Pre-implantation and placental development in humans and mice

He, N.

CHAPTER 7

General discussion and future perspectives

Human (extra)gonadal germ cell development

From the extragonadal location in which they originate, human primordial germ cells (PGCs) migrate and arrive at the developing gonadal primordia at approximately week 7 of gestation (W7, or week 5 post conception) [1-3]. Ectopic PGCs have been described in mouse and bovine adrenals [4-6]. Mostly, these ectopic germ cells are eliminated by apoptosis [7, 8]. However, some of them may escape from entering the normal apoptosis pathway and give rise to extragonadal germ cell tumors [9, 10]. Since the gonads have a common somatic origin as adrenal cortex [11-13], the aim of **Chapter 2** is to investigate whether there are human ectopic germ cells in adrenals and to study the development of human (extra)gonad germ cells in first and second trimesters.

Human germ cells (POU5F1-positive and/or DDX4-positive) are present in all W8.4-W22 adrenal glands, but not in kidney. Between first and second trimesters, germ cells gradually downregulate the pluripotency marker POU5F1 and upregulate late germ cell marker DDX4 in both male and female gonads. Human ovarian germ cells do not enter meiosis synchronously at W22, in agreement with previous reports [3, 14, 15]. Meiotic entry of mouse adrenal and gonadal PGCs has been described to be synchronous [6]. In contrast to mice, human adrenal germ cells show no meiotic entry at least until W21.5, although the possibility that they enter meiosis afterwards cannot be excluded. This work serves as a reference presenting evidence for the existence of human ectopic germ cells in adrenals. Our findings highlight differences in gonadal and extragonadal gametogenesis between humans and mice.

Human PGCs have been suggested to migrate along class III beta-tubulin (TUBB3)-positive neural crest derivations to reach gonadal primordia [2, 10]. Interestingly, early germ cells (POU5F1-positive) in gonads and adrenals are also TUBB3-positive whereas late germ cells (DDX4-positive) are TUBB3-negative. The PGCs that migrate along common routes with neural crest cells to reach the adrenal may thus become ectopic germ cells there. Because the gonads have a common somatic origin as adrenals [11-13], rat PGCs are shown to reach adrenals before gonadal and adrenal primordia are physically separated [16]. This can also explain the colonization of human ectopic germ cells in adrenals. Furthermore, it is important to study the fate of these ectopic germ cells during further development. Whether they will enter meiosis or will lose their germ cell identities?

Ectopic PGCs that escape from entering apoptosis are thought to account for the development of extragonadal germ cell tumors [9, 10, 17, 18]. Interestingly, single-nucleotide polymorphisms (SNPs) located in gene DDX4 have been found to be associated with neuroblastoma tumors [19]. Furthermore, TUBB3-positive neural crest derivatives are thought to be related to the origin of adrenal neuroblastoma tumors [20]. Since there is expression of TUBB3 in ovarian and adrenal POU5F1-positive germ cells, further studies are needed to investigate the potential relationship between ectopic adrenal germ cells and neuroblastoma by studying the expression of additional markers.

Epigenetic regulation in human pre-implantation embryos and first-trimester placental villi

X chromosome inactivation (XCI) and genomic imprinting are two essential specific epigenetic regulation processes established in early development [21], controlling the dosage of X-linked genes and parent-of-origin-specific genes respectively.

