치의과학박사 학위논문

Changes in Oral Mucosal MUC1 Expression and Salivary Hormones throughout the Menstrual Cycle

여성 생리주기에 따른 구강점막 MUC1 발현 및 타액 호르몬의 변화

2016년 2월

서울대학교 대학원

치의과학과 구강내과·진단학 전공

이 연 희

Changes in Oral Mucosal MUC1 Expression and Salivary Hormones throughout the Menstrual Cycle

여성 생리주기에 따른 구강점막 MUC1 발현 및 타액 호르몬의 변화

지도교수 고 홍 섭

이 논문을 치의과학박사 학위논문으로 제출함

2015년 10월

서울대학교 대학원

치의과학과 구강내과·진단학 전공

이 연 희

이연희의 치의과학박사 학위논문을 인준함

2015년 12월

위 원 장 _____

부위원장 _____

위 원 _____

위 원 _____

위 원 _____

ABSTRACT

Changes in Oral Mucosal MUC1 Expression and Salivary Hormones throughout the Menstrual Cycle

Yeon-Hee Lee, D.D.S., M.S.D.

Program in Oral Medicine and Oral Diagnosis, Dept. of Dental Science, Graduate School, Seoul National University

(Directed by Professor **Hong-Seop Kho**, D.D.S., M.S.D., Ph.D.)

The purpose of this study was to investigate relationships among oral mucosal epithelial MUC1 expression, salivary stress markers, and female gonadal hormones throughout the menstrual cycle.

Thirty healthy women (25.9 ± 2.1 years) with regular menstrual cycle were included. Unstimulated (UWS) and stimulated whole saliva (SWS) were collected during the menstrual cycle. The expression level of oral mucosal MUC1 was analyzed. 17β-Estradiol, progesterone, dehydroepiandrosterone (DHEA), cortisol, chromogranin A (CgA), and blood contamination levels were measured from unstimulated (UWS) and stimulated whole saliva (SWS).

In analyses using all data from the four phases combined, significant positive correlations

were observed between 17β-estradiol and DHEA in UWS, cortisol and CgA in UWS, MUC1 expression and DHEA in SWS, and among cortisol, progesterone, and DHEA in UWS and SWS. Significant negative correlations were observed between MUC1 and cortisol/DHEA ratio in UWS and SWS. When each phase was analyzed individually, MUC1 expression showed significant negative correlations with cortisol, progesterone, and cortisol/DHEA ratio in UWS and with progesterone and cortisol/DHEA ratio in SWS during the mid-luteal phase. A significant negative correlation was also observed between MUC1 and cortisol/DHEA ratio in UWS during the late-luteal phase.

In conclusions, stress-related psychoendocrinological interactions throughout the menstrual cycle resulted in a decrease in oral mucosal epithelial MUC1 expression and a weakening of oral mucosal defense.

Key words: MUC1, Stress, Steroid hormone, Saliva, Menstrual cycle

Student Number: 2012-31183

Changes in Oral Mucosal MUC1 Expression and Salivary Hormones throughout the Menstrual Cycle

여성 생리주기에 따른 구강점막 MUC1 발현 및 타액 호르몬의 변화

Yeon-Hee Lee, D.D.S., M.S.D.

Program in Oral Medicine and Oral Diagnosis, Dept. of Dental Science, Graduate School, Seoul National University

(Directed by Professor **Hong-Seop Kho**, D.D.S., M.S.D., Ph.D.)

이 연 희

서울대학교 대학원 치의과학과 구강내과·진단학 전공

(지도교수 고 홍 섭)

-CONTENTS-

I. INTRODUCTION

II. REVIEW OF LITERATURE

III. MATERIALS AND METHODS

IV. RESULTS

V. DISCUSSION

VI. CONCLUSIONS

REFERENCES

TABLES

KOREAN ABSTRACT

I. INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis has been the main focus of stress-related studies through the measurement of cortisol, dehydroepiandrosterone (DHEA), and cortisol/DHEA ratio (Hellhammer et al., 2009; Jeckel et al., 2010; Lennartsson et al., 2012; Tsigos and Chrousos, 2002). Female gonadal hormones also affect stress-related responses, thereby explaining differences in stress responses depending on sex and menstrual cycle (Childs et al., 2010; Kajantie and Phillips, 2006; Wolfram et al., 2011). It has been reported that the HPA axis response to acute stress was greater in the progesterone-dominated luteal phase (Kajantie and Phillips, 2006), and that cortisol awakening response was elevated in the ovulatory phase (Wolfram et al., 2011). Progesterone administration also affected the psychological responses to acute stress (Childs et al., 2010).

Stress and gonadal hormones can affect oral mucosal defense. Chronic stress decreases the salivary flow rate and sIgA levels (Hucklebridge et al., 1998; Somer et al., 1993). Many oral mucosal diseases have etiopathophysiological relationships with stress (Little et al, 2013). The estrogen receptor has been reported to be present in oral mucosal epithelium (Sawczuk et al., 2014). Additionally, the menstrual cycle affects the occurrence or exacerbation of recurrent aphthous stomatitis (Oh et al., 2009).

