Chapter 1

Physiology of Endometrial Development through the Cycle and Implantation

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1.1 Introduction

The monthly female reproductive cycle involves a pattern of cyclic sex steroid changes to prepare the endometrium for potential implantation of an embryo. This chapter outlines the physiological aspects of the reproductive cycle, including the production of gonadotrophic hormones, their relationship to the ovarian hormones, the development of follicles, release of the ovum and either potential implantation of the blastocyst or menstruation in the absence of pregnancy.

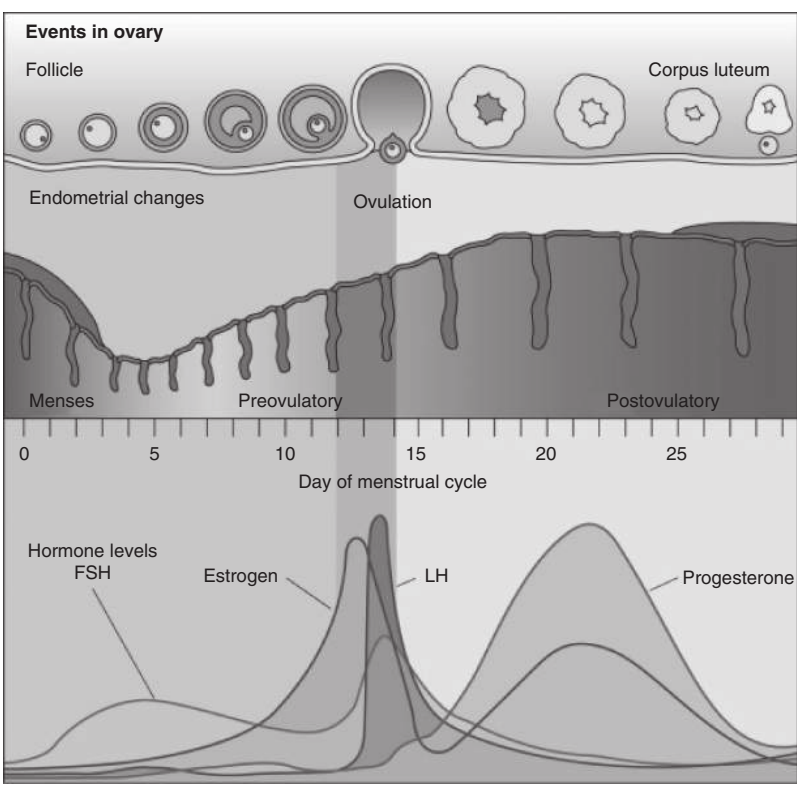

Figure 1.1 The female reproductive cycle – the follicular, endometrial and hormonal changes throughout the cycle are demonstrated
Source: Gartner, LP. *Textbook of Histology*, Fourth Edition. Chapter 20, Female Reproductive System (Elsevier, 2017).

The endometrial cycle has two distinct phases, each with different functions required of the tissue. This chapter utilises these endometrial phases as a framework to examine the reproductive cycle as a whole.

The preovulatory, or follicular, phase is a two-week period involving the development of the ovarian follicles usually resulting in ovulation. This phase is oestrogen dominant, repairing and priming a new endometrial layer after the previous menstruation.

The luteal phase is the two-week period following ovulation. This progesterone-dominant phase matures the endometrium, providing a nutritious site for potential implantation. In the absence of pregnancy, after withdrawal of hormones, the now-thickened endometrium is shed during menstruation and the cycle can commence again.

1.2 Endometrial Structure

The endometrium consists of three layers. The basal layer resting above the myometrium is the stratum basalis. The stratum compactum and stratum spongiosum lie above the basalis and together form the stratum functionalis. The functionalis layer undergoes cyclical change in response to hormone fluctuations and is shed with menstruation, leaving the stratum basalis to regenerate a new functionalis the following cycle. These layers are demonstrated in Figure 1.2.

Several cell types are contained within the endometrium. The most superficial layer, lining the uterine cavity, consists of simple columnar epithelium. Throughout the endometrium this luminal epithelium dips down into the underlying stroma to form glands, whose secretory function is intrinsically reliant on the monthly hormonal cycle.

