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The complexity of interactions between female sex hormones and *Chlamydia trachomatis* infections

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Abstract

Purpose of Review: This review focuses specifically on the mechanisms by which female sex hormones, estrogen and progesterone, affect *Chlamydia trachomatis* infections *in vivo* and *in vitro*.

Recent Findings: Recent data support previous work indicating that estrogen enhances chlamydial development via multiple mechanisms. Progesterone negatively impacts *Chlamydia* infections also through multiple mechanisms, particularly by altering the immune response. Conflicting data exist regarding the effect of synthetic hormones, such as those found in hormonal contraceptives, on chlamydial infections.

Summary: Numerous studies over the years have indicated that female sex hormones affect *C. trachomatis* infection. However, we still do not have a clear understanding of how these hormones alter *Chlamydia* disease transmission and progression. The studies reviewed here indicate that there are many variables that determine the outcome of *Chlamydia*/hormone interactions, including: 1) the specific hormone, 2) hormone concentration, 3) cell type or area of the genital tract, 4) hormone responsiveness of cell lines, and 5) animal models.

Keywords

Chlamydia trachomatis; female sex hormones; estrogen; progesterone; sexually transmitted infections

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Conflict of Interest

The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

1. Introduction

Concentrations of the female sex hormones (FSH), estrogen and progesterone naturally fluctuate with the menstrual cycle [1, 2]. Their presence in differing concentrations affects the physiology of the female genital tract (FGT), regulating ovulation, endometrial cell proliferation and maturation, and the immune response [1, 2]. Sex hormones even influence the composition of the vaginal microbiome [3, 4]. Because hormones are essential to the proper FGT function, the effects of estrogen and progesterone on the acquisition and progression of genital infections is an intriguing area of research. This review focuses specifically on the mechanisms by which endogenous and exogenous FSHs affect *Chlamydia trachomatis* infections.

Chlamydia trachomatis remains the world's predominant cause of bacterial sexually transmitted infection (STI) worldwide. Over one million chlamydial infections are reported to the Centers for Disease Control in the US each year. Although chlamydial infections are frequently reported in men, they are most commonly reported in young females [5]. Chlamydial infections initially present as cervicitis in women; however, many infections are asymptomatic. This is especially problematic as untreated chlamydial infections increase the patient's risk for developing pelvic inflammatory disease (PID), life-threatening ectopic pregnancy, and/or tubal infertility [6].

2. Influence of natural and synthetic FSH on *Chlamydia infections* in women

2.1 Complications of ascending chlamydial infections: salpingitis, and non-viable pregnancy

Few studies have directly examined the impact of estrogen or progesterone concentrations on transmission or progression of chlamydial infections in women. A 1986 study found that women infected with *C. trachomatis* or *Neisseria gonorrheae* were more likely to develop salpingitis in the first seven days following menses, corresponding to the estrogen-dominant proliferative phase [7]. These observations suggest a correlation between estrogen and ascending chlamydial infections [7]. However, other studies have reported increased detection of *Chlamydia* from female patients during the progesterone-dominant secretory/luteal phase of the menstrual cycle, including a recent retrospective analysis of patient medical records [8, 9]. Unfortunately, these studies are often limited by small sample size. It is also hard to determine if hormones directly affected pathogen transmission by examining the time of detection, as this only reflects the time at which the infection became symptomatic, not necessarily when it was acquired. For instance, a cervical infection that is detected in the progesterone-dominant third week of the menstrual cycle may have been transmitted during the estrogen-dominant second week.

