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The role of sex hormones in immune protection of the female reproductive tract

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Abstract

Within the human female reproductive tract (FRT), the challenge of protection against sexually transmitted infections (STIs) is coupled with the need to enable successful reproduction. Oestradiol and progesterone, which are secreted during the menstrual cycle, affect epithelial cells, fibroblasts and immune cells in the FRT to modify their functions and hence the individual's susceptibility to STIs in ways that are unique to specific sites in the FRT. The innate and adaptive immune systems are under hormonal control, and immune protection in the FRT varies with the phase of the menstrual cycle. Immune protection is dampened during the secretory phase of the cycle to optimize conditions for fertilization and pregnancy, which creates a 'window of vulnerability' during which potential pathogens can enter and infect the FRT.

Many challenges, including gynaecological cancers and sexually transmitted infections (STIs), threaten reproductive health by escaping the protection that is conferred by the mucosal immune system. In 2012, the worldwide incidences of ovarian cancer (239,000 cases), uterine cancer (320,000 cases) and cervical cancer (528,000 cases), the last of which is primarily caused by human papillomavirus (HPV) infection, were among the highest of all life-threatening diseases (see World Cancer Research Fund International — Data on Specific Cancers). The World Health Organization (WHO) estimates that in 2008 there were at least 498 million new cases of the more than 30 known STIs, including infection with *Trichomonas vaginalis* (276 million new cases), *Chlamydia trachomatis* (106 million new cases), *Treponema pallidum* (10 million new cases), HIV (2.7 million new cases) and *Neisseria gonorrhoeae* (106 million new cases); all of these infections can lead to reproductive failure and death[1]. Women are at a greater risk of STIs than men. Prevalence rates and total case numbers for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* infection are higher in women than in men[2]. In Sub-Saharan Africa, women account for two out of three new infections with HIV, and in the United States, genital herpes infects one in five women compared with one in ten men (see Genital Herpes — CDC Fact Sheet). Despite our growing understanding of the mucosal immune system in the female reproductive tract (FRT), much remains to be learnt about the underlying mechanisms that regulate susceptibility to STIs in the FRT.

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Competing interests statement

The authors declare no competing interests.

The mucosal immune system is the first line of defence against a complex range of viral, bacterial, fungal and parasitic pathogens. In common with other mucosal sites, the innate and adaptive (both cellular and humoral) elements of the mucosal immune system have evolved to meet the special challenges that are associated with the FRT. Unique among mucosal sites, the FRT has evolved to accept a semi-allogeneic fetus and to confer protection against potential pathogens. Important to this balance is the regulation of the FRT immune system by the sex hormones oestradiol (OE_2) and progesterone (P4). The FRT can be divided into a lower tract (vagina and ectocervix) and an upper tract (endocervix, uterus and Fallopian tubes) (FIG. 1). Each compartment has distinct reproductive responsibilities (sperm entry, ovum movement, nutrition or preparation for implantation) that coincide with distinct phases of the menstrual cycle. Sex hormones coordinate unique patterns of epithelial cell, stromal fibroblast and immune cell function, which optimize conditions for both maternal protection and fetal survival.

This Review focuses on current knowledge regarding the sentinel role of the mucosal immune system in the FRT, with a special emphasis on the interface between the immune system and the endocrine system. We describe the immune changes that occur *in vivo* during the menstrual cycle, as well as those that occur *in vitro* after treatment with sex hormones. As a result of the complexity of immune regulation in the human FRT, it is beyond the scope of this Review to examine the immune changes that occur during adolescence, pregnancy or menopause, or that are associated with sexual assault or gynaecological disorders. In the following sections, we define the changes in hormone levels that occur during the menstrual cycle, identify the cells responsible for innate and adaptive immune protection in the reproductive tract and focus on the role of sex hormones (particularly OE_2 and P4) in regulating epithelial, fibroblast and immune cell phenotype and function. Special emphasis is given to our limited, but growing, knowledge of the site-specific immune responses in the upper and lower FRT and how each cell type contributes — through the secretion of growth factors, cytokines and chemokines — to a tissue environment that maintains immune protection and reproductive potential. Finally, we discuss the concept of a ‘window of vulnerability’ in the menstrual cycle during which immune regulation, as a result of changes in hormone levels, optimizes conditions for fertilization and implantation but places women at increased risk of acquiring STIs[3].

Endocrine control of the menstrual cycle

The hypothalamic–pituitary axis regulates the cyclic secretion of OE_2 and P4 by the ovary during the menstrual cycle in women of reproductive age. In response to these hormones, changes take place throughout the reproductive tract in preparation for egg production, potential fertilization, implantation in the uterus and pregnancy. The menstrual cycle is divided into four stages: the menstrual phase, the proliferative phase (also known as the follicular phase), mid-cycle (during which ovulation occurs) and the secretory phase (also known as the luteal phase) (BOX 1; FIG. 2). The length of a menstrual cycle varies greatly among women (21–35 days), with 28 days used as the average length. At the beginning of the menstrual cycle (day 0), follicle-stimulating hormone (FSH) stimulates ovarian thecal and granulosa cells in the developing follicle to produce OE_2. Under the control of the hypothalamic–pituitary axis — through FSH and luteinizing hormone — OE_2 levels

increase during the proliferative phase to peak just before ovulation occurs at mid-cycle. This is followed by a surge in luteinizing hormone that is essential for ovulation, which occurs 24–36 hours after OE_2 levels peak in the blood. Following ovulation, the corpus luteum develops to become the primary source of OE_2 and P4 during the secretory phase. The concentrations of P4 and, to a lesser extent, OE_2 increase and peak at the mid-secretory phase. In the absence of fertilization, the corpus luteum degrades (in a process known as luteolysis), resulting in a decrease in OE_2 and P4 levels, which leads to endometrial shedding and the onset of menses[4]. As discussed below, these changes in hormone levels have a marked effect on the immune system in the FRT, and we propose that they lead to a window of vulnerability during which optimal conditions for fertilization and implantation increase an individual's susceptibility to STIs (FIG. 2).

Immune protection of the FRT

Cell types

The main cell types in the FRT that have immune capabilities are epithelial cells, stromal fibroblasts and leukocytes. Epithelial cells line the surface of the FRT, providing a barrier that separates the lumen from the underlying tissue (FIG. 1). Multi-layered squamous epithelial cells cover the lower FRT (vagina and ectocervix), whereas single-layer columnar epithelial cells cover the upper FRT (endocervix, uterus and Fallopian tubes). Beneath the epithelium is a dense layer of stromal fibroblasts, which provides structural tissue support. Distributed throughout the stroma is a dynamic population of leukocytes. These account for 6–20% of total cells in the human FRT, with more leukocytes being present in the upper tract than in the lower portions of the tract[5]. Most leukocyte subsets have a preferential distribution within the different sites in the FRT; for example, T cells ($CD3^+$), which are the most abundant leukocyte subset in the FRT, have higher proportions in the lower than in the upper tract, whereas granulocytes ($CD66b^+$) and natural killer (NK) cells are more abundant in the upper tract than in the lower tract (FIG. 3).

Pattern recognition receptors

Pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs), are essential for the initial detection and response to pathogens as they recognize conserved pathogen-associated molecular patterns (PAMPs). For example, TLR7 and RIG-I recognize HIV, whereas TLR2 and TLR4 recognize *C. trachomatis* and TLR2 recognizes *N. gonorrhoeae*[6–11]. PRR expression varies within the FRT[12]. Expression of the bacterial receptors TLR2, TLR4, nucleotide-binding oligomerization domain 1 (NOD1) and NOD2 is highest in the upper FRT and declines in the lower FRT, which suggests that the lower FRT might minimize responses against commensal bacteria, whereas the upper tract is very sensitive to bacterial pathogens[13,14]. A similar trend is seen for the cytoplasmic PRRs RIG-I and melanoma differentiation-associated protein 5 (MDA5; also known as IFIH1)[14]. By contrast, TLR7, TLR8 and TLR9 are evenly expressed throughout the FRT from the Fallopian tubes to the ectocervix, which suggests that immune recognition of viruses is fairly constant between the upper and the lower FRT[15].

Similar to other aspects of immune protection, PRR expression changes across the menstrual cycle and with hormone exposure. TLR2, TLR6, TLR9 and TLR10 expression is lower in human endometrial tissue recovered at the proliferative phase than in that recovered at the secretory phase[16–19]. OE_2 decreases *TLR4* mRNA expression by uterine fibroblasts and decreases TLR2 and TLR6 expression by the VK2 vaginal epithelial cell line *in vitro*, but it has no effect on the expression of other PRRs[20]. P4 increases TLR4 expression by fibroblasts, which suggests that these cells are more sensitive to bacterial pathogens in the secretory phase[17]. However, it has not been directly shown that the levels of PRR expression correlate with protection against pathogens in the FRT.

