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Vulvodynia and Hormonal Contraception: The Effects of Synthetic Hormones, Depression, and Age at Onset on the Diffusion and Type of Vulvar Pain

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ABSTRACT

Vulvodynia is defined as vulvar pain lasting at least three months without an identifiable cause, although various associated factors are identified. These potential factors include genetics, hormonal influences, inflammation, musculoskeletal issues, neurological mechanisms, psychosocial factors, and structural abnormalities. Research suggests that the persistence of vulvar pain, rather than remission, is more likely in patients experiencing diffuse pain across the vulva (as opposed to pain localized in a single area) and in those with an older age at symptom onset. This study examined the impact of several variables – hormonal contraception use, vulnerability to its adverse effects, depression, anxiety, participant age, and age at onset – on the spread of vulvar pain. Findings indicate that while the use of hormonal contraception and depression were not statistically linked to diffuse vulvar pain, vulnerability to negative effects from hormonal contraception, anxiety, current age, and age at onset were significantly associated with spread of vulvar pain symptoms. Additionally, we explored whether hormonal contraception use influences the way pain is reported by women suffering from vulvodynia. Results showed that women using hormonal contraception were more likely to describe their vulvar pain as cutting or burning compared to non-users. We believe that further research into the nature of these pain characteristics will shed light on the underlying mechanisms of vulvodynia, ultimately aiding in the development of more effective medical and psychological treatment approaches.

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INTRODUCTION

Various types of chronic pain affect millions of people worldwide each year. More precisely, chronic pain is defined as pain persisting for at least three months (WHO, 2021) and can impact any part of the body, such as the back, muscles, joints, or nerves, as seen in cases of neuropathy. Furthermore, due to its persistent nature, chronic pain often lowers quality of life and adversely affects mental health (Arnold et al., 2006). Among women, a common form of chronic pain is vulvodynia, which is characterized by enduring pain in the vulvar area lasting at least three months (Bornstein et al., 2016).

Vulvar pain symptoms are estimated to affect 7-15% of AFAB (i.e., assigned female at birth) individuals (Eppsteiner et al., 2014; Benoit-Piau et al., 2018). However, despite its prevalence, the underlying causes of vulvodynia remain unclear, and the condition is still under-researched. Moreover, given that vulvodynia affects an intimate area, it significantly impacts the sexuality, quality of life, and mental health of those affected (Arnold et al., 2006). Many vulvodynia patients also encounter skepticism from healthcare providers, as chronic pain in the female genital area is sometimes dismissed or minimized (Graziottin, 2015). For instance, women often report being told that their pain is “all in their head” during the diagnostic process, which can lead to feelings of frustration and invalidation. Consequently, obtaining an accurate diagnosis of vulvodynia can take years, making the diagnostic journey prolonged, demoralizing, and often more painful due to inadequate symptom management (Toeima & Nieto, 2011).

Nowadays, vulvodynia is understood as a multifactorial syndrome, with research identifying both biological and psychological factors, including anxiety and depression, as potential risk factors. Additionally, the ISSVD has pointed to hormone-related changes induced by medications, such as hormonal contraceptives (e.g., the pill and patch), as

potential contributors to vulvar pain (Bornstein et al., 2016). Nevertheless, although most women with vulvodynia experience symptom remission, research suggests that symptoms are more likely to persist when pain affects multiple vulvar areas rather than being localized to a single site, such as the vestibule (Pâquet et al., 2019). The age of symptom onset is also recognized as a potential factor influencing symptom persistence (Pâquet et al., 2019).

In response to these findings, this research investigates the relationship between hormonal contraceptive use and the risk of vulvar pain diffusion. It also examines other risk factors associated with vulvodynia, including age, age of symptom onset, anxiety, and depression. Additionally, this study explores the types of pain that hormonal contraceptives may induce in the vulvar area due to morphological changes in the vestibule (Johannesson et al., 2007). To address these objectives, this thesis is structured into five chapters. The first chapter introduces vulvodynia, covering vulvar anatomy, the classification of sexual pain in females, and the definitions and possible causes of vulvodynia. The second chapter examines steroid hormones, addressing both their natural production in the body and their role in hormonal contraceptives. Chapter three presents the research methods and analyses, which lead to the results discussed in chapter four. Successively, chapter five provides potential explanations for the findings.

Finally, it is important to note that this study does not aim to discourage the use of hormonal contraceptives. Indeed, these contraceptives can also have positive effects on the quality of life and sexual well-being for some women. However, investigating whether hormonal contraceptives are associated with an increased risk of developing chronic conditions, such as vulvodynia, is crucial. In fact, this knowledge allows

individuals to make more informed, well-rounded decisions about their health by understanding all potential risks.

Chapter 1: VULVODYNIA

This chapter aims to explore what vulvodynia is. Before delving into the definition of vulvodynia and distinguishing it from other conditions such as dyspareunia and vaginismus, a brief overview of the vulva anatomy – the external female genitalia, which includes both the urinary and reproductive systems (Nguyen & Duong, 2023) – will be provided. The second section will focus on Chronic Pelvic Pain (CPP), followed by a discussion on vulvodynia, a specific type of CPP. However, before examining vulvodynia in depth – its definition, prevalence, associated factors, and diagnosis – a brief description of the nosology of sexual pain will be given.

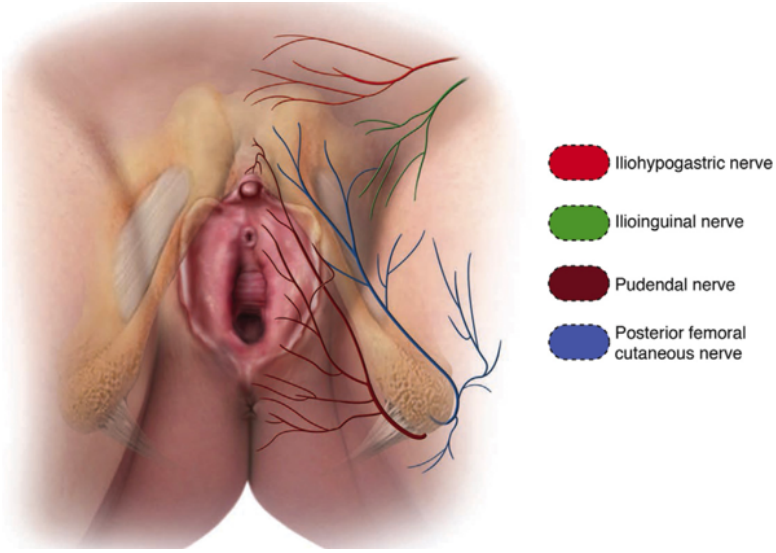
1.1 The Anatomy of the Vulva

This section provides a brief description of the anatomy of the vulva, given that understanding its anatomy is crucial for comprehending its physiology and distinguishing between different disorders, such as vulvodynia and dyspareunia, which will be defined later in the chapter (Deliveliotou & Creatsas, 2018). When conducting scientific research or evaluating a patient, it is important to remember that the vulva comprises different types of skin and changes throughout a woman's life cycle and hormonal status (Sacher, 2019). It is essential to spread awareness that there is no single "normal vulva". The common perception that non-protruding and symmetric labia minora are normal can lead to unnecessary surgeries (e.g., labiaplasty), damage female confidence, and decrease body image (Yurteri-Kaplan et al., 2012; Mazloomdoost et al., 2015; Yeung & Pauls, 2016). Despite ongoing debates about what should be considered part of the vulva (Zdilla, 2022), this section will include the mons pubis, labia majora, labia minora, clitoris,

urethra, vulvar vestibule, vestibular bulbs, Bartholin's glands, Skene's glands, and vaginal opening (Nguyen & Duong, 2023). Nevertheless, the innervation of the vulva is provided mainly by the pudendal nerve but also by the dorsal nerve of the clitoris (for the clitoris innervation), the lioinguinal nerve and the illiohypogastric nerve (Yeung & Pauls, 2016; Doucet et al., 2022). Figure 1 illustrates the nerve innervation of the vulva.

Figure 1

Innervation of the Vulva



Note. From Pauls, 2015.

The vulva lies between the inguinogluteal folds laterally and the anus posteriorly (Singh, 2013). At the top of the vulva is the mons pubis or mons Veneris, which consists of adipose tissue covered by pubic hair and contains sebaceous glands that release preprohormones (Nguyen & Duong, 2023). Starting anteriorly from the mons pubis and forming the posterior fourchette overlapping the perineum are the labia majora (Sacher, 2019). The labia majora are approximately 7-8 cm in length and 2-3 cm in width,

depending on the quantity of adipose tissue (Deliveliotou & Creatsas, 2018). Both the mons pubis and the labia majora primarily consist of adipose tissue, subcutaneous fat, and smooth muscles. The medial part of the labia majora is typically hairless and has a generally pink skin color (Singh, 2013). Furthermore, both the mons pubis and the labia majora derive embryologically from the ectoderm (Deliveliotou & Creatsas, 2018).

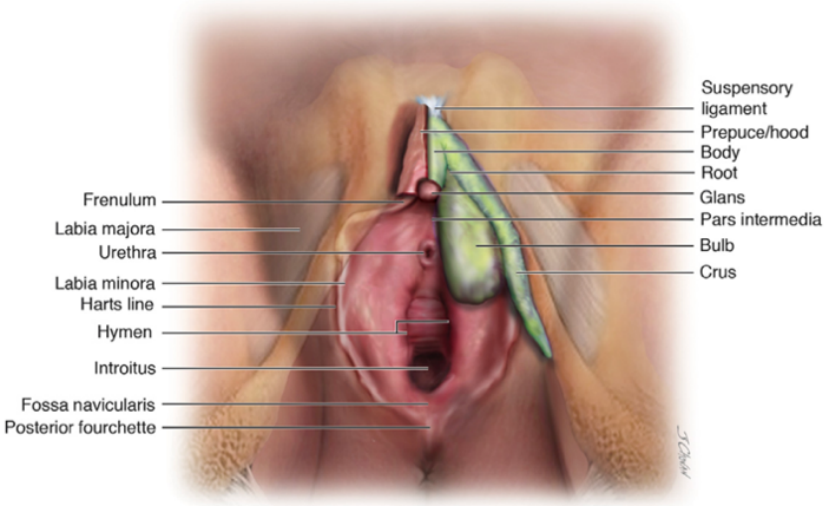
Medially to the labia majora and adjacent to the vestibule there are the labia minora (Yeung & Pauls, 2016). The labia minora are much shorter compared to the labia majora and there is a higher inter-individual variation between women in their size (Deliveliotou & Creatsas, 2018). The anterior section of the labia minora divides into two folds. The superior folds joins above the glans of the clitoris forming the prepuce, while the inferior folds on the labia minora unify below the glans of the clitoris giving rise to the frenulum (Sacher, 2019). Posteriorly, the labia minora unify together with the labia majora at the posterior fourchette (Yeung & Pauls, 2016). Interestingly, the Hart line define the keratinized epithelium of the labia minora which derives from the ectoderm versus the nonkeratinized epithelium of the vestibule of the vagina which comes from the endoderm (Robboy et al., 1978).

The vestibule, located between the clitoris and the posterior fourchette, comprises several structures: the vaginal orifice, external urethral meatus, clitoral bulbs, and the Bartholin and Skene glands (Deliveliotou & Creatsas, 2018; Yeung & Pauls, 2016). The vaginal orifice is the external opening of the vagina, which extends internally to the uterine cervix (Sacher, 2019). The walls of the vagina consist of three layers: the mucosal layer, the middle vascular layer, and the adventitial layer (Yavagal et al., 2011). The Bartholin glands are situated in the posterior portion of the vestibule and play a major role in lubricating the vagina and vulva (Lindeque, 2013). On the other hand, the role of

the Skene glands, located laterally to the external urethral meatus on both sides, is more debated (Dwyer, 2012). However, evidence suggests that they play a key role in urinary and sexual health (Cleveland Clinic, 2022). Interestingly, a histopathological study by Wernert et al. (1992) found that these glands were present in only two-thirds of females, which may explain differences in orgasmic experiences during intercourse (Dwyer, 2012). Just above the Skene glands and posterior to the clitoris lies the external urethral meatus, the external opening of the urethra (Singh, 2013). The urethra is "a membranous conduit for urine", and it extends from the bladder to the external urethral meatus in the vestibule and measures between 3.5 and 5 cm (Deliveliotou & Creatsas, 2018). Importantly, the shared embryological development of the bladder, urethra, and vestibule may explain the high comorbidity between urological pain symptoms (e.g., interstitial cystitis, IC) and vulvar pain (Cervigni & Natale, 2014). Finally, the clitoris is composed of two distinct neurovascular networks that are interconnected through a venous plexus known as the pars intermedia (Doucet et al., 2022). The internal structure of the clitoris consists of the corpora cavernosa and the corpora spongiosa, while the glans is the only external part, forming the tip of the clitoris (Sacher, 2019; Doucet et al., 2022). When discussing the clitoris, it is essential to consider it as a collection of structures collectively referred to as the "bulbo-clitoral organ" or the "clitoral complex" (Doucet et al., 2022). Furthermore, scientific literature consistently highlights the crucial role of the clitoral complex in female sexual arousal and orgasm, attributing this to its intricate vascularization, erectile properties, and its anatomical connections to both the urethra and vagina (Jannini et al., 2014; Doucet et al., 2022). Figure 2 shows the anatomy of the vulva.

Figure 2

Anatomy of the Vulva



Note. From Pauls, 2015.

1.2 Chronic pelvic pain

Chronic pelvic pain (CPP) is a broad term that encompasses pain originating from gynecological organs and other pelvic structures, including the bowel, bladder, blood vessels, nerves, and muscles (Rowen & Goldstein, 2021). Specifically, CPP is defined as "nonmalignant pain perceived in structures related to the pelvis of both males and females" lasting for at least six months (Fall et al., 2010). This pain can be non-cyclic or cyclic, for example appearing in relation to the menstrual cycle or intercourse (Gunter, 2011). CPP is a highly prevalent syndrome, affecting 4-16% of the general population (Taney & Tu, 2021).

The etiology of CPP can be broadly classified into gynecological, non-gynecological, and unidentifiable causes (Taney & Tu, 2021). The most common gynecological causes include endometriosis, adenomyosis, leiomyomas, pelvic inflammatory disease, and intra-abdominal adhesions (Taney & Tu, 2021). Conversely,

non-gynecological causes often associated with CPP primarily include irritable bowel syndrome (IBS) and bladder pain syndrome (BPS), also known as interstitial cystitis (IC; Zondervan et al., 2001). In the case of idiopathic CPP, where no identifiable cause is found, there may be a dysfunction in the central nervous system's ability to process pain, potentially leading to increased sensitivity to sensory stimulation (Gunter, 2011; Hellman et al., 2015). However, most patients experience multiple contributing factors, making CPP a multifactorial disorder (Zondervan et al., 2000). Additionally, CPP has been shown to increase the risk of anxiety and depressive disorders (Siqueira-Campos et al., 2019) and negatively impact cognition, behavior, and sexuality (Fall et al., 2010). These effects significantly reduce the quality of life for many patients suffering from CPP (Taney & Tu, 2021). Therefore, due to its multifactorial nature and psychological sequelae, it is crucial to manage this disorder with a multidisciplinary approach.

To reach the correct diagnosis, it is important to follow three main steps. The first step involves a thorough examination of the patient's medical history concerning CPP. This "history-taking" should answer a series of questions about the location of pain, radiation (whether pain originates in one location and spreads to another), quality (e.g., whether the pain is described as "burning" or "cramping"), timing (to determine if a traumatic event such as an injury triggered the pain), provocative and alleviating factors (e.g., exercise or defecation), and associated symptoms (Taney & Tu, 2021). The second crucial step is the physical examination, followed by an imaging examination and other procedures to rule out all possible causes of CPP (Lamvu et al., 2021). For example, a medical doctor might consider a urine pregnancy test or a vaginitis and other STI screening to exclude potential causes of CPP (Lamvu et al., 2021). In cases of idiopathic CPP, the diagnosis is based on exclusion criteria (Taney & Tu, 2021). Once the cause(s)

and exacerbating factors of CPP in the patient are understood, it is important to discuss the diagnosis with the patient, including pain education, setting expectations and goals, and establishing a plan for multimodal therapy and interdisciplinary treatment (Lamvu et al., 2021). Only after discussing the therapeutic approach's goals with the patient can the most suitable therapeutic plan be selected based on the patient's needs (Lamvu et al., 2021).

CPP treatment encompasses two main approaches, which are not mutually exclusive and can be combined based on the patient's situation (Howard et al., 2003). The first approach focuses on pain as a diagnosis in itself, while the second aims at treating disorders that may provoke or worsen the patient's CPP (Howard et al., 2003). However, the guidelines proposed by Lamvu and colleagues (2021) recommend a multimodal and interdisciplinary therapy approach that evaluates pharmacotherapy, nonpharmacological or interventional therapies (e.g., acupuncture or neuromodulation), physical therapy, psychological therapy, and self-care (e.g., a diet plan based on the patient's diagnosis). After 4-8 weeks, it is important to consider a follow-up to assess any improvement in pain symptoms or determine if the therapy needs to be adjusted (Lamvu et al., 2021).

In this thesis, the focus will be on a specific type of chronic pelvic pain that affects the external female genitalia, specifically the vulva, known as vulvodynia. The following section will provide an explanation of vulvodynia, including its prevalence, etiologies, and symptoms. Additionally, a brief overview of the nosology of sexual pain will be included to differentiate vulvodynia from dyspareunia and vaginismus.

1.3 The nosology of sexual pain: vulvodynia, dyspareunia, and vaginismus

Nosology refers to the science that classifies disorders or diseases (Derogatis et al., 2016). It is important not to confuse this term with *nomenclature*, which is a system used to assign names (Derogatis et al., 2016). In the specific context of female sexual pain disorders, the most commonly used terms are vulvodynia, dyspareunia, and vaginismus (Rowen & Goldstein, 2021). The term vulvodynia is derived from "vulva" and the Greek word "Odyne", meaning pain, thus translating to "vulvar pain" (Amalraj et al., 2009). The term dyspareunia was first coined by Barnes in 1873 and literally means "painful sexual intercourse" (Oxford English Dictionary, 2023). Meanwhile, vaginismus, first introduced by Sims in 1862, refers to the involuntary contraction of the vaginal muscles (Rowen & Goldstein, 2021). Nowadays, vulvodynia refers to pain in the vulva, dyspareunia describes painful sensations in the vagina (either superficial at the introitus or deep inside), usually occurring during penetrative intercourse, and vaginismus "involves persistent or recurrent difficulties in allowing vaginal penetration, whether by a penis, finger, or any object, despite the woman's desire to do so" (Basson et al., 2009; Chauhan et al., 2022). It is essential to note that these three conditions often co-occur, making it challenging to differentiate them during diagnosis (Revicky et al., 2012). Furthermore, the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) groups these three conditions together under the general diagnosis of Genito-Pelvic Pain/Penetration Disorder (GPPPD).

Dyspareunia is a highly prevalent condition among sexually active women, with estimates suggesting that it affects between 12% and 21% of this population (Landry & Bergeron, 2009). It can be classified as primary, occurring from the first sexual experience, or secondary, developing after a period of normal sexual function (Revicky

et al., 2012). The pain can be persistent, occurring in various sexual positions and with different partners, or conditional, manifesting only in certain positions (Revicky et al., 2012). The primary causes of dyspareunia include chronic pelvic pain (CPP), vulvodynia, endometriosis, hormonally induced changes, childbirth, and vaginismus (Revicky et al., 2012; Goldstein, 2009). For instance, research by Ballard and colleagues (2008) revealed that women with endometriosis – a condition characterized by the presence of endometrial-like tissue outside the uterus – are more than nine times as likely to experience dyspareunia compared to the general population. Understanding the underlying causes of dyspareunia is crucial for effective treatment (Ballard et al., 2008; Revicky et al., 2012). Moreover, women suffering from dyspareunia often report reduced sexual satisfaction, diminished psychological well-being, and a lower quality of life (Landry & Bergeron, 2009). This suggests that optimal treatment should combine medical intervention with psychological support.

In contrast, vaginismus is less common, with an estimated prevalence between 0.5% and 1% in the general female population (Simons & Carey, 2001). Compared to those suffering from vulvodynia and dyspareunia, individuals with vaginismus are more likely to have a history of childhood sexual trauma (Reissing et al., 2003). Negative perceptions of sex or upbringing in cultures where sex is taboo or virginity is highly valued are also significantly linked to the development of vaginismus (Crowley et al., 2009; Farnam et al., 2014). Studies have shown strong associations between vaginismus, phobias, and anxiety (Farnam et al., 2014). Biologically, vaginismus may be triggered by factors such as insufficient sexual arousal, vulvovaginitis, atrophic vaginitis, vulvar dermatoses, medications, radiotherapy, endometriosis, and pelvic inflammatory disease (Crowley et al., 2009; Weijmar Schultz et al., 2005).

As mentioned earlier, vulvodynia, dyspareunia, and vaginismus are distinct disorders, except in terms of the diagnostic criteria outlined in the DSM-5. However, not only can it sometimes be difficult to make a differential diagnosis, but these conditions may also exacerbate or trigger each other (Graziottin, 2010). For example, if a patient suffers from vulvodynia with persistent inflammation in the vestibule due to intercourse (i.e., mechanical trauma), she may also experience hyperactivation of mast cells (MC; Graziottin, 2010). This, in turn, leads to the proliferation and superficializing of nerve fibers (see "2.5 Inflammation in Vulvodynia: Mast Cells, Nerve Growth Factor and Steroid Hormone"), which can worsen or provoke pelvic floor muscle contraction, resulting in dyspareunia (Graziottin, 2010). A similar mechanism can occur in the reverse direction. For instance, a patient with dyspareunia and pelvic floor muscle contraction may experience sexual arousal difficulties, such as insufficient lubrication (Graziottin & Murina, 2011). This lack of lubrication can cause lesions in the vestibular mucosa, which hyperactivates MC, leading to the proliferation and superficializing of nerve fibers, ultimately causing vulvodynia (Graziottin & Murina, 2011).

In conclusion, while vulvodynia, dyspareunia, and vaginismus are distinct diagnoses, they often coexist in the same patient and can exacerbate one another. An accurate treatment plan addressing both medical and psychosexual needs is crucial to ensure the patient's well-being. The next section will focus on vulvodynia, the primary subject of this thesis.