MUC1, a large transmembrane glycoprotein, expressed in epithelial cell linings provides mucosal defense in the respiratory, reproductive, and gastrointestinal tracts (Gendler, 2001). Although the role of MUC1 may be somewhat different depending on anatomical location, epithelial MUC1 expression in both the oral cavity and female reproductive tract is up-regulated by the presence of microbial infections and pro-inflammatory cytokines (McAuley et al., 2007; Meseguer et al., 2001). Human endometrial MUC1 expression is also affected by

the menstrual cycle and is up-regulated during the progesterone-dominated luteal phase (Brayman et al., 2004; Hey et al., 1994; Meseguer et al., 2001). However, it is currently unknown whether oral mucosal epithelial MUC1 expression is affected by changes in female gonadal hormones throughout the menstrual cycle.

The hypothesis in the present study was oral mucosal epithelial MUC1 expression related to oral mucosal defense could be affected by stress and female gonadal hormonal changes throughout the menstrual cycle. Saliva samples were used because steroid hormone levels in saliva are well correlated with the free protein-unbound active hormone levels in blood (Streckfus and Bigler, 2002). The non-invasive nature of saliva collection provides an additional advantage for studies requiring repetitive sampling. The aim of the present study was to investigate whether changes in salivary stress markers and female gonadal hormones throughout the menstrual cycle affect oral mucosal epithelial MUC1 expression. The relationships among salivary stress markers and female gonadal hormones throughout the menstrual cycle were also investigated.

II. REVIEW OF LITERATURE

1. Stress markers and gonadal hormones

(1) Cortisol

Cortisol is a steroid hormone, in the glucocorticoid class of hormones, and is produced in humans by the zona fasciculatla of the adrenal cortex within the adrenal gland. It has been suggested that cortisol secretion is stimulated by psychological distress and social evaluative threats (Dickerson and Kemeny, 2004). Thus, glucocorticoid levels are frequently used to evaluate physiologic stress levels within and between individuals (Kanaley et al., 2001; Kunz-Ebrecht et al., 2004; Negrao et al., 2000). Many studies have also investigated the relationship between daily life stress and cortisol. For example, work stress (Steptoe et al., 2000), unemployment, and divorce (Ockenfels et al., 1995) were associated with increased morning or night-time cortisol levels. Recent studies also investigated the cortisol awakening response and the stress in daily life. Cortisol levels in human salivary and plasma fluid increased by 50–60% within 30 minutes after awakening. In addition, recent studies have shown that the cortisol awakening response is a useful indicator of HPA activity (Clow et al., 2004).

Cortisol is one of the most important end products of hypothalamic-pituitary-adrenal (HPA) axis activation (Kudielka and Kirschbaum, 2003). It is well known that physiologic response to stress is mediated by the HPA axis. HPA function is linked to critical metabolic tasks such as immune response, cardiovascular function, reproductive physiology, and general well-being. When stimulated by endogenous and exogenous challenges, the paraventricular

nucleus of the hypothalamus increases its production of corticotropin-releasing hormone (CRH), which in turn promotes the release of adrenocorticotropin (ACTH) by the anterior pituitary, leading to an increase in the secretion of glucocorticoids, including cortisol, by the adrenal cortex. Increases in cortisol levels trigger gluconeogenesis, resulting in higher levels of circulating glucose. Glucose provides energy to the tissues involved in responding to the challenges that trigger the activation of the HPA axis in the first place (Nepomnaschy et al., 2007). Thus, cortisol levels are frequently used to monitor HPA axis function and activation, and are interpreted as proxies of physiologic stress levels (Altemus et al., 2001; Kanaley et al., 2001; Padgett and Glaser, 2003). Furthermore, cortisol is considered as one of the endogenous hormones that modulate energy homeostasis. A major physiological role of cortisol is considered to be gluconeogenesis and maintenance of blood glucose (Miller, 1995). Hence it increases glucose in the bloodstream, increases the availability of substances that repair tissues (Kunz-Ebrecht et al., 2004). Cortisol also has inhibitory functions that would be nonessential or detrimental in a fight-or-flight situation. It alters immune system responses and suppresses the digestive system, the reproductive system and growth processes. This complex natural alarm system also communicates with regions of the brain that control mood, motivation and fear. Moreover, cortisol is associated with health-related variables such as psychoses and cardiovascular disease; therefore, cortisol is a mediator between psychosocial stress and health (McEwen, 2000).

(2) Dehydroepiandrosterone (DHEA)

DHEA, more correctly didehydroepiandrosterone, also known as androstenolone or prasterone, as well as 3β-hydroxyandrost-5-en-17-one or 5-androsten-3β-ol-17-one, is an

important endogenous steroid hormone (Mo et al., 2006). It is the most abundant circulating steroid hormone in human body (Ganong, 2005), in whom it is produced in the adrenal glands, the gonads, and the brain, where it functions predominantly as a metabolic intermediate in the biosynthesis of the androgen and estrogen sex steroids (Webb et al., 2006). DHEA and its sulfated form, DHEA-S, are circulating steroid hormones that are more abundant in humans, and despite not showing an intrinsic androgenic activity, both are precursors of potent androgens and estrogens (Nieschlag et al., 1973). DHEA also has a variety of potential biological effects in its own right, binding to an array of nuclear and cell surface receptors, and acting as a neurosteroid.