Well-studied genetic substrains of mice have revealed two separate XCI waves during mouse female embryo development [22, 23]. However, the XCI dynamics in human female pre-implantation embryos remain unknown, because the parental allelic origin of human embryos is often not available. Recent advances (i.e. single cell RNA-Seq) have enabled the allele-specific expression analysis in human pre-implantation embryos to study XCI status at single-cell resolution [24]. The main aim of **Chapter 3** is to understand the status of XCI in human pre-implantation embryos at single-cell level. Do trophectoderm (TE) cells have different XCI pattern from inner cell mass (ICM) at embryonic day (E)5-E7? If there is X inactivation, whether the XCI pattern is imprinted or random? In this study, X-linked genes are separated into X-sub (X-genes subject to X inactivation) and X-esc (escapees) categories since escapees have biallelic expression, even though some studies do not show significant differences in escapees expression level between sexes [25, 26]. As demonstrated in **Chapter 3**, equal proportion of biallelic expression in X-sub and in autosomal genes suggests that both X chromosomes are active in E3 cells. A 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 TE cells, whereas there is reactivation in E7 epiblast (EPI) and primitive endoderm (PE) cells. Additionally, equal DNA methylation of both maternal and paternal alleles in *AR* and *RP2* genes indicates non-imprinted X inactivation in human TE-derived cells, in contrast to the imprinted XCI in mouse TE cells [22]. Results strongly contrast with the previous hypothesis that both X chromosomes are active during human pre-implantation (E3-E7) development [24]. This is because in the allele-specific expression analysis of Petropoulos and colleagues [24], without genome sequencing data, only the SNPs with biallelic expression or random monoallelic expression could be detected as informative SNPs. Therefore, SNPs having imprinted monoallelic expression within an embryo would be missed in the analysis. In addition, those genes with biallelic expression can be X-esc genes at human pre-implantation stage. In another study using RNA FISH for X-linked genes to detect XCI, *ATRX* gene shows biallelic expression in most cells of E5 female blastocysts (without separation of ICM from TE) [27]; however, *ATRX* gene is an X-Y paired gene (thus an escapee) [28] which is supposed to have biallelic expression even on the inactive X chromosome. The first XCI wave in mouse pre-implantation embryos occurs at the two- to four-cell stage [23] while in humans it happens between E3-E4. This difference is probably due to the different timing of zygotic genome activation (ZGA) in mice (at two-cell stage [23]) and in humans (at E4 [24, 29]).

The imprinted *IGF2/H19* cluster domain, controlled by the *IGF2/H19* imprinting control region (ICR1), plays an essential role in placental and embryo growth and development in mammals [30, 31]. According to the parent-offspring conflict theory [32], paternally expressed

IGF2 gene enhances cell proliferation while maternally expressed *H19* gene inhibits embryo growth and somehow negatively affects size of the offspring [33]. Gain or loss of methylation at ICR1 and consequent dysregulation of *IGF2/H19* account for perinatal complications including small for gestational age embryos, intrauterine growth restriction, Beckwith–Wiedemann syndrome (BWS) [34] and Russell–Silver syndrome (RSS) [35]. Chorionic villus sampling (CVS) is a procedure used in prenatal diagnosis of fetal abnormalities. However, it remains unknown whether spatial variation in methylation exists in placental villi and whether the imprinting pattern in placental villi is representative for the fetus. Therefore, the focus of **Chapter 5** is to investigate whether the multiple sites of first-trimester placental villi show the same imprinting pattern of *IGF2/H19* and whether it is the same as in the embryo. In the multiple sites of collection in human first-trimester placental villi (and embryos) analyzed in two pregnancies, demethylated maternal alleles and methylated paternal alleles indicate that multi-site placental villi collections have the same imprinting pattern of the *IGF2/H19* ICR1, also being the same as in embryos. This contrasts with the variability reported between different placental villi at term [36]. The difference may be resulted from the fact that Turan et al analyzed a larger number of placentas, but only one CpG point was analyzed, whereas in our study the methylation status of all CpGs in two placentas are investigated by bisulfite sequencing. In humans, *H19*, but not *IGF2*, has been reported to be biallelically expressed at least in cytotrophoblasts before W10, but to become monoallelically expressed in placentas at term [37-40]. Since there is a mixture of two cell types within placental villi (TE-derived trophoblast cells and ICM-derived mesenchymal cells), it remains to be investigated whether the imprinting patterns of *IGF2/H19* ICR1 in these two cell types are the same as in the embryo.

The epigenome is known to be sensitive to environmental changes [41]. Studies have suggested that treatments and procedures (i.e. ovarian stimulation, intracytoplasmic sperm injection, pre-implantation embryo culture) in assisted reproductive technology (ART) may change epigenetic marks (including *IGF2/H19*) of gametes and embryos [42-46]. Moreover, children conceived by ART are reported to have a higher incidence of imprinted disorders (i.e. Angelman syndrome and BWS) [47-49]. Therefore, the epigenetic dynamics during pre-implantation development is expected to be better understood within the next decades, which shall provide evidence for embryo selection [via pre-implantation genetic diagnosis/screening (PGD/PGS)] and successful pregnancy in assisted reproduction. Although obtaining maternal and/or paternal materials has of course both ethical and practical issues, future work investigating (epi)genetics in human pre-implantation embryos may require embryonic genome sequencing, embryonic RNA-Seq as well as parental genome sequencing data. Furthermore, future investigations could extend to XCI dynamics and imprinting pattern in TE-derived trophoblast cells and ICM-derived mesenchymal cells in placental villi.