1.4 The Definition of Vulvodynia

The first definition of vulvodynia was proposed by Thomas in 1891, describing the syndrome as "excessive sensitivity of the nerves supplying the mucous membrane of

some portion of the vulva, sometimes confined to the vestibule and other times to one labium minus" (Thomas & Mundale, 1891; Rowen & Goldstein, 2021). Years later, in 1987, Friedrich defined vulvar vestibulitis syndrome (VVS) as "severe pain upon vestibular touch or attempted vaginal entry, tenderness to pressure localized within the vulvar vestibule, and physical findings of varying degrees of vestibular erythema". In 1970, Friedrich founded the International Society for the Study of Vulvovaginal Disease (ISSVD), which remains the main organization "for research, advocacy, and nosology related to vulvodynia" (Rowen & Goldstein, 2021).

In 2015, the ISSVD, in collaboration with the International Society for the Study of Women's Sexual Health (ISSWSH) and the International Pelvic Pain Society (IPPS), held an international meeting to redefine vulvodynia (Bornstein et al., 2016). The result of this meeting was the "2015 Consensus Terminology and Classification of Persistent Vulvar Pain and Vulvodynia" (Bornstein et al., 2016). This document classifies vulvar pain into two categories: the first being "Vulvar pain caused by a specific disorder", such as vulvovaginal atrophy due to menopause-related hormonal deficiencies (Portman & Gass, 2014; Bornstein et al., 2016). The second category is vulvodynia, defined as "vulvar pain lasting at least 3 months, without a clear identifiable cause, which may have potential associated factors" (Bornstein et al., 2016).

The classification system also allows for subtyping vulvodynia based on its presentation. It can be localized (i.e., affecting specific areas) or generalized (i.e., involving the entire vulva). For example, localized vulvodynia may manifest as vestibulodynia (i.e., pain in the vestibule) or clitorodynia (i.e., pain in the clitoris; Groysman, 2010). Additionally, vulvodynia can be further classified based on the nature of the pain: it may be provoked (e.g., insertional or contact pain), spontaneous, or mixed

(i.e., both provoked and spontaneous; Groysman, 2010; Bornstein et al., 2016). Provoked vestibulodynia (PVD) is the most common subtype, with a prevalence in the general population of up to 12% (Goldstein & Burrows, 2008).

Finally, Graziottin and Murina (2011) have critiqued the definition of vulvar pain as "without a clear identifiable cause", arguing that it is the responsibility of the medical professional to determine the underlying cause of the pain to provide the most effective treatment for the patient. Moreover, it is better to refer to vulvodynia as a syndrome rather than a chronic illness, given that it is often characterized by a combination of other disorders that need to be properly treated and lead the pain to be much more widespread instead of being focused on the vulva only (Graziottin & Murina, 2011; Rowen & Goldstein, 2020). The etiology of vulvodynia will be discussed in the next section.

1.5 Prevalence and Associated Factors of Vulvodynia

Vulvodynia is a prevalent and multifactorial syndrome (Harlow et al., 2001), with the prevalence of vulvar pain estimated to range between 8% and 16% among women (Eppsteiner et al., 2014; Benoit-Piau et al., 2018). However, pinpointing the exact prevalence is challenging due to the condition's frequent underdiagnosis (Toeima & Nieto, 2011). Moreover, Reed and colleagues (2012) examined the relationship between ethnicity and the prevalence of vulvodynia. Their findings indicate that Black women are at lower risk for vulvodynia than White women, while Hispanic women face a higher risk compared to White women (Reed et al., 2012). Although these differences may have genetic, environmental or biological explanations, the role of psychosocial influences on female sexual disorders must also be considered, and the exact reasons for these differences remain unclear (Shallcross et al., 2018).

As previously mentioned, vulvodynia is a multifactorial syndrome, making it difficult to identify the specific underlying causes. Potential contributing factors include genetics (see "2.7.1 The Role of Genetics"), hormonal influences (see Chapter 2), inflammation (see "2.5 Inflammation in Vulvodynia: Mast Cells, Nerve Growth Factor and Steroid Hormones"), musculoskeletal factors, neurological mechanisms, psychosocial influences, and structural abnormalities (Bornstein et al., 2016). The following sections briefly describe these factors, except for hormonal influences and inflammation, which will be discussed in detail in subsequent chapters.

1.5.1 Biological Factors

Regarding genetic factors, multiple studies suggest that certain women may have a genetic predisposition to vulvodynia through at least three overlapping mechanisms (Rowen & Goldstein, 2021). These include genetic polymorphisms that increase the risk of infections such as candidiasis, genetic alterations that lead to prolonged or heightened inflammatory responses, and greater sensitivity to hormonal fluctuations associated with oral contraceptive use (Babula et al., 2004; Foster et al., 2004; Goldstein et al., 2014).

Musculoskeletal factors associated with vulvodynia include pelvic floor muscle overactivity or hypertonicity (Rowen & Goldstein, 2021). When muscles attached to the posterior vestibule – such as the pubococcygeus, puborectalis, and superficial transverse perinei – become hypertonic, it can result in allodynia (i.e., "pain from a stimulus that would not normally provoke pain"; He & Kim, 2023), which is a common symptom of vestibulodynia (Rowen & Goldstein, 2021). In contrast, hypertonicity in deeper muscles like the iliococcygeus, coccygeus, and obturator internus may contribute to vaginal pain or deep dyspareunia during intercourse (King et al., 2014).

Regarding neurological mechanisms and vulvodynia-related allodynia, Pukall et al. (2005) conducted a significant study. The authors compared 14 women with vulvar pain to 14 controls during an fMRI study, applying mild and moderate pressure to the vulvar vestibule. Notably, results show that women with vulvar pain rated the pressure as significantly more intense and unpleasant compared to controls, with some experiencing pain even from mild pressure (Pukall et al., 2005). Interestingly, women with vulvodynia exhibited significantly higher activation in the insular and frontal cortical regions during painful stimuli (Pukall et al., 2005). Considering these findings, authors concluded that women with vulvodynia have heightened genital sensory processing, providing new evidence for top-down processing of painful stimuli in this syndrome (Pukall et al., 2005).

Finally, the relationship between infections and vulvodynia remains unclear in the scientific literature. For example, Edgardh and Abdelnoor (2007) found that infections such as Bacterial Vaginosis (BV) were a significant risk factor for vulvodynia, whereas STIs and frequent treatments for vulvovaginal candidiasis were not linked to vulvar pain. However, the two independent studies of Berglund et al. (2002) and Leusink et al. (2018) demonstrated an increased risk of developing PVD in patients with Candida.

In conclusion, scientific evidence points to multiple biological factors that may contribute to vulvodynia, emphasizing the syndrome's multifactorial nature, as several factors may coexist in the same patient. Nonetheless, Graziottin and Murina (2011) assert that while psychological factors can exacerbate vulvar pain, there is always an underlying biological cause that must be identified and treated – except in cases of grief.

1.5.2 Psychological Factors

Despite scientific evidence highlighting the significant role of psychosocial factors in pain conditions, including vulvodynia (Gatchel et al., 2007; Bergeron et al., 2011), these factors are often overlooked in favor of biomedical mechanisms (Bergeron & Rosen, 2020). As Bergeron and Rosen (2020) affirm, "pain is biopsychosocial in nature" (Gatchel et al., 2007). Indeed, anxiety and depression have been significantly associated with vulvodynia, both as risk factors, potential consequences and as factor enhancing pain (Masheb et al., 2005; Khandker et al., 2011)

In a pivotal study, Khandker and colleagues (2011) explored whether pre-existing depression and anxiety increase the risk of developing vulvodynia and if vulvodynia itself raises the likelihood of new or recurrent mood and anxiety disorders. Their results revealed that individuals with vulvodynia were significantly more likely to have experienced mood or anxiety disorders compared to controls (Khandker et al., 2011). Moreover, vulvodynia sufferers had an increased risk of developing mood or anxiety disorders at all ages (Khandker et al., 2011). These findings suggest that depression and anxiety may be risk factors for vulvodynia, while vulvodynia may, in turn, increase the risk of new or recurring psychological conditions (Khandker et al., 2011). Indeed, chronic stress may affect the HPA axis, leading to increased inflammation in the vestibule (Kemeny & Schedlowski, 2007; Weiss, 2007). Furthermore, the high comorbidity between vulvodynia and other chronic conditions, such as interstitial cystitis and irritable bowel syndrome, can exacerbate depressive symptoms (Arnold et al., 2006; Gardella et al., 2011). Consequently, women suffering from chronic vulvar pain experience a significantly reduced quality of life compared to healthy individuals (Arnold et al., 2006). Moreover, depression and anxiety may also amplify pain. For instance, Masheb et al.

(2005) found that vulvodynia patients with major depressive disorder (MDD) reported more intense pain than those without MDD. Notably, in most cases, depression preceded the onset of vulvodynia, supporting Khandker et al. (2011) hypothesis that depression and anxiety may be more of a risk factor for vulvodynia than a consequence of it.

As a result of the connection between depression and vulvodynia, vulvar pain is often linked to feelings of powerlessness, both in terms of control over one's body and life (Arnold et al., 2006). This has a detrimental effect on patients' sexuality. Specifically, individuals with vulvodynia report lower levels of sexual desire, arousal, and satisfaction, as well as reduced frequency of intercourse compared to healthy controls (Bergeron et al., 2014). Additionally, they frequently perceive themselves as being less sexually attractive than other women, experiencing significant distress related to body image and genital self-image (Bergeron et al., 2014). However, cognitive behavioral therapy (CBT) has been shown to significantly improve sexual interest, responsiveness, and activity (Lindström & Kvist, 2015). Moreover, six months of CBT may also lead to a reduction in pain severity (Lindström & Kvist, 2015).

Additional evidence for the link between vulvodynia and psychological factors comes from the work of Harlow and Stewart (2005). Authors examined whether childhood experiences of violence, including sexual and physical abuse, are linked to an increased risk of vulvodynia. While earlier studies did not find a direct correlation between childhood abuse and vulvodynia (Edwards et al., 1997; Dalton et al., 2002), Harlow and Stewart (2005) found that individuals who experienced little to no family support or felt unsafe in their home, neighborhood, or school during childhood were two to three times more likely to develop vulvodynia than controls. Furthermore, individuals who reported severe physical or sexual abuse had a four times increase in the risk of

developing vulvodynia (Harlow & Stewart, 2005). Nonetheless, when abuse was combined with childhood endangerment and a lack of family support, the risk increased of fourteen times (Harlow & Stewart, 2005). These findings suggest that psychological trauma may play a direct role in the vulvar inflammatory response, increasing the likelihood of vulvodynia in abuse survivors (Iglesias-Rios, 2015).

In summary, psychological factors play a crucial role in vulvodynia, acting both as risk factors and consequences of the condition. Anxiety and depression are strongly linked to vulvodynia, often intensifying pain and reducing quality of life. Childhood trauma, particularly abuse and lack of support, significantly raises the risk of developing vulvodynia. The condition also impacts patients' sexuality and emotional well-being, leading to feelings of powerlessness. However, cognitive behavioral therapy (CBT) has shown to improve both psychological and physical symptoms, emphasizing the need for a holistic, biopsychosocial approach to treatment.

1.6 Diagnosis of Vulvodynia

Vulvodynia is frequently underdiagnosed or misdiagnosed as other vulvar conditions (Schlaeger et al., 2022). Unfortunately, most patients must consult multiple doctors before receiving the correct diagnosis (Buchan et al., 2007). A key reason for this is that many healthcare providers lack adequate knowledge of vulvodynia (Toeima et al., 2011). Despite its high prevalence and significant impact on patients' quality of life, medical professionals often receive no basic training on this condition (Toeima et al., 2011). As a result, patients are often misdiagnosed – frequently with vulvovaginal candidiasis – and their condition worsens due to inappropriate treatment (Buchan et al., 2007; Leusink et al., 2018). On average, it is estimated that patients with vulvodynia see

between 3 and 15 doctors before receiving the correct diagnosis (Buchan et al., 2007; Leusink et al., 2018), with diagnostic delays ranging from 4 to 7 years also worsening the psychological distress in patients (Graziottin et al., 2015).

The first step in diagnosing vulvodynia is conducting a patient interview, which can be facilitated if the patient keeps a "pain diary" to record specific conditions that exacerbate or trigger the pain (Graziottin & Murina, 2011). According to Basson et al. (2004), the clinical interview should assess how the patient describes the pain (e.g., burning vs. stabbing), any pelvic floor tension during intercourse, changes in arousal (e.g., whether it has decreased compared to the past), the impact of pain on sexual life (e.g., sex avoidance or coping strategies), and the patient's medical history (e.g., past treatments and diagnoses). A key focus of this stage is understanding how the patient's sexual life has been affected (Sadownik, 2014). If necessary, the patient may be referred to a psychologist or sex therapist for further evaluation and support (Sadownik, 2014). Additionally, the gynecologist should inquire about urinary symptoms (e.g., frequency, hesitancy, sensation of incomplete emptying), defecation difficulties, and any musculoskeletal complaints, as these may indicate overactive pelvic floor dysfunction, which could be contributing to the vulvodynia (Goldstein, 2021).

The second step in diagnosing vulvodynia is the physical examination, which aims to gather evidence to better understand the etiology of the sexual pain (Goldstein, 2021). It is recommended to perform a vulvoscopy during this step, allowing the doctor to note any infections, trauma, or dermatitis observed (Goldstein, 2021). Next, the gynecologist conducts a sensory examination using a moistened cotton swab, also known as the "Q-tip test". This test begins by gently touching the medial thigh, buttocks, and mons pubis – areas typically painless – helping the patient become more comfortable with the

procedure (Goldstein, 2021). The Q-tip test on the vulva is performed in a clockwise manner, with the patient rating the pain intensity on a Likert scale from 0 to 10, where 0 represents no pain and 10 represents extreme pain (Sadownik, 2014). Following this, the gynecologist should proceed with a finger examination of the vagina to assess any pelvic floor tension, as well as a bimanual examination to evaluate the uterus, ovaries, and fallopian tubes (Goldstein, 2021). Lastly, based on the findings from the physical examination, additional testing – such as vaginal microbiome analysis and serum testing – should be considered (Graziottin & Murina, 2011; Goldstein, 2021).

In conclusion, a thorough clinical interview and physical examination are essential for identifying the underlying causes of vulvar pain, which, in turn, guide the appropriate treatment and management of vulvodynia. For example, if the patient exhibits pelvic floor tension, physiotherapy to rehabilitate the pelvic muscles should be considered (Schlaeger et al., 2022). Likewise, if the patient shows symptoms of distress or major depressive disorder, cognitive behavioral therapy or psychotherapy should be recommended (Lindström & Kvist, 2015).

Chapter 2: STEROID HORMONES

Hormones can be broadly defined as messengers released into the bloodstream to reach a target organ, initiating the endocrine process (Bernhard & Winfried, 2016; Stárka & Dušková, 2020). For the endocrine effect to occur, a hormone must be released in pulses and reach a certain threshold in the blood through the coordinated action of many cells (Bernhard & Winfried, 2016). The hormone must target only the intended organ; otherwise, it can cause serious conditions like endometriosis (Bernhard & Winfried, 2016). Additionally, it is important to distinguish hormones from neurotransmitters, which are also signals released in the blood but act in the synaptic cleft between the nerve cell and the target cell (Bernhard & Winfried, 2016).

This thesis will primarily focus on three steroid hormones which production starts from cholesterol (Osmosis, 2024; NIH, MedlinePlus). More precisely, the accent will be on estrogens, progesterone, and testosterone, which is included in the family of androgens (NIH, MedlinePlus). Specifically, the following sections will introduce these three classes of hormones, their mechanisms of production, their effects on the female body, and their role in the inflammatory process. Next, this work will explore contraception, synthetic hormones, and their potential association with vulvodynia. The final section will then present the research questions.

2.1 Estrogens, Progesterone and Testosterone

This section will introduce the different types of estrogens, along with progesterone and testosterone. Essentially, the aim is to answer the question: “What are estrogens, progesterone, and testosterone?”. Although this question seems simple, the answer is more complex. Moreover, it’s important to highlight the differences between

the various types of estrogens and other hormones like progesterone and testosterone, as understanding these differences can help identify specific inter-individual variations (Siiteri, 1987). Additionally, estrogens, progesterone, and testosterone play distinct roles in the inflammatory process (Schmidt et al., 2006; Straub, 2007). A better understanding of their types and synthesis can enhance our comprehension of this interaction (Cutolo et al., 2002).

As mentioned before, estrogens, progesterone (P4) and testosterone (T) are steroid hormones (DeMayo et al., 2002). Looking more deeply at the different types of estrogens, we find: estrone (E1), estradiol (E2), estriol (E3; Straub, 2007), and estetrol (E4; Coelingh Bennink et al., 2008). Estrone (E1) is synthesized in the adipose tissue through the conversion of androgens by the aromatase enzyme (Bernhard & Winfried, 2016). Regarding estradiol (E2), its synthesis mainly occurs in the granulosa and theca cells of the ovarian follicle and in the corpus luteum (Graziottin et al., 2022). Furthermore, it is the most highly present hormone in the female body during the reproductive age (Graziottin et al., 2022). Estriol (E3) is produced by hepatocytes in the liver starting from puberty (Graziottin et al., 2022). E3 levels are usually low, and it is typically linked to ERβ, mediating antiproliferative and reparative effects (Marín & Barbancho, 2006). This suggests an important role of E3 in autoimmunity, neurodegenerative, and inflammatory diseases (Marín & Barbancho, 2006). Finally, the last estrogen is estetrol (E4), which is synthesized only during pregnancy in the placenta and fetal liver (Coelingh Bennink et al., 2008). In simple terms, the highest level of estrogens in the female body is reached during the follicular phase of the menstrual cycle (Owen, 1975; Silberstein and Merriam, 2000).

Progesterone is a steroid hormone produced by the ovaries and adrenal glands (Csapo, 1958; Cable & Grider, 2023). Its concentration in the female body peaks during the luteal phase of the menstrual cycle and it plays a fundamental role in maintaining pregnancy (Osmosis, 2024). For example, it forms the mucus plug in the cervix and inhibits prolactin (Osmosis, 2024). Besides its major role during pregnancy and other functions in the female body, which I will discuss later, progesterone also plays a crucial role in enhancing repair in the central nervous system following neurodegenerative or traumatic injury (Stein, 2006).

Androgens are also sex steroid hormones, including Dehydroepiandrosterone (DHEA), Androstenedione, and Testosterone (T; Graziottin et al., 2022). In the female body, these hormones are synthesized by the ovaries and the adrenal gland (Burger, 2020). More precisely, T is produced 25% by the ovaries, 25% by the adrenal gland, and 50% by the conversion of DHEA and Androstenedione into T (Burger, 2020). Androgens are fundamental for our well-being, playing an important role in epithelial-stromal cross-talk, the "scarless" healing of the endometrium during menstruation, and regulation of cellular proliferation (Graziottin et al., 2022). Interestingly, all these functions are regulated by androgens through a single type of receptor (Graziottin et al., 2022), which suggests positive effects in diseases such as endometriosis thanks to pharmacological implementation (Gibson et al., 2018; Taylor et al., 2020; Gibson et al., 2020).

In conclusion, this work focuses on testosterone (T), estrogens (E), and progesterone (P4). These hormones, known as sex steroid hormones, are all produced from cholesterol (Osmosis, 2024; NIH, MedlinePlus). Despite their different roles, they are part of the intricate endocrine system, affecting the physical and psychological well-being of women (Castanho et al., 2014). The next section describes the production of

these hormones before puberty and during the menstrual cycle. Indeed, it is crucial to highlight the varying concentrations of these three hormones, as they not only have different effects on the inflammatory process, but their impact also changes based on the phase of the menstrual cycle (Schmidt et al., 2006; Straub, 2007).

2.2 The Role of Gonadotropins in Puberty and the Menstrual Cycle: the hypothalamic-pituitary-gonadal (HPG) axis

Before puberty, the hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the pituitary gland to release two hormones: the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH; Osmosis from Elsevier, 2022). The FSH and the LH are part of the gonadotropins family, and they are released by the anterior pituitary gland upon GnRH stimulation (Bernhard & Winfried, 2016). More precisely, at the onset of puberty, the hypothalamus begins to secrete GnRH in pulses, prompting the release of FSH and LH, which in turn cause the ovaries and ovarian follicles to develop and secrete hormones (Osmosis from Elsevier, 2022). Ovarian follicles are distributed throughout the ovaries, each consisting of a primary oocyte surrounded by a ring of follicular cells (Osmosis from Elsevier, 2022). As the ovarian follicles mature, the follicular cells differentiate into theca cells and granulosa cells, which play crucial roles in the synthesis of progesterone (by theca cells) and estrogens (by granulosa cells; Tomomi et al., 2015; Osmosis from Elsevier, 2022).

Nevertheless, the release of these hormones is regulated by the female menstrual cycle, which lasts approximately 28 days and is characterized by a surge in the FSH and the LH around day 14, facilitating ovulation (Tomomi et al., 2015; Osmosis from Elsevier, 2022). Moreover, the fluctuations in the FSH and the LH levels lead to

corresponding variations in estrogen and progesterone levels throughout the menstrual cycle (Tomomi et al., 2015; Osmosis from Elsevier, 2022). Indeed, the two weeks preceding ovulation constitute the follicular phase, during which estrogen production predominates (Owen, 1975; Silberstein & Merriam, 2000). On the other hand, the two weeks following ovulation constitute the luteal phase, during which progesterone production is more prevalent (Osmosis, 2024).

During the follicular phase of the menstrual cycle, estrogen induces significant changes in the uterus, particularly in the endometrium, promoting the thickening of the endometrial layer and the development of progesterone receptors (Tomomi et al., 2015; Osmosis from Elsevier, 2022). Simultaneously, the rising of estrogen levels exert a negative feedback effect on the pituitary gland, causing a reduction in the FSH secretion (Tomomi et al., 2015; Osmosis from Elsevier, 2022). However, as ovulation approaches, elevated estrogen levels increase the pituitary gland's sensitivity to GnRH released by the hypothalamus (Tomomi et al., 2015; Osmosis from Elsevier, 2022). Consequently, this triggers a positive feedback loop, leading to a surge in FSH and LH hormones, facilitating ovulation (Tomomi et al., 2015; Bernhard & Winfried, 2016; Osmosis from Elsevier, 2022).

