Pre-implantation and placental development in humans and mice

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CHAPTER 1

General introduction

Primordial germ cells and gametogenesis in humans and mice

The immortal life cycle of mammalian germ cells

In mammals, germ cells are immortal and highly specialized for transmitting genetic information through generations [1, 2]. Following the segregation of the germline from the somatic lineage early in embryogenesis [3], primordial germ cells (PGCs), the progenitors of haploid gametes, originate outside the early post-implantation embryo at the border with the extraembryonic part of the embryo. The PGCs then migrate across the embryo to reach the developing gonad [4, 5]. Through spermatogenesis or oogenesis in the gonads, PGCs differentiate into sperms or oocytes via meiosis (Figure 1) [3]. Following the fusion of sperm with an mature oocyte, the haploid genomes of each parent are unified, resulting in a totipotent one-cell zygote [6]. The zygote, which represents the earliest developmental stage of embryogenesis, will develop into an embryo and eventually a complete organism together with all of its extraembryonic structures [7]. These extraembryonic structures mediate implantation in the uterine wall and the following gas exchange and nutrient supply. From the post-implantation epiblast, PGCs are specified [8-10].

Origin and migration of human and mouse PGCs

Due to the limited access to very early human embryos and more importantly restrictions to the allowed time in culture (14 days), little is known about PGCs formation in humans [10]. During weeks 5-6 of gestation (W5-W6, or weeks 3-4 post conception), PGCs are observed in human embryos in the yolk sac wall close to the allantois [11]. Subsequently, they migrate through the dorsal hind gut mesentery to the gonadal primordia [12] and arrive at the prospective ovary or testis around W7 [13]. By contrast, mouse PGC precursors are known to be induced in epiblast-derived embryonic ectoderm at embryonic day (E)6.25, located between extraembryonic and embryonic ectoderm [14]. At E7.25, PGCs are present at the base of the mesodermal allantois [10, 15]. From there they migrate through the developing hindgut, along the midline of the embryo, and reach the genital ridges at around E10.5 [4, 16]. However, some PGCs may stop migrating on their way to the gonads or become lodged in extragonadal organs such as adrenal glands [17, 18]. Ectopic PGCs are often associated with extragonadal germ cell tumors in humans [19]. Therefore, the development of human extragonadal PGCs and their fate deserve further investigation.

Gametogenesis in humans and mice

Following gonadal colonization, both human and mouse PGCs differentiate into oogonia in females and spermatogonia in males upon sex determination [10, 20]. In the female ovaries, after mitotic arrest, oogonia enter meiotic prophase as primary oocytes and after birth arrest at the diplotene stage of meiosis [21]. After puberty, hormonal stimulation during ovulation of each menstrual cycle causes maturation and release of the dominant oocyte. After the first meiotic division is completed, the mature oocyte arrests at metaphase II stage, ready to be

fertilized [22, 23]. In the male testes, spermatogonia proliferate and then arrest in the G0/G1 phase of the mitotic division [24]. In contrast to females, male germ cells do not enter meiosis before birth [25]. After birth, the germ cells resume mitotic proliferation. Until puberty, some of spermatogonia in the seminiferous tubules enter meiosis I and differentiate into primary spermatocytes. Secondary spermatocytes are produced after meiosis I and then after the second meiotic division, haploid spermatids are formed. These spermatids subsequently differentiate morphologically into sperms through changes in cellular organization and shape [26].

Pre-implantation development and implantation in humans and mice

The pre-implantation development period, which includes fertilization, cell cleavage, morula and blastocyst formation, comprises the initial stages of mammalian development. It takes place before the embryo implants into the mother's uterus (Figure 2) [27]. Fertilization represents one of the most important and fascinating processes in biology, because the union of sperm and mature oocyte at fertilization triggers the recombination of paternal and maternal genetic information to form the diploid genome of a new and unique individual [7]. Understanding the stages of pre-implantation development and the underlying regulatory cellular and molecular mechanisms offers essential implications for assisted reproductive technology (ART).

In preparation for implantation and pregnancy, uterine endometrium transforms into a differentiated maternal tissue "decidua" through a remarkable event known as decidualization [28, 29] in response to estrogen and progesterone. The decidua is characterized by vascular remodelling, differentiation of stromal cells into large rounded decidual cells, angiogenesis and secretory transformation of uterine glands [28, 30].

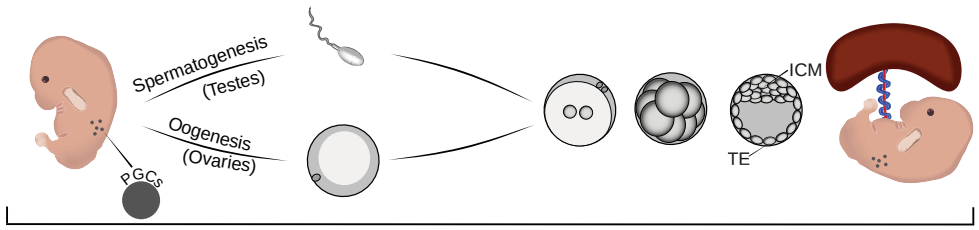

Figure 1. The immortal life cycle of mammalian germ cells.

Once the unipotent primordial germ cells (PGCs) are specified just outside the embryo proper during early embryogenesis, they migrate to the developing fetal gonads. There, they develop into female oogonia or male spermatogonia upon sex determination. Subsequently, germ cells undergo meiosis and produce mature sperms or oocytes depending on the sex. When the oocyte is fertilized by sperm, a totipotent zygote is formed. Through cell cleavage divisions and differentiation, the zygote develops into a blastocyst with pluripotent inner cell mass (ICM) and trophectoderm (TE). PGCs are specified from the ICM-derived epiblast cells, thus completing the cycle.

Human pre-implantation development

Pre-implantation embryos are morphologically similar in humans and mice, although the timing is quite different from each other (Figure 2) [31].

In humans, simultaneously with sperm-oocyte fusion, the second meiotic division of the oocyte is complete and the second polar body is extruded [26]. In the one-cell zygote, paternal and maternal haploid pronuclei move towards each other and they both undergo DNA replication before entering the first mitotic division [32] to produce a 2-cell embryo within one day after fertilization. Afterwards, the embryo undergoes a series of mitotic cell divisions (known as cleavage divisions) and produces sequentially smaller embryonic cells (called blastomeres).

As cleavage divisions continue, the embryo "compacts" to form a solid ball of cells (morula) by day 4 of development (Figure 2). Products of several genes are involved in this compaction and the following cavitation process, including the sodium-potassium ATPase transport system and tight junction proteins [33]. The sodium/potassium ATPases on the cell membrane transport sodium from outside environment into the morula, followed by water flow into the embryo and formation of blastocoelic fluid. When a large cavity (blastocoel) is formed, the embryonic cells form a compact mass (called the inner cell mass or ICM) at one side of this cavity and a thin outer shell of epithelium (called trophectoderm or TE) [34]. Now the embryo is referred to as a blastocyst. In the blastocyst, TE cells adjacent to the ICM are polar TE, whereas the cells surrounding the blastocoel are mural TE [35].

