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Function of adhesion molecules and signalling pathways in human endometrial and embryonic models

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Function of adhesion molecules and signalling pathways in human endometrial and embryonic models

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Institute of Molecular and Cell Biology, University of Tartu, Estonia

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LIST OF ORIGINAL PUBLICATIONS

The thesis contains the following publications, which are referred to in the text by Romain numerals:

I. **Stepanjuk, Artjom;** Koel, Mariann; Pook, Martin; Saare, Merli; Jääger, Kersti; Peters, Maire; Krjutškov, Kaarel; Ingerpuu, Sulev; Salumets, Andres (2019). MUC20 expression marks the receptive phase of the human endometrium. Reproductive Biomedicine Online, Vol. 39, Iss. 5, pp. 725–736. https://doi.org/10.1016/j.rbmo.2019.05.004.

II. Lavogina, Darja; **Stepanjuk, Artjom;** Peters, Maire; Samuel, Külli; Kasvandik, Sergo; Khatun Masuma; Arffman, Riika K; Enkvist, Erki; Viht, Kaido; Kopanchuk, Sergei; Lättekivi, Freddy, Velthut-Meikas; Agne; Uri, Asko; Piltonen, Terhi T; Rinken, Ago; Salumets, Andres (2021). Progesterone triggers Rho kinase-cofilin axis during in vitro and in vivo endometrial decidualisation. Human Reproduction, Vol. 36, Iss. 8, pp. 2230–2248. https://doi.org/10.1093/humrep/deab161.

III. Kallas-Kivi, Ade; Trei, Annika; **Stepanjuk, Artjom;** Ruisu, Katrin; Kask, Keiu; Pooga, Margus; Maimets, Toivo (2018). The role of integrin β1 in the heterogeneity of human embryonic stem cells culture. Biology Open, Vol. 7, Iss. 11. https://doi.org/10.1242/bio.034355.

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My contributions to the listed publications were as follows:

Ref. I: Involved in developing the primary conception and study design, conducted immunohistochemical experiments, data collection and analysis. Performed data analysis, visualised results and drafted the manuscript. Guided manuscript reviewing and editing.

Ref. II: Participated in the study design. Conducted tissue immunofluorescence experiments, Western blot and cell migration assay. Analysed data and participated in manuscript writing, figures preparation and final version editing.

Ref. III: Participated in conducting experiments and collecting data. Manuscript reviewing and editing.

LIST OF ABBREVIATIONS

8-Br-cAMP	–	8-Bromoadenosine 3',5'-cyclic adenosine monophosphate
AKT/PKB	–	Rho family serine/threonine-protein kinase or protein kinase B
ART	–	assisted reproductive technologies
ATP	–	adenosine triphosphate
cAMP	–	cyclical adenosine monophosphate
CFL1	–	non-muscle isoform of cofilin
CK2	–	casein kinase 2
CREB	–	cAMP response element-binding protein
E2	–	estradiol
ECM	–	extracellular matrix
EGF	–	epidermal growth factor
EGFR	–	epidermal growth factor receptor
EMT	–	epithelial-to-mesenchymal transition
EPAC	–	exchange factor directly activated by cAMP
ER	–	estrogen receptor
ERK1	–	extracellular signal-regulated kinase-1
ERK2	–	extracellular signal-regulated kinase-2
ERα	–	estrogen receptor alpha
ERβ	–	estrogen receptor beta
FACS	–	fluorescence-activated cell sorting
FOXO1	–	forkhead box protein O1
FSH	–	follicle-stimulating hormone
Fsk	–	forskolin
GAB1	–	GRB2-associated-binding protein 1
GnRH	–	gonadotropin-releasing hormone
GPCR	–	G-protein-coupled receptor
GRB2	–	growth factor receptor-bound protein 2
hCG	–	human chorionic gonadotropin
HGF	–	hepatocyte growth factor
HIF-1α	–	hypoxia-inducible factor 1-alpha
HPG	–	hypothalamic-pituitary-gonadal (axis)
IBMX	–	3-isobutyl-1-methylxanthine
ICM	–	inner cell mass
IGFBP-1	–	insulin-like growth factor-binding protein 1
IHC	–	immunohistochemistry
IL-15	–	interleukin-15
IVF	–	*in vitro* fertilisation
KNDy neurons	–	kisspeptin, neurokinin B and dynorphin coexpressing neurons in the hypothalamus
LH	–	luteinising hormone

LH+2	–	endometrial sample collected two days after luteinising hormone surge
LH+8	–	endometrial sample collected eight days after luteinising hormone surge
LIF	–	leukaemia inhibitory factor
LIMK	–	LIM domain kinase
MAPK	–	mitogen-activated protein kinase
MET	–	mesenchymal-to-epithelial transition
MET receptor or c-MET	–	tyrosine kinase receptor of HGF, or sometimes mesenchymal-to-epithelial transition factor receptor
MLC	–	myosin light chain
MMP	–	matrix metalloproteinase
mTOR	–	mammalian target of rapamycin kinase
NF-κB	–	nuclear factor kappa B
NK	–	natural killer
P4	–	progesterone
PI3K	–	phosphatidylinositol-3-kinase
PKA	–	protein kinase A or cAMP-dependent protein kinase
PR	–	progesterone receptor
PRA	–	progesterone receptor A
PRB	–	progesterone receptor B
PRKACA	–	catalytic subunit α of protein kinase A
PRKAR1A	–	cAMP-dependent protein kinase type I-alpha regulatory subunit
PRKAR2A	–	cAMP-dependent protein kinase type II-alpha regulatory subunit
PRKAR2B	–	cAMP-dependent protein kinase type II-beta regulatory subunit
PRL	–	prolactin
RIF	–	recurrent implantation failure
ROCK	–	Rho-associated protein kinase
ROS	–	reactive oxygen species
SOD	–	superoxide dismutase
SSEA-1	–	stage-specific embryonic antigen 1
STAT	–	signal transducer and activator of transcription
TIMP	–	tissue inhibitor of metalloproteinases
uNK	–	uterine natural killer
WOI	–	window of implantation
ZO-1	–	zonula occludence-1 or tight-junction protein-1

INTRODUCTION

The inner layer of the uterus or endometrium, consisting mainly of epithelial and stromal cell types, has a crucial role in female reproductive health. Under hormonal control of the hypothalamic-pituitary-gonadal (HPG) axis, the endometrium undergoes cyclic changes during the menstrual cycle. The thickness of the endometrial lining varies throughout the menstrual cycle, being thinnest after menstruation and thickest during the secretory phase. Estrogen and progesterone regulate endometrium growth, differentiation, and shedding. After each menstrual cycle, the endometrium regenerates and prepares for pregnancy during decidualisation. The endometrial lining is irreplaceable in establishing first contact between the maternal organism and the hatching blastocyst, supporting embryo implantation and nourishment during the initiation of pregnancy. Endometrial tissue is highly vascularised, providing nutrients and oxygen to the developing embryo.

Endometrial receptivity is a state of the endometrium when it is ready to accept and support an embryo for implantation, a critical step in achieving a successful pregnancy. The period during the menstrual cycle when the endometrium is most receptive is called the window of implantation (WOI), which typically occurs between days 19–21 of a 28-day menstrual cycle, but the exact timing can vary among women. During the receptive phase, the endometrium undergoes several molecular and cellular changes – increased expression of specific genes and proteins, such as integrins, leukaemia inhibitory factor and homeobox genes. The precise timing and molecular mechanisms underlying endometrial receptivity are complex and involve a coordinated interplay of hormonal, cellular, and molecular changes.

With the development of assisted reproduction technologies (ART) like *in vitro* fertilisation (IVF), the timing of embryo transfer became an essential factor in attempts to improve pregnancy rates in patients. Ultrasound can be used to evaluate endometrial thickness and visually determine the WOI. However, this method is non-specific and much less precise than histological or molecular methods. Histological endometrial dating is based on examining endometrial tissue samples under a microscope to assess structural changes. Modern molecular tests measure the expression levels of specific genes and proteins associated with receptivity. However, despite all attempts to improve pregnancy rates in patients undergoing IVF procedure, the success rate still fluctuates between 25–35% on average. The low success rate of IVF may be related to embryo-side factors, but increasing knowledge about the processes taking place in the endometrium could also help increase IVF's success rate.

Nevertheless, on the other hand, the number of children born after the IVF procedure is constantly growing, and in developed countries, it has reached up to 5% of all births. The average age of women giving birth to their first child is also constantly growing. Thus, in the foreseeable future, the number of problems associated with people’s reproductive health will most likely increase, and the

spread of ART will only expand. Therefore, research focused on the endometrium and the molecular interactions between the blastocyst and endometrial lining is critically important. Continued investigation in this area is crucial for developing more effective treatments for infertility and enhancing our knowledge of early embryonic development. Therefore, future studies are needed to elucidate these complex cellular and molecular interactions. The aim of this doctoral thesis is to expand our understanding of the mechanisms of endometrial receptivity and to highlight new molecular pathways involved, thereby shedding light on the fundamental biological interaction between the mother's body and the embryo that leads to pregnancy and, ultimately, the birth of a new human being.

This dissertation explores interactions between the two main cellular components of the endometrium – epithelial and stromal cells. It highlights the significance of physiological and morphological changes, particularly mesenchymal-to-epithelial transitions, in endometrial decidualisation and during the window of implantation (WOI). The findings emphasize the need for future research on epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions as key mechanisms in endometrial and implantation biology.

1. LITERATURE OVERVIEW

1.1. Female fertility

The female reproductive system consists of several vital organs, each playing a crucial role in reproduction. The main paired organs are the ovaries, which produce oocytes and hormones essential for reproduction. The ovaries are connected to the uterus by the Fallopian tubes, which serve as pathways for oocytes/embryos to travel from the ovaries to the uterus. The uterus is a hollow, muscular organ where foetal development occurs during pregnancy. It consists of the body and the cervix. The inner lining of the uterus called the endometrium, undergoes cyclic changes in response to hormonal fluctuations throughout the menstrual cycle. Hormonal regulation in the female reproductive system is orchestrated by a complex interplay of hormones produced by various glands and organs. The menstrual cycle, which typically lasts about 28 days, is controlled by hormones released by the hypothalamus, pituitary gland, and ovaries. The cyclical nature of the ovarian and menstrual cycles ensures the timely release of secondary oocytes and prepares the uterus for potential pregnancy. If fertilisation does not occur, hormone levels decline, leading to the shedding of the uterine lining and the onset of menstruation. Overall, the female reproductive system is intricately regulated by hormones and involves a coordinated series of events to facilitate fertilisation and support pregnancy.

1.1.1. Hormonal regulation of female fertility

Hormonal regulation of female fertility is under control of the central nervous system. Geoffrey W. Harris proposed the hypothesis of neuroendocrine control on gonadal function in his monograph in 1955 (Harris 1955). Further, this hypothesis evolved into the fundamental model of the hypothalamic-pituitary-gonadal (HPG) axis, which acts as a single endocrine gland system (Figure 1). In women, the HPG axis is activated during prepuberty, when central nervous system suppression of the pulsatile release of gonadotropin-releasing hormone (GnRH) is relieved, and the organism is subjected to cyclic fluctuations of sex hormone levels (Plant 2015). GnRH, isolated and identified in 1971, is secreted from the hypothalamus by GnRH-producing neurons (Amoss et al. 1971; Matsuo et al. 1971). GnRH-expressing neurons are located in the medial preoptic and arcuate nuclei of the hypothalamus and secrete GnRH into the hypophyseal plexus to the anterior pituitary (Figure 1A and 1B). GnRH acts through the G-protein-coupled receptor (GPCR) in the anterior pituitary to the gonadotroph cells and mediates the release of gonadotropins: luteinising hormone (LH) and follicle-stimulating hormone (FSH); (Figure 1C). Like GnRH, LH is released in a pulsatile manner (Perrett and McArdle 2013). In the case of female fertility regulation, the main targets of gonadotropins are ovaries (Figure 1D). Hormonal signalling in ovaries is mediated by gonadotropin receptors, members of the GPCRs family. Both LH

and FSH receptors have a large extracellular domain capable of hormone binding (Mcfarland et al. 1989; Milgrom E et al. 1997; Thu Vuhai-Luuthi et al. 1990; Vannier et al. 1995).

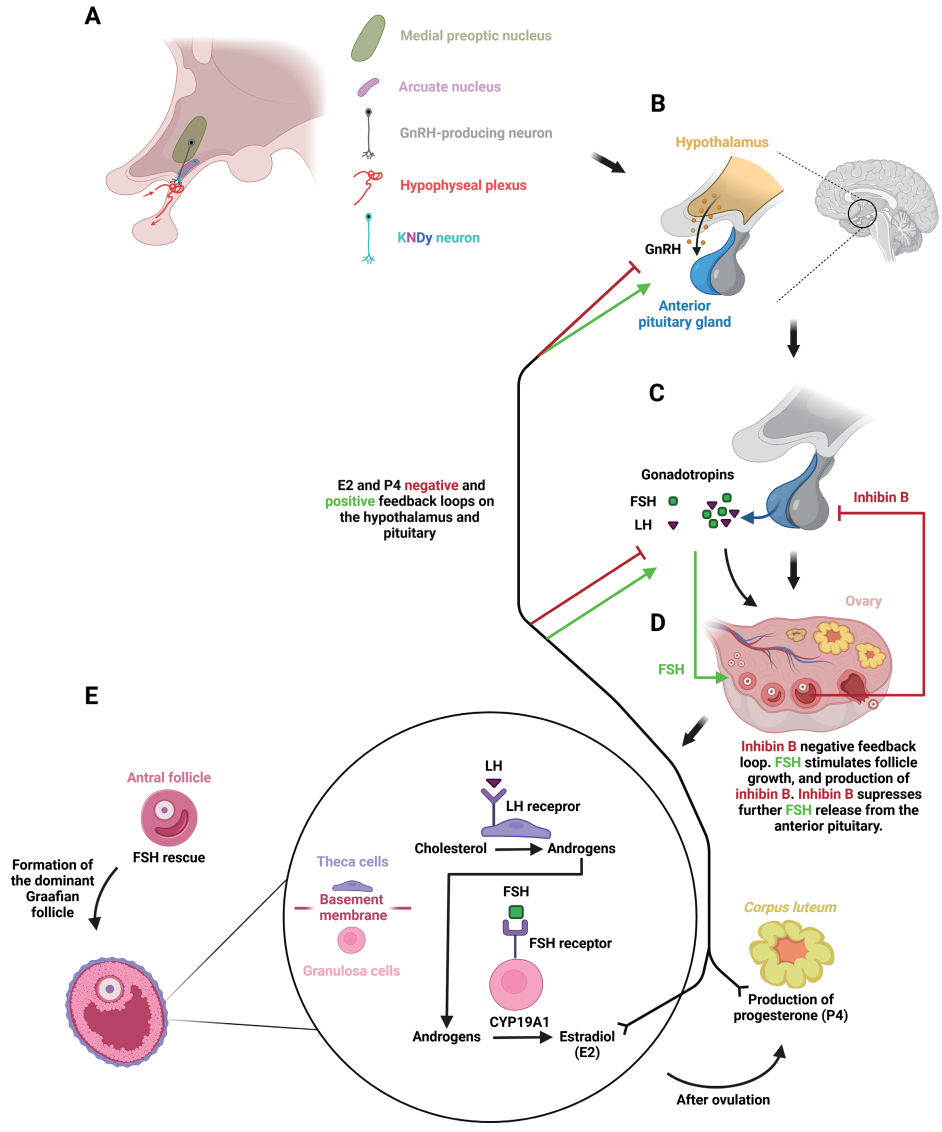

Figure 1. Female HPG axis. A – GnRH pulse generator in the hypothalamus. GnRH-producing neurons are mainly located in the medial preoptic nucleus of the hypothalamus, secreting GnRH into the hypophyseal plexus under the control of the regulatory network of KNDy neurons located mainly in the arcuate nucleus of the hypothalamus. B – Hypothalamic GnRH release. Secreted GnRH reaches the anterior pituitary gland through the hypophyseal plexus. C – Gonadotropins secreted by the anterior pituitary gland. GnRH reaches through hypophyseal plexus blood vessels gonadotrophic cells in

the anterior pituitary, which produce under the influence of GnRH gonadotropins – FSH and LH. D – Ovaries are the target of the FSH and LH in the female HPG axis. Gonadotropins reach ovarian tissue through circulation. E – Two-cell, two-gonadotropins model. A well-developed antral follicle is rescued from atresia by cyclic recruitment guided by FSH and transformed into the dominant preovulatory follicle, consisting of two well-differentiated cell types separated by a basement membrane: theca cells surrounding the follicle and granulosa cells inside the follicle. Theca cells express LH receptors and respond to LH synthesising androgens from cholesterol. Granulosa cells express FSH receptors and, as a response to FSH, express aromatase CYP19A1, converting androgen precursors into estradiol. After ovulation, a ruptured follicle further transforms into the *corpus luteum*, a progesterone-producing structure needed for maintaining pregnancy. HPG – hypothalamic-pituitary-gonadal, GnRH – gonadotropin-releasing hormone, KNDy – kisspeptin, neurokinin B and dynorphin coexpressing neurons, FSH – follicle-stimulating hormone, LH – luteinising hormone, E2 – estradiol, P4 – progesterone, CYP19A1 – aromatase cytochrome P450 family 19 subfamily A member 1. Created with BioRender.com

During the follicular phase of the ovarian cycle, a well-developed antral follicle is rescued from atresia by cyclic recruitment guided by FSH. This follicle becomes the dominant preovulatory follicle, transforming into a major producer of estrogens (Hannon and Curry 2018). Besides oocyte, the follicle consists of two well-differentiated cell types: theca cells surrounding the follicle and granulosa cells inside the follicle. Those two cell types are separated by a basement membrane and respond differently to gonadotropins. FSH receptors are expressed on granulosa cells during the whole stage of follicular maturation, whereas LH receptors are expressed on theca cells, and granulosa cells start to express LH receptors when the follicle reaches the early antral stage of development (Oktay, Briggs, and Gosden 1997; Yamato, Shima, and Nakano 1992; Yung et al. 2014). That model was named the two-cell, two-gonadotropins hypothesis. Theca cells express LH receptors and respond to LH by synthesising androgens called androstenedione and testosterone from cholesterol. Granulosa cells express FSH receptors and, as a response to FSH, express aromatase CYP19A1. CYP19A1 converts androgen precursor from theca cells into the primary estrogen named estradiol (E2) (Hillier, Whitelaw, and Smyth 1994; Moon et al. 1978; Kenneth J Ryan 1979; K J Ryan and Petro 1966). The additional functions of FSH are stimulating granulosa cells' proliferation during the follicular phase of the ovarian cycle, up-regulation of both FSH and LH receptors, and LH's additional function, luteinisation of granulosa cells. A high concentration of E2 from the dominant Graafian follicle induces ovulation by causing an LH surge via the positive feedback mechanism (Richards 1994; Ulloa-Aguirre et al. 2007). After ovulation, a ruptured follicle forms the *corpus luteum*, progesterone (P4) producing structure needed for maintaining pregnancy in the early stage (Figure 1E).

Ovarian steroid hormones, especially estradiol, also regulate the HPG axis by negative and positive feedback loops on the pituitary and hypothalamus, which is essential to maintaining the FSH and LH release pattern during the ovarian cycle (Figure 1). This influence can be direct and affect at the pituitary level by

modulating gonadotrophic cells response to GnRH or indirect at the hypothalamic level by regulating the amount of GnRH released into the circulation (Karsch et al. 1973; Plant 2012; Yen and Tsai 1972). The hormonal regulatory network of the HPG axis is more complicated and consists of other players. Inhibin B, which plays an important role in maintaining hormonal balance during the menstrual cycle, must be mentioned. Inhibin B, together with estrogen and progesterone, forms negative feedback loops to control the HPG axis. Under FSH control, stimulation and follicle growth occur, which produce inhibin B. During follicle maturation, the production of inhibin B increases, increasing amounts of inhibin B suppress further FSH release from the anterior pituitary (Figure 1). That negative feedback loop prevents overstimulation of follicles, and, as a rule, it helps to select only one dominant follicle, which continues its development and ends with ovulation (Burger 1993; Welt 2004).

The hypothalamic neuronal network, responsible for GnRH secreting, was named the GnRH pulse generator because of the pulsatile release of GnRH by the neurons. That concept was established in the 1980s, but it was unclear exactly how the amplitude of GnRH release was regulated for a very long time. At the hypothalamic level, the negative feedback loop of the E2 modulates the amplitude of the GnRH pulsatile release (Knobil 1980; Plant 2012; Pohl and Knobil 1982; Wilson et al. 1984). However, initially, it was demonstrated that GnRH neurons do not bind and accumulate estrogen, and further, it was shown that they also do not express estrogen receptors (Shivers et al. 1983). Thus, estrogen feedback at the hypothalamic level must be mediated indirectly by non-GnRH neurons or even glial cells, but this statement did not have any experimental support. The major breakthrough was made in 2003 when two independent research groups discovered that the malfunctioned kisspeptin receptor GPR54 causes hypogonadotropic hypogonadism in men and women. Further, it was established that kisspeptin peptide is a potent GnRH secretagogue and, that the kisspeptin-expressing neuronal network targets GnRH neurons, and that GnRH neurons also express GPR54 itself (de Roux et al. 2003; Seminara et al. 2003). Further, it was discovered that kisspeptin neurons also co-express two more peptides, neurokinin B and dynorphin. Those neurons expressing all three peptides were named (KNDy neurons), forming the KNDy neuronal network in the mammalian hypothalamus (G. Cheng et al. 2010). KNDy neurons express estrogen alpha (ERα) and progesterone receptors (PR), which explain how ovarian steroid hormones form positive and negative feedback loops on the GnRH pulse generator and regulate the HPG axis on the hypothalamic level (Figure 1A). During the follicular phase, GnRH release activity is limited by estrogen-negative feedback, and in the mid-cycle, it is switched to the positive feedback loop, which causes LH surge and ovulation in the end. Different KNDy network neuron populations react to the ovarian steroids and regulate GnRH neurons secretion activity by neuronal network intercalation mediated by three major peptides: kisspeptin, neurokinin B and dynorphin (Prashar et al. 2023; Skorupskaite, George, and Anderson 2014; Uenoyama et al. 2021). However, the precise mechanisms of the KNDy network regulatory role may vary in different mammal species. Studies

are limited in higher primates and, particularly, humans. Thus, it can be said that despite the important role of the hypothalamic KNDy neuronal network, in human females' ovulation is more emancipated from that control than in rodents or ruminants, which are often used as model organisms (Plant 2012; 2015; Prashar et al. 2023).

1.2. Endometrium

The inner lining of the human uterus, or endometrium, has many unique properties among other tissue types. It is the only tissue type in the human organism that is cyclically shed off during menstruation and after fully regenerating the total thickness of tissue (~8 mm) without forming scars and with restored functionality for embryo implantation and placentation (Salamonsen, Hutchison, and Gargett 2021). That cyclical process occurs approximately 400 times during reproductive life and also after parturition (McLennan and Rydell 1965). The normal idealised menstrual cycle of ~28 days is divided into three phases: menstrual, proliferative and secretory (Figure 2). Dating of the menstrual cycle starts from the day bleeding occurs, and the menstrual phase begins (~1–5 days), during which the functional layer of endometrial tissue is lost. Then begins the proliferative phase, which goes on until the ovulation event (day ~14), and during that cycle phase, cells actively proliferate and restore tissue thickness. After that, in the secretory phase, phenotypic changes in endometrial cells start, preparing the endometrium for embryo implantation and placenta formation if conception occurs. The secretory phase can be divided into early-, mid- and late-secretory phases. The major regulators of cyclic changes are ovarian steroid hormones, estrogen released from the developing ovarian follicle and progesterone produced in the *corpus luteum*, which forms after ovulation (Salamonsen, Hutchison, and Gargett 2021).

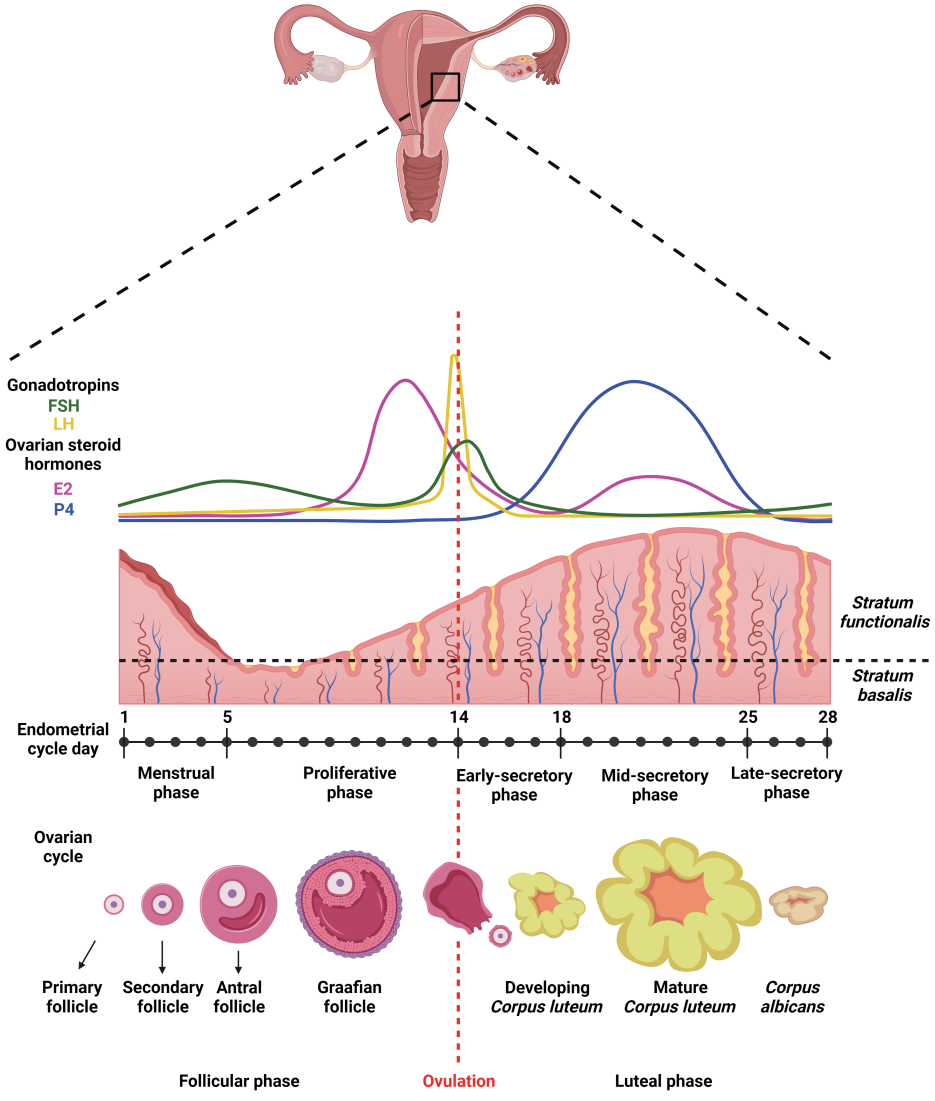

Figure 2. Human idealised menstrual cycle. Dating starts from the day bleeding occurs, signalling the beginning of the menstrual phase up to 5 days long, during which a functional layer of endometrial tissue *(stratum functionalis)* is lost. Until the ovulation at day 14, endometrial tissue thickness is restored during the proliferative phase under the control of estrogen from the developing ovarian follicle. After ovulation, the ruptured follicle is transformed into the progesterone-producing *corpus luteum* and starts the secretory cycle phase guided by progesterone, leading to the phenotypic changes in endometrial cells, preparing the endometrium for embryo implantation. The secretory phase can be divided into early-, mid- and late-secretory phases. It is necessary to remember that the endometrial cycle goes hand in hand with the ovarian cycle, which is part of the HPG axis and orchestrated by the gonadotropic and ovarian steroid hormone milieu. FSH – follicle-stimulating hormone, LH – luteinising hormone, E2 – estradiol, P4 – progesterone. Created with BioRender.com

1.2.1. Endometrial structure

Functionally, the endometrium is divided into two layers with different functions: the outer *stratum functionalis* or functional layer consisting of *stratum compactum* and *stratum spongiosum*, and the underlying *stratum basalis* or basal layer, which is located on the muscular compartment of the uterus wall or myometrium (Figure 2). Endometrial cellular composition is complex and consists of different cell types, which can be differentiated according to their biomarkers (Salamonsen, Hutchison, and Gargett 2021). The functional layer's primary function is to prepare a suitable microenvironment for possible embryo implantation if conception occurs. The functional layer consists of the outer luminal epithelium, which covers the whole uterine cavity, and the apical surface of the single layer of luminal epithelium cells interact with the uterine cavity environment. Numerous endometrial glands are opened on the luminal epithelium surface, penetrating vertically into the endometrial stroma. Glands consist of a single layer of glandular epithelium, which contiguously replaces luminal epithelial cells from the openings of the glands. Both luminal and glandular epithelium contain two morphologically different cell types: ciliated and non-ciliated epithelial cells. Glands in the proliferative endometrium are thinner and straighter, in contrast to the secretory endometrium, with more extended, wider, coiled structured and with wider lumens glands (Tempest et al. 2020). These changes in the endometrial gland morphology during the cycle are of functional significance because glands are an essential source of nutrients, growth factors and cytokines during embryo implantation and pregnancy in the first trimester when the placenta is not fully developed yet (Hempstock et al. 2004). The endometrial stroma consists of fibroblasts, vasculature and a variable number of leukocytes within an interstitial extracellular matrix.

The functional layer is shed off during the menstrual cycle and gradually restored from the basal layer. The basal layer's primary function is maintaining *stratum functionalis* regeneration after menstruation. *Stratum basalis* contains the majority of progenitor cells to restore the functional layer after menstruation. The basal layer also contains stromal cells, vasculature and immune cells, which are also present throughout the menstrual cycle (Salamonsen, Hutchison, and Gargett 2021). The main morphological difference between functional and basal layers is in the endometrial glands' structure, which is reflected in the presence of many horizontally branching networks of glands in the *stratum basalis*. These were found quite recently and suggest that those morphologically distant horizontally branching glands serve as a source of progenitor cells to restore glands and whole epithelium in the functional layer during the proliferative cycle phase (Figure 3). Vertically oriented glands expand from those horizontal networks during the proliferative phase (Tempest et al. 2020; Yamaguchi et al. 2021). That confirms the proposed old hypothesis of the role of somatic stem cells in endometrium regeneration (Prianishnikov 1978). However, the exact markers for all cell types and populations of those progenitor cells are unclear, and further intensive research is needed.

The endometrium has unique blood vessels, known as spiral arterioles, which can regenerate after menstruation and modify their permeability properties during a cycle under the influence of ovarian steroid hormones. The latter is essential during the onset of menstrual bleeding, embryo implantation, and placenta development and functioning (Hickey and Fraser 2000; Salamonsen, Hutchison, and Gargett 2021). Variable types of immune cells are present in the endometrium throughout the menstrual cycle, including tissue-resident immune cells called uterine natural killer (uNK) cells (Bulmer et al. 1991; Salamonsen, Hutchison, and Gargett 2021). uNK cells are morphologically distant from blood NK cells and form the major leukocyte population in the endometrium, up to 50–90% of all other leukocytes. Generally, those cells are in low abundance until the late-secretory phase, when there is a massive and selective migration of immune cells into the functional layer of the endometrium (Koopman et al. 2003; Zhou, Way, and Chen 2018). Populations of macrophages and neutrophils are also presented in the endometrium, with dynamic qualitative and quantitative changes during the endometrial cycle. The human endometrium also contains lymphoid aggregates consisting of B cells, T cells and macrophages, but the biological functions of those aggregates and the exact phenotypes of those cells are poorly understood (Salamonsen and Lathbury 2000; Zhou, Way, and Chen 2018). However, the detailed immunological regulation and modulation of the endometrial tissue are out of the scope of this thesis.

1.2.2. Endometrial cycle

The endometrium is highly responsive to ovarian steroid hormones' cyclical fluctuations, which act through specific receptors. During the menstrual cycle, the endometrium is controlled by three different hormonal conditions: the estrogen-dominated proliferative phase, the progesterone-dominated secretory phase and the reaction to progesterone withdrawal resulting in menstruation (King and Critchley 2010). There are two existing subtypes or isoforms of estrogen receptors (ER): ERα and ERβ, and two isoforms of progesterone receptors (PR): PRA and PRB (Salamonsen and Lathbury 2000). These receptors belong to a numerous superfamily of nuclear transcription factors, which regulate the expression of many genes. Due to the cholesterol origin of ovarian steroid hormones, they pass through the cell membrane and interact with the corresponding receptor in the cytoplasm. Binding to the receptors leads to receptor dimerisation and translocation to the cell nucleus, where it acts as a transcription factor, leading to the expression of ovarian steroid hormone-regulated genes (R. M. Evans 1988; Parker 1993; Yaşar et al. 2017). The dominant form of ER in all cell types throughout the menstrual cycle in the endometrium is ERα, but an expression of ERβ is also observed. The lowest expression of both isoforms of ER is noticed during menstruation (Matsuzaki et al. 1999). PRA and PRB are products of the same gene generated by alternative transcription via the usage of alternate promoter regions (Conneely 2000; Kastner et al. 1990). In human endometrial tissue, PRA is presented in glandular epithelial cells and stromal cell

nuclei during the proliferative phase, but only stroma cells express it at the end of the secretory phase. PRB is also expressed in both cellular components during the proliferative phase, but in contrast to PRA, it is absent in the late-secretory endometrium (H. Wang et al. 1998). More recent studies show that PRA and PRB play slightly different roles in the endometrium. In the case of human endometrium, PRB plays a dominant role during decidualisation – the process of achieving a receptive endometrial state for potential embryo implantation by controlling larger transcriptome and cistrome, including control of PRA expression (Bhurke, Bagchi, and Bagchi 2016; Kaya et al. 2015).

The endometrial cycle starts with menstruation, during which a functional layer of the endometrium is shed in a piecemeal manner (Figure 2). On the surface of the endometrium appear zones where cell contacts between epithelial cells are loose, the tissue starts to degrade, and bleeding occurs from the damaged endometrial blood vessels. Menstrual blood flushes off fragments of enzymatically degraded epithelium fragments, endometrial glands and decidualised stroma cells until the basal layer is exposed. It is important to note that tissue shedding occurs not simultaneously on the entire endometrium surface or in a generalised manner but at the local level with immediate re-epithelialisation of the exposed basal layer with newly formed cuboidal epithelium. The newly formed epithelium surface is very smooth with minor microvilli, and ciliated cells are rare. When the whole endometrium surface is re-epithelialized, the bleeding stops, and endometrial glands and stroma start restoring the functional layer's total thickness during the proliferative phase (Garry et al. 2009; Ludwig and Spornitz 1991; Salamonsen, Hutchison, and Gargett 2021).

In the 1980s, Finn proposed the theory that menstruation is a process closely related to inflammation (Finn 1986). Further, it was confirmed by many studies that an inflammatory cascade in the endometrium is triggered just before the start of menstrual bleeding in response to progesterone withdrawal (J. Evans and Salamonsen 2012; Salamonsen, Hutchison, and Gargett 2021). It was also connected to an initial study by Markee, who showed that progesterone withdrawal leads to endometrial transplant bleeding and vasoconstriction of spiral arterioles (Markee 1940). In endometrium exist a delicate balance between the reactive oxygen species (ROS) and the enzymes that deactivate them – superoxide dismutases (SODs). SODs activity in the endometrium decreases in response to progesterone withdrawal (Sugino et al. 1996; 2002). As a result, different ROS start to accumulate and play a role as mediators of inflammation and cytotoxicity through activation of the nuclear factor kappa B (NF-κB) pathway, which leads to the production of inflammatory factors like cytokines, chemokines, prostaglandins, and further tissue destruction and menstruation (Sugino 2007). Under the influence of progesterone during the secretory phase, NF-κB and its inhibitory complex are stable, which blocks the translocation of NF-κB to the nucleus. Removing the inhibitory complex from NF-κB at the end of the secretory phase due to progesterone withdrawal allows the transfer of NF-κB to the nucleus which is induced by ROS accumulation before the start of menstrual bleeding (Gloire, Legrand-Poels, and Piette 2006; Karin and Delhase 2000; Schoonbroodt

and Piette 2000; van der Burg and der Saag 1996; Sugino et al. 2004). NF-κB target genes that induce inflammation and have a role in menstruation are CXCL1, CXCL8, CCL1, CCL3, IL-1β, IL-6, COX-2, CXC, GM-CSF, TNF-α (J. Evans and Salamonsen 2012).

Progesterone withdrawal via the NF-κB pathway in the endometrium also induces the synthesis of prostaglandins and leukotrienes, which belong to the family of inflammatory eicosanoids. Both have specific roles in inflammation propagation, oedema, and leukocyte recruitment in endometrial tissue prior to menses. Most prostaglandins are produced in endometrial stroma cells and glandular epithelium, but perivascular cells are also an important site of their synthesis (Baird et al. 1996; Battersby et al. 2004; Catalano et al. 2011; L. Cheng et al. 1993; Jones, Kelly, and Critchley 1997). Prostaglandins also play an essential role in the vasoconstriction of endometrial blood vessels, which controls the bleeding intensity and induces hypoxia in the tissue. Induction of hypoxia, one of the most critical signals for menstruation in pre-menstrual endometrial tissue, leads to increased degradation of the functional layer and inflammation via elevated levels of hypoxia-inducible factor 1-alpha (HIF-1α), enhancing the expression of hypoxia-related genes (Critchley et al. 2006; Maybin et al. 2018). ROS-activated NF-κB and inflammation cascade caused by prostaglandins also induce the production and secretion of growth factors and different types of cytokines: chemokines, interleukins, and tumour necrosis factors (J. Evans and Salamonsen 2012). All these abovementioned inflammation factors and signal pathways are important in activating and recruiting different leukocytes.

uNKs additionally proliferate and migrate to the endometrium's functional layer, but a massive influx into *stratum functionalis* of blood leukocytes (like mast cells, neutrophils, eosinophils, and monocytes) through partially damaged spiral arterioles is also observed. Those different types and subpopulations of leukocytes migrate inside the functional layer and get activated there, which leads to degranulation and secretion of different degradative enzymes in tissue, such as elastase, cathepsin and matrix metalloproteinases (MMPs) (Salamonsen and Woolley 1999; J. Evans and Salamonsen 2012; Jeziorska, Salamonsen, and Woolley 1995; Salamonsen and Lathbury 2000). MMPs play an essential role in the progression of menstruation by degrading and modifying the extracellular matrix (ECM), leading to tissue fragmentation and breakdown. During the menstruation in the inflamed and shedding endometrial tissue, a balance exists between degradative proteolytic enzymes and their inhibitors through a complicated regulative network to avoid too deep tissue damage and destruction of the basal layer (Gaide Chevronnay et al. 2012; Henriet, Gaide Chevronnay, and Marbaix 2012; Marbaix et al. 1996; J Zhang 1997; Jin Zhang and Salamonsen 2002). In parallel to tissue shedding, fast re-epithelisation of denuded areas of endometrial tissue occurs. It is important to note that restoration of a functional layer is a different event that occurs later during the proliferative phase after total re-epithelisation of the denuded functional layer. The leading theory of re-epithelisation of an endometrial surface is based on the hypothesis that the new epithelial cells originate from the branches of endometrial glands that remain in

the basal layer. Exposed and partially degraded ECM facilitate luminal and glandular epithelial cells migration across the wounded endometrial surface during menstruation (J. Evans, Kaitu'u-Lino, and Salamonsen 2011; Henriet, Gaide Chevronnay, and Marbaix 2012; Salamonsen, Hutchison, and Gargett 2021).

The regeneration of *stratum functionalis* starts as a response to growing levels of E2 when the re-epithelisation of the endometrium after menstruation is complete, and the whole surface is covered with new luminal epithelium. The proliferative phase in the idealised cycle lasts approximately 10 days (Figure 2). During that time, the functional layer regrows and regenerates all lost cellular structures, including endometrial glands, stroma, vasculature and both ECM components – interstitial matrix between stroma cells and basal lamina underlying epithelial cells (Salamonsen, Hutchison, and Gargett 2021). E2 is a primary endometrial regeneration regulator. Furthermore, ERα and ERβ in endometrial epithelial and stromal cells are observed during the proliferative phase, and the rising level of E2 from ovarian follicles during that time plays a crucial role in the regeneration of endometrial tissue (Dupont et al. 2000; Hewitt, Winuthayanon, and Korach 2016; Toda et al. 2001). In addition to the results obtained in mouse models, severe abnormalities in the uterus and amenorrhea were also described in a female patient with a loss-of-function mutation in the *ESR1* gene encoding ERα (Quaynor et al. 2013). After menopause, the functional layer no longer regenerates due to a lack of circulating E2. However, hormone replacement therapy can stimulate the remaining atrophic endometrium to regenerate the functional layer (Salamonsen, Hutchison, and Gargett 2021; Ulrich et al. 2014). In recent years it was also shown that androgens contribute to epithelium proliferation and gland development, but androgen receptors were primarily detected in the basal layer of endometrium, which points to the possible role of androgens in the initiation of glandular regeneration; however, more research is crucial to evaluate the exact role of androgens during the proliferative phase of the human endometrium (Cousins et al. 2016; Gibson et al. 2020; Simitsidellis et al. 2019).

The high regenerative potential of the endometrium is supported by the presence of somatic stem or progenitor cells for both glandular epithelium and stromal compartments. The hypothesis that in the basal layer of endometrium exists a cell population with high regenerative potential responsible for cyclical endometrial regrowth was proposed in 1989 (Padykula et al. 1989; Salamonsen, Hutchison, and Gargett 2021). The presence of those cells in human endometrium was shown by grafting single-cell suspensions to the mouse model, where those cells responding to E2 and P4 treatment generated endometrial tissue with glands and stroma. Furthermore, blood-filled cysts were formed upon withdrawal of these hormones, similar to menstruation bleeding (H. Masuda et al. 2007). Several small populations of progenitor cells with properties similar to stem cells were identified in adult human endometrium (Salamonsen, Hutchison, and Gargett 2021). Intensive research continues to determine specific molecular markers of those progenitor cells and their specific niche, which supports and regulates the fate of these cells by modulating ECM components, paracrine factors and extracellular vesicles (Sagaradze et al. 2020). Epithelial progenitor cells are better

defined than stroma progenitors, generally described as mesenchymal stromal progenitor cell population. N-cadherin is the primary and most widely recognised molecular marker of human endometrial epithelial progenitor cells. Small populations of N-cadherin^{+} cells spread in the basal layer of the endometrium were found in the niches in the deep endometrial basalis glands (Nguyen et al. 2017). Another widely accepted epithelial molecular marker of progenitor cells is SSEA-1, but it is not always colocalised with N-cadherin (Valentijn et al. 2013). The simplified and widely accepted model of gland renewal can be described as the existence of four different cell types according to the expression of N-cadherin and SSEA-1. The most undifferentiated self-renewing progenitor cell population, N-cadherin^{+} and SSEA-1, is located in the deepest endometrial glands branches. A small cell population of N-cadherin+ SSEA-1+ rises from these progenitors and is located closer to the functional layer. In turn, those cells generate a population of N-cadherin^{-} SSEA-1^{+} epithelial cells, which form a junction between basal and functional epithelial layers. The most numerous and differentiated epithelial cells are N-cadherin^{-} SSEA-1^{-}, which mainly form the glandular epithelium of the functional layer (Figure 3). However, the exact role of those cells in endometrial regeneration needs to be established in the future. A small part of the progenitor cells also remains in the endometrium after menopause and can be activated by hormonal intervention (Salamonsen, Hutchison, and Gargett 2021; Ulrich et al. 2014). New cell subpopulations with unknown functions were also recently discovered in the endometrial tissue (Cousins et al. 2021; Salamonsen, Hutchison, and Gargett 2021).

