Charles University
Faculty of Science

Study programme: Biology
Branch of study: Biology

Nela Světlíková

Rhythmic function of placenta and the impact of disruption in maternal-placental-fetal axis

Rytmická funkce placenty a dopad narušení osy matka-placenta-fétus

Bachelor´s Thesis

Supervisor: Prof. PharmDr. Alena Sumová, DSc.

Prague, 2022

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 27.07.2022

Podpis

Poděkování:

Ráda bych poděkovala prof. PharmDr. Aleně Sumové, DSc., za její odborné vedení, cenné rady a podporu při psaní bakalářské práce a všem ostatním, kteří mě podporovali v průběhu celého studia.

Abstract

In mammals, the circadian rhythms result of a complex endogenous system consisting of hierarchically organized oscillators. The system enables the synchronization of the organism's internal processes with the external environment. It consists of the main component, the central clock, located in the suprachiasmatic nuclei in the hypothalamus, and peripheral clocks in other cells, tissues and organs. The placenta is a temporary, specialized mammalian organ that is part of the mother-placenta-fetus axis and exhibits rhythmicity in its functions. The aim of this thesis is to summarize the rhythmic functions of the placenta, such as immunity, protection, and production of hormones and other mediators that play an important role in fetal development and pregnancy. In addition, the thesis also describes rhythmic changes, that occur during pregnancy in the mother-placenta-fetus axis and how these rhythms influence each other.

Keywords

Placenta, circadian clock, ontogenesis, fetus, maternal synchronization, hormones, enzymes, immunity

Abstrakt

Cirkadiánní rytmy u savců jsou řízeny komplexním endogenně nastaveným systémem hierarchicky uspořádaných oscilátorů, které umožňují synchronizaci vnějšího prostředí s vnitřním prostředím organismu. Cirkadiánní systém se skládá z hlavní komponenty, a to z centrálních hodin, které se nacházejí v suprachiasmatických jádrech hypotalamu. Tyto centrální hodiny řídí periferní hodiny v buňkách různých orgánů v těle. Mezi orgány, kde se vyskytuje rytmicita, patří i savčí dočasný, specializovaný orgán, a to placenta, jež je součástí osy matka-placenta-fétus. Cílem této práce je nejen shrnout rytmicitu funkcí, jako je imunita, ochrana, produkce hormonů a dalších mediátorů, jež hrají důležitou roli při vývinu plodu a průběhu těhotenství, ale také popsat změny, které se týkají osy matka-placenta-fétus, kde se rytmicita každé části mění během těhotenství a vzájemně se ovlivňuje.

Klíčová slova

Placenta, cirkadiánní hodiny, ontogeneze, fétus, mateřská synchronizace, hormony, enzymy, imunita

List of used abbreviations

11-ßHSD2	11ß-hydroxysteroid dehydrogenase
AANAT	Aralkylamine N-acetyl transferase
ABCB	Atp-binding cassette protein subfamily b
ACTH	Adrenocorticotropic hormone
ASMT	N-acetylserotonin O-methyltransferase
AVP	Arginine vasopressin
bHLH-PAS	Basic helix-loop-helic/per-arnt-sim
Bmal1	Brain and muscle arnt-like 1
C-TGC	Canal trophoblast giant cells
CBP	Creb binding protein
CK1ε, CK1δ	Casein kinase 1 epsion, sigma
CLOCK	Circadian locomotor output cycles
CRY1	Cryptochrome
CT	Circadian time
CTB	Cytotrophoblast cells
Dbp	D-box binding PAR bZIP
DD	Dark/dark
Dec1	Deleted in esophageal cancer 1
DNA	Deoxyribonucleic acid
DPC	Days post coitus
E1	Estrone
E4PB4	E4-binding protein 4
FBXL	F-box protein
GD	Gestation day
GHT	Geniculo hypothalamic tract
GLUT	Glucose transporter
GRP	Gastrin releasing peptide
HAT	Histone acetyltrasferase
HBS	Hif-1 binding site
hCG	Human chorionic gonadotropin
HIF	Hypoxia inducible factor
HLA-G	Human leukocyte antigen G
HPA	Hypothalamic pituitary adrenal axis
hPL	Human placental lactogen
HSD11B2	Hydroxysteroid 11- ß dehydrogenase 2
ICM	Inner cell mass

IGF	Insulin-like growth factor
IgG	Immunoglobulin G
IGL	Intergeniculate leaflet
IL	Interleukin
JZ	Junctional zone
LD	Light/dark
LZ	Labyrinth zone
Mel_{1a}	Melatonin receptor type 1a
MHC I, II	Major histocompatibility complex
mRNA	Messenger ribonucleic acid
NAD+	Nikotinamidadenindinukleotid
NAMPT	Nicotinamidphosphoribotransferase
Nr3c1	Nuclear receptor subfamily 3 group C
Nr1d1	Nuclear receptor subfamily 1 group d member 1
P	Postnatal
PACAP	Pituitary adenylate cyclase-activating peptide
PAR bZIP	Proline and acidic amino acid – rich basic leucin zipper
PAS	Per-arnt-sim
PER	Period
PER2:LUC	PERIOD2:LUCIFERASE
PGH	Placental growth hormone
PL	Placenta lactogen
Pparγ	Peroxisome proliferator-activated receptor gamma
PRGC	Photosensitive retinal ganglion cells
RHT	Retinohypothalamic tract
ROR	Retinoic orphan receptor
SCF	Skp-Cull-F-Box
SCN	Suprachiasmatic nuclei
SIRT1	Sirtuin1
SpA-TGC	Spiral arteries trophoblast giant cells
STC	Spongiotrophoblast cells
TE	Trophectoderm
TGC	Trophoblast giant cells
TNF	Tummor necrosis factor
TTFL	Transcription translation feedback loops
VEGF	Vascular endothelial growth factor
VIP	Vasoactive intestinal peptide

INDEX

1 INTRODUCTION

Daily oscillations in mammals' physiology and behaviour are caused by an endogenous clock that developed in response to environmental changes in nature**.** Not only mammals but also other animals, plants, fungi, and bacteria have developed endogenous clock to temporally control their functions so that they are synchronized to the external cues. Rhythms that occur in the organisms with a period of approximately 24-hour are called circadian (from Latin circa-about, dies-day) rhythms. The circadian system in mammals consists of the central clock, also called central oscillator, and the peripheral clocks in various parts of the body. The central clock is located in the paired suprachiasmatic nuclei (SCN) of the hypothalamus and is comprised of neurons that are individual circadian oscillators synchronized by the synaptic and endocrine communication with each other. The peripheral tissues, organs, and cells have peripheral clocks that can oscillate autonomously and are synchronized via endocrine and neural pathways by the SCN. This fact that rhythmicity is present across peripheral tissues opened a question whether a specific mammalian organ crucial for gestation, that is placenta, also exhibits rhythmic functions. Placenta types greatly vary across mammalian species. Therefore, this thesis is focusing mainly on human and rodent placenta, which share the same placenta type, hemochorial, in which the maternal blood is in direct contact with fetus-derived tissue. The main aim of the thesis is to elucidate many aspects of placenta, including development, anatomy, and most important functions, such as respiratory, endocrine, excretory, and immune. The emphasis of this thesis is to describe rhythmicity in placental functions and the presence of clock genes in placental specific cells. Additionally, importance of these rhythmic functions for successful gestation and health of the mother and the fetus are discussed. This thesis focuses not only on rhythmic functions and functions regarding the clock machinery, but it also points out at the maternal-placenta-axis and its principal changes that are occurring during gestation. Ontogenesis of the circadian clock in the fetus is also briefly outlined.

2 CENTRAL CIRCADIAN CLOCK

Central circadian clock or the master pacemaker is located in the hypothalamus above the optic chiasma in a paired structure called the suprachiasmatic nuclei (SCN) (Moore & Eichler, 1972). Neurons comprising the SCN can generate circadian oscillations autonomously without any external cues (Welsh et al., 1995). During the 24-hour period, light is the strongest cue to entrain the central clock. It is perceived by specialized photosensitive retinal ganglion cells (pRGC). These specialized cells contain photopigment melanopsin (Berson et al., 2002; Hattar et al., 2002; Provencio et al., 2000). From the pRGCs the information is transferred by the monosynaptic retinohypothalamic tract (RHT) directly to the SCN (Moore et al., 1995).

The SCN consists of subpopulation of cells characterized by neurochemical content and localization. The core, located at the ventrolateral part, receives direct input from the RHT and is characterized by production of vasoactive intestinal peptide (VIP), calretinin, neurotensin and gastrin releasing peptide (GRP). Another subregion, found at the dorsomedial part, is called the shell. This subpopulation receives information from the core and can generate rhythmical signals. Neurotransmitters found in the shell are arginine vasopressin (AVP), angiotensin II and met-enkephalin (Abrahamson & Moore, 2001).

Robust rhythmicity and synchrony of SCN neurons is achieved as a result of a coupling mechanism (Aton & Herzog, 2005). The SCN receives information from afferent pathways. The RHT transfers the light information via releasing glutamate and pituitary adenylate cyclase-activating peptide (PACAP) (Hannibal, 2002) . The retinal projections terminate not only in the SCN but also in the intergeniculate leaflet (IGL), where photic and nonphotic information interact (Pickard, 1985). The information from the IGL is then projected to the SCN via the geniculo hypothalamic tract (GHT), where neuropeptide Y is the neurotransmitter (Moore, 1995). Another input to the SCN is from the midbrain, where the transmission is completed by serotonin (Pickard et al., 1996). The outputs from SCN are targeted at the brain regions that are related to emotions, sleep, food intake, such as thalamus and hypothalamus (Watts et al., 1987). Not only does the SCN synchronize clocks in other parts of the brain, but it also synchronizes clocks in peripheral tissues (Gachon et al., 2004).

3 PERIPHERAL CIRCADIAN CLOCK

Peripheral clocks are found in every cell (Balsalobre et al., 1998). We can find peripheral clock within tissues involved in the function of gastrointestinal, cardiovascular system, renal physiology, metabolism, and skeletal muscle. Additionally, circadian oscillations are found in mice splenic macrophages and dendritic cells. Lastly, clock genes are also present in the reproductive system (Reviewed in Richards & Gumz, 2012). Therefore, it is probable that rhythm takes an important role in physiology and separates important biological functions that might interfere with each other (Stratmann & Schibler, 2006).

Peripheral clocks are synchronized by neuronal and hormonal signals emanating from SCN. Mainly glucocorticoid hormones act as an entrainment signal that is important for phase synchronization of peripheral clocks (Balsalobre et al., 2000). The SCN clock-driven rhythmic activity of the autonomic nervous system also takes place in setting the phase of the peripheral clock, for example, it affects the clock in adrenal cortex and corticosteroid production (Ishida et al., 2005).

Indirect cues entraining the peripheral clock are driven by behaviour rhythms, such as feeding-fasting cycle and body temperature differences**.** Feeding-fasting cycle is in synchrony with sleep-wake cycle and under sufficient calorie intake, it does not affect the SCN clock. If the food intake occurs during the rest time, it acts as a dominant entrainment signal for peripheral clocks (Damiola et al., 2000)**.** The mechanism of how the feeding-fasting cycle affects the peripheral clocks on molecular level includes the ability of clock to control nicotiamidphosphoribotransferase (NAMPT) (Nakahata et al., 2009). The NAMPT enzyme produces nicotinamide adenine dinucleotide (NAD). The ability of CLOCK/BMAL1 to bind to E-elements of DNA is in fact influenced by the redox state of NAD cofactors (Rutter et al., 2001)**.** As the production of NAD and NADH by NAMPT is controlled by clock genes, the ratios of the NAD cofactors may affect the activity of circadian clock (Nakahata et al., 2009)**.** Temperature changes represent another entrainment signal that is in synchrony with sleep-wake cycle, because low amplitude temperature oscillations can entrain clock in the periphery (Brown et al., 2002). Altogether, the peripheral clocks are influenced by interconnected signals emanating directly from the SCN or indirectly via behavioural changes (Dibner et al., 2009).

4 MOLECULAR PRINCIPLES OF MAMMALIAN CIRCADIAN CLOCK

The molecular principle generating circadian signal of mammalian circadian clock is based on cell autonomous transcriptional-translational feedback loops (TTFL). Generation of 24 h period is driven by activity of protein products of genes whose transcription they inhibit via feedback loops. Among the canonical clock genes are Circadian locomotor output cycles kaput (*Clock*) and Brain and muscle ARNT-Like1 (*Bmal1*) which encode activator proteins CLOCK and BMAL1 (Takahashi, 2017). They bind to each other by the Per-Arnt-Sim (PAS) domain and form a heterodimer. CLOCK/BMAL1 heterodimer targets E-boxes of promoters of rhythmic genes (Gekakis et al., 1998). The targeted genes are *Period* (*Per1,2,3*) which encode repressor proteins PER1,2,3 (Shearman et al., 1997). Other targeted genes are *Cryptochrome* (*Cry1,2*), that encode repressor proteins CRY1,2 (Kume et al., 1999a). In the cytoplasm, protein products CRY and PER form a heterodimer, which translocates back to nucleus and inhibits CLOCK/BMAL1 activity and thus attenuates transcription of their own genes (Kume et al., 1999b; Lee et al., 2001; Sato et al., 2006).

The proper functioning of PER proteins is arranged mainly by phosphorylation facilitated by two serine and threonine kinases (casein kinase 1) CK1ε and CK1δ. The process is necessary to allow the PER/CRY heterodimer to enter the cell nucleus (Lee et al., 2001). The turnover of CRY is affected by F-box protein FBXL21, which serves in stabilization. The FBXL3 protein, that is part of Skp1-Cul1-F-box (SCF) E3 ligase sentences CRY for ubiquitination and degradation (Yoo et al., 2013). After the CRY and PER proteins undergo ubiquitination and degradation in the proteasome, the cycle is completed and the promoter of *Cry* and *Per* genes is freed from the repressor complex. CLOCK/BMAL1 heterodimer can activate transcription again, along with that a new cycle is ready to begin (Takahashi, 2017).

There are two additional loops for stabilization and coordination of the main cycle, and maintenance of the precise period. CLOCK/BMAL1 heterodimer also binds to promoters of genes coding nuclear receptors, such as repressor proteins REV-ERBα and REV-ERBβ (Zhang et al., 2015) and activators retinoic acid-related orphan receptor (RORα, β) (Sato et al., 2004). They target the RORes in promoter of *Bmal1*, where they cause either repression or activation of its transcription, respectively (Preitner et al., 2002). Lastly, PAR bZIP transcriptional factors such as activator Creb binding protein (CBP) and repressor E4PB4 bind to D-elements localized in promoters of *Per*, *Nr1D1* and *Ror* genes (Mitsui et al., 2001; Ohno et al., 2007; Sato et al., 2006) .