By day 6 in humans, the developing blastocyst "hatches" from the protective *zona pellucida* [36], an acellular membrane that has protected the embryo as it has moved from the

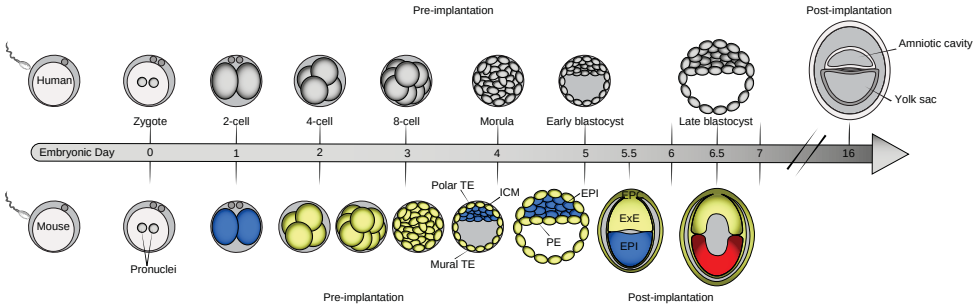

Figure 2. Stages and X chromosome status of pre-implantation and post-implantation development in humans and mice.

Simultaneously with sperm-oocyte fusion, the one-cell zygote is formed. By day 1, a 2-cell embryo is produced after the first mitotic division. As cleavage divisions continue, the embryo undergoes compaction and cell differentiation and develops into morula and then blastocyst. The blastocyst is composed of trophectoderm (TE) and the inner cell mass (ICM) which further differentiate into pluripotent epiblast (EPI) and primitive endoderm (PE). The implantation of the human hatched blastocyst occurs at around E6.5. During gastrulation at around E16, EPI gives rise to three primary germ layers while PE gives rise to the yolk sac. The extraembryonic cytotrophoblasts and syncytiotrophoblast cells are derived from TE. Being different from human post-implantation embryos, mouse post-implantation embryos become an egg cylinder within 1 day. The TE differentiates into giant cells, the extraembryonic ectoderm (ExE) and the ectoplacental cone (EPC). In the E6.5 embryo, epiblast cells give rise to the three germ layers. Colors indicate X chromosome inactivation status in cells. Yellow: imprinted X inactivation (Xp inactive, XaXi); blue: Xp reactivation (XaXa); red: random X inactivation (either Xm or Xp inactive).

oviduct where cleavage takes place, into the uterus. The blastocyst is then ready to attach to the endometrial lining of the uterus. At around 6.5 days, the polar TE cells undergo syncytial fusion of mononucleated cells and produce the first syncytiotrophoblast, which is able to invade the uterine lining [37]. Implantation is only facilitated during a limited reception-ready phase (the "implantation window"), on days 7-10 of development in humans [38]. This restricted period is regulated by appropriate levels of ovarian estrogen and progesterone, which synchronize the timing of embryo development with uterine receptivity [39]. The complex but highly organized implantation process can be classified into three phases [40]: (1) apposition - close apposition of the blastocyst to the uterine lining; (2) adhesion - attachment of the blastocyst to endometrial surface epithelium; and (3) penetration - invasion of the embryo through the epithelial surface and into the endometrial stroma. Successful implantation, a crucial step in the establishment and development of normal pregnancy, depends on the interaction between receptive decidua and a good-quality blastocyst at the right time [41].

Mouse pre-implantation development

While this pre-implantation period lasts approximately one week in humans, mouse embryos undergo the same events in 4.5 days (Figure 2) [31]. Embryonic cells start to compact and differentiate at the morula stage around E2.5. By E3.5, with lineage segregation and the formation of cavitation, a blastocyst is formed. Between E3.5 and E4.5, the ICM further differentiates and segregates into primitive endoderm (PE) and the pluripotent epiblast (EPI). These pre-implantation cell fate decisions are concisely regulated by cell signaling and subsequent lineage-specific transcription factors [42, 43]. At E4.5, the blastocyst with three segregated cell lineages (TE, EPI and PE) hatches out of the *zona pellucida* and initiates implantation [42].

Post-implantation development in humans and mice

Human post-implantation development

Following human implantation at day 7, the invasive syncytiotrophoblast, derived from polar TE, penetrates the uterine surface epithelium and thus draws the blastocyst deeper into the uterine wall [44]. The bilaminar embryonic disc comprises the EPI and the PE layers, both derived from ICM (Figure 2). By day 8, the amniotic cavity has appeared separating EPI from amnion while the syncytiotrophoblast grows and expands continuously. By day 9, the embryo becomes fully embedded within the uterine lining. The amniotic cavity expands, and trophoblastic *lacunae* appear in the syncytiotrophoblast which covers the embryo completely. On days 10-11, PE-derived primary yolk sac appears [45]. On days 11-13, the PE cells proliferate to produce the definitive (secondary) yolk sac whereas the primary yolk sac breaks up. An extraembryonic space, the chorionic cavity, is established. Its outer boundary, referred to as the chorion, is composed of trophoblasts and the underlying extraembryonic mesoderm [46]. During gastrulation on days 14-16, ingressing EPI cells converge at the midline and ingress at the primitive streak, replacing primitive endoderm to produce definitive endoderm [47]. Some EPI cells differentiate into embryonic mesoderm lying between the EPI layer and the definitive

endoderm layer. Cell movements in gastrulation transform the human embryo into a multi-layered organism with three primary germ layers (ectoderm, definitive endoderm and embryonic mesoderm) (Figure 2) [48].

Mouse post-implantation development

Being quite distinct from human post-implantation embryo structures (Figure 2) [48], cells of the mouse blastocyst proliferate rapidly and transform the embryo into an egg cylinder within 24 hours after implantation [42]. Mural TE differentiates into primary trophoblastic giant cells [49], whereas polar TE subsequently differentiates into the ectoplacental cone (EPC) and the extraembryonic ectoderm (ExE) [50]. As the egg cylinder elongates, a luminal space is established within the EPI through polarization and hollowing, thereby transforming the EPI into a cup-shaped polarized epithelium [51]. The same process of polarization and hollowing also occurs in the ExE, the lumen eventually fusing to form the pro-amniotic cavity during E5.5–E5.75. The PE differentiates into parietal endoderm covering inner surface of mural trophectoderm and visceral endoderm cells covering the elongating egg cylinder (Figure 2) [42]. In the E6.5 mouse embryo, the primitive streak is formed in the posterior region of the EPI, which indicates the initiation of gastrulation [52]. Epiblast cells proliferate to cells that pass through the primitive streak, thereby giving rise to the mesoderm and the definitive endoderm which replaces the visceral endoderm [53]. Those EPI cells not moving through the primitive streak become the ectoderm. Together, these are the three primary germ layers (embryonic ectoderm, embryonic mesoderm and definitive endoderm) of the mouse embryo (Figure 2) [54]. A set of signaling pathways, including the WNT, BMP, Nodal and FGF pathways have been shown to be involved in this gastrulation process largely through gene deletion studies [55, 56]. At E7.25, PGCs arise from the epiblast, at the base of the mesodermal allantois [10].