The endometrium restores full-thickness and functional layer during the proliferative phase, which ends after ovulation. Massive cell proliferation stops, and under the growing influence of progesterone from the *corpus luteum,* the secretory phase of the endometrial cycle occurs. Endometrium terminally differentiates and prepares for potential embryo implantation and placentation during the early- and mid-secretory phase (Figure 2). In the absence of implanting blastocyst during the late-secretory cycle stage, the *corpus luteum* will degenerate because of an absence of signal mediated by human chorionic gonadotropin (hCG) secreted from embryonic trophoblast cells. That leads to progesterone withdrawal, the start of menstruation, and the entire cycle will repeat (Salamonsen, Hutchison, and Gargett 2021).

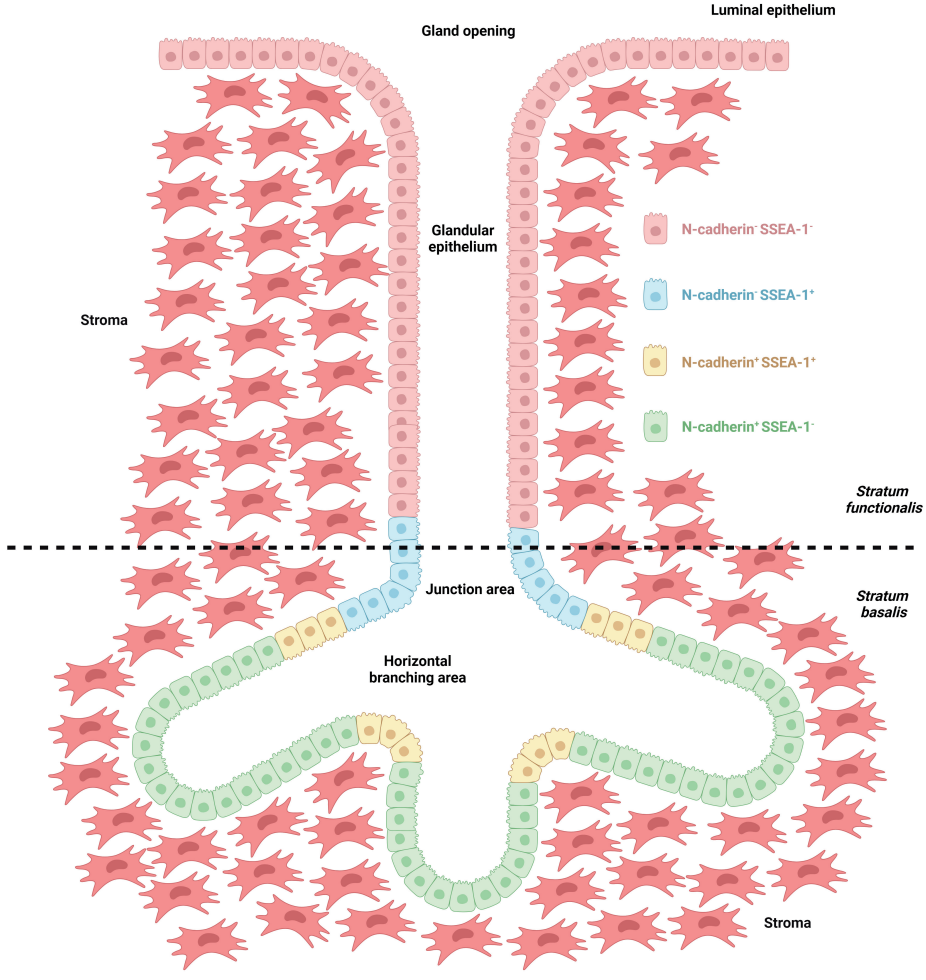

Figure 3. Endometrial regeneration. Horizontally branched networks of endometrial glands are located in the deep layers of endometrial tissue – *stratum basalis*. Those branching glands serve as a niche for progenitor cells, which rebuild the functional layer (*stratum functionalis*) during the proliferative cycle phase. According to the simplified model – vertically oriented glands expand from the networks of horizontal branches during the proliferation. Four different cell populations exist based on the expression of N-cadherin and SSEA-1. The most undifferentiated self-renewing progenitor cell population, N-cadherin^{+} and SSEA-1^{-}, is located in the deepest endometrial glands branches. A small cell population of N-cadherin+ SSEA-1+ rises from these progenitors and is located closer to the functional layer. In turn, those cells generate a population of N-cadherin^{-} SSEA-1^{+} epithelial cells, which form a junction between basal and functional epithelial layers. The most numerous and differentiated epithelial cells are N-cadherin^{-} SSEA-1^{-}, which mainly form the glandular epithelium of the functional layer. SSEA-1 – stage-specific embryonic antigen 1. Created with BioRender.com

1.2.3. Decidualisation

After ovulation, the endometrium enters the progesterone-dominated secretory phase, which can be divided into early-, mid-, and late secretory. At the end of the proliferative phase, progesterone receptor (PR) expression is increased in the endometrium by control of estrogen. Meanwhile, the *corpus luteum* formed after ovulation starts to produce progesterone. Progesterone level starts to rise gradually, increasing the effect on the endometrium, while estrogen level drops. In the PR knockout mice model, the uterus is non-receptive to embryo implantation, moreover, it is hypertrophic, inflamed and infiltrated with leukocytes. Thus, the endometrial phenotype of PR knockout mice is similar to endometrium prior to menstruation, when endometrial tissue reacts to progesterone withdrawal (Bhurke, Bagchi, and Bagchi 2016; Conneely 2000; Lydon et al. 1995; Salamonsen, Hutchison, and Gargett 2021). During the secretory phase, under the influence of progesterone, cells in the endometrial tissue start gradually preparing for possible conception and embryo implantation, called decidualisation. Decidualisation is a complex and dynamic remodelling of multicellular endometrial tissue to establish a suitable microenvironment for pregnancy development (Okada, Tsuzuki, and Murata 2018).

Decidualisation in humans occurs during each secretory phase of the menstrual cycle independently of conception, which is quite rare among mammalian species, but it is due to the type of placentation of human pregnancy. Human pregnancy has the most invasive hemochorial type of placentation combined with early immunological recognition of the allographic fetus and long gestational length. Thus, the endometrial lining must be well and timely prepared for potential embryo implantation and further development. Endometrial stroma cells differentiate terminally during decidualisation, leading to massive morphological and functional changes in the endometrial lining. Any impairment in the decidualisation process can lead to further pregnancy disorders like miscarriage, preterm birth, premature rupture of membranes, fetal death, preeclampsia, and fetal growth restriction due to abnormalities in the placenta development (I. Brosens et al. 2011; Burton and Jauniaux 2018; Emera, Romero, and Wagner 2012; Ng et al. 2020; Pavlicev and Norwitz 2018). Decidualisation begins approximately on the sixth day after ovulation, initiated during the mid-secretory phase of the menstrual cycle in the human organism. However, because of differences between individuals and even between cycles of the same person, the exact timing of the decidual reaction start may vary from 6 to 9 days after ovulation (Coulam 2016; Gellersen and Brosens 2003). Endometrial morphological changes begin in stromal cells surrounding spiral arteries in the upper two-thirds of the endometrium. Those changes were first noted during the development of endometrial dating methodology based on tissue morphological features (Noyes, Hertig, and Rock 1950; Okada, Tsuzuki, and Murata 2018; Rock and Bartlett 1937).

A critical network of factors essential for stromal cell decidualisation consists of the leading factor – progesterone, and proteins regulated by it. However, the

intracellular level of a well-known secondary messenger, cyclical adenosine monophosphate (cAMP), becomes important during the decidual reaction progression. Progesterone also increases cAMP synthesis in the decidualising cells through prostaglandin E2 induction. cAMP is produced from adenosine triphosphate (ATP) by membrane-bound adenylate cyclase, activated through G_s protein-coupled receptors. Increased adenylate cyclase activity raises the intracellular level of cAMP during the decidualisation in the stromal cells. However, in decidualised cells, a decreased degradation rate of cAMP is also observed because of the downregulation of cyclic nucleotide phosphodiesterases (Bartsch, Bartlick, and Ivell 2004; Gellersen and Brosens 2014; Houserman, Todd, and Hertelendy 1989; Tanaka et al. 1993). Most dramatic changes occur in the endometrial stroma, which differentiates terminally during decidualisation. Elongated fibroblast-like endometrial stromal cells change to rounded epithelioid-like cells. Ultrastructural characteristics of these cells have also changed after decidualisation: the nucleus is enlarged and rounded, the number of nucleoli is increased, the dense membrane-bound secretory granules appear, the rough cytoplasmic reticulum and Golgi complex are expanded, and glycogen and lipid droplets accumulate in the cytoplasm. Those ultrastructural changes lead stromal cells to acquire the secretory morphological phenotype (Cornillie, Lauweryns, and Brosens 1985; Gellersen and Brosens 2014; Kajihara et al. 2014; Okada, Tsuzuki, and Murata 2018). Decidualised stroma cells accumulate large amounts of reserve nutrients in their cytoplasm. Thus, those cells play an essential role during the first trimester of pregnancy when the placenta is not fully developed, and fetal growth and development are mainly supported by histotrophic nutrition provided by decidualised stromal cells and endometrial glands secretions. Under the influence of progesterone, changes occur in endometrial glands, which also acquire a secretory phenotype (Burton, Cindrova-Davies, and Turco 2020).

Two major molecular markers of decidualised stroma cells are prolactin (PRL) and insulin-like growth factor-binding protein 1 (IGFBP-1); their secretion means a successful decidual reaction (Maslar and Riddick 1979; Daly, Maslar, and Riddick 1983; Rutanen et al. 1986; Gellersen and Brosens 2014; Okada, Tsuzuki, and Murata 2018). Both play an essential role in further decidualisation and placentation by stimulating the growth and invasion of trophoblast cells, promoting angiogenesis, regulating uNK cells' survival and modulating the immune response to invading embryo. An influx of uNK cells attracted to the functional layer of the endometrium occurs, and they actively participate in the vascular remodelling to support blood supply to the growing conceptus and placenta development. During embryo implantation and placentation, trophoblast cells invade the endometrium and modify endometrial tissue on a microanatomical level (Jabbour and Critchley 2001; Gleeson et al. 2001; Corbacho, Escalera, and Clapp 2002; Ben-Jonathan, C. R. LaPensee, and E. W. LaPensee 2008; Stefanoska et al. 2013; Sharma, Godbole, and Modi 2016; Durairaj et al. 2017). Thus, hormonally-controlled ECM remodelling is one of the major events during decidualisation that prepares the endometrium for ECM-guided tissue reorganisation by various trophoblast cell subtypes. Decidual cells secrete and remodel

ECM proteins like fibronectin, laminin isoforms, type IV collagen, decorin and heparin sulfate-type proteoglycans, which interact with invading trophoblast cells and maintain a balance between promotion or vice versa, limiting too aggressive trophoblasts invasion (Khong, Lane, and Robertson 1986; Iwahashi et al. 1996; Gellersen and Brosens 2014). It was shown that the *FBLN1* gene product – secreted glycoprotein fibulin-1 is a key component of ECM that plays an important role in tissue remodelling by affecting cell adhesion, migration, proliferation and differentiation (Okada et al. 2003; Nakamoto et al. 2005; de Vega, Iwamoto, and Yamada 2009; Okada, Tsuzuki, and Murata 2018). Gap junction formation is considered an ultrastructural marker of decidualisation that also promotes trophoblastic invasion (Lawn, Wilson, and Finn 1971; Jahn et al. 1995; Yu et al. 2011; Laws et al. 2008). Invasion of trophoblast cells requires proteolytic degradation and remodelling of decidual ECM, achieved by secretion of MMPs. However, at the same time, decidua secretes tissue inhibitors of metalloproteinases (TIMPs) to limit the proteolytic activity of MMPs and avoid too-deep invasion and endometrial damage by trophoblasts (Pollheimer, Fock, and Knöfler 2014; Sharma, Godbole, and Modi 2016; Okada, Tsuzuki, and Murata 2018).

By complex interactions between decidua, decidual ECM and trophoblasts – physical and biochemical balance is achieved, which regulates proper embryo implantation and further placenta development. Decidua also plays an important role in modulating immunotolerance to the semi-allogenic embryo, protecting the fetus from the mother organism's immune system. Decidualised stroma makes a suitable microenvironment for immune cells' recruitment, distribution and function. For example, under progesterone regulation, decidualised stroma produces interleukin-15 (IL-15), an attractant and proliferation activator for certain leukocyte types (Kitaya et al. 2000; Ashkar et al. 2003; Mariee, Li, and Laird 2012; Okada, Tsuzuki, and Murata 2018). Numbers of uNK and regulatory T-cells increase during decidualisation, whereas uNK cells make up the largest population of immune cells, up to ~70% of total leukocytes in the endometrium. uNK cells play an important role in tissue remodelling, angiogenesis, and trophoblast invasion control due to their ability to secrete MMPs, angiogenic factors, and interferon-gamma. uNK cells also play a crucial role in endometrial vascular remodelling, which is necessary to support sufficient blood flow to the possible conceptus (Ashkar et al. 2003; Lobo et al. 2004; Santoni, Carlino, and Gismondi 2008; Gellersen and Brosens 2014; Okada, Tsuzuki, and Murata 2018). Blood vessels are critical in homeostasis, immune defence, oxygen transport, nutrition, excretion and fluid balance. Thus, remodelled vasculature is prepared to support embryonic growth in the decidua (Smith 2001; Gellersen and Brosens 2014; Okada, Tsuzuki, and Murata 2018).

To summarise, decidualisation is controlled by interactions of transcription factors, cytokines and signalling pathways orchestrated by progesterone. The decidualised stroma undergoes a series of changes to achieve a unique biosynthetic and secretory phenotype that protects the embryo from maternal immunological rejection and provides nutritional support for the developing embryo and placenta formation. During the endometrial decidualisation, ECM remodelling, modu-

lation of cell adhesion properties and cytoskeleton reorganisation, changes in cell metabolism, stress response, and inflammatory response take place. Thus, decidualised endometrium acquires unique microanatomical, biochemical and cellular properties that support blastocyst implantation.

1.2.3.1. Protein kinases in decidualisation

Protein kinases are a large group of enzymes that catalyse phosphate group transfer and covalent bonding to other proteins, a process named phosphorylation. The importance of phosphorylation is difficult to underestimate since it is one of the main regulatory elements of all cellular processes and pathways, especially signal transduction. In the human genome, about 500 genes code different kinases, which is approximately 2% of protein-coding genes (Manning et al. 2002). Protein kinases are also highly involved in the regulatory network of the decidualisation process. cAMP, the main intracellular metabolite responsible for decidualisation, is also involved in protein kinases activation and regulatory network. cAMP and its downstream targets represented by the exchange factor directly activated by cAMP (EPAC), cAMP-dependent protein kinase (PKA) and one of the substrates of the latter cAMP response element-binding protein (CREB) have been termed as the central players in decidualisation. CREB regulates the expression of many decidualisation-related genes, including the main decidualisation marker PRL (Telgmann et al. 1997; Brar et al. 1997; Telgmann and B. Gellersen 1998; Yoshino et al. 2003; Yoshie, Kusama, and Tamura 2015). Intracellular transcription factors that are members of the signal transducer and activator of transcription (STAT) protein family – STAT3 and STAT5 expression are up-regulated during decidualisation. The activation of STAT3 and STAT5 goes through their phosphorylation by nonreceptor Tyr kinase family Janus kinases, which leads to dimerization and translocation to the nucleus (Jabbour, Critchley, and Boddy 1998; Mak et al. 2002; Dimitriadis et al. 2006; Wei Wang et al. 2012). Other transcription factors up-regulated upon decidualisation include forkhead box Protein O1 (FOXO1). FOXO signalling resides downstream of the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt/PKB) pathway (Christian et al. 2002; Labied et al. 2006; Yoshino, Hirota, et al. 2003; S. Y. Lee et al. 2016; Fabi et al. 2017). Decidualisation-related progestin and interleukin signalling are involved in regulating the mitogen-activated protein kinases (MAPK) cascade (C. H. Lee et al. 2013). Other protein kinases have been less explored in the context of decidualisation, although given the extent of metabolic reprogramming and phenotype alteration, multiple cellular pathways are expected to contribute to the process.

1.2.3.2. ROCK kinase signalling in endometrium

Rho-associated protein kinase (ROCK) belongs to the family of serine-threonine-specific protein kinases and is involved mainly in regulating the shape and movement of the cells by acting on the cytoskeleton. There are two known isoenzymes of ROCK kinase – ROCK1 and ROCK2 (Matoba et al. 2020). In the context of decidualisation, the ROCK pathway is instead scarcely studied. However, considering the extensive changes in the cell structure and morphology of the stroma cells during decidualization, it seems clear that participation of the ROCK pathway is necessary. Increased phosphorylation of well-documented downstream target of ROCK, myosin light chain (MLC) was claimed to prevent *in vitro* decidualisation of endometrial stroma cells; however, the status of MLC phosphorylation is known to be regulated by several pathways (including cAMP/PKA), which tend to have opposing physiological roles due to complex interplay with MLC kinase and phosphatase (Ihnatovych et al. 2007; Álvarez-Santos et al. 2020). The same group noted that stabilisation of actin filaments by cyclic peptide jasplakinolide prevented in vitro decidualisation; still, dependent on the decidualisation-inducing mixture used, disruption of decidualisation by an actin assembly inhibitor latrunculin B was also evident (Ihnatovych et al. 2009). It has been shown that by promoting actin filament disassembly and creating new actin barbed ends, the important downstream element in the ROCK pathway – active cofilin, is required to maintain polarized protrusion and to set the direction of cell migration (Dawe et al. 2003; DesMarais et al. 2005; Svitkina 2018). Indeed, the mainstream paradigm views decidualization as a special case of mesenchymal-to-epithelial transition (MET) associated with decreased cell migration (X.-H. Zhang et al. 2013; Yu et al. 2016; Pan-Castillo et al. 2018).

1.3. Endometrial receptivity

The major conception of endometrial receptivity is based on the knowledge that a competent blastocyst needs a suitable endometrial microenvironment for further development. During decidualisation, the endometrium achieves a receptive phenotype suitable for blastocyst embedding (Craciunas et al. 2019). However, despite this, it is also worth remembering that the blastocyst can be attached to non-endometrial tissue, for example, in the case of an ectopic pregnancy (Marion and Meeks 2012). The basic concept is that the endometrium acquires the properties most suitable for successful blastocyst implantation for a short period. This short period of time is named the receptivity window. During the implantation window, the endometrium expresses a set of genes which are necessary for blastocyst apposition on the luminal epithelium, attachment to it, penetration through it and invasion to the decidualised stroma. In humans, the window of implantation occurs 6–9 days after the LH surge, which coincides with the mid-secretory 20–24 phase day of the regular endometrial cycle (Muter et al. 2023). That coincides nicely with the clinical observation that free-floating embryos can

be retrieved from the uterine cavity by flushing on days 5–7 after LH. Later, blastocysts are usually attached to the endometrial surface and cannot just be flushed away by the fluid (Formigli et al. 1990). By measuring hormones and hCG, it was established as well that 84% of conceptions can be detected between 8–10 days after ovulation (Wilcox, Baird, and Weinberg 1999; Erden et al. 2022). If endometrium fails to achieve the receptive state, the pregnancy will not be established with a high probability, or early pregnancy loss will occur. Thus, the correct endometrial dating and determination of the implantation window are essential for assisted reproductive technology to improve successful pregnancy rates. In the first attempts of endometrial dating, histological and ultrastructural characteristics of endometrial cells were used. Endometrial cycle dating based on histological features of endometrial tissue is widely used today. However, new approaches based on different types of omics have also been intensively introduced for endometrial dating (Muter et al. 2023; Lacconi et al. 2024). Despite this, molecular mechanisms of embryo implantation are still incompletely understood, and new molecular markers are needed to date the window of implantation more accurately and to understand the human endometrium receptivity mechanisms more deeply.

1.3.1. Mesenchymal epithelial transition (MET) in endometrial receptivity

Mesenchymal-epithelial transition (MET) and its reverse process epithelial-mesenchymal transition (EMT) are fundamental evolutionary conserved mechanisms important for different morphogenesis and organogenesis stages. Both mechanisms were initially revealed in the developing embryos but later discovered in some adult organs and tissues by Hay and colleagues in the 1980s. Transitions between the mesenchymal or stromal and epithelial phenotypes in adult organisms are essential to maintain organ homeostasis and regeneration in certain types of tissues (Greenburg and Hay 1982; Hay 1995; 2005; Lim and Thiery 2012; Owusu-Akyaw et al. 2019). The epithelial cells have tight contacts between the cells and a rigid position in the tissue. They attach to the specific form of ECM, named basement membrane, where they establish well-developed apical-basal polarity of the cells. Mesenchymal cells vice versa have transient intercellular contacts, allowing them to use ECM for moving rather than attaching to it, and they also have an elongated phenotype with a pronounced anterior-posterior polarity (Hay 1995; Owusu-Akyaw et al. 2019). Transitions between the epithelial and mesenchymal phenotypes through MET and EMT in an adult organism are related to wound healing and tissue regeneration. However, it must be mentioned that those phenotypes are not always wholly distinct, and cells may have characteristics in a kind of hybrid phenotype with both mesenchymal and epithelial characteristics (Nieto et al. 2016; Owusu-Akyaw et al. 2019).

During the MET, epithelial cells are generated from mesenchymal cells, which obtain properties characteristic of epithelial cells. Initially, mesenchymal cells establish apical-basal polarity, start to express polarity-related cell surface

receptors, form tight junctions and rearrange their cytoskeleton and organelles. MET of mesenchymal cells can be induced by neighbouring cells, changes in the composition of ECM, metabolic switching or cell fate change (Rodriguez-Boulan and Macara 2014; Pei et al. 2019). Due to the ability of the endometrial *functionalis* to repair cyclically without forming scar tissue, it was proposed that MET can play an important role in the re-epithelization of denuded areas of the basal layer after menstruation. It was hypothesised that stroma cells could transform into epithelioid cells and migrate into epithelial niches, especially in the case of luminal epithelium. Few mice models were developed to investigate that hypothesis, but no convincing results were obtained in adult mice. It is also worth noting that murine models were used, and no evidence of this hypothesis was still found in the human endometrium (Huang et al. 2012; Patterson et al. 2013; Cousins et al. 2014; Owusu-Akyaw et al. 2019; Ghosh et al. 2020; Salamonsen, Hutchison, and Gargett 2021).

It is much more intriguing to consider MET in the context of endometrial decidualisation, during which the mesenchymal stroma acquires epithelial features. During the decidualisation, fibroblast-like stroma cells differentiate into polygonal epithelioid cells and acquire properties similar to cells that are going through MET. Many decidualised stroma markers, like beta-catenin or tight-junction protein-1 (ZO-1), are expressed in the endometrial epithelial compartment during the proliferative phase, but their expression is shifted to stroma in the cycle's late-secretory phase (Gellersen and Brosens 2014; Yu et al. 2016). For example, cadherin-11 expression during the proliferative phase can be noticed only in the epithelial compartment of the endometrium, but during the decidualisation, expression can also be detected in the stroma. Moreover, the expression pattern of cadherin-11 mimics decidual reaction progression, beginning from the areas close to spiral arteries and further spreading to the more distant stroma (MacCalman et al. 1996). Interestingly, the widely accepted decidualisation marker PRL outside the decidualised stroma cells is also produced in specialised lactotropic epithelial cells in the adenohypophysis or anterior pituitary (Yu et al. 2016).

The cytoskeleton of decidualised stroma cells is modified in a similar way to the MET, which can be related to decreased motility of epitheliod decidua cells and rearrangements in their organelles distribution inside the cytoplasm, which is related to establishing apical-basal polarity (Pan-Castillo et al. 2018). Changes in the stroma cell morphology and molecular markers expression suggest that decidualisation can be considered a special case of MET (Gellersen and Brosens 2014). As mentioned above, there are many similarities between MET and decidualisation at the molecular level. During decidualisation in the stromal cells, the expression of the E-cadherin increases, and the expression of N-cadherin decreases. In the same manner, during the MET, the cadherin switch occurs, which is very important for establishing the epithelial phenotype. Through interactions with beta-catenin, Rac, Rho and their downstream effectors, E-cadherin modulates and stabilises the cortical actin network, strengthening adherence junctions between the cells. At the same time, the migratory capacity of the cells with high

E-cadherin expression is lower compared to cells with higher N-cadherin expression. Moreover, the E-cadherin knockout mice model established that E-cadherin is essential for fertility, endometrium development and functioning. Adherence and tight junction formation were severely affected in the uteri of those mice. Knockout mice were infertile due to defects during the decidualisation and embryo implantation (Reardon et al. 2012; X.-H. Zhang et al. 2013; Pei et al. 2019).

Gap junctions are another type of cell junction considered important during the endometrium preparation for pregnancy. Cells can very efficiently transfer their cytoplasmic content through gap junctions, which can be vital during pregnancy when a developing embryo depends on surrounding decidualised endometrium. During the decidualisation, the main gap junction protein connexin 43 expression is up-regulated. The same trend can be observed in cells during the MET when cells establish epithelial phenotype (Yu et al. 2011; 2014; 2016). Modulating cell-cell communication through adherence, tight, and gap junctions is very important in decidualisation and MET. Epitheloid phenotype and apical-basal polarity establishment during stromal cells decidualisation are associated with increased expression of junctional proteins to enhance cell-cell communication in the decidua, which is crucial for interacting with the embryo through the invasive trophoblast cells (Schumann et al. 2015; Owusu-Akyaw et al. 2019). Another feature of the decidualised stroma is a transition to the secretion of different types of ECM proteins, which is also typical for MET. Decidualised stroma decreases the production of fibronectin and starts to build around cells collagen type IV and laminin-rich ECM, which is similar to the basal membrane structure of epithelial cells (Aplin, Charlton, and Ayad 1988; Iwahashi et al. 1996; Yu et al. 2016). To sum up, it can be said that stroma decidualisation has a lot in common with MET at the molecular level and is considered a special case of MET.

However, there are also changes related to the MET and EMT balance in the epithelial compartment of the endometrium. In general, epithelial cells do not allow the adhesion of other cells on their apical surface, but trophoblast cells attach and later penetrate through the endometrial luminal epithelium during embryo implantation. It is speculated that before the embryo attaches to the endometrium, epithelial cells lose their apical-basal polarity through an EMT-related process and allow blastocyst attachment. Nevertheless, it is important to mention that studies with human embryos are very limited because of the hard accessibility of human implantation sites and related ethical controversies (Denker 1993; Murphy 2004; Schumann et al. 2015; Owusu-Akyaw et al. 2019). Some *in vitro* models were developed to study communication between the human embryo and maternal endometrium and possible EMT/MET process regulation. It was proposed that embryos can secrete different factors, like miRNAs, to modulate luminal endometrial phenotype in EMT. Despite some achievements, our understanding of these interactions is still limited, new reliable research models need to be established, and further intensive research is necessary (Simón et al. 1997; 1999; Z. Li et al. 2015; Liang, Wang, and Wang 2017; Owusu-Akyaw et al. 2019).

1.4. Mucins

Mucins form a diverse family of 21 highly glycosylated proteins, which are integral components of mucosal surfaces all over the body, having a great variety of functions, from the physical protection and lubrication of the epithelial lining to the molecular modulation of immunity and cell signalling. Mucins can be divided into two groups: secreted and membrane-associated or membrane-tethered. Secreted mucins can be divided into two subgroups: gel-forming – MUC2, MUC5AC, MUC5B, MUC6; and soluble mucins – MUC7, MUC8, MUC9, MUC19 (Fini et al. 2020). The primary function of secreted mucins is to form an extracellular mucus layer on the epithelial surface. That mucus forms a gel-like substance that coats, lubricates, holds water, and physically protects epithelium. In reproductive tracts, gel-forming mucins are secreted, creating a viscous mucous layer covering epithelial glycocalyx. Secreted mucins also provide a growth environment and nutrition for the microbiome in the tissues where interactions between microbiota and human cells occur (Hollingsworth and Swanson 2004; Corfield 2015; Fini et al. 2020). Membrane-associated mucins are MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC14, MUC15, MUC16, MUC17, MUC20, MUC21, MUC22 (Fini et al. 2020). Membrane-associated mucins are major components of the glycocalyx – molecular layer consisting of glycoproteins and glycolipids that can protrude hundreds of nanometres from the epithelial cell membrane. In general, mucins are proteins of very high molecular weight and a very high degree of glycosylation. The MUC family mucins molecule structure usually contains many tandem amino acid repeats of identical or high similarity sequences, which serve as sites of O-linked glycosylation. The complex glycosylation pattern consists of O-linked oligosaccharide chains, which may be 50–90% of the molecular weight of the mucin molecule. Thus, the biophysical properties of mucins depend more on the glycosylation pattern than the polypeptide core sequence itself (Argüeso and Gipson 2001; Hollingsworth and Swanson 2004; Hattrup and Gendler 2008; Corfield 2015; Fini et al. 2020). Membrane-associated mucins also play a role as cell surface receptors and signal transducers in molecular pathways related to inflammation regulation, cell-cell interactions, differentiation and apoptosis. Most of those signals are transduced intracellularly by the N-terminal cytoplasmic tail, which contains different functional domains for interacting with other proteins and phosphorylation sites (van Putten and Strijbis 2017; Fini et al. 2020). Membrane-associated mucins can also be detected in soluble form in various body fluids. It is proposed as a result of the shedding or cleavage of the large extracellular domains of mucins to the extracellular space. However, there is no complete understanding of the role and function of those fragments in the extracellular space (Thathiah, Blobel, and Carson 2003; Thathiah and Carson 2004; Govindarajan et al. 2012).

1.4.1. Mucins in the endometrium

The mucin barrier is hormonally regulated during the menstrual cycle in humans. Moreover, the apical surface of the endometrial epithelium is the first site of physical contact between the developing blastocyst and the uterus, which can be a crucial checkpoint during the implantation process. Thus, it is believed that mucins may play a significant role in regulating embryonic implantation. However, the only mucin that has been intensively investigated in the human endometrium is MUC1, which was also the first mucin detected in the human endometrium (Hey et al. 1994; 1995; Meseguer 1998). To a much lesser extent, in the endometrial receptivity context, MUC4 and MUC16 have been studied (Gipson et al. 1997; Alameda et al. 2007; Dharmaraj et al. 2014). Mucins are the largest membrane-associated glycoproteins – it was estimated that MUC1 can protrude 200–500 nm above the cell membrane, MUC4 can protrude 2 μm above the cell membrane and MUC16 up to 4 μm. In addition, densely packed glycan chains have antiadhesive properties on the cell surface (Jentoft 1990; Fini et al. 2020). In contrast, typical cell receptors like integrins protrude only 20–30 nm from the cell surface. Based on those differences, it can be speculated that mucin antiadhesive properties are based on the steric hindrance of access to cell-surface receptors (Thathiah and Carson 2004; Nishida et al. 2006).

1.4.2. MUC20

Compared to other mucins, MUC20 is a relatively small protein with a low level of glycosylation. That contrasts with other membrane-associated mucins that are highly glycosylated and can protrude hundreds of nanometres from the cell membrane. High MUC20 expression on the cell membrane is associated with ciliogenesis and ciliated cells in general; however, it can be a special case observed in the airway epithelium (Kesimer et al. 2013; Fini et al. 2020; Konstantinidi et al. 2022). MUC20 was initially discovered in renal tissue tubular epithelium (Higuchi, Orita, Nakanishi, et al. 2004). It was experimentally determined that MUC20 has an extracellular domain and cytoplasmic tail; however, the exact location of the transmembrane domain is still unknown. Sequence analysis revealed several hydrophobic regions suitable for association with the plasma membrane, but no alpha-helical transmembrane domain was identified. MUC20 was identified mainly in the membrane fraction and visualised on the membrane surface by immunoelectron microscopy (Higuchi, Orita, Nakanishi, et al. 2004; Higuchi, Orita, Katsuya, et al. 2004). In the human endometrium, MUC20 mRNA was detected for the first time by microarray-based gene expression analysis (G. E. Evans et al. 2012). Later, it was demonstrated that different types of endometrial cancers express MUC20. Moreover, high MUC20 protein expression was associated with a more aggressive tumour phenotype and poor survival rate (C.-H. Chen et al. 2013).

1.5. Embryo preimplantation development

It is very challenging to investigate human embryo development in *in vivo* conditions due to ethical considerations and technical difficulties. The human zygote is only 100 μm in diameter, making it very difficult to track the development of such a tiny object inside the maternal organism or collect enough material for proper analysis. However, our knowledge has expanded during the last decades because of the broader and further expanding use of assisted reproduction technologies in clinical practice, which stimulated intensive research in the field.

During ovulation, the human oocyte is released from the ovary and caught by fimbriae to be guided further to the oviduct. In the beginning, the oocyte is covered by the layer of cumulus cells, named *corona radiata,* which supports oocyte development and maturation. When the oocyte reaches the *ampulla* area of the oviduct, which is close to the ovary, it is ready for fertilisation. After the sperm entry, the meiosis is completed, and the first cleavage occurs one day later. The oviduct is covered with ciliated epithelial cells, which gently push the zygote forward through the oviduct to the uterus (Xie, Xu, and Liu 2023; Coticchio et al. 2015). During that journey, subsequent cleavages occur. The mammalian cleavage type is called rotational. Not all blastomeres divide exponentially from 2 to 4 to 8 cells simultaneously, so sometimes, an odd number of blastomeres can be detected. During the first cleavages, zygote development depends on the maternal proteins stored in the oocyte, and the zygote's genome is activated gradually. Only in the 8-cell stage does the human zygote fully activate its genome for active gene transcription (Kojima, Hoppe, and Giraldez 2024; Gauster et al. 2022). The first crucial morphological change becomes evident after zygote compaction when blastomeres form a compact ball of cells. Human embryos pass the compaction stage on day four after fertilisation when the embryo is in the 10-cell stage (Firmin et al. 2024; Gauster et al. 2022) (Figure 4).

Further compacted cells form a morula. First, blastomeres start to express cell adhesion proteins like E-cadherin, and further, cells on the surface of the morula become bigger and express tight junction proteins and cells within the morula start to express gap junction proteins (Canse, Yildirim, and Yaba 2023; Gauster et al. 2022). The morula starts forming the blastocoel – a small cavity filled with liquid, during the process known as cavitation and transforms into a blastocyst. During the blastocyst formation, first cell differentiation occurs – totipotent blastomeres divide into two groups: trophoblasts formed from the larger outer cells that further form extraembryonic tissues; and pluripotent inner cell mass (ICM) from smaller internal cells, which will give rise to the embryo. Separation into those two different groups of cells is the principal hallmark in the embryo's early development because it is the first differentiation and the basis for the further formation of all tissue types. At the time of blastocyst formation, it will finish moving through the oviduct and reach the uterus in 5–7 days after fertilisation (Shahbazi 2020; Rossant and Tam 2022).

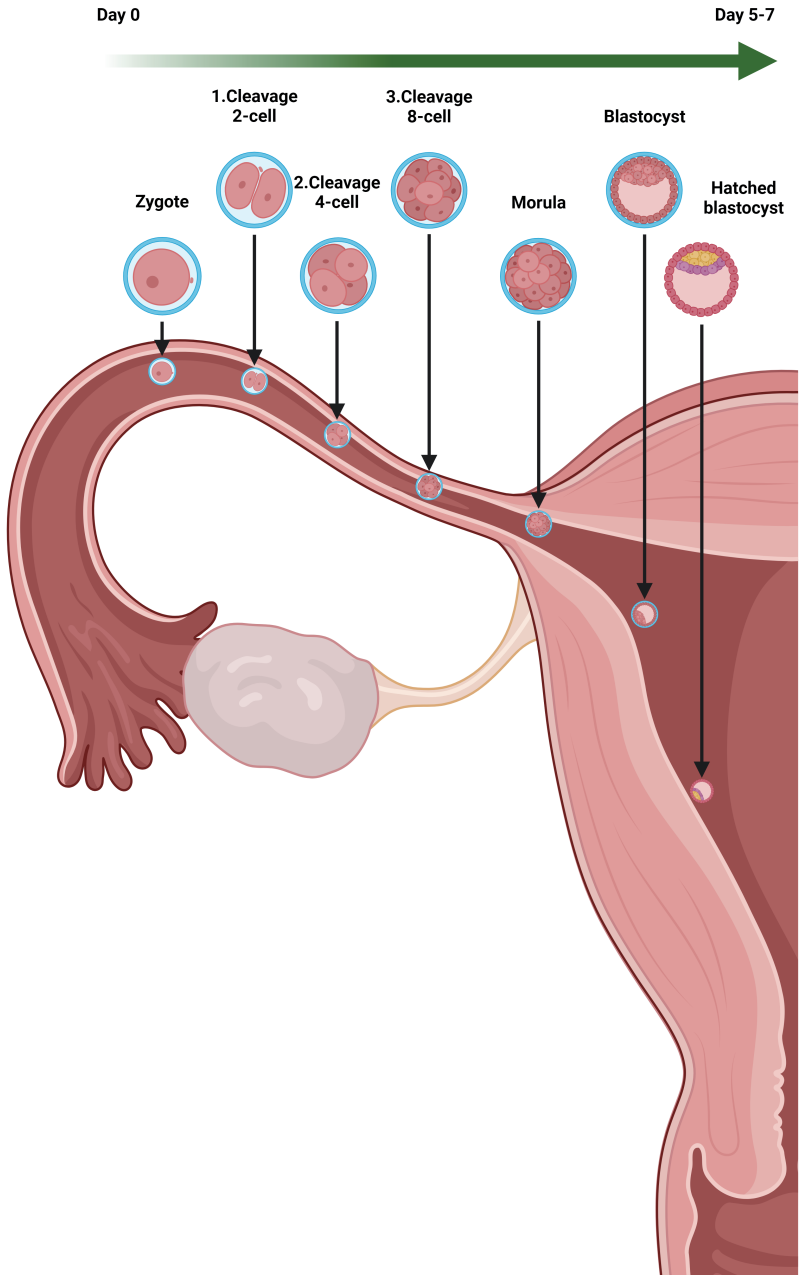

Figure 4. Embryo preimplantation development. After ovulation, the oocyte is fertilised in the oviduct, forming a zygote that undergoes several cleavages while moving toward the uterus. The zygote's genome is fully activated at the 8-cell stage, followed by compaction into a morula and subsequent transformation into a blastocyst. This blastocyst consists of trophoblasts, which form extraembryonic tissues, and an inner cell mass, which develops into the embryo. The blastocyst hatches from the *zona pellucida* and implants in the uterine wall 5–7 days after fertilisation. Created with BioRender.com

Until the blastocyst formation, the embryo is still the same size as the oocyte because cell dimensions decrease during the cleavage due to the new cell formation. In addition, the blastocyst is surrounded by a special dense extracellular matrix layer named *zona pellucida*. It protects the embryo and prevents blastocyst adhesion and implantation to the oviduct wall, which in the opposite case can lead to ectopic tubal pregnancy – a dangerous condition leading to embryo death and a life-threatening condition for a maternal organism. However, when the blastocyst reaches the uterus, it must escape from *zona pellucida* for successful implantation to the uterine wall. During the hatching, blastocyst trophoblast cells secrete trypsin-like protease, which digests holes in the zona pellucida, which in turn helps the expanding blastocyst to leave a tight envelope (Sathananthan, Gunasheela, and Menezes 2003; Syrkasheva et al. 2017). Hatched blastocyst interacts with the uterine wall's epithelial lining, leading to implantation 6–7 days after fertilisation (Rossant and Tam 2022; Gauster et al. 2022).

1.5.1. *In vitro* systems for studying embryo development

Due to the ethical and technical limitations of human embryo development studies, mentioned in the previous section, some *in vitro* systems were developed to overcome these circumstances. The main approach is to use corresponding cell lines to model uterine lining or embryo with invasive capacity. The most common cell lines representing endometrial epithelium are HEC-1-A, RL95-2 and Ishikawa (Hannan et al. 2010). Furthermore, corresponding trophoblast cell lines JAR and JEG-3 are used to produce spheroids that mimic invasive embryos. These cell lines were extensively used to investigate possible trophoblast-endometrium interactions (H. Wang et al. 2012; Xia Li et al. 2023). However, it needs to be mentioned that the cancerous origin of those cell lines can influence results and cannot be directly transferred to fundamental interactions that occur during embryo implantation. Primary endometrial stroma or epithelial cells grown in cell culture conditions stimulated with hormones corresponding to the modulated endometrial cycle can replace cancerous endometrial cell lines. However, cultured primary cells do not overcome all issues related to the reliability of i*n vitro* models because all *in vitro* cultured cell types change their physiology in culture conditions to adapt to an artificial two-dimensional growth environment (J. C. Chen et al. 2013; J. C. Chen and Roan 2015; J. C. Chen et al. 2016; A. Masuda et al. 2016; Riaz et al. 2024). The most recent approach to model endometrial tissue *in vitro* is to grow organoids from primary isolated stromal, epithelial, or both cell types. Organoids from primary endometrial cells in cell culture conditions most closely correspond to endometrial tissue under normal physiological state due to their ability to recreate the three-dimensional structure of the endometrial tissue (Xintong Li et al. 2022; Hewitt et al. 2023; Kleinová et al. 2024).

Developing an *in vitro* model that mimics the early human embryo is more challenging. Early embryo development shares similarities between different mammalian species, which can be transferred with some limitations to the human embryo. Mice embryos are used in most co-culture experimental models with

human endometrial tissue analogues, but embryos of other species are also studied (Aplin and Ruane 2017). For example, cattle embryos are also used to investigate the influence of IVF conditions on early development. However, there are also significant differences in physiology and developmental mechanisms between the species that need to be considered when those model organisms are used (Ménézo and Hérubel 2002; Tšuiko et al. 2017).

1.5.1.1. Embryoid bodies

An alternative approach to model a human embryo's early development is to use pluripotent embryonic stem cells originating from ICM. Stem cells can be induced to differentiate into trophoblast cells, or, under certain cell culture conditions, can form embryoid bodies (Ojosnegros et al. 2021). Embryoid bodies are three-dimensional organised clusters of pluripotent stem cells, which start to differentiate. Embryoid bodies can be induced to develop structures with a higher level of cell differentiation and structural organisation, which are named embryoids. Embryoids can mimic different early stages of human embryo development. Blastoids are embryoids with cavity and cell organisation and differentiation that resemble an embryo at the blastocyst stage (Ojosnegros et al. 2021; Kagawa et al. 2022). A similar model of gastrulating embryo is named gastruloid. An approach based on resembling early development stages with embryonic stem cells is the most promising model so far if we exclude work with actual human embryos grown up to 14 days (Simunovic and Brivanlou 2017; Ojosnegros et al. 2021).

1.6. Embryo implantation

When the endometrium is prepared for implantation, it achieves a receptive state named the window of implantation (WOI). During the WOI, the endometrium can interact for a limited time with the blastocyst hatched from *zona pellucida*, which follows with successful embryo implantation, placentation and further development. Human implantation occurs on 6–7 days after fertilisation. Implantation is a multi-stage process consisting of the following stages: apposition, adhesion or attachment and invasion or penetration. Already before implantation, embryo and endometrial epithelial cells start communicating at the molecular level (Aplin and Ruane 2017; Macklon and Brosens 2014; Craciunas et al. 2019).