OE_2 also modulates the signalling pathways downstream of PRRs and pro-inflammatory receptors. It inhibits the lipopolysaccharide (LPS)- and polyinosinic–polycytidylic acid (poly(I:C))-induced secretion of macrophage migration inhibitory factor (MIF), interleukin-6 (IL-6) and IL-8 by uterine epithelial cells and reverses the stimulatory effects of IL-1β on mRNA and protein expression of tumour necrosis factor (TNF), human β-defensin 2 (HBD2), IL-8 and nuclear factor-κB (NF-κB). This suggests that inflammatory responses to pathogens are decreased during periods of high OE_2 levels in the menstrual cycle[21,22]. OE_2 regulates the function of NF-κB, which is a key transcription factor involved in inflammatory gene expression, by restricting its cytoplasmic-to-nuclear translocation or by preventing the degradation of NF-κB inhibitors[23,24]. Furthermore, as secretory leukocyte protease inhibitor (SLPI; also known as antileukoproteinase) inhibits NF-κB expression, OE_2-mediated inhibition of pro-inflammatory cytokine expression may be mediated through the regulation of NF-κB by OE_2-induced SLPI[25]. Thus, OE_2 may reduce susceptibility to HIV infection in the FRT by creating an anti-inflammatory environment that is characterized by reduced target cell migration as a result of the decreased secretion of inflammatory cytokines, as well as by eliminating the immune-activated environment that is often associated with infections.

Secreted molecules

The lumen of the entire FRT is bathed in fluid, the composition of which differs both between the upper and the lower tract and across the menstrual cycle, and which represents the combined secretions of the different cell types in the FRT. Contained within the fluid are various immunomodulatory molecules including cytokines, chemokines, antimicrobial proteins, enzymes and growth factors. In cervico-vaginal lavage fluid (CVL fluid), the concentrations of antimicrobial proteins such as SLPI, HBD2, human neutrophil peptide 1 (HNP1; also known as neutrophil defensin 1), HNP2, HNP3, lysozyme, lactoferrin and surfactant A markedly decrease by mid-cycle (day 13) and remain low for 7–10 days during the secretory phase before returning to the higher levels found during the proliferative phase following menstruation[26,27]. These findings suggest that the antimicrobial contribution of the luminal fluid to overall immune protection in the FRT decreases during the secretory phase. Interestingly, total protein and transforming growth factor-β (TGFβ) levels remain unchanged throughout the cycle, which shows the selectivity of hormone effects. However, other studies have found no changes in secreted levels of various proteins at mid-cycle, possibly as a result of cycle length variation (BOX 1) and differences in sampling technique[28–32]. By contrast, IL-6 and IL-1β levels increase during the proliferative phase of

the cycle, which shows that concentration changes are specific to certain molecules at specific phases of the cycle[29].

Secretions from the upper FRT have a distinct proteomic profile compared with those from the lower FRT, with IL-1β, IL-6, IL-10, IL-18, CC-chemokine ligand 2 (CCL2; also known as MCP1) and vascular endothelial growth factor (VEGF) levels being markedly higher, and IL-12, IL-15 and MIF levels being markedly lower, in cervical secretions compared with in endometrial secretions[33]. This is probably representative of the unique functions of different FRT compartments — the upper FRT maintains a sterile environment, whereas the lower FRT hosts a population of commensal bacteria. Many of the proteins that are differentially expressed between the upper and the lower FRT and across the menstrual cycle, such as CCL2, IL-6 and IL-1β, are involved in immune cell trafficking and phenotype development. Thus, differences in the levels of specific proteins may account for variations in immune cell populations across the FRT.

Endocrine control of epithelial cells

Barrier function

Epithelial cells provide a protective barrier in the FRT that is responsive to hormonal and pathogenic stimuli. OE_2 increases the proliferation of epithelial cells in both the uterus and the vagina[34,35]. High levels of P4 are associated with thinning of the vaginal epithelium in animal models, although this has not been observed in humans[36]. Epithelial cells are linked by tight junction proteins, which regulate the movement of molecules across the epithelium. In the stratified squamous epithelium of the lower FRT, tight junctions are primarily present between basal epithelial cells[37] (FIG. 1). Their absence in the superficial epithelium results in weakly joined cells and may allow pathogens such as HIV to penetrate the epithelial layer, bringing them into proximity with immune cells in the basal epithelium and in the lamina propria. By contrast, the columnar epithelium in the upper FRT has strong networks of tight junctions. OE_2 modulates the expression of claudin and occludin proteins, which leads to a relaxation of tight junctions and greater flux across the epithelium[38,39]. The functional implications of this are unclear but may involve the movement of proteins across the epithelium as part of normal homeostasis in preparation for potential implantation or as part of the clearance of pathogens in immune defence. Whether alterations in barrier permeability throughout the menstrual cycle are associated with the increased movement of pathogens into the subepithelial tissue is unknown. However, pathogens and inflammatory conditions degrade tight junction integrity and thus barrier function, which leads to greater flux across the epithelium[40]. Therefore, a combination of high OE_2 levels and inflammation may degrade barrier function in the upper FRT and may increase susceptibility to infection.

Mucus production

Endocervical epithelial cells secrete negatively charged high-molecular-weight glycoproteins known as mucins, which are a major component of mucus and which trap pathogens and prevent their access to the epithelium. Mucin gene expression varies with menstrual status, which leads to changes in the overall properties of mucus[41,42]. Oestrogenic mucus is thin and watery with a low viscosity, which facilitates sperm movement into the

upper FRT. It is present during the proliferative phase and increases at ovulation. By contrast, progestational mucus is thick, viscous and present following ovulation and during the secretory phase, when it functions to impede the movement of material from the lower FRT into the upper FRT[43]. Mucus protects epithelial cells from direct contact with pathogens such as HIV. Cervico-vaginal mucus has recently been shown to interact with IgG antibodies to impede HIV mobility and thereby to enhance mucosal barrier function, but it is not known whether this property changes during the menstrual cycle[44,45].

Cytokines, chemokines and antimicrobial proteins

Epithelial cell secretion of cytokines, chemokines and antimicrobial proteins varies with location in the FRT and hormone exposure[46]. For example, OE_2 but not P4 suppresses the secretion of the antimicrobial proteins HBD2 and elafin (also known as peptidase inhibitor 3) by vaginal squamous epithelial cells[32,47]. By contrast, OE_2 increases uterine epithelial cell secretion of SLPI and HBD2, with preferential secretion towards the lumen from where incoming pathogens would meet the mucosal surface[22]. This may enable the FRT to host commensal bacteria in the lower tract and to maintain protection against infection in the upper tract, despite being exposed to potential pathogens throughout the menstrual cycle[148]. In addition, capacitated human sperm show directional movement towards CCL20 and HBD2 in chemoattractant assays as a result of their expression of CC-chemokine receptor 6 (CCR6)[48]. The fact that uterine but not vaginal epithelial cells secrete CCL20, at a time when HBD2 is suppressed in the lower FRT, provides a gradient that promotes sperm chemotaxis under non-inflammatory conditions towards the upper FRT, in which fertilization occurs.

Studies using vaginal epithelial cell lines show that OE_2 inhibits the expression of mRNA encoding the inflammatory proteins IL-1α and TNF, which suggests that inflammation and the resulting influx of immune cells to the epithelial surface are repressed before ovulation at the time that is most amenable for semen entry[20]. In other studies, apical secretions from uterine epithelial cells from premenopausal women, but not from postmenopausal women, had antibacterial activity against both Gram-positive and Gram-negative bacteria, and this was dependent on SLPI[49]. This may lead to decreased protection against bacterial pathogens in postmenopausal women as a result of the absence of OE_2.

Type I interferons (IFNs) mediate the antiviral response through their regulation of IFN-stimulated genes (ISGs). In the FRT, OE_2 regulates the expression of IFNε across the menstrual cycle but not of other type I IFNs such as IFNα and IFNβ[50,51]. Furthermore, whereas ISG expression by uterine epithelial cells in response to IFNβ is not affected by OE_2, OE_2 does reduce ISG levels in response to type III IFNs (IFN-λ1, IFN-λ2 and IFN-λ3)[50]. Together, these studies show the specificity of hormone action on epithelial cells and genes in the FRT, demonstrating their crucial role in homeostasis and antiviral defence in the FRT.