During the luteal phase, progesterone binds to receptors in the endometrium, stimulating the production of secretions from the endometrial glands that prepare the uterus for potential pregnancy (Tomomi et al., 2015; Osmosis from Elsevier, 2022). Progesterone then operates as a negative feedback signal, reducing LH secretion from the pituitary gland (Tomomi et al., 2015; Osmosis from Elsevier, 2022). Concurrently, progesterone levels decrease, triggering menstruation to commence (Osmosis from Elsevier, 2022).

2.3 Cholesterol as the starting point

This section provides a deeper explanation of the key processes involved in the synthesis of estrogens, progesterone, and testosterone starting from cholesterol. Indeed, understanding these processes is crucial to comprehending the initial stages of hormone production.

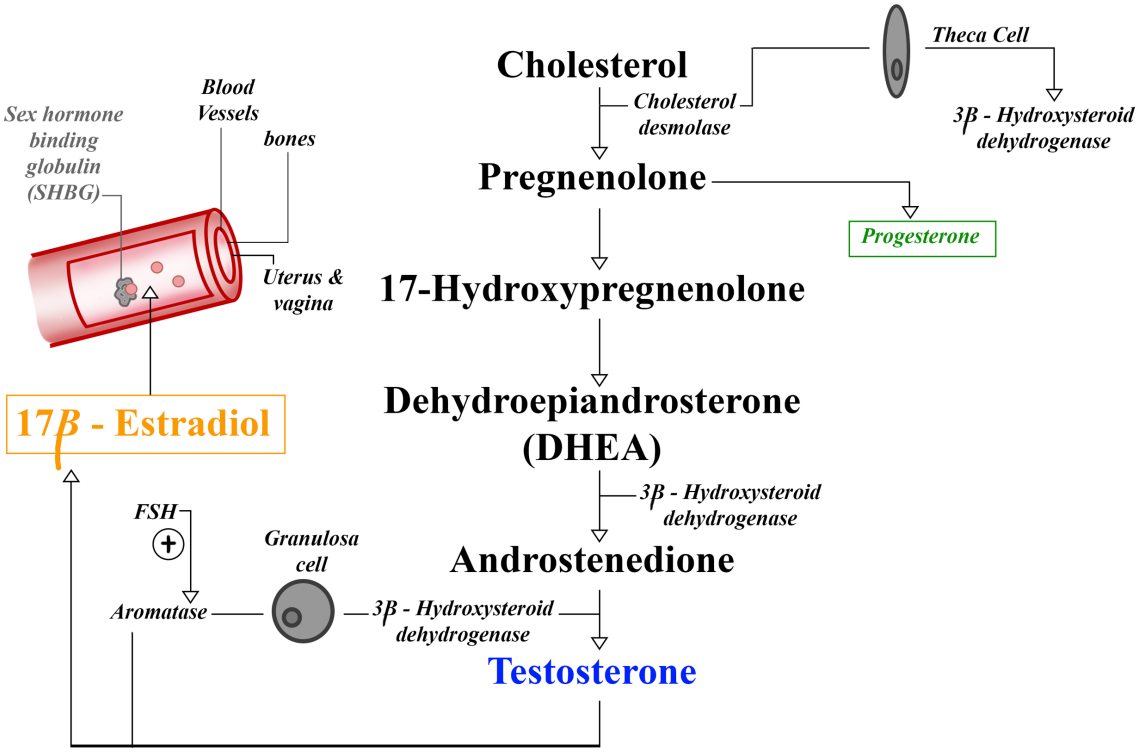
As reported before, estrogen and progesterone are two steroid hormones, meaning that their production starts with cholesterol (Bernhard & Winfried, 2016; Osmosis from Elsevier, 2022). Cholesterol is initially processed in the theca cells by the enzyme cholesterol desmolase, which converts cholesterol into pregnenolone (Osmosis from Elsevier, 2022). Additionally, theca cells release the enzyme 3β-hydroxysteroid dehydrogenase, which converts pregnenolone into progesterone (Tomomi et al., 2015; Burger, 2020; Osmosis from Elsevier, 2022). However, most pregnenolone is transformed into 17-hydroxypregnenolone and subsequently into dehydroepiandrosterone (DHEA), which becomes androstenedione – a precursor of testosterone (Tomomi et al., 2015; Osmosis from Elsevier, 2022). Furthermore, androstenedione diffuses into the granulosa cells, which produce two enzymes (Osmosis from Elsevier, 2022). First, the enzyme 17β-hydroxysteroid dehydrogenase converts androstenedione into testosterone, and second, the enzyme aromatase converts testosterone into 17β-estradiol (Tomomi et al., 2015; Osmosis from Elsevier, 2022).

Finally, FSH stimulates aromatase activity, leading to the release of 17β-estradiol into the bloodstream during the follicular phase (Osmosis from Elsevier, 2022). Nonetheless, this estrogen is predominantly bound to Sex Hormone Binding Globulin (SHBG), which transports it to nearby tissues such as the uterus, vagina, as well as other estrogen-responsive cells and tissues like bones and blood vessels (Osmosis from

Elsevier, 2022). Figure 3 represents the synthesis of progesterone, testosterone, and 17β-estradiol from cholesterol.

Figure 3

Synthetization of Progesterone, Testosterone and 17β-estradiol from Cholesterol



Note. Adapted from: Osmosis from Elsevier, 2022.

2.4 Estrogen and Progesterone effects in the body

Estrogen and progesterone exert both local and systemic effects throughout the body, affecting mood (Rector & Friedman, 2018; Wharton et al., 2012), physical health (Cauley, 2015) as well as the behavior (McEwen & Milner, 2016). Although early in life the development of female reproductive organs depends on the absence of testosterone and not on estrogens (Bernhard & Winfried, 2016), from puberty onward, these hormones play essential roles in the maturation of female reproductive organs such as the fallopian

tubes, uterus, cervix, and vagina (Osmosis from Elsevier, 2022). Indeed, estrogen also contributes significantly to the development of secondary sexual characteristics in females, including breast growth, hip widening, and the distribution of fat on the buttocks, hips, and thighs (Osmosis from Elsevier, 2022).

On a systemic level, estrogen provides protective effects on cardiovascular health by maintaining the flexibility of blood vessel walls and reducing levels of LDL cholesterol, thereby lowering the risk of cardiovascular diseases (Persson et al., 2011). It also supports bone health by preserving bone density (Baños de MacCarthy & Perez-Torres, 2011; Osmosis from Elsevier, 2022). Moreover, progesterone as well presents also non-reproductive functions, like maintaining bone strength and promoting skin elasticity (Osmosis from Elsevier, 2022). Nevertheless, most sex differences in the pulmonary system are primarily attributed to fluctuations in circulating hormones and structural changes (Harms et al., 2016). For example, Carlberg and colleagues (1984) showed that fluid retention and subsequent increases in pulmonary capillary blood volume, which affect gas exchange in the lungs, are influenced by estrogen levels.

Additionally, hormones play a significant role in emotional and cognitive processing (Toffoletto et al., 2014). Both estrogen receptors (ER) and progesterone receptors (PR) are present in brain regions crucial for the emotional and cognitive processing of information, such as the amygdala, cerebral cortex, cerebellum, and certain diencephalic structures (Brinton et al., 2008; Toffoletto et al., 2014). Notably, in 2005, Goldstein and colleagues examined two different phases of the menstrual cycle to study the effects of estrogens on emotional processing in premenopausal women. Through an fMRI paradigm, the researchers compared the brain activity of twelve women during the early follicular phase (characterized by low levels of estrogens and progesterone) and the

midcycle phase (characterized by low levels of progesterone but high levels of estrogen) while they viewed negative versus neutral stimuli (Goldstein et al., 2005). The results showed a greater BOLD signal during the early follicular phase compared to the midcycle phase in the central amygdala, paraventricular and ventromedial hypothalamic nuclei, hippocampus, orbitofrontal cortex (OFC), anterior cingulate gyrus, and peripeduncular nucleus of the brainstem when presented with negative stimuli (Goldstein et al., 2005). These findings suggest that estrogen may dampen arousal in women through cortical-subcortical control within the HPA circuitry (Goldstein et al., 2005).

In conclusion, these hormone-mediated effects underscore their critical roles not only in reproductive physiology but also in overall health and well-being (Osmosis from Elsevier, 2022). The complex relationship between hormones and their effects on the body and emotional processing must be considered, especially when patients experience changes in their hormonal systems, such as when taking oral contraceptives. This topic will be discussed further, particularly concerning vulvodynia, as (exogenous) hormones may directly affect this syndrome and influence other aspects of female health such as arousal and sexuality (Toffoletto et al., 2014; Caruso et al., 2014; Hahn & Cobey, 2019). It is crucial to avoid the bias of thinking that sex hormones only have localized effects. When studying hormones and vulvodynia, it is important to consider their impact on the entire body and mind to achieve the best possible outcome in terms of healing.

2.5 Inflammation in Vulvodynia: Mast Cells, Nerve Growth Factor and Steroid Hormones

In general terms, inflammation can be defined as the response of the immune system to potentially harmful stimuli such as pathogens, injury, and metabolic stress

(Antonelli & Kushner, 2017). According to Graziottin et al. (2022), inflammation is a crucial process for survival that can be categorized into two types: “Resolving Inflammation”, which aims for short-term, essential goals in the female organism, and “Non-resolving” or “Pathologic Inflammation”, leading to chronic tissue destruction. This biological process exists on a continuum of adaptiveness (Graziottin et al., 2022). Furthermore, inflammation is modulated by a psycho-neuro-immuno-endocrine loop, with female sexual hormones having a crucial role given their interaction with immune cells (Straub, 2007; Kovats, 2015).

Mast cells (MCs) play a crucial role in the inflammatory process (Frenzel & Hermine, 2013). Interestingly, this specific type of immune cell can be activated by a wide range of heterogeneous stimuli, including infections, chemical and physical insults, menstrual blood in tissues, mechanical trauma (such as intercourse), cell death, stress, and fluctuations in estrogen and progesterone levels (Gaudenzio et al., 2016; Kaur et al., 2017; Jensen et al., 2010; Graziottin, 2018). These cells are characterized by different types of vesicles that release a variety of molecules during degranulation (i.e., the process by which MCs release stored molecules in response to activating signals; Frenzel & Hermine, 2013; Gaudenzio et al., 2016). Molecules released during degranulation include bradykinin, vasoactive factors (responsible for edema and swelling), histamine, serotonin, and nerve growth factor (NGF; Frenzel & Hermine, 2013). NGF, in particular, stimulates nerve endings and induces their proliferation (Aloe, 2004).

Notably, when NGF is near the nerve fibers responsible for pain transmission, it promotes their multiplication and causes them to move closer to the surface (Bornstein et al., 2004; Awad-Igbaria et al., 2024). This process is essential for understanding the heightened pain perception experienced by patients suffering from vulvodynia

(Graziottin, 2018). These patients exhibit hypersensitivity in the vulvar region, which is linked to an increased number of MCs (Awad-Igbaria et al., 2024). Furthermore, these MCs are not only more numerous but also more active, leading to the release of greater amounts of NGF (Bornstein et al., 2004). As a result, pain-transmitting nerve fibers proliferate and become more superficial (Bornstein et al., 2004). Normally, these fibers would reside deeper within the tissue, but their migration to the surface causes the brain to interpret these signals as stemming from a more severe injury (Sarchielli et al., 2010; Graziottin, 2018). Indeed, patients with vulvodynia often describe their pain as a burning or stabbing sensation (Bornstein et al., 2016). Moreover, Weström and Willén (1998) found, through a histopathological study, that proliferating nerve cell fibers are mainly located between epithelial cells and surrounding the vessels. Nevertheless, in their study there was a statistically significant positive correlation between this proliferation and the status of inflammation in patients (Weström and Willén, 1998).

As mentioned earlier, MC degranulation can be triggered by various agonists, including hormonal fluctuations in estrogen and progesterone (Gaudenzio et al., 2016; Kaur et al., 2017; Jensen et al., 2010). This explains why many women experience a worsening of symptoms during menstruation (Estibeiro et al., 2022). Specifically, the phase preceding menstruation involves a decrease in estrogen levels (Tomomi et al., 2015). On the other hand, progesterone (P4) has anti-inflammatory properties and, along with testosterone, helps keep MC activity “silent” (Gibbons & Chang, 1972; Fedotcheva et al., 2022; Jensen et al., 2010). However, when P4 levels drop just before menstruation, MC activation occurs, leading to increased pain perception in affected patients (Graziottin, 2018).

Finally, the central nervous system (CNS) and the hypothalamic-pituitary axis (HPA) play crucial roles in this hormonal-inflammatory interplay (Straub, 2007). It is also important to note that stress can induce MC degranulation, highlighting the need for stress regulation in patients suffering from vulvodynia (Theoharides, 2020). The hyperactivity of MCs may lead to a shift from nociceptive pain (i.e., caused by tissue damage) to neuropathic pain, where pain signals are generated by the fibers and pathways themselves, rather than by an external stimulus (Vallvé-Juanico et al., 2019; Sarchielli et al., 2010). Neuropathic pain, in this context, becomes a disease in its own, rather than merely a symptom of injury (Graziottin, 2018). Nevertheless, mechanisms leading to neuropathic pain can extend resulting in both peripheral and central nervous system lesions (Sarchielli et al., 2010).

In conclusion, progesterone, estrogens, and androgens have different roles in the inflammatory process. Indeed, while progesterone and androgens inhibit and regulate inflammation, estrogens may have either anti-inflammatory or pro-inflammatory effects depending on the specific type of estrogen, as well as other factors mentioned above, and their levels in the body (Graziottin et al., 2022).

2.6 Contraception: What It Is and When to Use It

This section explores hormonal contraception, its definition, and its various applications. Although the term “contraception” suggests prevention of pregnancy, it is crucial to recognize that this is not the only purpose for which hormonal contraception is used (Brynhildsen, 2014). For instance, it is often an effective treatment for alleviating pain associated with endometriosis (Grandi et al., 2019) or dysmenorrhea (Brynhildsen,

2014). Additionally, postmenopausal women may consider hormone replacement therapy (HRT) to mitigate symptoms of menopause, such as hot flushes (Maclennan et al., 2004).

Contraceptive methods can broadly be categorized into two types: barrier and non-barrier contraception (Colquitt & Martin, 2015). Barrier contraception includes methods that physically prevent sperm from entering the uterus (Casey, 2023). Specifically, this category encompasses male and female condoms, diaphragms, cervical caps, contraceptive gels, contraceptive sponges, and spermicides (Casey, 2023). Notably, only male and female condoms offer protection against sexually transmitted infections (STIs; Vijayakumar et al., 2006; McKay, 2007). The diaphragm – a cup made of latex or silicone that covers the cervix (Secor, 1992) – is associated with an increased risk of urinary tract infections (UTIs) and incomplete bladder emptying (Colquitt & Martin, 2015; Fihn et al., 1985). This is particularly relevant in the context of vulvar pain, as there is a strong association between interstitial cystitis (IC), vulvodynia, and dyspareunia (Gardella et al., 2011). While this research does not focus on barrier contraception, it is essential to highlight that women today have a wide variety of contraceptive methods to choose from, depending on their individual circumstances and lifestyles (Colquitt & Martin, 2015). Indeed, clinicians must be prepared to explain the potential benefits and side effects associated with each contraceptive method and methods that may increase the risk of UTIs should be avoided in patients with vulvodynia.

Non-barrier contraception refers to methods that alter the production levels of endogenous sex steroid hormones in the female gonads – specifically, estrogens and progesterone – to prevent ovulation (Levin & Hammes, 2011; Hahn & Cobey, 2019). Synthetic progesterone, or progestin, suppresses GnRH in the hypothalamus, which in turn lowers LH levels, thereby preventing ovulation (Teal & Edelman, 2021). Similarly,

synthetic estrogens inhibit gonadotropins and FSH, which prevents the development of a dominant follicle (Teal & Edelman, 2021). The reduction of LH also leads to a decrease in testosterone (T), which is associated with increased hepatic production of SHBG and a reduction in free testosterone levels (Burrows et al., 2012; Zimmerman et al., 2024). Consequently, because these products function by suppressing ovulation through synthetic forms of estrogens and/or progestins, they require a prescription from a medical professional after a thorough evaluation of the patient's medical history, menstrual cycle, and adherence (Levin & Hammes, 2011).

Non-barrier contraceptive methods include spermicides, oral contraceptive pills, injections, implants, patches, and intravaginal and intrauterine devices (IUDs; Colquitt & Martin, 2015; Teal & Edelman, 2021). Generally, the effectiveness of non-barrier contraceptives for preventing pregnancy ranges from 78% to 99.9%, depending on the specific method used (Levin & Hammes, 2011; Colquitt & Martin, 2015). Commonly used synthetic progestins include norethindrone, drospirenone (DRSP), levonorgestrel (LNG), and medroxyprogesterone acetate (DMPA), which is an injectable form (Teal & Edelman, 2021). The most widely used synthetic estrogen in hormonal contraception is ethinylestradiol (EE; Teal & Edelman, 2021).

The oral contraceptive pill is the most commonly used form of hormonal contraception, with over half of women (65.3%) aged 15 to 49 in the United States currently using some form of contraception (Daniels and Abma, 2020). Specifically, 14% of these women use oral contraceptives (OC; Daniels and Abma, 2020). The pill must contain synthetic progestin, though it is not always combined with estrogens (Hahn & Cobey, 2019). Pills containing only progestin are known as the "minipill" (Levin & Hammes, 2011), while those containing both progestin and estrogens are called

"Combined (Oral) Contraceptives" (COCs; Hahn & Cobey, 2019). The typical pill pack includes 21 active pills and, optionally, 7 placebo pills to maintain the habit of daily intake while inducing a "fake menstruation" that mimics menstruation (Freeda, 2019).

In conclusion, there is a wide variety of contraceptive options available today, allowing individuals to choose the most suitable method based on their preferences and medical history. It is essential to recognize the unique characteristics of each contraceptive method and to consider individual differences when selecting the most appropriate option, whether for medical reasons or purely for contraception.

2.6.1 Positive and Negative Effects of Hormonal Contraception

This section will briefly discuss the *general* positive and negative side effects associated with hormonal contraception. The term "general" is used here because the next chapter will focus specifically on the interaction between vulvodynia and hormonal contraception, which will not be covered in this section. However, it is crucial to consider all potential advantages and disadvantages of hormonal contraception, even those not directly related to vulvar pain, such as diminished sexual arousal (Hahn & Cobey, 2019).

One of the main positive effects of hormonal contraception is the significant reduction in the risk of developing certain cancers, such as ovarian and endometrial cancer (Cibula et al., 2010; Hannaford et al., 2007; Hannaford et al., 2010). Additionally, hormonal contraceptives can enhance female sexual well-being by alleviating concerns about unwanted pregnancies (Graham et al., 2004) and boosting self-esteem, like in cases of acne caused by hormonal imbalances (Lucky et al., 1997; Redmond et al., 1997; Burrows et al., 2012). Moreover, as mentioned in the previous section, hormonal

contraceptives can also be beneficial for managing gynecological conditions such as dysmenorrhea or endometriosis (Burrows et al., 2012).

One of the major side effects of COCs frequently noted in the literature is the potential alteration of sexual behavior, such as decreased sexual satisfaction (Roberts et al., 2012; Hahn & Cobey, 2019). However, the impact of COCs on female sexuality remains a subject of mixed findings. For example, while it is established that androgen deficiency can lead to decreased female libido (Bachmann & Oza, 2002) or reduced lubrication, orgasm frequency, and relationship satisfaction, the effects on female sexuality are not consistent across studies (Both et al., 2019). It is also important to consider the variety of COCs available and that their effects may vary depending on the specific combination of hormones used (Mark et al., 2016). Furthermore, individual differences among women play a significant role, as evidenced by the review by Pastor and colleagues (2013), which found that while 15% of women reported a decrease in libido, 85% experienced either an increase or no change.

Another debated side effect of hormonal contraception is its potential impact on psychological well-being, particularly in relation to depressive symptoms. Indeed, the recent study of Johansson et al. (2023) confirms an increased risk of developing depressive symptoms in OC users compared to never users especially in the first 2 years. However, findings in this area remain inconsistent. For instance, O'Connell and colleagues (2007) conducted a three-month placebo-controlled trial to investigate the side effects of oral contraceptives (OCs) in adolescents and the potential benefits of OCs in treating dysmenorrhea. The study collected data using the pain subscale of the Moos Menstrual Distress Questionnaire (MMDQ) for assessing menstrual cycle pain, and the Center for Epidemiologic Studies Depression Scale (CES-D) to evaluate depression

levels (O'Connell et al., 2007). In addition to psychological assessments, physiological measures such as weight and height were also recorded. The study aimed to evaluate OC side effects by first asking participants to list any experienced side effects or changes, and then by having them assess their experience with 12 specific side effects, including headache, nausea, acne, abdominal pain, back pain, vomiting, breast tenderness, breast enlargement, mood swings, weight gain, premenstrual syndrome, and irregular bleeding (O'Connell et al., 2007). Overall, the results showed that both the placebo and OC groups reported similar types and quantities of side effects, suggesting that perceived rather than actual hormonal side effects often lead to discontinuation of OCs (O'Connell et al., 2007). This has significant implications, as discontinuing OCs without switching to another contraceptive method can increase the risk of unwanted pregnancies (O'Connell et al., 2007). However, incongruent findings might also be due to the different duration between studies.

Other negative effects associated with hormonal contraception include an increased risk of breast cancer (Fitzpatrick et al., 2023), a heightened risk of developing vulvodynia (Bouchard et al., 2002), a decrease in the volume and thickness of the labia minora (Battaglia et al., 2012), and an elevated risk of bacterial infections (Peebles et al., 2020). Specifically, users of IUDs have a higher risk of bacterial vaginosis (BV; Peebles et al., 2020), and COC use may increase the incidence of vaginal candidiasis and alter the vaginal microbiome (Van de Wijgert et al., 2013).

In conclusion, hormonal contraception is linked to both positive and negative effects, and findings in this area are often controversial. It is crucial to tailor hormonal contraception to the patient's individual needs and medical history. The next section will

focus on a specific risk associated with hormonal contraception: the risk of developing vulvodynia.

2.7 Hormonal contraception as a risk factor for vulvodynia

Pharmacologically induced hormonal changes (i.e., hormonal contraception) are listed as one of the primary potential factors associated with vulvodynia by the 2015 International Society for the Study of Vulvovaginal Disease (ISSVD), International Society for the Study of Women's Sexual Health (ISSWSH), and the International Pelvic Pain Society (IPPS) vulvar pain and vulvodynia consensus terminology (Goldstein, 2020; Bornstein et al., 2016). Consequently, this section will discuss the scientific evidence investigating the relationship between hormonal contraception and vulvodynia.