Early placentation in humans and mice

During human and mouse implantation, decidua has three portions renamed by their relationship to the implantation site [28, 57]: (1) Decidua basalis, which underlies the implantation site; (2) Decidua capsularis, which lies between the uterine lumen and the implantation site; (3) Decidua parietalis, which is the rest decidua. The decidua basalis forms the basal plate which is the maternal part of the placenta.

Human early placentation

In the first week of development, the human embryo obtains nutrients by simple diffusion [58]. As the blastocyst attaches to the uterine epithelium, the formation of the placenta begins [59]. From day 8, TE-derived invasive trophoblasts penetrate the decidual stroma and the trophoblastic *lacunae* within the syncytiotrophoblast start to contact with maternal capillaries [31, 37]. During days 11-13, the cytotrophoblasts, acting as stem cells, proliferate and bud into the overlying syncytiotrophoblasts. The cytotrophoblast cells, together with an outer layer of syncytiotrophoblasts, grow out into the *lacunae*, thus forming primary villi [60]. Thereafter, the extraembryonic mesoderm cells proliferate and penetrate into the primary villi, forming

secondary villi. By week 3, within the villous mesoderm core, hematopoietic progenitor cells develop and give rise to placental blood cells [61]. The villi containing the first placental blood vessels are referred to as tertiary villi [62].

Due to the rapid placental growth during early pregnancy as well as apoptosis in the syncytiotrophoblast layer [63], the villous cytotrophoblast cells continuously proliferate, differentiate and fuse into the outer syncytiotrophoblasts [64]. This differentiation and fusion process involves syncytin genes encoding proteins which can induce cell-cell fusion [65, 66]. In the distal part of the anchoring villi, cytotrophoblasts proliferate and differentiate into cell columns, where trophoblast cells acquire an invasive phenotype during differentiation. These invading trophoblasts, known as extravillous trophoblasts (EVTs), detach from the cell columns and start to migrate into the decidua and even the myometrium [67, 68]. This trophoblast differentiation process is tightly regulated by oxygen tension and proper interplay of transcription factors, hormones, growth factors and other signaling molecules [69]. Four subsets of EVTs have been identified: (1) Interstitial EVTs, which invade the decidual stroma and even into the inner third of the myometrium; (2) Endovascular EVTs, which can migrate into maternal remodelled spiral arteries in a retrograde fashion [70] and into maternal uterine veins [71]; (3) Intramural EVTs, which are present in vascular wall of the remodeled spiral arteries [72]; (4) "Epithelial" EVTs, which line the basal plate together with maternal endothelial cells [73].

EVTs are observed to "block" or "plug" the opening of spiral arteries into the intervillous space at W8-W12 [74]. Because of these "blocks" or "plugs", the maternal circulation to the placenta is restricted, thereby creating an hypoxic placental environment which is vital for regulating trophoblast cells differentiation during early pregnancy [75]. In addition, EVTs have been shown to be involved in the process of arterial remodelling together with macrophages and uterine natural killer (uNK) cells [76]. Initially, unremodelled decidual arteries consist of an intact endothelium and several well-organized smooth muscle cell (SMC) layers. Before EVT invasion of the spiral arteries, leukocytes infiltrate the vascular wall, synchronized with disorganization of the SMC layers and loss of endothelial cells. Under subsequent remodelling by interstitial and endovascular EVTs, decidual arteries lose endothelium and SMC layers entirely [77]. Remodelled decidual arteries lack maternal vasomotor control [78] and therefore become low-resistance, heavily dilated vessels that provide increased blood flow towards the placenta to meet the requirements of the growing fetus. At about W12, the open connection between maternal circulation and intervillous space is completely established [78, 79]. In this process, proper EVT invasion in decidual vasculature regulates oxygen tension for placental development [75] which is vital for successful pregnancy [80, 81]. On the contrary, insufficient EVTs invasion in maternal decidua is reported to result in inadequate blood perfusion of the placenta and subsequent pregnancy complications including preeclampsia and intrauterine growth restriction [82, 83]. Thus, characterizing the invasion of fetal EVTs in maternal decidua basalis in early pregnancy will help to identify the underlying mechanism and reveal potential therapeutic strategies for some pregnancy complications.

Mouse early placentation

In mice, the mesoderm-derived allantois contacts with chorion at E8.5 [84]. This event, called "chorioallantoic fusion", starts the formation of early placenta. Thereafter, fetal components of the placental vascular network undergo extensive branching and grow into the folds of the chorion [84]. At the same time, chorionic trophoblast cells, in direct apposition with endothelium of the fetal vessels, differentiate into multiple layers of trophoblast cells: two layers of syncytiotrophoblast cells and another layer of mononuclear trophoblast cells [85]. Maternal blood flows through the small spaces among trophoblast cells at around E10.5, resulting in a functional placenta exchanging maternal and fetal blood [86]. By E14.5, the placenta finally develops into the definitive placenta [87].

Mouse placentation shows many differences from humans [88]. Crucially, in contrast to human extensive trophoblast invasion into decidua and decidual vessels, mouse trophoblast invasion is very shallow [89]. Therefore, transformation of mouse uterine arteries is dependent on maternal (uNK) cells rather than fetal trophoblasts [90].

Late placentation in humans and mice

The placenta is the first fully-formed organ in mammals. It supports the directional and selective transport of nutrients, gases and waste between maternal and fetal circulations during intrauterine development [91]. It maintains pregnancy and supports fetal development and growth. Therefore, placental dysfunction may lead to pregnancy complications causing risk to mother and fetus [92]. Mouse and human placentae are haemochorial, meaning that the maternal blood directly contacts fetal trophoblast cells [93, 94]. The other two types of placenta, epitheliochorial placenta (as observed in horse, cow and pig) and endotheliochorial placenta (as described in cat and dog) have a barrier between fetal villi and maternal blood [94, 95].

Human late placentation

In humans, the chorionic surface is entirely covered by chorionic villi during the first 8 weeks. As the gestational sac enlarges, chorionic villi on the decidua capsularis undergoes progressive regression into a smooth surface called "chorion laeve" [96, 97]. The chorion laeve fuses with decidua parietalis by the end of the third month. Chorionic villi on the decidua basalis become larger and more extensively branched.