Endocrine control of stromal fibroblasts

Fibroblasts are essential structural components of the FRT, but their role in immune protection is poorly understood. Studies have shown that fibroblasts from other mucosal

surfaces are involved both in the recognition of pathogens and in the recruitment of immune cells to sites of infection[52]. Similarly to epithelial cells, fibroblasts have site-specific differences in their responses to sex hormones[53]. For example, during the secretory phase of the menstrual cycle under the influence of P4, and independently of the presence of a blastocyst in the uterine cavity, uterine fibroblasts undergo marked phenotypic changes known as decidualization in preparation for implantation. This response is not observed in fibroblasts from the cervix and the Fallopian tubes. OE_2 increases the secretion of hepatocyte growth factor (HGF) and CXC-chemokine ligand 12 (CXCL12; also known as SDF1α) and decreases CCL2 secretion from uterine fibroblasts but not from fibroblasts of the endocervix or ectocervix, which may account for differential recruitment of immune cells into the endometrium at different phases of the menstrual cycle[53,54]. Although both vaginal and uterine fibroblasts proliferate in response to OE_2 treatment *in vitro*, vaginal fibroblasts respond at OE_2 concentrations that are approximately 1,000-fold lower than those required for uterine fibroblasts to respond, which suggests that vaginal fibroblasts are more sensitive to the presence of OE_2 (REF. 55).

Fibroblasts can respond to pathogens that have breached the epithelial barrier by alerting immune cells and recruiting them to sites of infection. For example, uterine fibroblasts respond to the TLR3 and TLR4 ligands poly(I:C) and LPS by secreting cytokines such as TNF, IL-8, CCL2, CCL5 (also known as RANTES), CCL20 and HGF. OE_2 potentiates HGF secretion by fibroblasts in response to poly(I:C), which suggests that the intensity of the fibroblast immune response to viral pathogens varies with the stage of the menstrual cycle[53]. Secretions from uterine fibroblasts inhibit CCR5-tropic HIV infection of TZM-bl cells, whereas OE_2 pre-treatment increases antiviral activity against CXC-chemokine receptor 4 (CXCR4)-tropic HIV, which shows the potential importance of endocrine regulation of fibroblasts in protecting HIV-target cells in the FRT[54].

Endocrine control of immune cells

Immune cells in the FRT are regulated by sex hormones throughout the menstrual cycle to maintain the equilibrium between effectively fighting infection and the immune regulation and tissue remodelling that is required for successful implantation and pregnancy[3]. Immune cell number, distribution and function are tightly modulated throughout the menstrual cycle to achieve these goals (FIG. 3). The result is the migration and differentiation of unique immune cell phenotypes throughout the FRT, which are different from those of immune cells at other mucosal sites in the body and in peripheral blood.

Cell numbers, tissue distribution and trafficking

The proliferative phase of the menstrual cycle is characterized by the regeneration of the endometrial tissue. During this period, which is dominated by OE_2, angiogenesis occurs, as well as glandular epithelial cell and stromal fibroblast growth[56]. On the basis of multiple studies, immune cell numbers in the endometrium are known to increase during the late secretory phase and during menstruation[5,56–58]. By contrast, in the lower FRT, sex hormone fluctuations do not alter immune cell numbers, which remain constant throughout the cycle[59]. Around the time of ovulation, the peak in angiogenesis facilitates recruitment into the uterus of leukocytes, including NK cells, neutrophils and macrophages, which are

necessary should pregnancy occur. Cell recruitment is mediated through the cytokines, chemokines and growth factors that accumulate in the vicinity of the uterine blood vessels, such as CCL4 (also known as MIP1β), CCL14, CCL16 and CCL21, which are mainly produced by epithelial and stromal fibroblasts under the influence of sex hormones[56].

T cells constitute around 40–50% of leukocytes in the FRT[5,60–63]. During the proliferative phase, most T cells in the uterus are found as scattered T cells and small aggregates in the stroma or as intraepithelial lymphocytes[64,65]. During this time, uterine $CD8^+$ T cell numbers remain constant but undergo a uterine site-specific condensation in the lamina basalis, which results in the formation of lymphoid aggregates. Lymphoid aggregates consist of a B cell core surrounded by memory $CD8^+$ T cells and encapsulated by macrophages; they peak in size at mid-cycle and persist during the secretory phase[64]. In the absence of infection, lymphoid aggregates are found in the endometrium but not in the endocervix or lower FRT. Aggregate formation may be a mechanism to maintain the T cell repertoire and to prevent the loss of resident memory T cells during menstrual shedding. In the lower FRT, clusters of cells form in the vagina and cervix in response to herpes simplex virus 2 (HSV2) infection[66]. These clusters contain memory $CD4^+$ or $CD8^+$ T cells, B cells, dendritic cells (DCs) and macrophages, and may persist for months or years after viral clearance. Whether they are regulated by sex hormones is unknown. These cell clusters probably provide protection against secondary infections but, at the same time, may be a locus of increased susceptibility for other infections such as HIV[66,67].

Macrophages represent about 10–20% of the FRT leukocytes[5,63,64]. In the uterine endometrium, $CD68^+$ macrophages are found directly below the luminal epithelium and in the subepithelial stroma, as well as in clusters in the lamina basalis adjacent to the glandular epithelium[65]. Macrophages are more abundant in the endometrial stroma[68] than in the endocervix or ectocervix, and their numbers remain stable during the proliferative phase[69].

In the upper FRT, $CD1a^+$ and $CD11c^+$ DCs are located within the luminal epithelium, and $CD123^+$ plasmacytoid DCs are present in the stroma[65]. The functionalis layer and basalis layer of the endometrium contain $CD1a^+$ DCs and fewer numbers of $CD83^+$ mature DCs. Whereas numbers of $CD1a^+$ DCs remain constant and are similar in both layers during the proliferative phase, $CD83^+$ DCs are more abundant in the basal layer[70]. In the lower FRT, DCs are found mostly within the epithelium[71].

Uterine NK cells, which represent approximately 30% of leukocytes during the implantation window, increase in number during the secretory phase accompanying decidualization of the endometrium[56,72,73]. It is unclear whether uterine NK cell numbers increase as a result of the selective recruitment of $CD56^{hi}CD16^-$ NK cells from peripheral blood or as a result of *in situ* proliferation[56,72,73]. Chemokines and cytokines such as CXCL10, CXCL11 (REF. 74) and IL-15, the levels of which are regulated by sex hormones, selectively recruit NK cells. In addition, IL-15 can locally increase uterine NK cell proliferation[56].

P4 withdrawal initiates menstruation, which triggers an inflammatory response in the endometrium[58]. Chemokine, cytokine and growth factor secretion by the endometrial epithelium and stroma regulates the influx of leukocytes that mediate tissue breakdown and

repair. Neutrophils, NK cells, macrophages and smaller numbers of eosinophils and $CD1a^+$ DCs migrate into the uterus in response to hormonal changes[58,70]. The number of $CD68^+$ macrophages is increased during menstruation, particularly in the mid-menstrual phase (days 3–4), decreases towards the end of menstruation and remains stable throughout the proliferative phase[69].

Immune function and phenotype

Successful implantation is associated with immune cell regulation, in which an inflammatory response that attracts innate immune cell subsets specialized in tissue remodelling is integrated within a tolerogenic environment that prevents T cell-mediated allograft rejection[75,76]. Immune function is hormonally controlled in a site-specific manner, through growth factors, cytokines and chemokines that are present in the local tissue environment. In the lower FRT, $CD4^+$ and $CD8^+$ T cells are equally abundant, whereas in the endometrium, $CD8^+$ T cells predominate[60,61]. The increased presence of $CD4^+$ T cells in the lower tract suggests greater susceptibility to HIV infection at this site. In addition, $CD8^+$ cytotoxic T lymphocyte (CTL) activity is suppressed during the secretory phase of the cycle in the endometrium, presumably to minimize the recognition and the rejection of allogeneic sperm and the semi-allogeneic fetus. By contrast, CTL activity is maintained in the lower FRT, offering constant protection against potential incoming pathogens[77]. Interestingly, lymphoid aggregate formation correlates with the loss of CTL activity, in that lymphoid aggregates reach maximal size during the secretory phase of the cycle when CTL activity is suppressed[64,77]. These cell aggregates might therefore be a mechanism to prevent T cell-mediated rejection of the semi-allogeneic fetus.