Scientific research has indicated a link between synthetic hormone intake and an increased risk of developing vulvodynia (Berglund et al., 2002). For instance, the case study of Goldstein et al. (2010) reported the case of a 24-years-old woman who developed vestibulodynia with introital dyspareunia after the intake of OCs for 18 months (Goldstein et al., 2010). Notably, at the beginning of the study, the patient presented with a pain threshold of 10 grams, while after 3 months without taking OCs and by applying a compound of estradiol (0.03%) and testosterone (0.1%) on the vestibule twice daily, she showed a pain threshold of 400 grams (Goldstein et al., 2010). The changes observed in the patient throughout the study suggest that women using oral contraceptives (OCs) may experience decreased pain thresholds in the vulvar vestibule (Goldstein et al., 2010). In fact, Goldstein et al. (2010) concluded that women with low levels of free testosterone and estradiol who use OCs may fail to synthesize these receptors, leading to vestibulodynia.

However, the relationship between synthetic hormone intake and the risk of developing vulvodynia is complex. Both the duration of use and the composition of hormonal contraceptives must be considered when examining this potential link (Bouchard et al., 2002). Two independent studies showed that long-term OC use – more than two years – was associated with an increased risk of vulvar pain (Bouchard et al., 2002; Berglund et al., 2002). Additionally, the relative risk of developing vulvodynia tends to decrease with an older age at first use (Bazin et al., 1994; Bouchard et al., 2002). Furthermore, the progestogenic, estrogenic, and androgenic potency of the pills also influences the relative risk of developing vulvar vestibulitis (Bouchard et al., 2002; Greenstein et al., 2007). For example, Bouchard and colleagues (2002) identified a high-risk profile for OC use as pills with high progestogenic, low estrogenic, and high androgenic potency (with a relative risk of 19.0 for developing vulvar vestibulitis), whereas a low-risk profile was defined for OCs with low progestogenic, high estrogenic, and low androgenic potency (with a relative risk of 3.0). Reduced estrogen levels, in conjunction with stable progesterone levels, may induce structural changes in the vagina due to reactions to sex steroid hormone deprivation and substitution (Greenstein et al., 2007). Specifically, OCs may directly affect the vestibular epithelium through the action of steroid hormone receptors in the vulva, potentially altering the vulvar mucosa, increasing the inflammatory response, and exacerbating pain during touch and dyspareunia (Bouchard et al., 2002). These structural and mucosal alterations may contribute to an increased risk of developing vulvar vestibulitis syndrome (Greenstein et al., 2007).

Regarding morphological changes in the epithelium, Johannesson et al. (2007) compared 45 women – 25 non-COC users and 20 who had used COCs for at least one

year – to evaluate the impact of COCs on vestibular morphology. Their findings revealed that COC users had significantly greater distances between the dermal papillae and a higher number of superficial small vessels compared to non-users (Johannesson et al., 2007). This led to the conclusion that COCs may induce morphological alterations in the vulval vestibular mucosa, potentially making it more susceptible to mechanical stress and contributing to superficial dyspareunia. Similarly, both OC and depot-medroxyprogesterone have been shown to increase epithelial thickness (Ildgruben et al., 2003), with a higher number of immune cells observed in the epithelium of levonorgestrel and depot-medroxyprogesterone users (Ildgruben et al., 2003). However, Battaglia et al. (2012) reported the opposite effect in the vaginal introitus and labia minora of OC users, demonstrating a significant reduction in the thickness of both areas after three months of OC use. Nonetheless, the Pulsatility Index (PI) of the dorsal clitoral artery and posterior labial artery increased following three months of treatment (Battaglia et al., 2012).

Although the studies mentioned above suggest an increased risk of developing vulvar pain in users of synthetic hormones, these findings have not always been consistently replicated. For example, Edgardh and Abdelnoor (2007) did not observe any statistically significant increase in the risk of vulvar pain among OC users. Similarly, Reed et al. (2013) found no association between OC use and vulvodynia, nor did they find a link with the duration of contraceptive intake. As a matter of fact, this raises the question of why these differences in results exist. A possible answer can be found in the genetics differences of patients (Goldstein et al., 2014). This topic will be presented in the following section.

2.7.1 The Role of Genetics

The use of CHCs reduces serum-free testosterone (FT) leading Goldstein et al. (2014) to hypothesize that, because of androgenic effects, CHCs provoke a dysfunction of the vestibular glands, eventually resulting in PVD. Nevertheless, through the androgen receptor gene (AR), which contains a highly polymorphic cytosine-adenine-guanine (CAG) trinucleotide repeat sequence located on the X chromosome that regulates androgen signaling in steroid hormone-sensitive cells, testosterone exerts its effects on gene expressions (Goldstein et al., 2014).

Taking this all together, Goldstein et al. (2014) investigated the number of CAG repeats in the AR gene in women who developed PVD while using CHCs compared to women who did not develop PVD under CHCs. Notably, evidence shows that the overall number of CAG repeats was significantly smaller (p=0.025, d=0.53) in the control group (20.61±2.19) than in the study one (22.05±2.98; Goldstein et al., 2014). Finally, the analysis of free-testosterone levels, conducted exclusively on women who were taking CHCs containing drospirenone, demonstrated that the average of free testosterone in the control group was significantly lower (p=0.042, d=0.69) than the study group (0.189 ± 0.115 ng/dL vs. 0.127 ± 0.054 ng/dL, respectively; Goldstein et al., 2014).

Considering their results, authors infer that women who manifested PVD while taking CHCs were more likely to have an AR gene with more CAG repeats than women who didn't show any sign of PVD or dyspareunia (Goldstein et al., 2014). Stated differently, evidence sustains that PVD could be induced by CHCs in predisposed women and a predisposing factor is an AR gene with a higher number of CAG repeats (Goldstein et al., 2014). However, it has not yet been established whether the number of CAG repeats in the AR gene is the most important factor, but it suggests an important influence by the

genotype in provoking PVD that needs further investigation (Goldstein et al., 2014). In conclusion, Goldstein et al. (2014) hypothesized that the possible development of CHC-induced PVD is due to the combination of lowered FT with an ineffective AR receptor, explaining also why the control group didn't have PVD despite a longer intake of CHC compared to the study group (in years).

Finally, Graziottin and Murina (2011) emphasize the need for caution when attributing side effects to the pill, as these do not always occur. They recommend that when patients using hormonal contraception experience vaginal dryness or pain during intercourse, a thorough examination of the levator ani muscle and vaginal pH should be conducted (Graziottin & Murina, 2011). In conclusion, multiple factors must be considered when exploring the relationship between hormonal contraception and vulvodynia. This leads us to our research question, which is presented in the following section.

2.8 The Present Study

As demonstrated in the previous section, the relationship between synthetic hormones and the risk of developing vulvodynia is highly complex. On one hand, there is documented evidence of an increased risk of vulvodynia in patients who have used hormones, particularly for more than two years (Bouchard et al., 2002; Berglund et al., 2002). However, this relationship is not linear, as vulvodynia is, in most cases, a multifactorial condition. For instance, not all individuals with vulvodynia have taken synthetic hormones prior to the onset of vulvar pain symptoms. In fact, other factors and comorbidities, such as endometriosis, also play a significant role in the development of vulvodynia (Trutnovsky et al., 2018). Similarly, not all women who have taken oral

contraceptives (OC) develop vulvar pain syndromes, with one possible explanation being related to CAG repeat polymorphisms (Goldstein et al., 2014).

However, in our sample, previous analyses exploring the association between vulvar pain and hormonal contraception use have already been conducted. Hormonal contraception was categorized into combined hormonal contraceptives (CHC), progesterone-only contraceptives, and "unknown". Notably, the results indicate that both types of hormonal contraception were positively associated with vulvar pain and vaginal dryness. Furthermore, 53% of the participants reported continuing the same contraceptive method, even though it caused vulvar pain (Kiesner & Bittoni, 2024).

Nonetheless, as for vulvodynia, not all females taking hormonal contraception face negative effects. In fact, different hormonal doses and individual differences must be taken into account when examining the potential side effects of hormonal contraception (Pastor et al., 2013; Mark et al., 2016). For example, while some women report mood swings or worsening depression symptoms, others experience increased self-esteem and an improved sexual life (Redmond et al., 1997; Burrows et al., 2012; Johansson et al., 2023). However, it is possible that greater susceptibility to known side effects of hormonal contraception may also increase the risk of developing vulvar pain associated with its use.

That said, spontaneous remission of symptoms is also common in patients. Davis et al. (2013) demonstrated that in a sample of 239 patients, 41% experienced a reduction in pain symptoms without receiving any treatment over a two-year period. Further evidence is provided by Reed et al. (2016), where 50.6% of their sample showed remission without relapse. However, 39.7% of participants experienced remission followed by relapse, while 9.6% reported persistent vulvar pain (Reed et al., 2016).

Factors that were significantly associated with relapse and persistence included increased pain severity during or after intercourse, a longer duration of symptoms, and a diagnosis of fibromyalgia (Reed et al., 2016). In addition, factors associated only with relapse included provoked pain and a positive diagnosis of interstitial cystitis (IC) at the time of vulvodynia diagnosis (Reed et al., 2016). In contrast, factors significantly associated with persistent pain included more severe pain during intercourse and pain during oral sex or partner touch (Reed et al., 2016).

Building on the findings of Reed et al. (2016), Pâquet and colleagues (2019) conducted a seven-year longitudinal study aimed at identifying different profiles of women with persistent vulvar pain compared to those who experienced symptom remission. Their first finding reaffirmed that the majority of women (71.1%) showed a decrease in pain symptoms (Pâquet et al., 2019). However, a second group (28.9%) exhibited persistent vulvar pain, with factors significantly associated with persistence including older age at pain onset, pain in locations other than the vulvar vestibule, and anxiety (Pâquet et al., 2019). Nonetheless, depression has also been shown to be linked to vulvar pain symptoms (see "1.5.2 Psychological Factors"; Masheb et al., 2005; Khandker et al., 2011).

Depression is characterized by feelings of sadness, irritability, and emptiness (Dagostin Ferraz et al., 2024). It is well established that depression negatively affects cognitive functions, including memory (Kizilbash et al., 2002) and attention (Keller et al., 2019). Anxiety, on the other hand, is marked by persistent worry and fear (Ruscio et al., 2017), with anticipation being a key feature (LeDoux, 2015). Anxiety often involves the anticipation of threats that may not occur, leading to uncertainty (LeDoux, 2015). Like depression, anxiety has also been shown to impair cognitive functioning (Kizilbash et al.,

2002). Moreover, both conditions hyperactivate the hypothalamic-pituitary-adrenal (HPA) axis (Mello et al., 2003; Chen et al., 2014). This hyperactivity can trigger mast cell (MC) degranulation (Theoharides, 2020), which, as discussed in the section "*2.5 Inflammation in Vulvodynia: Mast Cells, Nerve Growth Factor and Steroid Hormones*", can result in neuropathic pain (Vallvé-Juanico et al., 2019; Sarchielli et al., 2010). Furthermore, in their meta-analysis, Dagostin Ferraz and colleagues (2024) found that women with vulvodynia had higher somatization scores compared to those without vulvar pain. In other words, depression and anxiety have a more pronounced impact on somatic pain in patients with vulvodynia compared to healthy individuals (Dagostin Ferraz et al., 2024).

Considering that Pâquet et al. (2019) found that the diffusion of vulvar pain symptoms is associated with persistent pain and knowing that hormonal contraception can lead to vulvar pain (Battaglia et al., 2012; Goldstein et al., 2014), we sought to investigate whether hormonal contraception increases the risk of spreading vulvar pain compared to pain localized only in the vestibule. Acknowledging the complex relationship between vulvar pain and hormones, we further examined whether this risk was higher among those who experienced negative side effects from hormonal contraception. Additionally, we explored the role of depression, anxiety, age at the onset of vulvar pain, and the patient's age as potential risk factors for the spread of vulvar pain symptoms versus localized vestibular pain. We focused on the diffusion of vulvar pain because it is recognized as a key factor in the persistence of vulvodynia. Indeed, investigating the underlying mechanisms may provide new insights and lead to novel treatment approaches, with the hope of alleviating symptoms for this population.

Moreover, evidence suggests that hormonal contraception can cause morphological changes in the vestibule, which could lead to pain characterized by vaginal dryness or other physical alterations (Johannesson et al., 2007). This type of pain may differ in sensation from vulvar pain caused by other factors. This raised the question of how hormonal contraception affects the characteristics of pain – whether as a burning, stabbing, or another type of sensation – and how these descriptions differ between women who use hormonal contraception and those who do not. The purpose of this second analysis was to distinguish between different causes of vulvar pain. If significant differences are found between the two groups in how they describe their pain, it could offer a new avenue for clinical investigation into the underlying causes of pain.

Taking all of this into account and recognizing the complexity of the relationships between these factors, our study aimed to:

1) Investigate the effects of hormonal contraception use and its negative side effects, along with depression, anxiety, age at onset of vulvar pain, and the subject's age, on the diffusion of vulvar pain compared to pain localized to one area.

2) Examine the reported pain sensations in women with vulvodynia who use hormonal contraception compared to those who suffer from vulvodynia but do not use hormonal contraception. We hypothesized differences in the type of pain reported between these two groups.

Chapter 3: METHODS

The analysis and results presented are part of the study "*Luci e Ombre della Sessualità e Salute Genitale Femminile*", conducted by the *Padova Sex Lab* at the University of Padua. The project aims to investigate the prevalence of female genital pain and the bio-psycho-social factors that influence female sexual behavior. Specifically, this study explores vulvar and vaginal pain from psychological, sociocultural, and medical perspectives, with the goal of improving the understanding of its prevalence and characteristics. This, in turn, will help identify risk factors, inform prevention strategies, and enhance treatment options. The data were collected online, and the research has been approved by the Ethical Committee for Psychological Research at the University of Padua.

3.1 Participants

The inclusion criteria for participating in the study were twofold: participants had to be assigned female at birth (AFAB) and be over 18 years old. The lab opted for broad inclusion criteria to estimate the prevalence of vulvar pain symptoms in the general female population. The first questionnaire was completed by 2'199 females. Only those participants who completed at least 75% of the questionnaire were included in the analyses, resulting in a starting sample of 1'923 participants. The participants' ages ranged from 18 to 64 years (mean age = 30±10). The majority of participants identified their gender as female (*n*=1'886; 98.08%), followed by non-binary (*n*=19; 1%). A total of 80.06% of the participants identified as heterosexual (*n*=1,538), 8.9% as bisexual (*n* =170), 4.5% as bi-curious (*n* =88), 2.5% as homosexual (*n* =47), and the remaining 4% identified as asexual, gender-fluid, pansexual, or other. Regarding religion, the majority

of participants were Christian (*n* =745; 38.80%), atheist (*n* =600; 31.25%), or agnostic (*n* =473; 24.64%). Moreover, most participants were Italian nationals (*n* =1,885; 97.97%).

In our sample, 978 (50.86%) subjects reported no genital pain, 292 (15.19%) reported vulvar pain, 155 (8.1%) experienced vaginal pain, and 498 (25.9%) reported both vulvar and vaginal pain. Regarding depression diagnoses, 245 participants (12.74%) reported having been diagnosed with depression at some point in their lives. Of these, 97 (39.59%) were in the group that reported no genital pain, while 148 (60.41%) of those with a depression diagnosis experienced vulvar and/or vaginal pain. Specifically, 43 (17.55%) reported vulvar pain, 21 (8.57%) reported vaginal pain, and 84 (34.29%) experienced both vulvar and vaginal pain. Regarding anxiety, 316 participants (16.43% of the total sample) had been diagnosed with anxiety at some point in their lives. Among them, 50 participants (15.82%) reported experiencing vulvar pain, while 123 (38.92%) suffered from vulvo-vaginal pain.

Concerning the age at onset of vulvar pain symptoms, most participants indicated that their painful symptoms began around the age of 20 (25.13%), followed by 25 (15.06%) and between the ages of 18 and 19 (12.62%). Interestingly, 54 participants (5.73%) reported experiencing genital pain before the age of 10, while 52 participants (5.51%) could not recall the exact age at symptom onset. Table 1 illustrates the percentage and number of participants for each age of onset.

Table 1

Distribution of Age at Onset of Genital Pain: Number and Percentage

Age at Onset	*N*	Percentage
Don’t remember	52	5.51%
Before 5	19	2.02%

Age at Onset	*N*	Percentage
6-7	21	2.23%
8-9	14	1.49%
10-11	21	2.23%
12-13	25	2.65%
14-15	42	4.45%
16-17	90	9.54%
18-19	119	12.62%
20	237	25.13%
25	142	15.06%
30	43	7.74%
35	39	4.14%
40	19	2.02%
45	9	0.95%
50	10	1.06%
Menopause	11	1.17%
Total	943	100%
N Missing	980	

Most of our sample, 1’344 participants (69.89%), reported having used hormonal contraception at some point in their lifetime. Of these, 611 (45.46%) were currently using hormonal contraception. Since the positive association between hormonal contraception use and vulvar pain symptoms in our sample was already addressed in a previous study (Kiesner & Bittoni, 2024), we focused directly on investigating the negative effects of hormonal contraception reported in our sample. The total number of negative side effects reported ranged from 0 to 4. Descriptive statistics indicate that 1’095 participants (57%) did not report any side effects, 672 participants (35%) declared experiencing one side effect, 136 participants (7%) reported two side effects, and 19 participants (1%) experienced three side effects. Additionally, 3 participants (0.15%) reported suffering from four side effects.

3.2 Procedure

The study was promoted both online and in person. For in-person recruitment, bookmarks from the *Padova Sex Lab* were distributed, which included a brief description of the study and a QR code linking directly to the *Qualtrics* platform, where participants could complete the questionnaire. These bookmarks were distributed in various buildings of the University of Padua and at several public events. Online recruitment was conducted through the lab's Instagram page, *@padovasexlab*. This page was created to promote discussions on social media regarding female, male, and transgender sexuality, as well as genital health. The online promotion also received support from various organizations and pages, including the Italian *Vulvodynia and Pudendal Neuropathy Committee*, which operates at a national level.

The study consists of three questionnaires. At the start of each questionnaire, participants are shown a video, created by some members of the *Padova Sex Lab*, which briefly explains the nature of the questions they will be answering. If participants choose to continue after viewing the video, they are asked to carefully read and sign the consent form, which outlines the study's aims and details regarding data processing. Participants declare that they are over 18 years old, voluntarily participating in the study, aware of the study's goals and how their data will be treated and acknowledge that their data cannot be returned once submitted. The first questionnaire includes the following sections:

- *Demographic Information and General Health (17 questions)*
- *Menstrual Health and Contraceptive Use (7 questions)*
- *Pain, Hygiene, and Genital Health (10 questions)*
- *Physical Symptoms (15 questions)*
- *Depressive Symptoms (12 questions)*

- *Anxiety (8 questions)*
- *Sexual Behaviors and Attitudes (25 questions)*
- *Negative Sexual Experiences (3 questions)*

Completing the first questionnaire takes approximately 15 minutes. If a participant report experiencing vulvar or genital pain, she is asked to complete the second questionnaire, which also takes around 15 minutes. Before accepting the consent form for the second questionnaire, the participant is required to enter her email address, which helps link the responses from the first and second questionnaires. The second questionnaire covers:

- *Romantic Relationship (9 questions)*
- *Emotions and Thoughts Related to Pain (24 questions)*
- *Concerns and Influences on Sexuality (26 questions)*
- *Influence on Daily Activities (5 questions)*
- *Strategies for Coping with Pain (21 questions)*
- *General Emotions (12 questions)*
- *Social Support (4 questions)*

Finally, participants are invited to complete the third part of the study. This requires downloading the "Vulvae" app, developed by the French research team *Les Observables SAS*. The app allows participants to map the areas of pain in the vulva each day. Additionally, participants complete a third questionnaire daily – taking about 3 minutes – over a two-month period to track the progression of pain. The third questionnaire includes queries on:

- *Genital Pain (8 questions)*
- *Menstruation (2 questions)*

- *Mood (13 questions)*
- *Physical Symptoms (12 questions)*
- *Sexual Response (16 questions)*
- *Daily Habits/Hygiene (10 questions)*
- *Treatment and Care of Genital Pain (6 questions)*

This research focuses solely on data collected from the first questionnaire. After a series of socio-demographic questions, some questions about the hormonal contraception are asked. The questions on hormonal contraception begin by asking whether the participant has ever used it, and if so, whether they are currently using it. Regarding the type of contraception, participants can choose from various options such as the vaginal ring, combined pill (estrogen-progestin), progestin-only pill, an unspecified pill, subcutaneous devices or implants, copper IUD, hormonal IUD, contraceptive patch, or specify another method in the "other" section. Additionally, participants are asked how long they had used hormonal contraception and whether they had used other types in the past (with the same list of options). The subsequent questions address any side effects experienced from hormonal contraception. Participants are asked to select the contraceptive method that caused side effects (including the option for "none") and then to identify which side effects they experienced. Multiple responses are allowed, with options such as:

- I don't remember
- Headache
- Joint pain
- Gastrointestinal problems
- Cellulite
- Swelling of limbs
- Swollen or painful breasts
- Cramps
- Kidney or back pain
- Acne
- Hirsutism (increased hair growth)
- Mood swings
- Depression
- Anxiety

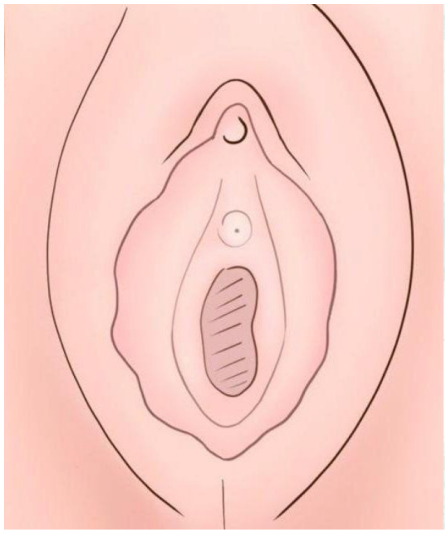
- Nervousness, irritability
- Anger
- Vulvar/genital pain or discomfort
- Vaginal dryness
- Excessive sexual desire
- Decreased sexual desire
- Intermenstrual spotting (spotting between periods)
- Nausea/vomiting/ dizziness
- Other

Moreover, participants are also asked whether they had experienced any positive effects from specific hormonal contraceptives, with the same options as those listed for negative effects. Additionally, they are asked to indicate how many times they have used hormonal contraceptives throughout their life (with options being never, once, twice, three times, four times, or more than four times). Finally, the last part of this section asks if the participant has ever been diagnosed with a psychiatric disorder. For the purposes of our research, we focused on diagnoses of depression and anxiety.