The associations between psychosocial stress and DHEA levels have also been recently investigated. The main secretagogue for DHEA is ACTH, and DHEA was reported to be secreted synchronously with cortisol during the night and day and had anti-glucocorticoid and anti-cortisol effects (Rosenfeld et al., 1971). DHEA may affect certain brain functions by modulating neurotransmitter receptors such as gamma-aminobutyric acid (GABA) or N-methyl-D-aspartic acid (NMDA) receptors, and be involved in the pathophysiology of cognitive decline and mood disorders (Wolf and Kirschbaum, 1999). Acute psychosocial stress was reported to increase DHEA concentration (Izawa et al., 2008; Shirtcliff et al., 2007). However, despite the well-documented effects of stress on HPA axis function, studies of stress-induced increases in DHEA and DHEA-S response have yielded mixed results. (Morgan et al., 2004; Oberbeck et al., 1998; Shirotsuki et al., 2009).

The mechanisms of action of DHEA in humans are not completely understood, although it is considered to be an important regulator of body fat, immune response, insulin sensitivity, and cognitive function (Corona et al., 2013). In addition, Kibaly et al. (2008) demonstrated

the involvement of this steroid in the modulation of sensory processes, especially neuropathic pain. Reductions in DHEA concentrations are found in some diseases, such as diabetes, schizophrenia, systemic lupus erythematosus, Alzheimer disease, depression, and other psychologic disturbances (Brooke et al., 2006; Chang et al., 2002; Rabkin et al., 2006; Villareal and Holloszy, 2006). DHEA, in addition to cortisol, is a major steroid produced by the zona reticularis of the adrenal cortex. Some studies have suggested that lower DHEA levels are associated with lower psychological well-being (van Niekerk et al., 2001).

Salivary concentrations of DHEA reflect those in serum. In saliva, the concentration of this steroid is independent of salivary flow rate and represents accurately the fraction of the free and biologically active hormone in the blood circulation (Chen et al., 2005; Gao et al., 2005). It is meaningful to examine the salivary DHEA response to situations causing social anxiety.

(3) Cortisol/DHEA ratio

Cortisol and DHEA are steroid hormones, both synthesized from pregnenelone, the master steroid hormone, which is derived from cholesterol. DHEA can counteract several adverse effects of glucocorticoid in different tissues (Ferrari et al., 2001; Kalimi et al., 1994). Its mechanism of action and physiological implications are still not fully understood. In the context of the immune system, DHEA could exert its anti-glucocorticoid activity by modulating glucocorticoid receptor (GR) beta expression and antagonizing the function of GR alpha (Pinto et al., 2015).

The effects of DHEA on brain functions are believed to be opposite to those of cortisol. For example, it was reported that the cortisol/DHEA ratio was elevated in depressive patients

(Young et al., 2002), and that a high cortisol/DHEA ratio in adolescents was predictive of persistent major depression (Goodyer et al., 2003). Moreover, under acutely stressful situations, the cortisol/DHEA ratio was reported to be correlated with a negative mood level (Izawa et al., 2008). Although the association between cortisol/DHEA ratio and mood under prolonged stress has not been investigated, DHEA could antagonize cortisol activity indicated that (Blauer et al., 1991). Therefore, it is considered that the cortisol to DHEA ratio may be a marker of endocrine imbalance. For example, persistently depressed individuals had a higher cortisol to DHEA ratio than non-depressed and remitted individuals (Goodyer et al., 2003). Goodyer et al. (2003) suggested that the high ratio might be a marker of persistent psychiatric disorders. Other studies have found that a higher morning cortisol to DHEA ratio is associated with higher anxiety (van Niekerk et al., 2001). Young et al. (2002) showed that the cortisol to DHEA ratio from saliva samples correlated with the length of the current depressive episode and suggested that the cortisol to DHEA ratio could be a marker of depressive states. Thus, it has been speculated that the cortisol to DHEA ratio would represent an endocrine imbalance of the HPA axis function and may be a marker of the state of other psychiatric disorders.

(4) Chromogranin A

Chromogranin A (CgA), an acidic glycoprotein initially isolated as the major soluble protein of adrenal medullary chromaffin granules, has been reported to localize in secretory granules of a wide variety of endocrine cells and neurons (Winkler and Fischer-Colbrie, 1992). In humans, CgA protein is encoded by the parathyroid secretory protein gene (Helman et al., 1988). CgA are produced from chromaffin cells of the adrenal medulla, paraganglia,

enterochromaffin-like cells, and beta cells of the pancreas, and it induces and promotes the generation of secretory granules such as those containing insulin in pancreatic islet beta cells. CgA is the precursor to several functional peptides including vasostatin-1, vasostatin-2, pancreastatin, catestatin, and parastatin. These peptides negatively modulate the neuroendocrine function of the releasing cell or nearby cells (Curry et al., 2002). Recently, Den et al. (2007) reported that the human salivary CgA concentration peaked upon awakening, quickly decreased to a nadir after 1 hour, then remained at a low level throughout the day.