The impact of hormones on fertility and pregnancy outcomes in infected patients has also been explored. A study examining *Chlamydia*-associated oxidative stress in infertile women found increased levels of both oxidative stress markers and luteinizing hormone in *Chlamydia*-infected women compared to both *Chlamydia* positive and negative fertile

women [10]. Non-viable pregnancies caused by spontaneous abortion or ectopic pregnancy are complications of chronic *Chlamydia* infections. There are multiple reasons that women experience spontaneous abortion, including an improper balance of hormones during pregnancy [11]. Decreased progesterone levels early during pregnancy can result in spontaneous abortion [12]. A prospective study measured estrogen and progesterone levels in *Chlamydia*-infected recurrent spontaneous aborters (RSA) during their first trimester. They found that these women had significantly increased estrogen and decreased progesterone levels compared to uninfected RSA and non-RSA control groups [12]. This study also demonstrated that *Chlamydia*-infected RSA had increased expression of contractile prostaglandin receptors, which are regulated by hormone concentrations during pregnancy and are involved in initiation of labor [12]. While there could be other reasons for these observations, these data suggest that *Chlamydia* directly or indirectly cause a shift in estrogen and progesterone levels that can result in spontaneous abortion. LPS has been shown to increase the ratio of estrogen to progesterone in mice [13]. Thus, it is possible that *Chlamydia* infection alters the levels of hormones in the host leading to negative pregnancy outcomes.

2.2 Contraceptives and chlamydial risk

Globally, over 150 million of women rely on hormonal contraceptives (HC) for safe and effective birth control, many of whom are in the 14–29 age range that is at the highest risk for chlamydial infections [5, 14, 15]. Thus, it is likely that *C. trachomatis* will encounter an FGT environment that is regulated by synthetic hormones. Forcey, *et al.*, demonstrated that HC use abolished the observed difference in detection of chlamydial DNA in the proliferative versus the secretory phases of the menstrual cycle, indicating that exposure to constant synthetic hormone levels alters the pattern of chlamydial infection compared to that in HC non-users [8]. Of the published studies examining the risk for chlamydial transmission with HC use, approximately 58% found a positive association [16]. Metaanalysis of 29 cross-sectional studies found a significant association between HC use and *Chlamydia* infection. Metaanlysis of 13 cross-sectional studies, which reported correction for some confounding variables, also found that HC use was associated with elevated chlamydial infection risk [17]. Three prospective studies noted a significant increase in chlamydial cervical infections with oral HC even after confounding variables, such as age, number of sexual partners and/or sexual behaviors, were taken into account [18–20]. Still, there are numerous studies that indicate no change in chlamydial infection rate with HC use. A recent review of prospective studies published in the last 10 years found inconclusive evidence that *Chlamydia* infections were impacted by HC use [21].

A major hurdle to interpreting and comparing data from these clinical studies is that they are often inconsistent in design and analysis methods, leading to conflicting conclusions. Some studies do not account for confounding variables, such as the number of sexual partners or use of barrier contraception. Many of the studies also do not specify the type of HC used. This is important because there is a wide range of HC formulations and delivery mechanisms. Oral contraceptives are often combinations of a synthetic estrogen and progestin or a progestin-only mini pill [2, 22]. Long-acting progestin-only HC may also be injected or implanted in the body. Studies that record the type of HC used report varying

conclusions. For example, one study found that the use of Depo-provera (DMPA) was associated with an increased risk of chlamydial infection, but found no increase in infection among women using oral contraceptives [23]. Conversely, another study found that combined oral contraceptives are a risk factor for ascending chlamydial infections where DMPA is not [24]. Levonorgestrel-intrauterine systems (LNG–IUS) release the synthetic progestin slowly over 3–5 years [25]. There have been concerns from obstetrician-gynecologists that LNG–IUS may result in increased risk of pelvic infection [26]. However, a study of US women showed that the risk of pelvic infection is not increased in the first few weeks after LNG–IUS placement [27]. Furthermore, STI testing and antibiotic treatment of *Chlamydia*-infected women at the time of LNG–IUS placement prevented pelvic infection [27]. These studies illustrate that HC use cannot be considered as a single experimental variable. To fully investigate the impact of HC on chlamydial infections, the type of HC must be known.