The vascular network supplying the endometrium involves a sophisticated plexus of vessels with varying hormonal sensitivities. The uterine arteries branching from the internal

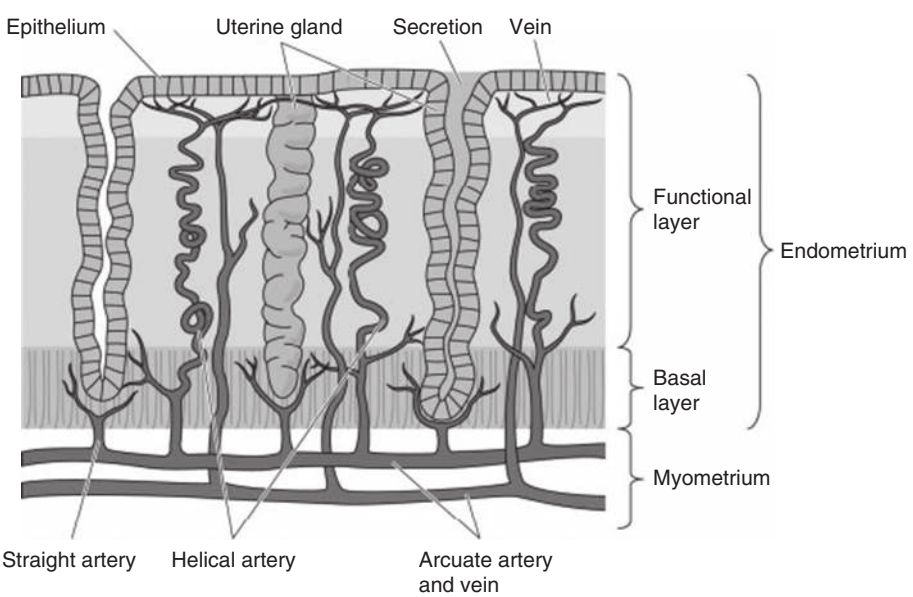

Figure 1.2 Endometrial histology – the basal and functional layers of the endometrium are demonstrated with the underlying vascular supply
Source: Gartner, LP. *Textbook of Histology*, Fourth Edition. Chapter 20, Female Reproductive System (Elsevier, 2017).

iliac vessels give rise to the radial arteries, which supply the underlying myometrium. These vessels branch into straight arterioles supplying the stratum basalis and coiled spiral, or helical, arterioles supplying the more superficial stratum functionalis. Blood from the subepithelial capillaries then drains into a venous plexus that mirrors the arterial supply. The vascular supply to the endometrium is demonstrated in Figure 1.2.

The basal vessels are surrounded by smooth muscle and are not influenced by cyclic sex steroid variation. However, the more superficial vessels consist mainly of endothelium. Without the surrounding smooth muscle support, these vessels are more easily influenced by endothelial growth factors, hormonal fluctuations, hypoxia and mechanical stress [1].

1.3 Follicular Phase

The hypothalamic-pituitary-gonadal axis regulates ovarian sex steroid production that leads to regular shedding of the superficial endometrium and blood loss when pregnancy does not occur. Release of hypothalamic gonadotropin-releasing hormone (GnRH) occurs in a rhythmic pulsatile pattern, approximately every one to two hours [2]. GnRH is transported to the anterior pituitary gland via a complex capillary bed, the hypothalamic-hypophyseal portal blood system. In response to the effect of GnRH, the anterior pituitary produces two hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

The prepubertal ovary contains a large collection of oocytes, each surrounded by a layer of granulosa cells and referred to as primordial follicles. With the onset of puberty, the increasing role of FSH and LH causes oocyte growth as well as the formation of additional layers of granulosa cells; the follicles are now termed 'primary follicles'. It is these primary follicles that undergo maturation and potential ovulation during the monthly ovarian cycle.

At the start of the follicular phase, FSH released from the anterior pituitary causes rapid growth of the granulosa cell layer in several primary follicles. A layer of thecal cells forms around the granulosa cell mass encapsulating the follicle.