The HAT and CBP stimulate the histones of chromatin in order to allow the transcription of *Bmal1* and *Clock* (Curtis et al., 2004; Hosoda et al., 2009). Inhibition of CLOCK/BMAL1

heterodimer is mediated by deacetylation, which is caused by sirtuin1 (SIRT1) (Nakahata et al., 2009). The interconnection of all three loops and their activators and repressors enables generating circadian rhythm on molecular level in mammalian cells.

5 DEVELOPMENT OF FETAL CLOCK

During rat fetal stages, the SCN begins to display expression of circadian clock genes by the gestational day (GD)20 to GD21. Firstly, the *Reverbα* stars to be rhythmically expressed at GD19, after that the *Per1* and *Bmal1* show circadian rhythm at GD20 and GD21 (Houdek & Sumová, 2014). That might be a result of continuous synaptogenesis during the SCN development (Moore & Bernstein, 1989). In vitro, monitoring cultured organotypic explants of fetal SCN showed PERIOD2:LUCIFERASE (PER2:LUC) bioluminescence rhythm in spare cells at GD15.5 in mouse (Carmona-Alcocer et al., 2018). Along with that, another apparent rhythm that develops during gestation is the circadian rhythm in expression of *Vip* and *Avp*, which show rhythms at GD21 (Houdek & Sumová, 2014). The development of clock continues during the postnatal period, and at 10th postnatal day (P10) it starts to exhibit adult-like expression rhythm (Sládek et al., 2004; Sumova et al., 2012).

Development of the fetal clock in the periphery has mainly focused on liver, adrenal gland, kidney and heart (Dolatshad et al., 2010; Torres-Farfan et al., 2011; Wharfe et al., 2011). The fetal liver expresses rhythm in circadian clock genes in vitro, however, the rhythm is absent in vivo (Dolatshad et al., 2010, Sládek at al., 2007). In another study, fetal hepatic clock showed rhythmicity by GD21, but their expression contradicted the classical antiphase relationship required for functional clock (Wharfe et al., 2011). Study of clock genes in the fetal adrenal gland at GD18 revealed rhythmic circadian oscillation of *Bmal1* and *Per2*, expressing antiphase relationship (Torres-Farfan et al., 2011). In vitro study of the heart clock showed a circadian rhythmicity, whereas the in vivo approach did not (Dolatshad et al., 2010).

The fetal clocks are entrained by signals emanating from the mother. Signals such as light information, feeding time and hormones, affect the rhythmicity of the fetus via maternal SCN clock. Recently, a great array of genes in the fetal SCN were found to be rhythmically driven by the maternal signals before the fetal SCN clock developed (Greiner et al., 2022). When function of the maternal SCN clock is disrupted, the offspring develops with mutually desynchronized clocks (Nováková et al., 2010; Reppert & Schwartz, 1986; Shibata & Moore, 1988; Weaver & Reppert, 1989). Furthermore, time of food intake seems to be a strong entraining signal of the fetal SCN, as their SCN clock rhythmicity shows to be rhythmically entrained by food intake of the mother that was kept under constant light exposure (Nováková et al., 2010; Olejníková et al., 2015). Hormones such as melatonin, dopamine and glucocorticoids likely impact the fetal clock.

As melatonin is secreted in circadian rhythm and crosses the placenta, it is a strong entrainment signal for the developing fetus (Okatani et al., 1998; Davis et al., 1988, Houdek et al., 2015). The maternal melatonin entrains the SCN clock of the fetus together with affecting the fetal adrenal clock. Firstly, Davis et al. (1988) discovered in hamsters that melatonin entrains rhythmicity of fetuses during last weeks of gestation in SCN lesioned mothers. The effect of melatonin on fetal clock was also shown in the pregnant rats, in which the exposure to constant light suppressed not only melatonin secretion, but also disrupted clock in the fetal adrenal gland. Melatonin injections to the mother at night-time restore the fetal adrenal clock (Mendez et al., 2012). Absence of melatonin signal leads to phase shift of the fetal clock gene expression profiles and the injection of melatonin into the fetus externally restores the phase (Torres-Farfan et al., 2006). On top of that, melatonin injections that were provided to pinealectomized pregnant rats were able to synchronize SCN of the fetuses, as documented by circadian rhythm in *c-foc* and *Avp* expression (Houdek et al., 2015). In contrast, melatonin had no effect on the clock in the fetal liver (Houdek et al., 2015).

Another hormone with high levels during the active phase, dopamine, seems to affect fetal clock (Bates & Herzog, 2020; Peleg et al., 1986). Fetal SCN expresses dopamine receptors from GD18. The cocaine application induces expression of the immediate early gene *c-fos* in the fetal SCN that in adults correlates with phase resetting of clock (Hastings et al., 1995; Weaver et al., 1992). Additionally, glucocorticoid receptors are present in the fetal SCN (Čečmanová et al., 2019). During gestation, glucocorticoids pass placenta in a rhythmic manner and enter the fetus, where they entrain the developing fetal SCN (Čečmanová et al., 2019; Pezük et al., 2012). Glucocorticoids were found to affect not only the amplitude, but also the phase of the rhythm of the fetal SCN clock in mPer2^{Luc} mice (Čečmanová et al., 2019).

6 DEVELOPMENT OF RODENT PLACENTA

First, the fertilization of the egg is essential, after that the blastulation begins. At 32-cell stage blastomeres of blastocyst divide into two distinct regions, the inner cell mass (ICM) and trophectoderm (TE) (Iwakura, 1989). At the implantation window, blastocyst undergoes the apposion, adhesion and invasion (Paria et al., 1993). That leads to decidualization of the endometrium (Enders & Schlafke, 1967). TE cells located above the ICM are called polar trophectoderm cells, they proliferate and form the ectoplacental cone and extraembryonic ectoderm. Moreover, the extraembryonic ectoderm is a precursor for the chorion formation. Whereas the ectoplacental cone is a precursor for the spongiotrophoblast layer of the junctional zone (JZ) (Reviewed in Soares et al., 2012).

TE surrounding blastocoel, the mural trophectoderm, also proliferates and forms the primary trophoblast giant cells (TGC), which contribute to making contact with maternal cells at

implantation site (Rossant & Cross, 2001). Whereas in humans the initial invasion is achieved by syncytium surrounded by cytotrophoblast cells (CTB). The CTBs are the source of the primary villi formation in human (Knöfler et al., 2019).

Furthermore, the mouse ICM is a precursor for the epiblast and primitive endoderm. Epiblast derives all embryonic tissues and extraembryonic mesoderm, precursor of allantois (Hemberger et al., 2020). The amnion is formed by fusion of extraembryonic mesoderm and embryonic ectoderm (Pereira et al., 2011). The formation of primitive streak is mediated by epithelial-mesenchymal transition of epiblast (Beddington & Robertson, 1999). Therefore, a small bud appearing from the primitive streak is allantois, which grows towards the chorion (Downs et al., 2009). After the chorioallantoic fusion, the chorionic part then develops primary villi, to which the vessels emanating from allantois grow (Rossant & Cross, 2001). Moreover, from these last steps the placenta forms the labyrinth zone (LZ), which is the location of the nutrient and gas exchange between maternal-fetal blood flow (Soares et al., 2012). Altogether, around the midgestation the final structure of placenta is evident in mice, and at the end of first trimester in humans (Georgiades, Ferguson-Smith, et al., 2002).

7 RODENT PLACENTA ANATOMY

Rodents and humans share the same discoidal shape of placenta, where the flat side is closest to the fetus, and the bulging part is embedded in the uterine wall. The structure itself is divided into three regions as seen in figure 1 and 2. Closest to the fetal side is the labyrinth, the most distal is the maternal decidua, and the third part, junctional zone, is inbetween. The labyrinth is the main region where the nutrient exchange between mother and fetus is located, in a humans this region is called fetal placenta (Georgiades, Fergyson-Smith, et al., 2002).

Closest to the fetus, the labyrinth is bordered by chorionic plate with umbilical cord transporting nutrients and oxygen to the fetus (Steven, 1975). The maternal blood is brought to the labyrinth from uterine tissue through the junctional zone (Brosens et al., 1967). The cells that line the blood vessels differ in human and rodent placentas, where only one layer of syncytiotrophoblast is found in humans, whereas there are three layers in rodents. The most adjacent cells to maternal sinusoids are cytotrophoblasts. The next two layers are multinucleated trophoblast cells, syncytiotrophoblast, they line the fetal blood (Enders, 1965) as seen in figure 1.

Bordering labyrinth is a junctional zone which is absent of fetal blood vessels but contains continuous maternal blood sinuses from the maternal uterine wall. Junctional zone has distinct two layers, the trophoblast giant cells (TGC), which borders uterine, and the spongiotrophoblast layer, composed of spongiotrophoblast and glycogen trophoblast cells (Simmons & Cross, 2005). Lastly, the maternal decidua is part which gets invaded by invasive trophoblast cells at the beginning of

implantation. The differences between the human and murine placenta are mainly their range of invasion, where murine placenta does not invade myometrium, whereas in humans it does (Adamson et al., 2002; Pijnenborg, 1996).

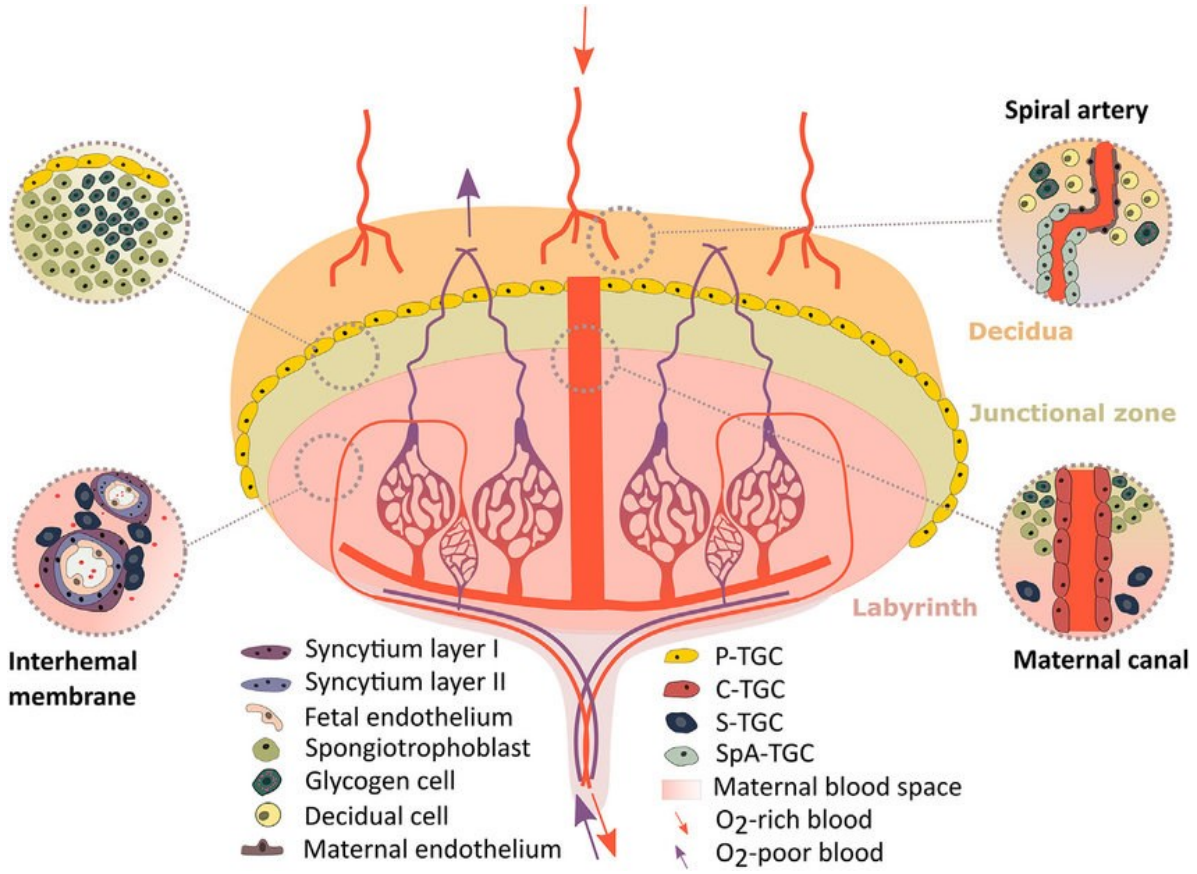

Figure 1: Murine placenta with three distinguished parts, the decidua (light orange), where the detail of spiral artery is described, labyrinth (pink), with detailed interhaemal membrane and maternal canal. Lastly the junctional zone (light green). The figure also represents cell types found in murine placenta and O_2 blood flow. At the interhaemal membrane detail, the two layers of syncytiotrophoblast (dark and light purple), next the maternal canal is lined by the canal trophoblast giant cells (C-TGC) (dark red) and the close up of spiral arteries show line up of spiral arteries trophoblast giant cells (SpA-TGC) (grey). Taken from (de Clercq & Vriens, 2018).

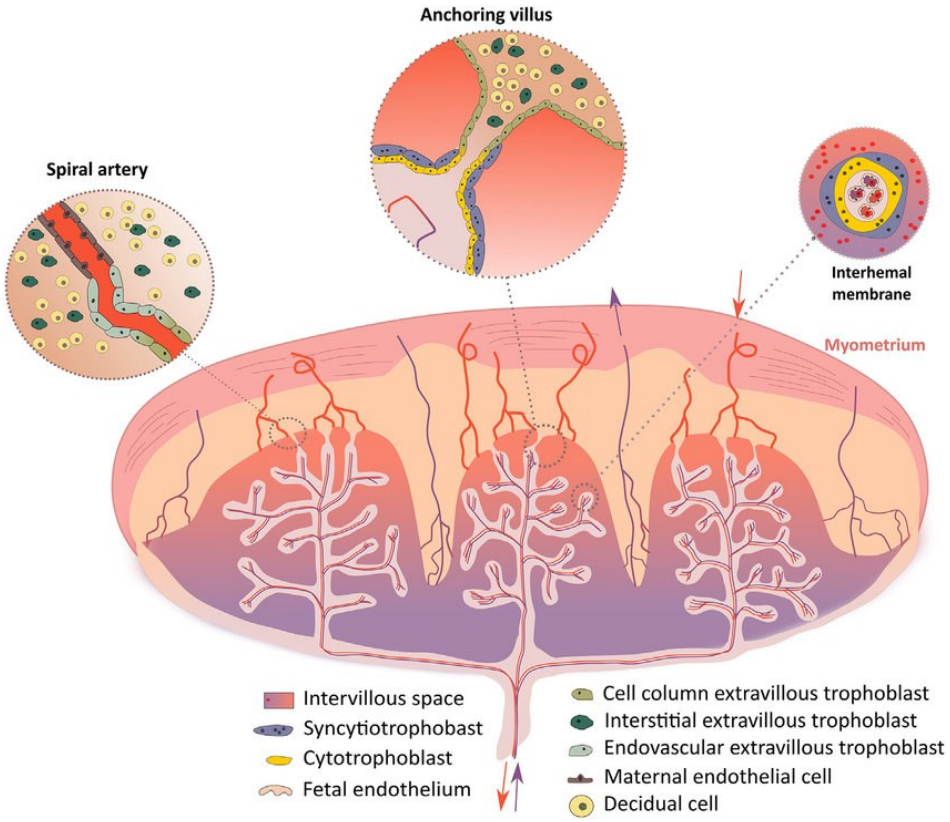

Figure 2: Human placenta with characteristic intervillous space and details of interhaemal membrane with only one syncytiotrophoblast layer (dark purple) and one cytotrophoblast layer (yellow). The difference here is shown by the placenta being embedded at the myometrium (pink). Here the spiral arteries are lined by the endovascular extravillous trophoblast cells (gray). Taken from (de Clercq & Vriens, 2018).