CgA is also produced by human submandibular glands and, in response to activation of the autonomic nervous system innervating the submandibular gland, is released directly from the exocrine cells of the granular convoluted tubules into the saliva. In addition, the highly concentrated salivary CgA is released in response to noradrenaline and acetylcholine in isolated and perfused rat submandibular glands (Kanno et al., 1999). Thus, salivary CgA has been proposed as a marker of sympathetic nervous activity involving the sympathetic-adrenomedullary system (Nakane et al., 2002) and might be used as an alternative to the measurement of plasma catecholamine concentration (Kanno et al., 1999).

Responding to stress, CgA and catecholamines are co-released into the extra-cellular environment. A prompt elevation in salivary CgA levels and a delayed increase in salivary cortisol levels when psychosomatic stress was induced by a test involving an oral presentation in front of an audience or a driving situation (Lee et al., 2006; Nakane et al., 2002). Lee et al. (2006) also found that salivary CgA concentration depicted an increase during the mental stress tasks and decrease during the intermissions, demonstrating the possible candidacy of CgA as a biomarker for a short-term mental workload. These studies

might suggest that salivary CgA may be a sensitive and promising index for psychosomatic stress.

Since researchers first applied noninvasive technology to measure CgA in saliva to the field of human science, substantial evidence of the psychological properties of salivary CgA has accumulated. In short-term stressful situations, such as public speaking, salivary CgA levels elevate and peak immediately before and decrease immediately after the event (Lee et al., 2006). In contrast, stress-reduction intervention, such as aromatherapy with lavender essential oil and exposure to negative air ions, has decreased elevated salivary CgA levels after stressful mental tasks (Matsumoto et al., 2014). Although CgA has been reported as a possible marker of stress, there were also inconsistent results showed that CgA levels were not increased in the stressful situation (Yamakoshi et al., 2009). Although CgA could serve as a potential neuropsychophysiological index for objectively assessing periodic emotional fluctuations, including late-luteal-specific emotional disturbances observed in women suffering from premenstrual syndrome, the scientific literature has failed to report on this aspect of the use of this biochemical stress marker.

(5) 17 beta-estradiol

Estradiol, or more precisely, 17β-estradiol, is a steroid and estrogen sex hormone, and the primary female sex hormone. It is named for and is important in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is essential for the development and maintenance of female reproductive tissues (Ryan, 1982), but it also has important effects in many other tissues including bone. While estrogen levels in men are lower compared to

women, estrogens have essential functions in men as well (Gooren et al., 1984). Estradiol is produced especially within the follicles of the female ovaries, but also in other endocrine and non-endocrine tissues including fat, liver, adrenal, breast, and neural tissues. Estradiol is biosynthesized from progesterone and arrived at in two steps from cholesterol, via intermediate pregnenolone (Saldanha et al., 2011). One principle pathway then converts progesterone to its 17-hydroxy derivative, 17-hydroxyprogesterone, and then to Δ4-androstenedione via sequential cytochrome P450-catalyzed oxidations. Alternatively, Δ4-androstenedione can be converted into the androgen, testosterone, which in turn can be converted directly into 17β-estradiol. In the female, estradiol acts as a growth hormone for tissue of the reproductive organs, supporting the lining of the vagina, the cervical glands, the endometrium, and the lining of the fallopian tubes. It enhances growth of the myometrium. Estradiol appears necessary to maintain oocytes in the ovary.

Women are more likely than men to show variations in HPA function in response to stressors (Weiss et al., 1999) and during depressive episodes (Young and Korszun, 2010). These differences in stress system response likely contribute to mood disorder risk in women (Weiss et al., 1999), and may be modulated by ovarian hormone fluctuations across the menstrual cycle (Kajantie and Phillips, 2006; Roca et al., 2005). While the role of corticosteroids in stress response and regulation is well known, the effects of estradiol along the hypothalamic-pituitary-gonadal (HPG) axis which can be stimulated by various stressors, including anxiety and depression are less well characterized. Brain activity related to processing negative emotional information is also modulated by changing estradiol levels across the menstrual cycle (Goldstein et al., 2005; Merz et al., 2012), suggesting that estrogen may alter the mood response to negative information, making this information more or less salient to cognitive processes and subsequently mood states.

Animal models suggest that female rodents do not gain the same beneficial effect of acute stress on hippocampally-mediated or prefrontal tasks as male animals, and that high estrogen levels enhance the negative effects of stress (Shansky et al., 2004; Shors and Leuner, 2003). However, estrogen replacement therapy increases resilience to stress in the learned helplessness model using ovariectomized rats, which might be related to hippocampal synaptic plasticity (Bredemann and McMahon, 2014; Smith et al., 2010). The modulation of stress effects on brain activity and function remain unclear and likely depend on the type of stress and measure of function (Shansky et al., 2004; Shors and Leuner, 2003). There is a sex difference in the endocrine response to psychosocial stress, and that cycling ovarian hormones may modulate this response in women (Kirschbaum et al., 1992; Kirschbaum et al., 1999).