1.6.1. Embryo implantation mechanisms

The first physical contact between the hatched blastocyst and receptive endometrium epithelial cell surface appears during the apposition, which is the first implantation step. During the apposition, the blastocyst can roll freely on the endometrial epithelial surface, and all receptor-ligand interactions are temporary

and reversible. Rolling on the endometrial surface, the blastocyst searches for the implantation site and orients into a position where ICM is closer to the endometrial epithelium (Ochoa-Bernal and Fazleabas 2020). The central receptor that plays an important role during the apposition is L-selectin. L-selectin binds reversibly to carbohydrate motifs, allowing blastocyst to roll on the endometrial surface and scan for a suitable implantation site. Thus, glycocalyx becomes very important during that step (Genbacev et al. 2003; Fazleabas and Kim 2003; Nejatbakhsh et al. 2012). Glycocalyx can also contain anti-adhesive molecules like MUC1, which hinder blastocyst attachment to the epithelial surface (Meseguer et al. 2001; Achache and Revel 2006). It is still not clear how a human blastocyst chooses an implantation site. Pinopodes – large cell membrane protrusions on epithelial cell surface that present suitable receptors-ligands motifs for blastocyst adhesion may play an important role in this process (Pantos et al. 2004; Jin et al. 2017).

The next implantation stage is blastocyst adhesion or attachment to the endometrial epithelium surface. The main difference from the apposition step is that the adhesion step cannot be reversed because molecular interactions during the blastocyst attachment are irreversible. It is still unclear how the blastocyst locates a suitable site for implantation (Ochoa-Bernal and Fazleabas 2020). It is supposed that some cytokines may work as an attractant for blastocysts. The most extensively studied in this context is a leukaemia inhibitory factor (LIF), which is considered important for successful embryo implantation in some studies. Pinopodes can also play a role in the localisation of the implantation site by exposing suitable receptors or ligands for blastocyst attachment (Nachtigall et al. 1996; Aghajanova et al. 2003; Kimber 2005). These molecules can be hidden oppositely under a thick layer of mucins and are poorly accessible for blastocyst without membrane protrusions from the epithelial surface. However, a blastocyst can penetrate through the glycocalyx layer to some extent by cleaving, for example, MUC1 and unmasking the epithelial surface (Meseguer et al. 2001). The primary receptors that play a role in blastocyst attachment belong to the integrin family. After integrins activation, the adhesion step is irreversible, and the blastocyst cannot change its location on the endometrial surface further (Lessey et al. 1995; Kimber and Spanswick 2000; Reddy and Mangale 2003; Singh and Aplin 2009).

After the blastocyst attachment, the invasion or penetration stage occurs. During the invasion, blastocyst penetrates through the endometrial epithelial surface into the decidualised stroma for further placentation. Trophoblast cells of the invasive blastocyst form invadopodia, the type of filopodia which secrete proteases from the tips and then penetrate between the endometrial epithelial cells. Trophoblast degrades basal membrane ECM proteins, allowing attachment and penetration into deeper layers of the endometrial stroma. Trophoblast cells start to proliferate and differentiate, forming two subgroups of cells: cytotrophoblasts, which will form villi in future, and syncytiotrophoblasts, which will cover the placenta (Bischof and Campana 1996; Giudice 1999; Carson et al. 2000; Fitzgerald et al. 2008).

1.6.2. Embryo implantation biomarkers

In clinical practice, the need for precise endometrial dating for WOI detection is increasing due to the widespread use of IVF in infertility treatment worldwide. The concept of the receptive endometrium is based on the theoretical assumption that WOI can be defined and reached at a predictable time that will be most suitable for embryo transfer and will support pregnancy development (Craciunas et al. 2019). The endometrial epithelial cell surface is the first site of physical interaction between the hatched blastocyst and the maternal organism. Endometrial tissue cells that respond to hormonal regulation can interact with blastocyst for a limited period of 6–7 days after fertilisation. This narrow time interval, when the endometrium is receptive, was first established in rodents but later confirmed in other species in the mid of 20th century (Hertig, Rock, and Adams 1956; Psychoyos 1986; Enders, Hendrickx, and Schlafke 1983; Aplin and Stevens 2022).

The first attempts to obtain endometrial dating were based on morphological features changes followed during the endometrium cycle. Based on Noyes's work, morphological criteria for endometrial dating evaluation were developed. This classical approach is still widely used for endometrial biopsy dating (Noyes, Hertig, and Rock 1950). Attempts were made to develop an immunohistological dating approach that combines tissue morphological parameters with receptivity biomarkers expression scoring – HSCORE. Integrins were used as receptive endometrium markers (Fuhrich, Lessey, and Savaris 2013; Germeyer et al. 2014). Integrins are transmembrane heterodimeric cell receptors responsible for interacting with various components of ECM or, in some cases, cell-cell adhesions and regulating cell shape, motility, signal transduction and cell cycle (Hynes 2002). The histological approach is suitable for endometrial cycle dating, but for precise WOI determination, it is not suitable. As mentioned above, the luminal epithelial cell surface is a site of initial contact and embryo attachment, but morphological changes are less pronounced in those cells (Aplin and Ruane 2017; Aplin and Stevens 2022). Pinopodes were also investigated as possible receptivity cellular morphological markers. Despite the promising results, especially in the case of patients with recurrent implantation failure (RIF), the main disadvantage of pinopode quantification is the need to use a complicated scanning electron microscopic technique (Jin et al. 2017; Qiong et al. 2017). However, with the development of technology, it became possible to use omics approaches for endometrial dating. The first studies were based on microarray investigation of endometrial transcriptome. The primary hypothesis was that transcriptome would reveal the molecular fingerprint of the receptive endometrium during the menstrual cycle (Popovici, Kao, and Giudice 2000; Brar et al. 2001; Díaz-Gimeno et al. 2011; Ruiz-Alonso et al. 2013). When RNA sequencing became available for broader usage, it was also applied to find endometrial receptivity fingerprint (Altmäe et al. 2017). However, smaller gene selection panels were also developed based on the classical RT-qPCR technology (Enciso et al. 2018; 2021; Haouzi et al. 2021). Like transcriptome analysis – endometrial proteome was also researched to identify receptivity or implantation biomarkers on the protein level (J. Evans et al.

2020). Endometrial biopsy contains a complex mixture of different cell types, which vary depending on the sampling depth, biopsy size and location on the uterine wall. Proportions between the stromal, glandular epithelium, luminal epithelium, vascular and different blood cell types may vary significantly, affecting endometrial dating (Aplin and Stevens 2022). Single-cell transcriptome analysis may overcome problems related to inconsistent biopsy cell composition. However, for now, the single-cell transcriptomic profile confirms the presence of known different cell types in biopsies from previous morphological studies: stromal cells, glandular and luminal epithelial cells with and without cilia. We do not know how receptivity may be affected by different ratios of ciliated and non-ciliated cells in the endometrial epithelium. Thus intensive research needs to be done to clarify that (W. Wang et al. 2020; Garcia-Alonso et al. 2021; Aplin and Stevens 2022). Another approach is to replace invasive biopsy-based endometrium dating with a more patient-friendly sampling of uterine secretome by analysing uterine flushings or extracellular vesicles containing uterine fluid (L. Wang et al. 2020; Kasvandik et al. 2020; Giacomini et al. 2021; Aplin and Stevens 2022; He et al. 2023).

In clinical practice, classical endometrial morphological parameters like endometrial thickness, volume, and histological evaluation were used for WOI determination. However, none of these parameters were sensitive or specific enough to increase clinical pregnancy rates (Craciunas et al. 2019). In recent years, the transition to molecular methods has taken place, and some commercially available receptivity tests have been developed, like The Endometrial Receptivity (ER) Map and ERA tests that were introduced to clinical practice (Díaz-Gimeno et al. 2013; Ruiz-Alonso et al. 2013; Enciso et al. 2018; 2021). An ERA is the most widely used omics-based test for endometrial receptivity assessment; initially, it was developed as an RNA expression microarray but later transferred to RNA-seq technology (Díaz-Gimeno et al. 2017). ERA test also passed clinical trials where test developers claimed that personalised embryo transfer with previous endometrial receptivity assessment by ERA test is more efficient and leads to increased pregnancy and live birth rates compared to frozen and fresh embryo transfer protocols (Simón et al. 2020). However, there is also a critical point of view on ERA test usage in all IVF patient groups and the superiority of personalised embryo transfer over conventional fresh or frozen embryo transfer cycles. More clinical trials are needed with larger patient cohorts and independent assessment of results to resolve that dispute (Scott 2021; Franasiak et al. 2021; Ben Rafael 2021; Aplin and Stevens 2022). Our understanding of endometrial receptivity is far from complete due to the complexity of molecular interactions and pathways in cyclical endometrial tissue, where cycles vary between women and different cycles of the same woman. Common endometrial pathologies like endometriosis and personal genetic background make it even more challenging to determine what receptive endometrium is and how to translate that knowledge into clinical practice (Franasiak et al. 2021; Lessey and Young 2019; Aplin and Stevens 2022). New molecular markers, pathways, and interactions between the endometrium and the implanting embryo must be

investigated in order to expand our knowledge of that process. IVF's father, Sir Robert Geoffrey Edwards, named endometrium the last barrier we must cross in assisted reproductive technologies. Thus, movement in this direction continues (Edwards 2006; Lessey and Young 2019; Aplin and Stevens 2022).

2. AIMS OF THE STUDY

The primary objective of the current doctoral thesis is to enhance our understanding of the molecular mechanisms governing female fertility by using the endometrial and embryonal models. This study concentrates on exploring adhesion and signalling pathways regulating endometrial function and embryo development, as modelled by the development of embryonic stem cell-derived embryonic bodies. The research has the potential to offer an updated view of molecular mechanisms of endometrial and early embryonal biology that are required to advance infertility diagnostics and improve the results of assisted reproduction. Ultimately, these studies will also advance our understanding of the complex molecular interplay between the receptive endometrium and the embryo, which is crucial for creating a favourable environment for embryo implantation and a critical milestone in establishing a conception.

The specific aims were as follows:

1) To study the expression pattern of mucins in the human endometrium and establish their roles in cell adhesion and as signalling molecules.
2) To investigate the function of MUC20 and its molecular interaction network in the human mid-secretory receptive endometrium.
3) To study the landscape of kinase activities in the human endometrium during the decidualisation process, which prepares the endometrium for blastocyst implantation.
4) To investigate the role of the ROCK2 kinase signalling pathway in the stromal cells of human endometrium.
5) To identify molecular factors affecting the adhesion of human embryonic stem cells and the formation of embryoid bodies in cell culture conditions.

3. METHODS

This section provides a brief summary of the key methods, sample collection protocols, and data analysis techniques employed in the studies, forming the basis of this dissertation. While the articles provide detailed protocols, this section focuses mainly on the methods directly implemented by the author. While all detailed information regarding chemicals, kits, and devices used in the experimental processes can be found in the corresponding articles, this section focuses on the methodological framework that guided the research. Technical aspects not central to the dissertation's focus are omitted but are fully detailed in the articles.

3.1. MUC20 expression in the human endometrium (Ref. I)

3.1.1. Study participants

The study was approved and prolonged by the Research Ethics Committee of the University of Tartu (protocol No 221/M-31; prolongation protocol No 276/M-15). Written informed consent was obtained from all participants. Endometrial biopsies were obtained from healthy volunteers at fertile age (≤35 years) with normal body mass index (BMI) (within a range of 19–25 kg/m^2). All women selected for the study had a regular menstrual cycle, were clinically examined by ultrasound for the absence of visible pelvic pathologies and polycystic ovary syndrome, and had no symptoms or complaints of endometriosis. The testosterone and prolactin levels measured from the blood plasma corresponded to the normal values of reproductive aga women. The level of progesterone measured from the blood plasma samples collected in the mid-secretory phase of the menstrual cycle corresponded to the expected levels at that cycle phase. The women were non-smokers, had not taken any hormonal treatments for 3 months before the study, had no previous infertility record, and had at least one live-born child. Endometrial biopsies were obtained using a Pipelle catheter on days 2 (LH2) and 8 (LH8) after the LH surge within the same natural cycle. Menstrual cycle dating was confirmed by combining menstrual cycle history and LH peak estimation by the BabyTime LH urine cassette, vaginal ultrasound and histological evaluation of biopsy according to the Noyes' criteria (Noyes, Hertig, and Rock 1950). Altogether, 10 women were recruited in whom mucins expression levels were detected in whole endometrial tissue biopsies, and 26 women were recruited in whom mucins expression levels were measured in sorted endometrial stromal and epithelial cells. An immunohistological evaluation was carried out on six additional women.

Western blot analysis was carried out on cultured primary epithelial and stromal cells obtained from two women. Two additional biopsies were obtained to isolate primary endometrial epithelial and stromal cell cultures. The first endometrial biopsy (proliferative phase, cycle day 12) was obtained from a 44-year-old woman (reproductive history: three pregnancies and three deliveries) with endometriosis (stage 3) undergoing laparoscopy at the Tartu University

Hospital Women's Clinic. The woman had a regular menstrual cycle (28±5 days), BMI 21 kg/m^2 and used no hormonal medications during the previous 3 months before laparoscopy. The second biopsy (early secretory phase, cycle day 19) was obtained from a 35-year-old woman (reproductive history: one pregnancy and one delivery) with secondary infertility undergoing laparoscopy at the Tartu University Hospital Women's Clinic. No signs of pelvic abnormalities (no adhesions, normal size of uterus and ovaries) or endometriosis were detected during laparoscopy. The woman had a regular menstrual cycle (28±5 days), BMI 26 kg/m^2 and used no hormonal medications during the previous 3 months before laparoscopy. The endometrial biopsy samples were collected during the laparoscopy using an endometrial suction Pipelle catheter. The simplified menstrual cycle scheme shows all samples used in our study (Figure 5).

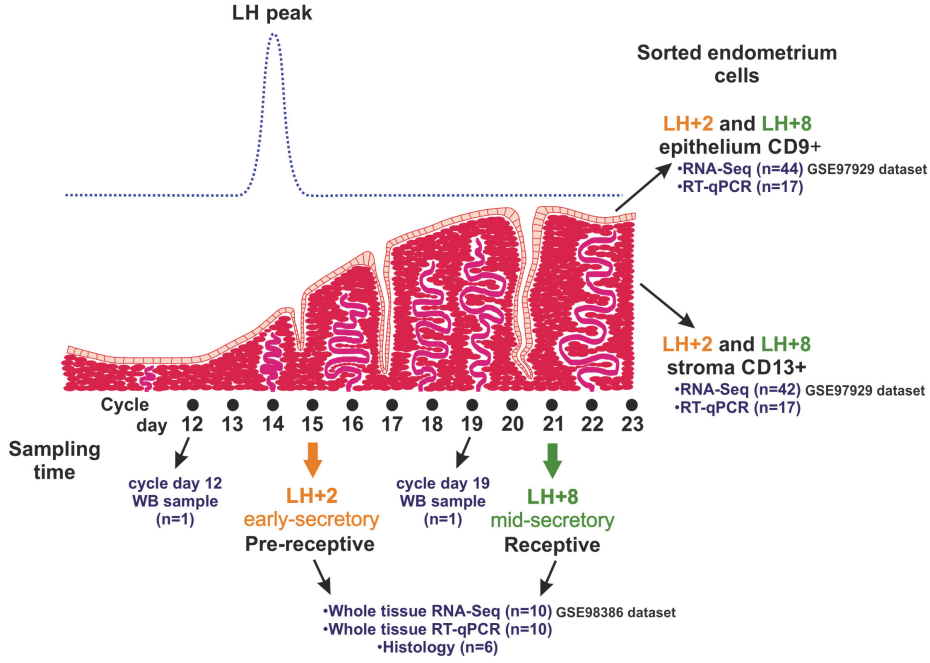

Figure 5. Scheme representing all samples used in (Ref. I) study. During the same cycle, endometrial biopsies were obtained on days 2 and 8 after LH surge (LH+2 and LH+8). Paired samples (LH+2 and LH+8) from 10 women were used for whole tissue analysis, and samples from 26 different women were used for endometrial epithelial and stromal cell analysis. Of the possible 104 samples from 26 women, the numbers suitable for RNA-seq and further validation were 18 for LH+2 epithelium, 26 for LH+8 epithelium, 20 for LH+2 stroma and 22 for LH+8 stroma. CD9 was considered as an epithelial marker, and CD13 as a stromal marker. Two separate biopsies were obtained on cycle day 12 and cycle day 19 for primary cell culture and further protein analysis. The number of analysed samples is shown in brackets (n). LH – luteinizing hormone, LH+2 – endometrial sample collected two days after luteinising hormone surge, LH+8 – endometrial sample collected eight days after luteinising hormone surge, CD – cluster of differentiation, RT-qPCR – quantitative reverse transcription polymerase chain reaction, RNA-Seq – RNA sequencing, WB – Western blot. (Modified Figure 1 in Ref. I).

3.1.2. Processing of biopsies

The endometrial tissue biopsies for the whole tissue study were placed into RNAlater solution and stored at –80 °C for further analysis. For cell–type specific analysis, the endometrial tissue samples were placed immediately into the cryopreservation medium (the exact composition is given in the article). The cryovials were put into a "Mr. Frosty" freezing container, placed into a –80 °C freezer overnight, and stored in liquid nitrogen until use. Total RNA from whole tissue was extracted using miRNeasy Mini kit following the manufacturer's protocol. Handling, dissociation and preparation of endometrial biopsies for fluorescence-activated cell sorting (FACS) have been described in detail (Krjutškov et al. 2016). Biopsied tissue samples were thawed and dissociated, and endometrial cells were stained with fluorochrome-conjugated antibodies. Stromal cells were stained with mouse anti-human CD13 monoclonal antibody, epithelial cells were stained simultaneously with mouse anti-human CD9 monoclonal antibody and all dead cells were stained with DAPI. CD13 or CD9 positive and DAPI negative (alive) cells were sorted directly into the QIAzol lysis reagent. Total RNA was isolated immediately using the RNeasy Micro kit.

The paired samples of (LH+2) and (LH+8) endometrial tissue biopsies from six healthy volunteers were used in immunohistochemistry (IHC). Tissue samples for histological assessment and IHC analysis were fixed and stored in a 10% formalin solution. Two separate biopsies for Western blot analysis were cryopreserved using the methodology mentioned earlier.

3.1.3. Transcriptome data analysis

Two of our previously published RNA sequencing (RNA-seq) datasets were used to determine the expression of mucins in the whole endometrium and sorted epithelial and stromal cells. Both datasets are accessible via Gene Expression Omnibus under accession numbers GSE98386 and GSE97929, respectively. The paired-end 100bp sequencing on the Illumina HiSeq2500 instrument was used for transcriptome profiling for whole tissue samples (GSE98386) for 10 women, as described in our previous study (Altmäe et al. 2017). The cell-type specific RNA-seq analysis was conducted with sorted epithelial and stromal cells (GSE97929) for 26 women, as described in the same study, following our single-cell tagged reverse transcription protocol with modifications for bulk RNA (Krjutškov et al. 2016).

In data analysis, the STRTprep pipeline (v3dev branch, available at https://github.com/shka/STRTprep) was used for pre-processing and differential transcriptome expression analyses. This pipeline uses the SAMstrt, which includes the variation of significance analysis of microarrays statistical test, adjusted to RNA-seq data (Katayama et al. 2013). As this is a non-parametrical statistical test, it is more robust to outliers and deviations from the assumptions of parametrical methods (J. Li and Tibshirani 2011).

3.1.4. Quantitative reverse transcription polymerase chain reaction

The *MUC1* and *MUC20* expression levels in whole endometrial biopsies were determined in 10 paired LH+2 and LH+8 endometrial samples. The cell-type specific expression pattern was determined in 17 out of 26 paired (LH+2 and LH+8) endometrial epithelial and stromal cell samples isolated by the FACS method described previously (Krjutškov et al. 2016). DNAse-treated RNA was converted into cDNA using the RevertAid First Strand cDNA Synthesis Kit. In accordance with conditions specified by the manufacturer, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was conducted using 1xHOT FIREPol EvaGreen qPCR Mix Plus. *MUC1* and *MUC20* primers were designed for analysis, and *SDHA* was used as an endogenous control. The expression differences between LH+2 and LH+8 were calculated using a Welch two-sample t-test and a P-value cut-off of $P < 0.05$. The $2^{-2\Delta\Delta Ct}$ method was used for calculating the relative expression and the fold change between LH+2 and LH+8 samples (Livak and Schmittgen 2001).

3.1.5. Cell culture

For isolation of primary cultures, the cryopreserved endometrial biopsy samples were thawed and washed twice with DMEM medium. The isolation and culturing of endometrial primary stromal and epithelial cells were carried out as described previously with minor modifications (J. C. Chen and Roan 2015). In our protocol, the primary endometrial epithelial cells were plated on fibronectin-coated flasks, and a trypsin-based detaching method was used for both stromal and epithelial cells. The primary epithelial cells' first passage and the stromal cells' second passage were used for protein isolation. MCF-7 and A431 cell lines were obtained from ATCC and cultured according to the received specifications (detailed description provided in the article). MCF-7 cell line lysate was used as a negative control, and A431 cell line lysate was used as a positive control for MUC20 protein expression in Western blot experiments. Cells were lysed in RIPA buffer containing Complete Protease Inhibitor Cocktail (exact composition is given in the article) and stored at –80 °C.

3.1.6. SDS-PAGE and Western blotting

The protein concentrations of samples in RIPA were measured with a BCA Protein Assay Kit, and equal protein amounts for each sample were loaded onto 4–10% gradient SDS-PAGE gels for electrophoresis. The material was transferred to a polyvinylidene difluoride membrane with a Mini Trans-Blot Cell system. The membranes were washed and blocked for 1 h with 2% (w/v) bovine serum albumin, dissolved in washing buffer. Further membranes were incubated overnight at 4 °C with primary anti-MUC20 antibody in the blocking solution. After

washing and additional blocking for 30 min with 2% (v/v) normal goat serum in the washing solution, membranes were incubated with corresponding secondary antibodies conjugated with horseradish peroxidase for 1 h at room temperature. Finally, membranes were rinsed three times for 15 min with washing buffer (all solutions and antibodies described in the details in the article). Membranes were incubated with Immobilon Western Chemiluminescent HRP Substrate solution, and a chemiluminescent signal was detected with BioSpectrum 510 Imaging System with VisionWorks LS software.

3.1.7. Immunohistochemistry

For immunohistochemical (IHC) experiments, 4-µm sections from paraffin-embedded tissue blocks were mounted on Superfrost Plus slides. Tissue sections on slides were deparaffinized and rehydrated according to standard protocol. Subsequently, sections were subjected to the antigen retrieval procedure. For antigen retrieval, slides were heated at 60 °C for 16 h in 10 mM Na-citrate buffer (pH 6.0) with 0.05% (v/v) Tween 20. After antigen retrieval, slides were cooled to room temperature and washed with tap water for 10 min. All following washing steps were done using 1×Tris-buffered saline (TBS) with 0.025% (v/v) Triton X-100 as a washing buffer. For the IHC procedure, a mouse and rabbit-specific HRP/DAB (ABC) detection kit was used. All steps were done according to the producer's protocol with only a few modifications. After protein non-specific blocking and washing steps, we applied additional blocking against possible tissue endogenous biotin. For that purpose, the endogenous avidin-biotin blocking kit was used. Mouse polyclonal antibodies at a concentration 5 µg/ml, obtained by immunisation against the full-length protein, were used as primary anti-MUC20 antibodies in IHC. Mouse IgG_1, IgG_{2A}, IgG_{2B}, and IgG_3 subclasses were mixed and used for isotype control at the same concentrations as the primary antibody. Primary antibody and mouse non-specific IgG subclasses were incubated overnight in a humidity chamber at 4 °C. For antibody dilution and incubation, 1×TBS buffer with 1% (w/v) BSA was used. After incubation with the primary antibody, we performed an additional blocking step with 4% (v/v) normal goat serum diluted in 1×TBS buffer. Further steps were performed according to the kit protocol. The chromogenic reaction was developed for 6 min and stopped after that. Cell nuclei were counterstained with Mayer's haematoxylin solution for 1 min, and slides were then washed for 10 min with tap water. Slides were dehydrated through 30 sec incubation in different ethanol and xylene solutions in the opposite order of the rehydration step. Cover glasses were mounted, and slides were investigated under an Olympus BX41 microscope with 10× and 40× magnification objectives, and tissue microphotos were taken with an Olympus DP71 camera and Cell B software. Semi-quantitative analysis of IHC (n=6) was performed with ImageJ package Fiji version 1.52e (Schindelin et al. 2012). DAB signal intensity was measured separately for epithelial and stromal components of the endometrium. The relative DAB intensity was calculated according to the formula: f=255-i, where f – relative DAB intensity, i – mean DAB intensity

obtained from the software; i ranges from 0 (zero – deep brown, highest expression), and 255 (total white). The method we used for the signal intensity measurements with ImageJ has been previously described for endometrial histological evaluation and consists of 10 image analysis steps precisely described in the following publication (Fuhrich, Lessey, and Savaris 2013).

3.1.8. Statistical analysis

Mann-Whitney U test or Welch two-sample t-test assessed gene expression for significant differences between the pre-receptive (LH+2) and receptive (LH+8) phases using RNA-seq or qRT-PCR data, respectively. For correlation analysis, the changes in the *MUC20* gene expression (log_2(fold-change) between LH+2 and LH+8) were compared with the changes in the expression of other epithelial cells' genes. We used the Spearman correlation test (Bonferroni corrected $P<0.05$, $R>0.8$) for that purpose. The enrichment of receptivity biomarkers in the human genome or in the *MUC20*-correlated gene subset was compared using Fisher's exact test. Statistical analyses and graphs were made in R (version 3.5.0). For semi-quantitative IHC analysis, DAB signal intensities were compared using a paired t-test with a statistically significant p-value cut-off of $p<0.05$. Statistical analysis and graphs were prepared in Microsoft Office Excel (365 ProPlus, version 1809).

3.2. ROCK2 mediated changes in the decidualised endometrial stroma (Ref. II)

3.2.1. Study participants characterisation and sample collection

Endometrial tissue samples were collected from 22 patients undergoing laparoscopy at the Tartu University Hospital Women's Clinic (Estonia) and 23 healthy women at the Department of Obstetrics and Gynecology, Oulu University Hospital (Finland). Only women who had not received any hormonal medications or contraception at least 3 months before surgery and sample collection were enrolled in this study. The study protocols and sample collection were approved by the Research Ethics Committee of the University of Tartu (Approval 276/M-13) and the Northern Ostrobothnia Hospital District ethics committee. All participants signed a written informed consent. The experiments were conducted in accordance with the principles of the Declaration of Helsinki. The collected samples were subdivided and further used in the study. The PCOS patients covered in the article are not part of the main scope of the current thesis and are not covered here in detail.

3.2.2. Isolation and culturing of endometrial stromal cells

Biopsies collected from two independent centres were processed according to a slightly different protocol at each centre, covered in the details in the corresponding article. However, a brief summary of the biopsies processing is given below. The protocol used for processing samples provided by the University of Oulu implies immediate digestion of biopsies and isolation of the endometrial stromal cells. The protocol of biopsies digestion and separation of the epithelial and stromal cells is based on previously published articles featuring some of the same authors (J. C. Chen et al. 2013; 2014). Further endometrial stromal cells were cultured until the desired confluency was reached. The same cell culture conditions were developed and used in previous studies (Piltonen et al. 2015; Khatun et al. 2020). Biopsies collected at the University of Tartu were subdivided, immersed into the cryopreservation medium, cooled down at –80 °C freezer overnight, and stored in liquid nitrogen until further use. This biopsies storage protocol was described in detail in the previously published work (Rekker et al. 2018). When stromal cells were needed for the research, tissue samples were thawed, washed and enzymatically dissociated. Stromal cells isolation was also previously described (Kasvandik et al. 2016). The purity of isolated stromal cells was verified by immunofluorescence analysis using an anti-cytokeratin 8/18 antibody as a negative control (epithelial marker) and an anti-vimentin antibody as a positive control (stromal marker).

Before the *in vitro* decidualisation studies, isolated endometrial stromal cells were cultured for two passages. After reaching confluency, the cells were seeded onto 6-well plates with a density of 100 000 – 170 000 cells per well; after 1–3 days, the growth medium was replaced with starvation medium (2% ccFBS), and after additional 24 hours, treatment was started. The solutions of hormones and chemicals in a starvation medium were applied. The *in vitro* decidualisation protocol lasted for 4 or 9 days; in the case of 9-day experiments, the solutions were replaced after 72 hours, and the collected aliquots of media were stored at –20 °C until further analysis. For the initial comparison of *in vitro* decidualisation protocols in control endometrial stromal cells, cells from a single individual were used and subjected to 6 different treatments in each independent experiment.

3.2.3. Prolactin concentration measurement

The total protein concentration in collected media aliquots was measured using Pierce™ Coomassie Plus Assay Reagent according to the manufacturer's instructions. The quantification of secreted prolactin content in media samples was done using the human PRL ELISA kit with subtle modifications in the manufacturer's protocol (80 µl of a standard or media sample and 80 µl of the antibody mixture were added to each well).

3.2.4. Protein kinase activity assay

The *in vitro* decidualised cells were rinsed with PBS, placed on ice and lysed directly in 6-well plates using the following lysis buffer: 50 mM HEPES pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1× protease inhibitor cocktail, and 0.5 mM PMSF. The non-cultured endometrial stromal cells isolated from the *in vivo* decidualised tissue were pelleted by 6 min centrifugation (205 g) at room temperature (rt), rinsed with PBS and lysed on ice using 100 µl of the aforementioned lysis buffer per pellet. In each independent experiment, one sample from the proliferative and one sample from the secretory phase was used. After 1–2 h lysis, the membranes were pelleted by centrifugation (10 000 *g*, 20 min, 4 °C) and the supernatants were collected.

The total protein concentration in obtained lysates was measured using a Coomassie Plus Assay Kit according to the manufacturer's instructions. Within each experiment, the total protein concentration of all collected lysates was equalised by suitable dilution with kinase assay buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 0.005% Tween-20, 5 mM dithiothreitol, and 0.5 mg/mL BSA fraction V). The equilibrium binding/displacement assay was carried out as previously described using the 2 nM final total concentrations for ARC-1139 (in case of PKA, ROCK, and Akt/PKB activity measurements) and ARC-1530 (in case of CK2 activity measurements) (Sinijarv et al. 2017; Khatun et al. 2020). For selective quantification of catalytic activity of the target protein kinase in lysates, competitive displacement of photoluminescent probe ARC-1139 or ARC-1530 with an excess of a well-characterized ATP-competitive selective inhibitor was performed (Enkvist et al. 2011; Vahter et al. 2018). The following inhibitors were used (the final total concentrations in the assay mixture are indicated in brackets): H89 (2 µM, 4 µM) for PKA studies, Y-27632 (2 µM, 4 µM) for ROCK studies, GSK690693 (0.2 µM, 0.4 µM) for Akt/PKB studies, and CX-4945 (2 µM, 4 µM) for CK2 studies. The same concentrated stock inhibitors were added sequentially into the same well, with measurements taken 30–60 min after each addition.

3.2.5. Mass spectrometry

Sample preparation for proteomics and phosphoproteomics is described in detail in the article. However, brief mass spectrometry settings are described as follows. Peptides were desalted with in-house-made C18 StageTips and reconstituted in 0.5% trifluoroacetic acid, according to the published method (Rappsilber, Mann, and Ishihama 2007). Phosphoproteome analysis and sample preparation were performed as described by the EasyPhos protocol (Humphrey, Azimifar, and Mann 2015). Samples were injected into a C18 cartridge trap-column from where they were eluted to an analytical C18 column. Peptides were separated at 200 nL/min (for phosphopeptides: 250 nL/min) with a 5–40% eluent B 240 min gradient in eluent A. A 90 min two-step separating gradient was used for the phosphopeptides, consisting of 5–15% 60 min and 15–30% 30 min steps. Eluent B was

80% acetonitrile + 0.1% formic acid, and eluent A was 0.1% formic acid in water. Eluted peptides were sprayed to a quadrupole-orbitrap MS/MS using a nano-electrospray source and a spray voltage of 2.5 kV (liquid junction connection). The MS instrument was operated with a top-10 data-dependent acquisition strategy. One 350–1400 m/z MS scan (at a resolution setting of 70 000 at 200 m/z) was followed by an MS/MS (R=17 500 at 200 m/z) of the 10 most intense ions using higher-energy collisional dissociation fragmentation (normalized collision energies of 26 and 27 for regular and phosphopeptides, respectively). For the total proteome analysis, the MS and MS/MS ion target and injection time values were 3×10^6 (50 ms) and 5×10^4 (50 ms), respectively. The MS and MS/MS ion target and injection time values for phosphopeptides were 1×10^6 (60 ms) and 2×10^4 (60 ms), respectively. The dynamic exclusion time was limited to 45 s and 70 s for phosphopeptide and full proteome samples, respectively. Only charge states +2 to +6 were subjected to MS/MS, and for phosphopeptides, the fixed first mass was set to 95 m/z.

3.2.6. Tissue immunofluorescent analysis

In brief, tissue sections were prepared using a standard procedure described below. Endometrial tissue biopsies were washed thrice with HBSS to remove excess blood and mucus from the samples, followed by fixation using 4% (w/v) PFA solution in PBS for 2 h at rt. The samples were then washed 3 × 10 min with PBS and dehydrated through a series of incubations in different sucrose solutions in PBS. The next day, biopsies were immersed in a 2:1 mixture of 20% sucrose and Tissue-Tek® O.C.T.™ compound, positioned in the cryomolds filled with Tissue-Tek® O.C.T.™ compound, and frozen 2-methylbutane cooled in liquid nitrogen. Frozen tissue blocks were covered with foil and stored at the –80 °C. Before sectioning, cryomicrotome was preincubated at –15 °C and frozen tissue blocks were then transferred into the apparatus to allow temperature equilibration. Cryomicrotome was set to cut 10 µm thick tissue slices, which were collected on microscope slides and dried for some hours at rt. The obtained slides with tissue samples were stored at –80 °C before further processing.

The slides with tissue sections were thawed and dried at rt after storage. Next, a hydrophobic barrier was drawn around a tissue sample, and the slides were washed 3×3 min with PBS. The subsequent incubations were carried out in the humidity chamber to avoid tissue drying. Endometrial tissue was permeabilized by 10 min treatment with 0.1% Triton X-100 in PBS (v/v) at rt. The washing procedure was then repeated, followed by 1 h blocking at rt with 1% BSA (w/v) in PBS. After 3 min wash with PBS, the solution of primary antibody (final concentration of 5 µg/mL) in 0.1% BSA/PBS (w/v) was applied, and samples were incubated overnight (16 h) at 4 °C. Next day, the slides were washed 4 × 3 min with PBS and the solution of secondary antibody (1:500, 4 µg/mL) in 0.1% BSA/PBS (w/v) was applied for 1 h at rt in the dark. The washing procedure was repeated; subsequently, the slides were incubated with phalloidin-Alexa Fluor® 488 dilution (5 U/mL) in 1% BSA/PBS (w/v) for 20 min at rt in the dark.

The slides were washed 3 × 3 min with PBS, counterstained with 1 μg/mL DAPI solution in PBS for 5 min, and washed again 2 × 3 min with PBS. After the final three rinses with MilliQ quality water, the cover glasses were mounted, and the prepared IF slides were left to harden overnight at 4 °C in the dark prior to imaging. The negative control slides were prepared exactly as described above, except that only 0.1% BSA/PBS (w/v) was used instead of incubation with primary antibody solution.

The imaging was performed using a 60× magnification oil immersion objective with settings adjusted according to the negative control (no primary antibody) to achieve minimal non-specific signal from the secondary antibody. Images were taken in randomly selected areas (3–8 per slide) where microanatomical tissue integrity was preserved without artefacts. The Z-stack layer thickness was set to 0.5 μm, and the number of layers was set according to the tissue thickness. For further analysis, the Z-stack layer with maximal mean signal intensity in the phospho-cofilin channel was chosen, provided that the area of interest contained endometrial stroma.

3.2.7. *In vitro* decidualised endometrial stroma cells immunofluorescent analysis

Passage 2 endometrial stromal cells were seeded onto 24-well Ibidi black m-plates and treated for 9 days with E2 (10 nM) or decidualisation-inducing mixture (10 nM E2; 200 μM 8-Br-cAMP; 100 μM IBMX) with or without 100 nM P4. *In vitro* decidualised and control stromal cells were rinsed with PBS and fixed directly on the plate using 4% PFA solution in PBS for 10 min at rt. Next, cells were washed 2 × 3 min with PBS and permeabilised with 0.1% Triton X-100 in PBS (10 min, rt). After another 2 × 3 min wash, 1 h blocking at rt with 1% BSA in PBS was carried out, followed by overnight incubation with a solution of primary antibody (5 μg/mL) in 1% BSA/PBS (w/v) at 4 °C. Next day, the samples were washed 3 × 5 min with 0.1% Triton X-100 in PBS and incubated for 3 h with the solution of secondary antibody (1:1000) in 1% BSA/PBS at 4 °C in the dark. The washing procedure was repeated; subsequently, the samples were incubated with phalloidin-Alexa Fluor® 488 dilution (3 U/mL) in 1% BSA/PBS for 20 min at rt in the dark. Next, counterstaining with 300 nM DAPI solution in PBS for 5 min was performed; the samples were finally rewashed 2 × 5 min with PBS, sealed with parafilm and stored at 4 °C in the dark until imaging.

For quantification of pS3 cofilin-1 signal intensity, the imaging was done in a single plane using manual focussing; two images per well were taken, one at the centre of the well and one at a different randomly chosen location. The settings (LED intensity/signal integration time/camera gain) were as follows: for nuclear stain, 4/110/9; for phalloidin-Alexa Fluor® 488, 6/120/7; for anti-pS3 cofilin-1, 9/1008/22.

3.2.8. mRNA sequencing

The mRNA isolation from snap-frozen endometrial biopsies was done using the miRNeasy mini kit and the QIAcube workstation according to the manufacturer's protocol. The total mRNA integrity was checked by Agilent 2100 Bioanalyzer using Agilent RNA 6000 Nano Kit. Libraries were prepared from 10 ng of RNA extracted from each sample with the QIAseq UPX 3' Transcriptome Kit. Samples were indexed on the 384-well single-use Cell ID RT Plate during reverse transcription. Indexed samples were pooled, and the final library was performed according to the manufacturer's instructions. Illumina adapters 3' Trans P12 from the above kit were ligated to the insert. The quality control of the library was performed on a 2200 TapeStation system using D1000 ScreenTape and TapeStation Analysis Software. The library was sequenced on the NextSeq 500 instrument, using Mid Output Kit v2.5 in paired-end mode with 100 bp + 27 bp length. In total, 150 million raw reads were obtained. For the given study, only data for ROCK2 was further used.

3.2.9. Western blot

Denatured protein samples (10 µg of the total protein) were loaded onto 8% SDS-PAGE gels for separation. Further, the tank blotting method was used to transfer proteins to the PVDF membrane (0.2 µm pore size). The membranes were blocked for 1 h at rt with a mixture of PBS and 0.05% (v/v) Tween 20 (PBS-T) supplemented with 5% (w/v) non-fat dried milk. After 3 washes with PBS-T, the membranes were incubated with primary antibodies (final concentration of 0.1 µg/ml in blocking solution) for 1 h at rt. After 3 washes with PBS-T, the membranes were incubated for 1 h with secondary antibodies (diluted to 1:5000 in blocking solution). The washing procedure was repeated, and the membranes were subsequently incubated with a chemiluminescent HRP substrate solution. A chemiluminescent signal was detected by exposing the membranes to X-ray film.

3.2.10. Cell migration assay

Passage 2 endometrial stroma cells were seeded onto 6-well plates and treated with E2 (10 nM) or decidualisation-inducing mixture (10 nM E2; 100 nM P4; 10 µM Fsk; 100 µM IBMX) as described above. In each independent experiment, cells from a different single individual were used. On day 7 of treatment, cruciform scratches were made on the bottoms of wells with the tip of the cell scraper (BD Falcon^{TM}) oriented in the scraper position. The medium with cell debris was aspirated, the cells were washed 3 × with PBS, and fresh media was added (containing the components of the original treatment mixtures). Immediately after that, the first set of pictures (0 h time point) of the scratches were taken. ESCs were cultured further for 2 days; during that time, the second (24 h time-point) and the third (48 h time-point) set of pictures were taken in the same positions.

3.2.11. Data analysis

The main data collection and analysis methodologies are briefly described here. The supplementary methods section of the corresponding article provides statistical analysis and data normalisation details for each experimental method (Ref. II). For general data analysis, GraphPad Prism 6 and Excel 2016 were used. MS raw data were processed with the MaxQuant (version 1.6.1.0) software package (Tyanova, Temu, and Cox 2016). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium *via* the PRIDE partner repository with the dataset identifier PXD026243 (Perez-Riverol et al. 2019). Imaris x64 (ver. 7.6.5) and Auto Quant X3 (ver. X3.0.4) were used for tissue microscopy data analysis. The image analysis of IF experiments with *in vitro* decidualised stromal cells was done using ImageJ software (Fiji package) (Schindelin et al. 2012). The mRNA sequencing data analysis is described under the supplementary methods. For signal quantification in Western blot, densitometry of the X-ray film images was performed using UVP™ Software (VisionWorks™ LS, ver. 8.0RC1.2) area density analysis.

In the case of all methods, at least three independent experiments were performed. Throughout the study, the grouped comparisons were carried out using 1-way ANOVA with Dunnett's test for multiple comparisons; unless indicated otherwise, the pairwise comparisons were carried out using the unpaired two-tailed t-test with Welch's correction. In all statistical tests throughout the study, the significance of comparisons is indicated as follows: *** indicates $P \leq 0.001$, ** indicates $P \leq 0.01$, * indicates $P \leq 0.05$.

3.3. The role of integrin β1 in the human embryonic stem cell culture (Ref. III)

3.3.1. Cell culture

This study used a commercially available human embryonic stem cell line (WA09 – H9). No *in vivo* experiments using animals or human subjects were performed, and therefore, approval from an ethics committee was unnecessary. According to the manufacturer's specifications, the H9 cell line was maintained on Matrigel-coated plates in a mTeSR1TM maintenance medium. The medium was replaced daily. After 3–4 days of growth, the colonies were detached mechanically using a micropipette tip (manual scraping technique) or dissociated with 10 mM EDTA in PBS for 3 min. After breaking up the colonies into smaller parts with gentle pipetting, the hES cell clumps were plated onto separate new Matrigel-coated plates. In the integrin β1 blocking experiments, the neutralising antibody against integrin β1 P5D2 was added to the cell culture media. A normal karyotype of cells was confirmed using G-banding.

3.3.2. Embryoid bodies assay

A suspension method was used to form embryoid bodies, where human embryonic stem cells were either manually scraped or dissociated with 10 mM EDTA-PBS for 3 min from the Matrigel plates. Then, embryoid bodies were left to form in Essential 6 medium on a low attachment culture plate. The medium was replaced with the new medium after two days. The formation of embryoid bodies was assessed visually using a microscope with a 37 °C heated stage. In the integrin β1 blocking experiments, the neutralising antibody against integrin β1 P5D2 was added (4 μg or 10 μg) into the medium during embryoid bodies formation. Formed embryoid bodies were collected on day 6 for histological assay, immunohistochemical analysis, flow cytometry and Western blot analysis.

3.3.3. Flow cytometry

For detection of integrins β1 and α6 on the surface of human embryonic stem cells, the cells were harvested either manually, with EDTA (10 mM, 3 min) or with 0.05% trypsin-EDTA solution for 5 min, washed with PBS containing 2% fetal bovine serum. The single cells were suspended in 100 μl PBS containing 1% of BSA, and 2 mM EDTA on a 96-well low adsorption microplate and the plate was lifted on ice. The cells were blocked using a 2% NGS in a PBS containing 1% BSA and 2 mM EDTA (10 min) and stained with the appropriate antibodies for detecting integrins β1 and α6 or their isotype control antibodies for 30 min on ice. After washing with PBS (1% BSA, 2 mM EDTA), the cells were incubated with goat anti-mouse Alexa Fluor 647 or chicken anti-rabbit Alexa Fluor 488 antibodies. Flow cytometry data were acquired with FACSAria using FACSDiva software. The positive or negative populations for specific markers were selected using density plots according to the population's borders using specific biological samples (trypsin-treated hES cells) and confirmed with specific isotype controls.