8 PLACENTA FUNCTIONS

The main functions of placenta include nutrient and gas exchange, protection and hormone production in order to achieve successful gestation (Soares et al., 2018). Transport of oxygen and carbon dioxide is facilitated by diffusion (G. J. Burton & Fowden, 2015). Another fundamental nutrient for the fetus is glucose, which is the main energy source of the fetus (Knopp, 1997). Not only is it transported by diffusion, additionally the transport is manifested by a family of GLUT transporters (Illsley & Illsley, 2000). Next, for the protein synthesis the amino acids are entering the fetal blood stream by specialized transporters for neutral, aniotic, catiotic amino acids, which mainly function with sodium as their cotransporter (Battaglia & Regnault, 2001). Other essential nutrients, such as fatty acids and vitamins, also enter the fetal blood stream (Baker et al., 1981; Duttaroy, 2009).

At the beginning of placenta formation, it is necessary to remodulate maternal blood stream, in which the TGCs in rodents and extravillous trophoblast (EVT) cells in humans are responsible for (Rai & Cross, 2014) The transport barrier in rodent placenta is built out of three layers, the cytotrophoblast layer interacting with the maternal blood and then two connected syncytial layers. In contrast, the human barrier consists of the syncytiotrophoblast layer that is in interaction with maternal blood supply and the second layer the cytotrophoblast (Georgiades, Fergyson-Smith, et al., 2002).

Furthermore, placenta is responsible for mediating humoral immunity from mother to fetus. The transfer of Immunoglobulin G (IgG) from mother to fetus is achieved by specialized Fc placental receptors, mainly located at the trophoblast layer at the maternal fetal interface (Saji et.al, 1999). The placenta also serves as a protective barrier. For example, the multidrug resistance protein family protect the fetus from xenobiotics and the enzyme 11ß-hydroxysteroid dehydrogenase (11-ßHSD2), which serves as protection of maternal stress hormone by oxidizing maternal cortisol into safe cortison (St-pierre et al., 2000; Mcternan et al., 2001).

The main differences in endocrinological aspects between human and rodent placenta is the involvement of pituitary gland, which is essential for regulating the progesterone production by corpus luteum in mice pregnancy, whereas in human its involvement is absent (Strauss et al., 1996; Jameson & Hollenberg, 1993). The main hormone synthesis site in placenta in human is the syncytiotrophoblast layer, whereas in rodents the hormones are mainly produced in junctional zone, analogue of basal plate in humans, by TGC and spongiotrophoblast cells (STC) (Malassiné et al., 2003; Simmons & Cross, 2005; Soares et al., 2012).

9 PLACENTA CLOCK GENES

The clock genes are present in peripheral organs of the mammalian body and their proper functioning is necessary for temporal regulation of the organ physiological functions (Balsalobre, 2002). The presence of clock genes in placenta was first mentioned by the Frigato et al. (2009). The placenta genes were studied in a first trimester human trophoblast cells HTR-8/SVneo in normoxic and hypoxic environments. The rhythmic expression of *Per2*, *Deleted in esophageal cancer 1* (*Dec1*) and *D-box binding PAR bZIP* (*Dbp*) clock genes appeared only after serum shock applicantion. Both *Per2* and *Dec1* mRNA showed period length around 24 hours, whereas the *Dbp* period was shorter, only 20 hours. In the study, the hypoxic environment was induced, because it is typically present at the beginning of pregnancy during the time of placentation (Soares et al., 2017). The expression of genes measured in hypoxic environment increased for those containing the HIF-binding site (HBS) motif in their promoter such as *Dec*, *Dbp*. The HBS is a motif to which the regulator of oxygen homeostasis binds (Kenneth & Rocha, 2008). Moreover, the expression in *Per2*

was delayed, as its promoter is absent from the HBS. This study presented a robust circadian clock at the beginning of gestation. The presence of the rhythmically expressed *Per2* clock gene in trophoblast cell line was confirmed by Demarez el al., (2012), who also measured expression of *Reverbα* and *Bma1l*.

The results of studies on expression of clock genes in human full-term placenta are not consistent. This might be caused by the different way of parturition, because one group of placentas was collected by the casarean section (Papacleovoulou et al., 2017) and the other by the vaginal birth (Pérez et al., 2015). The full-term human placenta delivered by vaginal birth had expressed *Clock*, *Bmal1*, *Cry1* and *Per2* genes. Both *Clock* and *Bmal1* expressed a circadian variation and peaked at 0800 h and 1200 h, respectively seen in figure 3. However, *Per2* exhibited two peaks at 0000h and 1200h and *Cry1* lacked any rhythm (Pérez et al., 2015). In contrast, in full-term human placenta delivered by the cesarean section, the expression profiles of clock genes were different and circadian rhythms for *Bmal1*, *Clock*, and *Per1* were not detected (Papacleovoulou et al., 2017).

In the whole mouse placenta (without differentiation of LZ and JZ) at 16.5 days post coitus (DPC), all canonical clock genes *Bmal1*, *Clock*, *Cry1*, *Cry2*, *Per1*, *Per2*, and also the *Dbp*, showed rhythmic expression. Moreover, expression of the clock genes of mice placenta also increased in later gestation together with expression of clock genes in uterus and fetal membranes, bringing up question, whether the increased expression is in correlation with the upcoming parturition (Ratajczak et al., 2010).

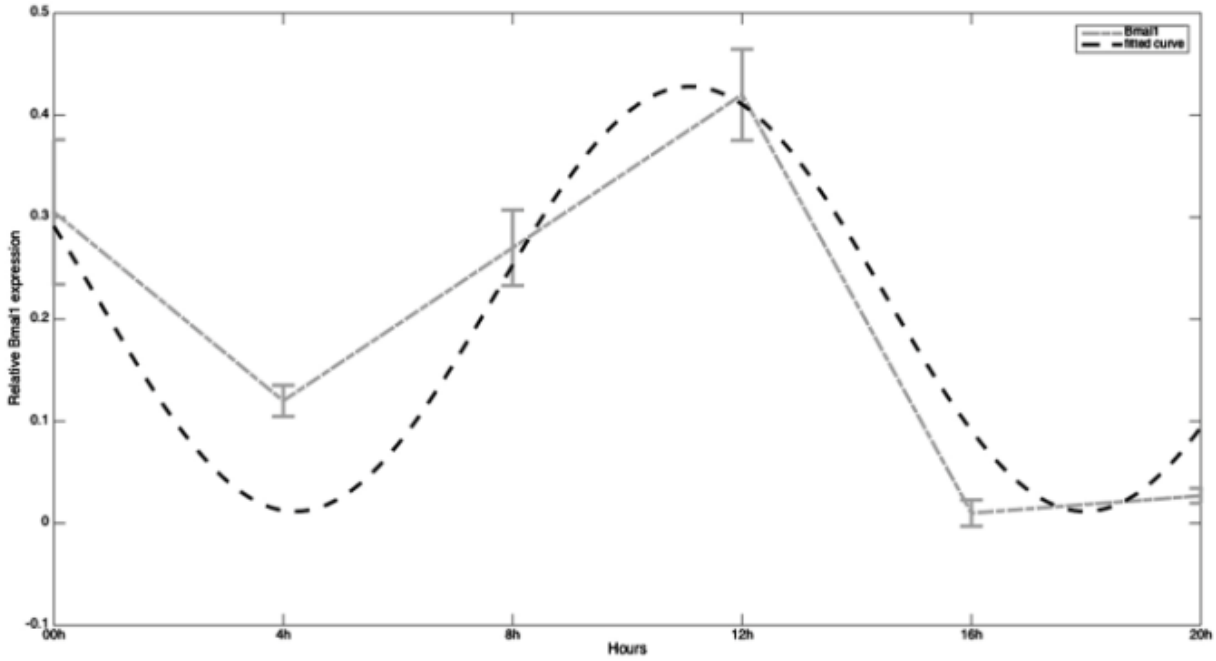

Figure 3: Expression of *Bmal1* mRNA measured in human full term placenta delivered by vaginal birth, at 00, 04, 08, 12, 16 and 20 hours of day. Taken from (Pérez et al., 2015).

9.1 SPATIAL DIFFERENTIATION OF CLOCK GENES

Studies on expression of clock genes and their oscillation vary in spatial localization within the placenta. Using in situ hybridization approach it was shown that in pregnant rats maintained on light/dark regime with 12h of light and 12h of darkness (LD12:12), the placenta collected on GD22 exhibits *Per1* expression in both labyrinth zone and decidua, with labyrinth zone lacking rhythmicity in clock gene expression. The circadian oscillation in the decidua zone persisted only one to two cycles of oscillation (Akiyama et al., 2010). Different results were found when the rats were maintained in constant darkness (DD; dark/dark) regime during pregnancy. *Per1* mRNA expression was detected after parturition in both decidua and labyrinth. The circadian oscillation of *Per1* mRNA was present in decidua at GD12 and also GD22 (Akiyama et al., 2010).

Another study that showed differences in spatial localization of clock genes in placenta was done by Wharfe et al., (2011). The authors showed that expressions of clock genes *Bmal1*, *Clock*, *Cry1*, *Cry2*, *Pe1r*, *Per2* were present in both junctional and labyrinth zones but with differences in expression levels. This result confirmed data of previous study by Ratajczak et al., (2010) on rhythmically expressed clock genes in the placenta. Where the labyrinth zone evinced *Clock*, *Per1*, *Cry2* with higher expression, whereas in the junctional zone the higher expression in *Bmal1*, *Per2*, *Per3* and *Cry1* was marked. In contrast to the previous study by Akiyama (2010), circadian variation of *Per1* expression in the labyrinth was detected. Furthermore, circadian fluctuation in expression of *Bmal1* and *Per2* in the junctional zone and *Per1* in the labyrinth zone exhibited peaks during dark the phase, the active phase of rodents (Ratajczak et al., 2010). This finding contradicts the circadian clock phase relationship, in which in other mammalian cells the two genes *Bmal1* and *Per* are in antiphase (Oishi et al., 1998).

At GD15 and GD21, the expression of *Clock*, *Cry1* and *Nr1d1* was present in the labyrinth zone of the rat placenta (Crew et al., 2018). Additionally, in that study an effect of obesity on the placental clock was found; the *Clock* expression was reduced at GD15 and *Cry1* was increased on the GD21. The effect of obesity-induced conditions was most prominent on the *Nr1d1*, owing to the fact that the *Nr1d1* is involved in the link between metabolism and molecular clock (Crew et al., 2018). Obesity impacts the course of gestation and maternal physiology which might affect the profiles of expression of clock genes in the placenta (Crew et al., 2018; Mariona, 2016).

Another study focused on placental clock in mice at GD15 (Demarez et al., 2021). In contrast to finding only *Per1* fluctuation of labyrinth (Wharfe et al., 2011), this study revealed circadian rhythms in expression of *Per2* and *Reverbα* in the labyrinth zone. *Per2* showed peaks at the beginning of the dark phase, whereas *Reverbα* peaked at the middle of the light phase. In this study, circadian rhythm in the expression of clock genes *Bmal1* and *Clock* in the labyrinth was not confirmed (Demarez et al., 2021).

Around the same time of gestation at GD18 and GD17 studies on rodent placentas kept under LD conditions, showed a circadian rhythm of clock genes *Nr1d1* and *Per2* in decidua (Čečmanová et al., 2019; Lužná et al., 2021). The circadian clock in decidua of mPer2^{Luc} mice maintained autonomous oscillations in vitro, as detected by rhythm in PER2-driven bioluminescence in the cultured placenta explants. The junctional zone exhibited also a bioluminescence signal, but with less intensity and lighter signal emanating, whereas labyrinth was lacking this signal (Lužná et al., 2021).

10 HORMONES

10.1 GLUCOCORTICOIDS

Glucocorticoids play a crucial role already at the beginning of gestation, during implantation (Mastorakos et al., 1996). They are important for fetal organ maturation and growth (T. J. Cole et al., 1995; Fowden et al., 1998). Nonetheless, excessive levels of glucocorticoids can be pathological, they can cause severe malfunctions in behaviour and physiological dysfunctions (Fowden et al., 1998; Uno et al., 1990). The syncytiotrophoblasts of placenta contain enzyme 11-hydroxysteroid dehydrogenase2 (HSD) and P-glycoprotein ATP binding cassette subfamily B member 1 (ABCB1), which influences the accessibility of glucocorticoids to reach the glucocorticoid receptors (Mark et al., 2009; Sun et al., 2006; Yang, 1997). Not only the ABCB1 reduces the entry of glucocorticoids to the fetus, but also it protects the fetus from harmful substances, such as xenobiotics (Sun et al., 2006). The placental 11ß-HSD2, also known as the placental glucocorticoid barrier, decreases the amount of glucocorticoids emanating from the maternal body into the fetus in rodents and humans (Salvante et al., 2017). The 11ßHSD2 is a NAD+ dependent enzyme that transforms the active corticosterone and cortisol into 11-dehydrocorticosterone and cortisone (Burton & Waddell, 1999).