(6) Progesterone

Progesterone is an endogenous steroid and sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species (Kumari et al., 2010). Progesterone is also a crucial metabolic intermediate in the production of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neurosteroid (Baulieu and Schumacher, 2000). Progesterone is produced in high amounts in the ovaries by the corpus luteum from the onset of puberty to menopause, and is also produced in smaller amounts by the adrenal glands after the onset of adrenarche in both males and females. During human pregnancy, progesterone is produced in increasingly high amounts by the ovaries and placenta by the presence of human chorionic gonadotropin. However, after the 8th week, production of progesterone shifts to the placenta. At term the

placenta produces about 250 mg progesterone per day. Whether a decrease in progesterone levels is critical for the initiation of labor has been argued and may be species-specific. After delivery of the placenta and during lactation, progesterone levels are very low. Adult males have levels similar to those in women during the follicular phase of the menstrual cycle.

Progesterone is the most important progestogen in the body, the result of its action as a potent agonist of the nuclear progesterone receptor (Daniel et al., 2010; Kumari et al., 2010). In addition, progesterone is an agonist of the more recently discovered membrane progesterone receptors (Thomas and Pang, 2012), as well as a ligand of the progesterone receptor membrane component 1, PGRMC1 (Meyer et al., 1998). Moreover, progesterone is also known to be an antagonist of the σ1 receptor (Maurice et al., 2001), and a negative allosteric modulator of the acetylcholine receptor (Baulieu and Schumacher, 2000), and a potent antagonist of the mineralocorticoid receptor (MR) (Rupprecht et al., 1993). Progesterone prevents MR activation by binding to this receptor with an affinity exceeding even those of aldosterone and glucocorticoids such as cortisol and corticosterone (Rupprecht et al., 1993), and produces anti-mineralocorticoid effects at physiological concentrations. In addition, progesterone binds to and behaves as a partial agonist of the glucocorticoid receptor (Attardi et al., 2007). Progesterone, through its neurosteroid active metabolites, acts indirectly as a positive allosteric modulator of the GABA receptor (Paul and Purdy, 1992) .

Progesterone has a number of physiological effects that are amplified in the presence of estrogens. Estrogens through estrogen receptors induce or up-regulate the expression of the progesterone receptor (Kastner et al., 1990). In addition, elevated levels of progesterone potently reduce the sodium-retaining activity of aldosterone, resulting in natriuresis and a reduction in extracellular fluid volume. Progesterone withdrawal, on the other hand, is

associated with a temporary increase in sodium retention due to the compensatory increase in aldosterone production, which combats the blockade of the mineralocorticoid receptor by the previously elevated level of progesterone.

2. Changes of gonadal hormones according to the menstrual cycle

The menstrual cycle is the regular natural changes that occurs in the uterus and ovaries that make pregnancy possible (Barbieri, 2014; Nagar and Msalati, 2013). The typical length of time between the first day of one period and the first day of the next is 21 to 45 days in young women and 21 to 31 days with an average of 28 days in adults. A woman's menstrual cycle typically follows a 28-day cycle and ends with the shedding of uterine lining leading to bleeding. The normal menstrual cycle indicates the proper functioning of hormones, having a normal menstrual cycle signifies a healthy HPA axis with a normal uterus. Menstruation stops occurring after menopause which usually occurs between 45 and 55 years of age. Bleeding usually lasts around 2 to 7 days (American Academy of Pediatrics et al., 2006).

The menstrual cycle is governed by hormonal changes. Each cycle can be divided into three phases based on events in the ovary or in the uterus. The ovarian cycle consists of the follicular phase, ovulation, and luteal phase whereas the uterine cycle is divided into menstruation, proliferative phase, and secretory phase. Stimulated by gradually increasing amounts of estrogen in the follicular phase, discharges of blood flow stop, and the lining of the uterus thickens. Approximately mid-cycle, 24–36 hours after the luteinizing hormone (LH) surges, the dominant follicle releases an ovocyte, in an event called ovulation. After ovulation, the ovocyte only lives for 24 hours or less without fertilization while the remains of the

dominant follicle in the ovary become a corpus luteum; this body has a primary function of producing large amounts of progesterone. Under the influence of progesterone, the uterine lining changes to prepare for potential implantation of an embryo to establish a pregnancy. If implantation does not occur within approximately two weeks, the corpus luteum will involute, causing a sharp drop in levels of both progesterone and estrogen. The hormone drop causes the uterus to shed its lining in a process termed menstruation.