By the end of the fourth month of pregnancy, the human placenta has attained its definitive structure and function (referred to as "definitive placenta"), consisting of fetal terminal chorionic villi and maternal decidua (Figure 3A) [98, 99]. The villi are composed of an inner EPI-derived mesenchymal core and two layers of TE-derived trophoblast epithelium (a layer of mononuclear cytotrophoblast cells and a layer of multinucleated syncytiotrophoblast cells) [37]. The chorionic villi either attach to the decidua basalis as anchoring villi or float within the intervillous space as floating villi [69].

The high-flow low-resistance maternal remodelled arteries accommodate massively

increased maternal blood perfusion into the intervillous space [78]. The relatively high arterial pressure allows exchange between maternal and fetal blood to take place. This must cross four layers of tissue (the placental barrier): two villous trophoblast layers (one layer of syncytiotrophoblasts and one layer of cytotrophoblasts), the connective tissue (extraembryonic mesoderm) in the core of villi and the fetal capillary endothelium [100]. After exchange of fetal and maternal blood at the external microvillous surface of chorionic villi, the single umbilical vein carries oxygenated blood and nutrient to the fetus. Then the two umbilical arteries transport deoxygenated fetal blood and waste product back to the placenta [101]. In-flowing maternal arterial blood pushes venous blood back to decidual veins through venous orifices in the basal plate. Thus, the maternal blood traverses the intervillous space of the placenta freely without preformed channels.

Mouse late placentation

Since E11.5, mouse definitive chorioallantoic placenta is established, composed of distinct structural layers [102]: maternal decidua with spiral arteries perfusion, an outer layer of trophoblast giant cells (TGCs) interfacing with the maternal decidua, followed by the junctional zone [glycogen cells (GCs) and spongiotrophoblasts], the labyrinth (trophoblast cells and extraembryonic mesoderm endothelial cells) and finally the chorionic plate facing the embryo [84, 103, 104] (Figure 3B). The GCs, which have high glycogen content and are sensitive to glucagon signaling, may be serving as a potential source of energy [105].

Maternal spiral arteries traverse the decidua basalis and converge to form centrally located arterial "canals" which carry maternal blood to the base of labyrinth [89] (Figure 3B). In the labyrinth, fetal and maternal blood exchanges, with the well-organized cell layers (a layer of mononuclear trophoblasts, a bilayer of syncytiotrophoblasts and an endothelial cell layer of the fetal vasculature) serving as the barrier between maternal-fetal exchange [101]. Afterwards, venous deoxygenated blood drains from the labyrinth through venous channels in the junctional zone into venous sinuses in the decidua basalis [89].

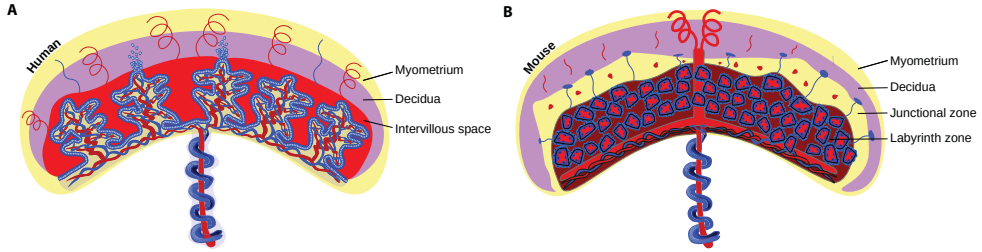

Figure 3. Anatomical comparison of definitive placenta in humans and mice.

(**A**) Structure of the human definitive placenta. The placenta is composed of maternal decidua and fetal chorionic villi. Chorionic villi consist of mesenchyme core, an inner cytotrophoblasts layer and an outer syncytiotrophoblasts layer. In the distal part of anchoring villi, extravillous trophoblast (EVT) cells detach from the distal part of cytotrophoblasts columns and invade the maternal decidua. (**B**) Structure of the mouse definitive placenta. The placenta is composed of maternal decidua, junctional zone and labyrinth zone. In the labyrinth, maternal blood is separated from fetal capillaries by three trophoblast layers: a mononuclear trophoblast cell layer and a bilayer of syncytiotrophoblasts.

Epigenetic regulation in germ cells, peri-implantation and placental development

Epigenetic regulation is crucial for various biological processes. Two epigenetic regulation processes established in early development [106], X chromosome inactivation (XCI) and genomic imprinting, are essential for dosage control of X-linked genes and parent-of-origin-specific genes in certain cells at certain stages.

X chromosome inactivation

In mammals, XCI is the mechanism to achieve dosage compensation of X-genes between females and males, in which one of the two X chromosomes becomes inactivated (Xi) in female somatic cells [106, 107]. XCI is regulated by X inactivation centre (Xic) [108, 109], which includes the non-coding locus *XIST* and its antisense transcript *TSIX* as a repressor [110]. *XIST* RNA coats the future inactive X chromosome, thus trigging the silencing of the whole X chromosome. This is followed by DNA methylation at CpG-rich promoters, histone H3 lysine 27 trimethylation (H3K27me3) and H4 hypoacetylation [106, 111]. Individuals, even with abnormal numbers of X chromosomes, silence all but one of the X chromosomes [112]. For example, Turner syndrome patients (45,XO) harboring a single X chromosome [113] show no inactivation of this unique X chromosome. Klinefelter syndrome patients (47, XXY) display one active and one inactive X chromosomes [114]. Additionally, a fraction of X-genes are revealed to escape X inactivation on the Xi and are known as “escapees”. A higher percentage of escapees along chromosome X are reported in humans (around 15%) than in mice (about 3%) [115, 116]. Many of the escapees are located in the pseudoautosomal regions [117], where genes are present on both X and Y chromosomes.

XCI has been investigated by several approaches including *XIST* RNA-FISH [118], H3K27 trimethylation [119], human androgen receptor gene assay (HUMARA) [120], retinitis pigmentosa 2 (*RP2*)-based assay [121] and allele-specific expression analysis of the informative single-nucleotide polymorphisms (SNPs) enabling parental origin to be assigned [122-124].

The dynamic XCI status during early development has been more extensively studied in mice than in humans, because of the availability of genetic substrains of mice [125-127]. After fertilization, X inactivation is initiated during pre-implantation development of mouse female embryos, closely related to the early lineage segregation [125]. The first XCI wave occurs at the two- to four-cell stage and results in the imprinted inactivation of Xp in all cells of the embryo (Figure 2). At the blastocyst stage, the Xp remains silent in the TE and PE [103, 126, 127] while the Xp becomes reactivated in the EPI at E3.5. Shortly thereafter, during the second XCI wave, either the Xm or the Xp is silenced in a random fashion, resulting in random XCI in epiblast cells (Figure 2) [128]. This is followed by reactivation of the silent X, in preparation for the equal segregation during meiosis, in female PGCs between E8.5 and E12.5, but not in the female somatic cells [119]. During placentation, mouse TE-derived cells maintain imprinted XCI. Therefore, TE-derived cells in the placenta contain an obligatorily silenced

Xp in females, including the TGCs, the spongiotrophoblasts and GCs in the outer zone and the syncytiotrophoblasts, mononuclear trophoblasts in the labyrinth zone [102]. By contrast, EPI-derived cells in the placenta, including the chorionic plate of the placenta and the fetal endothelial cells in the labyrinth zone, show random XCI.