10.1.1 HSD11B2

The placenta collected at GD19 from mice maintained under the LD cycle during gestation, shows a circadian variation of *Hsd11b2* gene. The expression of the *Hsd11b2* gene differs between two placental zones, fetal and maternal. Whereas the fetal part of placenta has a relatively constant expression of the *Hsd11b2* gene thorough a 24 h period, the maternal part of placenta shows a circadian rhythm, with acrophase around CT21 as seen in figure 4. The circadian variation of this gene implies an important role in regulating the access of glucocorticoids to placenta at given time, mainly at periods of gestation when the glucocorticoids emanating from mother are high (Čečmanová et al., 2019).

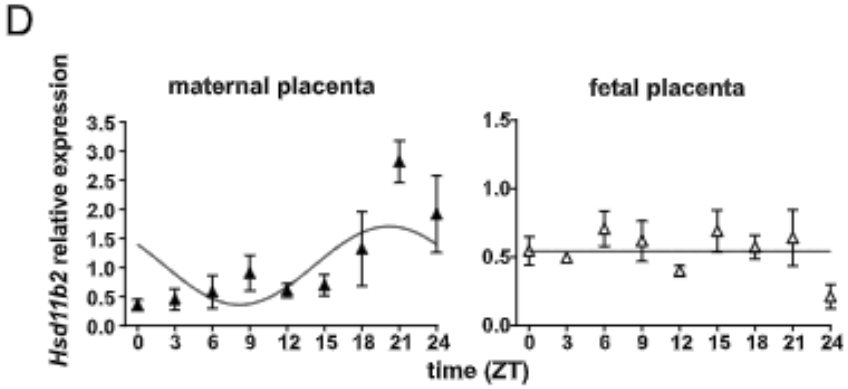

Figure 4: The circadian expression of *Hsd11b2* gene with differences between maternal part of placenta and fetal part accordingly. Taken from (Čečmanová et al., 2019).

10.1.2 ABCB1

In rodent placenta, *Abcb1* expression levels are higher in the labyrinth zone compared to the junctional zone. The circadian variation of the *Abcb1* in rats shows its higher expression in correlation with the active phase of mother, which is during the dark phase in rodents (Waddell et al., 2012). In mice models, genes *Abcb1a* and *Abcb1b* have a circadian profile expression, also peaking at dark phase. In mouse labyrinth, the rhythmicity of these two transporters is arranged not only by the glucocorticoids emanating from the maternal body, but also by the local labyrinth clock. In human BeWo trophoblast cells, the *Per2*, *Reverbα* and *Bmal1* have rhythmic expression in correlation with the *Abcb1* genes. Therefore, circadian rhythm in expression of *Abcb1* transporter is likely under the influence of rhythmically expressed clock genes present in trophoblast cells of human placenta (Demarez et al., 2021).

10.1.3 GLUCOCORTICOIDS AND PLACENTA CLOCK

Glucocorticoids are strong circadian entraining signals in the periphery (Pezük et al., 2012). The study of Čečmanová et.al. (2019) showed that in the mice kept under LD cycle, the placental glucocorticoid receptor *Nuclear receptor subfamily 3 group C* gene (*Nr3c1)* is expressed both in the fetal and the maternal part of placenta at GD19. The fetal part showed higher expression of *Nr3c1* but the expression did change throughout the 24 h period. In contrast, the maternal part expressed lower levels of *Nr3c1*, which however exhibited shallow rhythm with the peak around circadian time (CT)12, which corresponds to the time of lights off of the LD cycle. The in vitro study using mPer2^{Luc} mice showed that glucocorticoid treatment of arrhythmic part of placenta (fetal part) induces only an acute response by producing a maximum of 1-2 cycles of PER2-driven

bioluminescence. In the maternal part of placenta (decidua), the circadian rhythm of *Per2* mRNA was detected with a peak at CT12, and with the lowest expression at CT24. The glucocorticoid treatments to the cultured maternal placenta explants induced phase shifts of the PER2-driven bioluminescence rhythm. The magnitude of phase shift depended on the time of treatment. The highest phase shifts were induced at the time of the PER2 bioluminescence peak, and the lowest phase shifts were induced at the PER2 bioluminescence through (Čečmanová et al., 2019).

10.2 MELATONIN

The level of maternal serum melatonin is higher during pregnancy; the levels remain increased during the gestation and decrease after the parturition, indicating the additive production during gestation by placenta (Ejaz et al., 2021). Placenta harbors essential enzymes aralkylamine N-acetyltransferase (AANAT) and N-Acetylserotonin O-methyltransferase (ASMT), that synthesize melatonin (Iwasaki et al., 2005). Melatonin is produced in the villous trophoblast cells and the same cells harbor melatonin receptors Mel_{1a} and Mel_{1b} (Lanoix et al., 2008).

The circadian rhythm of melatonin produced by placenta has not been studied yet. Rat placenta has expressed the circadian rhythm of melatonin receptor Mel_{1a} in junctional and labyrinth zone (Lee et al., 2003). In rat placentas collected at GD19, the presence of *Mel1a* was found in spongiotrophoblast and TGC. The expression of *Mel1a* mRNA varied between 1600h of GD19 and 0400 h of GD20. The junctional zone at this time window expressed higher levels of *Mel1a* mRNA at daytime, whereas the expression in the labyrinth zone was the opposite. Altogether, data suggested temporal changes in expression of Mel1a in placenta. Data collected from the same placentas at two different times, 1600h and 2200h at GD19 and at 0400h and 1000 h GD20, confirmed circadian expression of the *Mel1a* in placenta. The measurements of expression of *Mel1a* in the junctional zone showed increased expression of *Mel1a* at day-time and decreased expression at night-time. The expression was in the opposite phase compared to the labyrinth zone. Altogether, the results imply that placenta rhythmically expresses *Mel1a* in each zone with a reverse phase of expression (Lee et al., 2003).

10.2.1 MELATONIN, DOPAMINE, LEPTIN, GHRELIN, INSULIN AND PLACENTA CLOCK

The hormonal effect of melatonin, dopamine, leptin, ghrelin and insulin on placental clock was studied in $mPer2^{Luc}$ mice that were kept under LD conditions (Lužná et al., 2021). The studied placentas were obtained on GD17. The study found that in the placenta, the maternal part (decidua) emits the strongest PER2-bioluminescence signal compared to other regions. Therefore, the decidua

region was used to study the effect of the five mentioned hormones on the clock in placenta. By applying melatonin or dopamine on the decidual clock of mouse placenta, circadian rhythm in PER2-driven bioluminescence responded by delaying or advancing, depending on when the treatment was applied. Treatment with melatonin just before the PER2-driven bioluminescence reached its peak caused phase advances. On the other hand, phase delays were observed when dopamine treatment was applied after the bioluminescence peak. Additionally, dopamine had an acute effect on the level of the bioluminescence, which declined immediately after its treatment, followed by an increase of the amplitude in the next cycle. The results suggest that these two hormones are affecting the clock in a complementary way during the day and night, as the effect of one ends, the effect of the other starts (Lužná et al., 2021).

Hormones, leptin, ghrelin and insulin affected the clock only marginally. Insulin had the least effect on the clock compared to the ghrelin and leptin; causing a small phase delay in the peak of PER2-driven bioluminescence. The treatments with ghrelin and leptin showed responses in phase delays after the bioluminescence peak and during its decline. The study pointed out that the effects take place in a time of attenuated foraging (Lužná et al., 2021).

10.3 CHORIONIC GONADOTROPIN

The human chorionic gonadotropin (hCG) is produced at the beginning of pregnancy by trophoblast cells of placenta, its levels increase at this time of pregnancy as it takes role in stimulating the production of progesterone by corpus luteum (L. A. Cole, 2010). The high hCG levels then slightly decrease in the middle of pregnancy, followed by an increase towards the term (Dfaz-Cuetot et al., 1994). The hormone has important role in vasculogenesis (Berndt et al., 2009) and stimulating the fusion of cytotrophoblast cells (Shi et al., 1993). The hCG decreases the immune response of the maternal body to placenta (Akoum et al., 2005), also it impacts the myometrium as it keeps it in relaxed state (Eta et al., 1994).

Few studies showed that the levels of hCG exhibit circadian variation (Ayala et al., 1984; Houghton et al., 1982). The 24-h patterns measured at 3 different periods during pregnancy showed that during the first trimester the hCG reached acrophase between 1100h and 1500h, respectively, and the acrophase shifted towards later hours of a day with the progress of pregnancy (Díaz-Cuetot et al., 1994). Food intake was taken into account as one of the external stimuli that might change the 24 h profile of hCG. The effect of food intake is apparent at first trimester pregnancies where the profile levels of hCG drop after meal (Díaz-Cuetot et al., 1994; Nakajimaf et al., 1990).

10.4 PLACENTAL LACTOGEN

A human placental lactogen (hPL), also known as human chorionic somatotropin, is a polypeptide hormone secreted by sycytiotrophoblast layer of placenta (Hoshina et al., 1982; Walker et al., 1991). hPL secretion starts around the second week of gestation and the secretion increases towards the end of gestation. In mice, the placental lactogen is produced by TGC, the production starts around GD12 and increases towards parturition (Soares et al., 1998) The polypeptide is involved in regulating the metabolism of the mother in order to increase nutrition availability for fetus (Handwerger & Freemark, 2000).

Detection of circadian variation of the hPL was not consistent among studies (Grumbach et al., 1968; Samaan et al., 1966; Teoh et al., 1971). In some of them the circadian variation of hPL secretion was reported (Gillmer et al., 1977; Houghton et al., 1982). Study by other authors (Lindberg & Nilsson, 1973) measured a 24 h variation of the hPL secretion, and the effect of sleep, meals and exercise, but it did not find any effects on the levels of hPL. In another study in mice, a daily pattern in fluctuation of hPL levels over a 24 h exhibited 2 peaks, one peak between 1500h and 2100h and the second peak between 0000h and 0600h (Markoff & Talamantes, 1981).

The team of Lee et al. (2003) measured circadian rhythmicity of mouse placenta lactogen II (PL-II) at GD19. PL-II was expressed in the junctional zone and with higher expression in the labyrinth zone. In the junctional zone the expression of *PL-II* was the highest during the night-time and the lowest at day-time and the labyrinth zone showed the strongest *PL-II* expression during the daytime and the weakest expression during the night-time. This study also measured circadian variation of the melatonin receptor (mentioned above in chapter 10.2: Melatonin) and found that its circadian expression is in the opposite phase in each of the placenta zones, similar to the PL-II expression. Importantly, the study found relationship between melatonin and PL-II, suggesting that melatonin acts as a regulatory factor of the *PL-II* gene (Lee et al., 2003).

10.5 PROGESTERONE

Progesterone is a steroid hormone firstly produced by corpus luteum and then by syncytiotrophoblasts of placenta through the gestation until term (Tuckey, 2005) Progesterone has impact on many functions during gestation, which include adjusting metabolism of mother in order to increase fat storage, stimulating the upcoming lactation and control its timing (Kalkhoff, 1982; Pang & Hartmann, 2007). At the beginning, it affects the implantation and adjusts maternal immune reaction by reducing it (Halasz et al., 2013; Laškarin et al., 2002). Throughout gestation, progesterone keeps the myometrium in a relaxed state (Fomin et al., 1999).

The rhythm in human and nonhuman primate pregnancies seems to be diurnal with peaks at night-time (Giussani et al., 2000; Magiakou et al., 1996; Walsh et al., 1984). At 18 to 25 weeks of gestation, the progesterone levels are highest at midnight and lowest during the daytime (Junkermann et al., 1982). As the pregnancy progresses, the levels of progesterone are becoming lower and lose their diurnal rhythm at the end of gestation (Challisa et al., 1983; Junkermann et al., 1982). In contrast, other study by Darne et al. (1989) showed the absence of diurnal rhythm of progesterone in the third trimester. The mechanism behind the nocturnal secretion of progesterone might be the process of steroidogenesis.

10.6 ESTROGENS

During pregnancy, placenta produces estrogen steroid hormones – estriol, estradiol, estrone. The placental estrogens have various functions. Estradiol is involved in many processes throughout the pregnancy; at the beginning the effect is seen at the implantation and endometrium growth (Groothuis et al., 2007). At the end of pregnancy estradiol stimulates the labor initiation and prepares maternal breast glands for breastfeeding (Di et al., 2001; Pang & Hartmann, 2007). Throughout the pregnancy its levels increase peaking just before parturition (Loriaux et al., 1972).

The diurnal pattern is characteristic for estrone towards the end of gestation, in which the estrone levels reach peak levels during morning, and through during late night and early morning (Challis et al., 1980). In one study, estradiol levels had no circadian variation at the end of gestation (Challis et al., 1980), but in another study circadian secretion of estradiol was detected at the same time of gestation (Zulu et al., 1978) . Estriol loses its circadian rhythm towards the end of gestation. 10 weeks before parturition, the estriol shows circadian rhythm with the peak between 0130h and 0330h, and the lowest levels between 0900 h and 1030h. Estriol continues to display circadian rhythm at 34-35 weeks of gestation, but the rhythm is shifted towards earlier hours (Challisa et al., 1983). It has been proposed that because the placenta secretes estrogens by aromatizing androgens from fetus, the levels of estrogens might also correlate with the circadian rhythm of dehydroepiandrosterone (Chatuphonprasert et al., 2018; Seron-Ferre et al., 1993).

11 IMMUNITY AND PROTECTION

During pregnancy, the crucial role of the placenta is to prevent the maternal body from immunological reaction. The placenta represses the complement activation, and in addition the trophoblast cells of placenta do not exhibit major histocompatibility complexes (MHCI) and MHC II (Holmes & Simpson, 1992; Weetman, 1999; Wood, 1994). Nevertheless, placental trophoblast cells contain HLA-G which downregulates the maternal immune reaction (Carosella et al., 1999). Another feature of the trophoblast cells is the expression of Fas ligand that serves in apoptosis of maternal T cells as it binds to Fas positive T-cells. The cytokines are involved in this process by increasing the Fas ligand trophoblast expression, thus increasing the apoptosis of mothers` T-cells (Yamada et al., 2017).

Many cytokines are produced by the placenta; during the beginning of gestation, expression of proinflammatory cytokines (Interleukin) IL-1ß, IL-6 and Tumor necrosis factor alpha (TNFα) is high and decreases over the gestation (reviewed in Bowen et al., 2002). The proinflammatory cytokines are mainly involved at the beginning of gestation because they play a role in proliferation and invasion of trophoblast cells into the decidua (Naruse et al., 2010). TNFα promotes secretion of vascular endothelial growth factor (VEGF), which plays a role in remodeling of spiral arteries (Athanassiades et al., 1998).

In the immune system, core circadian genes are involved in the T-cell antigen response (Fortier et al., 2011) and also B cells, macrophages and dendritic cells (Silver et al., 2012). The team of Astiz et al. (2021) proposed an idea of studying the presence of circadian clock genes involved in the immune system of placenta. Only a few studies were linked to the immune processes taking place in placenta.