Through the influence of a rise in follicle stimulating hormone (FSH) during the first days of the cycle, a few ovarian follicles are stimulated. These follicles, which were present at birth and have been developing. Ovulation is the second phase of the ovarian cycle in which a mature egg is released from the ovarian follicles into the oviduct. During the follicular phase, estradiol suppresses production of LH from the anterior pituitary gland. When the egg has nearly matured, levels of estradiol reach a threshold above which this effect is reversed and estrogen stimulates the production of a large amount of LH. This process, known as the LH surge, starts around day 12 of the average cycle and may last 48 hours. The exact mechanism of these opposite responses of LH levels to estradiol is not well understood. This may be enabled by the presence of two different estrogen receptors in the hypothalamus: estrogen receptor alpha, which is responsible for the negative feedback estradiol-LH loop, and estrogen receptor beta, which is responsible for the positive estradiol-LH relationship (Berg et al., 2008). However, in humans it has been shown that high levels of estradiol can provoke abrupt increases in LH suggesting that estrogen acts directly on the pituitary to provoke the LH surge.

The luteal phase is the final phase of the ovarian cycle and it corresponds to the secretory phase of the uterine cycle. During the luteal phase, the pituitary hormones FSH and LH cause

the remaining parts of the dominant follicle to transform into the corpus luteum, which produces progesterone. The increased progesterone in the adrenals starts to induce the production of estrogen. The hormones produced by the corpus luteum also suppress production of the FSH and LH that the corpus luteum needs to maintain itself. Consequently, the level of FSH and LH fall quickly over time, and the corpus luteum subsequently atrophies. Falling levels of progesterone trigger menstruation and the beginning of the next cycle. From the time of ovulation until progesterone withdrawal has caused menstruation to begin, the process typically takes about two weeks.

In the normal menstrual cycle, estradiol levels measure typically <50 pg/ml at menstruation, rise with follicular development (peak: 200 pg/ml), drop briefly at ovulation, and rise again during the luteal phase for a second peak. At the end of the luteal phase, estradiol levels drop to their menstrual levels unless there is a pregnancy. In women, progesterone levels are relatively low during the preovulatory phase of the menstrual cycle, rise after ovulation, and are elevated during the luteal phase. Progesterone levels tend to be < 2 ng/ml prior to ovulation, and > 5 ng/ml after ovulation (Barbieri, 2014).

3. Changes of stress hormone according to the menstrual cycle

Past research has successfully identified factors associated with the modulation of both the subjective and neuroendocrine stress responses in women (Kudielka et al., 2009). Menstrual cycle phases were examined in the association between subjective stress and physiological response (Kajantie and Phillips, 2006). Compared to the follicular phase, which is characterized by rising levels of circulating estrogens, many studies found the luteal phase,

which is characterized by high circulating levels of estrogens and progesterone, to be associated with increases in physiological responses to stress, although this is not consistently observed (Childs et al., 2010; Felmingham et al., 2012; Kirschbaum et al., 1999), Interestingly, most of the studies investigating the association between the subjective and neuroendocrine stress responses failed to control for possible effect of gender and/or cycle variations (Campbell and Ehlert, 2012).

It is often assumed that stress responsivity varies across the menstrual cycle. In addition, the psychosocial stress is associated with the experience of irregular menstrual cycles among college students. The students with high stress levels experienced irregular cycles more often than the ones with low stress levels (Yamamoto et al., 2009). Felmingham et al. (2012) reported that women in mid-luteal phase demonstrated a positive association between cortisol stress response and recall for threatening stimuli, which was not observed in other phases. According to the recent study, salivary CgA levels significantly increased in the late-luteal phase in women experiencing a cluster of severe negative emotional symptoms premenstrually (Matsumoto et al., 2014). Despite the importance of the assumption mentioned earlier to research on stress, however, the evidence suggesting that baseline cortisol levels vary across the menstrual cycle is contradictory.

It is obviously important to consider women's menstrual cycles when assessing variations in HPA axis function and physiologic stress levels. Nonetheless, our understanding of HPA axis function is quite incomplete as we still lack a proper characterization of the changes in HPA axis functioning across most reproductive transitions. Longitudinal changes in HPA axis functioning across women's menstrual cycles, for example, are yet to be properly characterized. Changes of stress hormones including DHEA and CgA in women across the

menstrual cycle did not have well-documented. Since the secretion of the stress hormone is affected by numerous factors its use as a biomarker of physiologic stress is not simple and should always be accompanied by proper controls.

4. Diagnostic use of saliva

Saliva is a clinically informative, biological fluid that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, and monitoring and management of patients with both oral and systemic diseases. Over the decade, the use of saliva as a vehicle for the determination of plasma steroid hormone levels has increased dramatically. Advantages of saliva sampling include the noninvasive nature of saliva collection; the convenience of multiple sampling; the ease of collection, storage, and shipment of individual samples for analysis; elimination of many ethical issued relating to studies directed at various groups including neonates, the elderly, the mentally and physically handicapped, etc. Over the past decade, new, highly sensitive analytical procedures have been developed for steroid determination. These newer procedures have permitted the automation of saliva analysis. In addition, it has been reported that saliva contains specific soluble biological markers. Most of the biomarkers present in blood and urine can also be detected in a sample of saliva (Hofman, 2001; Marti-Alamo et al., 2012).