Human extravillous trophoblast cells (EVTs) invasion in decidual vasculature during early pregnancy

Fetal EVTs have been described to block the entrance of uterine spiral arteries at W8-W12, thus preventing maternal blood flow into the intervillous space [50-52]. However, exosomes, placental particles [53, 54] and fetal cells [55] have been detected in the maternal blood from W6 onwards, without the presence of robust materno-placental vascular connection. For this reason, the aim of **Chapter 4** is to investigate whether there are alternative routes for EVTs to enter maternal circulation in early pregnancy (W5.5-W12) other than via spiral arteries. As shown in **Chapter 4**, fetal mononuclear EVTs enter veins and lymphatic vessels in decidua basalis directly from W5.5 onwards, much earlier than their remodelling decidual spiral arteries at W8. Same observation in a pregnancy with Klinefelter syndrome in the conceptus (W8.4, mosaic 46,XY and 47,XXY) indicates that efficient entry of maternal (blood and lymph) circulation by interstitial mononuclear EVTs occurs much earlier than previously reported W8 (via entering spiral arteries) [53]. These findings are supported by two very recent studies showing EVTs invasion in decidual veins in the first trimester, based on immunofluorescence for the EVT marker HLA-G [56, 57]. While attention should be paid to the different HLA-G isoforms when using HLA-G to determine EVTs [58], we are the first to combine chrX/chrY fluorescence in situ hybridization (FISH) and immunofluorescence to address the problem for unambiguous (male) EVTs identification. It could be important to investigate the EVTs invasion in decidua in pregnancies with other sex chromosomal disorders (i.e. Turner syndrome).

The entry of maternal circulation by EVTs via decidual veins and lymphatics since W5.5, occurring much earlier than the establishment of maternal-placental vascular connection at W8-W12 [53], enlarges the maternal-fetal interface during early pregnancy. Therefore, while maternal immune response in the uterus include crosstalk between immune cells (natural killer cells, lymphocytes) and EVTs [59, 60], EVTs invading maternal circulation contribute to the response that takes place outside the uterus.

Insufficient invasion of human fetal EVTs into maternal decidua may cause inadequate placental perfusion and consequent pregnancy complications including preeclampsia and intrauterine growth restriction [61-64]. Using the approach provided in **Chapter 4** to identify the (male) EVTs unambiguously in maternal decidua, defective EVTs invasion in pregnancy complications can be further defined and this may lead to identification of underlying mechanism and promising therapy strategies.

Question remaining to be answered is the underlying cellular/molecular mechanism of EVTs invasion in vein and lymphatics in first trimester. Other investigations could focus on the shared invasive characteristics (epithelial–mesenchymal transition) between EVTs and metastasizing malignant cells [65]. This may offer more knowledge on causes of tumor metastasis and provide valuable tumor therapies.

Abnormalities in mouse Turner syndrome placentas

Mice with a genetic form of Turner syndrome (39,XO) harboring either a single paternally inherited (Xp) or maternally inherited (Xm) chromosome show quite different survival rates to term [66-68]. Nevertheless, no detailed comparison of XpO and XmO placentas has been made to explain this difference. The aim of **Chapter 6** is to study whether the transcriptional level of Xp is equal to Xm, by comparing the consequences of Turner syndrome harboring either a single Xp or Xm in mouse E18.5 placenta. The larger area occupied by glycogen cells (GCs) and significantly increased expression level of *Ldha* in the outer zone (junctional zone, trophoblast giant cells and decidua) of XpO placenta indicate that the XpO genotype leads to a more severe placental phenotype than XmO at E18.5, and thus the transcriptional level of Xp is not equal to Xm.

During placental development, TE-derived cells in XX placentas have obligatory active Xm and inactive Xp [69]. The significantly larger amount of GCs in the XpO outer zone might be an adaptive response to defects in the epigenetic regulation of imprinted genes [70-72]. Moreover, abnormal amount of GCs, serving as a potential source of energy in mouse placenta [73], may indicate defects in glycogen metabolism and glucose transport [74, 75]. The higher *Ldha* expression, which encodes the enzyme converting lactate to pyruvate in anaerobic glycolysis, suggests increased anaerobic glycolysis and possible insufficient available oxygen in XpO placentas. This is in accordance with the reported increase in *Ldha* expression in human primary placental trophoblast cells cultured under hypoxic conditions [76].

