Effect of Ovarian Stimulation on the Endometrium

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Abstract

The endometrium is a dynamic endocrine organ. Its role in implantation is the single most vital step in the management of infertility, yet it is least understood.

In this chapter, we offer an insight into the endometrium. We discuss its physiology and functions, its molecular dynamics, the hormonal interplay involved in the menstrual cycle and its role in conception. We also discuss the factors regulating endometrial receptivity, how it is affected by various hormones, how the natural hormonal interplay affects the window of implantation and what effect different stimulation protocols have on its structure and functions.

Keywords

Endometrium • Implantation • Receptivity • Stimulation protocols • GnRH agonists • Antagonists

21.1 Introduction

Implantation is the single most important step in the management of infertility and is also the most poorly understood part of reproduction. The endometrium is an active endocrine organ. It synthesizes and secretes lipids like prostaglandins and thromboxane, cytokines like interleukin and interferon and peptides like prolactin, growth factors, relaxin and renin. The interplay of all these substances is essential to create a receptive environment for the implanting blastocyst. This environment is labile and easily disturbed by exogenous as well as

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endogenous hormonal fluctuations. To understand these factors, we must first review the natural mechanisms of the endometrium and its functions.

21.2 Physiology of the Endometrium and Hormonal Interplay

The growth of the endometrium in every cycle is stimulated by the rising levels of oestrogen. There is increase in stromal thickness as well as increase in ciliated and micro-villous cells of the endometrium. Spiral arteries, the end arterial branches of the uterine arteries, are sensitive to hormonal changes. Glandular formation begins under the influence of oestrogen, in this phase. Glands become enlarged, filled with vacuoles and tortuous. Endometrial thickness increases. Oestrogen also stimulates VEGF synthesis, which helps in angiogenesis.

Epithelial proliferation stops 3 days after ovulation. This inhibition is brought about by the rising levels of progesterone that begin in the secretory phase. Tortuosity of glands increases, and there is intensive coiling of spiral arterioles in response to the progesterone. This heralds the beginning of the secretory phase. At the time of implantation, there is increased oedema of the endometrial stroma. Oestrogen and progesterone at this stage cause increase in production of prostaglandins, which leads to increased capillary permeability and thus increased stromal oedema.

Decidualization of the endometrium takes place around day 21–23 under the influence of progesterone. This decidualization helps to control the invasion of trophoblast after implantation. If there is no implantation, endometrial breakdown begins. Drop in the oestrogen and progesterone levels leads to withdrawal of support leading to vasomotor reactions that cause apoptosis, and subsequently tissue loss, which in turn leads to menstruation. At a cellular level, MMP (matrix metalloproteinase) secretion caused by progesterone withdrawal leads to cell membrane breakdown and dissolution of the cell membranes. MMP expression is suppressed postmenstrually by the rising oestradiol levels [1].

21.3 Role of Endometrium in Conception

The endometrium performs numerous functions to achieve conception [2]:

- Sperm transport from cervix to oviducts
- Nourishment of blastocyst
- Removal of zona pellucida from fertilized ovum
- Attachment and implantation of blastocyst

21.4 Endometrial Receptivity and Window of Implantation [WOI]

Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to implantation of the embryo. The endometrium is normally a non-receptive environment for an embryo. The window of implantation is the window of time when the uterine environment is conducive to blastocyst acceptance and subsequent implantation. Embryo transfer data from assisted-conception cycles suggests a window lasting approximately 4 days, from days 20–24 of a 28-day normal cycle [3].

21.5 Markers of Endometrial Receptivity

21.5.1 Pinopodes

The beginning of the WOI is heralded by the progesterone-induced formation of pinopodes. Pinopodes are bleb-like short irregular surface projections found on the apical surface of the endometrial epithelium [4]. They are usually seen between the 19th and the 21st day of the menstrual cycle and persist for 24–48 h. They are considered transient markers of endometrial receptivity [5]. Their formation is stimulated by rising levels of progesterone seen in the luteal phase. Administration of oestradiol leads to their

rapid loss, usually within 24 h. Thus, their detection during the mid-secretory phase is useful as a marker for endometrial receptivity. Blastocyst attachment has been shown to occur on top of pinopodes.

There are numerous other markers that help to define endometrial receptivity.

21.5.2 Biochemical Markers

Adhesion molecules: Mainly αvβ3 integrin appears in endometrial glands and luminal surface on cycle days 20 to 21 and is among the best-described markers of endometrial receptivity.