The primary HC mechanism of action is to prevent release of luteinizing and follicle-stimulating hormones from the pituitary gland, preventing ovulation. However, localized effects on endometrial and cervical cells are also observed [1, 2]. Though all synthetic progestins act as progesterone receptor agonists, interaction with mineralocorticoid, glucocorticoid, androgen receptors, and ERs has been documented, suggesting ‘off-target’ interactions could influence chlamydial development and/or pathogenesis *in vivo* [1, 2, 28–30]. Fichorova, *et al.*, reported that immune biomarkers are differentally expressed in cervical samples from women that use oral HC versus DMPA and that infection with *C. trachomatis* can further alter the expression of inflammatory mediators under these hormonal conditions [14]. Therefore, HC may alter the immune response to sexually transmitted pathogens [31]. Several studies also speculate that HC-induced cervial ectopy is responsible for increased transmissiblity of *C. trachomatis*; however, no molecular mechanism has been provided for these predictions [16]. It is likely that HC impact chlamydial infections in various ways depending upon the formulation and delivery mechanism employed. Thus, more investigation is needed to elucidate the specific effects of common synthetic hormones found in HC, as this information could inform physician recommendations when choosing HC for high risk patients.

3. Animal models

Several animal models are used to study *Chlamydia* pathogenesis [32, 33]. Each of these models have advantages and disadvantages for examining the effects of hormones on chlamydial infections. Mice, due to their low cost, ease of use and extensive availability of reagents, are the most common animal model used in chlamydial research. However, mice have a short estrus cycle, lasting only 4–5 days, causing infected cells to be shed rapidly. To overcome this, mice are routinely treated with DMPA to prevent cycling [34]. Thus, most studies of chlamydial pathogenesis in mice are performed under conditions of synthetic progesterone exposure. Interestingly, Pal *et al.* demonstrated that infecting mice with *C. trachomatis* in the progesterone-dominant phase yielded 20% more ascending infections than in the estrogen-dominant phase [35]. Progesterone pre-treatment is also required for establishing chlamydial infections in rats [36]. Therefore, these studies indicate that progesterone dominant conditions are favorable to chlamydial infections in rodents. Another

caveat to rodent *Chlamydia*-infection models is that *C. muridarum* is often used, because it establishes a similar infection to humans when the animal is incoculated vaginally. To evaluate *C. trachomatis* infection in mice, the animals must be inoculated transcervically, bypassing natural ascension of the pathogen to the upper FGT from an initial cervical infection. Likewise, *Göttingen minipigs* must be transcervically inoculated with *C. trachomatis* during estrus to establish a lasting infection (>5 days) [37–39]. Intrauterine inoculation of minipigs with *C. trachomatis* during diestrus produces the longest duration of infection (≥10 days). The authors of these studies attributed increased IFNγ expression to faster clearance of *C. trachomatis* during estrus [37]. Conversely, studies evaluating *C. caviae* infection in guinea pigs found that pre-treatment with estrogen produced longer lasting infections with greater chlamydial shedding and pathology, while pre-treatment with progesterone had no effect on infection outcome [40–42]. Estrogen pretreatment is also essential for *C. trachomatis* serovars D and E infection in guinea pigs [43]. Unlike mice, the reproductive tract of guinea pigs has a more similar hormonal regulation to humans. Guinea pigs also have a longer estrus cycle (15–17 days), which eliminates the need for progesterone pre-treatment [32].

Non-human primate models serve as a physiologically relevant model for the study of *C. trachomatis* infections. The pig-tailed macaque has a menstrual cycle and vaginal microbiota that is very similar to humans [32]. Because they are naturally susceptible to *C. trachomatis*, macaques do not require pre-treatment with progesterone [44]. Baboons also establish chlamydial infections with disease progression similar to humans [44, 45]. Interestingly, Eastman, *et al.*, recently demonstrated that administration of Levonorgestrel via intrauterine system (LNG–IUS) increases *C. trachomatis* shedding, duration of infection and salpingitis in baboons [46]. These data suggest that chlamydial infections in non-human primates may be impacted by sex hormones, or at least synthetic progestins. While non-human primates are physiologically relevant models of human chlamydial infections, the effect of the endogenous hormonal environment on progression of chlamydial infection has not been investigated in these models.