11.1 CYTOKINES

From the point of view of circadian rhythmicity of initial steps of gestation, the expression of circadian variation of cytokines was studied. Proinflammatory cytokine the IL-1 has a circadian variation in the junctional zone, together with the IL-6, which showed a variation in the labyrinth zone of placenta. The junctional and labyrinth zones showed a circadian expression of TNFα, with higher expression across the daytime (Mark et al., 2012). It might be possible that the circadian rhythm of these cytokines plays important role in the timing of parturition (Waddell et al., 2012).

11.2 VASOACTIVE ENDOTHELIAL GROWTH FACTOR

The *Vasoactive endothelial growth factor* (*Vegf*) is a circadian output gene mainly regulated by the PER protein (Koyanagi et al., 2003). The daily variation of *Vegf* was only found in trophoblast HTR/Vsneo cells after the application of serum, and the expression lasted for 30 hours. During induced hypoxia, the *Vegf* showed an increase of mean expression. Such a response to hypoxia induced conditions was a result of a harbor promoter motif of *Vegf* (Frigato et al., 2009). The expression of *Vegf* in a rat placenta under normal conditions is zone dependent, the labyrinth has higher expression in contrast to the junctional zone. During the measurement throughout the day at 0800h, 1400h, 2000h, and 0200h, no variation of the *Vegf* mRNA was measured, only the highest expression was at 2000h. Therefore, the *Vegf* expression was stable over the period of 24 h without any circadian oscillation reaching significance (Frigato et al., 2009).

Hypoxia-inducible factor alpha (HIFα) is present in the placenta at the beginning of pregnancy due to a hypoxic environment (Burton et al., 2021). As a result of that, Frigato (2009) showed that as the *Vegf* promoter contains HBS, the HIFα induces a hypoxic response of the *Vegf* gene. In hypoxic conditions, the expression levels of *Vegf* were increased during two continuous circadian cycles (Wharfe et al., 2011).

11.3 KISSPEPTIN

Another regulator involved at the beginning of placenta formation is the neuropeptide kisspeptin (Hiden et al., 2007). The neuropeptide together with its receptor have higher expression pattern during the first trimester of gestation, and similarly to the proinflammatory cytokines, its function is to constrain the trophoblast invasion (Mor et al., 2011; Janneau et al., 2002). Additionally, kisspeptin might play a role at the end of gestation, as it might cause the trophoblast cells to undergo programmed cell death (Cindrova-Davies et al., 2007).

The circadian variation of kisspeptin was studied in a full-term placenta delivered by vaginal birth. The circadian variation in expression was tested in samples collected at 6 time points over 24 h interval, at 0000h, 0400h, 0800h, 1600h and 2000h. The circadian variation of kisspeptin was evident with peak at 0400h and the levels dropped to the trough at 2000h (de Pedro et al., 2015).

12 MATERNAL-PLACENTA-FETUS AXIS

12.1.1 MELATONIN SECRETION

Melatonin is released from the pineal gland at night-time (Reiter, 1993). At the beginning of gestation, the levels of melatonin in maternal serum decrease, but during pregnancy the levels markedly increase after 24 weeks, and even more towards the end of gestation (Nakamura et al., 2001). This might be involved in the intense production of superoxide free radicals at the beginning of gestation, because melatonin might take part in this process as it has antioxidative effects (McCarthy et al., 2019; Myatt & Cui, 2004; Tan et al., 2015). The increase in melatonin levels later in gestation suggests that the melatonin produced by placenta enters the maternal bloodstream (Nakamura et al., 2001).

As melatonin is produced by pineal gland, studies tested whether pinealectomy had any effect on the time of birth. During normal pregnancy, the parturition shows clear preferred time of day in early morning in humans and in daylight in rats (Rowland et al., 1991). The pinealectomy changed time of parturition, which was the opposite to the time in animals with intact pineal glands (Takayama et al., 2003).

12.1.2 HYPOTHALAMIC PITUITARY ADRENAL AXIS (HPA)

The hypothalamic-pituitary-adrenal axis (HPA) drives the production of glucocorticoids, and during gestation, the levels of corticosterone in rodents and cortisol in humans tend to increase towards term (Atkinson & Waddell, 1995a; Mastorakos & Ilias, 2003). The circadian rhythm in glucocorticoid levels exhibits the peak at morning hours with the lowest levels during late night in humans, and it is in opposite phase in nocturnal rodents (Atkinson & Waddell, 1995b; Patrick et al., 1980). During pregnancy, there is a change in expression of clock genes in hypothalamus in mice (Wharfe, Mark, et al., 2016). Clock genes *Bmal1*, *Reverbα* and *Per2* show high levels of expression throughout the whole pregnancy in maternal hypothalamus. Compared to that, clock genes *Clock, Cry1, Cry2* present fluctuations, their mesors decline from midgestation and peak close to term. Between GD14 and GD18, the acrophases of *Bmal1* and *Reverbα* is advanced and delayed, respectively. In other clock genes *Clock, Cry1, Cry2, Per1, Per2,* the change of acrophase is not significant. The amplitude of *Cry2* and *Per1* both increase by the GD10 and *Bmal1* amplitude increases on GD 14. The amplitude of genes *Clock*, *Per2*, *Cry1* declines toward term (Wharfe, et al., 2016).

During gestation, adrenocorticotropic hormone (ACTH) and corticosterone also exhibit strong interaction between time of day and stage of pregnancy. The overall levels of ACTH decrease towards the term, whereas corticosterone levels increase. ACTH and corticosterone

differ also in the shift of acrophase, ACTH profile delays after GD18, whereas corticosterone phase does not change (Wharfe et al., 2016).

Study by Yaw and colleagues (Yaw et al., 2021) showed a relationship between the pituitary gland and SCN. The team measured the phase of PER2:LUC rhythm of pituitary explant which showed the same phase of PER2:LUC as SCN around midgestation. Towards the end of gestation, a possible correlation between the change of the phase in pituitary rhythm and upcoming parturition was indicated. After GD18, at the peak phase of PER2:LUC of SCN, the pituitary clock showed a 13 h delay. The study suggested that such a relationship might exist as a preparation for parturition.

Taken altogether the HPA goes through rhythmic adaptation and changes during pregnancy. The reason behind the attenuated rhythmical secretion of ACTH might be because the fetal HPA is involved in gestation together with the absence of glucocorticoid barrier. It has been proposed that the circadian changes in the HPA axis could lead to metabolic changes in gestation (Wharfe, Mark, et al., 2016).

12.1.3 METABOLISM

Gestation is a metabolically challenging state of physiology, with high demands on glucose resources for developing fetuses (Bell et al., 1997). The maternal body adapts to meet those demands of fetus by changing the circadian clock of main metabolism-related organs. There has been significant change of hepatic clock genes during pregnancy as well as the change in expression of genes involved in glucose homeostasis. During gestation, the amplitude of circadian variation of clock genes *Clock*, *Bmal1*, *Per1*, *Per2*, *Cry1*, *Cry2* and *Reverbα* declines. As for that, the genes of glucose homeostasis show an attenuated rhythmicity at the end of gestation (Wharfe et al., 2016b).

In adipose tissue, circadian rhythm of clock genes *Bmal1*, *Cry1*, *Cry2*, *Reverbα Rorα* do not change during pregnancy, whereas the genes involved in lipid metabolism lose rhythmic variation at the beginning of gestation. The lost rhythmicity might be a result of different needs for metabolic products at different periods of gestation, as there is no correlation between clock genes and genes involved in lipid metabolism. The exception in metabolic genes is *Peroxisome proliferator-activated receptor gamma* (*Pparγ*), as its expression shows circadian rhythm during gestation in relation to the rhythm of clock genes (Wharfe et al., 2016a).

12.1.4 THERMOREGULATION

Humans have circadian regulation of core body temperature; the lowest temperature appears at night-time compared to day-time elevated levels (Refinetti et al., 1992). During gestation, the core temperature measured during midday remains high in the first trimester of pregnancy and falls towards the term (Hartgill et al., 2011). In nocturnal rodents, the circadian rhythm of core

temperature is the opposite, with higher temperature during night-time (Refinetti et al., 1992). During pregnancy the circadian rhythm in maternal body temperature changes, in pregnant rats, the body temperature decreases during the middle of gestation until term (Simrose et al., 1995). Not only does the average body temperature decrease, moreover, the night-time rise in temperature is lost on day 15 of gestation (Fewell, 1995). In mouse gestation, the circadian rhythm is also lost as seen in rats, the difference is the initial increase of mesor body temperature at the beginning of gestation, after midgestation the mesor declines together with decreasing amplitude of night-time body temperature (Wharfe et al., 2016a). After parturition the body temperature pattern is regained in both rodent species reaching the nonpregnant level (Simrose et al., 1995; Wharfe et al., 2016a). The decrease of body temperature of the mother is an adaptive approach as the fetus and placenta are characterized as a highly metabolic systems. By decreasing the temperature of the mother, the temperature emanating from the two systems easily spreads (Fewell, 1995; Wharfe et al., 2016a).

13 CONCLUSION

The emphasis of this thesis was on summarizing human and rodent haemochorial placenta characteristics, such as their structure, development, and functions. The aim was also to elucidate current knowledge about presence of circadian clock in placenta and rhythms in basic placenta functions. Moreover, the thesis briefly focused on development of the fetal circadian system together with changes of maternal rhythmicity during gestation.

From the available sources in literature it appears that the placenta harbors all canonical clock genes *Clock*, *Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1*, *Cry2*. The localization of clock genes and their circadian expression differs in each placenta zone. The placenta produces hormones such as progesterone, estrogen, hCG. The expression of these hormones changes throughout the pregnancy together with their 24 h profiles.

Melatonin and glucocorticoids showed to be heavily involved in pregnancy affecting the fetus, placenta and mother. This thesis pointed out that in the fetus, both melatonin and glucocorticoids entrain the developing fetal SCN. Moreover, maternal melatonin secretion changes during pregnancy, in a way that the levels of secretion increase. Following that, pinealectomy, aberration of the source of melatonin production, showed to impact developing fetal clock and the time of parturition. In placenta, the melatonin is also produced. There are no studies on its rhythmical secretion by placenta, but genes coding melatonin receptors showed to oscillate in circadian manner. The amount of glucocorticoids entering the placenta is controlled by HSD and ABCB1, as for that, they both exhibit circadian variation, both peaking at dark phase. Glucocorticoid receptors also exhibit circadian variation in placenta. As melatonin and glucocorticoids enter the placenta they both have an effect on the rhythmical expression of placental clock genes. Furthermore, dopamine was also shown to affect the clock in placenta, but in an opposite way compared to melatonin. Other hormones such as leptin, ghrelin and insulin that are also involved in pregnancy, display minor effects upon the placenta clock.

Furthermore, cytokines and neuropeptide kisspeptin, which are involved in important processes at the beginning of gestation, such as trophoblast invasion and adjustment of immunological reaction of the mother, all display circadian rhythmicity. In contrast, the *Vegf*, which is involved at the beginning of gestation, showed no circadian rhythm.

In summary, all data accumulated in this thesis provide an insight into the way of how the maternal circadian system changes during pregnancy. It includes changes in secretion of ACTH, thermoregulation and metabolism. These circadian changes likely result from the need for the mother to meet the demands of developing fetus. Signals emanating from the maternal body influence the developing fetus, together with gradually developing circadian clock.

Circadian rhythms of placenta functions represent a newly studied topic, as for that, further research on the subject may provide additional information that might change the prespective of the effects of placenta during gestation together with prevention of pregnancy complications in the future.

Literature

Abrahamson, E. E., Moore, R. Y. (2001). Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. *Brain Research, 916*(1-2), 172-191.

Adamson, S. L., Lu, Y., Whiteley, K. J., Holmyard, D., Hemberger, M., Pfarrer, C., & Cross, J. C. (2002). Interactions between Trophoblast Cells and the Maternal and Fetal Circulation in the Mouse Placenta. *Developmental Biology*, *250*(2), 358–373.

Akiyama, S., Ohta, H., Watanabe, S., Moriya, T., Hariu, A., Nakahata, N., Chisaka, H., Matsuda, T., Kimura, Y., Tsuchiya, S., Tei, H., Okamura, K., & Yaegashi, N. (2010). The uterus sustains stable biological clock during pregnancy. *The Tohoku Journal of Experimental Medicine*, *221*(4), 287–298.

Akoum, A., Metz, C. N., & Morin, M. (2005). Marked increase in macrophage migration inhibitory factor synthesis and secretion in human endometrial cells in response to human chorionic gonadotropin hormone. *The Journal of Clinical Endocrinology and Metabolism*, *90*(5), 2904–2910.

Athanassiades, A., Hamilton, G. S., & Lala, P. K. (1998). Vascular endothelial growth factor stimulates proliferation but not migration or invasiveness in human extravillous trophoblast. *Biology of Reproduction*, *59*(3), 643–654.

Atkinson, H. C., & Waddell, B. J. (1995). The Hypothalamic-Pituitary-Adrenal Axis in Rat Pregnancy and Lactation: Circadian Variation and Interrelationship of Plasma Adrenocorticotropin and Corticosterone. *Endocrinology, 136*(2), 512-520

Aton, S. J., & Herzog, E. D. (2005). Come together, right...now: synchronization of rhythms in a mammalian circadian clock. *Neuron*, *48*(4), 531–534.

Ayala, A. R., Bustos, H., & Ma, R. (1984). Daily rhythm of serum human chorionic gonadotropin and human chorionic somatomammotropin in normal pregnancy. *Int. J. Gynaecol. Obstet*,*22*(3), 173-176

Baker, H., Frank, O., Deangelis, B., Feingold, S., & Kaminetzky, H. A. (1981). Role of placenta in maternal-fetal vitamin transfer in humans. *American Journal of Obstetrics and Gynecology*, *141*(7), 792–796.

Balsalobre, A. (2002). Clock genes in mammalian peripheral tissues. *Cell and Tissue Research*, *309*(1), 193–199.

Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., Schutz, G., & Schibler, U. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science*, *289*(5488), 2344–2347.

Balsalobre, A., Damiola, F., & Schibler, U. (1998). A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell*, *93*(6), 929–937.

Bates, K., & Herzog, E. D. (2020). Maternal-Fetal Circadian Communication During Pregnancy. *Frontiers in Endocrinology*, *11*, 198.

Battaglia, F. C., & Regnault, T. R. H. (2001). Placental transport and metabolism of amino acids. *Placenta*, *22*(2–3), 145–161.

Bell, A. W., & Bauman, D. E. (1997). Adaptations of glucose metabolism during pregnancy and lactation. *Journal of mammary gland biology and neoplasia*, *2*(3), 265-278.