In analysing differentiation markers of cells from embryoid bodies, the embryoid bodies were dissociated into single cells by extensive pipetting and fixed using 1.6% paraformaldehyde solution for 10 min at room temperature. The cells were washed and stained using a permeabilisation buffer, blocked with 2% NGS in a permeabilisation buffer (10 min) and stained with the appropriate antibodies or their isotype control antibodies for 30 min at rt. For cell cycle analysis, the cells were stained with DAPI.

3.3.4. Western blot

The human embryonic stem cells and embryoid bodies were lysed in the RIPA buffer containing 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% SDS, 0.3% Triton X-100, 0.3% sodium deoxycholate, 2 mM EDTA, and the Complete protease inhibitor cocktail and stored at –20 °C. The concentration of the proteins in the samples was measured with the Protein Assay Kit, and equal amounts of

protein were electrophoresed in an 8% SDS polyacrylamide gel and transblotted with the Mini Trans-blot Cell system onto a polyvinylidene difluoride membrane. The membranes were blocked with a 5% (w/v) non-fat dry milk powder solution in PBS containing 0.1% Tween-20 (blocking solution). The incubation with primary antibodies was performed in the same blocking solution overnight at 4 °C, followed by incubation with a secondary antibody in a blocking solution for 1 h at rt. The membranes were probed with rabbit anti-integrin α6 antibodies, mouse anti-integrin β1 antibodies, and mouse anti-E-cadherin antibodies, which was followed by the inclusion of the horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies. The mouse anti-β-actin antibody was used for detecting the loading control. The binding of the antibodies was detected with the Immobilon Western Chemiluminescent HRP Substrate solution. The membranes were exposed to the X-ray films used for the chemiluminescent signal detection. Plots were analysed using densitometry with the ImageJ gel plot analysing tool.

3.3.5. Immunohistological assay

The 6-day-old embryoid bodies were fixed with 4% PFA in PBS for 30 min at room temperature, washed with PBS, embedded in 3% agarose gel, and processed for paraffin embedding by standard methods. Serial sections (~8 μm) were prepared using microtom Microm HM355S. Paraffin sections were stained using haematoxylin and eosin. The images of cross-sections were analysed using Cell^B image-acquisition software (Olympus). In paraffin sections of embryoid bodies, the localisation of analysed proteins was revealed using the following antibodies: mouse anti-Sox17, mouse anti-CD184, mouse anti-integrin β1, rabbit anti-integrin α6, and mouse anti-E-cadherin. The paraffin sections were stained with Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit (Abcam) according to the manufacturer's protocol. The sections were embedded into Histomount. The images of cross-sections were analysed using Cell^B image-acquisition software (Olympus).

3.3.6. Statistical analysis

A one-tailed paired t-test with a confidence interval of 95% was performed with GraphPad Prism 4 software. All results are presented as the mean ± of the standard deviation.

4. RESULTS AND DISCUSSION

4.1. Mucins expression pattern in the human endometrium

Mucins are a pretty little-studied group of proteins in the endometrial tissue. Most studies focus on MUC1 because it was the first mucin discovered in the endometrium (Hey et al. 1994). A model was proposed that MUC1 is an important receptivity regulator due to the ability to sterically block the interaction between the receptor-ligand complexes of the blastocyst and endometrial lining (Aplin and Ruane 2017). Few studies have also investigated the roles of MUC4 and MUC16 in the endometrium (Alameda et al. 2007; Dharmaraj et al. 2014; Gipson et al. 1997). Gene expression of the *MUC8* was also detected in the human endometrial tissue (Hebbar, Damera, and Sachdev 2005). However, no recent studies have been published about the possible role of secreted-type mucin MUC8 in the human endometrium.

Since mucins play a functionally important role in the endometrium and yet are little studied, we decided to re-evaluate the previously published RNA-seq datasets to determine mucin expression patterns in the whole endometrial tissue and by cell type. For that purpose, we used the data from the whole endometrial tissue (GSE98386) and separately sorted stromal and epithelial cells (GSE97929) for early-secretory (LH+2) and mid-secretory (LH+8) endometrial samples (Altmäe et al. 2017). Expression of the eight different mucins was detected in the epithelial and stromal cells – secreted *MUC6* and *MUC7*, and membrane-tethered *MUC1*, *MUC4*, *MUC13*, *MUC15*, *MUC20* and *MUC22* (Table 1). However, the expression of *MUC6* was detected in only one early-secretory stromal sample and *MUC7* in one mid-secretory epithelial sample. It coincided with earlier studies in which MUC6 and MUC7 were searched across the female reproductive tract. The endometrial tissue did not contain *MUC7* mRNA or protein molecules, and *MUC6* expression was generally very low, whereas increased MUC6 expression was associated with possible malignant transformations of endometrial tissue (Alameda et al. 2007; Gipson et al. 1997).

Further, we found that only a few early- and mid-secretory endometrium epithelial samples were positive for *MUC4* mRNA. Earlier histological studies also found that endometrial epithelial cells are weakly positive for MUC4 expression (Alameda et al. 2007; Dharmaraj et al. 2014; Koscinski et al. 2006). We also found that transcripts of transmembrane mucins *MUC13*, *MUC15* and *MUC22* were presented across our dataset. *MUC13* was primarily found in epithelial cells and only in one of the early- and mid-secretory stroma samples. *MUC15* and *MUC22* mRNAs were expressed across epithelial and stromal samples. However, those mucins were presented in less than half of the samples; only *MUC13* was presented in 53.8% of the mid-secretory epithelium samples. Information about mucins MUC13, MUC15, and MUC22 expression in the endometrium is absent in the literature, and their role in endometrial physiology needs to be established by future studies. Moreover, MUC15 was found to be an

important regulator of the trophoblast cells invasion in the placenta (Shyu et al. 2007).

Our data showed that *MUC1* mRNA was expressed in most of the epithelial (in 83% of early-secretory and 92.3% of mid-secretory epithelial samples) and approximately in half of the stromal samples (45% of early-secretory and 54.5% of mid-secretory stroma samples were positive for *MUC1*). The *MUC1* was the first among mucins, the expression of which was found, and it is the most studied in endometrial tissue. *MUC1* is primarily expressed in both glandular and luminal epithelium in human endometrium, and its expression is under progesterone control. Unlike in other species, human endometrium expresses high levels of MUC1 during the WOI (Hey et al. 1994; 1995; Meseguer 1998). MUC1 expression and glycosylation patterns were studied to reveal the relationship between MUC1 expression and endometrial receptivity. Decreased MUC1 expression during the WOI was associated with decreased endometrial receptivity (Bastu et al. 2015; Margarit et al. 2010; Wu et al. 2018; Xu et al. 2012). Glycosylation pattern may be important during the initial step of implantation – apposition, when the blastocyst can move on the epithelial surface of the endometrium without attachment to it due to temporary and reversible receptor-ligand interactions guided predominantly by L-selectin. Similar mechanisms are exploited by leukocytes when they role on the endothelial lining of blood vessels without permanent attachment to endothelial cells (Aplin and Jones 2012; Aplin and Kimber 2004; Genbacev et al. 2003; Nejatbakhsh et al. 2012). The glycosylation pattern of MUC1 is complex and does not allow to distinguish receptive endometrium or different endometrium-associated pathology groups based on it (Aplin 1999; Horne et al. 2005). However, these conclusions have been drawn based on relatively small study groups. Thus, to obtain more convincing evidence of the role of MUC1 in recurrent implantation failure, more samples from more carefully selected patient groups need to be analysed before any clinical conclusions can be made. Some studies have tried to clarify the exact MUC1 localisation on the surface of the luminal epithelium, and there is evidence that ciliated luminal epithelial cells express more MUC1 than other endometrial epithelial cell types. Blastocyst interaction with MUC1 as a part of the glycocalyx can play a role in implantation regulation (Horne et al. 2002; Jeschke et al. 2009; Wu et al. 2019). During blastocyst implantation, glycocalyx of luminal epithelial cells, including MUC1, is locally removed at the implantation site. The proposed model explains that MUC1 is removed from the implantation site prior to the embryo adhesion stage during implantation. Thus, there is no steric block between the receptor-ligand complexes of the blastocyst and endometrial lining that leads to blastocyst irreversible attachment to the endometrial epithelium and further invasion into the stromal part at the end of the implantation process (Aplin 2006; Meseguer et al. 2001; Singh et al. 2010). The exact mechanism of local MUC1 clearance from the epithelium surface is unknown. Proteases from the MMP family or ADAM17 secreted by epithelial cells can be involved in the partial glycocalyx disintegration before implantation (Julian, Dharmaraj, and Carson 2009; Thathiah, Blobel, and Carson 2003; Thathiah and Carson 2004). Embryos can

also achieve local clearance of MUC1 from the endometrial surface by blastocysts-produced proteases during *zona pellucida* hatching (Aplin and Ruane 2017; J. J. Brosens et al. 2014).

In contrast to other detected mucins, the *MUC20* expression pattern displayed a remarkable change in the expression between the pre-receptive and receptive endometrial samples. *MUC20* was expressed only in 5 out of 18 (27.8%) epithelial samples from early-secretory endometrium (LH+2), but in the mid-secretory endometrium (LH+8), most samples – 23 out of 26 (88.5%) became positive. Stroma samples also showed a trend that *MUC20* could be detected more in the receptive endometrium. However, stroma samples were less positive for *MUC20* mRNA detection; even in receptive samples, only 10 out of 22 (45.5%) were positive. Information about MUC20 expression and function in the endometrium is scarce. *MUC20* mRNA expression was documented previously in the endometrial tissue once using microarray-based gene expression analysis (G. E. Evans et al. 2012). One previously published interactome study also predicted that MUC20 might participate in a signalling network in the receptive endometrium or blastocyst-endometrium interaction (Altmäe et al. 2012). High mucins expression, in general, and MUC20 particularly, is associated with cancer or malignancy-associated changes in the tissue. Endometrial cancers with high MUC20 expression are known as more malignant and have a more aggressive and invasive phenotype (C.-H. Chen et al. 2013; Jonckheere and Van Seuningen 2018; Xiao et al. 2013; Zheng, Yu, and Lu 2019). However, information about the physiological role of MUC20 in the human endometrium is lacking, leading our further attention to this mucin.

Table 1. Expression of different mucin mRNAs in human endometrial cells. The upper sub-row indicates the ratio of samples with detected specific mucin mRNA compared to all samples analysed (detected/all samples). The lower sub-row represents the percentage of positive samples with referred mucin mRNA expression (Modified Table 1 in Ref. I).

	MUC1	***MUC4***	***MUC6***	***MUC7***	***MUC13***	***MUC15***	***MUC20***	***MUC22***
Epithelium LH+2	**15/18 (83.3%)**	1/18 (5.6%)	0/18 (0%)	0/18 (0%)	4/18 (22.2%)	8/18 (44.4%)	5/18 (27.8%)	3/18 (16.7%)
Epithelium LH+8	**24/26 (92.3%)**	3/26 (11.5%)	0/26 (0%)	1/26 (3.8%)	14/26 (53.8%)	11/26 (42.3%)	**23/26 (88.5%)**	1/26 (3.8%)
Stroma LH+2	9/20 (45%)	0/20 (0%)	1/20 (5%)	0/20 (0%)	1/20 (5%)	3/20 (15%)	2/20 (10%)	4/20 (20%)
Stroma LH+8	12/22 (54.5%)	0/22 (0%)	0/22 (0%)	0/22 (0%)	1/22 (4.5%)	4/22 (18.2%)	10/22 (45.5%)	2/22 (9.1%)

According to the literature, MUC16 is expressed in the endometrium, mainly located in the epithelial compartment of the endometrium (Gipson et al. 2008; Liu et al. 2020). We did not detect *MUC16* mRNA in the endometrial tissue, perhaps due to the low level of *MUC16* in our early- and mid-secretory samples.

The pattern of *MUC16* expression during the menstrual cycle is not clear. However, some studies indicate that MUC16 expression is down-regulated during the mid-secretory phase (Gipson et al. 2008; Liu et al. 2020). Nevertheless, there is also an opinion that the level of MUC16 is constitutive during the cycle, and differences can be explained by an increased epithelial cell amount in the endometrial tissue during the secretory phase (Dharmaraj et al. 2014). It is speculated that MUC16 can be similar to MUC1 with antiadhesive properties to blastocyst implantation due to its ability to block receptor-ligand interactions between the blastocyst and endometrial epithelium sterically (Gipson et al. 2008; Gnainsky et al. 2015; Liu et al. 2020). There is evidence that MUC16 can be a clinically relevant endometrial receptivity marker. However, more thorough studies with larger patient groups must be conducted (Liu et al. 2020). To summarise this part, we can say that mucins are an understudied family of proteins in the human endometrium, and their possible role in regulating endometrial receptivity needs further study.

4.2. *MUC1* and *MUC20* expression levels in secretory endometrium

To validate the elevated expression of *MUC20* in the mid-secretory endometrium, we decided to investigate and compare the expression level of *MUC20* in our samples. Because *MUC20* expression was never thoroughly reported in human endometrial tissue, we compared it in parallel with MUC1 expression level as a relatively well-described mucin in the endometrium. *MUC1* expression was similar between the early-secretory (LH+2) and mid-secretory (LH+8) samples in both epithelial and stroma cell populations (Figure 2 in Ref. I). However, if we compare *MUC1* expression between epithelial and stromal cells, irrespective of the cycle day, the expression of *MUC1* is significantly higher in the epithelial cells (Figure 2 in Ref. I). In contrast, the expression of *MUC20* was significantly higher in epithelial cells of the mid-secretory endometrium compared to early-secretory samples. Since *MUC20* mRNA was detected only in 2 out of 20 early-secretory stromal cell samples, comparing stromal cells in that context was impossible. We also used the RNA-seq dataset from the whole endometrial tissue (GSE98386) to reveal the expression of *MUC1* and *MUC20* (Altmäe et al. 2017). This independent dataset confirmed that in the whole endometrial tissue samples, *MUC1* expression levels were not statistically different between early- and mid-secretory samples, whereas *MUC20* expression was significantly higher ($P = 7.58\times10^{-5}$, Mann-Whitney test) in the mid-secretory (LH+8) endometrium (Ref. I).

For the RNA-seq results validation in the whole tissue and sorted endometrial stromal and epithelial cells, qRT-PCR was used. Validation results were in accordance with RNA-seq expression level data. *MUC1* expression was higher in the epithelial cells compared to stroma cells in both cycle phases. However, in stromal cells, the expression was higher only in the mid-secretory endometrium

samples, which was not seen based on RNA-seq data of sorted endometrial cells. *MUC20* expression was higher in the epithelial and whole endometrial tissue samples in the mid-secretory endometrium compared to the early-secretory endometrium (Figure 3 in Ref. I). *MUC1* expression results corresponded well with the literature data. High expression of the MUC1 was previously documented in the human endometrium during the cycle (Hey et al. 1994; 1995; Meseguer 1998; Aplin 1999; Horne et al. 2005). *MUC20*, in contrast, shows us a more dynamic mRNA expression pattern during the cycle, with increased expression in the mid-secretory endometrium when endometrial tissue is theoretically capable of interacting with the blastocyst. We also demonstrated that the *MUC20* mRNA expression level is increased in the epithelial compartment of the endometrium.

4.2.1. MUC20 protein expression and localisation in the endometrial tissue

We also wanted to confirm MUC20 expression in the endometrium on the protein level and to determine the exact MUC20 localisation in the tissue. We analysed MUC20 expression by Western blot assay in cultured primary endometrial epithelial and stromal cells. Both cell types were isolated from 2 endometrial biopsies – one from cycle day 12, which corresponds to the late-proliferative cycle phase, and another from cycle day 19, which corresponds to the mid-secretory cycle phase. MUC20 protein was expressed in the epithelial cells at a higher level than in stromal cells regardless of cycle day (Figure 4 in Ref. I). Moreover, stromal cells cultured from the cycle day 12 biopsy were negative for MUC20 protein expression, whereas those from the day 19 biopsy had a faint expression signal. At that moment, no samples were available to perform more analysis for sufficient statistical analysis. However, we observed from those samples that the MUC20 protein expression level was higher in epithelial cells and very low in the stroma, coinciding with a lower level of *MUC20* mRNA expression in the stromal samples. Therefore, we concluded that the epithelial compartment of the endometrium is the primary source of MUC20 protein.

To confirm our previous data about the *MUC20* elevated expression in the mid-secretory endometrium and determine the localisation of MUC20 in the intact endometrial tissue, we used immunohistochemistry (IHC). IHC confirmed that in the mid-secretory endometrium, the MUC20 expression was increased compared to the early-secretory (LH+2) cycle phase (Figure 5 in Ref. I). Moreover, the MUC20 protein was more abundant in the luminal and glandular epithelial cells. The semi-quantitative approach showed a statistically significant increase in MUC20 protein expression in the endometrial epithelial cells. In contrast, the stromal MUC20 level was similar regardless of the cycle stage (Figure 5 in Ref. I). Those results confirmed increased *MUC20* expression in the mid-secretory endometrium and that the *MUC20* expression occurs mainly in epithelial compartments of the endometrium. *MUC20* mRNA and protein expression

patterns turn out to be similar in the human endometrium. We demonstrated using Western blot and IHC methods that mid-secretory endometrial epithelial cells exhibit elevated *MUC20* expression. This finding was confirmed by our RNA-seq and RT-qPCR analyses, as well as by MUC20 protein expression analysis through Western blot and IHC. This new knowledge enhances our understanding of endometrial biology and mucins.

4.3. The difference between MUC20 and other MUC family members

The MUC20 belongs to the subgroup of membrane-bound or membrane-tethered mucins. However, compared to the other highly-glycosylated membrane-tethered mucins that can protrude from the cell surface hundreds of nanometres, MUC20 is a relatively small and compact protein with a low level of glycosylation (Figure 6) (Higuchi, Orita, Nakanishi, et al. 2004). MUC20 was discovered and initially described in the renal tubular epithelial cells (Higuchi, Orita, Nakanishi, et al. 2004). The predicted length of MUC20 was 503 amino acids. However, it was determined that MUC20 contains several characteristics of mucins tandem repeats of 19 amino acids, mainly consisting of threonine, serine, and proline residues, which are suitable for glycosylation. The number of these repeats can vary from 3 to 6, which suggests that different isoforms of MUC20 with varying lengths may exist (Higuchi, Orita, Nakanishi, et al. 2004). Consequently, the canonical MUC20 sequence in the UniProt database (accession number Q8N307) is 709 amino acids long. Based on the MUC20 sequence, it was also predicted that MUC20 contains few hydrophobic regions that can serve as transmembrane domains. Further, immunoelectron microscopy confirmed that MUC20 immuno-reactivity was observed on the plasma membrane of the cells (Higuchi, Orita, Nakanishi, et al. 2004). Western blot analysis of cell lysates also showed that MUC20 is enriched in the membrane fraction (Higuchi, Orita, Nakanishi, et al. 2004). Interestingly, already in the first published paper about the discovery of MUC20, authors also found that damage to the epithelial cells increases MUC20 expression level, which can be indirect evidence that MUC20 can have a role in the molecular pathways related to cell growth and differentiation (Higuchi, Orita, Nakanishi, et al. 2004). Recent works suggested that the high MUC20 expression can be associated with ciliogenesis and ciliated cells (Kesimer et al. 2013).

4.3.1. Model of MUC20 interaction with MET receptor in the human endometrium

As mentioned above, MUC20 belongs to the group of membrane-bound mucins. However, it differs from other membrane-tethered mucins by its relatively small size and low glycosylation level, which raises many questions about MUC20 molecular functions. Due to the physical properties of the MUC20 molecule

(contains only a few tandem repeats suitable for glycosylation), it is unlikely that it can be part of dense glycocalyx like other membrane-bound mucins. In contrast, other mucins can contain hundreds of tandem repeats, forming firm, dense, highly glycosylated structures that protrude hundreds of nanometres above the cell membrane (Fini et al. 2020; Kesimer et al. 2013; Konstantinidi et al. 2022; Woodward and Argüeso 2014). However, it is also known that membrane-bound mucins are not only a biological barrier on the cellular surface but can also transduce different kinds of molecular signals through the cytoplasmic C-terminal tail (Figure 6) (Fini et al. 2020).

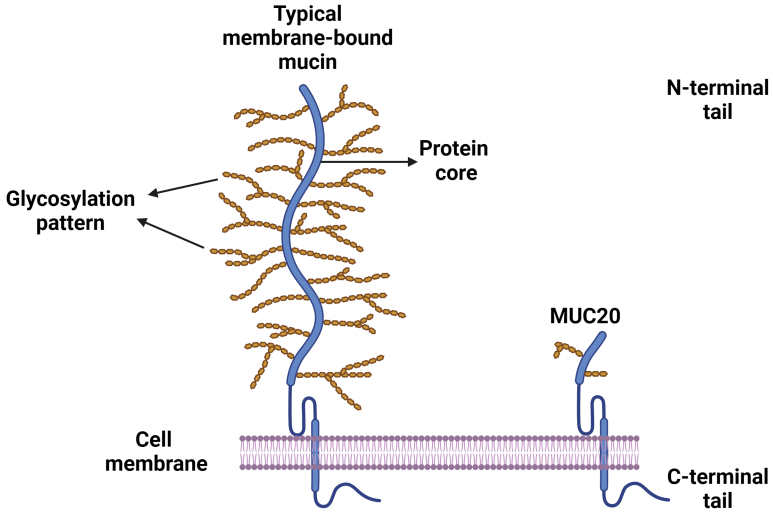

Figure 6. The MUC20 is different from other membrane-bound mucins. Typical membrane-bound mucins can contain hundreds of tandem repeats, forming firm, dense, highly glycosylated structures that protrude hundreds of nanometres above the cell membrane, forming the glycocalyx. MUC20 also belongs to the membrane-bound mucins group. However, MUC20 is a relatively small and compact protein with a low level of glycosylation. Created with BioRender.com

A first look at the molecular functions of MUC20 was proposed by the same team of authors who initially discovered MUC20. They found that MUC20 interacts with the mesenchymal-to-epithelial transition factor (MET/c-MET) receptor, modulating the signalling network emerging from the MET receptor (Higuchi, Orita, Katsuya, et al. 2004). Hepatocyte growth factor (HGF) is a multifunctional peptide produced in the liver and other tissues that regulates biological functions like cell proliferation, morphogenesis and survival. HGF acts on cells through the MET receptor, which belongs to the tyrosine kinases receptor superfamily (Petrini 2015). HGF is highly produced in mesenchymal or stromal cells but lacks in epithelial cells, whereas the MET receptor is predominantly expressed in cells of epithelial origin. HGF signalling is transduced by the MET receptor multi-functional docking site (MDS) in the C-terminal part of the receptor, where two phosphorylation sites are located (Petrini 2015). When those two tyrosine residues

are phosphorylated, MDS can interact with signal transducers like phosphatidylinositol 3-kinase (PI3K), growth factor receptor-bound protein 2 (GRB2) and GRB2-associated-binding protein 1 (GAB1). Different molecular pathways can be activated depending on the involved factor: GRB2-RAS or GAB1-PI3K, leading to various biological responses. GRB2-RAS pathway participates in regulating cell proliferation, whereas GAB1-PI3K is required to regulate cell survival, scattering and morphogenesis (Zhao et al. 2022; Sakai, Aoki, and Matsumoto 2015; Petrini 2015). MUC20 cytoplasmic tail interacts with the MET receptor MDS site and modulates the signalling cascade arising when the MET-HGF receptor-ligand complex is activated. When MUC20 interacts with the MDS domain of the MET receptor, it prevents GRB2 recruitment to the HGF-activated MET receptor complex and attenuates downstream extracellular signal-regulated kinase-1 and -2 (ERK1/2) activation in the GRB2-RAS pathway. However, the GAB1-PI3K pathway is not affected by MUC20 interaction with the MET receptor. The main biological effect mediated by MUC20 interaction with MET receptor is inhibiting cell proliferation, but simultaneously, processes related to scattering, morphogenesis, and cell survival are unaffected (Higuchi, Orita, Katsuya, et al. 2004).

Based on the knowledge that MUC20 can interact with the MET receptor in renal tubular epithelial cells, we investigated similar interactions in human endometrial tissue (Higuchi, Orita, Nakanishi, et al. 2004; Higuchi, Orita, Katsuya, et al. 2004). Moreover, endometrial tissue consists mainly of two components – stromal and epithelial cells, which is typical for the HGF-MET receptor-ligand interaction site where HGF is highly produced in the mesenchymal type of cells and MET receptor is expressed in the epithelium. From our cell-specific RNA-seq data set (GSE97929), we found that *MUC20* expression is highly correlated with the *MET* receptor expression in the epithelial cells (Ref. I). We also found that *HGF* mRNA in the stromal cells and *MET* receptor mRNA level in the epithelial cells are upregulated in the mid-secretory (LH+8) endometrium compared to the early-secretory phase (Figure 6 in Ref. I). We proposed the hypothetical model of MUC20 and HGF/c-MET complex interaction in a similar way that was shown before in the renal tissue (Figure 7 in Ref. I). However, further molecular studies need to be done to confirm or refute our hypothesis.

There is a lack of model systems where MUC20 molecular functions can be studied. Due to elevated levels of MUC20 expression in some cancerous tumours and their more aggressive phenotype, some cancer-origin cells were studied. In the case of pancreatic ductal adenocarcinoma cells, it was also found that MUC20 physically interacts with the MET receptor and could participate in the HGF/c-MET signalling network modulation (S. T. Chen et al. 2018). It was shown that the high level of MUC20 increases the malignant properties of the cells, such as elevated cell viability, invasion, and migration (S. T. Chen et al. 2018). However, the exact molecular mechanisms of MUC20 interaction with the HGF/c-MET receptor-ligand complex are still unclear. Authors have shown that MUC20 enhances HGF-induced phosphorylation of the MET receptor and stimulates c-MET/p-AKT activity (S. T. Chen et al. 2018). In contrast, in primary renal duct

cells, it was shown that MUC20 suppresses the HGF-induced Grb2-Ras-ERK1/2 pathway, whereas the activity of Gab1-PI3K/p-AKT is unaffected. Moreover, even truncated MUC20 interacts with the MET receptor in cancer cells, while in primary renal cells, it was shown that the C-terminal domain of the MUC20 is a primary binding domain to the MET receptor (Higuchi, Orita, Katsuya, et al. 2004). Those differences can be explained by the differences between normal and cancerous cells. However, to understand the modulatory effect of MUC20 on the MET receptor network, it is necessary to identify the exact binding site between MUC20 and the MET receptor.

It seems that MUC20 molecular interactions can be even more complicated. For example, in endometrial cancers, elevated MUC20 expression was found, and this was associated with higher malignancy and poor survival rate accompanied by higher cell invasiveness and migration capability (C.-H. Chen et al. 2013). However, the molecular mechanism exploited by endometrial cancer cells seems to differ from normal primary renal cells or pancreatic duct carcinoma cells. In the case of endometrial cancer, phenotype changes guided by high MUC20 protein expression level seem to be regulated through another receptor tyrosine kinase – epidermal growth factor (EGF) receptor (EGFR) (C.-H. Chen et al. 2013). Previous studies also showed that mucins – particularly MUC1 and MUC16 can interact with EGFR and modulate the activity of the pathway network connected to EGFR (Bitler, Goverdhan, and Schroeder 2010; X. Chen et al. 2024). It has also been found in epithelial ovarian cancer cells that MUC20 can regulate cell-ECM interactions and enhance migration, invasion and adhesion of the cells through activation of the integrin β1 pathway, leading to increased phosphorylation and activity of focal adhesion kinase (C.-H. Chen et al. 2016). Proliferation and migration of the thyroid cancer cells are also regulated through MUC20 and MET receptor signalling pathway (Hou et al. 2021). Some recent works also show that MUC20 could participate in proteosome capacity regulation in lymphoma cells through interaction with the MET receptor (X. Wang et al. 2021; 2024). Discrepancies among different types of tumours and normal primary cells may explain differences between possible MUC20 interaction with c-MET or EGFR. However, still more studies must be done to clarify that question.

4.4. Hypothetical role of MUC20 in the receptive endometrium

Analysis of the cell-specific RNA-seq data (GSE97929) showed us that the expression of several known endometrial receptivity marker genes was correlated with *MUC20* expression. Receptivity biomarkers were almost eight times over-represented among *MUC20*-correlated genes compared with the rest of the protein-coding genes in the human genome. Our study identified increased *MUC20* expression on the mRNA and protein levels in the endometrial epithelium during potential WOI. *MUC20* may be considered a new potential candidate for endometrial receptivity biomarkers because of its mid-secretory phase-specific

expression. However, we must be careful in our conclusions because *MUC20* expression correlation with other receptivity markers does not automatically mean that *MUC20* plays an important role in the endometrial receptivity process. *MUC20* needs to be further clinically validated, and functional studies must be done to determine its exact role in the receptive endometrium.

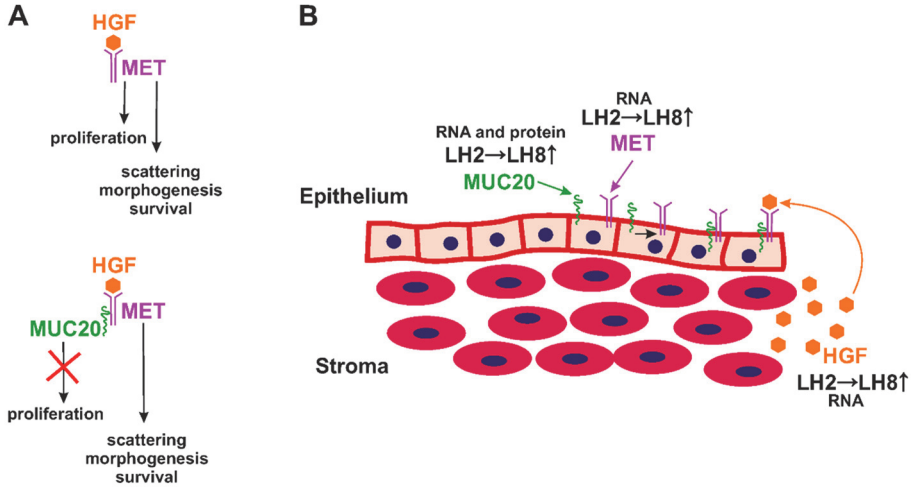

Figure 7. Model of MUC20 interaction with MET receptor in the human endometrium. A – the interaction of MUC20 with the MET receptor inhibits cell proliferation but does not affect cell scattering, morphogenesis and survival. B – in the receptive endometrium, expression of MET and MUC20 is increased in the epithelium, whereas HGF expression is increased in the stromal compartment. HGF – hepatocyte growth factor, MET – mesenchymal-to-epithelial transition factor receptor, LH2 – endometrial sample collected 2 days after luteinising hormone surge, LH8 – endometrial sample collected 8 days after luteinising hormone surge. (Modified Figure 7 in Ref. I).

Stromal cells residing in a wide variety of tissues produce HGF bound by epithelial cells via the MET receptor, leading to the activation signal cascade in the epithelial cells, transforming their physiology (Sakai, Aoki, and Matsumoto 2015; Zhao et al. 2022). We found that mRNA expression of *MET* in the epithelium and *HGF* in the stroma is increased in the mid-secretory phase compared to the early-secretory endometrium. *MET* mRNA up-regulation during the secretory phase in the glandular epithelium was also documented in the previous study in the human endometrium during the WOI (G. E. Evans et al. 2012). Based on the previously described MUC20 interaction with the MET receptor, we proposed a similar model that can also be utilised for the cell interactions in human endometrial tissue during the mid-secretory phase when cell proliferation is inhibited, but cell scattering and morphogenesis can be crucial for the interaction with the blastocyst (Figure 7). Interestingly, our model of the potential interaction of MUC20 and MET receptor is supported by the earlier interactome study of human implantation that also speculated potential signal networks within the endometrial tissue

(Altmäe et al. 2012). We reported for the first time cycle-dependent expression of the *MUC20* in the human endometrium at mRNA and protein levels. Further research should reveal the connection between MUC20 and HGF-activated MET receptor signalling in the human endometrium. In the future, it could be beneficial to determine the role of MUC20 in endometrial receptivity and the precise interaction of MUC20 with MET receptor for a better general understanding of endometrial receptivity mechanisms and more precise endometrial dating based on molecular biomarkers.

4.5. Changes in the protein kinases activity during stromal cell decidualisation

Decidualisation is a complex process that involves changes in gene expression profile, proliferation, metabolism, and morphology of the stromal cells, and it also affects the overall state of the endometrial tissue. Such enormous changes in the cells involve many different molecular pathways orchestrated by different protein kinases. One of the downstream targets of the main decidualisation regulator cAMP is cAMP-dependent protein kinase (PKA). cAMP response element-binding protein (CREB), one of the PKA substrates, plays an essential role in decidualisation. The expression of prolactin, a key decidualised stroma marker, is mediated by CREB (Telgmann et al., 1997; Yoshie et al., 2015). Other protein kinases are also involved in the decidualisation process in varying degrees. Non-receptor Tyr kinase family Janus kinases activate STAT family transcription regulators STAT3 and STAT5, which are upregulated during decidualisation. Moreover, the JAK-STAT pathway is involved in the cellular response to prolactin (Jabbour et al., 1998). PI3K and related kinase mammalian target of rapamycin (mTOR) and protein kinase B (Akt/PKB) pathways are also involved in decidualisation. PI3K and mTOR downregulate the activity of Akt/PKB, which leads to the dephosphorylation of the forkhead box Protein O1 (FOXO1) transcription factor. FOXO1 dephosphorylation causes translocation of the transcription factor to the nucleus and enhancement of its activity, resulting in upregulated transcription of *PRL* and *IGFBP1*, known as primary decidualised stroma cell markers (Baek et al., 2018; Fabi et al., 2017; S. Y. Lee et al., 2016; Yoshino, Hirota, et al., 2003). MAPK family kinases are also involved in different aspects of decidualisation. cAMP-induced p38 kinase dephosphorylation may modulate inflammatory response in the decidualised stroma. Inhibition of proinflammatory factor production plays an essential role in creating an immunosuppressed microenvironment for the implanting blastocyst (Yoshino, Osuga, et al. 2003). Other MAPK family members – ERK1/2 are also highly expressed in the decidua. Moreover, reducing ERK1/2 phosphorylation levels decreases the primary decidualisation marker genes *PRL* and *IGFBP1* expression (C. H. Lee et al. 2013). A central regulator of the secretory phase of the menstrual cycle, progesterone also interacts through its receptor with the MAPK pathway. When translocation of the activated progesterone receptor to the nucleus does not occur,

the non-genomic or rapid progesterone receptor pathway also involves a MAPK signal transduction cascade (Lee et al., 2013; Wetendorf and DeMayo, 2014; Yoshino et al., 2003). Other protein kinases are less explored in the context of decidualisation. However, considering extensive metabolic and phenotypic reprogramming of decidualised stromal cells, multiple cellular pathways regulated by protein kinases network are expected to contribute to that process. Four different protein kinases were in focus of our research: PKA – a well-established player in decidualisation, was included as a control; Akt/PKB – a central regulator of cellular metabolism; Rho-dependent protein kinase (ROCK) – cellular motility and cytoskeleton rearrangement regulator; and casein kinase 2 (CK2) – viability marker of the cells.

4.5.1. Establishment of the *in vitro* decidualisation protocol

Considering the complexity of decidualisation, it is reasonable to assume that many phosphorylation pathways are involved. The *in vitro* decidualisation approach allows artificial manipulation of stromal cells by treating them with hormones, cAMP by itself and cAMP intracellular level-modulating chemicals. We tested five different *in vitro* decidualisation protocols utilising combinations of progesterone, a cell-permeable analogue of cAMP – 8-Bromoadenosine 3',5'-cyclic adenosine monophosphate (8-Br-cAMP), an activator of endogenous adenyl cyclase – forskolin (Fsk), and an inhibitor of cAMP degrading phosphodiesterases – 3-isobutyl-1-methylxanthine (IBMX). Those decidualisation protocols were tested on the endometrial stromal cells isolated from the proliferative phase tissue samples. PRL secreted to the cell culture media was quantified and taken as a decidualisation marker. Adding only progesterone to the decidualisation media was not enough to induce decidualisation. However, adding the 8-Br-cAMP with or without IBMX was sufficient to induce the decidualisation (Figure 1A in Ref. II). Since prolactin is the primary marker of decidualisation, we got confirmation that we could manipulate stromal cells *in vitro* for further protein kinases activities investigation during the decidualisation process.

4.5.2. PKA, Akt/PKB and CK2 in decidualisation

After *in vitro* decidualisation, stromal cells were lysed, and selected protein kinases activities were measured. Both PKA and Akt/PKB activities were increased in the cells treated with decidualisation mixtures; moreover, adding the 8-Br-cAMP to the decidualisation mixture was enough to increase their activity, and Fsk or IBMX did not trigger a further rise in their activity. Conversely, the CK2 activity decreased under all decidualisation conditions (Figure 1B in Ref. II). Inhibition experiments were carried out to prove the role of the PKA and Akt/PKB in decidualisation. Protein kinase inhibitor H89 was added to the decidualisation media, and further, PRL secretion and kinases activities were measured. The PRL secretion was reduced in the media collected from the stromal cells treated with

H89 (Figure 3A in Ref. II). In the cell lysates, the measured activities of PKA and Akt/PKB were also significantly reduced, whereas the effect on CK2 was not observable (Figure 3B in Ref. II). Together, those results show that PKA and Akt/PKB can be essential players in establishing the decidua development.

However, results obtained from non-cultured stromal cells isolated directly from the secretory tissue samples did not correspond well with stromal cells decidualised *in vitro*. Stromal cells isolated from four patients in the proliferative phase (cycle days 6–11) were compared to four secretory samples (all from cycle day 23). Kinase activity profiles showed that PKA activity did not differ between proliferative and secretory cells. Similarly, CK2 activity was consistent across both proliferative and secretory samples, whereas Akt/PKB activity was lower in the secretory cells (Figure 4A in Ref. II). Akt/PKB activity drop was relatively small but still statistically significant. In the *in vitro* decidualised cells, the kinase activity profiles were quite opposite: PKA and Akt/PKB activity increased, and the CK2 activity decreased (Figure 1B in Ref. II).

We also used a broader approach and untargeted methods like proteomics and phosphoproteomics to investigate a regulatory network of the protein kinases during decidualisation. Proteomic and phosphoproteomic studies were conducted on lysates of non-cultured endometrial stromal cells isolated from proliferative or secretory cycle phases. Proteomics revealed a few known secretory phase markers (GBPI, MVP) and a well-known decidualisation marker (STAT3) up-regulated in the secretory phase samples (Table I in Ref. II). However, PKA, Akt/PKB or CK2 did not pass the cut-off criteria to be considered statistically significantly changed between proliferative and secretory phases. Phosphorylation sites were identified and compared in the proliferative and secretory endometrium using phosphoproteomics. We were particularly interested in determining whether the enrichment of these sites, which are targets of our selected kinases, occurs during the secretory phase. We found that phosphosites associated with nucleic acids binding, RNA processing, actin binding and cytoskeleton organisation were enriched in the secretory phase stroma (Table II in Ref. II). We have identified 6 upregulated phosphosites in the secretory stroma that are downstream members of the MAPK cascade, which are substrates of the MAPK1 (ERK2) and MAPK3 (ERK1). This data coincides well with previously published results because MAPK1 and MAPK3, also known as ERK2 and ERK1, were identified as essential regulators for endometrial decidualisation in humans (C. H. Lee et al., 2013). Also, increased phosphorylation of Akt/PKB kinase substrates was identified. Those phosphosites regulate the Akt/mTOR pathway, which is important for decidualisation, embryo implantation and early fetal-placental development in the animal model (Roberti et al., 2018).

This part of our work (Ref. II) mainly aimed to explore changes in the phosphorylation pathways during decidualisation using different complementary approaches. Few target protein kinase pathways were investigated using selective protein kinase probes. Those probes allowed us to quantify not the total protein amount but the biological activity of selected kinases and further combine that data with untargeted proteomic and phosphoproteomic methods. The central

decidualisation regulator – cAMP, is directly connected to the PKA signalling. Thus, the PKA signal network should be vital during decidua formation. Previously, *in vitro* decidualisation models have shown that PKA catalytical activity is important and utilising PKA inhibitors, decidualisation can be disrupted (Makrigiannakis et al., 1999; Telgmann et al., 1997; Yoshie et al., 2015; Yoshino, Hirota, et al., 2003). Our *in vitro* decidualisation results also showed increased PKA activity in the decidualised stroma (Figure 1B in Ref. II). Moreover, adding PKA inhibitor H89 to the decidualisation media decreased PKA activity and interfered with decidualisation because we observed a significant PRL secretion drop in the H89-treated cells (Figure 3 in Ref. II). However, we did not observe an increase in PKA activity in the secretory samples in the lysates of non-cultured stromal cells (Figure 4A in Ref. II). The previous study showed that the catalytic subunit amount of the PKA during *in vitro* conditions does not change, which implies that changes occur only at the regulatory level of kinase activity (Telgmann et al., 1997). The proteomic analysis detected the catalytic subunit α of protein kinase A (PRKACA) and several regulatory subunits (PRKAR1A, PRKAR2A, PRKAR2B) of PKA in lysates from non-cultured endometrial stroma. However, the observed trends varied, and the high inter-patient variability precluded any significant conclusions (Supplementary Table SII in Ref. II, available in electronic version). Furthermore, only two phosphosites were enriched in the secretory phase suitable for the PKA, and both were on the ribosomal protein 6 (Table II in Ref. II). However, based on the literature, those phosphosites are more suitable for Akt kinase and Akt/mTOR regulatory network (Roberti et al., 2018). Ribosomal protein 6 phosphorylation plays an important role not only in protein synthesis regulation but also in cell shape and size regulation (Ruvinsky and Meyuhas, 2006). Our results suggest that PKA activity during *in vivo* decidualisation does not increase significantly compared to *in vitro* conditions when we see an increase in enzyme activity. This controversy can be explained by differences between *in vivo* conditions when stromal cells during the initialisation of decidual reaction may have a temporary peak of PKA activity, which declines subsequently. During *in vitro* decidualisation, PKA is constantly activated because of the permanent presence of cAMP at high concentrations in the decidualisation media.

In the case of the Akt/PKB, there was also a big difference between stroma cells decidualised *in vitro* or *in vivo*. In cell lysates obtained from the *in vitro* decidualised stroma, we see an increase in the Akt/PKB activity (Figure 1B in Ref. II). On the contrary, in the case of *in vivo* decidualised cells, Akt/PKB activity was decreased (Figure 4A in Ref. II). The addition of the inhibitor H89 to the decidualisation media also decreased Akt/PKB activity, and PRL secretion was reduced by those cells. However, it is unclear whether PKA or Akt/PKB is primarily responsible for this effect, as H89 inhibits the activity of both kinases (Figure 3 in Ref. II). The elevated activity of Akt/PKB in the *in vitro* decidualised cells was inconsistent with literature data suggesting dephosphorylation of the Akt and a subsequent reduction in activity. Conversely, our *in vivo* decidualisation results, showing a decrease in the Akt/PKB activity, coincide well with

previously published observations by other authors (Fabi et al., 2017; S. Y. Lee et al., 2016; Yoshino, Hirota, et al., 2003). However, it must be considered that the Akt/PKB pathway has multiple negative feedback loops, so a connection between Akt phosphorylation level and catalytic activity is not always direct. Different Akt/PKB pathway components (mTORC1 versus mTORC2) can compete during decidualisation and play different roles in that process (Baek et al., 2018; Fabi et al., 2017; Han et al., 2007). Our phosphoproteomic data show a phosphorylation site suitable for the Akt/mTOR pathway activation during decidualisation. That phosphorylation substrate is also important in regulating the decidual function during early pregnancy, as shown in the rat model (Roberti et al., 2018). Akt/PKB may also be involved in decidualisation through alternative pathways, but more research must be done to obtain conclusive results.