4. In vitro studies

4.1 Potential interactions between Chlamydia and estrogen receptors

Estrogen receptors (ER) exist in two forms in the cell. First, there is the cytosolic receptor, which translocates to the nucleus to mediate gene transcription upon binding to the hormone ligand. Second, there are membrane ERs, which mediate non-genetic, rapid cellular signaling events in response to estrogen [47]. Membrane ERs and their membrane complex proteins have been associated with chlamydial entry into host cells [48–50]. *Chlamydia* enter host cells via clathrin-coated pits and caveolae, membrane structures known to contain ERs [48, 51–54]. Elegant studies by the Wryrick laboratory revealed that ERβ and protein disulfide isomerase (PDI), a member of the ER membrane complex, are associated with chlamydiae attached to the surface of host cells [50, 55]. Abromaitis and Stephens have shown that while PDI is not a receptor for attachment, its reductive functions are required for chlamydial entry into host cells [49]. Furthermore, inclusion development in cultured human endometrial cells was reduced by antibody blockage of membrane ERα/β or PDI, or

by exposure to the ER antagonist, tamoxifen, prior to *C. trachomatis* infection [50]. Additionally, inclusions observed in tamoxifen-exposed cultures were smaller than unexposed inclusions, suggesting that ERs are involved in inclusion development as well as EB attachment to the host cell [50].

In addition to aiding entry into host cells, there is evidence that chlamydiae may interact with ERs throughout the developmental cycle. CT441 is a protease secreted by *chlamydia* with a PDZ domain that interferes with the NF–κB pathway by cleaving the host p65 protein. Multiple research groups have postulated that CT441 aids chlamydial host immune evasion by inhibiting NF–κB-mediated cytokine expression [56]. Steroid receptor RNA activator 1 (SRA1), a co-activator of ERα, is a functional RNA that is also translated to produce the protein SRAP1. CT441 directly interacts with, but does not cleave, SRAP1 *in vitro* and *in vivo*. CT441/SRAP1 binding prevents activation of ERα, reducing ERα localization to the nucleus and estrogen-mediated changes in gene transcription [57]. Overall, these studies suggest that ERs influence chlamydial infection in multiple ways. Most studies indicate that chlamydiae benefit from ERs via direct interactions or through hormone-modulated host cell signaling and gene expression. However, it is possible that in some circumstances or at certain times in development, *Chlamydia* benefit from shutting off ER activity and have evolved mechanisms to alter the cellular environment via interaction with hormone receptors.

4.2 Physiological environment: Hormone modulation of host cell signaling and gene expression

In the mid-1980s, Moorman et al. found that exposing human primary endometrial cell cultures to 10^{-7}M estrogen or a combination of 10^{-8}M estrogen/10^{-7}M progesterone decreased the percentage of cells infected with *C. trachomatis* [58]. Conversely, studies using HeLa cells or explanted human endometrial cells found that exposure to 10^{-10}M estrogen increased attachment of chlamydiae to host cells, while exposure to 10^{-8}M estrogen or a combination of estrogen/progesterone had no effect or decreased infection with *Chlamydia trachomatis* [59, 60]. Subsequent studies demonstrated that *C. suis S45* infection in swine genital epithelial cells behaved similarly to *C. trachomatis* Serovar E infection in explanted human endometrial epithelial cells. They confirmed that cells harvested during the estrogen-dominant phase of the menstrual cycle were more susceptible to chlamydial infection than cells harvested during the progesterone-dominate phase [61]. Moreover, Guseva, *et al.*, noted that swine genital epithelial cells harvested during a particular phase of the menstrual cycle could not be reprogramed by exogenous hormone exposure [61]. These early data suggested that i) hormone concentration is a very important determinant of how estrogen impacts chlamydial infection, and ii) the varying physiological effects of hormones on different potential host cells may contribute to the enhancing or inhibitory nature of estrogen and progesterone on chlamydial infections.