The process of XCI in humans is less well-known than in mice. The inactivated X chromosomes in human PGCs are already reactivated at W4 [122]. During pre-implantation development at E3-E7, X-linked genes are thought to maintain biallelic expression in all lineages [118, 129]. Previous research regarding XCI in human placenta has resulted in conflicting views, supporting random [124, 130-133], non-random [134] and preferential paternal XCI [135-139]. These conflicting results may be due to high heterogeneity, clonal growth and a mixture of TE-derived and EPI-derived cells in placenta.

Genomic imprinting

In mammals, normally both maternal and paternal alleles of genes are expressed. However, genomic imprinting affects a subset of genes (known as imprinted genes) in both male and female offsprings, resulting in monoallelic expression of either maternal or paternal allele [106]. Most imprinted genes are located in clusters [140] that are regulated by transcription of a non-coding RNA. Imprinted genes play an essential role in embryonic and extraembryonic growth and development in mammals [141, 142].

IGF2/H19 locus, which is located on human chromosome 11 and on mouse chromosome 7, is one well-studied imprinted gene cluster [143, 144]. The location of its imprinting control region (ICR) is between protein-coding *IGF2* gene and noncoding *H19* gene [145]. The ICR on the maternal chromosome is bound by the insulator protein CTCF, and therefore is unmethylated. This allows activation of the *H19* promoter and inactivates of the *IGF2* promoter. As a result, the maternal chromosome has transcription of *H19* but not *IGF2*. In contrast, the ICR is methylated on the paternal chromosome and *IGF2* is transcribed [144].

A couple of techniques have been used to detect imprinting patterns [146], including DNA methylation at ICRs and allele-specific expression analysis of informative SNPs. Sperms and mature oocytes carry imprinted chromosomes. After fertilization, the imprint is maintained in each cell division during peri-implantation and placental development [147]. Genome-wide epigenetic reprogramming in human and mouse PGCs results in erasure of parental imprints before sex determination [147]. Upon sex determination, male and female germ cells acquire paternal and maternal imprints respectively on the chromosomes.

Aims and outline of this thesis

Pre-implantation and placental development in humans and mice has been investigated over several centuries. Although much is known about early development in mice, how differentiation and implantation are regulated at the molecular level in humans is limited. Since incorrect development and implantation is thought to be a cause of pregnancy failure and risk, the work in this thesis aimed to fill some of these gaps and specifically address the following aspects related to pre-implantation and placental development in humans and mice.

The main question of **Chapter 2** is what is the development of human (extra)gonadal germ cells between first and second trimesters regarding expression of germ cell markers and meiosis markers. Are there ectopic germ cells and do they enter meiosis in synchrony with the gonadal germ cells?

The main aim of **Chapter 3** is to investigate the status of XCI in human pre-implantation embryos. Do TE-derived cells show different XCI pattern from ICM-derived cells at E5-E7? If there is X inactivation, whether the XCI pattern is imprinted or random?

The focus of **Chapter 4** is on the invasion of decidual vasculature by human extravillous trophoblast cells from W5.5 till the end of first trimester. Do the EVTs invade arteries, veins and lymphatics? When does the invasion occur?

In **Chapter 5**, the investigation is extended towards the spatial pattern of methylation of the *IGF2/H19* imprinting control region in multiple sites of collection in human first-trimester placental villi. Do the multiple sites of placental villi show the same imprinting pattern? Is the imprinting pattern of *IGF2/H19* in human first-trimester placental villi the same as in the embryo?

The aim of **Chapter 6** is to compare the consequences of Turner syndrome harboring either a single paternally inherited (Xp) or maternally inherited (Xm) chromosome in mouse E18.5 placenta, in order to study whether the transcriptional level of Xp is equal to Xm.

Finally in **Chapter 7**, we combine and further discuss the findings obtained in chapters 2-6. Future perspectives are also proposed.

References

1. Surani MA, Hayashi K, Hajkova P: Genetic and epigenetic regulators of pluripotency. Cell 2007, 128:747-762.
2. Wylie C: Germ cells. Cell 1999, 96:165-174.
3. Lesch BJ, Page DC: Genetics of germ cell development. Nat Rev Genet 2012, 13:781-794.
4. Richardson BE, Lehmann R: Mechanisms guiding primordial germ cell migration: strategies from different organisms. Nat Rev Mol Cell Biol 2010, 11:37-49.
5. Starz-Gaiano M, Lehmann R: Moving towards the next generation. Mech Dev 2001, 105:5-18.
6. Flesch FM, Gadella BM: Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim Biophys Acta 2000, 1469:197-235.
7. Clift D, Schuh M: Restarting life: fertilization and the transition from meiosis to mitosis. Nat Rev Mol Cell Biol 2013, 14:549-562.
8. Hayashi Y, Saitou M, Yamanaka S: Germline development from human pluripotent stem cells toward disease modeling of infertility. Fertility and sterility 2012, 97:1250-1259.
9. Hayashi K, de Sousa Lopes SMC, Surani MA: Germ cell specification in mice. Science 2007, 316:394-396.
10. de Sousa Lopes SMC, Roelen BA: On the formation of germ cells: the good, the bad and the ugly. Differentiation 2010, 79:131-140.
11. Witschi E: Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. Contrib Embryol 1948, 32:67-80.
12. 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.
13. 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.
14. Johnson AD, Alberio R: Primordial germ cells: the first cell lineage or the last cells standing? Development 2015, 142:2730-2739.
15. Ginsburg M, Snow MH, McLaren A: Primordial germ cells in the mouse embryo during gastrulation. Development 1990, 110:521-528.
16. Saitou M, Yamaji M: Primordial germ cells in mice. Cold Spring Harb Perspect Biol 2012, 4.
17. 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.
18. 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.
19. Gobel U, Schneider DT, Calaminus G, Haas RJ, Schmidt P, Harms D: Germ-cell tumors in childhood and adolescence. GPOH MAKEI and the MAHO study groups. Ann Oncol 2000, 11:263-271.
20. McMurray CT: Mechanisms of trinucleotide repeat instability during human development. Nature Reviews Genetics 2010, 11:786-799.
21. Tam P, Snow M: Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. Development 1981, 64:133-147.
22. Nikolic A, Volarevic V, Armstrong L, Lako M, Stojkovic M: Primordial germ cells: current knowledge and perspectives. Stem cells international 2015, 2016.
23. Virant-Klun I: Postnatal oogenesis in humans: a review of recent findings. Stem cells and cloning: advances and applications 2015, 8:49.
24. McLaren A: Mammalian germ cells: birth, sex, and immortality. Cell structure and function 2001, 26:119-122.
25. Hassold T, Hunt P: To err (meiotically) is human: the genesis of human aneuploidy. Nature Reviews Genetics 2001, 2:280-291.