Another protein kinase with different activity profiles *in vitro* and *in vivo* was CK2. In contrast to other kinases from our selection, the *in vitro* decidualisation activity of the CK2 decreased (Figure 1B in Ref. II). CK2 is considered a viability marker, and the effect of cell culture conditions and artificial decidualisation on the viability of cells may explain the decreased activity of CK2. Non-cultured secretory stroma did not show a decrease in CK2 activity compared to the proliferative phase cells (Figure 4A in Ref. II). In the proteome of the proliferative and secretory endometrial stroma, different subunits of CK2 were identified; however, no statistically significant differences were found. Phosphorylation sites suitable for CK2 were found both in proliferative and secretory stroma. We did not find any particular phosphosites enriched in the secretory phase samples. We concluded that CK2 most likely does not play a significant role during the progression of decidual reaction. However, CK2 may be used as a marker of cell viability during the *in vitro* cultivation of human endometrial stroma cells.

When analysing various phosphorylation sites from the *in vivo* decidualised stroma, we found that most are associated with the MAPK pathway. MAPK signalling cascade has very complex hierarchical multilevel signal transduction and amplification. Due to the complexity of the MAPK signalling network organisation, contradictory results about MAPK cascade regulation during the decidualisation have been presented. According to the literature, MAPK signalling can be up- or down-regulated depending on protein kinase. p38, belonging to the MAPK family, is dephosphorylated under the influence of cAMP during decidualisation, leading to downregulation of the inflammatory factors (IL-6, IL-8, MCP-1, COX-2) produced in the decidua, creating an immunosuppressed microenvironment for the potential blastocyst implantation (Yoshino, Osuga, et al., 2003). On the other hand, MAPK1 (ERK2) and MAPK3 (ERK1) are highly expressed in the decidua, and their phosphorylation level is also elevated during decidualisation. Moreover, disrupting their phosphorylation using inhibitors leads to the down-regulation of decidualisation marker genes – *PRL* and *IGFBP1* (C. H. Lee et al., 2013). Unfortunately, we did not have specific probes to measure the activity of the MAPK cascade protein kinases and the possibility to compare results from *in vitr*o and *in vivo* decidualised cells with previously published literature data.

4.5.3. ROCK2 kinase in the decidualisation process

The above-described approach was used to identify the role of ROCK protein kinase in the decidualisation of human endometrium. First, ROCK activity was measured in the *in vivo* and *in vitro* decidualisation conditions, and further broader untargeted methods of proteomics and phosphoproteomics were applied to investigate the kinase signalling network during decidualisation. Results obtained from enzymatic activity measurements and omics methods were further validated using various molecular and microscopic techniques.

In the lysates of the *in vitro* decidualised cells, ROCK activity was significantly increased. Treating cells only with progesterone was not sufficient to increase ROCK activity. The highest ROCK activity was measured in the lysate obtained from the cells where decidualisation was induced with a mixture of P4, Fsk and IBMX without adding 8-Br-cAMP (Figure 1B in Ref. II). An additional *in vitro* decidualisation experiment was done utilising mixtures with and without P4 to reveal progesterone's role in the ROCK activation. Activation of the ROCK required the presence of progesterone because a decidualisation-inducing mixture containing only 8-Br-cAMP and IBMX was ineffective (Figure 2B in Ref. II). During *in vitro* decidualisation, both components are needed for ROCK pathway activation – progesterone and artificial cAMP or intracellular cAMP level increasing agents. Further, we performed an inhibition experiment to confirm that increased ROCK kinase activity affects decidualisation. We added various concentrations of the well-known ROCK inhibitor Y-27632 to the decidualisation mixture, which induced the highest ROCK activity in the previous experiment (Ishizaki et al. 2000). As a result, ROCK activity in the Y-27632 treated cell decreased dose-dependently (Figure 3A in Ref. II). Moreover, by implementing the highest inhibitor concentration, the PRL secretion decreased in treated cells, which means that ROCK directly affects the decidualisation process (Figure 3B in Ref. II). Compared to other studied kinases from our selection, the ROCK activity was exclusively similar *in vivo* as well as *in vitro* induced decidualisation conditions. ROCK activity was statistically significantly almost 2-fold higher in the secretory *in vivo* decidualised stroma cells compared to the proliferative stroma (Figure 4A in Ref. II).

Proteomic and phosphoproteomic analyses were applied to non-cultured proliferative and secretory samples to get clues about a regulatory network of the ROCK kinase during stroma decidualisation. We identified ROCK isoform 2 from our proteomic data, which passed statistical cut-off criteria and showed elevated expression in the secretory samples. ROCK2 was the only kinase from our selection that showed an increase in the activity and the quantity of the enzyme in the secretory stroma (Table I in Ref. II). We also had an exciting hit from our phosphoproteomic comparison between proliferative and secretory stroma. Namely, we identified a phosphorylation site, Ser3, enriched on the non-muscle isoform of cofilin (CFL1) in the secretory samples. This phosphosite is a substrate for LIMK1 and LIMK2 kinases, downstream targets of ROCK kinase

(Table II in Ref. II) (Prunier et al. 2017). The ROCK/LIMK pathway and the mentioned CFL1 Ser3 phosphosite are essential in regulating the cellular cytoskeleton dynamics (M.-H. Lee et al., 2019).

Further, we validated our proteomic and phosphoproteomic results by alternative methods. First, we decided to confirm our proteomic data, which showed us elevated ROCK2 expression in the secretory endometrium compared to the proliferative. Namely, we controlled quantitative changes of the ROCK2 expression on mRNA and protein levels under *in vivo* and *in vitro* decidualisation conditions. To investigate ROCK2 expression in the *in vivo* decidualised tissue sample, mRNA expression analysis was done using RNA-Seq from the snap-frozen endometrial biopsies. mRNA was isolated from the whole tissue samples, and after sequencing, the data for ROCK2 was used only. Indeed, ROCK2 mRNA expression was statistically significantly higher ($P \leq 0.05$) in the endometrial samples from the secretory phase of the cycle compared to proliferative (Supplementary Figure S6A in Ref. II). To investigate the ROCK2 protein level changes during decidualisation, the lysates of the *in vitro* decidualised stromal cells were used for Western blot analysis. Cells treated with a decidualisation mixture containing P4, 8-Br-cAMP, and IBMX showed elevated ROCK2 protein amount compared to controls (Supplementary Figure S6B and S6C in Ref. II). Those results coincide well with the activity studies *in vitro* and *in vivo*, which showed elevated ROCK2 activity in the decidualised stroma, as well as with proteomics results which also showed a quantitative increase of the ROCK2 enzyme in the *in vivo* decidualised cells.

The next step was to validate the phosphoproteomic result to check the enrichment of the phosphorylated pSer3 site of CFL1 in the secretory versus proliferative tissue samples and *in vitro* decidualised versus non-decidualised stroma cells. Tissue samples from proliferative and secretory endometrium were used for the immunofluorescent analysis of the presence of phosphorylated CFL1 in the tissue, representing *in vivo* decidualisation conditions. The pSer3 site of CFL1 was identified with a primary antibody that recognises phosphorylated epitope (Figure 4C in Ref. II). The fluorescent signal intensity from phospho-cofilin was quantified in the entire tissue and separately in the stromal and epithelial compartments. We found that the phosphorylated form of cofilin was enriched in the secretory endometrium. Signal intensity in secretory samples was higher in the total tissue as well as in epithelium and stroma taken separately (Figure 4B in Ref. II). The complementary experiment was conducted with cultured human endometrial stromal cells decidualised *in vitro* to compare CFL1 phosphorylation of the pSer3 site with control cell populations. The quantified immunofluorescence signal intensity showed that CFL1 phosphorylation in the *in vitro* decidualised cells is higher, whereas adding the P4 was also necessary to increase the phosphorylation (Figure 5 in Ref. II). We observed a similar pattern with ROCK2 in the *in vitro* decidualised cells when we analysed the protein expression levels by Western blot. Cells treated with a decidualisation mixture containing P4, 8-Br-cAMP and IBMX responded by elevated enzyme production (Supplementary Figure S6B and S6C in Ref. II). Those two results coincide well,

showing that the ROCK2 enzyme amount and downstream target CFL1 phosphorylation are increased in the *in vitro* decidualisation model. To summarise briefly, validation experiments confirmed proteomic and phosphoproteomic results, showing that the ROCK/LIMK/CFL pathway may be involved in decidualisation and that progesterone is needed for that pathway activation.

ROCK/LIMK/CFL pathway regulates the actin cytoskeleton, which is essential for cell motility and cellular shape backing or remodulation (Prunier et al. 2017). During the decidualisation, a phenotypical switch occurs in the stromal cells. Proliferative phase stromal cells have fibroblast-like morphology, but after decidualisation, the stromal cells achieve enlarged round-shaped epithelioid cell type morphology, which involves a lot of actin cytoskeleton remodulation (Pan-Castillo et al., 2018). It was previously reported that *in vitro* decidualised stromal cell motility is decreased compared to untreated controls (Cloke et al., 2008). We performed the functional experiment to estimate the motility of *in vitro* decidualised cells. We used a decidualisation mixture containing P4, Fsk and IBMX, which in our previous experiment was the most efficient for increasing ROCK activity (Figure 1B in Ref. II). Cell migration assay was chosen to track physiologically relevant motility properties of the decidualised cells compared to untreated control stromal cells. Closure of the scratch due to cell migration was observed under a microscope, and further cell-free area was quantified at fixed time points. Significant gradual reduction of the cell-free area by non-decidualised control cells was observed after 24h and 48h. In contrast, cells treated with a decidualisation mixture had very low motility (Figure 6 in Ref. II).

4.5.3.1. ROCK-mediated changes through the ROCK/LIMK/cofilin axis during decidualisation

To briefly sum up, the ROCK was the only kinase from our selection that showed a conserved elevated activity pattern for both *in vitro* and *in vivo* decidualisation conditions (Figure 1B and 4A in Ref. II). The inhibition experiment also showed that ROCK activity decreased in the lysates of the inhibitor-treated cells in a dose-dependent manner despite the ongoing influence of the decidualisation-inducing mixture. Moreover, at the highest (20μM) Y-27632 concentration, the decidualisation process was also disrupted whilst we detected decreased PRL secretion by those cells (Figure 3 in Ref. II). From the inhibition experiments, we conclude that ROCK activity is downstream of the initial cAMP-triggered signalling. This is evidenced by the fact that the selective PKA inhibitor H89 significantly reduces ROCK activity, while the selective ROCK inhibitor Y-27632 only has a minor inhibitory effect on PKA activity (Figure 3B in Ref. II). According to our proteomic data, ROCK2 isoform was more abundant in the secretory samples than in proliferative ones (Table I in Ref. II). In contrast, ROCK1 isoform was undetectable in most samples. Small GTPase RhoA is essential for the ROCK cascade activation (Riento and Ridley 2003). However, it was detected in both cycle phase samples, and there was no difference between the proliferative and

secretory samples (Supplementary Table SII in Ref. II, available in electronic version). Progesterone is needed to activate the ROCK axis and induce ROCK2 expression (Figure 2B and Supplementary Figures S6B and S6C in Ref. II). However, an *in vitro* decidualisation mixture containing only progesterone without 8-Br-cAMP or cAMP level modulating agents, like Fsk and IBMX, was inefficient enough to increase PRL secretion and elevate ROCK enzymatic activity (Figure 1 in Ref. II). Thus, we can hypothesise that the increase of ROCK activity might require activation of the PKA signalling, which is confirmed nicely by the inhibition experiment results.

In the secretory stroma samples, we identified enrichment of the pSer3 phosphorylation site on the CFL1 protein (Table II in Ref. II). That phosphosite is a substrate for the ROCK pathway downstream kinase LIMK (Prunier et al. 2017). According to the previous study, ROCK2 isoform is responsible for cofilin phosphorylation and stabilisation of the actin filaments (Shi et al., 2013). That coincides well with our results, which show that an increase in ROCK2 expression is detected in the *in vitro* decidualised stroma or secretory cycle samples. ROCK/LIMK/CFL pathway is a crucial regulator of the cellular actin cytoskeleton. Non-phosphorylated cofilin binds to F-actin and promotes actin filament depolymerisation, which allows active remodulation of the actin network by disassembly and assembly of the filaments. Phosphorylation of the cofilin in the pSer3 site leads to the loss of the phosphorylated cofilin affinity to actin filaments. In that case, a balance between the assembly and disassembly of the actin filaments is shifted, leading to stabilising the actin cytoskeleton. On the cellular level, it means that cell shape is stabilised and cell motility is decreased (Svitkina, 2018; K. Tanaka et al., 2018).

According to the literature, pathways related to the ROCK in the context of decidualisation have not been systematically studied. Few studies have been conducted that emphasize the role of the cell cytoskeleton changes during decidualisation (Tsuno et al. 2009; Ihnatovych et al. 2007; 2009). However, the results are contradictory because, *in vitro* decidualisation protocols, cell sources and cell culturing conditions are different across the studies, making any comparison difficult. For example, it was shown that ROCK1 and ROCK2 levels drop during *in vitro* decidualisation, but those cells were grown in a dense collagen gel mimicking 3D culture conditions (Tsuno et al., 2009). In contrast, our *in vitro* decidualisation data was obtained from regular 2D culture, making it hard to compare those results because, by default, cells grown in collagen gel will have different cytoskeletal organisation and cytoskeleton modulating enzyme activity levels and regulation compared to 2D culture. Two studies have been published where cell cytoskeleton organisation was investigated during decidualisation. It was found that elevated phosphorylated myosin light chain levels disturb *in vitro* decidualisation (Ihnatovych et al., 2007). Myosin light chain phosphorylation also follows a ROCK-dependent pathway. Their other finding was that artificially stabilised actin cytoskeleton by jasplakinolide, which enhances actin polymerisation, leads to *in vitro* decidualisation disruption. Nevertheless, actin assembly inhibitor latrunculin B, which in its molecular mechanism of action is the oppo-

site of jasplakinolide, also had an inhibitory effect on the *in vitro* decidualisation. It must be mentioned that the source of the stromal cells in that study was the placenta after delivery (Ihnatovych et al., 2009). Thus, those cells differ from stromal cells isolated directly from the endometrium, which we used in our study. Taken together, cytoskeleton reorganisation and associated signalling pathways are important during decidualisation; however, exact molecular mechanisms are poorly understood and not addressed in the literature at a detailed level.

We also accessed the structure of actin filaments and their orientation using high-resolution microscopy in the *in vitro* decidualised cells compared to controls. In the non-decidualised cells, actin filaments are primarily organised in a clear pattern of parallel lines forming stress fibres. The actin cytoskeleton of the *in vitro* decidualised cells looks more like a cross-linked mesh without a clear directionality (Figure 5 and Supplementary Figure S7 in Ref. II). It is known that the ROCK pathway plays a crucial role in the maintenance of stress fibres and regulates cellular motility (Prunier et al. 2017). Active unphosphorylated cofilin is needed to promote actin filaments disassembly and create actin barbed ends to maintain cell polarisation and migration motility (Dawe et al., 2003; DesMarais et al., 2005; Svitkina, 2018; K. Tanaka et al., 2018). A widely accepted hypothesis is that decidualisation is a special mesenchymal to epithelial transition case, making apparent large-scale cytoskeleton rearrangements during that process. Increased level of phosphorylated cofilin in the decidualised cells can explain morphological changes when the decidual reaction proceeds. During decidualisation, cells lose their front-rear polarity and reduce their motile capability. Decidulised epitheliod stroma cells establish phenotype with reduced motility and apical-basal cell polarity – characteristic of epithelium (Pan-Castillo et al., 2018; Yu et al., 2016; X.-H. Zhang et al., 2013). We showed that *in vitro* decidualised cell migration is decreased compared to untreated controls (Figure 6 in Ref. II). Previously, it was also shown that decidualised stroma motility is somewhat inhibited (Cloke et al., 2008). When chemotaxis of the stromal cells and stroma-based cell lines was investigated, it was also shown that inhibition of the ROCK kinase by Y-27632 increases the motility of the cells (Schwenke et al., 2013).

Based on our findings, we propose a model where decidualisation induces an increase in ROCK2 expression level and elevates enzymatic activity (Figure 8). *In vitro* decidualisation results show that both progesterone and cAMP are essential to increase ROCK2 expression and activity. Activation of the ROCK pathway leads to the downstream target CFL1 pSer3 phosphosite phosphorylation. Accumulation of the phosphorylated cofilin in the decidualised cells stabilizes the actin cytoskeleton and defines their epitheliod-like morphology and decreased motility. ROCK mediates changes through the ROCK/LIMK/cofilin axis during decidualisation, directing stromal cells through the mesenchymal to epithelial transition.

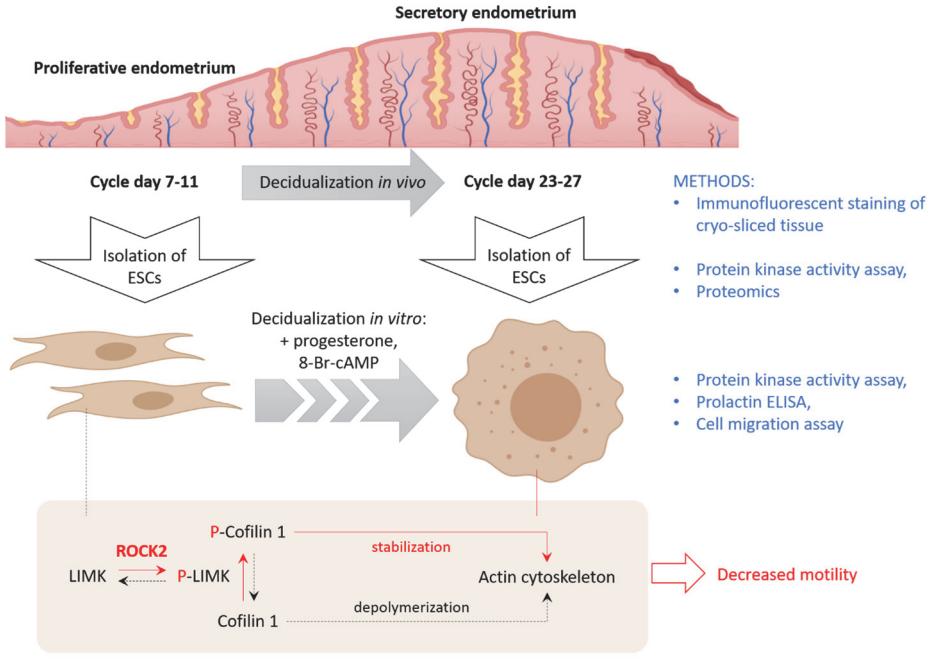

Figure 8. ROCK2-induced changes through ROCK/LIMK/cofilin axis during decidual reaction in the endometrial stroma. ROCK2 quantity and activity are elevated in the *in vitro* and *in vivo* decidualised cells. The ROCK/LIMK/cofilin axis is upregulated during decidualisation, leading to the remodelling of the actin cytoskeleton and decreased motility of decidualised stromal cells. Red arrows and red text indicate modifications carried out by ROCK2 in the stromal cells acquiring secretory phenotype after decidualisation. ROCK – Rho-dependent protein kinase, LIMK – LIM domain kinase, ESC – endometrial stromal cells, P – stands for phosphorylation, 8-Br-cAMP – 8-Bromoadenosine 3',5'-cyclic adenosine monophosphate. (Modified Figure 7 in Ref. II).

4.6. HGF/c-MET and ROCK2/LIMK/cofilin signalling pathways in the human endometrium

A widely accepted model suggests that in the many tissue types, stroma cells produce HGF, which is later utilised by the epithelial compartment of the tissue or by stroma cells themselves via autocrine signalling (Sakai, Aoki, and Matsumoto 2015; Totani et al. 2020; Zhao et al. 2022). Human endometrial tissue also actively produces HGF. In a recent study analysing the cytokines profile of menstrual blood, higher HGF levels were found in the menstrual blood compared to the peripheral blood samples (Naseri et al. 2023). Cytokine secretion profiles of endometrial stromal and epithelial cells, including HGF, depend on the interaction between different cell types and signals from the external cell environment, including hCG (Srivastava et al. 2013). However, the exact molecular mechanisms of HGF action in the endometrium are unclear. Special features of HGF

expression may cause several endometrial pathologies. In endometrial cancer, HGF produced by stromal cells promotes the proliferation and invasiveness of the epithelial cells via the HGF/c-MET/Akt signalling pathway (M. Li et al. 2015; Yoshida et al. 2002; 2004). HGF production and signalling changes were also noted in patients with endometriosis (Osuga et al. 1999). The invasiveness and proliferation of the endometriotic lesions can be partially explained by elevated HGF production levels by stromal cells (Ono et al. 2015). We found that MUC20 may play an important role in the HGF signalling regulation through MET receptor in the receptive mid-secretory endometrial epithelium cells (Ref. I).

Interestingly, ROCK kinase is one of the MET receptor downstream signalling targets (Royal et al. 2000; R. Li et al. 2015). We found increased ROCK2 activity in the decidualised stroma and the ROCK2/LIMK/cofilin pathway's involvement in the decidualisation process (Ref. II). We also saw increased *HGF* gene expression in the endometrial stroma in the receptive mid-secretory phase (LH+8) (Ref. I). Maybe stroma cell autocrine HGF/c-MET signalling also plays a role in the increased ROCK2 activity during stromal cell decidualisation. Moreover, decidualisation is closely related to the MET process. We can hypothesise that two signalling pathways, HGF/c-MET and ROCK2/LIMK/cofilin, may be involved in the complicated interplay and regulation of endometrial functions. However, further studies need to be done to confirm that hypothesis or disprove it. Further studies must reveal the possible role of the nature opposite MET and EMT processes in endometrial biology. Under especial interest are MET during stroma decidualisation and EMT of the endometrial epithelium during the WOI. These two processes could be important in the menstrual cycle progression and embryo implantation process, but their role must be resolved in future studies.

4.7. The role of integrin β1 in the maintaining of human embryonic stem cell culture and embryoid bodies formation

Various approaches have been developed to study the molecular-cellular interactions of the blastocyst with endometrial epithelial cells. Earlier models were based on using various cancer-derived cell lines to model endometrial lining and trophoblast spheroids to model blastocyst. More modern model systems use embryoid bodies derived from embryonic stem cells to mimic the blastocyst. However, maintaining pluripotent human embryonic stem cells requires very specific conditions for culturing. The complex interactions between the cells and extracellular matrix, guided by the integrins, are essential during stem cells culturing and embryoid bodies formation. Integrins regulate different stages of stem cell interactions with the extracellular matrix by establishing adhesion complexes and influencing cell migration, pluripotency, and differentiation potential (H. Wang, Luo, and Leighton 2015). The adhesion of cells is regulated by the integrins available on the cell surface that interact with ECM components (Meng

et al. 2010). Different combinations of integrin β and α chains form heterodimers which recognise and bind a specific ligand. Integrin β1 subunit combines with 12 α chains (LaFoya et al. 2018; Luo, Carman, and Springer 2007). Thus, the role of the integrin β1 subunit for the survival, migration and formation of embryoid bodies by embryonic stem cells is difficult to overestimate (Molè et al. 2021). The main aim of this part of our work (Ref. III) was to investigate the role of β1 integrin subunit in those interactions. Our study showed that the integrin β1 subunit is essential for assembling the adhesion complex and regulating the contractions of cells grown in culture.

Human embryonic stem cells require particular conditions for culturing, especially if large-scale production for embryoid bodies is needed or if even more complex blastoids and gastruloids are produced. Under standardised conditions, undifferentiated embryonic stem cells originating from the blastocyst inner cell mass grow in flat 2D colonies and can be manipulated to form spheroidal 3D embryoid bodies and differentiate into germ layer lineages. In cell culture conditions, human embryonic stem cells need a sufficient substitute for the extracellular matrix, which is usually replaced by Matrigel – a solubilised mixture of extracellular matrix proteins produced by mouse sarcoma cell line (Passaniti, Kleinman, and Martin 2022). The standard trypsinisation procedure is unsuitable for embryonic stem cell reseeding in the cell culture because it leads to massive cell death by disrupting contacts between cells and cell-ECM proteins. Embryonic stem cells are mostly manually reseeded as smaller colony fragments or as a single cell suspension obtained from the EDTA-induced dissociation. EDTA-obtained single cells are further cultured with the presence of ROCK inhibitor Y-27632. Inhibition of the ROCK pathway stimulates cell migration capability and the forming of new colonies. Integrins regulate different stages of stem cell interactions with the extracellular matrix by establishing adhesion complexes and influencing cell migration, pluripotency, and differentiation potential. The adhesion of cells is regulated by the integrins available on the cell surface that interact with Matrigel components. Different combinations of integrin β and α chains form heterodimers which recognise and bind a specific ligand. Thus, the role of integrin β1 for the survival, migration and formation of embryoid bodies by embryonic stem cells is difficult to overestimate. The purpose of our study was to show whether integrin β1 is essential for the assembly of adhesion complex during embryoid body formation.

First, we showed by the Fluorescence-Activated Cell Sorting (FACS) and Western blot analysis that the integrin β1 subunit is located on the surface of the human embryonic stem cells and, not surprisingly, that trypsinisation of the cells reduced significantly its expression on the cell surface (Figure 1B and Supplementary Figure S1 in Ref. III). The essential role of the integrin β1 subunit for the adhesion was confirmed by an experiment where embryonic stem cells were seeded on the Matrigel-coated plates in the presence of an integrin β1 subunit blocking antibody. Blocking antibody abolished the adhesion of the small colony fragments. When the ROCK kinase inhibitor Y-27632 was applied to the single-cell suspension, the blocking antibody hindering effect on adhesion was also

evident. However, the attachment of some cells could still be detected (Supplementary Figure S2A in Ref. III). That coincides with previous studies that showed that integrin heterodimer α6β1 is the central receptor of the embryonic stem cell for the interaction with laminins, one of the most crucial class of ECM proteins (Meng et al. 2010). Thus, blocking the β1 integrin chain severely affected the attachment of embryonic stem cells to the Matrigel, which, among other proteins, also contains laminins, especially laminin-111 (S. Kim et al. 2022).

As far as we demonstrated that the integrin β1 subunit is crucial for the embryonic stem cell adhesion on Matrigel, we studied in further experiments the role of the β1 subunit in forming embryoid bodies. Embryoid bodies can be produced from stem cell colony fragments acquired by manual reseeding or EDTA-detached single cells. Embryoid bodies obtained by these two methods have similar morphology, but embryoid bodies developed from the single cell were smaller than those gained from colony fragments. It is worth mentioning that embryoid bodies do not form from EDTA-obtained single cells without ROCK kinase inhibitor Y-27632 (Figure 7 in Ref. III). This indicates that the forming of embryoid bodies needs cell motility and contraction. We added the β1 integrin subunit-blocking antibody to the cell growth medium for 24h and followed the formation of embryoid bodies. While the integrin β1 subunit antibody concentration was increased, the number and size of the formed embryoid bodies decreased. Furthermore, when embryoid bodies were dissociated and analysed by FACS occurs that CD184 (endodermal marker) and nestin (ectodermal marker) expression was decreased, which indicates that the blocking of integrin β1 subunit affects the differentiation of cells to endodermal and ectodermal lineages (Figure 7 in Ref. III) (Holtzinger et al. 2015; Baba et al. 2022). Taken together, this data emphasises the important role of the integrin β1 subunit in embryoid bodies' formation and differentiation abilities.

We also developed a technique for the IHC analysis of embryoid bodies. To deal with such small objects, embryoid bodies were first immersed in 3% agarose for gelling before embedding them into the paraffin. Analysing 6-day-old embryoid bodies by IHC, we did not detect any signal which could show integrin β1 subunit expression (Figure 8E in Ref. III). However, the integrin β1 subunit was detected in the embryoid bodies' lysate (Supplementary Figure S1 in Ref. III). This controversy could be explained by the fact that the antibody was unsuitable for IHC. However, E-cadherin expression in the embryoid bodies showed somewhat interesting behaviour. The E-cadherin revealed the expression gradient within the embryoid body with an increase towards the outer layers of the cells (Figure 8 in Ref. III). It could be related to switching from E-cadherin to N-cadherin during the differentiation and epithelial-mesenchymal transition processes in the development of the embryoid body (Loh et al. 2019).

The culture of embryonic stem cells needs the proper microenvironment to maintain pluripotency and differentiation ability. Efficient adhesion of cells to ECM components is the first step towards cell survival and growth. We showed that the integrin β1 subunit is essential to maintain survival and differentiation of embryonic stem cells. The functionally active β1 subunit is needed for the stem

cells' adhesion to Matrigel, while blocking this integrin abolished cell adhesion and led to cell death in culture conditions. The embryoid bodies' formation was also impaired in the integrin β1 subunit blocking antibody presence. Adhesion of embryonic stem cells to the ECM also requires integrin β1 subunit expression on the cell plasma membrane. Attachment is a crucial step for the survival of embryonic stem cells and is necessary for maintaining the pluripotency and differentiation potential required for forming embryoid bodies. That is important to understand in the context of various protocols utilised for directed differentiation or producing different types of organoids required for research and translational therapy. In the context of reproductive biology research, the increasing trend is to use stem cell-derived embryo models (Y. Kim, Kim, and Shin 2023; Fu, Warmflash, and Lutolf 2021). Blastoids and gastruloids representing the early embryo developmental stages could be beneficial models for detailed investigation of molecular interactions between the endometrial lining and implanting blastocyst (Arias, Marikawa, and Moris 2022). Thus, research in this area will continue to develop. This is currently the best model for studying the interaction between the blastocyst and the endometrium if we exclude the use of human blastocysts.

SUMMARY AND CONCLUSIONS

This doctoral thesis explores molecular mechanisms that regulate female fertility, aiming to deepen our understanding of the fundamental biological process of blastocyst interaction with endometrial lining, which leads to embryo implantation and further pregnancy development. The study's objectives were focused on identifying the novel molecular pathways that regulate endometrial physiology and embryoid bodies' early development in cell culture. Through this research, we understand better how proceeds successful implantation which offers new insight into reproductive health. A combination of molecular biology techniques, including gene expression analysis, protein assays, *in vitro* models and histological approaches, were employed to elucidate the roles of various signalling pathways in regulating endometrial receptivity.

Key findings can be summarised as follows. The first aim was to investigate the expression pattern of different mucins on the endometrial surface during the WOI and determine their role in cell adhesion or signalling. It was found that MUC20 expression, particularly in the mid-secretory endometrium, is elevated. A model of MUC20 interaction with MET receptor was proposed that could regulate scattering and morphogenesis of endometrial epithelial cells. The second aim was to explore the landscape of kinase activities in the human endometrium during decidualisation, which prepares the endometrium for blastocyst implantation. The increased expression and activity of ROCK2 kinase were found during *in vitro* and *in vivo* decidualised stromal cells. ROCK2-mediated changes in the stromal cells during decidualisation are induced through the ROCK/LIMK/cofilin pathway, leading to the acquisition of the secretory phenotype. Due to the ethical limitations required for human embryo manipulation, it is important to understand different protocols utilised for organoid model systems production for research and translational therapy. Thus, the last aim of our work was to elucidate the formation of embryoid bodies from embryonic stem cells in cell culture conditions. Blocking of functionally active integrin β1 subunit by specific antibody abolished cell adhesion, and the formation of embryoid bodies was impaired in cell culture conditions.

This study has several limitations, even though we tried to use the most available research methods. Although new molecular pathways involved in endometrium decidualisation and preparation for embryo implantation have been identified, direct cell-to-cell signalling interactions were not investigated, and only hypothetical models of interactions were proposed. The investigation of the formation of embryoid bodies in cell culture was in its early stages in the current thesis. However, in recent years, significant progress has been made in the *in vitro* production of human blastoids and gastruloids, which serve as valuable models for studying early development and implantation in human embryos. This study cannot be viewed from a clinical perspective, as no clinical trials were conducted for validation. However, the new insights gained throughout this thesis enhance the understanding of the complex process of embryo implantation in humans and

may contribute to the development of improved prognostic biomarkers and therapeutic strategies for infertility.

This doctoral dissertation attempts to examine potential interactions both from the embryo's side and from the two main cellular parts of the endometrium – epithelial and stromal. Future research could build upon this work to investigate proposed molecular interactions in the decidualised endometrium and during embryo-endometrium interaction. Ultimately, this thesis has demonstrated that physiological and morphological changes in the endometrial cells related to mesenchymal to epithelial transition play an important role during decidualisation and WOI. Epithelial-to-mesenchymal and, vice versa, epithelial-to-mesenchymal transitions need to be considered as an important mechanism of endometrial and implantation biology and addressed in detail in future research. The biology of embryo implantation still has many questions that have no exact answer. This fascinating process during which the first long-term physical contact between the mother's body and the developing embryo occurs is of fundamental importance for every person, as it is a prerequisite for the development of pregnancy and will excite many generations of researchers.

SUMMARY IN ESTONIAN

Adhesioonimolekulide ja signaaliradade funktsioonide uurimine kasutades inimese endomeetriumi ja embrüo mudeleid

Käesolevas doktoritöös uuriti molekulaarseid mehhanisme, mis reguleerivad naiste viljakust, eesmärgiga mõista kuidas blastotsüst seostub endomeetriumi pinnaga, mille tulemusena toimub embrüo implanteerumine ning raseduse väljakujunemine. Meie eesmärgiks oli leida uusi molekulaarseid signaaliradu, mis reguleerivad endomeetriumi füsioloogiat ning lisaks sellele uurida embrüoidkehakeste arengut rakukultuuris. Meie uuringute tulemused lubavad paremini aru saada kuidas kulgeb embrüo edukas implanteerumine endomeetriumisse ehk pesastumine, mis on oluliseks eelduseks naise viljakuse väljakujunemises. Kombineerides molekulaarbioloogilisi meetodeid, kaasa arvatud geeniekspressiooni analüüsi, valkude tuvastamist, *in vitro* mudeleid ning immunohistokeemilist ja immunofluorestsents analüüse, õnnestus meil tuvastada mitmeid uusi signaaliradasid, mis reguleerivad endomeetriumi vastuvõtlikkust blastotsüsti vastu.

Meie esmaseks ülesandeks oli iseloomustada mutsiinide perekonna valkude ekspressiooni mustrit naise endomeetriumis spetsiifiliselt implantatsiooni akna kontekstis ning selgitada, milline on mutsiinide osa rakkude adhesiooni ja signaliseerimise protsessis. Selgus, et mutsiini MUC20 ekspressioon suureneb just endomeetriumi kesk-sekretoorses faasis. Me pakume välja mudeli, kus MUC20 ja MET retseptor toimivad koos, mis võib olla epiteelirakkude morfogeneesi reguleerimise aluseks. Teiseks me soovisime kaardistada detsidualiseerumise käigus toimuvaid kinaaside aktiivsuse muutusi, mis valmistavad inimese endomeetriumi ette blastotsüsti ehk lootepõiekese implanteerumiseks. Me täheldasime, et ROCK2 kinaasi ekspressioon ja aktiivsus tõusevad detsidualiseerunud endomeetriumi strooma rakkudes nii *in vitro* kui ka *in vivo* tingimustes. Detsidualiseerumise käigus põhjustab ROCK2 kinaas ROCK/LIMK/kofiliini signaaliraja kaudu strooma rakkude muutusi, mille tulemusel kujuneb välja endomeetriumi sekretoorne fenotüüp. Kuivõrd inimese embrüo uuringute puhul kehtivad eetilised piirangud, siis organoidsete mudelsüsteemide kasutamise korral saab uurida ka embrüo arengu kõige varasemaid etappe. Sellest lähtuvalt oli meie töö üheks eesmärgiks selgitada kuidas kulgeb embrüoidkehakeste teke embrüonaalsetest tüvirakkudestkoekultuuris. Kui me blokeerisime spetsiifilise antikehaga β1 integriini funktsionaalselt aktiivse alaühiku, siis oli nii rakkude adhesioon kui ka embrüoidkehakeste teke koekultuuris häiritud.

Meie töö tulemused ei anna siiski püstitatud küsimustele lõplikke vastuseid, kuigi me tuvastasime mõned uued signaalirajad (MUC20/MET-retseptor ja ROCK/LIMK/kofiliin), mis osalevad endomeetriumi detsidualiseerumises ja embrüo implanteerumiseks ettevalmistamises. Embrüoidkehakeste teke embrüonaalsetest tüvirakkudest koekultuuris jäi selle töö käigus uurimise algfaasi. Tuleb siiski märkida, et viimastel aastatel on tehtud märkimisväärseid edusamme saamaks *in vitro* tingimustes inimese blastoide ja gastruloide, mis on väärtuslikuks

materjaliks, et uurida inimese embrüo varajast arengut ja implanteerumist. See töö ei lähtu kliinilisest vaatepunktist, kuigi meie poolt saadud tulemused aitavad kindlasti laiemalt mõista inimese embrüo implanteerumise protsessi ning lisaks sellele me pakume välja mõned uued biomarkerite kandidaadid, mis võiksid sobida endomeetriumi dateerimiseks ja märklaudadeks tulevaste uuringute jaoks.

Selles doktoritöös vaadeldi rakkude omavahelisi seoseid ja suhtlemist nii embrüo kui ka endomeetriumi epiteeli ja strooma rakkude seisukohast. Edaspidise töö käigus tuleks uurida meie poolt tuvastatud molekulaarsete seoste olemasolu detsidualiseerunud endomeetriumis ning seda ka embrüo pesastumisel. Kokkuvõtteks võib saadud tulemuste põhjal öelda, et endomeetriumi rakkude füsioloogilised ja morfoloogilised muutused on väga sarnased mesenhümaalsele-epiteliaalsele üleminekule ja sellel on oluline osa detsidualiseerumises ning implantatsiooni akna reguleerimises. Epiteliaalsel-mesenhümaalsel ja vastupidi mesenhümaalsel-epiteliaalsel üleminekutel on ilmselt tähtis osa endomeetriumi funktsioneerimises ja implanteerumise kulgemises, kuid see vajaks kindlasti edasisi uuringuid. Embrüo implanteerumise biloogia hõlmab veel palju senini lahendamata küsimusi. See on mitmetahuline protsess, mille käigus tekib esimene pikaaegne kontakt ema keha ja areneva embrüo vahel ning sellel on määrav osa raseduse edasiseks edukaks kulgemiseks.

REFERENCES

Achache, Hanna, and Ariel Revel. 2006. 'Endometrial Receptivity Markers, the Journey to Successful Embryo Implantation'. *Human Reproduction Update* 12 (6): 731–46. https://doi.org/10.1093/humupd/dml004.

Aghajanova, Lusine, Anneli Stavreus-Evers, Yorgos Nikas, Outi Hovatta, and Britt-Marie Landgren. 2003. 'Coexpression of Pinopodes and Leukemia Inhibitory Factor, as Well as Its Receptor, in Human Endometrium'. *Fertility and Sterility* 79 (March):808–14. https://doi.org/10.1016/S0015-0282(02)04830-6.

Alameda, Francesc, Raquel Mejías-Luque, Marta Garrido, and Carme De Bolós. 2007. 'Mucin Genes (MUC2, MUC4, MUC5AC, and MUC6) Detection in Normal and Pathological Endometrial Tissues'. *International Journal of Gynecological Pathology* 26 (1): 61–65. https://doi.org/10.1097/01.pgp.0000225837.32719.c1.

Altmäe, Signe, Mariann Koel, Urmo Võsa, Priit Adler, Marina Suhorutšenko, Triin Laisk-Podar, Viktorija Kukushkina, et al. 2017. 'Meta-Signature of Human Endometrial Receptivity: A Meta-Analysis and Validation Study of Transcriptomic Biomarkers'. *Scientific Reports* 7 (1): 10077. https://doi.org/10.1038/s41598-017-10098-3.

Altmäe, Signe, Jüri Reimand, Outi Hovatta, Pu Zhang, Juha Kere, Triin Laisk, Merli Saare, et al. 2012. 'Research Resource: Interactome of Human Embryo Implantation: Identification of Gene Expression Pathways, Regulation, and Integrated Regulatory Networks'. *Molecular Endocrinology* 26 (1): 203–17. https://doi.org/10.1210/me.2011-1196.

Álvarez-Santos, Mayra D., Marisol Álvarez-González, Samuel Estrada-Soto, and Blanca Bazán-Perkins. 2020. 'Regulation of Myosin Light-Chain Phosphatase Activity to Generate Airway Smooth Muscle Hypercontractility'. *Frontiers in Physiology* 11 (June). https://doi.org/10.3389/fphys.2020.00701.

Amoss, Max, Roger Burgus, Richard Blackwell, Wylie Vale, Robert Fellows, and Roger Guillemin. 1971. 'Purification, Amino Acid Composition and N-Terminus of the Hypothalamic Luteinizing Hormone Releasing Factor (Lrf) of Ovine Origin'. *Biochemical and Biophysical Research Communication* 44 (1): 205–10.

Aplin, J. D. 1999. 'MUC-1 Glycosylation in Endometrium: Possible Roles of the Apical Glycocalyx at Implantation'. *Human Reproduction* 14 (suppl 2): 17–25. https://doi.org/10.1093/humrep/14.suppl_2.17.

———. 2006. 'Embryo Implantation: The Molecular Mechanism Remains Elusive'. *Reproductive BioMedicine Online* 13 (6): 833–39. https://doi.org/10.1016/S1472-6483(10)61032-2.

Aplin, J. D., and C. J. Jones. 2012. 'Fucose, Placental Evolution and the Glycocode'. *Glycobiology* 22 (4): 470–78. https://doi.org/10.1093/glycob/cwr156.

Aplin, J. D., and Susan J Kimber. 2004. 'Trophoblast-Uterine Interactions at Implantation.' *Reproductive Biology and Endocrinology : RB&E* 2 (July):48. https://doi.org/doi.org/10.1186/1477-7827-2-48.

Aplin, J. D., and Peter T. Ruane. 2017. 'Embryo–Epithelium Interactions during Implantation at a Glance'. Edited by Andrew Ewald. *Journal of Cell Science* 130 (1): 15–22. https://doi.org/10.1242/jcs.175943.

Aplin, J. D., and Adam Stevens. 2022. 'Use of 'omics for Endometrial Timing: The Cycle Moves On'. *Human Reproduction* 37 (4): 644–50. https://doi.org/10.1093/humrep/deac022.

Aplin, J.D., A.K. Charlton, and S. Ayad. 1988. 'An Immunohistochemical Study of Human Endometrial Extracellular Matrix during the Menstrual Cycle and First Trimester of Pregnancy'. *Cell and Tissue Research* 253 (1): 231–40. https://doi.org/10.1007/BF00221758.

Argüeso, Pablo, and Ilene K. Gipson. 2001. 'Epithelial Mucins of the Ocular Surface: Structure, Biosynthesis and Function'. *Experimental Eye Research* 73 (3): 281–89. https://doi.org/10.1006/exer.2001.1045.

Arias, Alfonso Martinez, Yusuke Marikawa, and Naomi Moris. 2022. 'Gastruloids: Pluripotent Stem Cell Models of Mammalian Gastrulation and Embryo Engineering'. *Developmental Biology* 488 (August):35–46. https://doi.org/10.1016/j.ydbio.2022.05.002.