Although it remains to be clarified how harboring either a single Xp or Xm affects mouse placental development and function, findings in **Chapter 6** contribute to the understanding of different effects of inheriting a single paternal or maternal X chromosome on mouse placental phenotypes and hints to a defect in glucose metabolism. The investigation of glucose metabolism in human Turner syndrome patients and their placentas is needed and may provide new therapeutic strategies for individuals.

Together, the results in this thesis provide new insights into normal development of human (extra)gonadal germ cells, human pre-implantation development as well as placental development in humans and mice. Furthermore, results offer more knowledge on how defects in these processes might cause abnormal pregnancy outcomes and placental functions.

References

1. Byskov AG: Differentiation of mammalian embryonic gonad. Physiol Rev 1986, 66:71-117.
2. Mollgard K, Jespersen A, Lutterodt MC, Yding Andersen C, Hoyer PE, Byskov AG: Human primordial germ cells migrate along nerve fibers and Schwann cells from the dorsal hind gut mesentery to the gonadal ridge. Mol Hum Reprod 2010, 16:621-631.
3. Heeren A, van Iperen L, Klootwijk DB, de Melo Bernardo A, Roost MS, Gomes Fernandes MM, Louwe LA, Hilders CG, Helmerhorst FM, van der Westerlaken LA, Chuva de Sousa Lopes SM: Development of the follicular basement membrane during human gametogenesis and early folliculogenesis. BMC Dev Biol 2015, 15:4.
4. Upadhyay S, Zamboni L: Ectopic germ cells: natural model for the study of germ cell sexual differentiation. Proc Natl Acad Sci U S A 1982, 79:6584-6588.
5. Wrobel KH, Suss F: On the origin and prenatal development of the bovine adrenal gland. Anat Embryol (Berl) 1999, 199:301-318.
6. Zamboni L, Upadhyay S: Germ cell differentiation in mouse adrenal glands. J Exp Zool 1983, 228:173-193.
7. Runyan C, Gu Y, Shoemaker A, Looijenga L, Wylie C: The distribution and behavior of extragonadal primordial germ cells in Bax mutant mice suggest a novel origin for sacrococcygeal germ cell tumors. Int J Dev Biol 2008, 52:333-344.
8. Stallock J, Molyneaux K, Schaible K, Knudson CM, Wylie C: The pro-apoptotic gene Bax is required for the death of ectopic primordial germ cells during their migration in the mouse embryo. Development 2003, 130:6589-6597.
9. Keegan CE, Hammer GD: Recent insights into organogenesis of the adrenal cortex. Trends Endocrinol Metab 2002, 13:200-208.
10. Mamsen LS, Brochner CB, Byskov AG, Mollgard K: The migration and loss of human primordial germ stem cells from the hind gut epithelium towards the gonadal ridge. Int J Dev Biol 2012, 56:771-778.
11. Hanley NA, Ball SG, Clement-Jones M, Hagan DM, Strachan T, Lindsay S, Robson S, Ostrer H, Parker KL, Wilson DI: Expression of steroidogenic factor 1 and Wilms' tumour 1 during early human gonadal development and sex determination. Mech Dev 1999, 87:175-180.
12. Hanley NA, Rainey WE, Wilson DI, Ball SG, Parker KL: Expression profiles of SF-1, DAX1, and CYP17 in the human fetal adrenal gland: potential interactions in gene regulation. Mol Endocrinol 2001, 15:57-68.
13. Morohashi K: The ontogenesis of the steroidogenic tissues. Genes Cells 1997, 2:95-106.
14. Anderson RA, Fulton N, Cowan G, Coutts S, Saunders PT: Conserved and divergent patterns of expression of DAZL, VASA and OCT4 in the germ cells of the human fetal ovary and testis. BMC Dev Biol 2007, 7:136.
15. Kurilo LF: Oogenesis in antenatal development in man. Hum Genet 1981, 57:86-92.
16. Hatano O, Takakusu A, Nomura M, Morohashi K: Identical origin of adrenal cortex and gonad revealed by expression profiles of Ad4BP/SF-1. Genes Cells 1996, 1:663-671.
17. Looijenga LH, Van Agthoven T, Biermann K: Development of malignant germ cells - the genvironmental hypothesis. Int J Dev Biol 2013, 57:241-253.
18. Schneider DT, Schuster AE, Fritsch MK, Hu J, Olson T, Lauer S, Gobel U, Perlman EJ: Multipoint imprinting analysis indicates a common precursor cell for gonadal and nongonadal pediatric germ cell tumors. Cancer Res 2001, 61:7268-7276.
19. Nguyen le B, Diskin SJ, Capasso M, Wang K, Diamond MA, Glessner J, Kim C, Attiyeh EF, Mosse YP, Cole K, et al: Phenotype restricted genome-wide association study using a gene-centric approach identifies three low-risk neuroblastoma susceptibility Loci. PLoS Genet 2011, 7:e1002026.
20. De Preter K, Vandesompele J, Heimann P, Yigit N, Beckman S, Schramm A, Eggert A, Stallings RL, Benoit Y, Renard M, et al: Human fetal neuroblast and neuroblastoma transcriptome analysis confirms neuroblast origin and highlights neuroblastoma candidate genes. Genome Biol 2006, 7:R84.
21. Inbar-Feigenberg M, Choufani S, Butcher DT, Roifman M, Weksberg R: Basic concepts of epigenetics. Fertil Steril 2013, 99:607-615.
22. Okamoto I, Otte AP, Allis CD, Reinberg D, Heard E: Epigenetic dynamics of imprinted X inactivation during