The third section of the questionnaire focuses on the participant's genital health. The initial questions address urinary infections (such as cystitis) and vaginal infections (like candida, chlamydia, or others). Most relevant to this study is the question: *"In general, do you experience discomfort or pain in the vulvar area (external genitals) and/or vaginal area (internal genitals)?"*. Participants can respond with one of the following options: no, yes (on the vulva), yes (inside the vagina), or both (on the vulva and inside the vagina). If the participant responds positively, an image of a stylized vulva is displayed (Figure 4), and they are asked to indicate, by tapping on the screen, the areas where they experience pain. The image allows the selection of up to 24 different parts of the vulva.

Figure 4

Stylized Vulva Presented in the Questionnaire



Another key question in this section of the questionnaire focuses on the type of pain described by participants. If a participant reported experiencing vulvar pain, after selecting the painful areas on the image, they are asked to describe the pain using specific descriptors. These include sensations of *cuts, itching, burning, irritation, pins* or *shocks, heaviness* in the bladder or lower abdomen, or pain/urgency during *urination.* Although the questionnaire also collects data on the type of vaginal pain reported, this information is not part of the analysis in this study.

The final question from the first questionnaire that we analyzed concerns the age at which painful symptoms first appeared *("Approximately when did the first symptoms of genital pain begin?")*. Nonetheless, the first questionnaire also includes additional queries related to sexual health and habits, diagnoses (such as the timing and questions on healthcare system interactions for vulvodynia or other female genital disorders), comorbidities (e.g., endometriosis and fibromyalgia), Persistent Genital Arousal Disorder (PGAD), the Female Sexual Function Index (FSFI) questionnaire, and experiences of sexual harassment and violence, but these topics are not part of the current analysis.

3.3 Measures and Analysis

The primary research question aimed to investigate, through multiple regression analysis, the effects of hormonal contraception use, its negative side effects, age, age at onset of genital pain, depression, and anxiety on the diffusion of vulvar pain, compared to women who experience pain localized to one area. Therefore, only the variables and measures relevant to our study are presented here. The primary dependent variable is the *diffusion of vulvar pain*. The variables for the first research question are the following:

Hormonal Contraception Use. This variable was measured by asking participants: "*Have you ever used hormonal contraceptives (e.g., pill, ring, patch, etc.)?*". Participants answered whether they had ever used such contraceptives, and if so, whether they were currently using them.

Negative Effects of Contraception. This variable represents the total number of side effects reported by the participant. For example, if a participant used the combined contraceptive pill and experienced two side effects, such as headaches and mood swings, and also used the patch, reporting decreased sexual desire (one side effect), the variable would total three reported side effects. In essence, this variable reflects the sum of all negative effects reported by each participant.

Diagnosis of Depression. This variable was created by selecting the option "depression" in response to the question: "*Have you ever received one or more of the following diagnoses? (You can select multiple options)*."

Diagnosis of Anxiety. This variable was created similarly, by selecting the option "anxiety" in response to the same question regarding diagnoses.

Age. This variable was derived from participants' answers to the first question of the questionnaire, which asked for their year of birth.

Age at Onset. This variable pertains to the age at which painful symptoms first appeared, as indicated by the question: "*Approximately when did the first symptoms of genital pain begin?*". The responses (in years) formed the independent variable for *Age at Onset*.

Diffusion of Vulvar Pain. The primary dependent variable, diffusion of vulvar pain, was measured by calculating the total number of areas selected on the image of the stylized vulva (cf. Figure 4). This variable ranges from 0 to 24 zones.

The second research question examined whether the use of hormonal contraception affects the described pain sensation in women suffering from vulvodynia. In order to create the variables related to each *descriptor* of pain, we selected the responses provided to describe pain such as *cuts, itching, burning, irritation, pins* or *shocks, heaviness* and *urination.* These pain descriptors were used to establish the dependent variables for our study. A normal logistic regression was performed to calculate the odds ratios and 95% confidence intervals, in order to determine whether there was a statistically significant difference in the pain descriptors reported by women using hormonal contraception compared to those who were not.

Finally, for addressing the first research question, we included participants who reported experiencing vulvar or vulvo-vaginal pain and had completed all questions related to the relevant variables. This resulted in a final sample of 745 participants. For the second research question, we compared participants who were currently using hormonal contraception with those who were not, focusing on how they reported sensations of vulvar pain. This resulted in a total sample of 1'190 subjects (n= 611 for those currently using hormonal contraception and n= 579 for those who had never used it).

3.4 Correlational Findings on Depression, Anxiety, and Vulvar Pain and Distribution of Painful Vulvar Zones and Types of Reported Pain

Before conducting the multiple regression analysis for our first research question, we performed a correlational analysis between a diagnosis of depression and vulvar pain. The results revealed a significant correlation between experiencing genital pain and having a depression diagnosis (X^2= 15.613, p= 0.0014), indicating that individuals with genital pain were more likely to have been diagnosed with depression. We then conducted a similar analysis for anxiety and vulvar pain. The findings again showed a significant association between reporting vulvar pain symptoms and having an anxiety diagnosis (X^2= 41.699, p< 0.0001), suggesting that women with genital pain were more likely to have been diagnosed with anxiety.

Regarding the number of painful zones in the vulva reported as painful, preliminary findings revealed that most subjects reported pain in 4 zones (n=91; 11.52%). Overall, most participants experienced pain in 3 to 6 vulvar zones (n=300; 37.97%). However, 20.91% (n=165) of participants reported pain in 10 or more zones, while 3.04% (n=24) indicated pain in as many as 20 to 24 zones. Table 2 presents the number of subjects and the corresponding percentages for the number of vulvar zones reported in the sample.

Table 2

Distribution of Participants by Number of Painful Vulvar Zones

Number of Painful Vulvar Zones	*N*	Percentage
0	57	7.22%
1	58	7.34%
2	77	9.75%

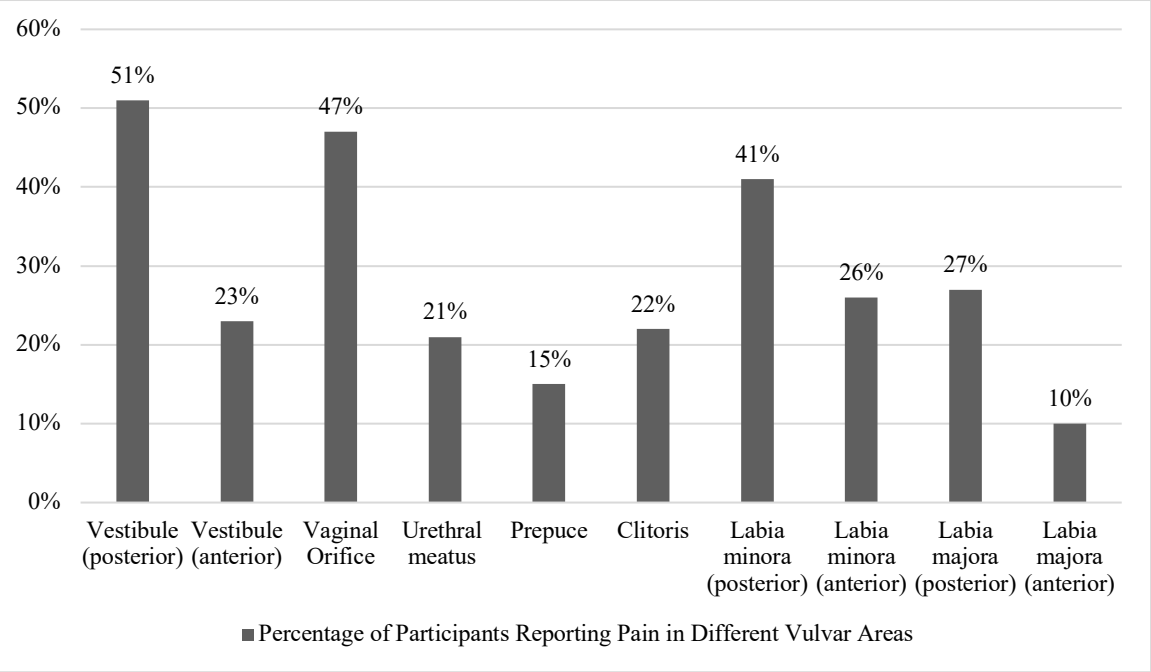
Number of Painful Vulvar Zones	*N*	Percentage
3	64	8.10%
4	91	11.52%
5	81	10.25%
6	64	8.10%
7	56	7.09%
8	47	5.95%
9	30	3.80%
10	28	3.54%
11	22	2.79%
12	27	3.42%
13	13	1.65%
14	15	1.90%
15	16	2.03%
16	3	0.38%
17	7	0.89%
18	6	0.76%
19	4	0.51%
20	2	0.25%
21	4	0.51%
22	3	0.38%
23	7	0.89%
24	8	1.01%
Total	790	100.00%
N Missing	1135	

Concerning the reported painful vulvar sites, the most frequently identified area was the posterior vestibule, reported by 51% (*n*= 406) of participants, while 23% (*n*= 178) of subjects experienced pain in the anterior vestibule. Additionally, 47% (*n*= 373) of participants indicated the vaginal orifice as a painful site. Within the vestibule, 21% (*n*=

165) reported pain at the external urethral meatus. In the labia minora, 26% (*n*= 202) reported anterior pain, and approximately 41% (*n*= 323) experienced pain posteriorly. The clitoris was painful for 22% (*n*= 177) of participants, and around 15% (*n*= 116) reported pain in the prepuce. For the labia majora, pain was reported by about 10% (*n*= 78) of participants in the anterior region, and 27% (*n*= 214) experienced pain in the posterior site. Figure 5 presents the percentages of the sample reporting pain in each vulvar zone.

Figure 5

Bar Graph Showing the Percentage of the Sample Reporting Pain in Each Vulvar Zone



Finally, the second research question in this study investigates differences in how participants using hormonal contraception reported vulvar pain sensations compared to those who were not using it. Interestingly, the most commonly reported sensation among participants with vulvar pain was *burning*, with 590 (74.68%) women indicating this

symptom. The second most frequent sensation was *itching*, reported by 435 (55.06%) participants, followed closely by *irritation* (*n*= 432, 54.68%) and a *pins-and-needles* sensation in the vulva (*n*= 413, 52.28%). Additionally, 330 (41.77%) women described a sensation of *cuts*, while 215 (27.22%) reported experiencing *shocks*. Regarding urinary symptoms, 279 participants (35.32%) experienced pain during *urination*, and 266 (33.67%) reported a feeling of *heaviness* in the bladder or lower abdomen.

Chapter 4: RESULTS

The primary aim of this study was to explore the association between the diffusion of vulvar pain and the use of hormonal contraception. Additionally, we examined whether vulnerability to the negative effects of hormonal contraception, as well as a history of depression or anxiety, were linked to the diffusion of vulvar pain, compared to pain localized to just one area of the vulva. We also investigated whether age and age at onset of vulvar pain symptoms influenced the spread of vulvar pain. Lastly, the second research question explored whether hormonal contraception use influences the way pain is reported by women suffering from vulvodynia.

4.1 Results of Multiple Regression Analysis on Predictors of Vulvar Pain Distribution

The first research question aimed to investigate the effects of hormonal contraception, depression and age at onset of genital pain on the spread of vulvar pain compared to pain localized in one area. To address this, we performed a multiple regression, considering the following predictors of vulvar pain distribution: participant age, diagnosis of depression (coded 0, 1), diagnosis of anxiety (coded 0, 1), age at onset of genital pain, hormonal contraception use (coded 0, 1), and negative side effects while using hormonal contraception. Overall, the model was statistically significant ($F(6, 738)=6.821$, $p<0.0001$), suggesting that the set of predictors explain a significant proportion of variation in genital pain diffusion ($R^2=4.5\%$). Concerning more precisely the results on our predictors, findings show that age had a statistically significant effect ($b=-0.054$; $t=-2.08$; $p=0.0382$), indicating that being older was negatively associated with the diffusion of vulvar pain symptoms. Moreover, even if having had a diagnosis of depression was not statistically significant ($b=-0.511$; $t=-1.93$; $p=0.0544$), the results

suggest that having a diagnosis of depression may be associated with less vulvar pain diffusion. However, a diagnosis of anxiety was statistically significant (*b*=-0.494; *t*=-2.11; *p*=0.0351), indicating that the spread of genital pain was less extensive among those without an anxiety diagnosis. Additionally, the age at onset of genital pain symptoms was statistically significant (*b*=-0.054; *t*=-2.15; *p*=0.0323), with older age at onset negatively associated with the diffusion of vulvar pain symptoms. Hormonal contraception use was not associated with the spread of genital pain symptoms (*b*=-0.386; *t*=-1.09; *p*=0.2751). However, experiencing side effects from hormonal contraception was significantly linked to genital pain diffusion (*b*= 0.747; *t*= 2.14; *p*=0.0326). Table 3 shows the results of the multiple regression analysis, including the estimates, standard errors, t-ratios, and p-values for each predictor.

Table 3

Results of Multiple Regression Analysis Predicting Diffusion of Vulvar Pain

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	9.57	0.74	12.88	<0.0001*
Age	-0.05	0.03	-2.08	0.0382*
Depression Diagnosis	-0.51	0.27	-1.93	0.0544
Anxiety Diagnosis	-0.49	0.23	-2.11	0.0351*
Age at Onset Genital Pain	-0.05	0.03	-2.15	0.0323*
Contraception Use	-0.37	0.34	-1.09	0.2751
Negative Effects Contraception	0.75	0.35	2.14	0.0326*

4.2 Results of Normal Logistic Regression Analysis Predicting Vulvar and Urinary Symptoms by Hormonal Contraception Use

To explore how hormonal contraception affects the characteristics of pain, we conducted a normal logistic regression to assess whether hormonal contraception use predicted specific types of pain symptoms. For example, the first type of pain we analyzed was the sensation of *cuts*. In other words, we aimed to determine if describing vulvar pain as *cuts* differed significantly between participants using hormonal contraception and those who were not. Notably, the results showed a statistically significant difference in the sensation of *cuts* between women using hormonal contraception and those who were not (OR=2.09, 95% CI [1.51, 2.87], $p<0.001$).

Next, we examined the sensation of *itching*, but no statistically significant difference was found between women using hormonal contraception and those who were not (OR=1.04, 95% CI [0.79, 1.37], $p=0.7732$). The third type of pain we investigated was the sensation of *burning* in the vulva. The results indicated a significant difference, with women using hormonal contraception being more likely to experience this type of pain (OR=1.31, 95% CI [1.02, 1.69], $p=0.0351$). However, no statistically significant differences were found for describing vulvar pain as *irritation* (OR=1.20, 95% CI [0.91, 1.59], $p=0.1850$), *pins-and-needles* (OR=1.32, 95% CI [1.00, 1.75], $p=0.0525$), or *shocks* (OR=1.32, 95% CI [0.91, 1.91], $p=0.1442$) between women using hormonal contraception and those who were not.

Finally, our analysis also included an investigation of the urinary symptoms reported. Specifically, we explored whether women using hormonal contraception had a higher risk of experiencing a sensation of *heaviness* in the bladder or lower abdomen, as well as pain during *urination*. However, there were no statistically significant differences

observed for *heaviness* (OR=1.12, 95% CI [0.79, 1.58], *p*=0.5292) or for pain during *urination* (OR=1.29, 95% CI [0.91, 1.81], *p*=0.1477). Table 4 summarizes the key findings regarding the types of vulvar and urinary symptoms reported by women currently using hormonal contraception compared to those who do not.

Table 4

Separate Normal Logistic Regression Models for Vulvar Pain Symptoms by Hormonal Contraception Status for Each Dependent Variable

			95% Confidence Interval	
Descriptor	Odds Ratio	Prob>Chisq	Lower	Upper
Cuts	2.09	<0.0001*	1.51	2.87
Itching	1.04	0.7732	0.79	1.37
Burning	1.31	0.0351*	1.02	1.69
Irritation	1.20	0.185	0.91	1.59
Pins-and-needles	1.32	0.0525	1.00	1.75
Shocks	1.32	0.1442	0.91	1.91
Heaviness	1.12	0.5292	0.79	1.58
Urination	1.29	0.1477	0.91	1.81

Chapter 5: DISCUSSION

The present study aimed to examine the effects of multiple variables on the diffusion of vulvar pain. Specifically, it investigated whether factors such as hormonal contraception use, susceptibility to its negative effects, participant age, and age at vulvar pain onset could partly explain the spread of vulvar pain symptoms. Additionally, we explored whether having a psychiatric condition, such as depression or anxiety, might exacerbate these symptoms. Our findings indicated that participant age, age at pain onset, an anxiety diagnosis, and vulnerability to negative effects from hormonal contraception were all statistically significant predictors of vulvar pain diffusion. In contrast, neither hormonal contraception use nor a depression diagnosis reached statistical significance, although depression approached significance.

The second part of the study examined whether morphological changes in the vulva due to hormonal contraception could result in different types of vulvar pain between current hormonal contraception users and non-users. Results indicate that such differences exist, particularly for sensations of *cutting* and *burning* in the vulva. The following sections will delve deeper into these findings, offering possible explanations for the observed effects.

5.1 Hormonal Contraception Use and Susceptibility to Negative Effects on Diffusion of Vulvar Pain

One of the key variables in the current study was the impact of hormonal contraception use and vulnerability to negative effects on the diffusion of vulvar pain. Although previous analyses found a positive correlation between hormonal contraception use and vulvar pain symptoms (Kiesner & Bittoni, 2024), the current analysis did not

show any statistically significant effect of this variable on the spread of vulvar pain. However, being susceptible to at least one negative effect from hormonal contraception was found to be statistically significant in predicting the diffusion of vulvar pain.

As mentioned earlier in this research, the relationship between vulvar pain symptoms and hormonal contraception is highly complex. Not all women with vulvodynia had used hormonal contraception prior to the onset of painful symptoms, and not all women who took hormonal contraception later developed vulvodynia. A possible explanation for this is offered by Goldstein and colleagues (2014), who found that women with fewer CAG repeats on their androgen receptor (AR) gene had a lower likelihood of developing vulvodynia due to hormonal contraception. Conversely, women with a higher number of CAG repeats were more prone to developing vulvar pain after using hormonal contraception. This suggests a predisposition to such side effects, as a higher number of CAG repeats on the AR gene reduces serum-free testosterone levels. The resulting hormonal imbalance, due to the androgenic effects of hormonal contraception, may lead to the development of vulvodynia (Goldstein et al., 2014).

Given this, it is plausible to hypothesize that the women in our sample who experienced negative effects from hormonal contraception were those genetically predisposed to such side effects. Among them, some may have developed more diffuse vulvar pain in response to hormonal contraception. This genetic predisposition could lead to pain spreading across multiple vulvar sites and potentially to the bladder, as the nerve fibers in both regions share a common embryological origin (Butrick, 2003; Theoharides et al., 2008; Birder et al., 2010; Gardella et al., 2011). There is a notable comorbidity between interstitial cystitis (IC) and vulvodynia, with prevalence being higher among oral contraceptive users and postmenopausal women, indicating a significant role of steroid

hormones (Gardella et al., 2011). Both vulvodynia and IC are characterized by altered or heightened pain sensitivity, which is linked to hyperactivation of afferent C-fibers in the dorsal horn (Butrick, 2003; Theoharides et al., 2008; Birder et al., 2010; Gardella et al., 2011).

Consequently, women with a genetic predisposition to develop vulvodynia after using hormonal contraception – due to a higher number of CAG repeats on the AR gene – are more likely to experience pain in multiple vulvar sites and the bladder, leading to comorbidity between vulvodynia and IC. This genetic vulnerability may help explain why negative side effects from hormonal contraception were significantly associated with the diffusion of vulvar pain in our study. In other words, this variable could reflect, at least in part, a genetic susceptibility to the side effects of hormonal contraception.

5.2 Effects of Depression and Anxiety on Vulvar Pain Diffusion

In our study, the variable *Diagnosis of Depression* approached significance but did not reach statistical significance in predicting the spread of vulvar pain compared to pain localized to a single vulvar site. In contrast, the variable *Diagnosis of Anxiety* was found to be statistically significant in predicting the diffusion of vulvar pain. Specifically, women who had received a diagnosis of anxiety at some point in their lives were more likely to experience widespread vulvar pain rather than pain confined to one region.

Depression and anxiety both affect the activity of the hypothalamic-pituitary-adrenal (HPA) axis (Mello et al., 2003; Chen et al., 2014). Anxiety, in particular, is a psychiatric condition known for its strong somatic effects (Buodo, 2022). The anticipation and fear of potential negative events, whether or not they occur, leads to a heightened and sustained production of cortisol, commonly known as the “stress

hormone" (Buodo, 2022). Cortisol is a direct product of the HPA axis. Specifically, when the body encounters a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH) to the pituitary gland (Tasker & Herman, 2011; Thiel & Dretsch, 2011). In response, the pituitary gland secretes adrenocorticotropic hormone (ACTH), which then travels to the adrenal glands located just above the kidneys (Tasker & Herman, 2011; Thiel & Dretsch, 2011). The adrenal glands, in turn, release cortisol, a hormone that triggers physiological changes to help the body cope with stress, such as increasing glucose storage for more energy (Tasker & Herman, 2011; Thiel & Dretsch, 2011).

However, this system functions optimally only when it is not constantly activated. Chronic stress has been linked to issues like neural death and neurological disorders, including Alzheimer's disease (Bachis et al., 2008; Ávila-Villanueva et al., 2020). Importantly, the body's immune response to chronic stress includes the release of pro-inflammatory cytokines (Harrison et al., 2014). Mast Cells (MCs) play a key role here, as they are major producers of cytokines, along with macrophages, endothelial cells, and Schwann cells (Zhang & An, 2007). When MCs degranulate, they not only release cytokines, which promote inflammation, but also Nerve Growth Factor (NGF; Frenzel & Hermine, 2013). The release of NGF leads to the proliferation and increased surface presence of nerve endings, which can cause pain to become neuropathic rather than nociceptive (Bornstein et al., 2004; Awad-Igbaria et al., 2024). In other words, the body can begin to signal pain in the absence of any harmful stimulus, such as during simple contact, a phenomenon known as hyperalgesia (Graziottin, 2018).

