Appendix 2

Foetal development

There are 26 stages in development and each of these stages, have clearly defined criteria which enables the researcher to identify the stage under investigation. When aiming for a range of stages, there are a number of factors to consider. Most importantly, the first morning a plug is noticed, is regarded as 0.5 dpc (0E), from this day to full term 17.5 - 19 days later, the embryo develops into a foetus at a rate determined by the mother's health and the number of pups in the uterine horns. The developing foetuses do not grow at a uniform rate and the resulting pups are often at slightly different developmental stages at a given time in the pregnancy. For example, if the uterine horns are dissected out at 13.5dpc, there will be a range of pups around this time within each horn. Clearly, when the pups are dissected out of their sacs it is easier to classify the developmental stage, however, if one is interested in the placenta and surrounding fluid then the embryo needs to be cryostated whilst in their sacs. Preliminary diagnosis of developmental stages comes prior to sectioning and requires the careful scrutinising of sections after cryostating, in addition to a surplus of sections to take into account those that are incorrectly diagnosed and cut at an odd angle.

The organs in mammalian development peak at different times during gestation and are often unrecognisable in the early stages, as the organ, which they later become. There follows a brief summary of the key characteristics, demonstrating the development of the main organs development, providing a reference for staging pups.

Differentiation of the **heart** begins with the splitting of the extra-embryonic mesoderm to form the intra-embryonic coelom at the 7 - 7.5 d.p.c. in the early headfold

stage embryo. Around 7.5 - 8 d.p.c. this division, forms the cardiogenic plate, spanning the ventral mid-line in the prospective pericardial region. However, it is not until 8.5 - 9 d.p.c that the primitive heart as an s shape can be seen to beat regularly with primitive red blood cells observed within the embryonic vasculature. During the period 9 - 10.5 d.p.c. there are little changes other than an accentuation of the s shape and the breakdown of a relatively small segment of dorsal mesocardium between the inflow and outflow regions of the heart, with the formation of the transverse pericardial sinus. At the 10 - 10.5 d.p.c. stage, the heart is the most prominent of all the organ and the first organ system to differentiate and function in the embryo.

The first major event in the differentiation of the **lungs** occurs at the 9 - 9.5 d.p.c. with the formation of the laryngo-tracheal grove, followed by the first indications of a pair of lung buds in the pericardio-peritoneal canals. At the 10 - 10.5 d.p.c. stage the canals extend either side of the heart, level with the lower part of the common atrial heart, in an uneven fashion initially with a single lobe on the left and four lobar bronchi on the right. By 12.5 - 13 d.p.c., the lungs have expanded to numerous segmental bronchi, consisting of homogenous cells, occupying a substantial volume of the peritoneal cavity. Detailed architecture of the future lung are first observed at 14.5 - 15 d.p.c., with the formation of terminal bronchioles dispersed throughout the lungs. During 15.5 - 17 d.p.c the lungs continue to branch such that by 17.5 - 18 d.p.c. the histological features resemble those at birth, with the exception of the cells that line the aveoli, which take on the squamous morphology at birth to facilitate gaseous exchange.

Primordial **germ** cells development is thought to originate during the early primitive streak stage (7 d.p.c.) as indicated by the presence of cells showing alkaline

phosphatase enzyme activity. In the slightly more advanced primitive streak stage these precursor germ cells are located in the mesodermal tissue of the viseral yolk sac, at the base of the allantois and at the caudal end of the primitive streak. By early somite stage (7.5 - 8 d.p.c) most primordial germ cells are located in association with the hindgut endoderm, some migrating to the mesentry of the hindgut by the 15 - 20 somite stage (9 - 9.5 d.p.c.), with the majority associated with the endoderm of the hindgut by somite stage 21 –30 (9.5 - 10.25 d.p.c.). By the 31 - 36 somite stage (10.25 - 10.5 d.p.c.) the majority of the primordial germ cells are found in the mesentry of the hindgut with a significant number having now migrated to the genital ridge.

Differentiation of the **urogenital** ridges, gonads and genital duct system begins around 9 d.p.c.. From 9.5 d.p.c. the urogenital ridges are first observed either side of the dorsal mesentry of the hindgut. By 11 d.p.c., the indifferent gonad can be seen, which increases in size and alkaline phosphatase activity over the following couple of days such that by 13 d.p.c. it is possible to distinquish between developing ovary and testis. At 13.5 d.p.c. early events associated with spermatogenosis occur including a number of type A spermatogonia in division, whilst the ovaries have a homogeneous cell morphology, first showing of egg clusters at 15.5 d.p.c.. By 17.5 - 18 d.p.c. the ovaries are in their final resting position with primary follicles and in the male the majority of the components of the reproductive system are recognisable.

Kidney differentiation is first noted with the appearance of mesonephric duct and tubules at 11.5 - 12 d.p.c., this system is later incorporated into the male reproductive system and completely regresses in the female, to be replaced by the formation of the metanephros by about 12.5 - 13 d.p.c. as an outgrowth of the ureteric bud. By 14.5 d.p.c.

primitive glomeruli are found throughout the early kidney and a well defined cortex and medulla region developed by 16.5 d.p.c.. By 17.5 d.p.c. the kidneys have ascended in relationship to the gonads (which have descended) and have a greater variety of cellular morphology.

The **extra embryonic** material of early stages (10 somites) consists of the amnion and the yolk sac. Not until 11.5 dpc is the placenta clearly seen, which matures over the following 6 - 7 days forming a labrynthine zone of trophoblast cells, endothelium cells and embryonic vessels.

In addition to the above criteria for organ development there is some organ movement within the confines of the developing foetus, to accommodate growing organs and tissues whilst they find their final resting place.