Ashkar, Ali A., Gordon P. Black, Qingxia Wei, Hong He, Luchuan Liang, Judith R. Head, and B. Anne Croy. 2003. 'Assessment of Requirements for IL-15 and IFN Regulatory Factors in Uterine NK Cell Differentiation and Function During Pregnancy 1'. *The Journal of Immunology* 171 (6): 2937–44. https://doi.org/10.4049/jimmunol.171.6.2937.

Baba, Yuta, Saki Onishi-Sakamoto, Kaori Ide, and Koji Nishifuji. 2022. 'Nestin Is a Marker of Unipotent Embryonic and Adult Progenitors Differentiating into an Epithelial Cell Lineage of the Hair Follicles'. *Scientific Reports* 12 (1): 17820. https://doi.org/10.1038/s41598-022-22427-2.

Baird, David T., S.T. Cameron, H.O.D. Critchley, T.A. Drudy, A. Howe, R.L. Jones, R.G. Lea, and R.W. Kelly. 1996. 'Prostaglandins and Menstruation'. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 70 (1): 15–17. https://doi.org/10.1016/S0301-2115(96)02568-7.

Bartsch, Olaf, Bettina Bartlick, and Richard Ivell. 2004. 'Phosphodiesterase 4 Inhibition Synergizes with Relaxin Signaling to Promote Decidualization of Human Endometrial Stromal Cells'. *The Journal of Clinical Endocrinology & Metabolism* 89 (1): 324–34. https://doi.org/10.1210/jc.2003-030498.

Bastu, Ercan, Mehmet F. Mutlu, Cenk Yasa, Ozlem Dural, Asli Nehir Aytan, Cem Celik, Faruk Buyru, and John Yeh. 2015. 'Role of Mucin 1 and Glycodelin A in Recurrent Implantation Failure'. *Fertility and Sterility* 103 (4): 1059-1064.e2. https://doi.org/10.1016/j.fertnstert.2015.01.025.

Battersby, S, H O D Critchley, A J de Brum-Fernandes, and H N Jabbour. 2004. 'Temporal Expression and Signalling of Prostacyclin Receptor in the Human Endometrium across the Menstrual Cycle'. *Reproduction* 127 (1): 79–86. https://doi.org/10.1530/rep.1.00038.

Ben Rafael, Zion. 2021. 'Endometrial Receptivity Analysis (ERA) Test: An Unproven Technology'. *Human Reproduction Open* 2021 (2). https://doi.org/10.1093/hropen/hoab010.

Ben-Jonathan, Nira, Christopher R. LaPensee, and Elizabeth W. LaPensee. 2008. 'What Can We Learn from Rodents about Prolactin in Humans?' *Endocrine Reviews* 29 (1): 1–41. https://doi.org/10.1210/er.2007-0017.

Bhurke, Arpita S., Indrani C. Bagchi, and Milan K. Bagchi. 2016. 'Progesterone-Regulated Endometrial Factors Controlling Implantation'. *American Journal of Reproductive Immunology* 75 (3): 237–45. https://doi.org/10.1111/aji.12473.

Bischof, P, and A Campana. 1996. 'A Model for Implantation of the Human Blastocyst and Early Placentation'. *Human Reproduction Update* 2 (3): 262–70. https://doi.org/10.1093/humupd/2.3.262.

Bitler, Benjamin G., Aarthi Goverdhan, and Joyce A. Schroeder. 2010. 'MUC1 Regulates Nuclear Localization and Function of the Epidermal Growth Factor Receptor'. *Journal of Cell Science* 123 (10): 1716–23. https://doi.org/10.1242/jcs.062661.

Brar, Anoop K., Graeme R. Frank, Cherie A. Kessler, Marcelle I. Cedars, and S. Handwerger. 1997. 'Progesterone-Dependent Decidualization of the Human Endometrium Is Mediated by cAMP'. *Endocrine* 6 (3): 301–7. https://doi.org/10.1007/BF02820507.

Brar, Anoop K., Stuart Handwerger, Cherie A. Kessler, and Bruce J. Aronow. 2001. 'Gene Induction and Categorical Reprogramming during in Vitro Human Endometrial Fibroblast Decidualization'. *Physiological Genomics* 7 (2): 135–48. https://doi.org/10.1152/physiolgenomics.00061.2001.

Brosens, Ivo, Robert Pijnenborg, Lisbeth Vercruysse, and Roberto Romero. 2011. 'The "Great Obstetrical Syndromes" Are Associated with Disorders of Deep Placentation'. *American Journal of Obstetrics and Gynecology* 204 (3): 193–201. https://doi.org/10.1016/j.ajog.2010.08.009.

Brosens, Jan J., Madhuri S. Salker, Gijs Teklenburg, Jaya Nautiyal, Scarlett Salter, Emma S. Lucas, Jennifer H. Steel, et al. 2014. 'Uterine Selection of Human Embryos at Implantation'. *Scientific Reports* 4 (1): 3894. https://doi.org/10.1038/srep03894.

Bulmer, Judith N, Lynn Morrison, Margaret Longfellow, Anne Ritson, and Denise Pace. 1991. 'Granulated Lymphocytes in Human Endometrium: Histochemical and Immunohistochemical Studies'. *Human Reproduction* 6 (6): 791–98.

Burg, Bart van der, and Paul T.van der Saag. 1996. 'Endocrinology and Paracrinology: Nuclear Factor-Kappa-B/Steroid Hormone Receptor Interactions as a Functional Basis of Anti-Inflammatory Action of Steroids in Reproductive Organs.' *Molecular Human Reproduction* 2 (6): 433–38. https://doi.org/10.1093/molehr/2.6.433.

Burger, H.G. 1993. 'Evidence for a Negative Feedback Role of Inhibin in Follicle Stimulating Hormone Regulation in Women'. *Human Reproduction* 8 (suppl_2): 129–32. https://doi.org/10.1093/humrep/8.suppl_2.129.

Burton, Graham J., Tereza Cindrova-Davies, and Margherita Y. Turco. 2020. 'Review: Histotrophic Nutrition and the Placental-Endometrial Dialogue during Human Early Pregnancy'. *Placenta* 102 (December):21–26. https://doi.org/10.1016/j.placenta.2020.02.008.

Burton, Graham J., and Eric Jauniaux. 2018. 'Pathophysiology of Placental-Derived Fetal Growth Restriction'. *American Journal of Obstetrics and Gynecology* 218 (2): S745–61. https://doi.org/10.1016/j.ajog.2017.11.577.

Canse, Ceren, Ecem Yildirim, and Aylin Yaba. 2023. 'Overview of Junctional Complexes during Mammalian Early Embryonic Development'. *Frontiers in Endocrinology* 14 (April). https://doi.org/10.3389/fendo.2023.1150017.

Carson, Daniel D., Indrani Bagchi, Sudhandsu K. Dey, Allen C. Enders, Asgerally T. Fazleabas, Bruce A. Lessey, and Koji Yoshinaga. 2000. 'Embryo Implantation'. *Developmental Biology* 223 (2): 217–37. https://doi.org/10.1006/dbio.2000.9767.

Catalano, R. D., M. R. Wilson, S. C. Boddy, and H. N. Jabbour. 2011. 'Comprehensive Expression Analysis of Prostanoid Enzymes and Receptors in the Human Endometrium across the Menstrual Cycle'. *Molecular Human Reproduction* 17 (3): 182–92. https://doi.org/10.1093/molehr/gaq094.

Chen, Chi-Hau, Ming-Kwang Shyu, Shu-Wei Wang, Chih-Hsing Chou, Miao-Juei Huang, Tzu-Chi Lin, Syue-Ting Chen, Ho-Hsiung Lin, and Min-Chuan Huang. 2016. 'MUC20 Promotes Aggressive Phenotypes of Epithelial Ovarian Cancer Cells via Activation of the Integrin B1 Pathway'. *Gynecologic Oncology* 140 (1): 131–37. https://doi.org/10.1016/j.ygyno.2015.11.025.

Chen, Chi-Hau, Shu-Wei Wang, Chih-Wei Chen, Miao-Ruei Huang, Ji-Shiang Hung, Hsiu-Chin Huang, Ho-Hsiung Lin, Ruey-Jien Chen, Ming-Kwang Shyu, and Min-Chuan Huang. 2013. 'MUC20 Overexpression Predicts Poor Prognosis and Enhances EGF-Induced Malignant Phenotypes via Activation of the EGFR–STAT3 Pathway in Endometrial Cancer'. *Gynecologic Oncology* 128 (3): 560–67. https://doi.org/10.1016/j.ygyno.2012.12.012.

Chen, Joseph C., David W. Erikson, Terhi T. Piltonen, Michelle R. Meyer, Fatima Barragan, Ramsey H. McIntire, John S. Tamaresis, Kim Chi Vo, Linda C. Giudice, and Juan C. Irwin. 2013. 'Coculturing Human Endometrial Epithelial Cells and Stromal Fibroblasts Alters Cell-Specific Gene Expression and Cytokine Production'. *Fertility and Sterility* 100 (4): 1132–43. https://doi.org/10.1016/j.fertnstert.2013.06.007.

Chen, Joseph C., Jacquelyn R. Hoffman, Ripla Arora, Lila A. Perrone, Christian J. Gonzalez-Gomez, Kim Chi Vo, Diana J. Laird, Juan C. Irwin, and Linda C. Giudice. 2016. 'Cryopreservation and Recovery of Human Endometrial Epithelial Cells with High Viability, Purity, and Functional Fidelity'. *Fertility and Sterility* 105 (2): 501-510.e1. https://doi.org/10.1016/j.fertnstert.2015.10.011.

Chen, Joseph C., Brittni A. Johnson, David W. Erikson, Terhi T. Piltonen, Fatima Barragan, Simon Chu, Nargis Kohgadai, et al. 2014. 'Seminal Plasma Induces Global Transcriptomic Changes Associated with Cell Migration, Proliferation and Viability in Endometrial Epithelial Cells and Stromal Fibroblasts'. *Human Reproduction* 29 (6): 1255–70. https://doi.org/10.1093/humrep/deu047.

Chen, Joseph C, and Nadia R Roan. 2015. 'Isolation and Culture of Human Endometrial Epithelial Cells and Stromal Fibroblasts'. *BIO-PROTOCOL* 5:1–10.

Chen, Syue Ting, Ting Chun Kuo, Ying Yu Liao, Mei Chun Lin, Yu Wen Tien, and Min Chuan Huang. 2018. 'Silencing of MUC20 Suppresses the Malignant Character of Pancreatic Ductal Adenocarcinoma Cells through Inhibition of the HGF/MET Pathway'. *Oncogene*, 1–13. https://doi.org/10.1038/s41388-018-0403-0.

Chen, Xinyi, Ineza Karambizi Sandrine, Mu Yang, Jingyao Tu, and Xianglin Yuan. 2024. 'MUC1 and MUC16: Critical for Immune Modulation in Cancer Therapeutics'. *Frontiers in Immunology* 15 (February). https://doi.org/10.3389/fimmu.2024.1356913.

Cheng, Guanliang, Lique M. Coolen, Vasantha Padmanabhan, Robert L. Goodman, and Michael N. Lehman. 2010. 'The Kisspeptin/Neurokinin B/Dynorphin (KNDy) Cell Population of the Arcuate Nucleus: Sex Differences and Effects of Prenatal Testosterone in Sheep'. *Endocrinology* 151 (1): 301–11. https://doi.org/10.1210/en.2009-0541.

Cheng, L., R W Kelly, K J Thong, R Hume, and D T Baird. 1993. 'The Effect of Mifepristone (RU486) on the Immunohistochemical Distribution of Prostaglandin E and Its Metabolite in Decidual and Chorionic Tissue in Early Pregnancy.' *The Journal of Clinical Endocrinology & Metabolism* 77 (3): 873–77. https://doi.org/10.1210/jcem.77.3.8370712.

Christian, Mark, Xiaohui Zhang, Tanja Schneider-Merck, Terry G. Unterman, Birgit Gellersen, John O. White, and Jan J. Brosens. 2002. 'Cyclic AMP-Induced Forkhead Transcription Factor, FKHR, Cooperates with CCAAT/Enhancer-Binding Protein β in Differentiating Human Endometrial Stromal Cells*'. *Journal of Biological Chemistry* 277 (23): 20825–32. https://doi.org/10.1074/jbc.M201018200.

Conneely, O. 2000. 'Progesterone Receptors in Reproduction: Functional Impact of the A and B Isoforms'. *Steroids* 65 (10–11): 571–77. https://doi.org/10.1016/S0039-128X(00)00115-X.

Corbacho, A. M., G. Martinez De La Escalera, and C. Clapp. 2002. 'Roles of Prolactin and Related Members of the Prolactin/Growth Hormone/Placental Lactogen Family in Angiogenesis', May. https://doi.org/10.1677/joe.0.1730219.

Corfield, Anthony P. 2015. 'Mucins: A Biologically Relevant Glycan Barrier in Mucosal Protection'. *Biochimica et Biophysica Acta – General Subjects* 1850 (1): 236–52. https://doi.org/10.1016/j.bbagen.2014.05.003.

Cornillie, F.J., J.M. Lauweryns, and I.A. Brosens. 1985. 'Normal Human Endometrium'. *Gynecologic and Obstetric Investigation* 20 (3): 113–29. https://doi.org/10.1159/000298983.

Coticchio, Giovanni, Mariabeatrice Dal Canto, Mario Mignini Renzini, Maria Cristina Guglielmo, Fausta Brambillasca, Diana Turchi, Paola Vittoria Novara, and Rubens Fadini. 2015. 'Oocyte Maturation: Gamete-Somatic Cells Interactions, Meiotic Resumption, Cytoskeletal Dynamics and Cytoplasmic Reorganization'. *Human Reproduction Update* 21 (4): 427–54. https://doi.org/10.1093/humupd/dmv011.

Coulam, Carolyn. 2016. 'What about Superfertility, Decidualization, and Natural Selection?' *Journal of Assisted Reproduction and Genetics* 33 (5): 577–80. https://doi.org/10.1007/s10815-016-0658-8.

Cousins, Fiona L., Phoebe M. Kirkwood, Alison A. Murray, Frances Collins, Douglas A. Gibson, and Philippa T.K. Saunders. 2016. 'Androgens Regulate Scarless Repair of the Endometrial "Wound" in a Mouse Model of Menstruation'. *FASEB Journal* 30 (8): 2802–11. https://doi.org/10.1096/fj.201600078R.

Cousins, Fiona L, Alison Murray, Arantza Esnal, Douglas A Gibson, Hilary O D Critchley, and Philippa T K Saunders. 2014. 'Evidence from a Mouse Model That Epithelial Cell Migration and Mesenchymal-Epithelial Transition Contribute to Rapid Restoration of Uterine Tissue Integrity during Menstruation.' *PLOS ONE* 9 (1): e86378. https://doi.org/10.1371/journal.pone.0086378.

Cousins, Fiona L., Ronald Pandoy, Shiying Jin, and Caroline E. Gargett. 2021. 'The Elusive Endometrial Epithelial Stem/Progenitor Cells'. *Frontiers in Cell and Developmental Biology* 9 (April). https://doi.org/10.3389/fcell.2021.640319.

Craciunas, Laurentiu, Ioannis Gallos, Justin Chu, Tom Bourne, Siobhan Quenby, Jan J Brosens, and Arri Coomarasamy. 2019. 'Conventional and Modern Markers of Endometrial Receptivity: A Systematic Review and Meta-Analysis'. *Human Reproduction Update* 25 (2): 202–23. https://doi.org/10.1093/humupd/dmy044.

Critchley, Hilary O. D., Julia Osei, Teresa A. Henderson, Lyndsey Boswell, Kurt J. Sales, Henry N. Jabbour, and Nik Hirani. 2006. 'Hypoxia-Inducible Factor-1α Expression in Human Endometrium and Its Regulation by Prostaglandin E-Series Prostanoid Receptor 2 (EP2)'. *Endocrinology* 147 (2): 744–53. https://doi.org/10.1210/en.2005-1153.

Daly, Douglas C., Ila A. Maslar, and Daniel H. Riddick. 1983. 'Prolactin Production during in Vitro Decidualization of Proliferative Endometrium'. *American Journal of Obstetrics and Gynecology* 145 (6): 672–78. https://doi.org/10.1016/0002-9378(83)90572-0.

Dawe, Helen R, Laurie S Minamide, James R Bamburg, and Louise P Cramer. 2003. 'ADF/Cofilin Controls Cell Polarity during Fibroblast Migration'. *Current Biology* 13 (3): 252–57. https://doi.org/10.1016/S0960-9822(03)00040-X.

Denker, H.-W. 1993. 'Implantation: A Cell Biological Paradox'. *Journal of Experimental Zoology* 266 (6): 541–58. https://doi.org/10.1002/jez.1402660606.

DesMarais, Vera, Mousumi Ghosh, Robert Eddy, and John Condeelis. 2005. 'Cofilin Takes the Lead'. *Journal of Cell Science* 118 (1): 19–26. https://doi.org/10.1242/jcs.01631.

Dharmaraj, N., P. J. Chapela, M. Morgado, S. M. Hawkins, B. A. Lessey, S. L. Young, and D. D. Carson. 2014. 'Expression of the Transmembrane Mucins, MUC1, MUC4 and MUC16, in Normal Endometrium and in Endometriosis'. *Human Reproduction* 29 (8): 1730–38. https://doi.org/10.1093/humrep/deu146.

Díaz-Gimeno, Patricia, José A. Horcajadas, José A. Martínez-Conejero, Francisco J. Esteban, Pilar Alamá, Antonio Pellicer, and Carlos Simón. 2011. 'A Genomic Diagnostic Tool for Human Endometrial Receptivity Based on the Transcriptomic Signature'. *Fertility and Sterility* 95 (1): 50-60.e15. https://doi.org/10.1016/j.fertnstert.2010.04.063.

Díaz-Gimeno, Patricia, Maria Ruiz-Alonso, David Blesa, Nuria Bosch, José A. Martínez-Conejero, Pilar Alamá, Nicolás Garrido, Antonio Pellicer, and Carlos Simón. 2013. 'The Accuracy and Reproducibility of the Endometrial Receptivity Array Is Superior to Histology as a Diagnostic Method for Endometrial Receptivity'. *Fertility and Sterility* 99 (2): 508–17. https://doi.org/10.1016/j.fertnstert.2012.09.046.

Díaz-Gimeno, Patricia, Maria Ruiz-Alonso, Patricia Sebastian-Leon, Antonio Pellicer, Diana Valbuena, and Carlos Simón. 2017. 'Window of Implantation Transcriptomic Stratification Reveals Different Endometrial Subsignatures Associated with Live Birth and Biochemical Pregnancy'. *Fertility and Sterility* 108 (4): 703-710.e3. https://doi.org/10.1016/j.fertnstert.2017.07.007.

Dimitriadis, Evdokia, Chelsea Stoikos, Yee-Lee Tan, and Lois A. Salamonsen. 2006. 'Interleukin 11 Signaling Components Signal Transducer and Activator of Transcription 3 (STAT3) and Suppressor of Cytokine Signaling 3 (SOCS3) Regulate Human Endometrial Stromal Cell Differentiation'. *Endocrinology* 147 (8): 3809–17. https://doi.org/10.1210/en.2006-0264.

Dupont, S., A. Krust, A. Gansmuller, A. Dierich, P. Chambon, and M. Mark. 2000. 'Effect of Single and Compound Knockouts of Estrogen Receptors Alpha (ERalpha) and Beta (ERbeta) on Mouse Reproductive Phenotypes'. *Development* 127 (19): 4277–91. https://doi.org/10.1242/dev.127.19.4277.

Durairaj, Ruban Rex Peter, Asma Aberkane, Lukasz Polanski, Yojiro Maruyama, Miriam Baumgarten, Emma S Lucas, Siobhan Quenby, et al. 2017. 'Deregulation of the Endometrial Stromal Cell Secretome Precedes Embryo Implantation Failure'. *Molecular Human Reproduction* 23 (7): 478–87. https://doi.org/10.1093/molehr/gax023.

Edwards, Robert G. 2006. 'Human Implantation: The Last Barrier in Assisted Reproduction Technologies?' *Reproductive BioMedicine Online* 13 (6): 887–904. https://doi.org/10.1016/S1472-6483(10)61039-5.

Emera, Deena, Roberto Romero, and Günter Wagner. 2012. 'The Evolution of Menstruation: A New Model for Genetic Assimilation'. *BioEssays* 34 (1): 26–35. https://doi.org/10.1002/bies.201100099.

Enciso, M., J. Aizpurua, B. Rodríguez-Estrada, I. Jurado, M. Ferrández-Rives, E. Rodríguez, E. Pérez-Larrea, A. B. Climent, K. Marron, and J. Sarasa. 2021. 'The Precise Determination of the Window of Implantation Significantly Improves ART Outcomes'. *Scientific Reports* 11 (1): 13420. https://doi.org/10.1038/s41598-021-92955-w.

Enciso, M, J P Carrascosa, J Sarasa, P A Martínez-Ortiz, S Munné, J A Horcajadas, and J Aizpurua. 2018. 'Development of a New Comprehensive and Reliable Endometrial Receptivity Map (ER Map/ER Grade) Based on RT-qPCR Gene Expression Analysis'. *Human Reproduction* 33 (2): 220–28. https://doi.org/10.1093/humrep/dex370.

Enders, Allen C., Andrew G. Hendrickx, and Sandra Schlafke. 1983. 'Implantation in the Rhesus Monkey: Initial Penetration of Endometrium'. *American Journal of Anatomy* 167 (3): 275–98. https://doi.org/10.1002/aja.1001670302.

Enkvist, Erki, Angela Vaasa, Marje Kasari, Marie Kriisa, Taavi Ivan, Kadri Ligi, Gerda Raidaru, and Asko Uri. 2011. 'Protein-Induced Long Lifetime Luminescence of Nonmetal Probes'. *ACS Chemical Biology* 6 (10): 1052–62. https://doi.org/10.1021/cb200120v.

Erden, Murat, Sezcan Mumusoglu, Mehtap Polat, Irem Yarali Ozbek, Sandro C Esteves, Peter Humaidan, and Hakan Yarali. 2022. 'The LH Surge and Ovulation Re-Visited: A Systematic Review and Meta-Analysis and Implications for True Natural Cycle Frozen Thawed Embryo Transfer'. *Human Reproduction Update* 28 (5): 717–32. https://doi.org/10.1093/humupd/dmac012.

Evans, G. E., G. T.M. Phillipson, I. L. Sin, C. M.A. Frampton, J. A. Kirker, S. M. Bigby, and J. J. Evans. 2012. 'Gene Expression Confirms a Potentially Receptive Endometrium Identified by Histology in Fertile Women'. *Human Reproduction* 27 (9): 2747–55. https://doi.org/10.1093/humrep/des233.

Evans, J., Jennifer Hutchison, Lois A. Salamonsen, and David W. Greening. 2020. 'Proteomic Insights into Endometrial Receptivity and Embryo-Endometrial Epithelium Interaction for Implantation Reveal Critical Determinants of Fertility'. *Proteomics* 20 (1): 1900250. https://doi.org/10.1002/pmic.201900250.

Evans, J., T. Kaitu'u-Lino, and L. A. Salamonsen. 2011. 'Extracellular Matrix Dynamics in Scar-Free Endometrial Repair: Perspectives from Mouse In Vivo and Human In Vitro Studies'. *Biology of Reproduction* 85 (3): 511–23. https://doi.org/10.1095/biolreprod.111.090993.

Evans, J., and Lois A. Salamonsen. 2012. 'Inflammation, Leukocytes and Menstruation'. *Reviews in Endocrine and Metabolic Disorders* 13 (4): 277–88. https://doi.org/10.1007/s11154-012-9223-7.

Evans, Ronald M. 1988. 'The Steroid and Thyroid Hormone Receptor Superfamily'. *Science* 240 (4854): 889–95.

Fabi, François, Kathy Grenier, Sophie Parent, Pascal Adam, Laurence Tardif, Valérie Leblanc, and Eric Asselin. 2017. 'Regulation of the PI3K/Akt Pathway during Decidualization of Endometrial Stromal Cells'. Edited by Zeng-Ming Yang. *PLOS ONE* 12 (5). https://doi.org/10.1371/journal.pone.0177387.

Fazleabas, Asgerally T., and J. Julie Kim. 2003. 'What Makes an Embryo Stick?' *Science* 299 (5605): 355–56. https://doi.org/10.1126/science.1081277.

Fini, M. Elizabeth, Shinwu Jeong, Haiyan Gong, Rafael Martinez-Carrasco, Nora M.V. Laver, Minako Hijikata, Naoto Keicho, and Pablo Argüeso. 2020. 'Membrane-Associated Mucins of the Ocular Surface: New Genes, New Protein Functions and New Biological Roles in Human and Mouse'. *Progress in Retinal and Eye Research* 75 (March):100777. https://doi.org/10.1016/j.preteyeres.2019.100777.

Finn, C.A. 1986. 'Implantation, Menstruation and Inflammation'. *Biological Reviews of the Cambridge Philosophical Society* 61 (4): 313–28.

Firmin, Julie, Nicolas Ecker, Diane Rivet Danon, Özge Özgüç, Virginie Barraud Lange, Hervé Turlier, Catherine Patrat, and Jean-Léon Maître. 2024. 'Mechanics of Human Embryo Compaction'. *Nature* 629 (8012): 646–51. https://doi.org/10.1038/s41586-024-07351-x.

Fitzgerald, Justine S., Tobias G. Poehlmann, Ekkehard Schleussner, and Udo R. Markert. 2008. 'Trophoblast Invasion: The Role of Intracellular Cytokine Signalling via Signal Transducer and Activator of Transcription 3 (STAT3)'. *Human Reproduction Update* 14 (4): 335–44. https://doi.org/10.1093/humupd/dmn010.

Formigli, L., C. Roccio, G. Belotti, A. Stangalini, M. T. Coglitore, and G. Formigli. 1990. 'Non-Surgical Flushing of the Uterus for Pre-Embryo Recovery: Possible Clinical Applications'. *Human Reproduction* 5 (3): 329–35. https://doi.org/10.1093/oxfordjournals.humrep.a137099.

Franasiak, Jason M., Diana Alecsandru, Eric J. Forman, Laura C. Gemmell, Jeffrey M. Goldberg, Natalia Llarena, Cheri Margolis, Joop Laven, Sam Schoenmakers, and Emre Seli. 2021. 'A Review of the Pathophysiology of Recurrent Implantation Failure'. *Fertility and Sterility* 116 (6): 1436–48. https://doi.org/10.1016/j.fertnstert.2021.09.014.

Fu, Jianping, Aryeh Warmflash, and Matthias P. Lutolf. 2021. 'Stem-Cell-Based Embryo Models for Fundamental Research and Translation'. *Nature Materials* 20 (2): 132–44. https://doi.org/10.1038/s41563-020-00829-9.

Fuhrich, Daniele G, Bruce A Lessey, and Ricardo F Savaris. 2013. 'Comparison of HSCORE Assessment of Endometrial B3 Integrin Subunit Expression with Digital HSCORE Using Computerized Image Analysis (ImageJ)'. *Anal Quant Cytopathol Histpathol* 35 (4): 210–16.

Gaide Chevronnay, Héloïse P., Charlotte Selvais, Hervé Emonard, Christine Galant, Etienne Marbaix, and Patrick Henriet. 2012. 'Regulation of Matrix Metalloproteinases Activity Studied in Human Endometrium as a Paradigm of Cyclic Tissue Breakdown and Regeneration'. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1824 (1): 146–56. https://doi.org/10.1016/j.bbapap.2011.09.003.

Garcia-Alonso, Luz, Louis François Handfield, Kenny Roberts, Konstantina Nikolakopoulou, Ridma C. Fernando, Lucy Gardner, Benjamin Woodhams, et al. 2021. 'Mapping the Temporal and Spatial Dynamics of the Human Endometrium in Vivo and in Vitro'. *Nature Genetics* 53 (12): 1698–1711. https://doi.org/10.1038/s41588-021-00972-2.

Garry, R., R. Hart, K. A. Karthigasu, and C. Burke. 2009. 'A Re-Appraisal of the Morphological Changes within the Endometrium during Menstruation: A Hysteroscopic, Histological and Scanning Electron Microscopic Study'. *Human Reproduction* 24 (6): 1393–1401. https://doi.org/10.1093/humrep/dep036.

Gauster, Martin, Gerit Moser, Stefan Wernitznig, Nadja Kupper, and Berthold Huppertz. 2022. 'Early Human Trophoblast Development: From Morphology to Function'. *Cellular and Molecular Life Sciences* 79 (6): 345. https://doi.org/10.1007/s00018-022-04377-0.

Gellersen, B., and J Brosens. 2003. 'Cyclic AMP and Progesterone Receptor Cross-Talk in Human Endometrium: A Decidualizing Affair'. *Journal of Endocrinology* 178 (3): 357–72. https://doi.org/10.1677/joe.0.1780357.

Gellersen, B., and Jan J. Brosens. 2014. 'Cyclic Decidualization of the Human Endometrium in Reproductive Health and Failure'. *Endocrine Reviews* 35 (6): 851–905. https://doi.org/10.1210/er.2014-1045.

Genbacev, Olga D., Akraporn Prakobphol, Russell A. Foulk, Ana R. Krtolica, Dusko Ilic, Mark S. Singer, Zhi-Qiang Yang, Laura L. Kiessling, Steven D. Rosen, and Susan J. Fisher. 2003. 'Trophoblast L-Selectin-Mediated Adhesion at the Maternal-Fetal Interface'. *Science* 299 (5605): 405–8. https://doi.org/10.1126/science.1079546.

Germeyer, Ariane, Ricardo F Savaris, Julia Jauckus, and Bruce Lessey. 2014. 'Endometrial Beta3 Integrin Profile Reflects Endometrial Receptivity Defects in Women with Unexplained Recurrent Pregnancy Loss'. *Reproductive Biology and Endocrinology* 12 (1): 53. https://doi.org/10.1186/1477-7827-12-53.

Ghosh, Arnab, Shafiq M. Syed, Manish Kumar, Tyler J. Carpenter, Jose M. Teixeira, Nathaniel Houairia, Sumedha Negi, and Pradeep S. Tanwar. 2020. 'In Vivo Cell Fate Tracing Provides No Evidence for Mesenchymal to Epithelial Transition in Adult Fallopian Tube and Uterus'. *Cell Reports* 31 (6): 107631. https://doi.org/10.1016/j.celrep.2020.107631.

Giacomini, E., G. M. Scotti, V. S. Vanni, D. Lazarevic, S. Makieva, L. Privitera, S. Signorelli, et al. 2021. 'Global Transcriptomic Changes Occur in Uterine Fluid-Derived Extracellular Vesicles during the Endometrial Window for Embryo Implantation'. *Human Reproduction* 36 (8): 2249–74. https://doi.org/10.1093/humrep/deab123.

Gibson, Douglas A., Ioannis Simitsidellis, Frances Collins, and Philippa T.K. Saunders. 2020. 'Androgens, Oestrogens and Endometrium: A Fine Balance between Perfection and Pathology'. *Journal of Endocrinology* 246 (3): R75–93. https://doi.org/10.1530/JOE-20-0106.

Gipson, Ilene K., Timothy Blalock, Ann Tisdale, Sandra Spurr-Michaud, Sara Allcorn, Anneli Stavreus-Evers, and Kristina Gemzell. 2008. 'MUC16 Is Lost from the Uterodome (Pinopode) Surface of the Receptive Human Endometrium: In Vitro Evidence That MUC16 Is a Barrier to Trophoblast Adherence'. *Biology of Reproduction* 78 (1): 134–42. https://doi.org/10.1095/biolreprod.106.058347.

Gipson, Ilene K., Samuel B. Ho, Sandra J. Spurr-Michaud, Ann S. Tisdale, Qian Zhan, Emina Torlakovic, Jeffrey Pudney, Deborah J. Anderson, Neil W. Toribara, and Joseph A. Hill. 1997. 'Mucin Genes Expressed by Human Female Reproductive Tract Epithelia'. *Biology of Reproduction* 56 (4): 999–1011. https://doi.org/10.1095/biolreprod56.4.999.

Giudice, Linda C. 1999. 'Potential Biochemical Markers of Uterine Receptivity'. *Human Reproduction* 14 (suppl_2): 3–16. https://doi.org/10.1093/humrep/14.suppl_2.3.

Gleeson, Louise M., Chandan Chakraborty, Timothy McKinnon, and Peeyush K. Lala. 2001. 'Insulin-Like Growth Factor-Binding Protein 1 Stimulates Human Trophoblast Migration by Signaling through A5β1 Integrin via Mitogen-Activated Protein Kinase Pathway1'. *The Journal of Clinical Endocrinology & Metabolism* 86 (6): 2484–93. https://doi.org/10.1210/jcem.86.6.7532.

Gloire, Geoffrey, Sylvie Legrand-Poels, and Jacques Piette. 2006. 'NF-κB Activation by Reactive Oxygen Species: Fifteen Years Later'. *Biochemical Pharmacology* 72 (11): 1493–1505. https://doi.org/10.1016/j.bcp.2006.04.011.

Gnainsky, Y, I Granot, P Aldo, A Barash, Y Or, G Mor, and N Dekel. 2015. 'Biopsy-Induced Inflammatory Conditions Improve Endometrial Receptivity: The Mechanism of Action'. *Reproduction* 149 (1): 75–85. https://doi.org/10.1530/REP-14-0395.

Govindarajan, Bharathi, Balaraj B. Menon, Sandra Spurr-Michaud, Komal Rastogi, Michael S. Gilmore, Pablo Argüeso, and Ilene K. Gipson. 2012. 'A Metalloproteinase Secreted by Streptococcus Pneumoniae Removes Membrane Mucin MUC16 from the Epithelial Glycocalyx Barrier'. Edited by Paulo Lee Ho. *PLoS ONE* 7 (3): e32418. https://doi.org/10.1371/journal.pone.0032418.

Greenburg, G, and E. D. Hay. 1982. ‘Epithelia Suspended in Collagen Gels Can Lose Polarity and Express Characteristics of Migrating Mesenchymal Cells.’ *Journal of Cell Biology* 95 (1): 333–39. https://doi.org/10.1083/jcb.95.1.333.

Hannan, Natalie J., Premila Paiva, Evdokia Dimitriadis, and Lois A. Salamonsen. 2010. ‘Models for Study of Human Embryo Implantation: Choice of Cell Lines?1’. *Biology of Reproduction* 82 (2): 235–45. https://doi.org/10.1095/biolreprod.109.077800.

Hannon, Patrick R., and Thomas E. Curry. 2018. ‘Folliculogenesis’. In *Encyclopedia of Reproduction*, 72–79. Elsevier. https://doi.org/10.1016/B978-0-12-801238-3.64628-7.

Haouzi, Delphine, Frida Entezami, Antoine Torre, Charlène Innocenti, Yannick Antoine, Charlotte Mauries, Claire Vincens, et al. 2021. ‘Customized Frozen Embryo Transfer after Identification of the Receptivity Window with a Transcriptomic Approach Improves the Implantation and Live Birth Rates in Patients with Repeated Implantation Failure’. *Reproductive Sciences* 28 (1): 69–78. https://doi.org/10.1007/s43032-020-00252-0.

Harris, G. W. 1955. *Neural Control Of The Pituitary Gland*. http://archive.org/details/in.ernet.dli.2015.548105.

Hattrup, Christine L., and Sandra J. Gendler. 2008. ‘Structure and Function of the Cell Surface (Tethered) Mucins’. *Annual Review of Physiology* 70 (1): 431–57. https://doi.org/10.1146/annurev.physiol.70.113006.100659.

Hay, E. D. 1995. ‘An Overview of Epithelio-Mesenchymal Transformation’. *Acta Anatomica* 154 (1): 8–20. https://doi.org/10.1159/000147748.

———. 2005. ‘The Mesenchymal Cell, Its Role in the Embryo, and the Remarkable Signaling Mechanisms That Create It’. *Developmental Dynamics* 233 (3): 706–20. https://doi.org/10.1002/dvdy.20345.

He, Aihua, Hong Wu, Yangyun Zou, Cheng Wan, Jing Zhao, Qiong Zhang, Nenghui Liu, et al. 2023. ‘Can Biomarkers Identified from the Uterine Fluid Transcriptome Be Used to Establish a Noninvasive Endometrial Receptivity Prediction Tool? A Proof-of-Concept Study’. *Reproductive Biology and Endocrinology* 21 (1). https://doi.org/10.1186/s12958-023-01070-0.

Hebbar, Vidya, Gautam Damera, and Goverdhan P. Sachdev. 2005. ‘Differential Expression of MUC Genes in Endometrial and Cervical Tissues and Tumors’. *BMC Cancer* 5 (1): 124. https://doi.org/10.1186/1471-2407-5-124.

Hempstock, Joanne, Tereza Cindrova-Davies, Eric Jauniaux, and Graham J. Burton. 2004. ‘Endometrial Glands as a Source of Nutrients, Growth Factors and Cytokines during the First Trimester of Human Pregnancy: A Morphological and Immunohistochemical Study’. *Reproductive Biology and Endocrinology* 2:58 (July). https://doi.org/10.1186/1477-7827-2-58.

Henriet, Patrick, Héloïse P. Gaide Chevronnay, and Etienne Marbaix. 2012. ‘The Endocrine and Paracrine Control of Menstruation’. *Molecular and Cellular Endocrinology* 358 (2): 197–207. https://doi.org/10.1016/j.mce.2011.07.042.

Hertig, Arthur T., John Rock, and Eleanor C. Adams. 1956. ‘A Description of 34 Human Ova within the First 17 Days of Development’. *American Journal of Anatomy* 98 (3): 435–93. https://doi.org/10.1002/aja.1000980306.

Hewitt, Sylvia C., Mackenzie J. Dickson, Nicole Edwards, Kathleen Hampton, Stavros Garantziotis, and Francesco J. DeMayo. 2023. ‘From Cup to Dish: How to Make and Use Endometrial Organoid and Stromal Cultures Derived from Menstrual Fluid’. *Frontiers in Endocrinology* 14 (September). https://doi.org/10.3389/fendo.2023.1220622.

Hewitt, Sylvia C., Wipawee Winuthayanon, and Kenneth S. Korach. 2016. 'What's New in Estrogen Receptor Action in the Female Reproductive Tract'. *Journal of Molecular Endocrinology* 56 (2): R55–71. https://doi.org/10.1530/JME-15-0254.

Hey, N. A., R A Graham, M W Seif, and J D Aplin. 1994. 'The Polymorphic Epithelial Mucin MUC1 in Human Endometrium Is Regulated with Maximal Expression in the Implantation Phase.' *The Journal of Clinical Endocrinology & Metabolism* 78 (2): 337–42. https://doi.org/10.1210/jcem.78.2.8106621.

Hey, N. A., T.C. Li, P.L. Devine, R.A. Graham, H. Saravelos, and J.D. Aplin. 1995. 'MUC1 in Secretory Phase Endometrium: Expression in Precisely Dated Biopsies and Flushings from Normal and Recurrent Miscarriage Patients'. *Human Reproduction* 10 (10): 2655–62. https://doi.org/10.1093/oxfordjournals.humrep.a135762.

Hickey, M, and I S Fraser. 2000. 'The Structure of Endometrial Microvessels'. *Human Reproduction* 15 (Suppl. 3): 57–66.

Higuchi, Toshio, Takuya Orita, Ken Katsuya, Yoshiki Yamasaki, Kiyotaka Akiyama, Huiping Li, Tadashi Yamamoto, Yutaka Saito, and Motonao Nakamura. 2004. 'MUC20 Suppresses the Hepatocyte Growth Factor-Induced Grb2-Ras Pathway by Binding to a Multifunctional Docking Site of Met.' *Molecular and Cellular Biology* 24 (17): 7456–68. https://doi.org/10.1128/MCB.24.17.7456-7468.2004.

Higuchi, Toshio, Takuya Orita, Setsuko Nakanishi, Ken Katsuya, Hirotaka Watanabe, Yoshiki Yamasaki, Iwao Waga, et al. 2004. 'Molecular Cloning, Genomic Structure, and Expression Analysis of MUC20, a Novel Mucin Protein, Up-Regulated in Injured Kidney'. *Journal of Biological Chemistry* 279 (3): 1968–79. https://doi.org/10.1074/jbc.M304558200.

Hillier, Stephen G, Philippa F Whitelaw, and Christopher D Smyth. 1994. 'Follicular Oestrogen Synthesis: The "two-Cell, Two-Gonadotrophin" Model Revisited'. *Molecular and Cellular Endocrinology* 100:51–54.

Hollingsworth, Michael A., and Benjamin J. Swanson. 2004. 'Mucins in Cancer: Protection and Control of the Cell Surface'. *Nature Reviews Cancer* 4 (1): 45–60. https://doi.org/10.1038/nrc1251.

Holtzinger, Audrey, Philip R. Streeter, Farida Sarangi, Scott Hillborn, Maryam Niapour, Shinichiro Ogawa, and Gordon Keller. 2015. 'New Markers for Tracking Endoderm Induction and Hepatocyte Differentiation from Human Pluripotent Stem Cells'. *Development* 142 (24): 4253–65. https://doi.org/10.1242/dev.121020.

Horne, A. W., E.-N. Lalani, R.A. Margara, T.A. Ryder, M.A. Mobberley, and J.O. White. 2005. 'The Expression Pattern of MUC1 Glycoforms and Other Biomarkers of Endometrial Receptivity in Fertile and Infertile Women'. *Molecular Reproduction and Development* 72 (2): 216–29. https://doi.org/10.1002/mrd.20307.

Horne, A. W., John O. White, El-Nasir Lalani, Margaret A. Mobberley, Raul A. Margara, Geoffrey H. Trew, and Timothy A. Ryder. 2002. 'Analysis of Epitopes on Endometrial Epithelium by Scanning Immunoelectron Microscopy'. *Biochemical and Biophysical Research Communications* 292 (1): 102–8. https://doi.org/10.1006/bbrc.2002.6624.

Hou, Shasha, Xiaorui Xie, Jing Zhao, Cailan Wu, Ning Li, Zhaowei Meng, Chunquan Cai, and Jian Tan. 2021. 'Downregulation of miR-146b-3p Inhibits Proliferation and Migration and Modulates the Expression and Location of Sodium/Iodide Symporter in Dedifferentiated Thyroid Cancer by Potentially Targeting MUC20'. *Frontiers in Oncology* 10 (January). https://doi.org/10.3389/fonc.2020.566365.

Houserman, V. L., H. Todd, and F. Hertelendy. 1989. 'Progesterone Treatment in Vitro Enhances Prostaglandin E and Forskolin-Promoted Cyclic AMP Production in Human Endometrial Stromal Cells'. *Journal of Reproduction and Fertility* 85 (1): 195–202. https://doi.org/10.1530/jrf.0.0850195.

Huang, Cheng Chiu, Grant D. Orvis, Ying Wang, and Richard R. Behringer. 2012. 'Stromal-to-Epithelial Transition during Postpartum Endometrial Regeneration'. *PLOS ONE* 7 (8). https://doi.org/10.1371/journal.pone.0044285.

Humphrey, Sean J., S. Babak Azimifar, and Matthias Mann. 2015. 'High-Throughput Phosphoproteomics Reveals in Vivo Insulin Signaling Dynamics'. *Nature Biotechnology* 33 (9): 990–95. https://doi.org/10.1038/nbt.3327.

Hynes, Richard O. 2002. 'Integrins: Bidirectional, Allosteric Signaling Machines'. *Cell* 110 (6): 673–87. https://doi.org/10.1016/S0092-8674(02)00971-6.

Ihnatovych, Ivanna, WenYang Hu, Jody L. Martin, Asgerally T. Fazleabas, Primal de Lanerolle, and Zuzana Strakova. 2007. 'Increased Phosphorylation of Myosin Light Chain Prevents in Vitro Decidualization'. *Endocrinology* 148 (7): 3176–84. https://doi.org/10.1210/en.2006-1673.