MCs are also highly sensitive to hormonal fluctuations (Gaudenzio et al., 2016; Kaur et al., 2017; Jensen et al., 2010). During menstruation, when estrogen and progesterone levels drop, women with vulvodynia may experience increased pain in the

vulva due to the heightened activity of MCs (Estibeiro et al., 2022). Furthermore, it is important to note that anxiety and depressive symptoms tend to increase in women with a “normal” menstrual cycle during the premenstrual phase (Lane & Francis, 2003). Given this, it can be hypothesized that in women with vulvodynia who also suffer from anxiety, hyperactivation of the HPA axis, followed by MC degranulation and the release of pro-inflammatory cytokines and NGF, may lead to the spread of vulvar pain. The combined effect of cytokines and the proliferation and increased surface presence of nerve endings could cause pain to spread from, for example, the vestibule to other areas of the vulva.

If our findings confirm a significant role of anxiety in predicting diffuse vulvar pain, the explanation provided here offers a possible mechanism, though further investigation is needed. It is important to remember that one of the primary reasons we examined this variable was its identification by Pâquet et al. (2019) as a key factor in the persistence of pain. Therefore, understanding the specific origins of anxiety in each patient and addressing it properly is crucial. For example, anxiety may stem from the anticipation of pain during intercourse, which could be exacerbated during the premenstrual phase due to hormonal fluctuations. Tailoring treatment to the individual causes of anxiety in each patient is fundamental for providing the best care and, ideally, achieving a remission of vulvar pain symptoms.

5.3 Effects of Participant Age and Age at Onset on Vulvar Pain Diffusion

Both age and age at onset of vulvar pain have been identified as statistically significant predictors of the spread of vulvar pain symptoms. Specifically, the later the onset of vulvar pain, the less extensive the symptoms are likely to be. Similarly, older individuals are less likely to experience widespread vulvar pain, which tends to be more

localized. Interestingly, experiencing vulvar pain at an older age is associated with a greater likelihood of symptom remission (Pâquet et al., 2019). Despite this, vulvodynia is relatively common among sexually active women across all age groups, with an estimated prevalence of 8% to 15% (Graziottin & Murina, 2010; Reed et al., 2012). In our sample, the majority of participants reported the onset of painful symptoms between the ages of 18 and 25, which can be attributed to factors such as sexual activity and hormonal changes during this developmental stage.

In Italy, the average age for first penetrative sexual intercourse ranges from 17 to 25 years (Statista Research Department, 2024). During this same period, the production of estrogens, particularly estradiol (E2), peaks (Graziottin et al., 2022). This surge in hormones, combined with the mechanical trauma associated with penetrative intercourse, may lead to inflammation in the vulvar tissue, as some estrogens are known to act as "pro-inflammatory hormones" (Straub, 2007). Consequently, this interaction can result in the continuous degranulation of mast cells (MCs), which – as previously discussed in relation to depression and anxiety – may contribute to neuropathic pain (Vallvé-Juanico et al., 2019; Sarchielli et al., 2010).

Notably, 5.73% of our participants reported the onset of genital pain before the age of 10 (*n*=54), with 2.02% (*n*=19) experiencing pain prior to age 5. The underlying causes of vulvar pain in very young children likely differ from those in individuals who report pain later in life. Common etiologies of vulvar pain in childhood include *unintentional* or *intentional* traumatic lesions and *Lichen Sclerosus (LS)* – an inflammatory dermatosis primarily affecting the ano-genital area (Graziottin & Murina, 2017; Krapf, 2020). When considering *unintentional* traumatic genital lesions, it is important to note that such incidents are relatively rare, accounting for only 0.6% of all

pediatric injuries. Among these, bicycle-related accidents are the most frequent, representing 14.7% of pediatric genital injuries (Casey et al., 2013). Typically, 94.7% of these cases are treated and released from the hospital (Casey et al., 2013).

In contrast, *intentional* traumatic genital lesions in children are unfortunately more prevalent. For instance, Gilbert and colleagues (2009) estimated that in high-income countries, between 5% and 10% of girls and 5% of boys experience sexual abuse – including penetrative sex – each year. Consequently, these alarming figures demand serious attention. Indeed, the genital injuries and psychological trauma resulting from such abuse must not be overlooked in clinical practice (Johnson, 2004). As emphasized by Johnson (2004), a forensic interview conducted by a trained professional is essential when there are suspicions of sexual abuse in children.

Another critical issue regarding *intentional* genital trauma is *Female Genital Mutilation/Cutting (FGM/C*; Graziottin & Murina, 2017). Globally, five girls undergo cutting or infibulation every minute, amounting to 6'000 per day and two million annually (Ly Tall, 2020). In Italy, it is estimated that 35'000 women currently live with the adverse effects of FGM/C (Abdulcadir, 2019). Given this high prevalence, proper medical and psychological training is crucial to improving the psychological and sexual quality of life for these women (Abdulcadir et al., 2017).

In conclusion, the statistically significant effects observed for the variables of *age* and *age at onset* can be attributed to multiple factors. Generally, older age is associated with a greater likelihood of remission from vulvar pain symptoms and a reduced spread of these symptoms. One potential explanation for these findings is the varying levels of estrogens produced in the female body. Conversely, the factors contributing to the onset of vulvar pain in very young females warrant further investigation, particularly regarding

critical issues such as child sexual abuse, which must never be overlooked in clinical practice.

5.4 The Impact of Hormonal Contraception on Vulvar Pain Perception

The second aim of this study was to investigate the effects of hormonal contraception on various types of vulvar pain. The results indicate that women who use hormonal contraception are twice as likely to report vulvar pain characterized as *cuts* compared to those who have never used hormonal contraceptives. Additionally, there is a statistically significant increase in the likelihood of experiencing a *burning* sensation among users of hormonal contraception compared to those who have never used it. However, the sensation of *pins-and-needles* approaches statistical significance but does not reach the threshold. Other descriptors, such as *itching, irritation, shocks, heaviness*, and pain during *urination*, did not show statistically significant differences.

A possible explanation for these findings is the vaginal dryness often associated with hormonal contraception. In our sample, most hormonal contraceptive users reported experiencing vaginal dryness (Kiesner & Bittoni, 2024). The existing literature also supports an association between vaginal dryness and hormonal contraceptive use. Specifically, hormonal contraceptives are linked to decreased androgen levels, which subsequently reduce free testosterone (Goldstein et al., 2014). This occurs because the suppression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) leads to reduced androgen production by the ovaries, resulting in increased levels of sex hormone-binding globulin (SHBG; Burrows et al., 2012). Elevated SHBG levels bind more testosterone, leading to a decreased availability of free testosterone in the female

body (Burrows et al., 2012). Lower testosterone levels are known to be associated with vaginal dryness (Maseroli & Vignozzi, 2020).

Hormonal contraceptives also reduce estrogen levels, which is significant because estrogens play a crucial role in providing vaginal lubrication (Simon, 2011). While some combined hormonal contraceptives (CHCs) contain synthetic estrogens, these cannot fully replicate the effects of naturally produced estrogens. Moreover, pills with high progesterone and low estrogen levels exacerbate vaginal dryness (Bourchard et al., 2002). Nonetheless, all estradiol produced in the female body results from the aromatization of testosterone into 17β-estradiol (Tomomi et al., 2015; Osmosis from Elsevier, 2022). Therefore, a reduction in androgen levels leads to decreased estrogen production.

In summary, hormonal contraception contributes to vaginal dryness through three mechanisms: first, by increasing SHBG levels, which decreases free testosterone availability; second, by significantly lowering estrogen levels, especially in progestin-only pills or those with high progestin and low estrogen; and third, by reducing the androgens available for aromatization into estradiol, further diminishing estrogen production. Returning to our study, vaginal dryness may lead to micro-lesions, which could explain the increased likelihood of women using hormonal contraception reporting vulvar pain as *cuts*.

Regarding the *burning* sensation, additional explanations may be relevant. Research has shown that the connective tissue in women using hormonal contraception has a significantly higher density of superficial small blood vessels (Johannesson et al., 2007). This increased vascularization in the vulvar connective tissue may contribute to sensations of warmth or burning. Furthermore, vaginal dryness induced by hormonal contraception, along with a higher number and superficialization of nerve fiber endings

in the vulva due to the activity of MC, may also contribute to the *burning* sensation and the *pins-and-needles* feeling (Awad-Igbaria et al., 2024).

5.5 Limitations and Future Directions

The previous section discussed possible explanations for the effects observed in this study. However, it is important to emphasize that these hypotheses require further investigation for confirmation. Additionally, this study has several limitations that should be considered in future research. Addressing these limitations could lead to a more nuanced understanding of the complex relationship between hormonal contraception and vulvar pain.

The first limitation is the lack of analysis of interaction effects among the key predictors of diffusion of vulvar pain, specifically hormonal contraception and its side effects, depression or anxiety diagnosis, participant age, and age of vulvar pain onset. For instance, there may be an interaction effect between depression and anxiety diagnoses, as these factors frequently co-occur in women with vulvodynia, possibly leading to more widespread vulvar pain rather than pain localized to a single site (Khandker et al., 2011). Another omission in this study is the measurement of free testosterone and estrogens levels in participants. Indeed, given the study's second objective – exploring the effects of hormonal contraception on the type of vulvar pain – comparing free testosterone and estrogens levels between hormonal contraceptive users and non-users would have provided valuable insights into the role of testosterone in vaginal dryness. Furthermore, the effects of hormonal contraception are known to vary over time (Johansson et al., 2023), so the absence of analysis on the duration of contraceptive use represents an

additional limitation. Nonetheless, future research could include an analysis of different hormone types and their specific effects.

Moreover, the study sample includes women who reported experiencing vulvar pain but had not received a formal diagnosis. This has implications from two perspectives. For scientific rigor, it may have been preferable to include only women formally diagnosed with vulvodynia, ideally with specific areas of the vulva identified. However, given the high prevalence of undiagnosed vulvodynia and the extended time often required to reach a diagnosis (Toeima & Nieto, 2011), this study also contributes to a broader understanding of the prevalence of genital pain in the female population. Nonetheless, another limitation is the use of online recruitment for participants. While this approach enabled a large sample size, it may have excluded women without electronic devices or internet access, such as older women, potentially limiting the generalizability of the findings.

Nevertheless, it is worth noting that the primary aim of the study, *"Luci e Ombre della Sessualità e Salute Genitale Femminile",* was not to specifically examine the relationship between hormones and vulvodynia. Rather, it aimed to investigate genital pain experiences in the female population and their associated psychological and social factors. Consequently, more targeted research on hormone use and its effects on vulvar health could incorporate more specific analyses, such as those mentioned above.

In conclusion, future research should aim to address the limitations identified in this study to provide a clearer understanding of how hormonal contraception and other factors contribute to vulvar pain. In fact, longitudinal studies examining hormonal, psychological, and lifestyle factors over time would offer insights into both the onset and progression of vulvar pain conditions. Investigating the interaction between hormonal

contraception and mental health diagnoses, such as depression and anxiety, could uncover potential effects that exacerbate pain. Furthermore, measuring free testosterone and estrogen levels in participants using different types and durations of hormonal contraception could clarify the role of hormonal changes in vulvar pain and vaginal dryness. Finally, recruiting diverse samples – including formally diagnosed patients and underrepresented groups – would enhance the generalizability of findings and inform more comprehensive healthcare interventions.

CONCLUSION

The aim of this research has been to contribute new scientific insights into potential risk factors that characterize, exacerbate, or sustain vulvar pain. Indeed, our primary focus was on the diffusion of vulvar pain – a key factor linked to the persistence of vulvodynia (Pâquet et al., 2019) – and we identified several statistically significant predictors: susceptibility to the adverse effects of hormonal contraception, anxiety, age, and age at onset of pain. Additionally, we found that hormonal contraceptives can induce morphological changes in the vulvar epithelium, leading to distinct types of pain – often described as cutting or burning – among users of hormonal contraception compared to non-users. However, these findings require further research to confirm the proposed explanations, taking into account the study's limitations and suggested directions for future exploration.

Importantly, current scientific literature agrees that vulvodynia is a multifactorial condition, which partly explains the challenges in studying it. In other words, vulvar pain may be influenced by a range of factors, from biological to psychological. Clinical assessments should, therefore, consider a broad spectrum of potential causes, incorporating lifestyle habits and socio-demographic variables as well. Nonetheless, healthcare providers must recognize that this pain is localized in the female genital area, necessitating a thorough and respectful investigation of all possible contributing factors without trivializing the patient's experience. Indeed, it is essential for healthcare professionals to be well-informed not only to diagnose vulvodynia but also to educate and advise patients on factors that could potentially worsen their symptoms. Patient care should be approached collaboratively, with a clear exchange of information between the patient's concerns and the doctor's expertise. Informing patients about potential risks

associated with hormonal contraceptive use – such as an increased likelihood of developing vulvar pain – enables them to make informed decisions and to be attentive to any changes or signals from their bodies.

Finally, there is a pressing need for national healthcare systems worldwide to formally recognize vulvodynia. The current invisibility of this condition reflects societal inequalities, as it impacts an estimated up to 15% of women, whose pain often goes unacknowledged. Recognition by healthcare systems could result in financial support for affected individuals, who currently bear the full cost of treatment if they can afford it, and ideally, it would drive improved medical training for the diagnosis and management of this condition. When such issues remain hidden, they persist unaddressed, perpetuating stigma and neglecting the needs of a significant segment of society.

REFERENCES

Abdulcadir, J. (2019). *La rinascita delle bambine e delle donne con mutilazioni genitali* [TEDx Talk]. TEDx Padova. YouTube. https://www.youtube.com/watch?v=O10PZg74muk

Abdulcadir, J., Alexander, S., Dubuc, E., Pallitto, C., Petignat, P., & Say, L. (2017). Female genital mutilation/cutting: sharing data and experiences to accelerate eradication and improve care. *Reproductive Health, 14*(S1). https://doi.org/10.1186/s12978-017-0361-y

Ali, E.S., Mangold, C. & Peiris, A.N. (2017). Estriol: emerging clinical benefits. *Menopause 24*(9):1081–1085. doi: 10.1097/GME.0000000000000855

Aloe, L. (2004). Rita Levi-Montalcini: the discovery of nerve growth factor and modern neurobiology. *Trends in Cell Biology, 14*(7), 395-399. https://doi.org/10.1016/j.tcb.2004.05.011

Alvergne, A., & Lummaa, V. (2010). Does the contraceptive pill alter mate choice in humans? *Trends in Ecology & Evolution, 25*(3), 171–179. http://doi.org/10.1016/j.tree.2009.08.003

Amalraj, P., Kelly, S., & Bachmann, G.A. (2009). Historical Perspective of Vulvodynia. In Goldstein, A.T., Pukall, C.F., & Goldstein, I. (Eds.), *Female sexual pain disorders* (pp. 1-3). John Wiley & Sons

American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, DSM-5.* Arlington, VA. pp.437-440.

Antonelli, M. & Kushner, I. (2017). It's time to redefine inflammation. *The FASEB Journal, 31*(5), 1787-1791. https://doi.org/10.1096/fj.201601326R

Ávila-Villanueva, M., Gómez-Ramírez, J., Maestú, F., Venero, C., Ávila, J. & Fernández-Blázquez, M.A. (2020). The Role of Chronic Stress as a Trigger for the Alzheimer Disease Continuum. *Frontiers in Aging Neuroscience, 12*. doi: 10.3389/fnagi.2020.561504

Awad-Igbaria, Y., Edelman, D., Ianshin, E., Abu-Ata, S., Shamir, A., Bornstein, J., & Palzur, E. (2024). Inflammation-induced mast cell-derived nerve growth factor: a key player in chronic vulvar pain? *Brain*. https://doi.org/10.1093/brain/awae228

Babula, O., Danielsson, I., Sjoberg, I., Ledger, W.J. & Witkin, S.S. (2004). Altered distribution of mannose-binding lectin alleles at exon I codon 54 in women with vulvar vestibulitis syndrome. *American Journal of Obstetrics & Gynecology, 191*(3), 762–766. doi: 10.1016/j.ajog.2004.03.073

Bachis, A., Cruz, M.I., Nosheny, R.L. & Mocchetti, I. (2008). Chronic unpredictable stress promotes neuronal apoptosis in the cerebral cortex. *Neuroscience Letters, 442*(2), 104-108. Doi: 10.1016/j.neulet.2008.06.081

Baggish, M.S., & Miklos, J.R. (1995). Vulvar pain syndrome: a review. *Obstetrical & Gynecological Survey, 50*(8), 618-627. DOI: 10.1097/00006254-199508000-00023

Ballard, K.D., Seaman, H.E., de Vries, C.S. & Wright, J.T. (2008). Can symptomatology help in the diagnosis of endometriosis? Findings from a national case-

control study--Part 1. *BJOG, 115*(11), 1382-1391. doi: 10.1111/j.1471-0528.2008.01878.x

Baños de MacCarthy, G. & Perez-Torres, I. (2011). Non Reproductive Effects of Sex Hormones and the Cardiovascular System. In Hoffmann, A.B. (Ed.), *Sex Hormones: Development, Regulation and Disorders* (pp. 31-51). New York: Nova Science Publishers.

Basson, R., Althof, S., Davis, S., Fugl-Meyer, K., Goldstein, I., Leiblum, S., Meston, C., Rosen, R. & Wagner, G. (2004). Summary of the recommendations on sexual dysfunctions in women. *The Journal of Sexual Medicine, 1*(1), 24-34. DOI: 10.1111/j.1743-6109.2004.10105.x

Bazin, S., Bouchard, C., Brisson, J., Morin, C., Meisels, A. & Fortier M. Vulvar vestibulitis syndrome: An exploratory case-control study. (1994). *Obstetrics and Gynecology, 83*(1) 47–50.

Benoit-Piau, J., Bergeron, S., Brassard, A., Dumoulin, C., Khalifé, S., Waddell, G., & Bergeron, S., Likes, W.M. & Steben, M. (2014). Psychosexual aspects of vulvovaginal pain. *Best Practice & Research Clinical Obstetrics & Gynaecology, 28*(7), 991-999. doi: 10.1016/j.bpobgyn.2014.07.007

Berglund A-L., Nigaard L., & Rylander E. (2002). Vulvar pain, sexual behavior and genital infections in a young population: a pilot study. *Acta Obstetricia et Gynecologica Scandinavica, 81*(8), 738-42. doi: 10.1034/j.1600-0412.2002.810809.x

Bernhard, K. & Winfried, G.R. (2016). Anterior Pituitary Hormones. In Bernhard, K. & Winfried, G.R. (Eds.), *Hormones and the Endocrine System: Textbook of Endocrinology* (pp. 69-73). Cham: Springer International Publishing.

Birder, L., de Groat, W., Mills, I., Morrison, J., Thor, K. & Drake, M. (2010). Neural control of the lower urinary tract: peripheral and spinal mechanisms. *Neurourology and Urodynamics, 29*(1), 128-139. doi: 10.1002/nau.20837

Bornstein, J., Goldschmid, N. & Sabo, E. (2004). Hyperinnervation and Mast Cell Activation May Be Used as Histopathologic Diagnostic Criteria for Vulvar Vestibulitis. *Gynecologic and Obstetric Investigation, 58*(3), 171-8. doi: 10.1159/000079663

Bosio, S., Perossini, S., Torella, M., Braga, A., Salvatore, S., Serati, M., Frigerio, M., & Manodoro, S. (2024). The association between vulvodynia and interstitial cystitis/bladder pain syndrome: A systematic review. *International Journal of Gynecology & Obstetrics*, *167*(1), 1–15. https://doi.org/10.1002/ijgo.15538

Both, S., Lew-Starowicz, M., Luria, M., Sartorius, G., Maseroli, E., Tripodi, F., Lowenstein, L., Nappi, R.E., Corona, G., Reisman, Y. & Vignozzi, L. (2019). Hormonal Contraception and Female Sexuality: Position Statements from the European Society of Sexual Medicine (ESSM). *The Journal of Sexual Medicine, 16*(11), 1681–1695. https://doi.org/10.1016/j.jsxm.2019.08.005

Bouchard C, Brisson J, Fortier M, Morin C, & Blanchette C. (2002). Use of Oral Contraceptive Pills and Vulvar Vestibulitis: A Case-Control Study. *American Journal of Epidemiology, 156*(3), 254–61. https://doi.org/10.1093/aje/kwf037.

Brinton, R.D., Thompson, R.F., Foy, M.R., Baudry, M., Wang, J., Finch, C.E., Morgan, T.E., Pike, C.J., Mack, W.J., Stanczyk, F.Z. & Nilsen, J. (2008). Progesterone

receptors: form and function in brain. *Frontiers in Neuroendocrinology, 29*(2), 313–339. doi: 10.1016/j.yfrne.2008.02.001

Brynhildsen J. (2014). Combined hormonal contraceptives: prescribing patterns, compliance, and benefits versus risks. *Therapeutic Advances in Drug Safety, 5*(5), 201-213. doi:10.1177/2042098614548857

Buchan, A., Munday, P., Ravenhill, G., Wiggs, A. & Brooks. F. (2007). A qualitative study of women with vulvodynia: I. The journey into treatment. *Journal of Reproductive Medicine, 52*(1), 15-18.

Buodo, G. (2022). *Depression* [Slides]. Affective Neuroscience, University of Padova

Burger, H.G. (2020). Androgen production in women. *Fertility and Sterility, 77*(4), 3-5. https://doi.org/10.1016/S0015-0282(02)02985-0

Burrows, L.J., Basha, M., and Goldstein, A.T. (2012). The Effects of Hormonal Contraceptives on Female Sexuality: A Review. *The Journal of Sexual Medicine, 9*(9), 2213-23. doi: 10.1111/j.1743-6109.2012.02848.x.

Butrick, C.W. (2003). Interstitial cystitis and chronic pelvic pain: new insights in neuropathology, diagnosis, and treatment. *Clinical Obstetrics and Gynecology, 46*(4), 811-823. doi: 10.1097/00003081-200312000-00011.