Regulatory T (T_{Reg}) cell subsets are also hormonally regulated. In the endometrium, forkhead box P3 $(FOXP3)^+$ T_{Reg} cell numbers increase throughout the proliferative phase and then decrease at the beginning of the secretory phase[78]. In peripheral blood, the number of $CD4^+CD25^+FOXP3^+$ T_{Reg} cells follows the same pattern during the menstrual cycle[79]. Interestingly, the decrease in T_{Reg} cell number during the secretory phase does not occur in pathological circumstances such as in recurrent spontaneous abortions or in endometriosis, which indicates that the increased number of T_{Reg} cells before ovulation may be necessary to induce immune tolerance for successful implantation and for tissue breakdown and repair[78,79]. Furthermore, these findings indicate that CTL suppression during the secretory phase is not mediated by T_{Reg} cells.

We recently reported a decreased number of $CD4^+$ T helper 17 (T_H17) cells in the endometrium compared with the cervix from premenopausal women[61]. T_H17 cells are involved in host defence against extracellular bacteria and fungi, and their increased number in peripheral blood has been linked to recurrent pregnancy loss[80]. Experiments using ovariectomized mice show that OE_2 deficiency induces T_H17 cell differentiation[81]. Furthermore, mouse models have shown the induction of T_H17 cell responses by sperm antigens, and that this response is inhibited by OE_2 at oestrus[82]. Although variations in T_H17 cell number in the FRT throughout the menstrual cycle were not addressed in our study[61], we found decreased numbers of T_H17 cells in the endometrium from premenopausal women compared with postmenopausal women. Decreasing the number of

T_H17 cells in the endometrium, which is possibly mediated by sex hormones, may be necessary for successful fertilization and implantation, whereas higher T_H17 cell numbers are required in the lower FRT to prevent bacterial and fungal infections. As T_H17 cells are susceptible to HIV infection, their presence places the lower FRT at greater risk of HIV infection than the endometrium.

Whereas B cells are a minor cell population in all FRT tissues, IgG- and IgA-producing plasma cells are predominantly found in the cervix and, to a lesser extent, the vagina[83]. In FRT secretions, IgG is partly locally produced and partly derived from the circulation. Cervico-vaginal secretions are characterized by greater amounts of IgG than IgA. Interestingly, in both humans and rodents, uterine and cervico-vaginal levels of IgA and IgG are hormonally regulated[83]. Despite the low numbers of IgA-producing plasma cells in the endometrium[84–86], levels of stromal IgA and IgG increase during ovulation[87,88]. By contrast, in cervical secretions, both IgG and IgA levels are lowest at the mid-secretory phase of the menstrual cycle[89,90]. Suppression of IgG and IgA levels at mid-cycle in the lower FRT is thought to reduce the levels of sperm-specific antibodies, which would otherwise contribute to infertility. As discussed elsewhere, immunoglobulin changes during the menstrual cycle at each site are probably the result of endocrine regulation of receptors for IgA (the polymeric IgA receptor (pIgR)) and IgG (the neonatal Fc receptor (FcRn)) in epithelial cells, of plasma cell synthesis and of transudation of immunoglobulins from blood into FRT tissues[91,92]. In humans, pIgR production by uterine epithelial cells varies with the stage of the menstrual cycle, with the greatest quantities being produced during the secretory phase[93]. By contrast, pIgR production in vaginal epithelium is minimal[94]. In rodents, OE_2 stimulates the production of secretory component (the external domain of pIgR) by uterine epithelial cells but inhibits production by vaginal epithelial cells[95]. Overall, these findings suggest that antibodies secreted into the upper tract contribute to the removal of potential pathogens by inhibiting cell entry and/or by neutralizing the biological activity of a pathogen. Antibody binding further mediates pathogen removal through phagocytosis by macrophages or through the complement system. The lack of immunoglobulin-producing cells in the upper FRT suggests that antibodies of the upper tract are less likely to be of local origin and that they are possibly derived from the gut. Evidence for a gut origin comes from studies of IgA^+ cell traffic from the gut via the mesenteric lymph nodes to the blood and into mucosal sites[96–99]. Interestingly, differences between species have been reported; for example, immunoglobulin-secreting plasma cells are found in the mouse uterus but not in the human uterus[84–86,100]. An explanation for this difference is that, whereas semen is deposited into the vagina of humans, it is placed directly into the uterus of mice, where it possibly elicits a more pronounced inflammatory response than that seen in the human uterus (reviewed in REF. 92).

NK cells in the FRT express CD9 and have distinct site-specific phenotypes[73]. Ectocervical and vaginal NK cells are $CD56^+CD16^+$ but lack expression of CD94 and CD69, which is similar to the phenotype of $CD56^{low}CD16^+$ cytotoxic NK cells in the blood[72,73]. By contrast, NK cells of the upper FRT are $CD56^+CD16^-$ $CD94^+CD69^+$ and express the activating receptors natural killer group 2 member D (NKG2D) and NKp30 (also known as NCR3), but not NKp44 (also known as NCR2) or NKp46 (also known as NCR1), which differentiates these cells from the decidual NK cells that are found during pregnancy[72].

Endometrial NK cells have low levels of cytotoxic activity, cytokine secretion and pro-angiogenic factor production. Decidual NK cells and endometrial NK cells are different cell subsets, and it is unclear whether endometrial NK cells have tissue-remodelling functions (similarly to decidual NK cells) or whether they are inactive cells awaiting pregnancy[72,101,102]. Intracellular expression of IFNγ by uterine NK cells is suppressed by epithelial cell production of TGFβ[103], and NK cell cytotoxicity and perforin production are inhibited by P4 (REF. 58). Thus, the cytolytic activity of $CD8^+$ T cells and of uterine NK cells is suppressed in the endometrium during the secretory phase of the menstrual cycle.

Additional innate lymphoid cell (ILC) subsets other than NK cells have recently been described in human decidua, but their presence in the FRT of non-pregnant subjects has not been investigated[104]. Considering the central role of ILCs in tissue remodelling, antimicrobial responses and maintenance of epithelial barrier integrity (reviewed in REF. 105), their characterization, function and hormonal regulation in the FRT is an important area for future studies.

Further indication of immune cells adopting a specialized cell phenotype in the endometrium comes from the high proportion of $CD163^+CD14^{low}$ macrophages, which are known as alternatively activated macrophages[106]. These cells are distinct from those in the cervix and vagina, which express high levels of CD14 (REFS 63,107). Macrophages sustain HIV infection in the lower and upper tract[107,108]. By contrast, $CD4^+$ T cells in the endometrium are poorly susceptible to HIV infection[61], which suggests that macrophages may be the main HIV-target cell in the upper FRT rather than $CD4^+$ T cells.

Endocrine control of the mucosal environment

It is important to view the immune system of the FRT not as a set of isolated cell types but rather as part of a mutually interdependent network. The multidirectional interactions between epithelial cells, fibroblasts and immune cells are essential for maintaining reproductive health and immune protection. Epithelial cell interactions with underlying stromal fibroblasts and immune cells are essential in facilitating sex hormone-induced changes (FIG. 4). Epithelial cells contribute to the tissue environment by basolaterally secreting a range of growth factors and cytokines in response to OE_2, including TGFβ, granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), IL-4, IL-6, TNF, IL-8 and IL-10 (REFS 109–111). Uterine fibroblasts respond to OE_2 by secreting paracrine factors such as HGF that mediate hormone effects on epithelial cell growth and differentiation, as well as by increasing blood supply to the endometrium[112,113]. Fibroblasts throughout the FRT secrete CCL2 and IL-8, and there is some evidence that their secretion is partially controlled by OE_2 (REFS 114,115). Cytokines and chemokines produced by immune cells, such as TNF, CCL5, fibroblast growth factor 2 (FGF2) and GM-CSF, also affect other immune cells as well as fibroblasts and epithelial cells[106]. These findings indicate that epithelial cells, fibroblasts and immune cells in the FRT are under hormonal control to create an optimal tissue environment at the time of implantation (the secretory phase of the cycle) to enable successful reproduction.