26. Sathananthan AH: Ultrastructure of human gametes, fertilization and embryos in assisted reproduction: a personal survey. Micron 2013, 44:1-20.
27. Garcia-Herrero S, Cervero A, Mateu E, Mir P, Póo M, Rodrigo L, Vera M, Rubio C: Genetic Analysis of Human Preimplantation Embryos. Current Topics in Developmental Biology 2016.
28. Plaisier M: Decidualisation and angiogenesis. Best Pract Res Clin Obstet Gynaecol 2011, 25:259-271.
29. Ramathal CY, Bagchi IC, Taylor RN, Bagchi MK: Endometrial decidualization: of mice and men. In Seminars in reproductive medicine. © Thieme Medical Publishers; 2010: 017-026.
30. Gellersen B, Brosens IA, Brosens JJ: Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. In Seminars in reproductive medicine. © Thieme Medical Publishers; 2007: 445-453.
31. Niakan KK, Han J, Pedersen RA, Simon C, Pera RAR: Human pre-implantation embryo development. Development 2012, 139:829-841.
32. Hamatani T, Carter MG, Sharov AA, Ko MS: Dynamics of global gene expression changes during mouse preimplantation development. Developmental cell 2004, 6:117-131.
33. Watson AJ, Barcroft LC: Regulation of blastocyst formation. Frontiers in bioscience: a journal and virtual library 2001, 6:708.
34. Hardy K, Handyside AH, Winston R: The human blastocyst: cell number, death and allocation during late preimplantation development in vitro. Development 1989, 107:597-604.
35. Chia CM, Winston R, Handyside AH: EGF, TGF-alpha and EGFR expression in human preimplantation embryos. Development 1995, 121:299-307.
36. Krupinski P, Chickarmane V, Peterson C: Simulating the mammalian blastocyst--molecular and mechanical interactions pattern the embryo. PLoS Comput Biol 2011, 7:e1001128.
37. Huppertz B: The anatomy of the normal placenta. Journal of clinical pathology 2008, 61:1296-1302.
38. Xiao Y, Sun X, Yang X, Zhang J, Xue Q, Cai B, Zhou Y: Leukemia inhibitory factor is dysregulated in the endometrium and uterine flushing fluid of patients with adenomyosis during implantation window. Fertil Steril 2010, 94:85-89.
39. Smith SK: Regulation of angiogenesis in the endometrium. Trends in Endocrinology & Metabolism 2001, 12:147-151.
40. Wang H, Dey SK: Roadmap to embryo implantation: clues from mouse models. Nature Reviews Genetics 2006, 7:185-199.
41. Kapiteijn K, Koolwijk P, van der Weiden RM, van Nieuw Amerongen G, Plaisier M, van Hinsbergh VW, Helmerhorst FM: Human embryo-conditioned medium stimulates in vitro endometrial angiogenesis. Fertil Steril 2006, 85 Suppl 1:1232-1239.
42. Bedzhov I, Graham SJ, Leung CY, Zernicka-Goetz M: Developmental plasticity, cell fate specification and morphogenesis in the early mouse embryo. Phil Trans R Soc B 2014, 369:20130538.
43. Frum T, Ralston A: Cell signaling and transcription factors regulating cell fate during formation of the mouse blastocyst. Trends in Genetics 2015, 31:402-410.
44. Benirschke K, Burton GJ, Baergen RN: Early development of the human placenta. In Pathology of the human placenta. Springer; 2012: 41-53
45. Enders A, King B: Development of the human yolk sac. In The human yolk sac and yolk sac tumors. Springer; 1993: 33-47
46. Bourne G: The foetal membranes: a review of the anatomy of normal amnion and chorion and some aspects of their function. Postgraduate medical journal 1962, 38:193.
47. Chu L-F, Leng N, Zhang J, Hou Z, Mamott D, Vereide DT, Choi J, Kendziorski C, Stewart R, Thomson JA: Single-cell RNA-seq reveals novel regulators of human embryonic stem cell differentiation to definitive endoderm. Genome biology 2016, 17:173.
48. Tang WW, Kobayashi T, Irie N, Dietmann S, Surani MA: Specification and epigenetic programming of the human germ line. Nature Reviews Genetics 2016, 17:585-600.
49. Cross J: How to make a placenta: mechanisms of trophoblast cell differentiation in mice–a review. Placenta 2005, 26:S3-S9.

50. Carney E, Prideaux V, Lye S, Rossant J: Progressive expression of trophoblast-specific genes during formation of mouse trophoblast giant cells in vitro. Molecular reproduction and development 1993, 34:357-368.
51. Bedzhov I, Zernicka-Goetz M: Self-organizing properties of mouse pluripotent cells initiate morphogenesis upon implantation. Cell 2014, 156:1032-1044.
52. Kojima Y, Tam OH, Tam PP: Timing of developmental events in the early mouse embryo. In Seminars in cell & developmental biology. Elsevier; 2014: 65-75.
53. Robb L, Tam PP: Gastrula organiser and embryonic patterning in the mouse. In Seminars in cell & developmental biology. Elsevier; 2004: 543-554.
54. Tam PP, Loebel DA: Gene function in mouse embryogenesis: get set for gastrulation. Nature Reviews Genetics 2007, 8:368-381.
55. Olivera-Martinez I, Storey KG: Wnt signals provide a timing mechanism for the FGF-retinoid differentiation switch during vertebrate body axis extension. Development 2007, 134:2125-2135.
56. Kojima Y, Kaufman-Francis K, Studdert JB, Steiner KA, Power MD, Loebel DA, Jones V, Hor A, de Alencastro G, Logan GJ: The transcriptional and functional properties of mouse epiblast stem cells resemble the anterior primitive streak. Cell Stem Cell 2014, 14:107-120.
57. Lim HJ, Wang H: Uterine disorders and pregnancy complications: insights from mouse models. The Journal of clinical investigation 2010, 120:1004-1015.
58. Harris SE, Gopichandran N, Picton HM, Leese HJ, Orsi NM: Nutrient concentrations in murine follicular fluid and the female reproductive tract. Theriogenology 2005, 64:992-1006.
59. Aplin JD: The cell biological basis of human implantation. Best Practice & Research Clinical Obstetrics & Gynaecology 2000, 14:757-764.
60. Handwerger S: New insights into the regulation of human cytotrophoblast cell differentiation. Molecular and cellular endocrinology 2010, 323:94.
61. Dempsey EW: The development of capillaries in the villi of early human placentas. Developmental Dynamics 1972, 134:221-237.
62. Demir R, Kaufmann P, Castellucci M, Erbengi T, Kotowski A: Fetal vasculogenesis and angiogenesis in human placental villi. Cells Tissues Organs 1989, 136:190-203.
63. Huppertz B, Kadyrov M, Kingdom JCP: Apoptosis and its role in the trophoblast. American Journal of Obstetrics and Gynecology 2006, 195:29-39.
64. Simpson R, Mayhew T, Barnes P: From 13 weeks to term, the trophoblast of human placenta grows by the continuous recruitment of new proliferative units: a study of nuclear number using the disector. Placenta 1992, 13:501-512.
65. Mi S, Lee X, Li X-p, Veldman GM, Finnerty H, Racie L, Lavallie E, Tang X-Y, Edouard P, Howes S: Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. Nature 2000, 403:785-789.
66. Blaise S, de Parseval N, Bénit L, Heidmann T: Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. Proceedings of the National Academy of Sciences 2003, 100:13013-13018.
67. Brosens JJ, Pijnenborg R, Brosens IA: The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. Am J Obstet Gynecol 2002, 187:1416-1423.
68. Knofler M: Critical growth factors and signalling pathways controlling human trophoblast invasion. Int J Dev Biol 2010, 54:269-280.
69. Ji L, Brkić J, Liu M, Fu G, Peng C, Wang Y-L: Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. Molecular aspects of medicine 2013, 34:981-1023.
70. Harris LK: Review: Trophoblast-vascular cell interactions in early pregnancy: how to remodel a vessel. Placenta 2010, 31 Suppl:S93-98.
71. 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.
72. Lyall F, Robson SC, Bulmer JN: Spiral Artery Remodeling and Trophoblast Invasion in Preeclampsia and Fetal Growth RestrictionNovelty and Significance. Hypertension 2013, 62:1046-1054.