Cable, J.K. & Grider, M.H. (2023). Physiology, Progesterone. StatPearls [Internet]. https://www.ncbi.nlm.nih.gov/books

Carlberg, K.A., Fregly, M.J. & Fahey, M. (1984). Effects of chronic estrogen treatment on water exchange in rats. *American Journal of Physiology, 247*(1):E101–10. DOI: 10.1152/ajpendo.1984.247.1.E101

Caruso, S., Agnello, C., Malandrino, C., Lo Presti, L., Cicero, C. & Cianci, S. (2014). Do Hormones Influence Women's Sex? Sexual Activity over the Menstrual Cycle. *The Journal of Sexual Medicine, 11*(1), 211–221. https://doi.org/10.1111/jsm.12348

Casey, F.E. (2023). Barrier Contraceptives. *MSD Manual Consumer version*. https://www.msdmanuals.com/home/women-s-health-issues/family-planning/barrier-contraceptives

Casey, J.T., Bjurlin, M.A. & Cheng, E.Y. (2013). Pediatric genital injury: an analysis of the National Electronic Injury Surveillance System. *Urology, 82*(5), 1125-1130. doi: 10.1016/j.urology.2013.05.042

Castanho, T.C., Moreira, P.S., Portugal-Nunes, C., Novais, A., Costa, P.S., Palha, J.A., Sousa, N. & Correia Santos, N. (2014). The role of sex and sex-related hormones in cognition, mood and well-being in older men and women. *Biological Psychology, 103*, 158-166. https://doi.org/10.1016/j.biopsycho.2014.08.015

Cauley, J.A. (2015). Estrogen and bone health in men and women. *Steroids, 99*(Part A), 11-15. https://doi.org/10.1016/j.steroids.2014.12.010

Cervigni, M. & Natale, F. (2014). Gynecological disorders in bladder pain syndrome/interstitial cystitis patients. *International Journal of Urology, 21*, 85-88. doi: 10.1111/iju.12379.

Chauhan, S., More, A., Chauhan, V. & Kathane A. (2022). Endometriosis: A Review of Clinical Diagnosis, Treatment, and Pathogenesis. *Cureus, 14*(9):e28864. doi: 10.7759/cureus.28864

Chen, F., Zhou, L., Bai, Y., Zhou, R. & Chen L. (2014). Hypothalamic-pituitary-adrenal axis hyperactivity accounts for anxiety- and depression-like behaviors in rats perinatally exposed to bisphenol. *American Journal of Biomedical Science and Research, 29*(3), 250-258. doi: 10.7555/JBR.29.20140058

Cibula, D., Gompel, A., Mueck, A.O., La Vecchia, C., Hannaford, P.C., Skouby, S.O., Zikan, M., & Dusek, L. (2010). Hormonal contraception and risk of cancer. *Human Reproduction Update, 16*(6), 631–50. DOI: 10.1093/humupd/dmq022

Cleveland Clinic. (2022). *Skene's Gland*. [Internet]. https://my.clevelandclinic.org/health/body/24089-skenes-gland

Coelingh Bennink, H. J. T., Holinka, C. F., & Diczfalusy, E. (2008). Estetrol review: profile and potential clinical applications. *Climacteric, 11*(sup1), 47–58. https://doi-org.proxy.insermbiblio.inist.fr/10.1080/13697130802073425

Colquitt, C.W. & Martin, T.S. (2015). Contraceptive Methods: A Review of Nonbarrier and Barrier Products. *Journal of Pharmacy Practice*, 1-6. DOI: 10.1177/0897190015585751

Crowley, T., Goldmeier, D. & Hiller, J. (2009). Clinical Review: Diagnosing and managing vaginismus. BMJ, 338:b2284. doi: https://doi.org/10.1136/bmj.b2284

Csapo, A. (1958). Progesterone. *Scientific American, 198*(4), pp. 40-47.

Cutolo, M., Seriolo, B., Villaggio, B., Pizzorni, C., Craviotto, C. & Sulli, A. (2002). Androgens and Estrogens Modulate the Immune and Inflammatory Responses in Rheumatoid Arthritis. *Annals of the New York Academy of Science, 966*(1), 131–142. https://doi.org/10.1111/j.1749-6632.2002.tb04210.x

Dagostin Ferraz, S., Rodrigues Candido, A. C., Rodrigues Uggioni, M. L., Colonetti, T., Santina Dagostin, V., & Rosa, M. I. (2024). Assessment of anxiety, depression and somatization in women with vulvodynia: A systematic review and META-analysis. *Journal of Affective Disorders*, *344*, 122–131. https://doi.org/10.1016/j.jad.2023.10.025

Daniels, K. & Abma, J. C. (2020). *Current Contraceptive Status Among Women Aged 15–49: United States, 2017–2019*. National Center For Health Statistics.

Deliveliotou, A. & Creatsas, G. (2018). Anatomy of the vulva. In Farage, M.A. & Maibach, H.I. (Eds.), *The Vulva: Physiology and Clinical Management* (2nd Edition), pp.3-5. Boca Raton, FL: CRC Press.

DeMayo, F.J., Zhao, B., Takamoto, N. & Tsai, S.Y. (2002). Mechanisms of action of estrogen and progesterone. *Annals of the New York Academy of Sciences, 955*, 48-59. DOI: 10.1111/j.1749-6632.2002.tb02765.x

Derogatis, L.R., Sand M., Balon, R., Rosen, R. & Parish, S.J. (2016). Toward a More Evidence-Based Nosology and Nomenclature for Female Sexual Dysfunctions—Part I. *The Journal of Sexual Medicine, 13*(12), 1881-1887. https://doi.org/10.1016/j.jsxm.2016.09.014

Desai, R., Popa, V., Gillespie, S., Marshall, T. & Rowsome, P. (2024). Endocrine System: Anatomy and Physiology. *Osmosis from Elsevier*.

Doucet, L., Badre, M., Kielholz, M.A., Arena, F., Silveira, P., Brockmann, C.D. & Abdulcadir, J. (2022). Le clitoris, état des lieux pluridisciplinaire. *Obstetrica, 5*, 44-49. https://archive-ouverte.unige.ch//unige:160459

Dwyer, P.L. (2012). Skene's gland revisited: function, dysfunction and the G spot. *International Urogynecology Journal, 2*3, 135–137. https://doi.org/10.1007/s00192-011-1558-1

Edgardh K. & Abdelnoor M. (2007). Vulvar Vestibulitis and Risk Factors: a Population-based Case-control Study in Oslo. *Acta DermatoVenereologica, 87*(4): 350–354. doi: 10.2340/00015555-0250

Eppsteiner, E., Boardman, L. & Stockdale, C.K. (2014). Vulvodynia. *Best Practice & Research Clinical Obstetrics & Gynaecology, 28*(7), 1000–1012. doi: 10.1016/j.bpobgyn.2014.07.009

Estibeiro, V., Juntunen, A., Bond, J.C. & Harlow, B.L. (2022). Menstrual Cycle Characteristics and Vulvodynia. *Journal of Women's Health, 31*(8), 1127–1136. https://doi.org/10.1089/jwh.2020.9011

Fall, M., Baranowski, A.P., Elneil, S., Engeler, D., Hughes, J., Messelink, E.J., Oberpenning, F. & de C Williams, A.C. (2010). EAU guidelines on chronic pelvic pain. *European Urology, 57*(1), 35-48. doi: 10.1016/j.eururo.2009.08.020.

Farnam, F., Janghorbani, M., Merghati Khoei, E. & Raisi, F. (2014). Vaginismus and its correlates in an Iranian clinical sample. *International Journal of Impotence Research 26*(6), DOI: 10.1038/ijir.2014.16

Fedotcheva, T.A., Fedotcheva, N.I. & Shimanovsky, N.L. (2022). Progesterone as an Anti-Inflammatory Drug and Immunomodulator: New Aspects in Hormonal Regulation of the Inflammation. *Biomolecules, 12*(9):1299. https://doi.org/10.3390/biom12091299

Fihn, S.D., Latham, R.H., Roberts, P., Running, K. & Stamm, W.E. (1985). Association Between Diaphragm Use and Urinary Tract Infection. *Journal of the American Medical Association, 254*(2):240–245. doi:10.1001/jama.1985.03360020072027

First sexual intercourse in Italy 2017, by age. (2024). *Statista Research Department*. https://www.statista.com/statistics/784002/first-sexual-intercourse-by-age-in-italy/

Fitzpatrick, D., Pirie, K., Reeves, G., Green, J., & Beral, V. (2023). Combined and progestagen-only hormonal contraceptives and breast cancer risk: A UK nested case–control study and meta-analysis. *PLOS Medicine, 20*(3). https://doi.org/10.1371/journal.pmed.1004188

Foster, D.C., Sazenski, T.M., & Stodgell, C.J. (2004). Impact of genetic variation in interleukin-1 receptor antagonist and melanocortin-1 receptor genes on vulvar vestibulitis syndrome. *Journal of Reproductive Medicine, 49*(7), 503–509.

Freeda. (October 7, 2019). *Pillola, anello vaginale, cerotto: quali sono e come funzionano i contraccettivi ormonali* [Video]. YouTube. https://www.youtube.com/watch?v=y3c2wCjwMLk

Frenzel, L. & Hermine, O. (2013). Mast cells and inflammation. *Joint Bone Spine, 80*(2), *80*(2), 141-145. https://doi.org/10.1016/j.jbspin.2012.08.013

Friedrich E.G. (1987). Vulvar vestibulitis syndrome. *Journal of Reproductive Medicine, 32*(2):110-4. PMID: 3560069

Gardella, B., Porru, D., Nappi, R.E., Daccò, M.D., Chiesa, A. & Spinillo, A. (2011). Interstitial Cystitis is Associated with Vulvodynia and Sexual Dysfunction—A Case-Control Study. *The Journal of Sex Medicine, 8,* 1726–1734. DOI: 10.1111/j.1743-6109.2011.02251.x

Gaudenzio, N., Sibilano, R., Marichal, T., Starkl, P., Reber, L.L, Cenac, N., McNeil, B.D., Dong, X., Hernandez, J.D., Sagi-Eisenberg, R., Hammel, I., Roers, A., Valitutti, S., Tsai, M., Espinosa, E. & Galli, S.J. (2016). Different activation signals induce distinct mast cell degranulation strategies. *The Journal of Clinical Investigations, 126*(10), 3981-3998. https://doi.org/10.1172/JCI85538.

Gibbons, A.F. & Chang, M.C. (1972). Number of mast cells in the rat uterus with special reference to its relation to hormonal treatment and decidual response. *Biology of Reproduction, 6*(2), 193-203. doi: 10.1093/biolreprod/6.2.193

Gilbert, R., Widom, C. S., Browne, K., Fergusson, D., Webb, E., & Janson, S. (2009). Burden and consequences of child maltreatment in high-income countries. *The Lancet*, *373*(9657), 68–81. https://doi.org/10.1016/s0140-6736(08)61706-7

Goldstein A.T. & Burrows L. (2008). Vulvodynia. *The Journal of Sexual Medicine 5*(1), 5–14, quiz 5. DOI: 10.1111/j.1743-6109.2007.00679.x

Goldstein A.T., et al. (2014). Polymorphisms of the Androgen Receptor Gene and Hormonal Contraceptive Induced Provoked Vestibulodynia. *The Journal of Sexual Medicine, 11*(11), 2764-2771. DOI: 10.1111/jsm.12668.

Goldstein, A., Burrows, L., & Goldstein, I. (2010). Can Oral Contraceptives Cause Vestibulodynia?. *The Journal of Sex Medicine, 7*(4):1585–1587. DOI: 10.1111/j.1743-6109.2009.01685.x

Goldstein, A.T. (2009). Hormonal causes of dyspaurenia. In Goldstein, A.T., Pukall, C.F., & Goldstein, I. (Eds.), *Female sexual pain disorders* (pp. 63-68). John Wiley & Son

Goldstein, A.T. (2020). Hormonal causes of dyspaurenia. In John Wiley & Sons (Eds.), *Female sexual pain disorders: Evaluation and management* (pp. 63-68). John Wiley & Sons

Goldstein, J.M., Jerram, M., Poldrack, R., Ahern, T., Kennedy, D.N., Seidman, L.J. & Makris, N. (2005). Hormonal Cycle Modulates Arousal Circuitry in Women Using Functional Magnetic Resonance Imaging. *The Journal of Neuroscience, 25*(40), 9309 – 9316. DOI: 10.1523/JNEUROSCI.2239-05.2005

Graham, C.A., Sanders, S.A., Milhausen, R.R., & McBride, K.R. (2004). Turning on and turning off: A focus group study of the factors that affect women's sexual arousal. *Archives of Sexual Behavior, 33*(6), 527–38. doi: 10.1023/B:ASEB.0000044737.62561.fd

Grandi, G., Barra, F., Ferrero, S., Sileo, F.G., Bertucci, E., Napolitano, A. & Facchinetti, F. (2019). Hormonal contraception in women with endometriosis: a systematic review. *The European Journal of Contraception & Reproductive Health Care, 24*(1), 61–70. https://doi.org/10.1080/13625187.2018.1550576

Graziottin A., Cuccarollo, A., Uccella, S. & Franchi, M.P. (2022). Estrogeni e infiammazione. *L'Endocrinologo, 23*:281–289. https://doi.org/10.1007/s40619-022-01073-w

Graziottin, A. (2018). *Mestruazione: una differenza di genere per eccellenza - 2: Mastociti e infiammazione*. In *Medicina di Genere: oltre la Pillola Rosa e la Pillola Blu* [FAD]. By-Business Center. https://www.alessandragraziottin.it/it/div_audio.php/Mestruazione-una-differenza-di-genere-per-eccellenza-2-Mastociti-e-infiammazione?ID=23666

Graziottin, A. & Murina F. (2011). *Vulvodinia*. Milano: Springer-Verlag.

Graziottin, A., & Murina, F. (2017). *Vulvar pain: From childhood to old age*. Springer. pp.53-70.

Greenstein, A., Ben-Aroya, Z., Fass, O., Militscher, I., Roslik, Y., Chen, J., & Abramov, L. (2007). Vulvar Vestibulitis Syndrome and Estrogen Dose of Oral Contraceptive Pills. *The Journal of Sexual Medicine, 4*(6), 1679-1683. doi: 10.1111/j.1743-6109.2007.00621.x

Groysman, V. (2010). Vulvodynia: New Concepts and Review of the Literature. *Dermatologic Clinics (28)*4, 681-696.

Gunter, J. (2011) Neurobiology of chronic pelvic pain. In Vercellini, P. (Ed.), *Chronic Pelvic Pain* (pp. 1-6). John Wiley & Sons.

Hahn, A.C. & Cobey, K.D. (2019). Chapter 14. Synthetic Hormones: The Influence of Hormonal Contraceptives and Hormonal Replacement Therapy on Aspects of Women's Mating Psychology. In Welling, L.L.M. & Shackelford, T.K. (Eds.*), The Oxford Handbook of Evolutionary Psychology and Behavioral Endocrinology* (pp.237-256). New York NY: Oxford University Press.

Hannaford, P.C., Iversen, L., Macfarlane, T.V., Elliott, A.M., Angus, V., & Lee, A.J. (2010). Mortality among contraceptive pill users: Cohort evidence from Royal College of General Practitioners' Oral Contraception Study. *British Medical Journal*, *340*:c927. doi:10.1136/bmj.c927

Hannaford, P.C., Selvaraj, S., Elliott, A.M., Angus, V., Iversen, L., & Lee, A.J. (2007). Cancer risk among users of oral contraceptives: Cohort data from the Royal College of General Practitioner's Oral Contraception Study. *British Medical Journal, 335*(7621). doi: 10.1136/bmj.39289.649410.55

Harlow, B.L., Wise, L.A. & Stewart, E.G. (2001). Prevalence and predictors of chronic lower genital tract discomfort. *American Journal of Obstetrics & Gynecology, 185*(3), 545-50. doi: 10.1067/mob.2001.116748

Harms, C.A., Smith, J.R. & Kurti, S.P. (2016). Sex Differences in Normal Pulmonary Structure and Function at Rest and During Exercise. In Hemnes, A.R. (Ed.), *Gender, Sex Hormones and Respiratory Disease: A Comprehensive Guide* (pp.1-26).

Harrison, N. A., Cercignani, M., Voon, V., & Critchley, H. D. (2014). Effects of Inflammation on Hippocampus and Substantia Nigra Responses to Novelty in Healthy Human Participants. *Neuropsychopharmacology*, *40*(4), 831–838. https://doi.org/10.1038/npp.2014.222

Hellman, K.M., Patanwala, I.Y., Pozolo, K.E. & Tu, F.F. (2015). Multimodal nociceptive mechanisms underlying chronic pelvic pain. *American Journal of Obstetrics & Gynecology, 213*(6), 827.e1-9. doi: 10.1016/j.ajog.2015.08.038.

Howard, F.M. (2003). Chronic Pelvic Pain. *Obstetrics & Gynecology, 101*(3), 594-611. https://doi.org/10.1016/S0029-7844(02)02723-0

Jannini, E.A., Buisson, O. & Rubio-Casillas, A. (2014). Beyond the G-spot: clitourethrovaginal complex anatomy in female orgasm. *Nature Reviews Urology, 11*(9), 531-538. doi: 10.1038/nrurol.2014.193.

Jensen, F., Woudwyk, M., Teles, A., Woidacki, K., Taran, F., Costa, S., Malfertheiner, S.F. & Zenclussen, A.C. (2010). Estradiol and progesterone regulate the migration of mast cells from the periphery to the uterus and induce their maturation and degranulation. *PLoS One, 5*(12):e14409. doi: 10.1371/journal.pone.0014409

Johannesson, U., Blomgren, B., Hilliges, M., Rylander, E. and Bohm-Starke, N. (2007). The vulval vestibular mucosa – morphological effects of oral contraceptives and menstrual cycle. *British Journal of Dermatology, 157*(3):487-93. doi: 10.1111/j.1365-2133.2007.08066.x

Johansson, T., Vinther Larsen, S., Bui, M., Ek, W.E., Karlsson, T. & Johansson, Å. (2023). Population-based cohort study of oral contraceptive use and risk of depression. *Epidemiology and Psychiatric Sciences, 32*:e39. doi:10.1017/S2045796023000525

Johnson, C. F. (2004). Child sexual abuse. *The Lancet*, *364*(9432), 462–470. https://doi.org/10.1016/s0140-6736(04)16771-8

Kaur, G., Singh, N. & Jaggi, A. (2017). Mast cells in neuropathic pain: an increasing spectrum of their involvement in pathophysiology. *Reviews in the Neurosciences, 28*(7), 759-766. https://doi.org/10.1515/revneuro-2017-0007

Keller, A. S., Leikauf, J. E., Holt-Gosselin, B., Staveland, B. R., & Williams, L. M. (2019). Paying attention to attention in depression. *Translational Psychiatry, 9*(1). https://doi.org/10.1038/s41398-019-0616-1

Kiesner, J. & Bittoni, C. (2024). *Hormonal Contraceptive (HC) Use and Risk for Vulvodynia: Are Side Effects of HC Use Early Signs of Risk?* Abstract presented at ISSVD, Ljubljana

King, M., Rubin, R., & Goldstein, A. (2014). Current uses of surgery for the treatment of genital pain. *Current Sexual Health Reports, 6* (4), 252–258.

Kizilbash, A. H., Vanderploeg, R. D., & Curtiss, G. (2002). The effects of depression and anxiety on memory performance. *Archives of Clinical Neuropsychology*, *17*(1), 57–67. https://doi.org/10.1093/arclin/17.1.57

Kovats, S. (2015). Estrogen receptors regulate innate immune cells and signaling pathways. *Cellular Immunology, 294*(2), 63–69. doi: 10.1016/j.cellimm.2015.01.018

Krapf, J. M., Mitchell, L., Holton, M. A., & Goldstein, A. T. (2020). Vulvar Lichen Sclerosus: Current Perspectives. *International Journal of Women's Health, 12*, 11–20. https://doi.org/10.2147/IJWH.S191200

Lamvu, G., Carrillo, J., Ouyang, C. & Rapkin. A. (2021). Chronic Pelvic Pain in Women: A Review. *JAMA, 325*(23), 2381–2391. doi:10.1001/jama.2021.2631

Landry, T. & Bergeron, S. (2009). How Young does Vulvo-Vaginal Pain Begin? Prevalence and Characteristics of Dyspareunia in Adolescents. *The Journal of Sexual Medicine, 6*(4), 927–935. https://doi.org/10.1111/j.1743-6109.2008.01166.x

Lane, T. & Francis, A. (2003). Premenstrual symptomatology, locus of control, anxiety and depression in women with normal menstrual cycles. *Archives of Women's Mental Health, 6*(2), 127–138. https://doi.org/10.1007/s00737-003-0165-7

LeDoux, J.E. (2015). *Anxious: Using the brain to understand and treat fear and anxiety*. Penguin.

Leusink, P., Teunissen, D., Lucassen, P. L., Laan, E. T., & Lagro-Janssen, A. L. (2018). Facilitators and barriers in the diagnostic process of vulvovaginal complaints (vulvodynia) in general practice: a qualitative study. *European Journal of General Practice, 24*(1), 92–98. https://doi.org/10.1080/13814788.2017.1420774

Leusink, P., Van de Pasch, S., Teunissen, D., Laan, E.T., Lagro-Janssen, A.L. (2018). The Relationship Between Vulvovaginal Candidiasis and Provoked Vulvodynia: A Systematic Review. *The Journal of Sexual Medicine, 15*(9), 1310–1321. https://doi.org/10.1016/j.jsxm.2018.07.011

Levin, E.R. & Hammes, S.R. (2011). Chapter 40. Estrogens and progestins. In Brunton, L.L., Chabner, B.A. & Knollmann, B.C. (Eds.), *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12th ed. New York, NY: McGraw-Hill.

Lindeque, L.X. (2013). The Bartholin gland: an overview of anatomy, physiology and disease: review. *Obstetrics and Gynaecology Forum, 23*(1). https://hdl.handle.net/10520/EJC130953

Lindström, S. & Kvist, L.J. (2015). Treatment of Provoked Vulvodynia in a Swedish cohort using desensitization exercises and cognitive behavioral therapy. *BMC Women's Health 15*, 108. https://doi.org/10.1186/s12905-015-0265-3

Lucky, A.W., Henderson, T.A., Olson, W.H., Robisch, D.M., Lebwohl, M., & Swinyer, L.J. (1997). Effectiveness of norgestimate and ethinyl estradiol in treating moderate acne vulgaris. *Journal of the American Academy of Dermatology;37*(5 Pt 1), 746–54.

Ly Tall, A.B. (2020). *La pratique des mutilations génitales féminines: Valeur culturelle ou répression sexuelle ?* L'Harmattan. p. 21.

