Chapter 5

Investigation into the cause of prenatal lethality
INVESTIGATION INTO THE CAUSE OF PRENATAL LETHALITY

Introduction

The survival and growth of the embryo is critically dependent on the placenta which forms an interface for maternal and fetal blood circulation, mediating nutrient and gas exchange and fetal waste disposal. Hmox1 plays an important role in placental development and function (Reviewed in Chapter 1). Defect in placentation was also reported in Hmox1 gene-targeted mice but the histology of the 12.5 dpc KO placenta was compared with the corresponding HET placenta (Poss, 1998). Subsequently another group compared placental weights from WT, HET and KO embryos at 12.5 dpc, 15.5 dpc and 18.5 dpc and reported it as a developmental delay in Hmox1 deficient mice (Zhao et al., 2009). The authors also compared the histology of 14.5 dpc placenta obtained from HET embryos with that from wild type matings. They found the spongiotrophoblast layer to be much thinner in these embryos as compared to the placenta from wild type matings. As no comparison was made with the KO placenta I decided to carry out a systematic analysis of the placentas from the gestational age 11.5 dpc to 18.5 dpc and the results are presented in this chapter.

During the course of my work the placental weights and histology of only 14.5 dpc WT, HET and KO placentas were reported (Zenclussen et al., 2011a) in Hmox1 mice on the BALB/c genetic background. All the studies mentioned have addressed the role of maternal Hmox1 in pregnancy. In this part of my study I addressed the role of placentation in the prenatal lethality of the KO embryos.
Results and Discussion

• Placental weights

The placental weights of the embryos (WT♦) from the wild type matings were compared with that in WT, HET and KO embryos from heterozygous matings. Due to the small size of the conceptus, dissection of intact placenta from 9.5 dpc and 10.5 dpc is difficult, so the placenta of these embryos were not collected. The placental weights are shown as dot density plots in Figures 47 to 49.

At 11.5 dpc (Figure 47), the placental weights of 95% WT♦ embryos were distributed between 28 mg to 65 mg. Within this range 80% WT and 92% HET placental weights were also distributed but 75% of the KO placental weights were clustered over a narrower range of 36 mg to 43 mg. At 12.5 dpc, ~ 82% of the WT♦ placental weights and all WT and HET placental weights were distributed over a range of 40 mg to 73 mg. The WT placental weights were distributed uniformly in this range while a clustered distribution was observed in the other two groups. KO placental weights were clustered together over a narrower range of 35 mg to 47 mg and were significantly lower than the HET and WT♦ (p < 0.05). It is concluded that at these two gestational ages the KO placentas were probably smaller and hence lighter.

The distribution of placental weights at 13.5 dpc, 14.5 dpc and 15.5 dpc is shown in Figure 48. At 13.5 dpc, the weights of placenta from WT♦ embryos vary between 44 mg to 88 mg and the WT placentas were also in this range; the distribution was uniform for both these groups. The HET and especially the KO placental weights were distributed sparsely over a larger range of 31 mg to 93 mg. The average placental weights of the WT and WT♦ embryos were higher than that of the KO. At 14.5 dpc, the placental weights of the WT♦ embryos form a dense cluster over a smaller range (68 mg to 97 mg). The three groups of placental weights from the heterozygous matings were uniformly distributed over a larger range (60 mg to 118 mg). The average placental weights were similar in the three groups and were higher than that from the WT♦ embryos. At 15.5 dpc, 90% placental weights from the WT♦ embryos and 70% WT from the heterozygous matings form a cluster within the
Figure 47: Dot density graphs and box plots of the placental weights of embryos at 11.5 dpc and 12.5 dpc. Four groups of embryos from two types of matings were – Wild type embryos (WT♦) from wild type matings, and three groups of embryos from heterozygous matings - wild type (WT), heterozygous (HET) and knockout (KO). In the box plot, the red and the black bars represent the mean and median respectively.
**Figure 48**: Dot density graphs and box plots of the placental weights of embryos at 13.5 dpc, 14.5 dpc and 15.5 dpc. Four groups of embryos from two types of matings were – Wild type embryos (WT♦) from wild type matings, and three groups of embryos from heterozygous matings - wild type (WT), heterozygous (HET) and knockout (KO). In the box plot, the red and the black bars represent the mean and median respectively.
Figure 48

13.5 dpc

Placenta weight (mg)

14.5 dpc

Placenta weight (mg)

15.5 dpc

Placenta weight (mg)

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range 76 mg to 106 mg. All the KO and HET placental weights were distributed in a higher range (80 mg to 127 mg) as compared to the other two groups. The average placental weight from HET embryos was significantly higher than that from the WT embryos (p < 0.05). The trend of lower average placental weight in KO embryos was observed only at 13.5 dpc. No other consistent pattern of average placental weights were observed at these three gestational ages.

The dot density plots in Figure 49, show the distribution of the placental weights at late gestation. At 16.5 dpc, the weights of the placenta from the WT embryos were distributed uniformly between 69 mg to 113 mg. The distribution range of WT and KO placental weights was higher (84 mg to 127 mg) while a much larger range was observed for HET placental weights (88 mg to 162 mg). The significantly higher average weight of the HET placenta was an unusual observation. At 18.5 dpc, the placental weights of the WT embryos range from 72 mg to 165 mg; the WT and HET placental weights were also found to be distributed in this range. The average placental weight of the WT and HET groups were similar and both were lower than the WT. The only KO embryo obtained had a lighter placenta as compared to the other three groups.