Ihnatovych, Ivanna, Mark Livak, Jennifer Reed, Primal de Lanerolle, and Zuzana Strakova. 2009. 'Manipulating Actin Dynamics Affects Human In Vitro Decidualization1'. *Biology of Reproduction* 81 (1): 222–30. https://doi.org/10.1095/biolreprod.108.074666.

Ishizaki, Toshimasa, Masayoshi Uehata, Ichiro Tamechika, Jeongsin Keel, Kimiko Nonomura, Midori Maekawa, and Shuh Narumiya. 2000. 'Pharmacological Properties of Y-27632, a Specific Inhibitor of Rho-Associated Kinases'. *Molecular Pharmacology* 57 (5): 976–83. https://doi.org/10.1016/S0026-895X(24)26508-1.

Iwahashi, M., Y. Muragaki, A. Ooshima, M. Yamoto, and R. Nakano. 1996. 'Alterations in Distribution and Composition of the Extracellular Matrix during Decidualization of the Human Endometrium'. *Reproduction* 108 (1): 147–55. https://doi.org/10.1530/jrf.0.1080147.

Jabbour, H. N., and H. O. Critchley. 2001. 'Potential Roles of Decidual Prolactin in Early Pregnancy'. *Reproduction (Cambridge, England)* 121 (2): 197–205. https://doi.org/10.1530/rep.0.1210197.

Jabbour, H. N., H. O. D. Critchley, and S. C. Boddy. 1998. 'Expression of Functional Prolactin Receptors in Nonpregnant Human Endometrium: Janus Kinase-2, Signal Transducer and Activator of Transcription-1 (STAT1), and STAT5 Proteins Are Phosphorylated after Stimulation with Prolactin'. *The Journal of Clinical Endocrinology & Metabolism* 83 (7): 2545–53. https://doi.org/10.1210/jcem.83.7.4989.

Jahn, E., I. Classen-Linke, M. Kusche, H.M. Beier, O. Traub, R. Grummer, and E. Winterhager. 1995. 'Expression of Gap Junction Connexins in the Human Endometrium throughout the Menstrual Cycle'. *Human Reproduction* 10 (10): 2666–70. https://doi.org/10.1093/oxfordjournals.humrep.a135764.

Jentoft, Neil. 1990. 'Why Are Proteins O-Glycosylated?' *Trends in Biochemical Sciences* 15 (8): 291–94. https://doi.org/10.1016/0968-0004(90)90014-3.

Jeschke, Udo, Hermann Walzel, Ioannis Mylonas, Panos Papadopoulos, Naim Shabani, Christina Kuhn, Sandra Schulze, et al. 2009. 'The Human Endometrium Expresses the Glycoprotein Mucin-1 and Shows Positive Correlation for Thomsen-Friedenreich Epitope Expression and Galectin-1 Binding'. *Journal of Histochemistry and Cytochemistry* 57 (9): 871–81. https://doi.org/10.1369/jhc.2009.952085.

Jeziorska, Maria, Lois A. Salamonsen, and David E. Woolley. 1995. 'Mast Cell and Eosinophil Distribution and Activation in Human Endometrium throughout the Menstrual Cycle1'. *Biology of Reproduction* 53 (2): 312–20. https://doi.org/10.1095/biolreprod53.2.312.

Jin, X. Y., L. J. Zhao, D. H. Luo, L. Liu, Y. D. Dai, X. X. Hu, Y. Y. Wang, et al. 2017. 'Pinopode Score around the Time of Implantation Is Predictive of Successful Implantation Following Frozen Embryo Transfer in Hormone Replacement Cycles'. *Human Reproduction (Oxford, England)* 32 (12): 2394–2403. https://doi.org/10.1093/humrep/dex312.

Jonckheere, Nicolas, and Isabelle Van Seuningen. 2018. 'Integrative Analysis of the Cancer Genome Atlas and Cancer Cell Lines Encyclopedia Large-Scale Genomic Databases: MUC4/MUC16/MUC20 Signature Is Associated with Poor Survival in Human Carcinomas'. *Journal of Translational Medicine* 16 (1): 259. https://doi.org/10.1186/s12967-018-1632-2.

Jones, R. L., R. W. Kelly, and H. O. Critchley. 1997. 'Chemokine and Cyclooxygenase-2 Expression in Human Endometrium Coincides with Leukocyte Accumulation'. *Human Reproduction* 12 (6): 1300–1306. https://doi.org/10.1093/humrep/12.6.1300.

Julian, JoAnne, Neeraja Dharmaraj, and Daniel D. Carson. 2009. 'MUC1 Is a Substrate for γ-Secretase'. *Journal of Cellular Biochemistry* 108 (4): 802–15. https://doi.org/10.1002/jcb.22292.

Kagawa, Harunobu, Alok Javali, Heidar Heidari Khoei, Theresa Maria Sommer, Giovanni Sestini, Maria Novatchkova, Yvonne Scholte op Reimer, et al. 2022. 'Human Blastoids Model Blastocyst Development and Implantation'. *Nature* 601 (7894): 600–605. https://doi.org/10.1038/s41586-021-04267-8.

Kajihara, Takeshi, Kayoko Tanaka, Tatsuo Oguro, Hideno Tochigi, Japarath Prechapanich, Satomi Uchino, Atsuo Itakura, et al. 2014. 'Androgens Modulate the Morphological Characteristics of Human Endometrial Stromal Cells Decidualized In Vitro'. *Reproductive Sciences* 21 (3): 372–80. https://doi.org/10.1177/1933719113497280.

Karin, Michael, and Mireille Delhase. 2000. 'The IκB Kinase (IKK) and NF-κB: Key Elements of Proinflammatory Signalling'. *Seminars in Immunology* 12 (1): 85–98. https://doi.org/10.1006/smim.2000.0210.

Karsch, F J, R F Weick, W R Butler, D J Dierschke, L C Krey, / G Weiss, J Hotchkiss, T Yamaji, and E Knobil. 1973. 'Induced LH Surges in the Rhesus Monkey: Strength-Duration Characteristics of the Estrogen Stimulus'. *Endocrinology* 92 (6): 1740–47.

Kastner, P., A. Krust, B. Turcotte, U. Stropp, L. Tora, H. Gronemeyer, and P. Chambon. 1990. 'Two Distinct Estrogen-Regulated Promoters Generate Transcripts Encoding the Two Functionally Different Human Progesterone Receptor Forms A and B.' *The EMBO Journal* 9 (5): 1603–14. https://doi.org/10.1002/j.1460-2075.1990.tb08280.x.

Kasvandik, Sergo, Merilin Saarma, Tanel Kaart, Ilmatar Rooda, Agne Velthut-Meikas, Aivar Ehrenberg, Kristina Gemzell, Parameswaran Grace Lalitkumar, Andres Salumets, and Maire Peters. 2020. 'Uterine Fluid Proteins for Minimally Invasive Assessment of Endometrial Receptivity'. *Journal of Clinical Endocrinology and Metabolism* 105 (1): 219–30. https://doi.org/10.1210/clinem/dgz019.

Kasvandik, Sergo, Külli Samuel, Maire Peters, Margus Eimre, Nadežda Peet, Anne Mari Roost, Lee Padrik, Kalju Paju, Lauri Peil, and Andres Salumets. 2016. 'Deep Quantitative Proteomics Reveals Extensive Metabolic Reprogramming and Cancer-Like Changes of Ectopic Endometriotic Stromal Cells'. *Journal of Proteome Research* 15 (2): 572–84. https://doi.org/10.1021/acs.jproteome.5b00965.

Katayama, Shintaro, Virpi Töhönen, Sten Linnarsson, and Juha Kere. 2013. 'SAMstrt: Statistical Test for Differential Expression in Single-Cell Transcriptome with Spike-in Normalization'. *Bioinformatics* 29 (22): 2943–45. https://doi.org/10.1093/bioinformatics/btt511.

Kaya, Hatice S., Alison M. Hantak, Lisa J. Stubbs, Robert N. Taylor, Indrani C. Bagchi, and Milan K. Bagchi. 2015. 'Roles of Progesterone Receptor A and B Isoforms During Human Endometrial Decidualization'. *Molecular Endocrinology* 29 (6): 882–95. https://doi.org/10.1210/me.2014-1363.

Kesimer, M, C Ehre, K A Burns, C W Davis, J K Sheehan, and R J Pickles. 2013. 'Molecular Organization of the Mucins and Glycocalyx Underlying Mucus Transport over Mucosal Surfaces of the Airways'. *Mucosal Immunology* 6 (2): 379–92. https://doi.org/10.1038/mi.2012.81.

Khatun, Masuma, Riikka K Arffman, Darja Lavogina, Marika Kangasniemi, Johanna Laru, Anne Ahtikoski, Siri Lehtonen, et al. 2020. 'Women with Polycystic Ovary Syndrome Present with Altered Endometrial Expression of Stanniocalcin-1†'. *Biology of Reproduction* 102 (2): 306–15. https://doi.org/10.1093/biolre/ioz180.

Khong, T. Y., E. B. Lane, and W. B. Robertson. 1986. 'An Immunocytochemical Study of Fetal Cells at the Maternal-Placental Interface Using Monoclonal Antibodies to Keratins, Vimentin and Desmin'. *Cell and Tissue Research* 246 (1): 189–95. https://doi.org/10.1007/BF00219017.

Kim, Suran, Sungjin Min, Yi Sun Choi, Sung-Hyun Jo, Jae Hun Jung, Kyusun Han, Jin Kim, et al. 2022. 'Tissue Extracellular Matrix Hydrogels as Alternatives to Matrigel for Culturing Gastrointestinal Organoids'. *Nature Communications* 13 (1): 1692. https://doi.org/10.1038/s41467-022-29279-4.

Kim, Yunhee, Inha Kim, and Kunyoo Shin. 2023. 'A New Era of Stem Cell and Developmental Biology: From Blastoids to Synthetic Embryos and Beyond'. *Experimental & Molecular Medicine* 55 (10): 2127–37. https://doi.org/10.1038/s12276-023-01097-8.

Kimber, Susan J. 2005. 'Leukaemia Inhibitory Factor in Implantation and Uterine Biology', August. https://doi.org/10.1530/rep.1.00304.

Kimber, Susan J., and Catherine Spanswick. 2000. 'Blastocyst Implantation: The Adhesion Cascade'. *Seminars in Cell & Developmental Biology* 11 (2): 77–92. https://doi.org/10.1006/scdb.2000.0154.

King, Anne E., and Hilary O.D. Critchley. 2010. 'Oestrogen and Progesterone Regulation of Inflammatory Processes in the Human Endometrium'. *The Journal of Steroid Biochemistry and Molecular Biology* 120 (2–3): 116–26. https://doi.org/10.1016/j.jsbmb.2010.01.003.

Kitaya, Kotaro, Jinsuke Yasuda, Izumi Yagi, Yoshihiro Tada, Shinji Fushiki, and Hideo Honjo. 2000. 'IL-15 Expression at Human Endometrium and Decidua'. *Biology of Reproduction* 63 (3): 683–87. https://doi.org/10.1095/biolreprod63.3.683.

Kleinová, Mária, Ivan Varga, Michaela Čeháková, Martin Valent, and Martin Klein. 2024. 'Exploring the Black Box of Human Reproduction: Endometrial Organoids and Assembloids – Generation, Implantation Modeling, and Future Clinical Perspectives'. *Frontiers in Cell and Developmental Biology* 12 (October). https://doi.org/10.3389/fcell.2024.1482054.

Knobil, E. 1980. 'The Neuroendocrine Control of the Menstrual Cycle'. *Recent Progress in Hormone Research* 36:53–88. https://doi.org/10.1016/b978-0-12-571136-4.50008-5.

Kojima, Mina L., Caroline Hoppe, and Antonio J. Giraldez. 2024. 'The Maternal-to-Zygotic Transition: Reprogramming of the Cytoplasm and Nucleus'. *Nature Reviews Genetics*, November, 1–23. https://doi.org/10.1038/s41576-024-00792-0.

Konstantinidi, Andriana, Rebecca Nason, Tomislav Čaval, Lingbo Sun, Daniel M. Sørensen, Sanae Furukawa, Zilu Ye, et al. 2022. 'Exploring the Glycosylation of Mucins by Use of O-Glycodomain Reporters Recombinantly Expressed in Glyco-engineered HEK293 Cells'. *Journal of Biological Chemistry* 298 (4): 101784. https://doi.org/10.1016/j.jbc.2022.101784.

Koopman, Louise A., Hernan D. Kopcow, Basya Rybalov, Jonathan E. Boyson, Jordan S. Orange, Frederick Schatz, Rachel Masch, et al. 2003. 'Human Decidual Natural Killer Cells Are a Unique NK Cell Subset with Immunomodulatory Potential'. *Journal of Experimental Medicine* 198 (8): 1201–12. https://doi.org/10.1084/jem.20030305.

Koscinski, Isabelle, Stephane Viville, Nicole Porchet, Annie Bernigaud, Fabienne Escande, Andre Defossez, and Marie Pierre Buisine. 2006. 'MUC4 Gene Polymorphism and Expression in Women with Implantation Failure'. *Human Reproduction* 21 (9): 2238–45. https://doi.org/10.1093/humrep/del189.

Krjutškov, K;, S; Katayama, M; Saare, M; Vera-Rodriguez, D; Lubenets, K; Samuel, T; Laisk-Podar, et al. 2016. 'Single-Cell Transcriptome Analysis of Endometrial Tissue'. *Human Reproduction* 31 (4): 844–53. https://doi.org/10.1093/humrep/dew008.

Labied, Soraya, Takeshi Kajihara, Patricia A. Madureira, Luca Fusi, Marius C. Jones, Jenny M. Higham, Rana Varshochi, et al. 2006. 'Progestins Regulate the Expression and Activity of the Forkhead Transcription Factor FOXO1 in Differentiating Human Endometrium'. *Molecular Endocrinology* 20 (1): 35–44. https://doi.org/10.1210/me.2005-0275.

Lacconi, Valentina, Micol Massimiani, Ilenia Carriero, Claudia Bianco, Carlo Ticconi, Valentina Pavone, Alessandra Alteri, et al. 2024. 'When the Embryo Meets the Endometrium: Identifying the Features Required for Successful Embryo Implantation'. *International Journal of Molecular Sciences* 25 (5): 2834. https://doi.org/10.3390/ijms25052834.

LaFoya, Bryce, Jordan A. Munroe, Alison Miyamoto, Michael A. Detweiler, Jacob J. Crow, Tana Gazdik, and Allan R. Albig. 2018. 'Beyond the Matrix: The Many Non-ECM Ligands for Integrins'. *International Journal of Molecular Sciences* 19 (2): 449. https://doi.org/10.3390/ijms19020449.

Lawn, A. M., E. W. Wilson, and C. A. Finn. 1971. 'The Ultrastructure of Human Decidual and Predecidual Cells'. *Journal of Reproduction and Fertility* 26 (1): 85–90. https://doi.org/10.1530/jrf.0.0260085.

Laws, Mary J., Robert N. Taylor, Neil Sidell, Francesco J. DeMayo, John P. Lydon, David E. Gutstein, Milan K. Bagchi, and Indrani C. Bagchi. 2008. 'Gap Junction Communication between Uterine Stromal Cells Plays a Critical Role in Pregnancy-Associated Neovascularization and Embryo Survival'. *Development* 135 (15): 2659–68. https://doi.org/10.1242/dev.019810.

Lee, Chae Hyun, Tae Hoon Kim, Jae Hee Lee, Seo Jin Oh, Jung-Yoon Yoo, Hyo Suk Kwon, Young Im Kim, et al. 2013. 'Extracellular Signal-Regulated Kinase 1/2 Signaling Pathway Is Required for Endometrial Decidualization in Mice and Human'. Edited by Bin He. *PLoS ONE* 8 (9): e75282. https://doi.org/10.1371/journal.pone.0075282.

Lee, So Young, Yun Young Lee, Joong Sub Choi, Mee-Sup Yoon, and Joong-Soo Han. 2016. 'Phosphatidic Acid Induces Decidualization by Stimulating Akt-PP2A Binding in Human Endometrial Stromal Cells'. *The FEBS Journal* 283 (22): 4163–75. https://doi.org/10.1111/febs.13914.

Lessey, Bruce A., Arthur J. Castelbaum, Stephen W. Sawin, and Jinghai Sun. 1995. 'Integrins as Markers of Uterine Receptivity in Women with Primary Unexplained Infertility*†'. *Fertility and Sterility* 63 (3): 535–42. https://doi.org/10.1016/S0015-0282(16)57422-6.

Lessey, Bruce A., and Steven L. Young. 2019. 'What Exactly Is Endometrial Receptivity?' *Fertility and Sterility* 111 (4): 611–17. https://doi.org/10.1016/j.fertnstert.2019.02.009.

Li, Jun, and Robert Tibshirani. 2011. 'Finding Consistent Patterns: A Nonparametric Approach for Identifying Differential Expression in RNA-Seq Data'. *Statistical Methods in Medical Research* 22 (5): 519–36. https://doi.org/10.1177/0962280211428386.

Li, Min, Xiaoyan Xin, Tingting Wu, Teng Hua, Hongbin Wang, and Hongbo Wang. 2015. 'Stromal Cells of Endometrial Carcinoma Promotes Proliferation of Epithelial Cells through the HGF/c-Met/Akt Signaling Pathway'. *Tumor Biology* 36 (8): 6239–48. https://doi.org/10.1007/s13277-015-3309-2.

Li, Ran, Jennifer F. Knight, Morag Park, and Ann Marie Pendergast. 2015. 'Abl Kinases Regulate HGF/Met Signaling Required for Epithelial Cell Scattering, Tubulogenesis and Motility'. *PLOS ONE* 10 (5): e0124960. https://doi.org/10.1371/journal.pone.0124960.

Li, Xia, Zhuo-Hang Li, Ying-Xiong Wang, and Tai-Hang Liu. 2023. 'A Comprehensive Review of Human Trophoblast Fusion Models: Recent Developments and Challenges'. *Cell Death Discovery* 9 (1): 1–14. https://doi.org/10.1038/s41420-023-01670-0.

Li, Xintong, Suranga P. Kodithuwakku, Rachel W. S. Chan, William S. B. Yeung, Yuanqing Yao, Ernest H. Y. Ng, Philip C. N. Chiu, and Cheuk-Lun Lee. 2022. 'Three-Dimensional Culture Models of Human Endometrium for Studying Trophoblast-Endometrium Interaction during Implantation'. *Reproductive Biology and Endocrinology* 20 (1): 120. https://doi.org/10.1186/s12958-022-00973-8.

Li, Zhengyu, Jinhai Gou, Jia Jia, and Xia Zhao. 2015. 'MicroRNA-429 Functions as a Regulator of Epithelial–Mesenchymal Transition by Targeting Pcdh8 during Murine Embryo Implantation'. *Human Reproduction* 30 (3): 507–18. https://doi.org/10.1093/humrep/dev001.

Liang, Jingjie, Shaoyu Wang, and Zhengguang Wang. 2017. 'Role of microRNAs in Embryo Implantation'. *Reproductive Biology and Endocrinology* 15 (1): 90. https://doi.org/10.1186/s12958-017-0309-7.

Lim, Jormay, and Jean Paul Thiery. 2012. 'Epithelial-Mesenchymal Transitions: Insights from Development'. *Development* 139 (19): 3471–86. https://doi.org/10.1242/dev.071209.

Liu, Liu, Yangyang Wang, Xiaoyan Chen, Yichao Tian, Tin Chiu Li, Lijuan Zhao, Qiaoqiao Chen, Mingling Wei, and Songying Zhang. 2020. 'Evidence from Three Cohort Studies on the Expression of MUC16 around the Time of Implantation Suggests It Is an Inhibitor of Implantation'. *Journal of Assisted Reproduction and Genetics* 37 (5): 1105–15. https://doi.org/10.1007/s10815-020-01764-z.

Livak, Kenneth J, and Thomas D Schmittgen. 2001. 'Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method'. *Methods* 25 (4): 402–8. https://doi.org/10.1006/meth.2001.1262.

Lobo, Shalini C., S.-T. Joseph Huang, Ariane Germeyer, Chrysoula Dosiou, Kim Chi Vo, Suzana Tulac, Nihar R. Nayak, and Linda C. Giudice. 2004. 'The Immune Environment in Human Endometrium during the Window of Implantation'. *American Journal of Reproductive Immunology* 52 (4): 244–51. https://doi.org/10.1111/j.1600-0897.2004.00217.x.

Loh, Chin-Yap, Jian Yi Chai, Ting Fang Tang, Won Fen Wong, Gautam Sethi, Muthu Kumaraswamy Shanmugam, Pei Pei Chong, and Chung Yeng Looi. 2019. 'The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges'. *Cells* 8 (10): 1118. https://doi.org/10.3390/cells8101118.

Ludwig, H., and U. M. Spornitz. 1991. 'Microarchitecture of the Human Endometrium by Scanning Electron Microscopy: Menstrual Desquamation and Remodeling'. *Annals of the New York Academy of Sciences* 622 (1): 28–46. https://doi.org/10.1111/j.1749-6632.1991.tb37848.x.

Luo, Bing-Hao, Christopher V. Carman, and Timothy A. Springer. 2007. 'Structural Basis of Integrin Regulation and Signaling'. *Annual Review of Immunology* 25 (Volume 25, 2007): 619–47. https://doi.org/10.1146/annurev.immunol.25.022106.141618.

Lydon, J P, F J DeMayo, C R Funk, S K Mani, A R Hughes, C A Montgomery, G Shyamala, O M Conneely, and B W O'Malley. 1995. 'Mice Lacking Progesterone Receptor Exhibit Pleiotropic Reproductive Abnormalities.' *Genes & Development* 9 (18): 2266–78. https://doi.org/10.1101/gad.9.18.2266.

MacCalman, Colin D., Emma E. Furth, Akinyinka Omigbodun, Mary Bronner, Christos Coutifaris, and Jerome F. Strauss. 1996. 'Regulated Expression of Cadherin-11 in Human Epithelial Cells: A Role for Cadherin-11 in Trophoblast-Endometrium Interactions?' *Developmental Dynamics* 206 (2): 201–11. https://doi.org/10.1002/(SICI)1097-0177(199606)206:2<201::AID-AJA9>3.0.CO;2-M.

Macklon, Nick S., and Jan J. Brosens. 2014. 'The Human Endometrium as a Sensor of Embryo Quality1'. *Biology of Reproduction* 91 (4): 98, 1–8. https://doi.org/10.1095/biolreprod.114.122846.

Mak, I. Y. H., J. J. Brosens, M. Christian, F. A. Hills, L. Chamley, L. Regan, and J. O. White. 2002. 'Regulated Expression of Signal Transducer and Activator of Transcription, Stat5, and Its Enhancement of PRL Expression in Human Endometrial Stromal Cells in Vitro'. *The Journal of Clinical Endocrinology & Metabolism* 87 (6): 2581–88. https://doi.org/10.1210/jcem.87.6.8576.

Manning, G., D. B. Whyte, R. Martinez, T. Hunter, and S. Sudarsanam. 2002. 'The Protein Kinase Complement of the Human Genome'. *Science* 298 (5600): 1912–34. https://doi.org/10.1126/science.1075762.

Marbaix, E, I Kokorine, P Moulin, J Donnez, Y Eeckhout, and P J Courtoy. 1996. 'Menstrual Breakdown of Human Endometrium Can Be Mimicked in Vitro and Is Selectively and Reversibly Blocked by Inhibitors of Matrix Metalloproteinases.' *Proceedings of the National Academy of Sciences* 93 (17): 9120–25. https://doi.org/10.1073/pnas.93.17.9120.

Margarit, L., A. Taylor, M. H. Roberts, L. Hopkins, C. Davies, A. G. Brenton, R. S. Conlan, et al. 2010. 'MUC1 as a Discriminator between Endometrium from Fertile and Infertile Patients with PCOS and Endometriosis'. *The Journal of Clinical Endocrinology & Metabolism* 95 (12): 5320–29. https://doi.org/10.1210/jc.2010-0603.

Mariee, N., T.C. Li, and S.M. Laird. 2012. 'Expression of Leukaemia Inhibitory Factor and Interleukin 15 in Endometrium of Women with Recurrent Implantation Failure after IVF; Correlation with the Number of Endometrial Natural Killer Cells'. *Human Reproduction* 27 (7): 1946–54. https://doi.org/10.1093/humrep/des134.

Marion, Laura L., and George Rodney Meeks. 2012. 'Ectopic Pregnancy: History, Incidence, Epidemiology, and Risk Factors'. *Clinical Obstetrics and Gynecology* 55 (2): 376. https://doi.org/10.1097/GRF.0b013e3182516d7b.

Markee, J.E. 1940. 'Menstruation in Intraocular Transplants in the Rhesus Monkey'. In *Contributions to Embryology of the Carnegie Institution*, 28:219–308.

Maslar, Ila A., and Daniel H. Riddick. 1979. 'Prolactin Production by Human Endometrium during the Normal Menstrual Cycle'. *American Journal of Obstetrics and Gynecology* 135 (6): 751–54. https://doi.org/10.1016/0002-9378(79)90386-7.

Masuda, Ayako, Noriko Katoh, Kazuhiko Nakabayashi, Kiyoko Kato, Kenzo Sonoda, Mari Kitade, Satoru Takeda, Kenichiro Hata, and Junko Tomikawa. 2016. 'An Improved Method for Isolation of Epithelial and Stromal Cells from the Human Endometrium'. *Journal of Reproduction and Development* 62 (2): 213–18. https://doi.org/10.1262/jrd.2015-137.

Masuda, Hirotaka, Tetsuo Maruyama, Emi Hiratsu, Junichi Yamane, Akio Iwanami, Takashi Nagashima, Masanori Ono, et al. 2007. 'Noninvasive and Real-Time Assessment of Reconstructed Functional Human Endometrium in NOD/SCID/Γcnull Immunodeficient Mice'. *Proceedings of the National Academy of Sciences* 104 (6): 1925–30. https://doi.org/10.1073/pnas.0604310104.

Matoba, Keiichiro, Yusuke Takeda, Yosuke Nagai, Kensuke Sekiguchi, Tamotsu Yokota, Kazunori Utsunomiya, and Rimei Nishimura. 2020. 'The Physiology, Pathology, and Therapeutic Interventions for ROCK Isoforms in Diabetic Kidney Disease'. *Frontiers in Pharmacology* 11 (September). https://doi.org/10.3389/fphar.2020.585633.

Matsuo, H, Y Baba, R M G Nair, A Arimura, and A V Schb11y. 1971. 'Structure of the Porcine Lh- and Fsh-Releasing Hormone, I. the Proposed Amino Acid Sequence'. *Biochemical and Biophysical Research Communication* 43 (6): 1334–39.

Matsuzaki, Sachiko, Takao Fukaya, Takashi Suzuki, Takashi Murakami, Hironobu Sasano, and Akira Yajima. 1999. 'Oestrogen Receptor α and β mRNA Expression in Human Endometrium throughout the Menstrual Cycle'. *Molecular Human Reproduction* 5 (6): 559–64.

Maybin, Jacqueline A., Alison A. Murray, Philippa T. K. Saunders, Nikhil Hirani, Peter Carmeliet, and Hilary O. D. Critchley. 2018. 'Hypoxia and Hypoxia Inducible Factor-1α Are Required for Normal Endometrial Repair during Menstruation'. *Nature Communications* 9 (1): 295. https://doi.org/10.1038/s41467-017-02375-6.

Mcfarland, K C, Rolf Sprengel, Heidi S Phillips, Martin Kohler, Nora Rosemblit, Karoly Nikolics, Deborah L Segaloff, and Peter H Seeburg. 1989. 'Lutropin-Choriogonadotropin Receptor: An Unusual Member of the G Protein-Coupled Receptor Family'. *Science* 245:525–28.

McLennan, C E, and A H Rydell. 1965. 'Extent of Endometrial Shedding during Normal Menstruation.' *Obstetrics and Gynecology* 26 (5): 605–21.

Ménézo, Yves JR, and François Hérubel. 2002. 'Mouse and Bovine Models for Human IVF*'. *Reproductive BioMedicine Online* 4 (2): 170–75. https://doi.org/10.1016/S1472-6483(10)61936-0.

Meng, Ying, Shawdee Eshghi, Ying J. Li, Ray Schmidt, David V. Schaffer, and Kevin E. Healy. 2010. 'Characterization of Integrin Engagement during Defined Human Embryonic Stem Cell Culture'. *The FASEB Journal* 24 (4): 1056–65. https://doi.org/10.1096/fj.08-126821.

Meseguer, M. 1998. 'MUC1 and Endometrial Receptivity'. *Molecular Human Reproduction* 4 (12): 1089–98. https://doi.org/10.1093/molehr/4.12.1089.

Meseguer, M., John D. Aplin, Pedro Caballero-Campo, J. Enrique O'Connor, Julio C. Martín, José Remohí, Antonio Pellicer, and Carlos Simón. 2001. 'Human Endometrial Mucin MUC1 Is Up-Regulated by Progesterone and Down-Regulated In Vitro by the Human Blastocyst1'. *Biology of Reproduction* 64 (2): 590–601. https://doi.org/10.1095/biolreprod64.2.590.

Milgrom E, de Roux N, Ghinea N, Beau I, Loosfelt H, Vannier B, Savouret J F, and Misrahi M. 1997. 'Gonadotrophin and Thyrotrophin Receptors'. *Hormone Research* 48 (suppl 4): 33–37.

Molè, Matteo Amitaba, Antonia Weberling, Reinhard Fässler, Alison Campbell, Simon Fishel, and Magdalena Zernicka-Goetz. 2021. 'Integrin B1 Coordinates Survival and Morphogenesis of the Embryonic Lineage upon Implantation and Pluripotency Transition'. *Cell Reports* 34 (10): 108834. https://doi.org/10.1016/j.celrep.2021.108834.

Moon, Y S, B K Tsang, C Simpson, and D T Armstrongf. 1978. '17beeta-Estradiol Biosynthesis in Cultured Granulosa and Thecal Cells of Human Ovarian Follicles: Stimulation by Follicle-Stimulating Hormone*'. *Journal of Clinical Endocrinology and Metabolism* 47 (2): 263–67.

Murphy, Christopher R. 2004. 'Uterine Receptivity and the Plasma Membrane Transformation'. *Cell Research* 14 (4): 259–67. https://doi.org/10.1038/sj.cr.7290227.

Muter, Joanne, Vincent J. Lynch, Rajiv C. McCoy, and Jan J. Brosens. 2023. 'Human Embryo Implantation'. *Development* 150 (10): dev201507. https://doi.org/10.1242/dev.201507.

Nachtigall, M J, H J Kliman, R F Feinberg, D L Olive, O Engin, and A Arici. 1996. 'The Effect of Leukemia Inhibitory Factor (LIF) on Trophoblast Differentiation: A Potential Role in Human Implantation'. *The Journal of Clinical Endocrinology & Metabolism* 81 (2): 801–6. https://doi.org/10.1210/jcem.81.2.8636307.

Nakamoto, T., H. Okada, T. Nakajima, A. Ikuta, K. Yasuda, and H. Kanzaki. 2005. 'Progesterone Induces the Fibulin-1 Expression in Human Endometrial Stromal Cells'. *Human Reproduction* 20 (6): 1447–55. https://doi.org/10.1093/humrep/deh841.

Naseri, Sara, Yael Rosenberg-Hasson, Holden T. Maecker, Maria I. Avrutsky, and Paul D. Blumenthal. 2023. 'A Cross-sectional Study Comparing the Inflammatory Profile of Menstrual Effluent vs. Peripheral Blood'. *Health Science Reports* 6 (1). https://doi.org/10.1002/hsr2.1038.

Nejatbakhsh, Reza, Maryam Kabir-Salmani, Eva Dimitriadis, Ahmad Hosseini, Robabeh Taheripanah, Yousef Sadeghi, Yoshihiro Akimoto, and Mitsutoshi Iwashita. 2012. 'Subcellular Localization of L-Selectin Ligand in the Endometrium Implies a Novel Function for Pinopodes in Endometrial Receptivity'. *Reproductive Biology and Endocrinology* 10 (1): 46. https://doi.org/10.1186/1477-7827-10-46.

Ng, Shu-Wing, Gabriella A. Norwitz, Mihaela Pavlicev, Tamara Tilburgs, Carlos Simón, and Errol R. Norwitz. 2020. 'Endometrial Decidualization: The Primary Driver of Pregnancy Health'. *International Journal of Molecular Sciences* 21 (11): 4092. https://doi.org/10.3390/ijms21114092.

Nguyen, Hong P.T., Li Xiao, James A. Deane, Ker Sin Tan, Fiona L. Cousins, Hirotaka Masuda, Carl N. Sprung, Anna Rosamilia, and Caroline E. Gargett. 2017. 'N-Cadherin Identifies Human Endometrial Epithelial Progenitor Cells by in Vitro Stem Cell Assays'. *Human Reproduction* 32 (11): 2254–68. https://doi.org/10.1093/humrep/dex289.

Nieto, M. Angela, Ruby Yun-Ju Huang, Rebecca A. Jackson, and Jean Paul Thiery. 2016. 'EMT: 2016'. *Cell* 166 (1): 21–45. https://doi.org/10.1016/j.cell.2016.06.028.

Nishida, Noritaka, Can Xie, Motomu Shimaoka, Yifan Cheng, Thomas Walz, and Timothy A. Springer. 2006. 'Activation of Leukocyte B2 Integrins by Conversion from Bent to Extended Conformations'. *Immunity* 25 (4): 583–94. https://doi.org/10.1016/j.immuni.2006.07.016.

Noyes, R.W., A.T. Hertig, and J. Rock. 1950. 'Dating the Endometrial Biopsy'. *Fertility and Sterility* 1 (1): 3–25. https://doi.org/10.1016/S0015-0282(16)30062-0.

Ochoa-Bernal, Maria Ariadna, and Asgerally T. Fazleabas. 2020. 'Physiologic Events of Embryo Implantation and Decidualization in Human and Non-Human Primates'. *International Journal of Molecular Sciences* 21 (6): 1973. https://doi.org/10.3390/ijms21061973.

Ojosnegros, Samuel, Anna Seriola, Amélie L Godeau, and Anna Veiga. 2021. 'Embryo Implantation in the Laboratory: An Update on Current Techniques'. *Human Reproduction Update* 27 (3): 501–30. https://doi.org/10.1093/humupd/dmaa054.

Okada, H., T. Nakajima, T. Yoshimura, K. Yasuda, and H. Kanzaki. 2003. 'Microarray Analysis of Genes Controlled by Progesterone in Human Endometrial Stromal Cells in Vitro'. *Gynecological Endocrinology* 17 (4): 271–80. https://doi.org/10.1080/gye.17.4.271.280.

Okada, H., Tomoko Tsuzuki, and Hiromi Murata. 2018. 'Decidualization of the Human Endometrium'. *Reproductive Medicine and Biology* 17 (3): 220–27. https://doi.org/10.1002/rmb2.12088.

Oktay, Kutluk, David Briggs, and Roger G Gosden. 1997. 'Ontogeny of Follicle-Stimulating Hormone Receptor Gene Expression in Isolated Human Ovarian Follicles*'. *Journal of Clinical Endocrinology and Metabolism* 82 (11): 3748–51.

Ono, Yoshihiro J., Masami Hayashi, Akiko Tanabe, Atsushi Hayashi, Masanori Kanemura, Yoshito Terai, and Masahide Ohmichi. 2015. 'Estradiol-Mediated Hepatocyte Growth Factor Is Involved in the Implantation of Endometriotic Cells via the Mesothelial-to-Mesenchymal Transition in the Peritoneum'. *American Journal of Physiology-Endocrinology and Metabolism* 308 (11): E950–59. https://doi.org/10.1152/ajpendo.00573.2014.

Osuga, Y., O. Tsutsumi, R. Okagaki, Y. Takai, A. Fujimoto, A. Suenaga, M. Maruyama, M. Momoeda, T. Yano, and Y. Taketani. 1999. 'Hepatocyte Growth Factor Concentrations Are Elevated in Peritoneal Fluid of Women with Endometriosis'. *Human Reproduction* 14 (6): 1611–13. https://doi.org/10.1093/humrep/14.6.1611.

Owusu-Akyaw, Amma, Kavitha Krishnamoorthy, Laura T Goldsmith, and Sara S Morelli. 2019. 'The Role of Mesenchymal–Epithelial Transition in Endometrial Function'. *Human Reproduction Update* 25 (1): 114–33. https://doi.org/10.1093/humupd/dmy035.

Padykula, Helen A., Linda G. Coles, William C. Okulicz, Steven I. Rapaport, John A. McCracken, Norval W. Jr. King, Christopher Longcope, and Ita R. Kaiserman-Abramof. 1989. 'The Basalis of the Primate Endometrium: A Bifunctional Germinal Compartment'. *Biology of Reproduction* 40 (3): 681–90. https://doi.org/10.1095/biolreprod40.3.681.

Pan-Castillo, Belen, Salvatore A. Gazze, Samantha Thomas, Christopher Lucas, Lavinia Margarit, Deyarina Gonzalez, Lewis W. Francis, and Robert Steven Conlan. 2018. 'Morphophysical Dynamics of Human Endometrial Cells during Decidualization'. *Nanomedicine: Nanotechnology, Biology and Medicine* 14 (7): 2235–45. https://doi.org/10.1016/j.nano.2018.07.004.

Pantos, Konstantinos, George Nikas, Evangelos Makrakis, Dimitrios Stavrou, Panagiotis Karantzis, and Michael Grammatis. 2004. 'Clinical Value of Endometrial Pinopodes Detection in Artificial Donation Cycles'. *Reproductive BioMedicine Online* 9 (1): 86–90. https://doi.org/10.1016/S1472-6483(10)62115-3.

Parker, Malcolm G. 1993. 'Steroid and Related Receptors'. *Current Opinion in Cell Biology* 5 (3): 499–504.

Passaniti, Antonino, Hynda K. Kleinman, and George R. Martin. 2022. 'Matrigel: History/Background, Uses, and Future Applications'. *Journal of Cell Communication and Signaling* 16 (4): 621–26. https://doi.org/10.1007/s12079-021-00643-1.

Patterson, Amanda L., Ling Zhang, Nelson A. Arango, Jose Teixeira, and James K. Pru. 2013. 'Mesenchymal-to-Epithelial Transition Contributes to Endometrial Regeneration Following Natural and Artificial Decidualization'. *Stem Cells and Development* 22 (6): 964–74. https://doi.org/10.1089/scd.2012.0435.

Pavlicev, Mihaela, and Errol R. Norwitz. 2018. 'Human Parturition: Nothing More Than a Delayed Menstruation'. *Reproductive Sciences* 25 (2): 166–73. https://doi.org/10.1177/1933719117725830.

Pei, Duanqing, Xiaodong Shu, Ama Gassama-Diagne, and Jean Paul Thiery. 2019. 'Mesenchymal–Epithelial Transition in Development and Reprogramming'. *Nature Cell Biology* 21 (1): 44–53. https://doi.org/10.1038/s41556-018-0195-z.

Perez-Riverol, Yasset, Attila Csordas, Jingwen Bai, Manuel Bernal-Llinares, Suresh Hewapathirana, Deepti J Kundu, Avinash Inuganti, et al. 2019. 'The PRIDE Database and Related Tools and Resources in 2019: Improving Support for Quantification Data'. *Nucleic Acids Research* 47 (D1): D442–50. https://doi.org/10.1093/nar/gky1106.

Perrett, Rebecca M., and Craig A. McArdle. 2013. 'Molecular Mechanisms of Gonadotropin-Releasing Hormone Signaling: Integrating Cyclic Nucleotides into the Network'. *Frontiers in Endocrinology* 4 (NOV). https://doi.org/10.3389/fendo.2013.00180.

Petrini, Iacopo. 2015. 'Biology of MET: A Double Life between Normal Tissue Repair and Tumor Progression'. *Annals of Translational Medicine* 3 (6): 82–82. https://doi.org/10.3978/j.issn.2305-5839.2015.03.58.

Piltonen, T.T., J.C. Chen, M. Khatun, M. Kangasniemi, A. Liakka, T. Spitzer, N. Tran, H. Huddleston, J.C. Irwin, and L.C. Giudice. 2015. 'Endometrial Stromal Fibroblasts from Women with Polycystic Ovary Syndrome Have Impaired Progesterone-Mediated Decidualization, Aberrant Cytokine Profiles and Promote Enhanced Immune Cell Migration in Vitro'. *Human Reproduction* 30 (5): 1203–15. https://doi.org/10.1093/humrep/dev055.

Plant, Tony M. 2012. 'A Comparison of the Neuroendocrine Mechanisms Underlying the Initiation of the Preovulatory LH Surge in the Human, Old World Monkey and Rodent'. *Frontiers in Neuroendocrinology* 33 (2): 160–68. https://doi.org/10.1016/j.yfrne.2012.02.002.

———. 2015. 'The Hypothalamo-Pituitary-Gonadal Axis'. *Journal of Endocrinology* 226 (2): T41–54. https://doi.org/10.1530/JOE-15-0113.

Pohl, C R, and E Knobil. 1982. 'The Role of the Central Nervous System in the Control of Ovarian Function in Higher Primates'. *Annual Reviews of Physiology* 44:583–93.

Pollheimer, J., V. Fock, and M. Knöfler. 2014. 'Review: The ADAM Metalloproteinases – Novel Regulators of Trophoblast Invasion?' *Placenta*, Models for Molecular Understanding of Placental Development and Associated Disorders, 35 (February):S57–63. https://doi.org/10.1016/j.placenta.2013.10.012.

Popovici, Rozana M., Lee-Chuan Kao, and Linda C. Giudice. 2000. 'Discovery of New Inducible Genes in in Vitro Decidualized Human Endometrial Stromal Cells Using Microarray Technology'. *Endocrinology* 141 (9): 3510–15. https://doi.org/10.1210/endo.141.9.7789.

Prashar, Vikash, Tania Arora, Randeep Singh, Arti Sharma, and Jyoti Parkash. 2023. 'Hypothalamic Kisspeptin Neurons: Integral Elements of the GnRH System'. *Reproductive Sciences* 30 (3): 802–22. https://doi.org/10.1007/s43032-022-01027-5.

Prianishnikov, V. A. 1978. 'On the Concept of Stem Cell and a Model of Functional-Morphological Structure of the Endometrium'. *Contraception* 18 (3): 213–23.

Prunier, Chloé, Renaud Prudent, Reuben Kapur, Karin Sadoul, and Laurence Lafanechère. 2017. 'LIM Kinases: Cofilin and Beyond'. *Oncotarget* 8 (25): 41749–63. https://doi.org/10.18632/oncotarget.16978.

Psychoyos, Alexandre. 1986. 'Uterine Receptivity for Nidation'. *Annals of the New York Academy of Sciences* 476 (1 Nidation): 36–42. https://doi.org/10.1111/j.1749-6632.1986.tb20920.x.

Putten, Jos P.M. van, and Karin Strijbis. 2017. 'Transmembrane Mucins: Signaling Receptors at the Intersection of Inflammation and Cancer'. *Journal of Innate Immunity* 9 (3): 281–99. https://doi.org/10.1159/000453594.

Qiong, Zhang, Hao Jie, Wang Yonggang, Xu Bin, Zhao Jing, and Li Yanping. 2017. 'Clinical Validation of Pinopode as a Marker of Endometrial Receptivity: A Randomized Controlled Trial'. *Fertility and Sterility* 108 (3): 513-517.e2. https://doi.org/10.1016/j.fertnstert.2017.07.006.