Recently, we studied the effects of estrogen and progesterone on chlamydial infection using a co-culture model of immortalized human endometrial epithelial cells (Ishikawa, IK) and stromal (SHT–290) cells. This model more closely mimics an *in vivo* environment than previous studies using exogenous hormone supplementation of genital epithelial

monocultures. Hormonal regulation of the endometrial epithelial cell cycle relies on a combination of direct interaction of hormones with epithelial cell receptors as well as paracrine signals released by underlying stromal cells in response to hormonal stimulation [62]. *C. trachomatis*-infected IK/SHT–290 co-cultures exposed to 10^{-8}M estrogen produced significantly more inclusions and progeny chlamydiae compared to hormone-free controls [50, 63]. We also exposed IK/SHT–290 co-cultures to progesterone dominant conditions mimicking those in the secretory phase. To do this, co-cultures were primed with 10^{-8}M estrogen prior to adding a combination of 10^{-9}M estrogen/10^{-7}M progesterone. When infected under progesterone dominant conditions, chlamydial infection was decreased compared to estrogen-exposed samples [63]. Interestingly, the hormone-mediated positive or negative effects on chlamydial infection in IK cells were only observed when the stromal cells were present, suggesting that secreted stromal cell effectors are important components of the observed changes in chlamydial infection [50, 63]. Stromal cells release a variety of cell signaling molecules that, along with direct estrogen and/or progesterone signaling, regulate proliferation and maturation of endometrial epithelial cells. Notably, estrogen increased phosphorylation of ERK, a member of the MAPK pathway, in IK cells during IK/SHT–290 co-culture, but not in IK cultures alone. Cytokine expression in the presence of estrogen was also reduced in IK/SHT–290 and HEC-1B/SHT–290 co-cultures [50]. These data suggest that paracrine stromal cell signaling influences the impact of hormones on chlamydial infection and may act through regulation of host cell signaling pathways.

Studies have demonstrated that human FSHs also mediate gene expression of a significant proportion of chlamydial genes. The expression of approximately 25% of the chlamydial transcriptome was altered 2-fold or greater in response to estrogen and/or progesterone exposure in infected ECC–1 cells. Specifically, estrogen-exposure downregulated chlamydial genes involved in fatty acid and nucleotide biosynthesis. Additionally, estrogen upregulated genes involved in chlamydial persistence, suggesting that estrogen may promote the chlamydial stress response in ECC–1 cells [64]. This is an interesting finding given that other human cell culture models of estrogen exposure demonstrate that estrogen promotes chlamydial infection and progeny production [40, 50, 59–61]. Progesterone did not affect expression of the same chlamydial genes as estrogen in infected ECC–1 cells, but did alter accumulation of a substantial number of chlamydial transcripts, including those involved in the TCA cycle, glycolysis, and carbohydrate and amino acid metabolism. In all, progesterone upregulated expression of 85 and down-regulated expression of 135 chlamydial genes [64].

As with *C. trachomatis*/ER interactions, these studies collectively confirm that hormone modulation of the host cell environment can have different effects on chlamydial infection depending on several factors, including cell line or model system, primary vs immortalized cells, and concentration of hormones. This make data interpretation and comparisons between studies difficult. However, regardless of the experimental variables, the results from these studies support the conclusion that sex hormones impact *in vitro* chlamydial infection in multiple ways.