early mouse development. Science 2004, 303:644-649.

23. Deng Q, Ramskold D, Reinius B, Sandberg R: Single-cell RNA-seq reveals dynamic, random monoallelic gene expression in mammalian cells. Science 2014, 343:193-196.

24. Petropoulos S, Edsgard D, Reinius B, Deng Q, Panula SP, Codeluppi S, Plaza Reyes A, Linnarsson S, Sandberg R, Lanner F: Single-Cell RNA-Seq Reveals Lineage and X Chromosome Dynamics in Human Preimplantation Embryos. Cell 2016, 165:1012-1026.

25. Xiong Y, Chen X, Chen Z, Wang X, Shi S, Wang X, Zhang J, He X: RNA sequencing shows no dosage compensation of the active X-chromosome. Nature genetics 2010, 42:1043-1047.

26. Disteche CM: Dosage compensation of the active X chromosome in mammals. Nature genetics 2006, 38:47-53.

27. Okamoto I, Patrat C, Thepot D, Peynot N, Fauque P, Daniel N, Diabangouaya P, Wolf JP, Renard JP, Duranthon V, Heard E: Eutherian mammals use diverse strategies to initiate X-chromosome inactivation during development. Nature 2011, 472:370-374.

28. Bellott DW, Hughes JF, Skaletsky H, Brown LG, Pyntikova T, Cho TJ, Koutseva N, Zaghlul S, Graves T, Rock S, et al: Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. Nature 2014, 508:494-499.

29. Yan L, Yang M, Guo H, Yang L, Wu J, Li R, Liu P, Lian Y, Zheng X, Yan J, et al: Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat Struct Mol Biol 2013, 20:1131-1139.

30. Frost JM, Moore GE: The importance of imprinting in the human placenta. PLoS Genet 2010, 6:e1001015.

31. Demars J, Shmela ME, Rossignol S, Okabe J, Netchine I, Azzi S, Cabrol S, Le Caignec C, David A, Le Bouc Y, et al: Analysis of the IGF2/H19 imprinting control region uncovers new genetic defects, including mutations of OCT-binding sequences, in patients with 11p15 fetal growth disorders. Hum Mol Genet 2010, 19:803-814.

32. Moore T, Haig D: Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet 1991, 7:45-49.

33. Reik W, Walter J: Genomic imprinting: parental influence on the genome. Nat Rev Genet 2001, 2:21-32.

34. Weksberg R, Shuman C, Beckwith JB: Beckwith-Wiedemann syndrome. Eur J Hum Genet 2010, 18:8-14.

35. Azzi S, Steunou V, Tost J, Rossignol S, Thibaud N, Das Neves C, Le Jule M, Habib WA, Blaise A, Koudou Y, et al: Exhaustive methylation analysis revealed uneven profiles of methylation at IGF2/ICR1/H19 11p15 loci in Russell Silver syndrome. J Med Genet 2015, 52:53-60.