Quaynor, Samuel D., Earl W. Stradtman, Hyung-Goo Kim, Yiping Shen, Lynn P. Chorich, Derek A. Schreihofer, and Lawrence C. Layman. 2013. 'Delayed Puberty and Estrogen Resistance in a Woman with Estrogen Receptor α Variant'. *The New England Journal of Medicine* 369 (2): 164–71. https://doi.org/10.1056/NEJMoa1303611.

Rappsilber, Juri, Matthias Mann, and Yasushi Ishihama. 2007. 'Protocol for Micro-Purification, Enrichment, Pre-Fractionation and Storage of Peptides for Proteomics Using StageTips'. *Nature Protocols* 2 (8): 1896–1906. https://doi.org/10.1038/nprot.2007.261.

Reardon, Sarah N., Mandy L. King, James A. MacLean, Jordan L. Mann, Francesco J. DeMayo, John P. Lydon, and Kanako Hayashi. 2012. 'Cdh1 Is Essential for Endometrial Differentiation, Gland Development, and Adult Function in the Mouse Uterus1'. *Biology of Reproduction* 86 (5). https://doi.org/10.1095/biolreprod.112.098871.

Reddy, K. V. R., and Sachin S. Mangale. 2003. 'Integrin Receptors: The Dynamic Modulators of Endometrial Function'. *Tissue and Cell* 35 (4): 260–73. https://doi.org/10.1016/S0040-8166(03)00039-9.

Rekker, Kadri, Tõnis Tasa, Merli Saare, Külli Samuel, Ülle Kadastik, Helle Karro, Martin Götte, Andres Salumets, and Maire Peters. 2018. 'Differentially-Expressed miRNAs in Ectopic Stromal Cells Contribute to Endometriosis Development: The Plausible Role of miR-139-5p and miR-375'. *International Journal of Molecular Sciences* 19 (12): 3789. https://doi.org/10.3390/ijms19123789.

Riaz, Muhammad Assad, Franziska Louisa Kary, Alexandra Jensen, Felix Zeppernick, Ivo Meinhold-Heerlein, and Lutz Konrad. 2024. 'Long-Term Maintenance of Viable Human Endometrial Epithelial Cells to Analyze Estrogen and Progestin Effects'. *Cells* 13 (10): 811. https://doi.org/10.3390/cells13100811.

Richards, Joanne S. 1994. 'Hormonal Control of Gene Expression in the Ovary'. *Endocrine Reviews* 15 (6): 725–51.

Riento, Kirsi, and Anne J. Ridley. 2003. 'ROCKs: Multifunctional Kinases in Cell Behaviour'. *Nature Reviews Molecular Cell Biology* 4 (6): 446–56. https://doi.org/10.1038/nrm1128.

Rock, J, and M K Bartlett. 1937. 'Biopsy Studies of Human Endometrium: Criteria of Dating and Information about Amenorrhea, Menorrhagia, and Time of Ovulation'. *Journal of the American Medical Association* 108 (24): 2022. https://doi.org/10.1001/jama.1937.02780240014006.

Rodriguez-Boulan, Enrique, and Ian G. Macara. 2014. 'Organization and Execution of the Epithelial Polarity Programme'. *Nature Reviews Molecular Cell Biology* 15 (4): 225–42. https://doi.org/10.1038/nrm3775.

Rossant, Janet, and Patrick P. L. Tam. 2022. 'Early Human Embryonic Development: Blastocyst Formation to Gastrulation'. *Developmental Cell* 57 (2): 152–65. https://doi.org/10.1016/j.devcel.2021.12.022.

Roux, Nicolas de, Emmanuelle Genin, Jean-Claude Carel, Fumihiko Matsuda, Jean-Louis Chaussain, and Edwin Milgrom. 2003. 'Hypogonadotropic Hypogonadism Due to Loss of Function of the KiSS1-Derived Peptide Receptor GPR54'. *PNAS* 100 (19): 10972–76.

Royal, Isabelle, Nathalie Lamarche-Vane, Louie Lamorte, Kozo Kaibuchi, and Morag Park. 2000. 'Activation of Cdc42, Rac, PAK, and Rho-Kinase in Response to Hepatocyte Growth Factor Differentially Regulates Epithelial Cell Colony Spreading and Dissociation'. *Molecular Biology of the Cell* 11 (5): 1709–25. https://doi.org/10.1091/mbc.11.5.1709.

Ruiz-Alonso, Maria, David Blesa, Patricia Díaz-Gimeno, Eva Gómez, Manuel Fernández-Sánchez, Francisco Carranza, Joan Carrera, Felip Vilella, Antonio Pellicer, and Carlos Simón. 2013. 'The Endometrial Receptivity Array for Diagnosis and Personalized Embryo Transfer as a Treatment for Patients with Repeated Implantation Failure'. *Fertility and Sterility* 100 (3): 818–24. https://doi.org/10.1016/j.fertnstert.2013.05.004.

Rutanen, Eeva-Marja, Riitta Koistinen, Jari Sjöberg, Mervi Julkunen, Torsten Wahlström, Hans Bohn, and Markku Seppälä. 1986. 'Synthesis of Placental Protein 12 by Human Endometrium*'. *Endocrinology* 118 (3): 1067–71. https://doi.org/10.1210/endo-118-3-1067.

Ryan, K J, and Z Petro. 1966. 'Steroid Biosynthesis by Human Ovarian Granulosa and Thecal Cells'. *The Journal of Clinical Endocrinology and Metabolism* 26 (1): 46–52.

Ryan, Kenneth J. 1979. 'Granulosa-Thecal Cell Interaction in Ovarian Steroidogenesis'. *Journal of Steroid Biochemistry* 11:799–800.

Sagaradze, Georgy D., Nataliya A. Basalova, Anastasia Yu. Efimenko, and Vsevolod A. Tkachuk. 2020. 'Mesenchymal Stromal Cells as Critical Contributors to Tissue Regeneration'. *Frontiers in Cell and Developmental Biology* 8 (September). https://doi.org/10.3389/fcell.2020.576176.

Sakai, Katsuya, Shunsuke Aoki, and Kunio Matsumoto. 2015. 'Hepatocyte Growth Factor and Met in Drug Discovery'. *The Journal of Biochemistry* 157 (5): 271–84. https://doi.org/10.1093/jb/mvv027.

Salamonsen, Lois A., Jennifer C. Hutchison, and Caroline E. Gargett. 2021. 'Cyclical Endometrial Repair and Regeneration'. *Development* 148 (17). https://doi.org/10.1242/DEV.199577.

Salamonsen, Lois A, and Louise J Lathbury. 2000. 'Endometrial Leukocytes and Menstruation'. *Human Reproduction Update* 6 (1): 16–27.

Salamonsen, Lois A., and David E. Woolley. 1999. 'Menstruation: Induction by Matrix Metalloproteinases and Inflammatory Cells'. *Journal of Reproductive Immunology* 44 (1–2): 1–27. https://doi.org/10.1016/S0165-0378(99)00002-9.

Santoni, Angela, Claudia Carlino, and Angela Gismondi. 2008. 'Uterine NK Cell Development, Migration and Function'. *Reproductive BioMedicine Online* 16 (2): 202–10. https://doi.org/10.1016/S1472-6483(10)60575-5.

Sathananthan, Dr Henry, Sulochana Gunasheela, and Judith Menezes. 2003. 'Mechanics of Human Blastocyst Hatching *in Vitro*'. *Reproductive BioMedicine Online* 7 (2): 228–34. https://doi.org/10.1016/S1472-6483(10)61757-9.

Schindelin, Johannes, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, Stephan Preibisch, et al. 2012. 'Fiji: An Open-Source Platform for Biological-Image Analysis'. *Nature Methods* 9 (7): 676–82. https://doi.org/10.1038/nmeth.2019.

Schoonbroodt, Sonia, and Jacques Piette. 2000. 'Oxidative Stress Interference with the Nuclear Factor-κB Activation Pathways'. *Biochemical Pharmacology* 60 (8): 1075–83. https://doi.org/10.1016/S0006-2952(00)00371-3.

Schumann, S., V. U. Buck, I. Classen-Linke, G. Wennemuth, and R. Grümmer. 2015. 'Claudin-3, Claudin-7, and Claudin-10 Show Different Distribution Patterns during Decidualization and Trophoblast Invasion in Mouse and Human'. *Histochemistry and Cell Biology* 144 (6): 571–85. https://doi.org/10.1007/s00418-015-1361-z.

Scott, Richard T. 2021. 'Endometrial Receptivity Screening in the General Assisted Reproductive Technology Population'. *Fertility and Sterility* 115 (4): 895–96. https://doi.org/10.1016/j.fertnstert.2021.02.018.

Seminara, Stephanie B, Sophie Messager, Emmanouella E Chatzidaki, Rosemary R Thresher, James S Acierno, Jenna K Shagoury, Yousef Bo-Abbas, et al. 2003. 'The GPR54 Gene as a Regulator of Puberty From the Reproductive Endocrine Unit'. *The NEW ENGLAND JOURNAL of MEDICINE* 349 (17): 1614–27.

Shahbazi, Marta N. 2020. 'Mechanisms of Human Embryo Development: From Cell Fate to Tissue Shape and Back'. *Development* 147 (14): dev190629. https://doi.org/10.1242/dev.190629.

Sharma, Shipra, Geeta Godbole, and Deepak Modi. 2016. 'Decidual Control of Trophoblast Invasion'. *American Journal of Reproductive Immunology* 75 (3): 341–50. https://doi.org/10.1111/aji.12466.

Shivers, B. D., R. E. Harlan, J. I. Morrell, and D. W. Pfaff. 1983. 'Absence of Oestradiol Concentration in Cell Nuclei of LHRH-Immunoreactive Neurons'. *Nature* 304:345–47.

Shyu, Ming-Kwang, Mei-Chun Lin, Jin-Chung Shih, Chien-Nan Lee, John Huang, Chian-Hui Liao, I-Fang Huang, Hsiao-Yu Chen, Min-Chuan Huang, and Fon-Jou Hsieh. 2007. 'Mucin 15 Is Expressed in Human Placenta and Suppresses Invasion of Trophoblast-like Cells in Vitro'. *Human Reproduction* 22 (10): 2723–32. https://doi.org/10.1093/humrep/dem249.

Simitsidellis, Ioannis, A. Esnal-Zuffiaure, Olympia Kelepouri, Elisabeth O'Flaherty, Douglas A. Gibson, and Philippa T.K. Saunders. 2019. 'Selective Androgen Receptor Modulators (SARMs) Have Specific Impacts on the Mouse Uterus'. *Journal of Endocrinology* 242 (3): 227–39. https://doi.org/10.1530/JOE-19-0153.

Simón, Carlos, MarÍa José Gimeno, Amparo Mercader, José Enrique O'Connor, José RemohÍ, Mary Lake Polan, and Antonio Pellicer. 1997. 'Embryonic Regulation of Integrins B3,α 4, and A1 in Human Endometrial Epithelial Cells in Vitro1'. *The Journal of Clinical Endocrinology & Metabolism* 82 (8): 2607–16. https://doi.org/10.1210/jcem.82.8.4153.

Simón, Carlos, Carlos Gómez, Sergio Cabanillas, Iavor Vladimirov, Gemma Castillón, Juan Giles, Kubra Boynukalin, et al. 2020. 'A 5-Year Multicentre Randomized Controlled Trial Comparing Personalized, Frozen and Fresh Blastocyst Transfer in IVF'. *Reproductive BioMedicine Online* 41 (3): 402–15. https://doi.org/10.1016/j.rbmo.2020.06.002.

Simón, Carlos, Amparo Mercader, Juan Garcia-Velasco, George Nikas, Carlos Moreno, Jose Remohí, and Antonio Pellicer. 1999. 'Coculture of Human Embryos with Autologous Human Endometrial Epithelial Cells in Patients with Implantation Failure1'. *The Journal of Clinical Endocrinology & Metabolism* 84 (8): 2638–46. https://doi.org/10.1210/jcem.84.8.5873.

Simunovic, Mijo, and Ali H. Brivanlou. 2017. 'Embryoids, Organoids and Gastruloids: New Approaches to Understanding Embryogenesis'. *Development* 144 (6): 976–85. https://doi.org/10.1242/dev.143529.

Singh, H., and John D. Aplin. 2009. 'Adhesion Molecules in Endometrial Epithelium: Tissue Integrity and Embryo Implantation'. *Journal of Anatomy* 215 (1): 3–13. https://doi.org/10.1111/j.1469-7580.2008.01034.x.

Singh, H, L Nardo, S J Kimber, and J D Aplin. 2010. 'Early Stages of Implantation as Revealed by an in Vitro Model'. *Reproduction* 139 (5): 905–14 . https://doi.org/10.1530/REP-09-0271.

Sinijarv, Hedi, Shanshan Wu, Taavi Ivan, Tonis Laasfeld, Kaido Viht, and Asko Uri. 2017. 'Binding Assay for Characterization of Protein Kinase Inhibitors Possessing Sub-Picomolar to Sub-Millimolar Affinity'. *Analytical Biochemistry* 531 (August):67–77. https://doi.org/10.1016/j.ab.2017.05.017.

Skorupskaite, Karolina, Jyothis T. George, and Richard A. Anderson. 2014. 'The Kisspeptin-GnRH Pathway in Human Reproductive Health and Disease'. *Human Reproduction Update* 20 (4): 485–500. https://doi.org/10.1093/humupd/dmu009.

Smith, Stephen K. 2001. 'Regulation of Angiogenesis in the Endometrium'. *Trends in Endocrinology & Metabolism* 12 (4): 147–51. https://doi.org/10.1016/S1043-2760(01)00379-4.

Srivastava, Akhilesh, Jayasree Sengupta, Alka Kriplani, Kallol K Roy, and Debabrata Ghosh. 2013. 'Profiles of Cytokines Secreted by Isolated Human Endometrial Cells under the Influence of Chorionic Gonadotropin during the Window of Embryo Implantation'. *Reproductive Biology and Endocrinology* 11 (1). https://doi.org/10.1186/1477-7827-11-116.

Stefanoska, I., M. Jovanović Krivokuća, S. Vasilijić, D. Ćujić, and L. Vićovac. 2013. 'Prolactin Stimulates Cell Migration and Invasion by Human Trophoblast *in Vitro*'. *Placenta* 34 (9): 775–83. https://doi.org/10.1016/j.placenta.2013.06.305.

Sugino, N. 2007. 'The Role of Oxygen Radical-Mediated Signaling Pathways in Endometrial Function'. *Placenta* 28 (Supplement A, Trophoblast Research vol. 21): S133–36. https://doi.org/10.1016/j.placenta.2006.12.002.

Sugino, N., Ayako Karube-Harada, Shiro Kashida, Shuji Takiguchi, and Hiroshi Kato. 2002. 'Differential Regulation of Copper-Zinc Superoxide Dismutase and Manganese Superoxide Dismutase by Progesterone Withdrawal in Human Endometrial Stromal Cells'. *Molecular Human Reproduction* 8 (1): 68–74.

Sugino, N., Ayako Karube-Harada, Toshiaki Taketani, Aki Sakata, and Yasuhiko Nakamura. 2004. 'Withdrawal of Ovarian Steroids Stimulates Prostaglandin F2alpha Production Through Nuclear Factor-kappaB Activation via Oxygen Radicals in Human Endometrial Stromal Cells: Potential Relevance to Menstruation'. *Journal of Reproduction and Development* 50 (2): 215–25. https://doi.org/10.1262/jrd.50.215.

Sugino, N., K Shimamura, S Takiguchi, H Tamura, M Ono, M Nakata, Y Nakamura, K Ogino, T Uda, and H Kato. 1996. 'Changes in Activity of Superoxide Dismutase in the Human Endometrium throughout the Menstrual Cycle and in Early Pregnancy'. *Human Reproduction* 11 (5): 1073–78.

Svitkina, Tatyana. 2018. 'The Actin Cytoskeleton and Actin-Based Motility'. *Cold Spring Harbor Perspectives in Biology* 10 (1). https://doi.org/10.1101/cshperspect.a018267.

Syrkasheva, Anastasiya G., Nataliya V. Dolgushina, Andrey Yu Romanov, Olga V. Burmenskaya, Nataliya P. Makarova, Espet O. Ibragimova, Elena A. Kalinina, and Gennady T. Sukhikh. 2017. 'Cell and Genetic Predictors of Human Blastocyst Hatching Success in Assisted Reproduction'. *Zygote* 25 (5): 631–36. https://doi.org/10.1017/S0967199417000508.

Tanaka, N., K. Miyazaki, H. Tashiro, H. Mizutani, and H. Okamura. 1993. 'Changes in Adenylyl Cyclase Activity in Human Endometrium during the Menstrual Cycle and in Human Decidua during Pregnancy'. *Journal of Reproduction and Fertility* 98 (1): 33–39. https://doi.org/10.1530/jrf.0.0980033.

Telgmann, Ralph, and Birgit Gellersen. 1998. 'Marker Genes of Decidualization: Activation of the Decidual Prolactin Gene'. *Human Reproduction Update* 4 (5): 472–79. https://doi.org/10.1093/humupd/4.5.472.

Telgmann, Ralph, Erik Maronde, Kjetil Taskén, and Birgit Gellersen. 1997. 'Activated Protein Kinase A Is Required for Differentiation-Dependent Transcription of the Decidual Prolactin Gene in Human Endometrial Stromal Cells'. *Endocrinology* 138 (3): 929–37. https://doi.org/10.1210/endo.138.3.5004.

Tempest, Nicola, Marnix Jansen, Ann Marie Baker, Christopher J. Hill, Mike Hale, Derek Magee, Darren Treanor, Nicholas A. Wright, and Dharani K. Hapangama. 2020. 'Histological 3D Reconstruction and in Vivo Lineage Tracing of the Human Endometrium'. *Journal of Pathology* 251 (4): 440–51. https://doi.org/10.1002/path.5478.

Thathiah, Amantha, Carl P. Blobel, and Daniel D. Carson. 2003. 'Tumor Necrosis Factor-α Converting Enzyme/ADAM 17 Mediates MUC1 Shedding'. *Journal of Biological Chemistry* 278 (5): 3386–94. https://doi.org/10.1074/jbc.M208326200.

Thathiah, Amantha, and Daniel D. Carson. 2004. 'MT1-MMP Mediates MUC1 Shedding Independent of TACE/ADAM17'. *Biochemical Journal* 382 (1): 363–73. https://doi.org/10.1042/BJ20040513.

Thu Vuhai-Luuthi, Mai, Andre Jolivet, Bahija Jallal, Roland Salesse, Jean-Michel Bidart, Anne Houllier, Anne Guiochon-Mantel, Jean Garnier, and Edwin Milgrom. 1990. 'Monoclonal Antibodies against Luteinizing Hormone Receptor. Immunochemical Characterization of the Receptor*'. *Endocrinology* 127 (5): 2090–98.

Toda, K, K Takeda, T Okada, S Akira, T Saibara, T Kaname, K Yamamura, S Onishi, and Y Shizuta. 2001. 'Targeted Disruption of the Aromatase P450 Gene (Cyp19) in Mice and Their Ovarian and Uterine Responses to 17beta-Oestradiol'. *Journal of Endocrinology* 170 (1): 99–111. https://doi.org/10.1677/joe.0.1700099.

Totani, Haruhito, Keiko Shinjo, Miho Suzuki, Keisuke Katsushima, Shoko Mase, Ayako Masaki, Asahi Ito, et al. 2020. 'Autocrine HGF/c-Met Signaling Pathway Confers Aggressiveness in Lymph Node Adult T-Cell Leukemia/Lymphoma'. *Oncogene* 39 (35): 5782–94. https://doi.org/10.1038/s41388-020-01393-x.

Tšuiko, Olga, Maaike Catteeuw, Masoud Zamani Esteki, Aspasia Destouni, Osvaldo Bogado Pascottini, Urban Besenfelder, Vitezslav Havlicek, et al. 2017. 'Genome Stability of Bovine in Vivo-Conceived Cleavage-Stage Embryos Is Higher Compared to in Vitro-Produced Embryos'. *Human Reproduction* 32 (11): 2348–57. https://doi.org/10.1093/humrep/dex286.

Tsuno, Akitoshi, Kaei Nasu, Akitoshi Yuge, Harunobu Matsumoto, Masakazu Nishida, and Hisashi Narahara. 2009. 'Decidualization Attenuates the Contractility of Eutopic and Ectopic Endometrial Stromal Cells: Implications for Hormone Therapy of

Endometriosis.' *The Journal of Clinical Endocrinology and Metabolism* 94 (7): 2516–23. https://doi.org/10.1210/jc.2009-0207.

Tyanova, Stefka, Tikira Temu, and Juergen Cox. 2016. 'The MaxQuant Computational Platform for Mass Spectrometry-Based Shotgun Proteomics'. *Nature Protocols* 11 (12): 2301–19. https://doi.org/10.1038/nprot.2016.136.

Uenoyama, Yoshihisa, Mayuko Nagae, Hitomi Tsuchida, Naoko Inoue, and Hiroko Tsukamura. 2021. 'Role of KNDy Neurons Expressing Kisspeptin, Neurokinin B, and Dynorphin A as a GnRH Pulse Generator Controlling Mammalian Reproduction'. *Frontiers in Endocrinology* 12 (September). https://doi.org/10.3389/fendo.2021.724632.

Ulloa-Aguirre, Alfredo, Teresa Zariñán, Ana Ma Pasapera, Patricia Casas-González, and James A. Dias. 2007. 'Multiple Facets of Follicle-Stimulating Hormone Receptor Function'. *Endocrine* 32 (3): 251–63. https://doi.org/10.1007/s12020-008-9041-6.

Ulrich, D., K. S. Tan, J. Deane, K. Schwab, A. Cheong, A. Rosamilia, and C. E. Gargett. 2014. 'Mesenchymal Stem/Stromal Cells in Post-Menopausal Endometrium'. *Human Reproduction* 29 (9): 1895–1905. https://doi.org/10.1093/humrep/deu159.

Vahter, Jürgen, Kaido Viht, Asko Uri, Ganesh babu Manoharan, and Erki Enkvist. 2018. 'Thiazole- and Selenazole-Comprising High-Affinity Inhibitors Possess Bright Microsecond-Scale Photoluminescence in Complex with Protein Kinase CK2'. *Bio-organic & Medicinal Chemistry* 26 (18): 5062–68. https://doi.org/10.1016/j.bmc.2018.09.003.

Valentijn, A.J., K. Palial, H. Al-lamee, N. Tempest, J. Drury, T. Von Zglinicki, G. Saretzki, P. Murray, C.E. Gargett, and D.K. Hapangama. 2013. 'SSEA-1 Isolates Human Endometrial Basal Glandular Epithelial Cells: Phenotypic and Functional Characterization and Implications in the Pathogenesis of Endometriosis'. *Human Reproduction* 28 (10): 2695–2708. https://doi.org/10.1093/humrep/det285.

Vannier, Brigitte, Hugues Loosfelt, Geri Meduri, Christophe Pichon, and Edwin Milgrom. 1995. 'Anti-Human FSH Receptor Monoclonal Antibodies: Immunochemical and Immunocytochemical Characterization of the Receptor †'. *Biochemistry* 35:1358–66.

Vega, S. de, T. Iwamoto, and Y. Yamada. 2009. 'Fibulins: Multiple Roles in Matrix Structures and Tissue Functions'. *Cellular and Molecular Life Sciences* 66 (11): 1890–1902. https://doi.org/10.1007/s00018-009-8632-6.

Wang, H, H O D Critchley, R W Kelly, D Shen, and D T Baird. 1998. 'Progesterone Receptor Subtype B Is Differentially Regulated in Human Endometrial Stroma'. *Molecular Human Reproduction* 4 (4): 407–12.

Wang, H., Xie Luo, and Jake Leighton. 2015. 'Extracellular Matrix and Integrins in Embryonic Stem Cell Differentiation'. *Biochemistry Insights* 8s2 (January):BCI. S30377. https://doi.org/10.4137/BCI.S30377.

Wang, H., Federica Pilla, Sandra Anderson, Sebastián Martínez-Escribano, Isabel Herrer, Juan M. Moreno-Moya, Sirisha Musti, Silvina Bocca, Sergio Oehninger, and José A. Horcajadas. 2012. 'A Novel Model of Human Implantation: 3D Endometrium-like Culture System to Study Attachment of Human Trophoblast (Jar) Cell Spheroids'. *Molecular Human Reproduction* 18 (1): 33–43. https://doi.org/10.1093/molehr/gar064.

Wang, L., Shulan Lv, Wenjun Mao, Meili Pei, and Xiaofeng Yang. 2020. 'Assessment of Endometrial Receptivity during Implantation Window in Women with Unexplained Infertility'. *Gynecological Endocrinology* 36 (10): 917–21. https://doi.org/10.1080/09513590.2020.1727433.

Wang, W., Felipe Vilella, Pilar Alama, Inmaculada Moreno, Marco Mignardi, Alina Isakova, Wenying Pan, Carlos Simon, and Stephen R. Quake. 2020. 'Single-Cell Transcriptomic Atlas of the Human Endometrium during the Menstrual Cycle'. *Nature Medicine* 26 (10): 1644–53. https://doi.org/10.1038/s41591-020-1040-z.

Wang, Wei, Robert N. Taylor, Indrani C. Bagchi, and Milan K. Bagchi. 2012. 'Regulation of Human Endometrial Stromal Proliferation and Differentiation by C/EBPβ Involves Cyclin E-Cdk2 and STAT3'. *Molecular Endocrinology* 26 (12): 2016–30. https://doi.org/10.1210/me.2012-1169.

Wang, Xiaobin, Yingqing Shi, Hua Shi, Xiaoyu Liu, Aijun Liao, Zhuogang Liu, Robert Z. Orlowski, Rui Zhang, and Huihan Wang. 2024. 'MUC20 Regulated by Extrachromosomal Circular DNA Attenuates Proteasome Inhibitor Resistance of Multiple Myeloma by Modulating Cuproptosis'. *Journal of Experimental & Clinical Cancer Research* 43 (1): 68. https://doi.org/10.1186/s13046-024-02972-6.

Wang, Xiaobin, Fazal Shirazi, Wei Yan, Xiaoyu Liu, Hua Wang, Robert Z. Orlowski, and Huihan Wang. 2021. 'Mucin 20 Modulates Proteasome Capacity through C-Met Signalling to Increase Carfilzomib Sensitivity in Mantle Cell Lymphoma'. *Journal of Cellular and Molecular Medicine* 25 (21): 10164–74. https://doi.org/10.1111/jcmm.16953.

Welt, Corrine K. 2004. 'Regulation and Function of Inhibins in the Normal Menstrual Cycle'. *Seminars in Reproductive Medicine* 22 (August):187–93. https://doi.org/10.1055/s-2004-831894.

Wilcox, Allen J., Donna Day Baird, and Clarice R. Weinberg. 1999. 'Time of Implantation of the Conceptus and Loss of Pregnancy'. *New England Journal of Medicine* 340 (23): 1796–99. https://doi.org/10.1056/NEJM199906103402304.

Wilson, Richard C., James S. Kesner, Jean-Marc Kaufman, Tsuguo Vemura, Tatsuo Akema, and Ernst Knobil. 1984. 'Central Electrophysiologic Correlates of Pulsatile Luteinizing Hormone Secretion in the Resus Monkey'. *Neuroendocrinology* 39:256–60.

Woodward, Ashley M., and Pablo Argüeso. 2014. 'Expression Analysis of the Transmembrane Mucin MUC20 in Human Corneal and Conjunctival Epithelia'. *Investigative Ophthalmology & Visual Science* 55 (10): 6132–38. https://doi.org/10.1167/iovs.14-15269.

Wu, Fangrong, Xiaoyan Chen, Yingyu Liu, Bo Liang, Hui Xu, Tin Chiu Li, and Chi Chiu Wang. 2018. 'Decreased MUC1 in Endometrium Is an Independent Receptivity Marker in Recurrent Implantation Failure during Implantation Window'. *Reproductive Biology and Endocrinology* 16 (1): 60. https://doi.org/10.1186/s12958-018-0379-1.

Wu, Fangrong, Di Mao, Yingyu Liu, Xiaoyan Chen, Hui Xu, Tin Chiu Li, and Chi Chiu Wang. 2019. 'Localization of Mucin 1 in Endometrial Luminal Epithelium and Its Expression in Women with Reproductive Failure during Implantation Window'. *Journal of Molecular Histology* 50 (6): 563–72. https://doi.org/10.1007/s10735-019-09848-6.

Xiao, Xiuying, Lisha Wang, Ping Wei, Yayun Chi, Dali Li, Qifeng Wang, Shujuan Ni, et al. 2013. 'Role of MUC20 Overexpression as a Predictor of Recurrence and Poor Outcome in Colorectal Cancer'. *Journal of Translational Medicine* 11 (1): 151. https://doi.org/10.1186/1479-5876-11-151.

Xie, Jun, Xiao Xu, and Suying Liu. 2023. 'Intercellular Communication in the Cumulus–Oocyte Complex during Folliculogenesis: A Review'. *Frontiers in Cell and Developmental Biology* 11 (January). https://doi.org/10.3389/fcell.2023.1087612.

Xu, Bufang, Xiaoxi Sun, Lu Li, Ling Wu, Aijun Zhang, and Yun Feng. 2012. 'Pinopodes, Leukemia Inhibitory Factor, Integrin-B3, and Mucin-1 Expression in the Peri-Implantation Endometrium of Women with Unexplained Recurrent Pregnancy Loss'. *Fertility and Sterility* 98 (2): 389–95. https://doi.org/10.1016/j.fertnstert.2012.04.032.

Yamaguchi, Manako, Kosuke Yoshihara, Kazuaki Suda, Hirofumi Nakaoka, Nozomi Yachida, Haruka Ueda, Kentaro Sugino, et al. 2021. 'Three-Dimensional Understanding of the Morphological Complexity of the Human Uterine Endometrium'. *iScience* 24 (4): 1–41. https://doi.org/10.1016/j.isci.2021.102258.

Yamato, M, K Shima, and R Nakano. 1992. 'Gonadotropin Receptors in Human Ovarian Follicles and Corpora Lutea throughout the Menstrual Cycle'. *Hormone Research* 37 (suppl 1): 5–11.

Yaşar, Pelin, Gamze Ayaz, Sırma Damla User, Gizem Güpür, and Mesut Muyan. 2017. 'Molecular Mechanism of Estrogen–Estrogen Receptor Signaling'. *Reproductive Medicine and Biology* 16 (1): 4–20. https://doi.org/10.1002/rmb2.12006.

Yen, S S C, and C C Tsai. 1972. 'Acute Gonadotropin Release Induced by Exogenous Estradiol During the Mid-Follicular Phase of the Menstrual Cycle'. *The Journal of Clinical Endocrinology and Metabolism* 34 (2): 298–305.

Yoshida, Souichi, Tasuku Harada, Tomio Iwabe, Fuminori Taniguchi, Akiko Fujii, Yasuko Sakamoto, Nobuhiro Yamauchi, Goshi Shiota, and Naoki Terakawa. 2002. 'Induction of Hepatocyte Growth Factor in Stromal Cells by Tumor-Derived Basic Fibroblast Growth Factor Enhances Growth and Invasion of Endometrial Cancer'. *The Journal of Clinical Endocrinology & Metabolism* 87 (5): 2376–83. https://doi.org/10.1210/jcem.87.5.8483.

Yoshida, Souichi, Tasuku Harada, Masahiro Mitsunari, Tomio Iwabe, Yasuko Sakamoto, Satoru Tsukihara, Yumiko Iba, Sayako Horie, and Naoki Terakawa. 2004. 'Hepatocyte Growth Factor/Met System Promotes Endometrial and Endometriotic Stromal Cell Invasion via Autocrine and Paracrine Pathways'. *Journal of Clinical Endocrinology and Metabolism* 89 (2): 823–32. https://doi.org/10.1210/jc.2003-030874.

Yoshie, Mikihiro, Kazuya Kusama, and Kazuhiro Tamura. 2015. 'Molecular Mechanisms of Human Endometrial Decidualization Activated by Cyclic Adenosine Monophosphate Signaling Pathways'. *Journal of Mammalian Ova Research* 32 (3): 95–102. https://doi.org/10.1274/jmor.32.95.

Yoshino, O, Y Hirota, K Koga, T Yano, O Tsutsumi, and Y Taketani. 2003. 'Akt as a Possible Intracellular Mediator for Decidualization in Human Endometrial Stromal Cells'. *Molecular Human Reproduction* 9 (5): 265–69. https://doi.org/10.1093/molehr/gag035.

Yoshino, O, Yutaka Osuga, Yasushi Hirota, Kaori Koga, Tetsuya Hirata, Tetsu Yano, Takuya Ayabe, Osamu Tsutsumi, and Yuji Taketani. 2003. 'Endometrial Stromal Cells Undergoing Decidualization Down-Regulate Their Properties to Produce Proinflammatory Cytokines in Response to Interleukin-1β via Reduced P38 Mitogen-Activated Protein Kinase Phosphorylation'. *The Journal of Clinical Endocrinology & Metabolism* 88 (5): 2236–41. https://doi.org/10.1210/jc.2002-021788.

Yu, Jie, Sarah L. Berga, Erika B. Johnston-MacAnanny, Neil Sidell, Indrani C. Bagchi, Milan K. Bagchi, and Robert N. Taylor. 2016. 'Endometrial Stromal Decidualization Responds Reversibly to Hormone Stimulation and Withdrawal'. *Endocrinology* 157 (6): 2432–46. https://doi.org/10.1210/en.2015-1942.

Yu, Jie, Anisoara Boicea, Kara L. Barrett, Christopher O. James, Indrani C. Bagchi, Milan K. Bagchi, Ceana Nezhat, Neil Sidell, and Robert N. Taylor. 2014. 'Reduced Connexin 43 in Eutopic Endometrium and Cultured Endometrial Stromal Cells from

Subjects with Endometriosis'. *Molecular Human Reproduction* 20 (3): 260–70. https://doi.org/10.1093/molehr/gat087.

Yu, Jie, Juanjuan Wu, Indrani C. Bagchi, Milan K. Bagchi, Neil Sidell, and Robert N. Taylor. 2011. 'Disruption of Gap Junctions Reduces Biomarkers of Decidualization and Angiogenesis and Increases Inflammatory Mediators in Human Endometrial Stromal Cell Cultures'. *Molecular and Cellular Endocrinology* 344 (1): 25–34. https://doi.org/10.1016/j.mce.2011.04.011.

Yung, Y., S. Aviel-Ronen, E. Maman, N. Rubinstein, C. Avivi, R. Orvieto, and A. Hourvitz. 2014. 'Localization of Luteinizing Hormone Receptor Protein in the Human Ovary'. *Molecular Human Reproduction* 20 (9): 844–49. https://doi.org/10.1093/molehr/gau041.

Zhang, J. 1997. 'Tissue Inhibitor of Metalloproteinases (TIMP)-1, -2 and -3 in Human Endometrium during the Menstrual Cycle'. *Molecular Human Reproduction* 3 (9): 735–41. https://doi.org/10.1093/molehr/3.9.735.

Zhang, Jin, and Lois A. Salamonsen. 2002. 'In Vivo Evidence for Active Matrix Metalloproteinases in Human Endometrium Supports Their Role in Tissue Break-down at Menstruation'. *The Journal of Clinical Endocrinology & Metabolism* 87 (5): 2346–51. https://doi.org/10.1210/jcem.87.5.8487.

Zhang, Xiu-Hong, Xuan Liang, Xiao-Huan Liang, Tong-Song Wang, Qian-Rong Qi, Wen-Bo Deng, Ai-Guo Sha, and Zeng-Ming Yang. 2013. 'The Mesenchymal—Epithelial Transition During In Vitro Decidualization'. *Reproductive Sciences* 20 (4): 354–60. https://doi.org/10.1177/1933719112472738.

Zhao, Yang, Wenling Ye, Yan-Dong Wang, and Wei-Dong Chen. 2022. 'HGF/c-Met: A Key Promoter in Liver Regeneration'. *Frontiers in Pharmacology* 13 (March). https://doi.org/10.3389/fphar.2022.808855.

Zheng, Fei, Huimin Yu, and Jingjing Lu. 2019. 'High Expression of MUC20 Drives Tumorigenesis and Predicts Poor Survival in Endometrial Cancer'. *Journal of Cellular Biochemistry* 120 (7): 11859–66. https://doi.org/10.1002/jcb.28466.

Zhou, Jordan Z., Sing Sing Way, and Kang Chen. 2018. 'Immunology of the Uterine and Vaginal Mucosae'. *Trends in Immunology* 39 (4): 302–14. https://doi.org/10.1016/j.it.2018.01.007.

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PUBLICATIONS

CURRICULUM VITAE

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Education:

2015–…	PhD studies in molecular and cell biology, University of Tartu
2013–2015	MSc in Biology (molecular and cell biology), University of Tartu, *cum laude*
2010–2013	BSc in Biology, University of Tartu
2007–2010	Secondary education, Kohtla-Järve Järve Russian Gymnasium

Institutions and positions held:

2019–2023	Celvia CC AS, specialist
2018–2019	Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, specialist
2016–2018	Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu, specialist
2015–2016	Celvia CC AS (former CCHT), specialist
2015–2015	Celvia CC AS (former CCHT), research fellow

Special courses:

2018	Intensive PhD course "Comparative aspects of reproduction and its failure in humans and animals", Tartu
2018	International Federation of Fertility Societies (IFFS) 2018 Workshop "The Management of Infertility", Tartu
2017	University of Tartu Institute of Clinical Medicine Happy Pregnancy project 4th workshop "Linking scientific advances with clinical practice in reproductive biomedicine", Tartu
2016	University of Tartu Institute of Clinical Medicine special course "Immunohistochemistry and immunofluorescence", Tartu

Scientific awards:

RIIKLIKULT TUNNUSTATUD
TEADUSE POPULARISEERIJA 2022

2022 Science Popularizer 2022 Award (2nd Prize, series of popular science articles titled "A Guide for Teachers" led by Sulev Kuuse, published in *Eesti Loodus* magazine). My article: *Electron Microscopy Reveals the Intricacies of Cell Structures*. *Eesti Loodus*, December 2021.

2015 The master's thesis *"Modification of Integrins in Microvesicles Secreted by Trophoblasts and the Development of the Tetraspanin CD63 and CFP Fusion Protein"* received third prize in the bio- and environmental sciences category for master's level students at the 2015 National Student Research Competition.

Teaching at the university:

Member of the author team for the university textbook *"Cell Biology"* (published by the University of Tartu Press), contributing to the chapter *"Selection of Cell Biology Methods: The Electron Microscopy Section."* The textbook was awarded the 2023 University of Tartu Language Deed Laureate and the Grand Prize at the 2023 National Language Deed Competition.

Participated in teaching cell biology practical courses.

2021 Supervised Irina Guljavina master's thesis, *"Extracellular Vesicles as Biomarkers for Psoriasis and Psoriatic Arthritis,"* defended at the Institute of Molecular and Cell Biology, University of Tartu, in 2021.

2017 Supervised Helen Kiik bachelor's thesis, *"Expression of Laminin Chains in the Human Endometrium,"* defended at the Institute of Molecular and Cell Biology, University of Tartu, in 2017. This thesis received a certificate of recognition at the National Student Research Competition in the bio- and environmental sciences category for applied higher education and bachelor's level students.

Publications:

2024

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2019

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2018

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Haridus:

2015–… Doktoriõpe, Molekulaar- ja rakubioloogia, Tartu ülikool

2013–2015 Magistriõpe, Bioloogia (molekulaar- ja rakubioloogia), Tartu ülikool, *cum laude*

2010–2013 Bakalaureuseõpe, Bioloogia, Tartu ülikool

2007–2010 Kohtla-Järve Järve Vene Gümnaasium

Töökogemus:

2019–2023 Celvia CC AS, spetsialist

2018–2019 Tartu Ülikool, Meditsiiniteaduste valdkond, bio- ja siirdemeditsiini instituut, spetsialist

2016–2018 Tartu Ülikool, Loodus- ja täppisteaduste valdkond, molekulaar- ja rakubioloogia instituut, spetsialist

2015–2016 Celvia CC AS (endine Tervisetehnoloogiate Arenduskeskus AS), spetsialist

2015–2015 Celvia CC AS (endine Tervisetehnoloogiate Arenduskeskus AS), teadur

Enesetäiendus:

2018 Intensive PhD course “Comparative aspects of reproduction and its failure in humans and animals“, Tartu

2018 International Federation of Fertility Societies (IFFS) 2018 Workshop “The Management of Infertility“, Tartu

2017 Tartu ülikooli kliinilise meditsiini instituudi täiendusõppeprogramm Happy Pregnancy 4. töötuba “Linking scientific advances with clinical practice in reproductive biomedicine”, Tartu

2016 Tartu ülikooli kliinilise meditsiini instituudi täienduskeskuse täiendkursus “Immunohistokeemia ja immunofluorestsents”, Tartu

Teaduspreemiad ja tunnustused:

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TEADUSE POPULARISEERIJA 2022

2022 Teaduse populariseerija 2022 auhind, (2. preemia, Sulev Kuuse juhitud populaarteaduslike artiklite sari „Abiks õpetajale“ Eesti Looduse ajakirjas), Minu artikkel: Elektronmikroskoopia lubab näha rakkude siseehituse pisiasju. Eesti loodus, Detsember 2021

2015 Magistritöö „Integriinide modifitseerimine trofoblastide poolt sekreteeritud mikrovesiikulites ning tetraspaniin CD63 ja CFP liitvalgu saamine“ sai 2015. a üliõpilaste teadustööde riiklikul konkursil bio- ja keskkonnateaduste valdkonnas magistriõppe üliõpilaste astmes III. preemia

Õppetöö:

Kõrgkooli õpiku “Rakubioloogia” (Tartu Ülikooli kirjastus) autorite kollektiivi liige (peatükk: valik rakubioloogia meetodeid, elektronmikroskoopia osa). Antud õpik on 2023. aasta Tartu Ülikooli keeleteo laureaat ja 2023. aasta riikliku keeleteo konkursi peaauhinna laureaat.

Osalenud rakubioloogia praktikumi läbiviimisel.

2021 Juhendanud Irina Guljavina magistri tööd „Ekstratsellulaarsed vesiikulid biomarkeritena psoriaasi ja psoriaatilise artriidi korral”, kaitstud Tartu Ülikooli Molekulaar- ja rakubioloogia instituudis 2021. aastal.

2017 Juhendanud Helen Kiige bakalaureuse tööd „Laminiini ahelate ekspressioon inimese endomeetriumis“, kaitstud Tartu Ülikooli Molekulaar- ja rakubioloogia instituudis 2017. aastal, antud töö sai üliõpilaste teadustööde riiklikul konkursil bio- ja keskkonnateaduste valdkonnas rakenduskõrgharidusõppe ja bakalaureuseõppe üliõpilaste astmes tänukirja.

Publikatsioonid:

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Darja Lavogina, **Artjom Stepanjuk**, Maire Peters, Külli Samuel, Sergo Kasvandik, Masuma Khatun, Riikka K Arffman, Erki Enkvist, Kaido Viht, Sergei Kopanchuk, Freddy Lättekivi, Agne Velthut-Meikas, Asko Uri, Terhi T Piltonen,

Ago Rinken, Andres Salumets. 2021. “Progesterone triggers Rho kinase-cofilin axis during *in vitro* and *in vivo* endometrial decidualization”, *Human Reproduction*, Volume 36, Issue 8, August 2021, Pages 2230–2248. https://doi.org/10.1093/humrep/deab161

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2018

Ade Kallas-Kivi, Annika Trei, **Artjom Stepanjuk**, Katrin Ruisu, Keiu Kask, Margus Pooga, Toivo Maimets. 2018. “The role of integrin β1 in the heterogeneity of human embryonic stem cells culture”, *Biol Open*, 15 November 2018, 7 (11), bio034355. doi: https://doi.org/10.1242/bio.034355

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