At the same time, secretions in the tissue environment function to modulate immune protection. For example, epithelial cell secretions have marked effects on immune cell phenotype, such as conferring a more tolerogenic phenotype on DCs[116,117]. TGFβ is a particularly potent immunomodulator that is responsible for the downregulation of expression of DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN; also known as CD209) on immature DCs, which reduces HIV transinfection to target cells[116,117]. CCL20 directly inactivates HIV but also attracts $CCR6^+$ cells to the mucosal surface, some of which are uniquely susceptible to HIV infection[118,119]. For example, $CD4^+CCR6^+$ T cells express the HIV co-receptor CCR5 and have increased susceptibility to *in vitro* HIV infection compared with $CD4^+CCR6^-$ T cells[61]. Thus, CCL20 can function as both an inhibitor and a promoter of HIV infection. Other antimicrobial proteins can also modulate intracellular cell signalling pathways in addition to their HIV-specific effects. For example, elafin and SLPI, secretion of which by uterine epithelial cells is increased by OE_2, dampen the inflammatory response to TLR3 and TLR4 ligands by decreasing TLR expression and/or by reducing NF-κB activation and nuclear translocation[25,120]. HBD2, another antimicrobial factor that is regulated by OE_2, functions as a ligand for TLR4 in immature DCs, leading to DC maturation[121]. Thus, OE_2 can potentially increase antiviral activity in secretions, can induce signalling in immune cells and can alter the response to PRR ligands, as well as regulate the influx of immune cells through a restrained inflammatory response.

Endocrine control of a window of vulnerability

At the time of fertilization, during the secretory phase of the menstrual cycle, the FRT must distinguish between a semi-allogeneic fetal placental unit and potential pathogens that are dispersed throughout the FRT during copulation[3]. In preparation for implantation, potential pathogens within the FRT are removed or inactivated, but specific aspects of the innate and adaptive immune responses are regulated to prevent rejection of the fetus. Without these essential conditions being met, successful fertilization, implantation and pregnancy are unlikely to occur. On the basis of our studies and those of others, we proposed that such regulated immune activity results in increased susceptibility to STIs, including HIV[3]. By examining multiple immunological parameters in the lower and upper FRT (FIG. 5), we hypothesized that, during the secretory phase of the menstrual cycle, there is a period lasting 7–10 days that overlaps with the time of implantation, during which important components of innate, humoral and cell-mediated immunity are regulated by OE_2 and P4 in a manner that limits the response to STIs[3].

Various studies have supported the concept of a ‘window’ for HIV infection. Repeated vaginal exposure of pigtail macaques to low doses of simian–human immunodeficiency virus (SHIV) during normal menstrual cycles[122,123] showed that the majority of macaques first showed signs of viraemia in the proliferative phase. Taking into account a viral eclipse phase of 7–14 days before viraemia could be detected, these studies estimated a window of most frequent virus transmission between days 24 and 31 of the menstrual cycle (the late secretory phase). In other studies, *ex vivo* incubation of human cervical explants with HIV showed that productive infection does not occur in tissues from the proliferative phase of the menstrual cycle (which are OE_2 dominated) but only in tissues from the secretory phase[124].

In vitro studies indicate that OE_2 reduces susceptibility to HIV infection in $CD4^+$ T cells and macrophages[125–127]. Although experimental models indicate that OE_2 reduces susceptibility to HIV infection, definitive evidence that OE_2 prevents HIV acquisition in women remains to be shown.

Several studies indicate that the window of vulnerability may exist for other STIs. For example, gonococcal pelvic infection and chlamydial pelvic inflammatory disease are more likely to occur just before or at the time of menstruation[128]. Mice are most susceptible to *N. gonorrhoeae* when OE_2 levels are rising, and treatment with OE_2 increases *T. vaginalis* or *C. albicans* infection, whereas P4 promotes *C. tracho-matis* infection of the lower FRT[129–131]. Furthermore, OE_2 increases the attachment of *C. trachomatis*, *T. vaginalis* or *N. gonorrhoeae* to epithelial cells, which is an important factor for establishing infection[132]. OE_2 treatment of ovariectomized mice protects them from HSV2 infection with no demonstrable vaginal pathology or viral shedding[133]. By contrast, P4 treatment of ovariectomized mice makes them highly susceptible to HSV2 infection, with marked pathology, high viral titres in vaginal secretions and persistent inflammation and neutrophil infiltration. This is similar to results observed in non-human primates in which pre-treatment with P4 leads to higher levels of SIV infection[36]. Studies showing different patterns in the hormonal regulation of susceptibility to infection probably reflect variations between animal models, as well as the pathogen target of infection and immune responses to each pathogen. Interestingly, recent studies suggest that immune protection during the proliferative phase of the cycle can be compromised by STI co-infection (BOX 2). Although the exact endocrine conditions responsible for successful infection vary with animal models and cells studied, it is evident that the window of vulnerability provides a useful concept from which to examine the range of pathogens that compromise reproductive health and the lives of women worldwide.

Conclusions

The complexity of immune protection in the FRT requires an understanding of reproductive function and its control by an endocrine system that supports fertilization, implantation and pregnancy. The FRT consists of distinct anatomical sites (Fallopian tubes, uterus, endocervix, ectocervix and vagina) that function separately but in a coordinated manner under the influence of OE_2 and P4. Immune protection throughout the FRT is also precisely regulated by OE_2 and P4. The net result is integrated immune protection that complements the reproductive requirements of each site in the FRT. By examining immune protection in the upper and the lower FRT during the menstrual cycle, a pattern evolves in which aspects of innate, humoral and cellular immunity are either enhanced or suppressed to support both maternal protection and reproductive success. Immune cells, epithelial cells and fibroblasts contribute to a distinct tissue environment in response to OE_2 and P4 that regulates specific immune cell functions throughout the FRT. As a result, the immune conditions that are optimal for fertilization, implantation and pregnancy create a window of vulnerability during the secretory phase of the menstrual cycle, thereby increasing the likelihood of infection by HIV and other STIs. Despite considerable progress in understanding the interface of endocrinology and mucosal immunity in the FRT, much remains to be done to identify the complex mechanisms involved in successful fertility that are proposed to increase the risk of

infection by STIs during certain stages of the menstrual cycle. This knowledge will be essential for protecting women from bacterial, fungal and viral pathogens (including HIV) that compromise reproductive health and threaten the lives of women worldwide. Understanding mucosal immune regulation in the FRT will lead to new concepts for therapeutics to enhance tissue and intracellular antimicrobial activity, as well as to the optimization and the development of vaccines and microbicides to prevent sexual transmission of HIV and other STIs to women without compromising reproductive potential.

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Glossary

Implantation	The binding to and invasion of the uterine endometrium by the blastocyst, which occurs 5–12 days after fertilization.
Proliferative phase	Days 5–14 of the classical menstrual cycle. Defined as the period between the end of menstrual bleeding and ovulation. Characterized by rising serum levels of oestradiol and very low levels of progesterone.
Secretory phase	Days 14–28 of the classical menstrual cycle. Defined as the period between ovulation and the initiation of menstrual bleeding. Characterized by high levels of both oestradiol and progesterone.
Corpus luteum	The tissue formed after ovulation by thecal and granulosa cells from the remains of the collapsed ovarian follicle; it is responsible for progesterone and oestradiol secretion during the secretory phase of the menstrual cycle. In the absence of fertilization, the corpus luteum degrades, thus decreasing hormone synthesis and signalling the initiation of menstruation.
Pattern-recognition receptors	(PRRs). Multiple families of conserved receptors, such as Toll-like receptors (TLRs), that are present on the cell surface or within intracellular compartments. PRRs recognize conserved structures that are present on pathogens or that are produced as part of their life cycle.
Pathogen-associated molecular patterns	(PAMPs). Conserved structures that are an integral part of pathogens but not mammalian cells and that are recognized by pattern-recognition receptors. Examples include viral and bacterial components such as double- and single-stranded RNA, bacterial lipopolysaccharide and hypomethylated DNA.
Cervico-vaginal lavage fluid	(CVL fluid). The fluid recovered after gently washing the vaginal walls and external cervix; it contains the cellular secretions present in the lower female reproductive tract.