36. Turan N, Katari S, Gerson LF, Chalian R, Foster MW, Gaughan JP, Coutifaris C, Sapienza C: Inter- and intra-individual variation in allele-specific DNA methylation and gene expression in children conceived using assisted reproductive technology. PLoS Genet 2010, 6:e1001033.

37. Adam GI, Cui H, Miller SJ, Flam F, Ohlsson R: Allele-specific in situ hybridization (ASISH) analysis: a novel technique which resolves differential allelic usage of H19 within the same cell lineage during human placental development. Development 1996, 122:839-847.

38. Buckberry S, Bianco-Miotto T, Hiendleder S, Roberts CT: Quantitative allele-specific expression and DNA methylation analysis of H19, IGF2 and IGF2R in the human placenta across gestation reveals H19 imprinting plasticity. PLoS One 2012, 7:e51210.

39. Jinno Y, Ikeda Y, Yun K, Maw M, Masuzaki H, Fukuda H, Inuzuka K, Fujishita A, Ohtani Y, Okimoto T, et al.: Establishment of functional imprinting of the H19 gene in human developing placentae. Nat Genet 1995, 10:318-324.

40. Monk D, Arnaud P, Apostolidou S, Hills FA, Kelsey G, Stanier P, Feil R, Moore GE: Limited evolutionary conservation of imprinting in the human placenta. Proc Natl Acad Sci U S A 2006, 103:6623-6628.

41. el Hajj N, Haaf T: Epigenetic disturbances in in vitro cultured gametes and embryos: implications for human assisted reproduction. Fertility and sterility 2013, 99:632-641.

42. Stouder C, Deutsch S, Paoloni-Giacobino A: Superovulation in mice alters the methylation pattern of imprinted genes in the sperm of the offspring. Reproductive Toxicology 2009, 28:536-541.

43. Sato A, Otsu E, Negishi H, Utsunomiya T, Arima T: Aberrant DNA methylation of imprinted loci in superovulated oocytes. Human reproduction 2007, 22:26-35.

44. Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM: Differential Effects of Culture

on Imprinted H19 Expression in the Preimplantation Mouse Embryo 1. Biology of reproduction 2000, 62:1526-1535.

45. Rivera RM, Stein P, Weaver JR, Mager J, Schultz RM, Bartolomei MS: Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. Human molecular genetics 2008, 17:1-14.

46. Fernández-Gonzalez R, Moreira P, Bilbao A, Jiménez A, Pérez-Crespo M, Ramírez MA, De Fonseca FR, Pintado B, Gutiérrez-Adán A: Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. Proceedings of the National Academy of Sciences of the United States of America 2004, 101:5880-5885.

47. Pinborg A, Loft A, Romundstad LB, Wennerholm UB, Söderström-Anttila V, Bergh C, Aittomäki K: Epigenetics and assisted reproductive technologies. Acta obstetricia et gynecologica Scandinavica 2016, 95:10-15.

48. DeBaun MR, Niemitz EL, Feinberg AP: Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. The American Journal of Human Genetics 2003, 72:156-160.

49. Bowdin S, Allen C, Kirby G, Brueton L, Afnan M, Barratt C, Kirkman-Brown J, Harrison R, Maher ER, Reardon W: A survey of assisted reproductive technology births and imprinting disorders. Human Reproduction 2007, 22:3237-3240.

50. Burton GJ, Jauniaux E, Watson AL: Maternal arterial connections to the placental intervillous space during the first trimester of human pregnancy: the Boyd collection revisited. Am J Obstet Gynecol 1999, 181:718-724.

51. Pijnenborg R, Vercruysse L, Hanssens M: The uterine spiral arteries in human pregnancy: facts and controversies. Placenta 2006, 27:939-958.

52. Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ: Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. Am J Pathol 2000, 157:2111-2122.

53. Chamley LW, Holland OJ, Chen Q, Viall CA, Stone PR, Abumaree M: Review: where is the maternofetal interface? Placenta 2014, 35 Suppl:S74-80.

54. Sarker S, Scholz-Romero K, Perez A, Illanes SE, Mitchell MD, Rice GE, Salomon C: Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. J Transl Med 2014, 12:204.

55. Merviel P, Aractingi S, Uzan S: [Detection of fetal cells in maternal blood: myth or reality?]. Gynecol Obstet Fertil 2001, 29:371-376.

