Pre-implantation and placental development in humans and mice

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Summary

Studies aimed at understanding pre-implantation and placental development in humans and mice have provided essential implications for assisted reproductive technology and demonstrated the underlying causes and therapies for infertility, recurrent miscarriage and pregnancy complications. In this thesis, **Chapter 1** presents the state of the literature on early development in both humans and mice, including primordial germ cells (PGCs) development, pre-implantation development, implantation, early placentation and placental development. It also provides a review of two epigenetic regulation processes established in early development: X chromosome inactivation (XCI) and genomic imprinting.

Chapter 2 describes the development of gonadal and extragonadal PGCs in humans, using immunofluorescence for germ cells markers and meiotic markers. Between first and second trimesters, there is a shift towards POU5F1−/DDX4+ germ cells in gonads. Human ectopic germ cells are present in both first- and second-trimester adrenals. Moreover, the asynchronous meiotic entry of human 'adrenal' and 'ovarian' germ cells highlights species-specific differences between human and mouse early gonadal and extragonadal gametogenesis.

Chapter 3 investigates the status of XCI in human pre-implantation embryos at single-cell level, by analyzing online available RNA-seq data of human embryonic day (E)3-E7 embryos. Equal proportion of biallelic expression in "X-sub" (X-genes subject to X inactivation) and in autosomal genes suggests that both X chromosomes are active in E3 cells. Smaller proportion of biallelic expression in "X-sub" than in autosomal genes indicates that one X chromosome is inactive in E4-E6 cells and E7 trophectoderm (TE) cells, whereas it becomes reactivated in E7 epiblast and primitive endoderm cells. In addition, combined human androgen receptor (*AR*)/retinitis pigmentosa 2 (*RP2*) DNA methylation assay shows equal methylation of paternal and maternal alleles in female TE-derived cells, indicating non-imprinted X inactivation in human TE-derived cells, in contrast to the imprinted XCI in mouse TE-derived cells.

Chapter 4 describes in detail the invasion of human fetal extravillous trophoblast cells (EVTs) in maternal decidua and decidual vasculature during early pregnancy (W5.5-W12), using combined immunofluorescence and fluorescence in situ hybridization (FISH) for chrX/chrY to identify (male) EVTs unambiguously. This study serves as a reference for the entering of EVTs in maternal circulation (via decidual veins and lymphatic vessels) since W5.5, much earlier than previously accepted W8 (via decidual spiral arteries). The placental decidua interface of one Klinefelter syndrome embryo (mosaic 47,XXY and 46,XY) is also described.

Chapter 5 studies the spatial imprinting pattern of *IGF2/H19* in multiple-site collection of human first-trimester placental villi, using bisulfite DNA sequencing and allele-specific expression analysis of two informative non-CpG single-nucleotide polymorphism (SNP) sites allowing to discriminate the maternal and paternal alleles. Demethylated maternal alleles and methylated paternal alleles suggest a normal imprinting pattern of *IGF2/H19* in multi-site placental villi collections as in the embryo.

Chapter 6 explores different consequences of Turner syndrome, using a mouse model that harbors either a single maternally inherited (Xm) or paternally inherited (Xp) chromosome, by a detailed analysis of morphology and glucose metabolism in E18.5 XmO and XpO placentas compared to wild type placentas. The significantly larger area occupied by glycogen cells in XpO placental outer zone (junctional zone, trophoblast giant cells and decidua) and the significantly higher expression of *Ldha* in XpO labyrinth zone suggest a more severe placental phenotype in E18.5 XpO placentas than in XmO placentas, with increased anaerobic glycolysis and underlying defects in oxygen availability in XpO placentas.

Finally, **Chapter 7** provides general discussion about the findings described in this thesis. This section also presents and discusses the future perspectives on embryo selection in assisted reproduction and new treatment strategies for infertility, pregnancy complications and sex chromosome related syndromes.

Together, the work in this thesis provides novel insights into gonadal and extragonadal PGCs development in humans and XCI in human pre-implantation embryos. Also we have demonstrated the invasion of decidual vasculature by human extravillous trophoblast cells during early pregnancy, the spatial imprinting pattern of *IGF2/H19* in human first-trimester placental villi and finally the consequences of harboring different combinations of sex chromosomes in the placenta.