Beddington, R. S. P., & Robertson, E. J. (1999). Axis development and early asymmetry in mammals. *Cell*, *96*(2), 195–209.

Berndt, S., Blacher, S., D'Hauterive, S. P., Thiry, M., Tsampalas, M., Cruz, A., Pèqueux, C., Lorquet, S., Munaut, C., Noël, A., & Foidart, J. M. (2009). Chorionic gonadotropin stimulation of angiogenesis and pericyte recruitment. *The Journal of Clinical Endocrinology and Metabolism*, *94*(11), 4567–4574.

Berson, D. M., Dunn, F. A., & Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science (New York, N.Y.)*, *295*(5557), 1070–1073.

Bowen, J. M., Chamley, L., Mitchell, M. D., & Keelan, J. A. (2002). Cytokines of the Placenta and Extra-placental Membranes: Biosynthesis, Secretion and Roles in Establishment of Pregnancy in Women. *Placenta*, *23*(4), 239–256.

Brosens, I., Robertson, W. B., & Dixon, H. G. (1967). The physiological response of the vessels of the placental bed to normal pregnancy. *The Journal of Pathology and Bacteriology*, *93*(2), 569–579.

Brown, S. A., Zumbrunn, G., Fleury-Olela, F., Preitner, N., & Schibler, U. (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Current Biology*, *12*(18), 1574–1583.

Burton, G. J., Cindrova-Davies, T., Yung, H. W., & Jauniaux, E. (2021). Hypoxia and reproductive health: Oxygen and development of the human placenta. *Reproduction (Cambridge, England)*, *161*(1), F53–F65.

Burton, G. J., & Fowden, A. L. (2015). The placenta: A multifaceted, transient organ. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1663).

Burton, P. J., & Waddell, B. J. (1999). M i n i rev i ew Dual Function of 11-Hydroxysteroid Dehydrogenase in Placenta: Modulating Placental Glucocorticoid Passage and Local Steroid Action 1. *Biology of reproduction*, *60*, 234–240.

Carmona-Alcocer, V., Abel, J. H., Sun, T. C., Petzold, L. R., Doyle, F. J., Simms, C. L., & Herzog, E. D. (2018). Ontogeny of circadian rhythms and synchrony in the suprachiasmatic nucleus. *Journal of Neuroscience*, *38*(6), 1326–1334.

Carosella, E. D., Dausset, J., & Rouas-Freiss, N. (1999). Immunotolerant functions of HLA-G. *Cellular and Molecular Life Sciences CMLS 1999 55:3*, *55*(3), 327–333.

Čečmanová, V., Houdek, P., Šuchmanová, K., Sládek, M., & Sumová, A. (2019). Development and Entrainment of the Fetal Clock in the Suprachiasmatic Nuclei: The Role of Glucocorticoids. *Journal of Biological Rhythms*, *34*(3), 307–322.

Challis, J. R. G., Patrick, J. E., Campbell, K., Natale, R., & Richardson, B. (1980). Diurnal changes in maternal plasma oestrone and oestradiol at 30 to 31, 34 to 35 and 38 to 39 weeks gestational age. *British Journal of Obstetrics and Gynaecology*, *87*(11), 983–988.

Challisa, J. R. G., Spraguea, C., & Patrick, J. E. (1983). Relation between Diurnal Changes in Peripheral Plasma Progesterone, Cortisol, and Estriol in Normal Women at 30-31, 34-35, and 38-39 Weeks of Gestation. *British Journal of Obstetrics and Gynaecology*, *16*(1), 33-44

Chatuphonprasert, W., Jarukamjorn, K., & Ellinger, I. (2018). Physiology and pathophysiology of steroid biosynthesis, transport and metabolism in the human placenta. *Frontiers in Pharmacology*, *9*, 1027.

Cindrova-Davies, T., Yung, H. W., Johns, J., Spasic-Boskovic, O., Korolchuk, S., Jauniaux, E., Burton, G. J., & Charnock-Jones, D. S. (2007). Oxidative Stress, Gene Expression, and Protein Changes Induced in the Human Placenta during Labor. *The American Journal of Pathology*, *171*(4), 1168.

Cole, L. A. (2010). Biological functions of hCG and hCG-related molecules. *Reproductive Biology and Endocrinology 2010 8:1*, *8*(1), 1–14.

Cole, T. J., Blendy, J. A., Monaghan, A. P., Krieglstein, K., Schmid, W., Aguzzi, A., Fantuzzi, G., Hummler, E., Unsicker, K., & Schütz, G. (1995). Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. *Genes & Development*, *9*(13), 1608–1621.

Crew, R. C., Waddell, B. J., & Mark, P. J. (2018). Obesity-induced changes in hepatic and placental clock gene networks in rat pregnancy. *Biology of Reproduction*, *98*(1), 75–88.

Curtis, A. M., Seo, S. B., Westgate, E. J., Rudic, R. D., Smyth, E. M., Chakravarti, D., FitzGerald, G. A., & McNamara, P. (2004). Histone Acetyltransferase-dependent Chromatin Remodeling and the Vascular Clock. *Journal of Biological Chemistry*, *279*(8), 7091–7097.

Damiola, F., le Minli, N., Preitner, N., Kornmann, B., Fleury-Olela, F., & Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes and Development*, *14*(23), 2950–2961.

Darne, F. J., McGarrigle, H. H. G., & Lachelin, G. C. L. (1989). Diurnal variation of plasma and saliva oestrogen, progesterone, cortisol and plasma dehydroepiandrosterone sulphate in late pregnancy. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, *32*(2), 57–66.

Davis, F. C., Mannion, J., & MANNION Entrainment, J. (1988). Entrainment of hamster pup circadian rhythms by prenatal melatonin injections to the mother. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *255*(3), R439-R448.

de Clercq, K., & Vriens, J. (2018). Establishing life is a calcium-dependent TRiP: Transient receptor potential channels in reproduction. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1865*(11), 1815–1829.

de Pedro, M. A., Morán, J., Díaz, I., Murias, L., Fernández- Plaza, C., González, C., & Díaz, E. (2015). Circadian Kisspeptin expression in human term placenta. *Placenta*, *36*(11), 1337–1339.

Demarez, C., de Assis, L. V. M., Krohn, M., Ramella, N., Schwaninger, M., Oster, H., & Astiz, M. (2021). The trophoblast clock controls transport across placenta in mice. *Development (Cambridge)*, *148*(8).

Díaz-Cuetot, L., Méndez, J. P., Barrios-De-Tomasi, J., Lee, J.-Y., Wide, L., Veldhuis, J. D., Ulloa-Aguirre, A. (1994). Amplitude regulation of episodic release, in vitro biological to immunological ratio, and median charge of human chorionic gonadotropin in pregnancy. *The Journal of Clinical Endocrinology & Metabolism*, *78*(4), 890-897.

Di, W. L., Lachelin, G. C. L., McGarrigle, H. H. G., Thomas, N. S. B., & Becker, D. L. (2001). Oestriol and oestradiol increase cell to cell communication and connexin43 protein expression in human myometrium. *Molecular Human Reproduction*, *7*(7), 671–679.

Dibner, C., Schibler, U., & Albrecht, U. (2009). The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. In *Annual Review of Physiology* (Vol. 72, pp. 517–549).

Dolatshad, H., Cary, A. J., & Davis, F. C. (2010). Differential Expression of the Circadian Clock in Maternal and Embryonic Tissues of Mice. *PLoS ONE*, *5*(3).

Downs, K. M., Inman, K. E., Jin, D. X., & Enders, A. C. (2009). The Allantoic Core Domain: New Insights Into Development of the Murine Allantois and Its Relation to the Primitive Streak. *Developmental Dynamics : An Official Publication of the American Association of Anatomists*, *238*(3), 532.

Duttaroy, A. K. (2009). Transport of fatty acids across the human placenta: A review. *Progress in Lipid Research*, *48*(1), 52–61.

Ejaz, H., Figaro, J. K., Woolner, A. M. F., Thottakam, B. M. V., & Galley, H. F. (2021). Maternal Serum Melatonin Increases During Pregnancy and Falls Immediately After Delivery Implicating the Placenta as a Major Source of Melatonin. *Frontiers in Endocrinology*, *11*.

Enders, A. C. (1965). A comparative study of the fine structure of the trophoblast in several hemochorial placentas. *American Journal of Anatomy*, *116*(1), 29–67.

Enders, A. C., & Schlafke, S. (1967). A morphological analysis of the early implantation stages in the rat. *American Journal of Anatomy*, *120*(2), 185-225.

Eta, E., Ambruss, G., & Rao, C. v. (1994). Direct regulation of human myometrial contractions by human chorionic gonadotropin. *The Journal of Clinical Endocrinology and Metabolism*, *79*(6), 1582–1586.

Fewell, J. E. (1995). Body temperature regulation in rats near term of pregnancy. *Canadian Journal of Physiology and Pharmacology*, *73*(3), 364–368.

Fomin, V. P., Cox, B. E., & Ann Word, R. (1999). Effect of progesterone on intracellular Ca2+ homeostasis in human myometrial smooth muscle cells. *American Journal of Physiology - Cell Physiology*, *276*(2 45-2).

Fortier, E. E., Rooney, J., Dardente, H., Hardy, M.-P., Labrecque, N., & Cermakian, N. (2011). Circadian variation of the response of T cells to antigen. *Journal of Immunology (Baltimore, Md. : 1950)*, *187*(12), 6291–6300.

Fowden, A. L., Li, J., & Forhead, A. J. (1998). Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proceedings of the Nutrition Society*, *57*(01), 113–122.

Frigato, E., Lunghi, L., Ferretti, M. E., Biondi, C., & Bertolucci, C. (2009). Evidence for circadian rhythms in human trophoblast cell line that persist in hypoxia. *Biochemical and Biophysical Research Communications*, *378*(1), 108–111.

Gachon, F., Nagoshi, E., Brown, S. A., Ripperger, J., & Schibler, U. (2004). The mammalian circadian timing sytem: From gene expression to physiology. *Chromosoma*, *113*(3), 103–112.

Gekakis, N., Staknis, D., Nguyen, H. B., Davis, F. C., Wilsbacner, L. D., King, D. P., Takahashi, J. S., & Weitz, C. J. (1998). Role of the CLOCK protein in the mammalian circadian mechanism. *Science*, *280*(5369), 1564–1569.

Georgiades, P., Fergyson-Smith, A. C., & Burton, G. J. (2002). Comparative developmental anatomy of the murine and human definitive placentae. *Placenta*, *23*(1), 3–19.

Gillmer, M. D. G., M. D. G., Beard, R. W., Oakley, N. W., Brooke, F. M., Brudenell, M., & Chard, T. (1977). Plasma human placental lactogen profiles over 24 hours in normal and diabetic pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology*, *84*(3), 197-204

Giussani, D. A., Jenkins, S. L., Winter, J. A., Tame, J. D., & Nathanielsz, P. W. (2000). Androstenedione treatment of pregnant baboons at 0.7-0.8 of gestation promotes a premature forward shift in the nocturnal maternal plasma estradiol surge relative to progesterone and increases myometrial contraction activity. *Endocrinology*, *141*(9), 3296–3303.

Groothuis, P. G., Dassen, H. H. N. M., Romano, A., & Punyadeera, C. (2007). Estrogen and the endometrium: lessons learned from gene expression profiling in rodents and human. *Human Reproduction Update*, *13*(4), 405–417.

Grumbach, M. M., Kaplan, S. L., Sciarra, J. J., & Burr, I. M. (1968). Chorionic growth hormone-prolactin (CGP): secretion, disposition, biologic activity in man, and postulated function as the "growth hormone" of the 2d half of pregnancy. *Annals of the New York Academy of Sciences*, *148*(2), 501–531.

Halasz, M., & Szekeres-Bartho, J. (2013). The role of progesterone in implantation and trophoblast invasion. *Journal of Reproductive Immunology*, *97*(1), 43–50.

Handwerger, S., & Freemark, M. (2000). The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. *Journal of Pediatric Endocrinology & Metabolism : JPEM*, *13*(4), 343–356.

Hannibal, J. (2002). Neurotransmitters of the retino-hypothalamic tract. In *Cell and Tissue Research* (Vol. 309, Issue 1, pp. 73–88).

Hartgill, T. W., Bergersen, T. K., & Pirhonen, J. (2011). Core body temperature and the thermoneutral zone: A longitudinal study of normal human pregnancy. *Acta Physiologica*, *201*(4), 467–474

Hastings, M. H., Ebling, F. J., Grosse, J., Herbert, J., Maywood, E. S., Mikkelsen, J. D., & Sumova, A. (1995). Immediate-Early Genes and the Neural Bases of Photic and Non-Photic Entrainment. *Ciba Foundation Symposium*, *183*, 175–197.

Hattar, S., Liao, H. W., Takao, M., Berson, D. M., & Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science (New York, N.Y.)*, *295*(5557), 1065–1070.

Hemberger, M., Hanna, C. W., & Dean, W. (2020). Mechanisms of early placental development in mouse and humans. *Nature Reviews Genetics*, *21*(1), 27-43

Hiden, U., Bilban, M., Knöfler, M., & Desoye, G. (2007). Kisspeptins and the placenta: regulation of trophoblast invasion. *Reviews in Endocrine & Metabolic Disorders*, *8*(1), 31–39.

Holmes, C. H., & Simpson, K. L. (1992). Complement and pregnancy: new insights into the immunobiology of the fetomaternal relationship. *Bailliere's Clinical Obstetrics and Gynaecology*, *6*(3), 439–460.

Hoshina, M., Boothby, M., & Boime, I. (1982). Cytological localization of chorionic gonadotropin alpha and placental lactogen mRNAs during development of the human placenta. *The Journal of Cell Biology*, *93*(1), 190–198.

Hosoda, H., Asano, H., Ito, M., Kato, H., Iwamoto, T., Suzuki, A., ... & Kida, S. (2009). CBP/p300 is a cell type-specific modulator of CLOCK/BMAL1-mediated transcription. *Molecular brain*, *2*(1), 1-18.

Houdek, P., Polidarová, L., Nováková, M., Matějů, K., Kubík, Š., & Sumová, A. (2015a). Melatonin administered during the fetal stage affects circadian clock in the suprachiasmatic nucleus but not in the liver. *Developmental Neurobiology*, *75*(2), 131–144.

Houdek, P., & Sumová, A. (2014). In vivo initiation of clock gene expression rhythmicity in fetal rat suprachiasmatic nuclei. *PLoS ONE*, *9*(9).

HOUGHTON, D. J., NEWNHAM, J. P., LO, K., RICE, A., & CHARD, T. (1982). Circadian variation of circulating levels of four placental proteins. *British Journal of Obstetrics and Gynaecology*, *89*(10), 831–835.