Mac Bride M.B., Rhodes D.J., & Shuster L.T. (2010). Vulvovaginal Atrophy. *Mayo Clin Proc, 85*(1), 87-94. DOI:https://doi.org/10.4065/mcp.2009.0413

Maclennan, A.H., Broadbent, J.L., Lester, S. & Moore, V. (2004). Oral oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. *Cochrane Database Systematic Review,* (4):CD002978. doi: 10.1002/14651858.CD002978.pub2.

Marín, F. & Barbancho, M. C. (2006). Action of Selective Estrogen Receptor Modulators (SERMs) Through the Classical Mechanism of Estrogen Action. In Sanchez, A. C., Alsina, J. C. & Dueñas-Díez, J-L. (Eds.), *Selective Estrogen Receptor Modulators: A New Brand of Multitarget Drugs* (pp. 71-77). Springer.

Mark, K. P., Leistner, C. E., & Garcia, J. R. (2016). Impact of contraceptive type on sexual desire of women and of men partnered to contraceptive users. *Journal of Sexual Medicine, 13*(9), 1359–1368. http://doi.org/10.1016/j.jsxm.2016.06.011

Maseroli, E., & Vignozzi, L. (2020). Testosterone and Vaginal Function. *Sexual Medicine Reviews*, *8*(3), 379–392. https://doi.org/10.1016/j.sxmr.2020.03.003

Mazloomdoost, D., Crisp, C.C., Westermann, L.B., Benbouajili, J.M., Kleeman, S.D. & Pauls, R.N. (2015). Survey of male perceptions regarding the vulva. *American Journal of Obstetrics & Gynecology,* 213(5), 731.e1-9. doi: 10.1016/j.ajog.2015.05.063.

McEwen, B.S. & Milner, T.A. (2016). Understanding the broad influence of sex hormones and sex differences in the brain. *Journal of Neuroscience Research, 95*(1-2), 24-39. https://doi.org/10.1002/jnr.23809

McKay, A. (2007). The effectiveness of latex condoms for prevention of STI/HIV. The *Canadian Journal of Human Sexuality, 16*(1-2). https://link.gale.com/apps/doc/A169458822/AONE?u=anon~f1bcebfb&sid=googleScholar&xid=88ab8b16

MedilinePlus. n.d. *Testosterone Levels Test*. National Library of Medicine. https://medlineplus.gov/hormones.html

Mello, A. de A. F. de, Mello, M. F. de, Carpenter, L. L., & Price, L. H. (2003). Update on stress and depression: the role of the hypothalamic-pituitary-adrenal (HPA) axis. *Revista Brasileira de Psiquiatria, 25*(4), 231–238. https://doi.org/10.1590/s1516-44462003000400010

Morin, M. (2018). Fear-avoidance and Pelvic Floor Muscle Function are Associated With Pain Intensity in Women With Vulvodynia. *The clinical journal of pain 34*(9), 804-810. Doi: 10.1097/AJP.0000000000000604.

Nguyen, J.D. & Duong, H. (2023). Anatomy, Abdomen and Pelvis: Female External Genitalia. *StatPearls [Internet]*. https://www.ncbi.nlm.nih.gov/books/

O'Connell, K., Davis, A.R. & Kern, J. (2007). Oral contraceptives: side effects and depression in adolescent girls. *Contraception, 75*(4), 299-304. doi: 10.1016/j.contraception.2006.09.008

Osmosis. (2024). *Female reproductive system Notes: Diagrams & Illustrations*. https://www.osmosis.org/

Owen J. (1975). Physiology of the menstrual cycle. *The American Journal of Clinical Nutrition. 28*(4), 333-338. https://doi.org/10.1093/ajcn/28.4.333

Oxford English Dictionary, s.v. "dyspareunia (n.), Etymology," December 2023, https://doi.org/10.1093/OED/2868626679.

Pastor, Z., Holla, K., & Chmel, R. (2013). The influence of combined oral contraceptives on female sexual desire: A systematic review. *European Journal of Contraception & Reproductive Health Care, 18*(1), 27–43. http://doi.org/10.3109/13625187.2012.728643

Pauls, R. N. (2015). Anatomy of the clitoris and the female sexual response. *Clinical Anatomy*, *28*(3), 376–384. https://doi.org/10.1002/ca.22524

Peebles, K., Kiweewa, F.M., Palanee-Phillips, T., Chappell, C., Singh, D., Bunge, K.E., et al. (2020). Elevated Risk of Bacterial Vaginosis Among Users of the Copper Intrauterine Device: A Prospective Longitudinal Cohort Study. *Clinical Infectious Diseases, 73*(3), 513–520. https://doi.org/10.1093/cid/ciaa703

Persson, L., Henriksson, P., Westerlund, E., Hovatta, O., Angelin, B. & Rudling, M. (2011). Endogenous Estrogens Lower Plasma PCSK9 and LDL Cholesterol But Not

Lp(a) or Bile Acid Synthesis in Women. *Arteriosclerosis, Thrombosis, and Vascular Biology, 32*(3). https://doi.org/10.1161/ATVBAHA.111.242461

Portman, D.J. & Gass, M.L. (2014). Vulvovaginal atrophy terminology consensus conference Panel. Genitourinary syndrome of menopause: a new terminology for vulvovaginal atrophy from the international society for the study of women's sexual health and the North american menopause society. *Menopause, 21*(10), 1063-8. doi: 10.1097/GME.0000000000000329

Pukall, C.F. Goldstein, A.T., Bergeron, S., Foster, D., Stein, A., Kellogg-Spadt, S. & Bachmann, G. (2016). Vulvodynia: Definition, Prevalence, Impact, and Pathophysiological Factors. *The Journal of Sexual Medicine, 13*(3), 291–304, https://doi.org/10.1016/j.jsxm.2015.12.021

Pukall, C.F., Strigo, I.A., Binik, Y.M., Amsel, R., Khalifé, S. & Bushnell, M.C. (2005). Neural correlates of painful genital touch in women with vulvar vestibulitis syndrome. *Pain, 115*(1-2), 118-27. doi: 10.1016/j.pain.2005.02.020

Rector, J. L., & Friedman, E. M. (2018). Hormones and well-being. In E. Diener, S. Oishi, & L. Tay (Eds.), *Handbook of well-being*. Salt Lake City, UT: DEF Publishers. DOI:nobascholar.com

Redmond, G.P., Olson, W.H., Lippman, J.S., Kafrissen, M.E., Jones, T.M., & Jorizzo, J.L. (1997). Norgestimate and ethinyl estradiol in the treatment of acne vulgaris: A randomized, placebo-controlled trial. *Obstetrics and Gynecology, 89*(4), 615–22. DOI: 10.1016/S0029-7844(97)00059-8

Reed, B. D., Harlow, S. D., Sen, A., Legocki, L. J., Edwards, R. M., Arato, N., & Haefner, H. K. (2012). Prevalence and demographic characteristics of vulvodynia in a population-based sample. *American Journal of Obstetrics and Gynecology*, *206*(2), 170.e1-170.e9. https://doi.org/10.1016/j.ajog.2011.08.012

Reed, B.D., Haefner, H.K., Harlow, S.D., Gorenflo, D.W. & Sen, A. (2006). Reliability and validity of self-reported symptoms for predicting vulvodynia. *Obstetrics and Gynecology, 108*(4), 906-13. doi: 10.1097/01.AOG.0000237102.70485.5d

Reed, B.D., Haefner, H.K., Sen, A. & Gorenflo, D.W. (2008). Vulvodynia incidence and remission rates among adult women: a 2-year follow-up study. *Obstetrics and Gynecology, 112*(2 Pt 1), 231-7. doi: 10.1097/AOG.0b013e318180965b

Reed, B.D., Harlow, S.D., Legocki, L.J., Helmuth, M.E., Haefner, H.K., Gillespie, B.W. & Sen, A. (2013). Oral contraceptive use and risk of vulvodynia: a population-based longitudinal study. *Epidemiology, 120*(13):1678-84. doi: 10.1111/1471-0528.12407

Reissing, E., Binik, Y., Khalife, S., Cohen, D. & Amsel R. (2003). Etiological correlates of vaginismus: sexual and physical abuse, sexual knowledge, sexual self-schema, and relationship adjustment. *Journal of Sex & Marital Therapy, 29*(1), 47-59. DOI: 10.1080/713847095

Revicky, V., Mukhopadhyay, S. & Morris, E. (2012). Dyspareunia in gynaecological practice. *Obstetrics, gynaecology and reproductive medicine, 22*(6), 148-154. https://doi.org/10.1016/j.ogrm.2012.02.010

Ridley, C.M. (1998). Vulvodynia: Theory and Management. *Dermatologic Clinics, 16*(4):775-778. DOI: 10.1016/s0733-8635(05)70045-0

Robboy, S.J., Ross, J.S., Prat, J., Keh, P.C. & Welch, W.R. (1978). Urogenital sinus origin of mucinous and ciliated cysts of the vulva. *Obstetrics and Gynecology, 51*(3), 347-51. doi: 10.1097/00006250-197803000-00020.

Roberts, S. C., Klapilová, K., Little, A. C., Burriss, R. P., Jones, B. C., DeBruine, L. M., et al. (2012). Relationship satisfaction and outcome in women who meet their partner while using oral contraception. *Proceedings of the Royal Society of London B: Biological Sciences, 279*(1732), 1430–1436. http://doi.org/10.1098/rspb.2011.1647

Rosenberg, M.J., Meyers, A., & Roy, V. (1999). Efficacy, Cycle Control, and Side Effects of Low- and Lower-Dose Oral Contraceptives: A Randomized Trial of 20 mg and 35 mg Estrogen Preparations. *Contraception, 60*(6), 321–329. https://doi.org/10.1016/S0010-7824(99)00109-2.

Rowen, T.S. & Goldstein, A.T. (2021). Nosology of Pelvic Pain and Vulvodynia. In John Wiley & Sons (Eds.), *Female sexual pain disorders: Evaluation and management* (pp. 1-8). John Wiley & Sons

Ruscio, A.M., Hallion, L.S., Lim, C.C.W., Aguilar-Gaxiola, S., Al-Hamzawi, A., Alonso, J., Andrade, L.H., Borges, G., Bromet, E.J., Bunting, B., Caldas de Almeida, J.M., Demyttenaere, K., Florescu, S., de Girolamo, G., Gureje, O., Haro, J.M., He, Y., Hinkov, H., Hu, C., ... Scott, K.M. (2017). Cross-sectional comparison of the epidemiology of DSM-5 generalized anxiety disorder across the globe. *JAMA Psychiatry, 74*(5), 465-475. https://doi.org/10.1001/jamapsychiatry.2017.0056

Sacher, B.C. (2019). The Normal Vulva and Vagina. In Bornstein, J. (Ed.), *Vulvar Disease: Breaking the Myths*, pp.7-19. Cham, Switzerland: Springer Nature.

Sadownik, L. (2014). Etiology, diagnosis, and clinical management of vulvodynia. *International Journal of Women's Health*, 437. https://doi.org/10.2147/ijwh.s37660

Sarchielli, P., Nardi, K., Caproni, S., Chiasserini, D., Pieroni, A., Corbelli, I., & Calabres, P. (2010). Pathophysiological mechanisms of inflammatory and neuropathic pain. In P. Sarchielli, K. Nardi, S. Caproni, D. Chiasserini, A. Pieroni, I. Corbelli, & P. Calabres (Eds.), *Pain and its origins, diagnosis and treatments: Nerve growth factor and pain* (pp. 9-12). Nova Science Publishers.

Schlaeger, J.M., Glayzer, J.E., Villegas-Downs, M., Li, H., Glayzer, E.J., He, Y., Takayama, M., Yajima, H., Takakura, N., Kobak, W.H. & McFarlin, B.L. (2022). Evaluation and Treatment of Vulvodynia: State of the Science. *Journal of Midwifery & Women's Health, 68*(1), 9-34. doi: 10.1111/jmwh.13456

Schmidt, M., Naumann, H., Weidler, C., Schellenberg, M., Anders, S. & Straub, R.H. (2006). Inflammation and Sex Hormone Metabolism. *Annals of the New York Academy of Science, 1069*(1), 236-246. https://doi.org/10.1196/annals.1351.021

Secor R. (1992). The cervical cap. *NAACOGS Clin Issue Perinatal Women's Health Nurse, 3*(2), 236-245. PMID: 1596432.

Shallcross, R., Dickson, J.M., Nunns, D., Mackenzie, C. & Kiemle, G. (2018). Women's Subjective Experiences of Living with Vulvodynia: A Systematic Review and Meta-Ethnography. *Archives of Sexual Behavior, 47*, 577–595. https://doi.org/10.1007/s10508-017-1026-1

Siiteri, P.K. (1987). Adipose tissue as a source of hormones. *The American Journal of Clinical Nutrition, 45*(1), 283-289. https://doi.org/10.1093/ajcn/45.1.283

Silberstein, S.D. & Merriam, G.R. (2000). Physiology of the menstrual cycle. *Cephalalgia, 20*(3), 148-154. https://doi-org.proxy.insermbiblio.inist.fr/10.1046/j.1468-2982.2000.00034.x

Simon, J. A. (2011). Identifying and Treating Sexual Dysfunction in Postmenopausal Women: The Role of Estrogen. *Journal of Women's Health*, *20*(10), 1453–1465. https://doi.org/10.1089/jwh.2010.2151

Simons, J.S. & Carey, M.P. (2001). Prevalence of sexual dysfunctions: results from a decade of research. *Archives of Sexual Behavior, 30*(2), 177-217. DOI: 10.1023/a:1002729318254

Singh, N. (2013). Development and Anatomy: 1 Disorders of Development. In Brown, L. (Ed.), *Pathology of the Vulva and Vagina* (pp. 1-10).

Siqueira-Campos, V.M., Da Luz, R.A., de Deus, J.M., Martinez, E.Z., & Conde, D.M. (2019). Anxiety and depression in women with and without chronic pelvic pain: prevalence and associated factors. *Journal of Pain Research, 12*, 1223–1233. https://doi.org/10.2147/JPR.S195317

Sjöberg I., Cajander S., & Rylander E. (1998). Morphometric charac- teristics of vaginal epithelium during the menstrual cycle. *Gynecol Obstet Invest, 26*(2), 136–44. DOI: 10.1159/000293685

Spinillo A., Capuzzo E., Nicola S., Baltaro F., Ferrari A., Monaco A. (1995). The impact of oral contraception on vulvovaginal candidiasis. *Contraception, 51*(5), 293–7. DOI: 10.1016/0010-7824(95)00079-p

Stárka, L. & Dušková M. (2020). What is a hormone? *Physiological Research, 69*(Suppl 2):S183-S185. doi: 10.33549/physiolres.934509.

Stein, D.G. (2006). The case of progesterone. *Annals of the New York Academy of Sciences, 1052*(1), 152-169. https://doi.org/10.1196/annals.1347.011

Straub, R.H. (2007). The Complex Role of Estrogens in Inflammation. *Endocrine Reviews, 28*(5), 521–574. https://doi.org/10.1210/er.2007-0001

Strauhal, M.J., Frahm, J., Morrison, P., Featherstone, W., Hartman, D., Florendo, J. & Parker, S. (2007). Vulvar Pain: A Comprehensive Review. *Journal of Women's Health Physical Therapy, 31*(3), 7-26. doi:10.1097/01274882-200731030-00003

Sundell, M. (2023). Background. In *Epidemiological and clinical aspects of hormonal contraception and menopausal hormone therapy in women*. Linköping University medical dissertation Nr. 1849, 14-27.

Taney, J. & Tu, F. (2021). Chronic Pelvic Pain. In John Wiley & Sons (Eds.), *Female sexual pain disorders: Evaluation and management* (pp. 313-321). John Wiley & Sons

Tasker, J.G. & Herman, J.P. (2011). Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis. *Stress, 14*(4), 398-406. doi: 10.3109/10253890.2011.586446

Teal, S. & Edelman, A. (2021). Contraception Selection, Effectiveness, and Adverse Effects: A Review. *Journal of the American Medical Association, 326*(24), 2507–2518. doi:10.1001/jama.2021.21392

Theoharides, T.C. (2020). The impact of psychological stress on mast cells. *Annals of Allergy, Asthma & Immunology, 125*(4), 388-392. https://doi.org/10.1016/j.anai.2020.07.007

Theoharides, T.C., Whitmore, K., Stanford, E., Moldwin, R. & O'Leary, M.P. (2008). Interstitial cystitis: bladder pain and beyond. *Expert Opinion on Pharmacotherapy, 9*(17), 2979-2994. doi: 10.1517/14656560802519845

Thiel, K. J., & Dretsch, M. N. (2011). Basics of the stress response. In C. D. Conrad (Ed.), *The handbook of stress: Neuropsychological effects on the brain* (pp. 1-28). Wiley-Blackwell.

Thomas, T., & Mundale, P. (1891). *A practical treatise on the diseases of women* (Vol. 6). Lea Brothers and Company.

Toeima, E., & Nieto, J. (2010). Junior doctors' understanding of vulval pain/Vulvodynia: a qualitative survey. *Archives of Gynecology and Obstetrics*, *283*(S1), 101–104. https://doi.org/10.1007/s00404-010-1513-2

Toffoletto, S., Lanzenberger, R., Gingnellc, M., Sundström-Poromaad, I. & Comasco, E. (2014). Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: A systematic review. *Psychoneuroendocrinology, 50*, 28–52. http://dx.doi.org/10.1016/j.psyneuen.2014.07.025

Tomomi, S., Shinichi, M. & Taisen, I. (2015). Progesterone. In Yoshio, T., Hironori, A. & Kazuyoshi, T (Eds.), *Handbook of hormones: comparative endocrinology for basic and clinical research* (pp. 507-508). Elsevier Science & Technology

Trutnovsky, G., Plieseis, C., Bjelic-Radisic, V., BertholinyGalvez, M. C., Tamussino, K., & Ulrich, D. (2018). Vulvodynia and chronic pelvic pain in a gynecologic outpatient clinic. Journal of *Psychosomatic Obstetrics & Gynecology, 40*(3), 243–247. https://doi.org/10.1080/0167482X.2018.1477753

Tugrul, C. & Kabakci, E. (1997). Vaginismus and its correlates. *Journal of Sex & Marital Therapy*, 12:23-4

Vallvé-Juanico, J., Houshdaran, S. & Giudice, L.C. (2019). The endometrial immune environment of women with endometriosis. *Human Reproduction Update, 25*(5):564–591. doi: 10.1093/humupd/dmz018

Van de Wijgert, J.H.H.M, Verwijs, M.C.; Turner, A.N. & Morrison, C.S. (2013). Hormonal contraception decreases bacterial vaginosis but oral contraception may increase candidiasis: implications for HIV transmission. *AIDS, 27*(13), p 2141-2153. DOI: 10.1097/QAD.0b013e32836290b6

Vijayakumar, G., Mabude, Z., Smit, J., Beksinska, M. & Lurie, M. (2006). A review of female-condom effectiveness: patterns of use and impact on protected sex acts and STI incidence. *International Journal of STD & AIDS, 17*(10), 652-659. doi:10.1258/095646206780071036

Weijmar Schultz, W., Basson, R., Binik, Y., Eschenbach, D., Wesselmann, U. & Van Lankveld, J. (2005). Women's sexual pain and its management. *Journal of Sexual Medicine, 2*(3), 301-316. doi: 10.1111/j.1743-6109.2005.20347.x

Wernert, N., Albrech, M., Sesterhenn, I., Goebbels, R., Bonkhoff, H., Seitz, G., Inniger, R. & Remberger, K. (1992). The "female prostate": location, morphology,

immunohistochemical characteristics and significance. *European Urology, 22*(1), 64-69. doi: 10.1159/000474724.

Weström, L.V. & Willén, R. (1998). Vestibular Nerve Fiber Proliferation in Vulvar Vestibulitis Syndrome. *Obstetrics & Gynecology, 91*(4), 572-576.

Wharton, W., Gleason, C. E., Olson, S. R., Carlsson, C. M., & Asthana, S. (2012). Neurobiological underpinnings of the estrogen - mood relationship. *Current Psychiatry Research and Reviews, 8*(3), 247-256. doi: 10.2174/157340012800792957

World Health Organization. (2021). *Chronic pain.* https://www.who.int/news/item/01-02-2021-who-issues-new-guidelines-on-the-management-of-chronic-pain-in-children

Yavagal, S., De Farias, T.F., Medina, C.A. & Takacs, P. (2011). Normal vulvovaginal, perineal, and pelvic anatomy with reconstructive considerations. *Seminars in Plastic Surgery, 25*(2), 121-129. doi: 10.1055/s-0031-1281481.

Yeung, J. & Pauls, R.N. (2016). Anatomy of the Vulva and the Female Sexual Response. *Obstetrics and Gynecology Clinics of North America, 43*(1), 27-44. https://doi.org/10.1016/j.ogc.2015.10.011

Yurteri-Kaplan, L.A., Antosh, D.D., Sokol, A.I., Park, A.J., Gutman, R.E., Kingsberg, S.A. & Iglesia, C.B. (2012). Interest in cosmetic vulvar surgery and perception of vulvar appearance. *American Journal of Obstetrics & Gynecology, 207*(5), 428.e1-7. doi: 10.1016/j.ajog.2012.06.056.

Zdilla, M.J. (2022). What is a vulva?. *Anatomical Science International, 97*, 323–346. https://doi.org/10.1007/s12565-022-00674-7

Zhang, J.M. & An, J. (2007). Cytokines, inflammation, and pain. *International Anesthesiology Clinics, 45*(2), 27-37. doi: 10.1097/AIA.0b013e318034194e

Zimmerman, Y., Eijkemans, M. J. C., Coelingh Bennink, H. J. T., Blankenstein, M. A., & Fauser, B. C. J. M. (2014). The effect of combined oral contraception on testosterone levels in healthy women: A systematic review and meta-analysis. *Human Reproduction Update*, *20*(1), 76–105. http://doi.org/10.1093/humupd/dmt038

Zondervan, K.T. & Barlow, D.H. (2000). Epidemiology of chronic pelvic pain. *Best Practice & Research Clinical Obstetrics & Gynaecology, 14*(3), 403-414. https://doi.org/10.1053/beog.1999.0083

Zondervan, K.T., Yudkin, P.L., Vessey, M.P., Jenkinson, C.P., Dawes, M.G., Barlow, D.H. & Kennedy, S.H. (2001). The community prevalence of chronic pelvic pain in women and associated illness behaviour. *British Journal of General Practice, 51*(468), 541-547.