5. Immune Response

Immunity in the FGT is uniquely designed so that it protects the genital tract from infection, while allowing a fetus to develop during pregnancy. Estrogen and progesterone's role in regulating the FGT immune system has been extensively reviewed by others [31, 65–67]. It is also hypothesized that HC influence STIs by altering the host's immune response [3]. Several studies indicate that sex hormones alter the expression of immune factors or immune cell responses during chlamydial infection. Like previous studies, progesterone exposure significantly reduced chlamydial infection in ECC–1 cells, as measured by decreased accumulation of *C. trachomatis* DNA. Cytokine and chemokine expression was increased in progesterone-exposed *C. trachomatis*-infected ECC–1 cultures compared to estrogen-exposed cultures, suggesting that progesterone's inhibitory effect on *Chlamydia* is immune-mediated [68]. Argrawal, *et al*., showed that *C. trachomatis*-infected cells pre-exposed to estrogen had reduced TLR4 expression and Th1–associated cytokines compared to infected cells not exposed to estrogen. The anti-inflammatory cytokine IL–10, however, was significantly increased in infected, estrogen-exposed cells, suggesting that estrogen may promote a less effective Th2 immune response to *C. trachomatis* infections [69]. Conversely, other studies suggest that estrogen-mediated immune factors protect against chlamydial infections. Interferon ε (IFNε) is a type 1 interferon expressed in the FGT whose function is not well understood. Its expression varies throughout the menstrual cycle with the highest expression occurring during estrogen-dominant conditions. *C. muridarum-infected* IFNε $^{-/-}$ mice had more severe infections and bacterial shedding than wild type mice. These data suggest that estrogen-stimulated expression of IFNε may promote clearance of *C. muridarum* from mice [70]

Hormones also affect antibody responses during chlamydial infection. The role of secretory IgA in clearance of chlamydial infection has been debated in several studies. Armitage, *et al*. showed that murine expression of the polymeric immunoglobulin receptor plgR, which is essential for transcytosis of IgA in the FGT, is increased during estrus, whereas DMPA decreased plgR expression and IgA accumulation in the tissues. Although *C. muridarum* infection increased plgR expression in DMPA pre-treated mice, the availability of IgA at the time of inoculation was lower than it would have been had the mice been infected during estrus. These data raise the possibility that DMPA pre-treatment masks the contributions of IgA to infection clearance in the mouse model [71].

While clinical studies show that LNG–IUS use is not associated with increased pelvic infection incidence [27], *in vitro* studies show that LNG affects immune cells during *Chlamydia* infection. Primary human dendritic cells (DCs) exposed to LNG had decreased expression of CD80, CD86 and CD40 and were inhibited in their ability to activate naïve T cells *in vitro*. Additionally, incubating LNG–exposed DCs with inactivated *C. trachomatis* significantly reduced the DC CD40 expression, suggesting that LNG alone as well as LNG in the presence of *Chlamydia* alters expression of immune-associated factors [72]. Mice implanted with LNG pellets and infected intra-nasally with *C. trachomatis* had significantly fewer CD40–expressing DCs in their cervical lymph nodes than mice that received placebo pellets. LNG-treated mice were also not able to clear infection from the lungs compared to placebo-treated mice. Interestingly, clearance of infection by mice without CD4+ T cells

was similar to that of LNG-treated mice [72]. Overall, these studies show that LNG reduces the ability of DCs to mature and activate T cells *in vitro* and *in vivo*, both in the presence and absence of *C. trachomatis*. *C. trachomatis*-infected baboons implanted with a LNG–IUS responded to infection with a Th2 response, while animals without LNG–IUS responded with a cell- mediated Th1 response. Baboons with a LNG–IUS shed greater numbers of *C. trachomatis* for a longer period of time and were more likely to develop PID than animals without the LNG–IUS. These results indicate that LNG–IUS contraception increased the risk of PID by hormonal-modulation of the immune response [46].

Overall, these studies highlight the numerous effects of natural and synthetic hormones on immune function. It is likely that hormone-induced changes in the immune response impacts chlamydial infections. Indeed, hormonal modulation of the immune system is hypothesized to alter transmission and disease progression for other STI pathogens, notably HIV [19, 73]. When considering these studies, it is important to note that different areas of the FGT (uterus, Fallopian tubes, cervix) do not respond to hormone-stimulated signals in the same manner. For example, cytotoxic T lymphocyte activity in the uterus and Fallopian tubes is suppressed during the secretory phase, where as others found no difference in cytotoxic T lymphocyte activity in the cervix between the proliferative secretory phases [67]. Similar differences have also been observed for other immune functions, like secretory IgA and chemokine receptor expression [67]. Therefore, it is possible that hormones impact *C. trachomatis* infection differently at varying sites within the FGT by tissue specific changes in the immune response.