Tight junction proteins	A group of proteins, including claudins and occludin, that form complexes to link adjacent epithelial cells, creating a polarized epithelium that provides a barrier and regulates the movement of molecules.
Decidualization	The changes to the endometrium that occur as it transitions to a pregnant state under the influence of progesterone. Characterized by vascular, stromal and epithelial changes that create a permissive uterine environment for implantation.
TZM-bl cells	Modified HeLa cells that express high levels of the HIV receptor CD4, the co-receptors CC-chemokine receptor 5 (CCR5) and CXC-chemokine receptor 4 (CXCR4), and a Tat-induced β-galactosidase cassette.
Lamina basalis	The lower layer of the uterine endometrium that is not shed at menses and from which the functionalis layer is reconstituted during the proliferative phase of the menstrual cycle.
Functionalis layer	The upper layer of the uterine endometrium that is shed at menses.
Innate lymphoid cell	(ILC). An innate immune cell with classical lymphoid morphology that lacks cell lineage markers and antigen specificity. ILCs are heterogeneous and include cytotoxic natural killer cells and cytokine-producing non-cytotoxic helper ILC populations.
Alternatively activated macrophages	Macrophages that have been activated by the T helper 2 cell-type cytokines interleukin-4 (IL-4) and IL-13, as opposed to the classical interferon-γ (IFNγ) activation pathway. Alternative activation confers a phenotype that is instrumental in immune regulation and tissue repair.
Viral eclipse phase	The interval of time after viral infection during which the virus cannot be detected.

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Box 1

Length and hormone dynamics of the menstrual cycle

Only 10% of women have the classical 28-day cycle consisting of 14-day proliferative and secretory phases; lengths of these phases in the remaining women range from 10–23 days and 7–19 days, respectively[134]. Given the marked changes in hormone levels that occur over short periods, particularly at ovulation when oestradiol (OE_2) levels surge and recede in 72–96 hours, a shift of 2–3 days between menstrual cycles may represent a different endocrine environment in the female reproductive tract (FRT). There is also considerable variation in hormone levels between sequential menstrual cycles in an individual woman[32,135,136].

Multiple factors affect hormone responsiveness in the FRT, including receptor expression, co-regulator expression and hormone concentration. At the cellular level, OE_2 and progesterone (P4) exert their effects via the intracellular and plasma membrane oestrogen and progesterone receptors, which are expressed by multiple cell types within the FRT. There are two cytoplasmic isoforms of each receptor, the expression level of which in endometrial tissue peaks in the late proliferative phase before declining in the secretory phase[137,138].

Hormone concentrations in the FRT are distinct from the peripheral circulation. There is a steep gradient in hormone concentration between follicular fluid, ovarian veins and peripheral circulation during the menstrual cycle, with highest levels being detected in the ovaries and lowest levels in the general circulation. For example, in the proliferative phase, OE_2 levels reach more than 1,500 ng ml^{-1} in follicular fluid from the ovary containing the dominant follicle, 10–70 ng ml^{-1} in its innervating ovarian vein, 40–250 ng ml^{-1} in the opposite ovary and 0.05–0.8 ng ml^{-1} in its ovarian vein and in the peripheral circulation[139,140]. Similarly, in the secretory phase, P4 concentration markedly increases in ovarian venous plasma (~500 ng ml^{-1}), uterine circulation (~20 ng ml^{-1}) and peripheral circulation (~10 ng ml^{-1}) compared with in the proliferative phase (~5–11 ng ml^{-1})[141].

Box 2

STIs modify the window of vulnerability for HIV

The presence of pre-existing infections in the female reproductive tract can increase susceptibility to subsequent HIV infection[142] and can potentially expand the 'window of vulnerability'. Mechanisms involved in this effect include increased inflammation, upregulation of cytokine and chemokine expression, and recruitment of susceptible target cells. For example, herpes simplex virus 2 infection increases the likelihood of acquiring HIV by 3- to 5-fold[143]. Foci of $CD4^+$ T cells, $CD8^+$ T cells and dendritic cells (DCs) expressing the HIV co-receptors CC-chemokine receptor 5 (CCR5) and DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) form at the site of herpetic lesions and remain for several months after viral clearance and healing[66]. In *ex vivo* experiments, these sites have a greater susceptibility to HIV infection.

Macaques co-infected with *Chlamydia trachomatis* and *Trichonomas vaginalis* become infected with simian–human immunodeficiency virus (SHIV) during the proliferative phase of the menstrual cycle rather than during the secretory phase, as has been described for animals without co-infections[144]. Women with chlamydial infection have an increased number of HIV-susceptible $CD4^+CCR5^+$ T cells in the endocervix compared with uninfected women[145]. Similarly, gonococcal infection increases the number of $CD4^+$ T cells in the endocervix[146]. As $CD4^+CCR5^+$ T cells are the main target cells of HIV, this could be one of the mechanisms by which chlamydial and gonococcal infections predispose women to HIV acquisition. In addition, analysis of cervico-vaginal lavage fluid in women co-infected with HIV and *C. trachomatis* showed an increased white blood cell count[147], which suggests increased risk for HIV transmission. These findings suggest that changes induced in the mucosal environment by sexually transmitted infections can overcome the immune defences that are normally present during the proliferative phase of the menstrual cycle.

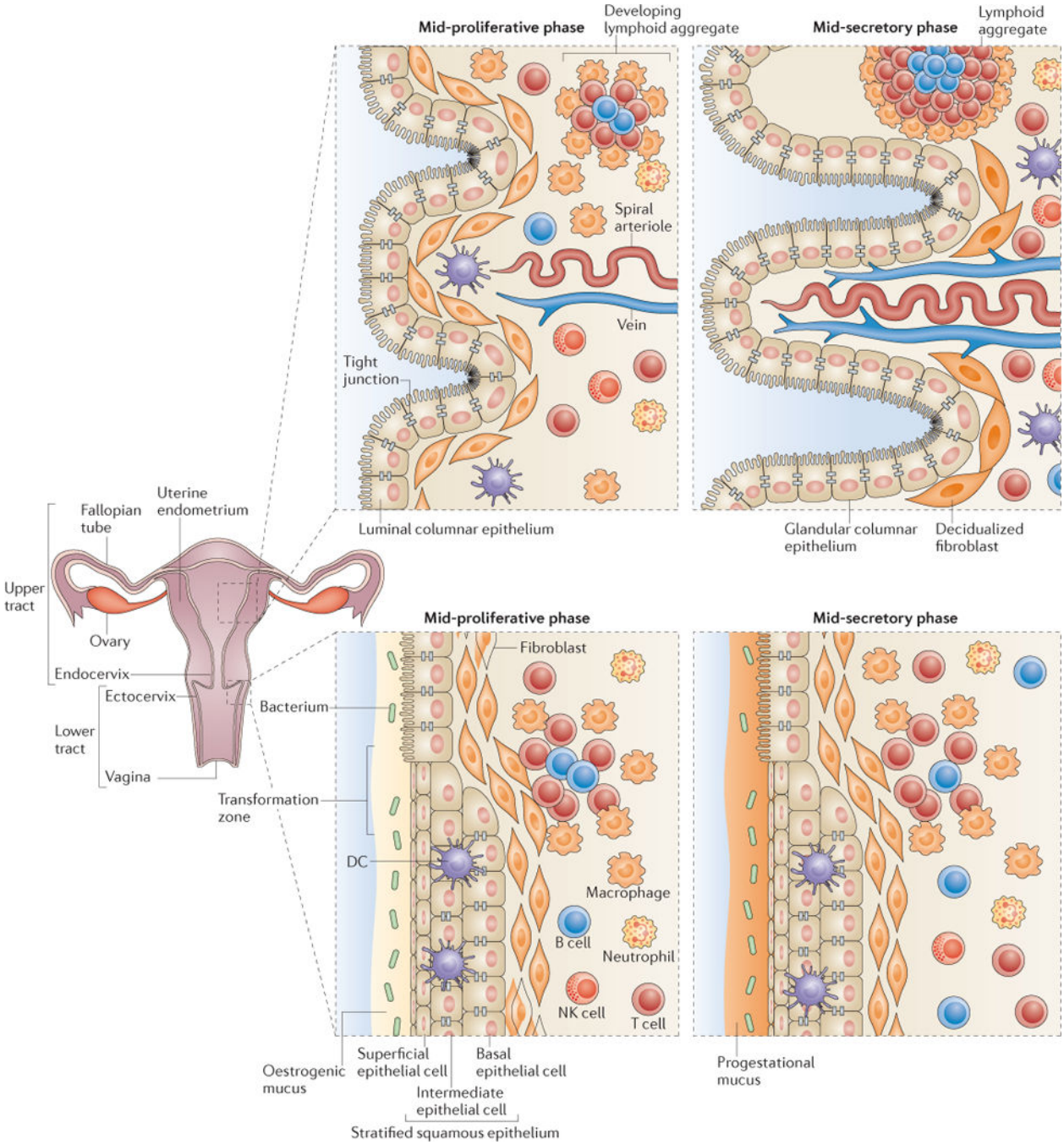


Figure 1. Anatomy and histology of the FRT

The female reproductive tract (FRT) is composed of distinct anatomical regions that undergo morphological changes during the menstrual cycle. The lower FRT consists of the vagina and ectocervix and is protected by a stratified squamous epithelium, which is composed of superficial, intermediate and basal epithelial cells. The thickness of the squamous epithelium remains fairly constant in humans during the menstrual cycle. By contrast, the upper FRT, which consists of the endocervix, endometrium and Fallopian tubes, is covered by a single-layer columnar epithelium. In the endometrium, the columnar