Some hormones have specific protein carriers that have relatively high affinity for the respective hormone, whereas much of the nonspecific binding is to albumin. Therefore, the free concentration of a particular steroid hormone is dependent on the affinity and total binding capacity of the various binding proteins in plasma. The primary entry of the steroid

hormones into saliva is via passive diffusion through the salivary gland epithelium. The free concentration of the hormone in the plasma provides the concentration gradient of the steroid through the epithelial membrane, and the movement into the primary secretory fluid within the acinar intercalated duct complex. Because of the slow dissociation rate of the steroid or weakly bound steroid hormone that disassociates and passes through these various membrane structures as the blood passes through the salivary gland during saliva formation. The saliva levels will therefore reflect the free concentration of hormones in plasma (Cardoso et al., 2009; Hofman, 2001). However, if a particular steroid hormone does not have a high-affinity, high-capacity binding protein in the plasma, the saliva levels will correlate with the total plasma hormone concentration.

The steroid hormone can be metabolized by the salivary gland epithelial cells and during transcellular movement of the hormone. Metabolism of the hormone can also occur by the oral bacteria in the saliva at the time of collection and, therefore, appropriate sample collection and storage conditions may need to be developed. Despite the aforementioned complications, noninvasive saliva collection is an excellent medium for the monitoring of plasma steroid levels. For example, saliva has been successfully used in combination with experimental stress challenges to assess stress reactivity in different phases of the menstrual cycle (Nepomnaschy et al., 2011). The literature has reported cortisol in saliva as a useful marker for objectively assessing stress (Hansen et al., 2008). Most studies consider salivary cortisol levels a reliable measure of HPA axis adaptation to stress. However, the stress response of the HPA axis is rather complex and modulated by numerous factors. In addition, measuring salivary catecholamine proves rather difficult because of its low concentration and rapid degradation (Nakane et al., 2002). In saliva, the concentration of DHEA is independent of salivary flow rate and represents accurately the fraction of the free and biologically active

hormone in the blood circulation (Chen et al., 2005; Gao et al., 2005). In other words, salivary concentrations of DHEA reflect those in serum. In addition, the collection of saliva is less expensive without causing stress to the patient, which could in turn cause alterations in DHEA levels. Saliva sampling has permitted the evaluation and assessment of a multitude of endocrine studies that would have been extremely difficult.

5. MUC1

(1) Structure

MUC1 (Mucin1) is a member of the mucin family and encodes a membrane bound, glycosylated phosphoprotein. MUC1 has a core protein mass of 120-225 kDa which increases to 250-500 kDa with glycosylation. It extends 200-500 nm beyond the surface of the cell (Brayman et al., 2004). MUC1 is cleaved in the endoplasmic reticulum into two pieces, the cytoplasmic tail including the transmembrane domain and the extracellular domain. It anchored to the apical surface of many epithelia by a transmembrane domain. The cytoplasmic tail of MUC1 is 72 amino acids long and contains several phosphorylation sites (Singh and Hollingsworth, 2006). Beyond the transmembrane domain is a SEA domain that contains a cleavage site for release of the large extracellular domain. The release of mucins is performed by sheddases (Hattrup and Gendler, 2008). The extracellular domain includes a 20 amino acid variable number tandem repeat (VNTR) domain, with the number of repeats varying from 20 to 120 in different individuals. These repeats are rich in serine, threonine, and proline residues which permits heavy O-glycosylation (Brayman et al., 2004). Multiple alternatively spliced transcript variants that encode different isoforms of this gene have been

reported, but the full-length nature of only some has been determined.

(2) Functions

MUC1 is ubiquitously expressed and lines the apical surface of epithelial cells in the lungs, stomach, intestines, eyes and several other organs (Hollingsworth and Swanson, 2004). MUC1 is primarily involved in the protection of epithelial surfaces. It protects the body from infection by pathogen binding to oligosaccharides in the extracellular domain, preventing the pathogen from reaching the cell surface (Moncada et al., 2003). The cell-surface MUC1 is highly expressed on the mucosal surface and limits the density of *Helicobacter pylori* in a murine infection model (McAuley et al., 2007). Although cleavage by unknown MUC1 sheddase cannot be definitively excluded, the most likely scenario is disassociation of the non-covalent interaction between the transmembrane and extracellular domains at the SEA module, a site of cleavage during synthesis found in most cell surface mucins. Disassociation could occur due to conformational changes in MUC1 following binding or due to shear forces following binding to the highly motile bacteria.

MUC1 also functions in a cell signaling capacity (Singh and Hollingsworth, 2006). The cytoplasmic tail of MUC1 is involved in several signaling pathways, including those involving Ras, β-catenin, p120 catenin, p53 and estrogen receptor α. The cytoplasmic domain of MUC1 also forms complexes with transcription factors, and then translocates to the nucleus by an unknown mechanism, where it is believed to influence the transcription of their target genes. It has also been proposed to localize to mitochondrial membranes under conditions of genotoxic stress, where it attenuates the apoptotic pathway in response and

confers resistance to apoptosis-inducing drugs. Overexpression and changes in glycosylation of MUC1 are often associated with colon, breast, ovarian, lung, and pancreatic cancers (Gendler, 2001).