Illsley, N. P., & Illsley, N. P. (2000). Glucose transporters in the human placenta The epithelial-mesenchymal transition in the differentiation of human cyto-to extravillous trophoblast View project Trophoblast invasion in Abnormally Invasive Placenta (AIP) View project CURRENT TOPIC Glucose Transporters in the Human Placenta. *Placenta*, *21*, 14–22

Ishida, A., Mutoh, T., Ueyama, T., Bando, H., Masubuchi, S., Nakahara, D., Tsujimoto, G., & Okamura, H. (2005). Light activates the adrenal gland: Timing of gene expression and glucocorticoid release. *Cell Metabolism*, *2*(5), 297–307.

Iwakura, Y. (1989). Mechanism of Blastocyst Formation of the Mouse Embryo. *Development, Growth & Differentiation*, *31*(6), 523–529.

Iwasaki, S., Nakazawa, K., Sakai, J., Kometani, K., Iwashita, M., Yoshimura, Y., & Maruyama, T. (2005). Melatonin as a local regulator of human placental function. *Journal of Pineal Research*, *39*(3), 261–265

Jameson, J. L., & Hollenberg, A. N. (1993). Regulation of chorionic gonadotropin gene expression. *Endocrine Reviews*, *14*(2), 203-221

Janneau, J. L., Maldonado-Estrada, J., Tachdjian, G., Miran, I., Motté, N., Saulnier, P., Sabourin, J. C., Coté, J. F., Simon, B., Frydman, R., Chaouat, G., & Bellet, D. (2002). Transcriptional expression of genes involved in cell invasion and migration by normal and tumoral trophoblast cells. *The Journal of Clinical Endocrinology and Metabolism*, *87*(11), 5336–5339.

Junkermann , Mangold, H., Vecsei, P., & Runnebaum, B. (1982). Circadian rhythm of serum progesterone levels in human pregnancy and its relation to the rhythm of cortisol. *European Journal of Endocrinology*, *101*(1), 98-104.

Kalkhoff, R. K. (1982). Metabolic effects of progesterone. *American Journal of Obstetrics and Gynecology*, *142*(6 Pt 2), 735–738.

Kenneth, N. S., & Rocha, S. (2008). Regulation of gene expression by hypoxia. *The Biochemical Journal*, *414*(1), 19–29.

Knöfler, M., Haider, S., Saleh, L., Pollheimer, J., Gamage, T. K. J. B., & James, J. (2019). Human placenta and trophoblast development: key molecular mechanisms and model systems. *Cellular and Molecular Life Sciences*, *76*(18), 3479–3496.

Knopp, R. H. (1997). Hormone-mediated Changes in Nutrient Metabolism in Pregnancy: A Physiological Basis for Normal Fetal Developmenta. *Annals of the New York Academy of Sciences*, *817*(1), 251–271.

Koyanagi, S., Kuramoto, Y., Nakagawa, H., Aramaki, H., Ohdo, S., Soeda, S., & Shimeno, H. (2003). A molecular mechanism regulating circadian expression of vascular endothelial growth factor in tumor cells. *Cancer research*, *63*(21), 7277-7283.

Kume, K., Zylka, M. J., Sriram, S., Shearman, L. P., Weaver, D. R., Jin, X., Maywood, E. S., Hastings, M. H., & Reppert, S. M. (1999). mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. *Cell*, *98*(2), 193–205.

Lanoix, D., Beghdadi, H., Lafond, J., & Vaillancourt, C. (2008). Human placental trophoblasts synthesize melatonin and express its receptors. *Journal of Pineal Research*, *45*(1), 50–60.

Laškarin, G., Tokmadži, V. S., Štrbo, N., Bogovi, T., Szekeres-Bartho, J., Randi, L., Podack, E. R., & Rukavina, D. (2002). Progesterone induced blocking factor (PIBF) mediates progesterone induced suppression of decidual lymphocyte cytotoxicity. *American Journal of Reproductive Immunology (New York, N.Y. : 1989)*, *48*(4), 201–209.

Lee, C., Etchegaray, J. P., Cagampang, F. R. A., Loudon, A. S. I., & Reppert, S. M. (2001). Posttranslational mechanisms regulate the mammalian circadian clock. *Cell*, *107*(7), 855–867.

Lee, C. K., Moon, D. H., Shin, C. S., Kim, H., Yoon, Y. D., Kang, H. S., Lee, B. J., & Kang, S. G. (2003). Circadian expression of Mel1a and PL-II genes in placenta: effects of melatonin on the PL-II gene expression in the rat placenta. *Molecular and Cellular Endocrinology*, *200*(1–2), 57–66.

Lindberg, B. S., & Nilsson, B. A. (1973). Variations in maternal plasma levels of human placental lactogen (HPL) in normal pregnancy and labour. *BJOG: An International Journal of Obstetrics & Gynaecology*, *80*(7), 619-626.

Loriaux, D. L., Ruder, H. J., Knab, D. R., & Lipsett, M. B. (1972). Estrone Sulfate, Estrone, Estradiol and Estriol Plasma Levels in Human Pregnancy. *The Journal of Clinical Endocrinology & Metabolism*, *35*(6), 887–891.

Lužná, V., Liška, K., Sládek, M., & Sumová, A. (2021). Hormonal fine-tuning of clock in decidual region of mouse placenta by dopamine, melatonin, insulin, leptin and ghrelin. *Placenta*, *108*, 55–63

Magiakou, M. A., Mastorakos, G., Rabin, D., Margioris, A. N., Dubbert, B., Calogero, A. E., Tsigos, C., Munson, P. J., & Chrousos, G. P. (1996). The maternal hypothalamic-pituitary-adrenal axis in the third trimester of human pregnancy. *Clinical Endocrinology*, *44*(4), 419–428

Malassiné, A., Frendo, J. L., & Evain-Brion, D. (2003). A comparison of placental development and endocrine functions between the human and mouse model. *Human Reproduction Update*, *9*(6), 531–539.

Mariona, F. G. (2016). Perspectives in obesity and pregnancy. *Women's Health*, *12*(6), 523–532.

Mark, P. J., Augustus, S., Lewis, J. L., Hewitt, D. P., & Waddell, B. J. (2009). Changes in the Placental Glucocorticoid Barrier During Rat Pregnancy: Impact on Placental Corticosterone Levels and Regulation by Progesterone. *Biology of Reproduction*, *80*(6), 1209.

Markoff, E., & Talamantes, F. (1981). Serum placental lactogen in mice in relation to day of gestation and number of conceptuses. *Biology of Reproduction*, *24*(4), 846–851.

Mastorakos, G., & Ilias, I. (2003). Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Annals of the New York Academy of Sciences*, *997*, 136–149

Mastorakos, G., Scopa, C. D., Kao, L. C., Vryonidou, A., Friedman, T. C., Kattis, D., Phenekos, C., Rabin, D., & Chrousos, G. P. (1996). Presence of Immunoreactive

Corticotropin-Releasing Hormone in Human Endometrium. *Journal of Clinical Endocrinology and Metabolism Copyright*, *0*(3).

McCarthy, R., Jungheim, E. S., Fay, J. C., Bates, K., Herzog, E. D., & England, S. K. (2019). Riding the Rhythm of Melatonin Through Pregnancy to Deliver on Time. *Frontiers in Endocrinology*, *10*.

McTernan, C. L., Draper, N., Nicholson, H., Chalder, S. M., Driver, P., Hewison, M., ... & Stewart, P. M. (2001). Reduced placental 11β-hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: an analysis of possible mechanisms. *The Journal of Clinical Endocrinology & Metabolism*, *86*(10), 4979-4983.

.

Mendez, N., Abarzua-Catalan, L., Vilches, N., Galdames, H. A., Spichiger, C., Richter, H. G., Valenzuela, G. J., Seron-Ferre, M., & Torres-Farfan, C. (2012). Timed maternal melatonin treatment reverses circadian disruption of the fetal adrenal clock imposed by exposure to constant light. *PLoS ONE*, *7*(8).

Mitsui, S., Yamaguchi, S., Matsuo, T., Ishida, Y., & Okamura, H. (2001). Antagonistic role of E4BP4 and PAR proteins in the circadian oscillatory mechanism. *Genes and Development*, *15*(8), 995–1006.

Moore, R. Y., & Eichler, V. B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain research*.

Moore, R. Y., & Bernstein, M. E. (1989). Synaptogenesis in the rat suprachiasmatic nucleus demonstrated by electron microscopy and synapsin I immunoreactivity. *The Journal of Neuroscience*, *9*(6), 2151.

Moore, R. Y., Speh, J. C., & Patrick Card, J. (1995). The retinohypothalamic tract originates from a distinct subset of retinal ganglion cells. *Journal of comparative neurology*, *352*(3), 351-366.

Mor, G., Cardenas, I., Abrahams, V., & Guller, S. (2011). Inflammation and pregnancy: the role of the immune system at the implantation site. *Annals of the New York Academy of Sciences*, *1221*(1), 80

Myatt, L., & Cui, X. (2004). Oxidative stress in the placenta. *Histochemistry and Cell Biology*, *122*(4), 369–382.

Nagoshi, E., Saini, C., Bauer, C., Laroche, T., Naef, F., & Schibler, U. (2004). Circadian gene expression in individual fibroblasts: Cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell*, *119*(5), 693–705.

Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M., & Sassone-Corsi, P. (2009a). Circadian Control of the NAD+ Salvage Pathway by CLOCK-SIRT1. *Science*, *324*(5927), 654–657.

Nakajimaf, S. T., Mcauliffe, T., & Gibson, M. (1990). The 24-hour pattern of the levels of serum progesterone and immunoreactive human chorionic gonadotropin in normal early pregnancy. *The Journal of Clinical Endocrinology & Metabolism*, *71*(2), 345-353.

Nakamura, Y., Tamura, H., Kashida, S., Takayama, H., Yamagata, Y., Karube, A., Sugino, N., & Kato, H. (2001). Changes of serum melatonin level and its relationship to feto-placental unit during pregnancy. *Journal of Pineal Research*, *30*(1), 29–33.

Naruse, K., Innes, B. A., Bulmer, J. N., Robson, S. C., Searle, R. F., & Lash, G. E. (2010). Secretion of cytokines by villous cytotrophoblast and extravillous trophoblast in the first trimester of human pregnancy. *Journal of Reproductive Immunology*, *86*(2), 148–150.

Nováková, M., Sládek, M., & Sumová, A. (2010). Exposure of pregnant rats to restricted feeding schedule synchronizes the SCN clocks of their fetuses under constant light but not under a light-dark regime. *Journal of Biological Rhythms*, *25*(5), 350–360.

Ohno, T., Onishi, Y., & Ishida, N. (2007). A novel E4BP4 element drives circadian expression of mPeriod2. *Nucleic Acids Research*, *35*(2), 648–655.

Oishi, K., Sakamoto, K., Okada, T., Nagase, T., & Ishida, N. (1998). Antiphase Circadian Expression betweenBMAL1andperiodHomologue mRNA in the Suprachiasmatic Nucleus and Peripheral Tissues of Rats. *Biochemical and Biophysical Research Communications*, *253*(2), 199–203.

Olejníková, L., Polidarová, L., Paušlyová, L., Sládek, M., & Sumová, A. (2015). Diverse development and higher sensitivity of the circadian clocks to changes in maternal-feeding regime in a rat model of cardio-metabolic disease. *Chronobiology International*, *32*(4), 531–547.

Okatani, Y., Okamoto, K., Hayashi, K., Wakatsuki, A., Tamura, S., & Sagara, Y. (1998). Maternal-fetal transfer of melatonin in pregnant women near term. *Journal of pineal research*, *25*(3), 129-134.

Owens Kenneth J Ryan, D. M., & Tulchinsky, D. (1981). Episodic secretion of human chorionic gonadotropin in early pregnancy. *The Journal of Clinical Endocrinology & Metabolism*, *53*(6),

Pando, M. P., Morse, D., Cermakian, N., & Sassone-Corsi, P. (2002). Phenotypic rescue of a peripheral clock genetic defect via SCN hierarchical dominance. *Cell*, *110*(1), 107–117.

Pang, W. W., & Hartmann, P. E. (2007). Initiation of human lactation: secretory differentiation and secretory activation. *Journal of Mammary Gland Biology and Neoplasia*, *12*(4), 211–221.

Papacleovoulou, G., Nikolova, V., Oduwole, O., Chambers, J., Vazquez-Lopez, M., Jansen, E., Nicolaides, K., Parker, M., & Williamson, C. (2017). Gestational disruptions in metabolic rhythmicity of the liver, muscle, and placenta affect fetal size. *FASEB Journal*, *31*(4), 1698–1708.

Paria, B. C., Huet-Hudsont, Y. M., & Dey, S. K. (1993). Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. *Proc. Natl. Acad. Sci. USA*, *90*, 10159–10162.

Patrick, J., Challis, J., Campbell, K., Carmichael, L., Natale, R., & Richardson, B. (1980). Circadian rhythms in maternal plasma cortisol and estriol concentrations at 30 to 31, 34 to 35, and 38 to 39 weeks' gestational age. *American Journal of Obstetrics and Gynecology*, *136*(3), 325–334.

Peleg, D., Munsick, R. A., Diker, D., Goldman, J. A., & Ben-Jonathan, N. (1986). Distribution of Catecholamines between Fetal and Maternal Compartments During Human Pregnancy with Emphasis on L-Dopa and Dopamine. *The Journal of Clinical Endocrinology & Metabolism*, *62*(5), 911–914.

Pereira, P. N., Dobreva, M. P., Graham, L., Huylebroeck, D., Lawson, K. A., & Zwijsen, A. (2011). Amnion formation in the mouse embryo: The single amniochorionic fold model. *BMC Developmental Biology*, *11*(1), 1–13

Pérez, S., Murias, L., Fernández-Plaza, C., Díaz, I., González, C., Otero, J., & Díaz, E. (2015). Evidence for clock genes circadian rhythms in human full-term placenta. *Systems Biology in Reproductive Medicine*, *61*(6), 360–366.

Pezük, P., Mohawk, J. A., Wang, L. A., & Menaker, M. (2012a). Glucocorticoids as Entraining Signals for Peripheral Circadian Oscillators. *Endocrinology*, *153*(10), 4775.

Pickard, G. E. (1985). Bifurcating axons of retinal ganglion cells terminate in the hypothalamic suppachiasmatic nucleus and the intergeniculate leaflet of the thalamus. *Neuroscience letters*55.2 (1985): 211-217.