Anti-adhesion molecules: MUC-1 (mucin 1)
Cytokines: Leukaemia inhibitory factor (LIF)
Endometrial growth factors:
- Heparin-binding epidermal growth factor (HB-EGF)
- Insulin-like growth factor-binding protein-1 (IGFBP-1)
- Endometrial immune markers

21.5.3 Genetic Markers

Hoxa10 gene expression in the endometrium rises at the time of ovulation and has been shown to be essential for human implantation [6]. The uterine sensitization-associated gene-1 (USAG-1) is preferentially expressed in the maximal duration of endometrial receptivity [7]. Endometrial bleeding associated factor (EBAF) is found to be expressed in the late secretory and menstrual phase of the endometrium [8].

21.6 Hormonal Interplay

During stimulation, both gonadotropin (LH and FSH) and steroid hormone (oestrodiol and progesterone) levels vary. This may negatively or positively impact the endometrium according to the rise and timing of rise of these hormones.

21.6.1 Effect of Oestradiol on the Endometrium

A study by Basir et al. [9] in 2001 studied the effect of oestradiol in high and low responders in patients undergoing ovarian stimulation. They found that there was a much greater endometrial glandular volume in natural cycles as compared to stimulated cycles. The glands were more tortuous and numerous and occupied a greater area at the time of implantation.

In high responders, they observed a decline in glandular volume and an increase in the diameter of the glands, which was in direct proportion to the rise in oestradiol levels. This led to prolonged retention of glandular secretions and retarded emptying. This caused asynchronous secretory transformation of the endometrium due to reduced volume and insufficient secretions, leading to reduced endometrial receptivity. They also observed that stromal oedema was marked in such cases.

21.6.2 Effect of Progesterone

A study conducted by Bell et al. [10] has demonstrated the changes occurring in the endometrium due to the effects of progesterone. They divided the proteins that are secreted and synthesized by the endometrium into three groups, depending on their response to external stimulation.

Group 1: EP6, EP12
Group 2: EP 13, EP14, EP15
Group 3: EP 9, EP11

Of these three groups, group 2 is the one that is dependent on the histological endometrial type and is unaffected by short-term changes in levels of progesterone. According to the study, EP 14 and 15 are the two major proteins of pregnancy. EP 15 is associated with the decidua spongiosa region of the decidua parietalis during pregnancy and originates in the secretory glandular epithelium. During the menstrual cycle, it is said to be present in the uterine lumen and thus play a role in the implantation of the blastocyst. Whereas

EP14 is not secreted in significant amounts during the menstrual cycle, in pregnancy, it is the major secretory protein of the decidua compacta layer of the decidua parietalis. Both these proteins are mainly progesterone dependent because their synthesis depends on the stage of differentiation of the endometrium. The rate of synthesis of group 3 endometrial proteins is dependent on variations in the progesterone levels, independent of the stage of differentiation. The study suggests that its presence in the peripheral sera may be a way to examine the response of the endometrium to progesterone.

21.7 Effect of Various Stimulation Protocols

Controlled ovarian stimulation interrupts natural physiological processes. It affects the levels of oestrogen and progesterone, the timing of their expression and their ratios as well as the endometrial expression of their receptors. All this is likely to alter the extent and timing of endometrial receptivity [11]. COH also has a profound effect on endometrial gene expression. The pattern of expression depends upon the type of protocol used (agonist or antagonist). On a genomic level, implantation is affected due to dysregulation of genes in response to changing hormone levels, especially progesterone.

A study by Laberta et al. [12] showed that in patients of COH with high progesterone levels, there is dysregulation of 140 and 370 genes respectively (depending on the method used), which has an impact on the biological functions they represent, mainly cell adhesion, immune system and organ development. High progesterone also has a secondary impact on the E2 receptors and can lead to desensitization of the receptors to E2. The higher the E2 levels rise, the higher the progesterone levels appear to be, indicating a dependency on the number of follicles formed with COH. The study also noted that in cases with high progesterone, where the endometrium was out of phase on day 3, no pregnancies were obtained. However, if a day 5 transfer was done in these same patients, the pregnancy rates were decent. This gave credence to the theory that given enough time, the endometrium recovers from the effects of high steroid hormones.

21.7.1 Clomiphene Citrate and Endometrium

Clomiphene is an oestrogen receptor blocker that acts by competitively binding to oestrogen receptors [13]. It remains in the bound form for a longer duration than oestrogen and thus reduces the receptor concentrations. This in turn reduces the effect of the negative feedback mechanism on gonadotropin production. However, this competitive binding also leads to endometrial thinning in 15–50 % patients. The mechanism responsible for this is the ER down-regulation that leads to suppression of pinopode formation. It has been observed that this endometrial suppression is not dose dependent and recurred in repeat cycles in the same woman.