epithelial cells proliferate during the menstrual cycle and form glands in the secretory phase. The transformation zone is where the columnar epithelium of the upper FRT meets the squamous epithelium of the lower FRT. Overlying the epithelial surface in the lower FRT and endocervix is mucus, the consistency of which changes across the cycle, becoming thick and viscous in the secretory phase. Also present is a dynamic population of bacteria, primarily composed of lactobacilli in most women, that acidify the lumen of the lower FRT. Underlying the epithelium is a dense layer of fibroblasts, interspersed with immune cells (T cells, macrophages, B cells, neutrophils, natural killer (NK) cells and dendritic cells (DCs)). The transformation zone contains a particularly high number of immune cells compared with the rest of the FRT. In the endometrium, immune cells form lymphoid aggregates that reach peak size around ovulation and during the secretory phase of the cycle.

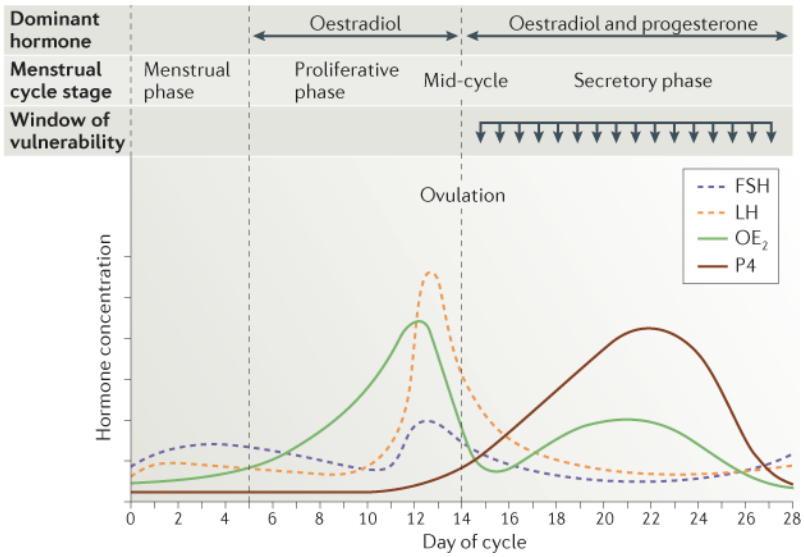


Figure 2. The menstrual cycle

The 28-day menstrual (ovarian) cycle is divided into four stages — menstrual phase, proliferative phase, mid-cycle (ovulation) and secretory phase — that are characterized by cyclic changes in hormone levels. Day 0 is defined by the onset of menstrual bleeding, which lasts for 3–5 days in most women. Menses is followed by the proliferative phase, during which the endometrial lining is reconstituted. Follicle-stimulating hormone (FSH) produced by the anterior pituitary gland induces oestradiol (OE_2) production by the ovary. OE_2 levels increase during the proliferative phase and peak before mid-cycle (ovulation), followed by a rapid drop in concentration. Rising OE_2 levels stimulate luteinizing hormone (LH) production by the anterior pituitary, the levels of which surge in the late-proliferative phase within 24–36 hours of the OE_2 peak, leading to ovulation and increasing progesterone (P4) synthesis. At the same time, FSH levels increase by a smaller amount. Both LH and FSH levels rapidly drop in the early secretory phase. After ovulation, the concentrations of P4, and to a lesser extent OE_2, which are both produced by the corpus luteum in response to LH, steadily increase before peaking at mid-secretory phase. Both FSH and LH levels remain low throughout the secretory phase. In the absence of fertilization, OE_2 and P4 levels drop, which leads to endometrial shedding and the onset of menses. Immune changes in the FRT that occur as a result of cyclic changes in hormone levels create an optimal environment for successful fertilization and implantation during the secretory phase. This environment of regulated immune responses creates a ‘window of vulnerability’ during this phase, with permissive conditions for the entry and survival of pathogens.

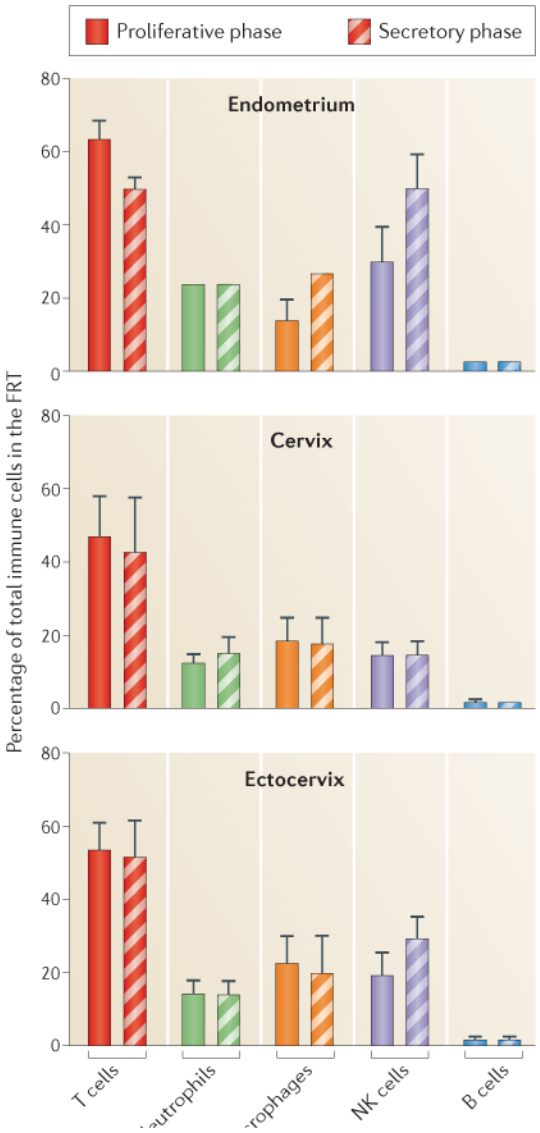


Figure 3. Fluctuations in immune cell populations in the FRT during the menstrual cycle

Bars represent the mean ± standard error of the mean (SEM) from different studies analysing immune cell subsets in the female reproductive tract (FRT; endometrium[5,61,72,73,108], endocervix and ectocervix[5,60,61,63,73]). Studies included in the figure are limited to those that used flow cytometry, as microscopic analysis of tissues does not enable immune cell frequency to be accurately obtained. Statistical analyses are not possible because of the limited number of flow cytometry studies that have been carried out. NK, natural killer.

