minipigs also have far larger litters than primates and are amenable to cross-fostering and other litter interventions.

Most mammals share many aspects of the reproductive system in terms of the regulatory hypothalamic system that releases gonadotropin releasing hormone in pulses, the pituitary secretion of follicle stimulating hormone and luteinizing hormone and the ovarian release of sex hormones. However, there are significant interspecies differences in detailed functioning. In respect of minipigs and primates the most obvious difference being that pigs have oestrous cycles and reabsorb the endometrium if conception does not occur during that cycle whereas primates have a menstrual cycle.

The evidence suggests that menstruation is not beneficial in itself, and that it is probable that the foetal development of menstruating species requires a more developed endometrium, which is too thick to reabsorb completely. Ignoring the period of menses the reproductive cycle of the pig is almost identical to man.

For studies, including general toxicity studies, where effects on reproductive organs are a key consideration, economics demands that the use of animals at the earliest practicable age is advantageous. It is suggested that a minipig at 4 months of age is a fair model for the human teenager, and should be sexually mature even by the end of a one month toxicity study. Hence, unless the clinical usage would specifically exclude young adults as a target group, this is the recommended age range at the start of most standard toxicity studies. Here, there is a substantial advantage in using the minipig because the difficulties of sourcing older dogs that will be sexually mature during short-term toxicity studies can be avoided.

In reproductive toxicity studies, the minipig has many advantages over existing models. The reproductive physiology is close enough to man to allow results or false positives.

Conclusion

At 90 days the ovaries of the gilt have an adult appearance in terms of follicles. Many researchers consider that the appearance of Corpora Lutea at 6 months is the essential indicator of reproductive maturity. The appearance at 6 months is not substantively different to the appearance at 90 days, at which stage gilts are known to be reproductively capable, prior to the appearance of substantial numbers of Corpora Lutea. There are parallels with this appearance and the condition of polycystic ovaries which is common in teenagers. This stage in the gilt probably corresponds to the female teenager.

In the testes at 90 days the germinal epithelium looks anatomically complete. Sperm production appears to be in full flow with plenty of sperm in the lumen. Although, there are fewer sperm than seen in the mature boar this is essentially the adult appearance. The histology suggests that boars of this age should be reproductively capable from 3 months.

Quoted estimates of 4.5 months appear to be a little high.

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In for use in reproductive toxicity studies, the minipig has many advantages over existing models. The reproductive physiology is close enough to man to be meaningful, and litter size and age to sexual maturity have big advantages over primates. Animal size means that investigations such as ultrasound and semen samples can be performed in addition to the traditional fertility and developmental toxicity measurements, including behavioural assessments.