Granulosa cells produce several hormones, including oestrogen, and rapid follicular growth results in increasing oestrogen levels. Oestrogen release then stimulates development of more FSH and LH receptors, increasing the cells' sensitivity to the hormones and triggering further proliferation of both granulosa and thecal cell layers. Meanwhile, LH stimulates thecal production of androstenedione, which the granulosa cells assist in converting to oestrogen, further contributing to the rapid rise in oestrogen levels.

Prior to ovulation, one follicle begins to grow more rapidly than the others (dominant or leading follicle), potentially due to increased receptor expression and thus sensitivity to FSH [3]. Within the dominant follicle, the oestrogen-containing follicular fluid secreted by the granulosa cells accumulates in a sac referred to as the follicular antrum. The increasing oestrogen production acts as negative feedback to the anterior pituitary to reduce the production of both FSH and LH, which accounts for the dips in gonadotrophic hormone production (Figure 1.1). The withdrawal of gonadotrophic stimulation causes the remaining follicles to undergo atresia, while the intrinsic oestrogen from the dominant follicle is sufficient for its ongoing growth.

However, when oestrogen production from the dominant follicle reaches peak levels, it contrastingly acts as positive feedback to the anterior pituitary, stimulating a sudden surge in LH and, to a lesser extent, FSH production. Meanwhile, the increasing LH levels stimulate

the granulosa cells to produce two other sex steroids, progesterone and inhibin. Inhibin reduces production of FSH from the anterior pituitary, accounting for the lower peak of FSH compared to LH observed (Figure 1.1).

The high levels of LH and increasing production of progesterone reduce the secretion of oestrogen and stimulate ovulation. Follicular angiogenesis and prostaglandin release cause swelling of the follicle. Simultaneous release of enzymes from thecal cells begins to weaken the follicle capsule, until it eventually ruptures, releasing the ovum. In a normal 28-day cycle, this process occurs at day 14.

1.3.1 Follicular Phase Endometrium

Following menstruation signalling the end of the previous cycle, the endometrial layer is thin. The endometrium must repair before the next ovulation to provide opportunity for implantation. The follicular phase involves proliferation of both stromal and glandular layers of the endometrium with accompanying angiogenesis.

It appears that endometrial vascular cells contain both oestrogen and progesterone receptors [4]. However, the initial repair of the endometrium commences shortly after the onset of menstruation, when the circulating ovarian hormone levels remain low. This would suggest that local factors, including angiogenic growth factors and prostaglandins, are essential to endometrial repair. Interestingly, many of these factors are released in the pro-inflammatory and intermittently hypoxic environment of the late luteal phase prior to menstruation. This suggests that progesterone withdrawal is not only an important trigger of menstruation but also indirectly relevant to the subsequent repair of endometrial tissue [5].

The growth factors and chemical mediators present in the tissue work to re-epithelialise the endometrium in a number of days. Angiogenesis and vascular permeability are vital in ensuring adequate delivery of nutrients to the newly forming endometrium. In addition, increasing oestrogen levels cause proliferation and growth of the stromal and glandular cells. However, the secretory function of the endometrial glands does not mature without the presence of progesterone during the luteal phase.

The aim of the oestrogen-dominant follicular phase is to repair the previously shed stratum functionalis. A thickened, oedematous endometrium is formed in preparation for ovulation and potential implantation.

1.4 Luteal Phase

Following the release of the ovum during ovulation, the follicle contains the remnant granulosa and theca cells. Under the influence of the high levels of LH at this time, these cells undergo a process of luteinisation involving cholesterol deposition vital for steroidogenesis. The cells are now referred to as lutein cells and the cell mass is termed the ‘corpus luteum’, meaning ‘yellow body’, referring to the characteristic yellow appearance of the cells caused by intracellular cholesterol.

The primary hormonal focus of the follicular phase is the rising oestrogen levels, which prime the endometrium and initiate the essential preovulatory LH surge. During the late follicular phase, LH alters the function of the granulosa cells, changing the hormonal environment to one of mainly progesterone secretion. This effect continues during the luteal phase, with the corpus luteum producing increasing amounts of progesterone and, to a lesser extent, oestrogen. This change in sex steroid production can be observed in Figure 1.1.