56. Moser G, Weiss G, Sundl M, Gauster M, Siwetz M, Lang-Olip I, Huppertz B: Extravillous trophoblasts invade more than uterine arteries: evidence for the invasion of uterine veins. Histochem Cell Biol 2016.

57. Windsperger K, Dekan S, Pils S, Golletz C, Kunihs V, Fiala C, Kristiansen G, Knöfler M, Pollheimer J: Extravillous trophoblast invasion of venous as well as lymphatic vessels is altered in idiopathic, recurrent, spontaneous abortions. Human Reproduction 2017, 32:1208-1217.

58. James JL, Chamley LW: A caution on the use of HLA-G isoforms as markers of extravillous trophoblasts. Placenta 2008, 29:305-306; author reply 307.

59. Gregori S, Amodio G, Quattrone F, Panina-Bordignon P: HLA-G Orchestrates the Early Interaction of Human Trophoblasts with the Maternal Niche. Front Immunol 2015, 6:128.

60. Lash GE: Molecular Cross-Talk at the Feto-Maternal Interface. Cold Spring Harb Perspect Med 2015, 5.

61. Burke SD, Karumanchi SA: Spiral artery remodeling in preeclampsia revisited. Hypertension 2013, 62:1013-1014.

62. Fisher SJ: Why is placentation abnormal in preeclampsia? Am J Obstet Gynecol 2015, 213:S115-122.

63. Plaisier M, Dennert I, Rost E, Koolwijk P, van Hinsbergh VW, Helmerhorst FM: Decidual vascularization and the expression of angiogenic growth factors and proteases in first trimester spontaneous abortions. Hum Reprod 2009, 24:185-197.

64. Plaisier M, Rodrigues S, Willems F, Koolwijk P, van Hinsbergh VW, Helmerhorst FM: Different degrees of vascularization and their relationship to the expression of vascular endothelial growth factor, placental growth factor, angiopoietins, and their receptors in first-trimester decidual tissues. Fertil Steril 2007, 88:176-187.

65. Kokkinos M, Murthi P, Wafai R, Thompson E, Newgreen D: Cadherins in the human placenta–epithelial–mesenchymal transition (EMT) and placental development. Placenta 2010, 31:747-755.

66. Burgoyne PS, Evans EP, Holland K: XO monosomy is associated with reduced birthweight and lowered weight gain in the mouse. J Reprod Fertil 1983, 68:381-385.

67. Burgoyne PS, Tam PP, Evans EP: Retarded development of XO conceptuses during early pregnancy in the mouse. J Reprod Fertil 1983, 68:387-393.

68. Hunt PA: Survival of XO mouse fetuses: effect of parental origin of the X chromosome or uterine environment? Development 1991, 111:1137-1141.

69. Harper MI, Fosten M, Monk M: Preferential paternal X inactivation in extraembryonic tissues of early mouse embryos. J Embryol Exp Morphol 1982, 67:127-135.

70. Hemberger M: The role of the X chromosome in mammalian extra embryonic development. Cytogenet Genome Res 2002, 99:210-217.

71. Lefebvre L: The placental imprintome and imprinted gene function in the trophoblast glycogen cell lineage. Reprod Biomed Online 2012, 25:44-57.

72. Sandovici I, Hoelle K, Angiolini E, Constancia M: Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming. Reprod Biomed Online 2012, 25:68-89.

73. Coan PM, Conroy N, Burton GJ, Ferguson-Smith AC: Origin and characteristics of glycogen cells in the developing murine placenta. Dev Dyn 2006, 235:3280-3294.

74. Redline RW, Chernicky CL, Tan HQ, Ilan J, Ilan J: Differential expression of insulin-like growth factor-II in specific regions of the late (post day 9.5) murine placenta. Molecular reproduction and development 1993, 36:121-129.

75. Sibley C, Coan P, Ferguson-Smith A, Dean W, Hughes J, Smith P, Reik W, Burton G, Fowden A, Constancia M: Placental-specific insulin-like growth factor 2 (IGF2) regulates the diffusional exchange characteristics of the mouse placenta. Proceedings of the National Academy of Sciences of the United States of America 2004, 101:8204-8208.

76. Kay HH, Zhu S, Tsoi S: Hypoxia and lactate production in trophoblast cells. Placenta 2007, 28:854-860.

7