Pickard, G. E., Weber, E. T., Scott, P. A., Riberdy, A. F., & Rea, M. A. (1996). 5HT1B Receptor Agonists Inhibit Light-Induced Phase Shifts of Behavioral Circadian Rhythms and Expression of the Immediate–Early Gene c-fos in the Suprachiasmatic Nucleus. *The Journal of Neuroscience*, *16*(24), 8208.

Pijnenborg, R. (1996). The placental bed. *Hypertension in Pregnancy*, *15*(1), 7–23.

Pittendrigh, C. S., & Daan, S. (1976). A functional Analysis of circadian pacemakers in nocturnal Rodents. *Journal od comparative Physiol*ogy, *106*, 333-355

Preitner, N., Damiola, F., Luis-Lopez-Molina, Zakany, J., Duboule, D., Albrecht, U., & Schibler, U. (2002). The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell*, *110*(2), 251–260.

Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F., & Rollag, M. D. (2000). A novel human opsin in the inner retina. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(2), 600–605.

Rai, A., & Cross, J. C. (2014). Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. *Developmental Biology*, *387*(2), 131–141.

Ratajczak, C. K., Herzog, E. D., & Muglia, L. J. (2010). Clock Gene Expression in Gravid Uterus and Extra-Embryonic Tissues During Late Gestation in the Mouse. *Reproduction, Fertility, and Development*, *22*(5), 743–750.

Refinetti, R., & Menaker, M. (1992). The circadian rhythm of body temperature. *Physiology & Behavior*, *51*(3), 613–637.

Reiter, R. J. (1993). The melatonin rhythm: both a clock and a calendar. *Experientia*, *49*(8), 654–664.

Reppert, S. M., & Schwartz, W. J. (1986). Maternal suprachiasmatic nuclei are necessary for maternal coordination of the developing circadian system. *Journal of Neuroscience*, *6*(9), 2724–2729.

Richards, J., & Gumz, M. L. (2012). Advances in understanding the peripheral circadian clocks. *The FASEB Journal*, *26*(9), 3602–3613.

Rossant, J., & Cross, J. C. (2001). Placental development: Lessons from mouse mutants. *Nature Reviews Genetics 2001 2:7*, *2*(7), 538–548.

Rowland, D. L., Wagonblast, A. L., & Dykstra, T. A. (1991). Timing of parturition in the rat: an analysis of successive births. *Chronobiologia*, *18*(1), 31–38.

Ruoff, P. (1992). Introducing temperature-compensation in any reaction kinetic oscillator model. *Journal of Interdisiplinary Cycle Research*, *23*(2), 92–99.

Rutter, J., Reick, M., Wu, L. C., & McKnight, S. L. (2001). Regulation of crock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science*, *293*(5529), 510–514.

Saji, F., Samejima, Y., Kamiura, S., & Koyama, M. (1999). Dynamics of immunoglobulins at the feto-maternal interface. *Reviews of reproduction*, *4*, 81-89.

Salvante, K. G., Milano, K., Kliman, H. J., & Nepomnaschy, P. A. (2017). Placental 11 β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) expression very early during human pregnancy. *Journal of Developmental Origins of Health and Disease*, *8*(2), 149-154.

Samaan, N., Yen, S. C., Friesen, H., & Pearson, O. H. (1966). Serum placental lactogen levels during pregnancy and in trophoblastic disease. *The Journal of Clinical Endocrinology and Metabolism*, *26*(12), 1303–1308.

Sato, T. K., Panda, S., Miraglia, L. J., Reyes, T. M., Rudic, R. D., McNamara, P., Naik, K. A., Fitzgerald, G. A., Kay, S. A., & Hogenesch, J. B. (2004). A functional genomics strategy reveals rora as a component of the mammalian circadian clock. *Neuron*, *43*(4), 527–537.

Sato, T. K., Yamada, R. G., Ukai, H., Baggs, J. E., Miraglia, L. J., Kobayashi, T. J., Welsh, D. K., Kay, S. A., Ueda, H. R., & Hogenesch, J. B. (2006). Feedback repression is required for mammalian circadian clock function. *Nature Genetics*, *38*(3), 312.

Seron-Ferre, M., Ducsay, C. A., & Valenzuela, G. J. (1993). Circadian rhythms during pregnancy. *Endocrine Reviews*, *14*(5), 594-609.

Shearman, L. P., Zylka, M. J., Weaver, D. R., Kolakowski, L. F., & Reppert, S. M. (1997). Two period homologs: Circadian expression and photic regulation in the suprachiasmatic nuclei. *Neuron*, *19*(6), 1261–1269.

Shi, Q. J., Lei, Z. M., Rao, C. v., & Lin, J. (1993). Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. *Endocrinology*, *132*(3), 1387–1395.

Shibata, S., & Moore, R. Y. (1988). Development of a fetal circadian rhythm after disruption of the maternal circadian system. *Developmental Brain Research*, *41*(1–2), 313–317.

Silver, A. C., Arjona, A., Hughes, M. E., Nitabach, M. N., & Fikrig, E. (2012). Circadian expression of clock genes in mouse macrophages, dendritic cells, and B cells. *Brain, Behavior, and Immunity*, *26*(3), 407–413.

Simmons, D. G., & Cross, J. C. (2005). Determinants of trophoblast lineage and cell subtype specification in the mouse placenta. *Developmental Biology*, *284*(1), 12–24.

Simrose, R. L., & Fewell, J. E. (1995). Body temperature response to IL-1 beta in pregnant rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *269*(5), R1179-R1182.

Sládek, M., Sumová, A., Kováčiková, Z., Bendová, Z., Laurinová, K., & Illnerová, H. (2004). Insight into molecular core clock mechanism of embryonic and early postnatal rat suprachiasmatic nucleus. *Proceedings of the National Academy of Sciences*, *101*(16), 6231-6236.

Soares, M. J., Chakraborty, D., Karim Rumi, M. A., Konno, T., & Renaud, S. J. (2012). Rat placentation: An experimental model for investigating the hemochorial maternal-fetal interface. *Placenta*, *33*(4), 233–243.

Soares, M. J., Iqbal, K., & Kozai, K. (2017). Hypoxia and Placental Development. *Birth Defects Research*, *109*(17), 1309–1329.

Soares, M. J., Miiller, H., Orwig, K. E., Peters, T. J., & Dai, G. (1998). The Uteroplacental Prolactin Family and Pregnancy'. *Biology of reproduction*, *58*, 273–284.

Soares, M. J., Varberg, K. M., & Iqbal, K. (2018). Hemochorial placentation: development, function, and adaptations. *Biology of reproduction*, *99*(1), 196-211.

St-pierre, M. V., Serrano, M. A., Macias, R. I. R., Dubs, U., Hoechli, M., Lauper, U., ... & Marin, J. J. G. (2000). Expression of members of the multidrug resistance protein family in human term placenta. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *279*(4), R1495-R1503

Stratmann, M., & Schibler, U. (2006). Properties, entrainment, and physiological functions of mammalian peripheral oscillators. *Journal of Biological Rhythms*, *21*(6), 494–506.

Strauss, J. F., Martinez, F., & Kiriakidou, M. (1996). Placental Steroid Hormone Synthesis: Unique Features and Unanswered Questions'. *Biology of reproduction*, *54*, 303–311.

Sumova, A., Sladek, M., Polidarova, L., Novakova, M., & Houdek, P. (2012). Circadian system from conception till adulthood. *Progress in Brain Research*, *199*, 83–103.

Sun, M., Kingdom, J., Baczyk, D., Lye, S. J., Matthews, S. G., & Gibb, W. (2006). Expression of the Multidrug Resistance P-Glycoprotein, (ABCB1 glycoprotein) in the Human Placenta Decreases with Advancing Gestation. *Placenta*, *27*(6–7), 602–609.

Takahashi, J. S. (2017). Transcriptional architecture of the mammalian circadian clock. *Nature Reviews Genetics*, *18*(3), 164–179.

Takayama, H., Nakamura, Y., Tamura, H., Yamagata, Y., Harada, A., Nakata, M., Sugino, N., & Kato, H. (2003). Pineal gland (melatonin) affects the parturition time, but not luteal function and fetal growth, in pregnant rats. *Endocrine Journal*, *50*(1), 37–43.

Tan, D. X., Manchester, L. C., Esteban-Zubero, E., Zhou, Z., & Reiter, R. J. (2015). Melatonin as a Potent and Inducible Endogenous Antioxidant: Synthesis and Metabolism. *Molecules (Basel, Switzerland)*, *20*(10), 18886–18906.

Teoh, E. S., Spellacy, W. N., & Buhi, W. C. (1971). Human chorionic somatomammotrophin (hcs): a new index of placental function. *BJOG: An International Journal of Obstetrics & Gynaecology*, *78*(8), 673–685.

Torres-Farfan, C., Mendez, N., Abarzua-Catalan, L., Vilches, N., Valenzuela, G. J., & Seron-Ferre, M. (2011). A circadian clock entrained by melatonin is ticking in the rat fetal adrenal. *Endocrinology*, *152*(5), 1891-1900.

Torres-Farfan, C., Rocco, V., Monsó, C., Valenzuela, F. J., Campino, C., Germain, A., Torrealba, F., Valenzuela, G. J., & Seron-Ferre, M. (2006). Maternal melatonin effects on clock gene expression in a nonhuman primate fetus. *Endocrinology*, *147*(10), 4618–4626.

Tuckey, R. C. (2005). Progesterone synthesis by the human placenta. *Placenta*, *26*(4), 273–281.

Uno, H., Lohmiller, L., Thieme, C., Kemnitz, J. W., Engle, M. J., Roecker, E. B., & Farrell, P. M. (1990). Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. I. Hippocampus. *Brain Research. Developmental Brain Research*, *53*(2), 157–167.

Waddell, B. J., Wharfe, M. D., Crew, R. C., & Mark, P. J. (2012). A rhythmic placenta? Circadian variation, clock genes and placental function. *Placenta*, *33*(7), 533–539.

Walker, W. H., Fitzpatrick, S. L., Saunders, G. F., Barrera-Saldaña, H. A., & Resendez-Perez, D. (1991). The Human Placental Lactogen Genes: Structure, Function, Evolution and Transcriptional Regulation. *Endocrine Reviews*, *12*(4), 316–328.

Walsh, S. W., Ducsay, C. A., & Novy, M. J. (1984). Circadian hormonal interactions among the mother, fetus, and amniotic fluid. *American Journal of Obstetrics and Gynecology*, *150*(6), 745–753.

Watts, A. G., Swanson, L. W. (1987). Efferent Projections of the Suprachiasmatic Nucleus: I. Studies Using Anterograde Transport of Phaseolus vulgaris leucoagglutinin in the rat. *Journal of comparative neurology*, *258*(2), 204-229

Weaver, D. R., & Reppert, S. M. (1989). Periodic feeding of SCN-lesioned pregnant rats entrains the fetal biological clock. *Brain Research. Developmental Brain Research*, *46*(2), 291–295.

Weaver, D. R., Rivkees, S. A., & Reppert, S. M. (1992). D1-dopamine receptors activate c-fos expression in the fetal suprachiasmatic nuclei. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(19), 9201–9204.

Weetman, A. P. (1999). The immunology of pregnancy. *Thyroid : Official Journal of the American Thyroid Association*, *9*(7), 643–646.

Welsh, D. K., Logothetis, D. E., Meister, M., & Reppert, S. M. (1995). Individual Neurons Dissociated from Rat Suprachiasmatic Nucleus Express Independently Phased Circadian Firing Rhythms. *Neuron*, *14*, 697–706.

Wharfe, M. D., Mark, P. J., & Waddell, B. J. (2011). Circadian variation in placental and hepatic clock genes in rat pregnancy. *Endocrinology*, *152*(9), 3552–3560.

Wharfe, M. D., Mark, P. J., Wyrwoll, C. S., Smith, J. T., Yap, C., Clarke, M. W., & Waddell, B. J. (2016). Pregnancy-induced adaptations of the central circadian clock and maternal glucocorticoids. *Journal of Endocrinology*, *228*(3), 135–147.

Wharfe, M. D., Wyrwoll, C. S., Waddell, B. J., & Mark, P. J. (2016a). Pregnancy suppresses the daily rhythmicity of core body temperature and adipose metabolic gene expression in the mouse. *Endocrinology*, *157*(9), 3320–3331.

Wharfe, M. D., Wyrwoll, C. S., Waddell, B. J., & Mark, P. J. (2016b). Pregnancy-induced changes in the circadian expression of hepatic clock genes: Implications for maternal glucose homeostasis. *American Journal of Physiology - Endocrinology and Metabolism*, *311*(3), E575–E586.

Wood, G. W. (1994). Is restricted antigen presentation the explanation for fetal allograft survival? *Immunology Today*, *15*(1), 15–18.

Yamada, A., Arakaki, R., Saito, M., Kudo, Y., & Ishimaru, N. (2017). Dual Role of Fas/FasL-Mediated Signal in Peripheral Immune Tolerance. *Frontiers in Immunology*, *8*(APR), 403.

Yang, K. (1997). Placental 11β-hydroxysteroid dehydrogenase: Barrier to maternal glucocorticoids. *Reviews of Reproduction*, *2*(3), 129–132.

Yaw, A. M., Duong, T. v., Nguyen, D., & Hoffmann, H. M. (2021). Circadian rhythms in the mouse reproductive axis during the estrous cycle and pregnancy. *Journal of Neuroscience Research*, *99*(1), 294–308.

Yoo, S. H., Mohawk, J. A., Siepka, S. M., Shan, Y., Huh, S. K., Hong, H. K., Kornblum, I., Kumar, V., Koike, N., Xu, M., Nussbaum, J., Liu, X., Chen, Z., Chen, Z. J., Green, C. B., & Takahashi, J. S. (2013). Competing E3 Ubiquitin Ligases Determine Circadian Period by Regulated Degradation of CRY in Nucleus and Cytoplasm. *Cell*, *152*(5), 1091.

Yoo, S. H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepka, S. M., Hong, H. K., Oh, W. J., Yoo, O. J., Menaker, M., & Takahashi, J. S. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(15), 5339–5346.

Zhang, Y., Fang, B., Emmett, M. J., Damle, M., Sun, Z., Feng, D., Armour, S. M., Remsberg, J. R., Jager, J., Soccio, R. E., Steger, D. J., & Lazar, M. A. (2015). Discrete functions of nuclear receptor Rev-erbα couple metabolism to the clock. *Science*, *348*(6242), 1488–1492.

Zulu, P., & di Nisio, Q. (1978). Circadian variations in serum concentrations of human chorionic gonadotropin, human chorionic somatomammotropin, oestradiol and progesterone during pregnancy. *Clin. Exp. Obstet. Gynecol.* 5(3-4),87-92