The importance of progesterone during the luteal phase lies in maturation of the endometrium. Progesterone enhances development of the endometrial glands and initiates secretions. It stimulates angiogenesis with the formation of tight, coiled vessels, which become full and permeable, creating oedema within the endometrial stroma. These characteristic histological findings of the luteal-phase endometrium reflect the nutrient-rich, thickened endometrium prepared for potential implantation of the fertilised ovum.

During the luteal phase, elevated levels of progesterone and oestrogen released from the corpus luteum act as negative feedback on the anterior pituitary to minimise secretion of FSH and LH. Furthermore, the release of inhibin from the lutein cells further suppresses gonadotrophin secretion, mainly FSH.

However, the withdrawal of gonadotrophic stimulation eventually causes degeneration of the corpus luteum. As the cells atrophy and the cell mass degenerates, the production of progesterone and oestrogen dwindles, removing the negative feedback effect on the anterior pituitary stimulating the release of both FSH and LH.

1.4.1 Luteal Phase Endometrium

During the mid-luteal phase, the progesterone-dominant environment is one focused on haemostasis. The hormone encourages a stable endometrium for blastocyst implantation, protecting against haemorrhage that could compromise the success of the pregnancy.

Haemostasis is encouraged via several mechanisms. Following the oestrogen-stimulated rapid growth of the endometrium during the proliferative phase, progesterone acts to limit uncontrolled angiogenesis. It also stimulates the release of tissue factor, a high-affinity receptor for factor VII, playing a vital role in the initiation of the clotting cascade [6].

Progesterone is also involved in the inhibition of matrix metalloproteinases (MMP). MMP are enzymes responsible for the degradation of the structural extracellular matrix. They are largely released from the endometrial stromal cells and, to a lesser extent, endometrial leucocytes [7,8,9,10]. The inhibition of MMP expression stabilises the endometrial stroma and the underlying vascular network, preventing haemorrhage.

By the late luteal phase, the stromal cells are the only endometrial cellular component with progesterone receptors [9]. In the absence of pregnancy, degeneration of the corpus luteum and progesterone withdrawal therefore removes the stabilising effect of progesterone on the cells of the perivascular stroma. Progesterone withdrawal allows the perivascular stromal cells to produce pro-inflammatory chemokines, including prostaglandins, interleukins and tumour necrosis factor-α, which stimulate vascular permeability and attract endometrial leucocytes, including neutrophils, macrophages and mast cells.

Furthermore, progesterone withdrawal likely stimulates a period of vasospasm through its effect on the perivascular stroma; vasoconstriction and tissue hypoxia are followed by vasodilation and subsequent reperfusion injury [11]. This environment up-regulates the pro-inflammatory state with further release of inflammatory chemical mediators and their cellular recruits.

Proliferation of leucocytes occurs within the endometrium as well as migration from the peripheral circulation [8]. The subsequent release of lytic enzymes and MMP destabilises the endometrial matrix. In particular, degradation of the perivascular matrix causes

weakening and disruption to the vasculature and results in haemorrhage. The vascular fragility and propensity to bleed are exacerbated by the concomitant oestrogen withdrawal of the late luteal phase [12]. Furthermore, proteolytic destruction of the remaining stromal matrix causes cellular breakdown and tissue sloughing.

These changes are accompanied by the loss of other progesterone-based haemostatic measures. Hormone withdrawal reduces the expression of coagulant factors, including tissue factor and plasminogen activator inhibitor-l, and impairs platelet function [13,14]. There is additional up-regulation of anticoagulant factors such as tissue plasminogen activator and urokinase. The result is a haemorrhagic environment favouring tissue destruction. The presence of desquamated cells and the associated chemical mediators triggers uterine contractility to promote expulsion of the tissue.

1.5 Implantation

Following fertilisation of the ovum, successful implantation requires a viable blastocyst to communicate effectively with a receptive endometrium, which can support the growing embryo until the placenta is able to supply adequate nutrition. The endometrium is considered receptive for a relatively short period of time each cycle, likely several days. This period is referred to as the 'window of implantation', beyond which the blastocyst cannot adhere and menstruation results [15,16].

The initial communication between the developing blastocyst and the endometrium is mediated by a range of factors secreted by the epithelial cells, guiding the orientation and attachment of the blastocyst. The complex role and interaction of these factors are discussed elsewhere in this book.