73. Byrne S, Challis E, Williams JL, Pringle JH, Hennessy JM, Ockleford CD: A mosaic cell layer in human pregnancy. Placenta 2010, 31:373-379.
74. 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.
75. James JL, Stone PR, Chamley LW: The regulation of trophoblast differentiation by oxygen in the first trimester of pregnancy. Hum Reprod Update 2006, 12:137-144.
76. Lyall F: Mechanisms regulating cytotrophoblast invasion in normal pregnancy and pre-eclampsia. Australian and New Zealand journal of obstetrics and gynaecology 2006, 46:266-273.
77. Harris L: Review: trophoblast-vascular cell interactions in early pregnancy: how to remodel a vessel. Placenta 2010, 31:S93-S98.
78. Pijnenborg R, Vercruysse L, Hanssens M: The uterine spiral arteries in human pregnancy: facts and controversies. Placenta 2006, 27:939-958.
79. 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.
80. 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.
81. 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.
82. Burke SD, Karumanchi SA: Spiral artery remodeling in preeclampsia revisited. Hypertension 2013, 62:1013-1014.
83. Fisher SJ: Why is placentation abnormal in preeclampsia? Am J Obstet Gynecol 2015, 213:S115-122.
84. Rossant J, Cross JC: Placental development: lessons from mouse mutants. Nat Rev Genet 2001, 2:538-548.
85. Cross JC: Genetic insights into trophoblast differentiation and placental morphogenesis. In Seminars in cell & developmental biology. Elsevier; 2000: 105-113.
86. Dzierzak E, Robin C: Placenta as a source of hematopoietic stem cells. Trends in molecular medicine 2010, 16:361-367.
87. Latos PA, Hemberger M: From the stem of the placental tree: trophoblast stem cells and their progeny. Development 2016, 143:3650-3660.
88. Carter AM: Animal models of human placentation–a review. Placenta 2007, 28:S41-S47.
89. Adamson SL, Lu Y, Whiteley KJ, Holmyard D, Hemberger M, Pfarrer C, Cross JC: Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. Dev Biol 2002, 250:358-373.
90. Moffett A, Loke C: Immunology of placentation in eutherian mammals. Nature Reviews Immunology 2006, 6:584-594.
91. Jansson T: Placenta plays a critical role in maternal-fetal resource allocation. Proc Natl Acad Sci U S A 2016, 113:11066-11068.
92. Ray JG, Vermeulen MJ, Schull MJ, McDonald S, Redelmeier DA: Metabolic syndrome and the risk of placental dysfunction. Journal of Obstetrics and Gynaecology Canada 2005, 27:1095-1101.
93. Enders AC, Blankenship TN: Comparative placental structure. Advanced drug delivery reviews 1999, 38:3-15.
94. Carter AM, Enders AC: Comparative aspects of trophoblast development and placentation. Reproductive Biology and Endocrinology 2004, 2:46.
95. PrabhuDas M, Bonney E, Caron K, Dey S, Erlebacher A, Fazleabas A, Fisher S, Golos T, Matzuk M, McCune JM: Immune mechanisms at the maternal-fetal interface: perspectives and challenges. Nature immunology 2015, 16:328-334.
96. Pijnenborg R, Vercruysse L, Brosens I: Deep placentation. Best Pract Res Clin Obstet Gynaecol 2011, 25:273-285.

97. Ilancheran S, Moodley Y, Manuelpillai U: Human fetal membranes: a source of stem cells for tissue regeneration and repair? Placenta 2009, 30:2-10.

98. Gude NM, Roberts CT, Kalionis B, King RG: Growth and function of the normal human placenta. Thrombosis research 2004, 114:397-407.

99. Georgiades P, Ferguson-Smith A, Burton G: Comparative developmental anatomy of the murine and human definitive placentae. Placenta 2002, 23:3-19.

100. Kulvietis V, Zalgeviciene V, Didziapetriene J, Rotomskis R: Transport of nanoparticles through the placental barrier. The Tohoku journal of experimental medicine 2011, 225:225-234.

101. Dilworth M, Sibley C: Review: Transport across the placenta of mice and women. Placenta 2013, 34:S34-S39.

102. Rai A, Cross JC: Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. Dev Biol 2014, 387:131-141.

103. Cross JC, Baczyk D, Dobric N, Hemberger M, Hughes M, Simmons DG, Yamamoto H, Kingdom JC: Genes, development and evolution of the placenta. Placenta 2003, 24:123-130.

104. Malassine A, Frendo JL, Evain-Brion D: A comparison of placental development and endocrine functions between the human and mouse model. Hum Reprod Update 2003, 9:531-539.

105. 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.

106. Inbar-Feigenberg M, Choufani S, Butcher DT, Roifman M, Weksberg R: Basic concepts of epigenetics. Fertil Steril 2013, 99:607-615.

107. Lyon MF: Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature 1961, 190:372-373.

108. Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, Willard HF: A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 1991, 349:38-44.