This study revealed that the placental weights of the embryos from heterozygous mating were more variable than those from the WT embryos. At the gestational ages 11.5 dpc, 12.5 dpc and 13.5 dpc the placenta from the KO embryos were lighter than those of the WT embryos. At all the other gestational ages, a consistent pattern of average placental weights was not observed in the four groups. My results at 14.5 dpc are not consistent with that reported recently (Zenclussen et al., 2011a)

- **Histological characterization of Hmox1 placenta**

Placental defects observed in mid-gestation KO embryos were reduced labyrinth area and placental size. Histological examination revealed placental hypoplasia at 12.5 dpc in KO embryos (Poss, 1998). Low level of Hmox1 in heterozygotes condition is associated with pathological changes in the placenta (Zhao
Figure 49: Dot density graphs and box plots of the placental weights of embryos at 16.5 dpc and 18.5 dpc. Four groups of embryos from two types of matings were – Wild type embryos (WT♦) from wild type matings, and three groups of embryos from heterozygous matings - wild type (WT), heterozygous (HET) and knockout (KO). In the box plot, the red and the black bars represent the mean and median respectively.
et al., 2009). At 14.5 dpc, the HET placenta had reduced spiral arteries and reduced spongiotrophoblast zone (due to apoptosis). Reduced giant cell number and junctional area in HET and KO placentas were also reported recently (Zenclussen et al., 2011a).

To characterize the morphology and tissue organization of the placenta, histological analysis was done at 14.5 dpc. This stage was chosen as the placenta is fully matured and thus any differences in tissue organization between the different genotypes would be visible. The schematic representation of mouse placenta at 14.5 dpc is shown in Figure 50. It is composed of three major layers - (1) the outer maternal decidua basalis which includes uterine decidual cells as well as maternal spiral artery; (2) a middle junctional zone which connects the innermost part of placenta to uterus and consists of many cell types, majorly spongiotrophoblast cells and glycogen trophoblast cells and (3) the inner labyrinth zone consisting of highly branched villi (Watson & Cross, 2005). This inner zone functions for efficient nutrient exchange between the mother and fetus.

Three placentas each from the WT♦, WT and KO embryos were used for analysis. These embryos were in TS22 which is the characteristic developmental stage of 14.5 dpc. In addition, the embryos had similar body and placental weights. The placentas were serially sectioned in the radial plane and the sections were examined to locate the site of umbilical attachment. This site was used as a reference point for comparing the sections as it is at the midpoint of the placenta.

A representative of each genotype (WT♦, WT and KO) is shown in Figure 51. The comparative histological analysis of the mid-radial sections at 2X magnification revealed that the typical morphology was observed in all placentas of the WT♦ embryos with well defined junctional and labyrinth zones, and distinct interdigitating organization between the two. In contrast, all the placentas of the WT and KO embryos had disorganized tissue architecture. These placentas had an inconspicuous junctional zone and the labyrinth area appeared enlarged.

At 10X magnification (Figure 52) all the major placental cell types: trophoblast giant cells, glycogen cells and spongiotrophoblasts were visible in the WT and KO placentas but the distinct organization of junctional zone was lacking. It was
Figure 50: Schematic representation of tissue organization in mature mouse placenta at 14.5 dpc showing the three major zones - decidua basalis (DB), junctional zone (JZ) and labyrinth zone (LZ). The arrows represent the orientation while sectioning the placenta in radial plane. (Adapted from Watson ED et. al. 2005)
Figure 51: Mid-radial sections of a 14.5 dpc placenta at 2X magnification were compared from wild type and heterozygous matings - WT♦ placentas of embryos from wild type matings; WT and KO embryos from the heterozygous matings are shown to same scale. The three major layers of placenta – decidua basalis (DB), junctional zone (JZ) and labyrinth zone (LZ) are clearly visible in the WT♦ placentas. In placentas of WT and KO embryos the typical structural organization is absent and the regions are indicated by “→“.
Figure 51
Figure 52: Mid-radial sections of the 14.5 dpc placentas at low (2X) and high (10X) magnifications. The area indicated in 2X image is shown at 10X magnification. The placentas from WT♦, WT and KO embryos are shown to the same scale. The junctional zone (JZ) and labyrinth zone (LZ) are clearly visible in the WT♦ placentas and the different types of cells identified are indicated in 10X magnification: A- trophoblast giant cells, B – glycogen cells and C - spongiotrophoblasts
Figure 52
also observed that spongiotrophoblast like cells abnormally invaded the labyrinth zone and thus the distinct boundary of the junctional zone was not defined.

The overall conclusion of results presented in this section is that both WT and KO placentas from the heterozygous matings have a disorganized tissue structure indicating that maternal Hmox1 level is an important factor in the normal development of placenta. In a recent publication (Zhao et al., 2011), malformation of the fetoplacental interface independent of the genotype of the embryos was observed in case of heterozygous maternal background. Taken together with my results I conclude that defective placentation probably affects the size of the embryos, and may not be the cause of prenatal lethality of KO embryos.
List of the placentas of 14.5 dpc embryos used for histological analysis.

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