The luminal epithelium of the endometrium forms projections termed 'pinopodes', which assist in the adhesion of the blastocyst to the endometrial lining [17]. Once attached, the trophoblast cells strongly adhere to the epithelium and release a range of lytic enzymes allowing invasion of the blastocyst into the deeper endometrial stroma and absorption of nutrients [18,19].

The blastocyst is surrounded by a layer of cells, trophoblasts. Implantation is associated with proliferation of the trophoblasts into two distinct layers. The cytotrophoblasts form the inner layer and fuse to form an outer layer of multinucleated cells termed 'syncitiotrophoblasts'.

In the absence of pregnancy, the degeneration of the corpus luteum causes progesterone withdrawal, resulting in menstruation and a new reproductive cycle. A successful pregnancy therefore requires that the endometrial tissue has sufficient exposure to progesterone to stabilise the endometrial lining and prevent expulsion of the trophoblast.

Consequently, the syncitiotrophoblast cells secrete human chorionic gonadotrophin (HCG), a hormone structurally and functionally similar to LH. HCG thereby maintains the corpus luteum, allowing continued production of progesterone. The importance of progesterone at this point lies in the development of a supportive endometrium and prevention of uterine contractility that could expel the implanting blastocyst. The corpus luteum is the main source of progesterone and oestrogens for the developing pregnancy for the first two to three months, beyond which the placenta provides sufficient hormone production to maintain the pregnancy and the corpus luteum involutes.

The process of blastocyst invasion in the progesterone-dominant environment triggers the decidualisation of the endometrium. The formation of the decidua is a process of stromal cell growth and intracellular accumulation of nutrients, activation of the glandular secretory function and angiogenesis to support the developing blastocyst.

During invasion, the syncitiotrophoblasts form villous projections into the stroma. Until blood flow develops, the blastocyst is maintained by absorption of nutrients across the villi from the glandular secretions and decidual cells [19]. Blastocyst invasion and decidualisation then trigger the release of various angiogenic factors. This process stimulates angiogenesis of fetal vessels and vascular remodelling of the maternal uterine spiral arterioles by invasive trophoblasts to form feto-maternal circulatory connections allowing diffusion of nutrients and waste [20,21,22,23,24].

Invasion of maternal vessels by trophoblasts creates obvious potential for haemorrhage compromising implantation and placentation. The decidual cells play an important role in maintaining haemostasis via increased production of tissue factor and plasminogen activator inhibitor-l. These haemostatic mediators promote thrombin formation and prevent fibrinolysis, creating an anticoagulant environment [14].

The endometrium appears to play an active role in blastocyst selection, rather than passively accepting the attaching embryo. Abnormal embryos trigger a chemically mediated signal within the endometrium. Following the process of decidualisation, the stromal cells appear to have the ability to respond to these signals by engulfing the blastocyst and inhibiting the release of biochemical implantation factors, ultimately causing expulsion of the blastocyst with endometrial bleeding. Consequently, it is suggested that inadequate decidualisation results in loss of embryo selection and may be implicated in early or recurrent pregnancy loss [25,26].

1.6 Conclusion

The monthly female reproductive cycle involves a complex cascade of hormones to allow ovulation, fertilisation and potential implantation of a blastocyst. The multilayer structure of the endometrium is intrinsic to its cyclical function. Each month the superficial layers are primed for potential implantation and then, in the absence of pregnancy, shed and renewed to allow the cycle to commence again.

Many of the processes involved in this cycle are triggered directly by rhythmical hormonal fluctuations, relying on either hormonal stimulation or withdrawal to occur. The development of the ovum, ovulation and maturation of the endometrium are all examples. There are also some aspects that are indirectly related to hormone function through the flow-on effects of intermediate messengers. The premenstrual inflammatory tissue response and the initial repair of the endometrium are examples where tissue changes are indirectly influenced by hormones via the release of local chemical mediators.

The physiological principles behind the monthly reproductive cycle have long been understood. Yet increasing knowledge of the complexity of chemical pathways, including molecular and genetic involvement, has assisted in identifying possible contributors to infertility and early pregnancy loss. The result has been a greater understanding of potential areas for medical intervention.

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