6. Future Directions and Conclusions

While the effects of estrogen and progesterone on chlamydia infection have been investigated for decades, we still do not have a clear understanding of how these hormones affect *Chlamydia* disease transmission and progression. This is not surprising given the complexity of hormone signaling in the FGT. The data presented thus far indicate that FSHs affect chlamydia via multiple mechanisms. The estrogen receptor may directly or indirectly aid EB host cell entry [48, 50]. Endogenous estrogen and progesterone often have opposing effects on chlamydial infection by altering the physiological environment through regulation of host-cell signaling pathways, paracrine stromal cell signals, chlamydial gene transcription and the immune response [41–43, 50, 60, 61, 63, 74]. Synthetic hormones found in HCs also impact chlamydial infection, but not necessarily in the same manner as endogenous hormones. Additionally, the published studies reviewed here indicate that there are many variables that determine the outcome of *Chlamydia*/hormone interactions, including: 1) the specific hormone used (natural or synthetic compounds), 2) hormone concentration, 3) cell type or area of the genital tract examined, 4) hormone responsiveness of tissue culture cell lines, and 5) the animal model used. Future research should be targeted to determine the exact mechanisms by which natural and synthetic forms of FSHs influence chlamydial development, keeping the stated experimental variables in mind.

To date, most studies examining hormonal effects on *Chlamydia* focused on hormone-host cell/immune cell-pathogen interactions. However, it is important to remember that sex hormones influence the entire body, not just genital tract cells. Hormones affect the

availability of nutrients in the environment. For example, estrogen regulates iron levels in the body, a key nutrient for *C. trachomatis* [75, 76]. Microbial endocrinology is a relatively new but emerging field of research because of the widespread involvement of the microbiome with the host. It has been suggested that bacteria interact with eukaryotic hosts through an interplay of quorum sensing molecules and host hormones [77]. Hormonal fluctuations during the menstrual cycle may alter the vaginal microbiome composition, which is known to influence the outcome of vaginal infections through direct (Ex: maintenance of low vaginal pH) and indirect mechanisms (Ex: modulation of immune responses) [78]. The role of the host vaginal microbiota is an understudied factor in the interactions with FSHs and *Chlamydia*. Studies have suggested that *C. trachomatis* infection is associated with altered diversity in the vaginal microbiome, as reviewed by Molenaar, *et al*. and others [73, 79]. In baboons, *C. trachomatis* infection in the presence of the LNG–IUS decreased the total number of microbiome-associated bacteria, but not the diversity of the vaginal microbiome [78]. Therefore, it is possible that hormones indirectly influence *Chlamydia* transmission by altering the vaginal microbiota.

Another aspect of hormone-*Chlamydia* studies that is worth further investigation is the relevance of the most common animal models used for *Chlamydia* research. Because rodents require progesterone pre-treatment for the establishment of chlamydial infection, the majority of *in vivo* studies on *Chlamydia* are under the influence of DMPA. Although these studies have provided a wealth of knowledge about chlamydia infections, there is evidence that DMPA influences the outcome of mouse infection studies. As noted above, DMPA reversed estrogen-stimulated expression of plgR, possibly masking the value of IgA in clearing chlamydial infections [71]. Kaushic, *et al*., demonstrated that when mice were treated with DMPA prior to vaccination with an attenuated strain of Herpes Simplex Virus (HSV) and subsequent challenge, none of the animals were protected. However, when mice were exposed to natural progesterone before vaccination, they were protected from lethal HSV challenge [80]. Thus, it is worth determining if DMPA treatment in mice skews the progression and outcome of chlamydial infection, particularly in the case of vaccine research.

Although numerous studies have investigated the effects of FSHs on chlamydial infection, the exact mechanisms of these interactions have not completely been elucidated. Future research is crucial to increase our understanding of the roles of sex hormones in the entry, establishment, and pathogenesis of *Chlamydia*. A better understanding of these interactions will help researchers and medical professionals improve treatments, contraceptive recommendations and develop vaccines against *Chlamydia*.

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•• Of major importance

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