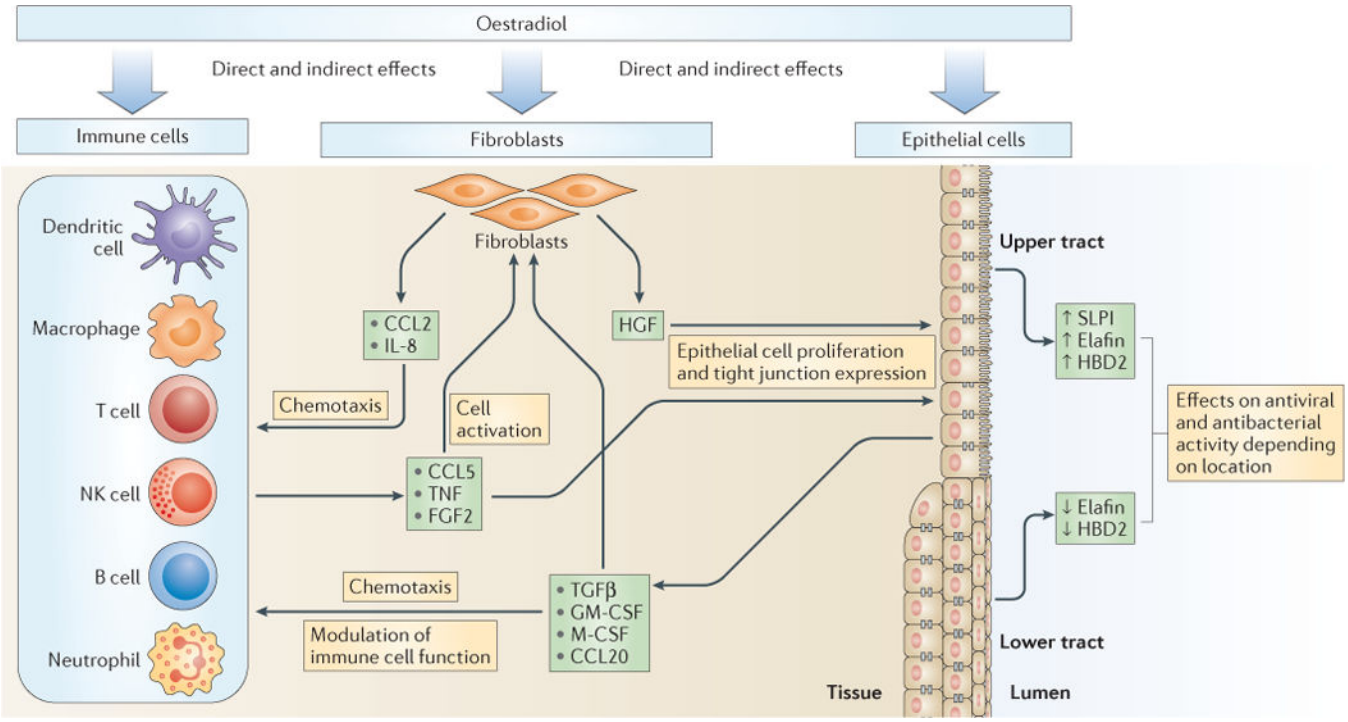


Figure 4. Oestradiol-mediated control of interactions between epithelial cells, fibroblasts and immune cells in the FRT

Oestradiol (OE_2) functions directly through receptor expression on multiple cell types of the female reproductive tract (FRT) or indirectly through intermediary molecules to regulate gene transcription and protein expression, and to alter the number, distribution and phenotype of cells in the FRT. OE_2 induces the expression of multiple cytokines, chemokines, growth factors and antimicrobial proteins. For example, OE_2-mediated stimulation of epithelial cells increases the luminal secretion of antimicrobial proteins (such as secretory leukocyte protease inhibitor (SLPI), elafin and human β-defensin 2 (HBD2)) in the uterus but decreases the secretion of elafin and HBD2 in the vagina, possibly leading to differences in antiviral and antibacterial activity in the FRT lumen depending on anatomical location. Transforming growth factor-β (TGFβ), granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and CC-chemokine ligand 20 (CCL20) are secreted by epithelial cells into the tissue environment under the influence of OE_2, where they modulate immune cell chemotaxis and function — for example, leading to changes in dendritic cell (DC) responses to Toll-like receptor (TLR) ligands. In contrast to its direct effects on epithelial cells, OE_2 can indirectly alter the proliferation and the barrier function of uterine epithelial cells by stimulating the secretion of hepatocyte growth factor (HGF) by uterine fibroblasts, which in turn modulates tight junction expression and cell replication. OE_2 also directly affects uterine fibroblasts to increase their secretion of CCL2 and interleukin-8 (IL-8), which leads to increased chemotaxis of neutrophils, monocytes and DCs. Less clear is the role of OE_2 in regulating the contributions of immune cells to the mucosal environment in the FRT; these cells secrete CCL5, tumour necrosis factor (TNF) and fibroblast growth factor 2 (FGF2). TNF, which is a pro-inflammatory cytokine, activates fibroblasts and degrades tight junction integrity (and

thus the barrier function) of epithelial cells. Similarly, FGF2 stimulates growth of uterine epithelial cells and fibroblasts, and also alters epithelial structure and integrity (not shown). NK, natural killer.

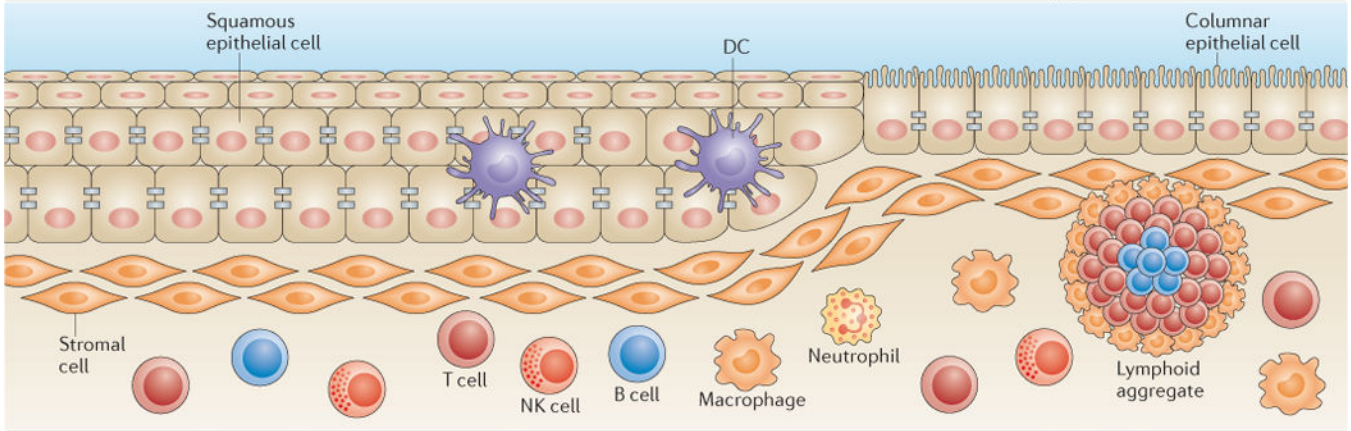
	Immune function	Effects of oestradiol and progesterone	
		Lower FRT	Upper FRT
Lumen	Pathogen killing and inactivation	↓ HBD2, elafin, IgA and IgG	↑ CCL20, HBD2, SLPI, elafin, IgA and IgG (pIgR and FcRn)
			
Epithelial cells	Immune cell recruitment, innate immune protection and tolerogenic nature	↓ CCL2, IL-8, HBD2 and elafin	↑ CX_3CL1, CCL2, IL-8, HBD2, CCL20, SLPI, elafin and TGFβ
Fibroblasts	Immune cell recruitment	↔ CXCL12	↑ CXCL12
	Regulation of epithelial cells	↔ HGF	↑ HGF
Immune cells	Pathogen killing	↔ $CD8^+$ CTL activity	↓ $CD8^+$ CTL activity
	Protection against viruses	↔ NK cell activity (IFNγ)	↓ NK cell activity (IFNγ)
	Protection against bacteria	$CD14^+$ macrophages	$CD163^+$ macrophages
	Infection by HIV	↓ $CD4^+$ T cell susceptibility	↓ $CD4^+$ T cell susceptibility

Figure 5. Influence of sex hormones on mucosal immunity in the lower and upper FRT during the window of vulnerability

This figure depicts the key immunological mechanisms present in the female reproductive tract (FRT) that are essential for successful reproduction and that directly or indirectly affect pathogens that enter the FRT and threaten reproductive health. These immune mechanisms are under hormonal control. During the 'window of vulnerability', oestradiol (OE_2) and progesterone (P4), selectively stimulate and/or suppress aspects of the innate and adaptive immune systems as shown, in ways that vary according to the FRT site. For example, in the lower FRT, innate components (such as human β-defensin 2 (HBD2)) in the lumen are suppressed at a time when $CD8^+$ cytotoxic T lymphocyte (CTL) activity and natural killer (NK) cell cytotoxic activity are maintained. By contrast, $CD8^+$ CTL and NK cell activities are suppressed in the uterus at a time when luminal innate components are enhanced. These uterine changes are consistent with increased luminal pathogen killing and/or inactivation at a time when semi-allogeneic blastocyst rejection might otherwise occur. The resulting alterations in immune protection optimize conditions for successful implantation but also lead to an increased risk of acquiring sexually transmitted infections (STIs)[3]. In the table, double-headed arrows indicate that there is no change. CCL, CC-chemokine ligand; CXCL12, CXC-chemokine ligand 12; CX_3CL1, CX_3C-chemokine ligand 1; DC, dendritic cell; FcRn, neonatal Fc receptor; HGF, hepatocyte growth factor; IFNγ, interferon-γ; IL-8, interleukin-8; pIgR, polymeric IgA receptor; SLPI, secretory leukocyte protease inhibitor; TGFβ, transforming growth factor-β.