21.7.2 Letrozole and Endometrium

Letrozole has some advantages over clomiphene in its action over the endometrium. This aromatase inhibitor works on an enzymatic level, without affecting or blocking the oestrogen receptors. Thus, endometrial thickness remains unaltered. It was especially preferred in patients in which clomiphene caused endometrial thinning. However, this has now been banned by the FDA for use in ovulation induction.

21.7.3 Gonadotropins and Endometrium

A study conducted by Kolibianakis et al. in 2002 [14] studied the effects of gonadotropins on the endometrium in COH cycles. They found that endometrium advancement was noted at the time of oocyte pickup which was directly related to the level of LH at the initiation of treatment and the duration of FSH stimulation before addition of the antagonist. This is explained on the basis

of the two-cell–two-gonadotropin theory [14]: the higher LH level at the start of treatment leads to increased androgen production in the theca cells, which in turn will cause increased oestrogen production by the granulosa cells, due to FSH stimulation. The higher circulating oestrogen levels in turn cause earlier appearance of progesterone receptors in the endometrium, thus leading to endometrial advancement.

21.7.4 GnRH Agonists and Antagonists

The prolonged pituitary suppression caused by administration of agonists appears to affect the implantation window as well, causing it to shift forwards. A study by Hernandez [15] demonstrated that antagonist decreases the oestradiol production by the granulosa cells, which in turn affects endometrial development by affecting the mitosis of endometrial cells. In antagonist cycles, there is increased frequency of endometrial advancement, probably due to the fact that unlike agonist cycles, complete pituitary suppression does not occur in antagonist cycles, leading to a higher starting LH level, as described above, leading to increased oestradiol levels at an earlier stage [14]. Extreme endometrial advancement, of more than 3 days, is seen in higher frequency in antagonist cycles, leading to lower pregnancy rates in these cases. On a molecular level, a study by Rackow et al. [16] showed that HOXA 10 gene expression in endometrial stromal cells was impaired in antagonist cycles, as compared to agonists, thereby affecting endometrial receptivity.

As a counterview to this, a study by Saadat et al. [17] demonstrated that endometrial advancement takes place in all COH cycles, irrespective of the protocol used. They proved this both ultra-structurally, as well as by electron microscopy. According to them, the main cause appears to be due to increased progesterone levels leading to premature luteinization. A study by Simon et al. [18] has compared the standard step-up stimulation protocols with a step-down regimen. Their study was based on the theory that lower oestradiol levels during COH help to improve endometrial receptivity in patients undergoing IVF. They showed that oestradiol levels on the day of hCG trigger were significantly lower in the step-down regimes as compared to the step-up protocols. The implantation and pregnancy rates were also higher in these patients.

Early rise in progesterone levels is another factor seen in COH cycles. Rise in progesterone takes place especially in high responders, and although this does not affect oocyte quality, a level above 1.5 mg/dl [10] has a negative impact on endometrial receptivity, with precocious secretory endometrium formation and an out-of-phase endometrium on the day of implantation.

21.8 The Luteal Phase

Even though the agonist or antagonist treatment stops on the day of the hCG trigger, their effect on the suppression of endogenous LH continues, lasting for as long as 10 days after stopping stimulation [19]. Abnormally low LH levels may be insufficient to stimulate and maintain the corpus luteal function, leading to a luteal phase defect.

In high responders on antagonist protocol, an agonist trigger is often used to induce the LH surge for final oocyte maturation. Because of the agonist's longer-lasting action, LH insufficiency is common, leading to an out-of-phase endometrium, which is prematurely secretory in nature. This reduces implantation and pregnancy rates drastically. To bypass this, modified luteal support regimes have come into practice. Also, many clinicians prefer to vitrify the embryos formed and transfer them in a subsequent natural or hormone replacement cycle [20].

Conclusion

This chapter summarizes the physiology and functioning of the endometrium, the interplay of hormones that takes place on a day-to-day basis, how this differs when it is exposed to exogenous hormones and what can be done to optimize its functioning. This knowledge can help to improve pregnancy rates and increase the live birth rates in patients being treated for

infertility. Numerous tests and diagnostic methods are now being derived based on this, and treatment of the endometrial factor in infertility is now the wave of the future.

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