109. Brown CJ, Lafreniere RG, Powers VE, Sebastio G, Ballabio A, Pettigrew AL, Ledbetter DH, Levy E, Craig IW, Willard HF: Localization of the X inactivation centre on the human X chromosome in Xq13. Nature 1991, 349:82-84.

110. Galupa R, Heard E: X-chromosome inactivation: new insights into cis and trans regulation. Curr Opin Genet Dev 2015, 31:57-66.

111. Okamoto I, Heard E: Lessons from comparative analysis of X-chromosome inactivation in mammals. Chromosome research 2009, 17:659-669.

112. Augui S, Nora EP, Heard E: Regulation of X-chromosome inactivation by the X-inactivation centre. Nature Reviews Genetics 2011, 12:429-442.

113. Cockwell A, MacKenzie M, Youings S, Jacobs P: A cytogenetic and molecular study of a series of 45, X fetuses and their parents. Journal of medical genetics 1991, 28:151-155.

114. Iitsuka Y, Bock A, Nguyen DD, Samango-Sprouse CA, Simpson JL, Bischoff FZ: Evidence of skewed X-chromosome inactivation in 47, XXY and 48, XXYY Klinefelter patients. American journal of medical genetics 2001, 98:25-31.

115. Carrel L, Willard HF: X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 2005, 434:400-404.

116. Berletch JB, Yang F, Disteche CM: Escape from X inactivation in mice and humans. Genome biology 2010, 11:213.

117. Gray KA, Yates B, Seal RL, Wright MW, Bruford EA: Genenames.org: the HGNC resources in 2015. Nucleic Acids Res 2015, 43:D1079-1085.

118. 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.

119. de Sousa Lopes SMC, Hayashi K, Shovlin TC, Mifsud W, Surani MA, McLaren A: X chromosome activity in mouse XX primordial germ cells. PLoS Genet 2008, 4:e30.

120. Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW: Methylation of HpaII and HhaI sites near

the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 1992, 51:1229-1239.

121. Machado FB, Machado FB, Faria MA, Lovatel VL, da Silva AFA, Radic CP, De Brasi CD, Rios ÁFL, de Sousa Lopes SMC, da Silveira LS: 5 me CpG Epigenetic Marks Neighboring a Primate-Conserved Core Promoter Short Tandem Repeat Indicate X-Chromosome Inactivation. PloS one 2014, 9:e103714.

122. Guo F, Yan L, Guo H, Li L, Hu B, Zhao Y, Yong J, Hu Y, Wang X, Wei Y, et al: The Transcriptome and DNA Methylome Landscapes of Human Primordial Germ Cells. Cell 2015, 161:1437-1452.

123. 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.

124. Moreira de Mello JC, de Araujo ES, Stabellini R, Fraga AM, de Souza JE, Sumita DR, Camargo AA, Pereira LV: Random X inactivation and extensive mosaicism in human placenta revealed by analysis of allele-specific gene expression along the X chromosome. PLoS One 2010, 5:e10947.

125. 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.

126. Payer B, Lee JT: X chromosome dosage compensation: how mammals keep the balance. Annu Rev Genet 2008, 42:733-772.

127. Reik W, Lewis A: Co-evolution of X-chromosome inactivation and imprinting in mammals. Nat Rev Genet 2005, 6:403-410.

128. Silva J, Nichols J, Theunissen TW, Guo G, van Oosten AL, Barrandon O, Wray J, Yamanaka S, Chambers I, Smith A: Nanog is the gateway to the pluripotent ground state. Cell 2009, 138:722-737.

129. Sahakyan A, Plath K: Transcriptome Encyclopedia of Early Human Development. Cell 2016, 165:777-779.

130. Looijenga LH, Gillis AJ, Verkerk AJ, van Putten WL, Oosterhuis JW: Heterogeneous X inactivation in trophoblastic cells of human full-term female placentas. Am J Hum Genet 1999, 64:1445-1452.

131. Migeon BR, Do TT: In search of nonrandom X inactivation: studies of the placenta from newborns heterozygous for glucose-6-phosphate dehydrogenase. Basic Life Sci 1978, 12:379-391.

132. Mohandas TK, Passage MB, Williams JW, 3rd, Sparkes RS, Yen PH, Shapiro LJ: X-chromosome inactivation in cultured cells from human chorionic villi. Somat Cell Mol Genet 1989, 15:131-136.

133. Willemsen R, Bontekoe CJ, Severijnen LA, Oostra BA: Timing of the absence of FMR1 expression in full mutation chorionic villi. Hum Genet 2002, 110:601-605.

134. Dhara SK, Benvenisty N: Gene trap as a tool for genome annotation and analysis of X chromosome inactivation in human embryonic stem cells. Nucleic Acids Res 2004, 32:3995-4002.

135. Goto T, Wright E, Monk M: Paternal X-chromosome inactivation in human trophoblastic cells. Mol Hum Reprod 1997, 3:77-80.

136. Harrison KB: X-chromosome inactivation in the human cytotrophoblast. Cytogenet Cell Genet 1989, 52:37-41.

137. Harrison KB, Warburton D: Preferential X-chromosome activity in human female placental tissues. Cytogenet Cell Genet 1986, 41:163-168.

138. Ropers HH, Wolff G, Hitzeroth HW: Preferential X inactivation in human placenta membranes: is the paternal X inactive in early embryonic development of female mammals? Hum Genet 1978, 43:265-273.

139. Uehara S, Tamura M, Nata M, Ji G, Yaegashi N, Okamura K, Yajima A: X-chromosome inactivation in the human trophoblast of early pregnancy. J Hum Genet 2000, 45:119-126.

140. Delaval K, Feil R: Epigenetic regulation of mammalian genomic imprinting. Current opinion in genetics & development 2004, 14:188-195.

141. Tycko B, Morison IM: Physiological functions of imprinted genes. Journal of cellular physiology 2002, 192:245-258.

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

143. van den Berg IM, Laven JS, Stevens M, Jonkers I, Galjaard RJ, Gribnau J, van Doorninck JH: X chromosome inactivation is initiated in human preimplantation embryos. Am J Hum Genet 2009, 84:771-779.

144. Autuoro JM, Pirnie SP, Carmichael GG: Long noncoding RNAs in imprinting and X chromosome inactivation. Biomolecules 2014, 4:76-100.

145. Abramowitz LK, Bartolomei MS: Genomic imprinting: recognition and marking of imprinted loci. Current opinion in genetics & development 2012, 22:72-78.

146. Tost J, Jammes H, Dupont J-M, Buffat C, Robert B, Mignot T-M, Mondon F, Carbonne B, Siméoni U, Grangé G: Non-random, individual-specific methylation profiles are present at the sixth CTCF binding site in the human H19/IGF2 imprinting control region. Nucleic acids research 2006, 34:5438-5448.

147. Barlow DP, Bartolomei MS: Genomic imprinting in mammals. Cold Spring Harbor perspectives in biology 2014, 6:a018382.