(3) MUC1 expression in the oral cavity

MUC1 is primarily involved in the protection of epithelial surfaces. The protective role of MUC1 can be important in oral mucosal defense because epithelial surfaces are constantly attacked by a variety of both pathogenic and commensal microbes (Li et al., 2003). In fact, up-regulation of MUC1 expression in oral epithelial cells has been reported to result from *Porphyromonas gingivalis* infection and increases of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. These results suggested the possibility that up-regulation of MUC1 could be a component of the host response to bacterial infection in the oral cavity. In addition, there is a possibility that MUC1 expression in the oral mucosal epithelial cells is affected by changes of pro-inflammatory cytokine levels in saliva. (Li et al., 2003; McAuley et al., 2007). In the elderly, there was decreased MUC1 expression in oral mucosal epithelial cells compared with young adults. This decrease of oral mucosal defense provided by MUC1 can be a predisposing factor for oral mucosal diseases in the aged population (Chang et al., 2011). There were increased oral mucosal MUC1 transcripts in BMS patients compared with oral lichen planus patients as well as healthy controls (Kho et al., 2013). Lectin-binding studies on oral lesions such as leukoplakias and carcinomas demonstrate that there is aberrant glycosylation associated with increasing dysplasia in the oral glycocalyx. These lectin-labeled carbohydrate residues may be from MUC1 in the oral epithelium (Saussez et al., 1998; Vedtofte et al., 1984)

(4) Perspectives in MUC1 research

Although much work is still needed to fully understand the relationship between MUC1 and oral mucosal defense, significant progress towards to clarify the relationship has been made in recent years. Better insights of MUC1 expression in oral cavity have arisen from human and also animal models. The molecular framework and biophysical properties of MUC1 might involve in the protection of oral cavity. Altered MUC1 glycosylation and phosphorylation, and changes of saliva rheological properties can affect protection against a variety of pathogens in the mouth. Alternative splicing may regulate MUC1 expression and possibly function. It might be explained with alternative splice variants of MUC1 including a form lacking the mucin-like repeat domain which have been identified in the field of cancer immunology. Further research should consider these concepts to comprehensively understand functions and properties of oral mucosal MUC1. Additionally, clarifying a molecular mechanism of MUC1 expression in oral epithelium can provide the crucial information about various diseases and conditions of oral cavity.

III. MATERIALS AND METHODS

1. Participants

Thirty healthy women (mean age ± SD, 25.9 ± 2.1 years, range: 22 to 32 years), with regular menstrual cycles of 28-32 days, were included. Women were recruited from the Medical, Dental, and Nursing campus of the Seoul National University and participated voluntarily. The average length and regularity of their menstrual cycles over the past 3 months were monitored. Inclusion criteria were; age of greater than 20 and less than 40 years,

no oral mucosal pain and diseases, no history of serious illness, and no history of taking medication known to affect the salivary flow rate for the past 3 months. Exclusion criteria were; smokers, wearing removable dentures or orthodontic appliances, having a treatment history for cancer, having a history of hormonal therapy, taking oral contraceptives, or being incapable of communication. The research protocol was approved by the IRB of the Seoul National University Dental Hospital (#CRI13012) on 8 Aug, 2013 and informed consent was obtained from all participants.

2. Overall procedures according to the menstrual cycle

All participants were evaluated 4 times during the menstrual cycle, (1) 5 to 7 days after the start of the cycle (follicular), (2) 13 to 15 days (ovulatory), (3) 21 to 23 days (mid-luteal), and (4) 26 to 28 days (late-luteal). These phases were determined on the basis of self-reported menstrual cycle day-counts.

On the first examination day, the follicular phase, whole saliva samples were collected and salivary flow rates were measured. Then, an intra-oral examination, including measurements of gingival index (GI) and periodontal probing depth (PPD), was performed. During the 3 subsequent visits, collections of whole saliva samples and measurements of flow rates were done.

3. Determination of periodontal health

The extent of gingival inflammation was evaluated using the GI (Löe, 1967; Newman *et al*, 2002) and measurement of PPD by a single examiner (YHL). To determine the GI, four

gingival areas (facial, mesial, distal, and lingual) adjacent to each tooth were assessed and were given a score from 0 to 3. The scores from the four areas of each tooth were totaled and divided by four to give an average GI score for each tooth. By adding the average GI scores from all teeth, the sum of GI scores for each individual was obtained. The PPD was measured with the Michigan-O probe (Hu-Friedy Mfg. Co., Chicago, IL, USA) in six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) of each tooth. The average PPD of each tooth and the sum of PPDs for each individual were obtained as described for the GI score. The sum of GI scores and sum of PPDs were used as representatives of the overall status of periodontal inflammation in individuals.

4. Collection of whole saliva samples

Saliva samples were collected between 7:30 a.m. and 9:30 a.m., to minimize diurnal variability. The times of awakening reported by the participants varied between 5:30 a.m. and 7:30 a.m., and the mean time-difference between waking up and collection was 1.5 h. All participants refrained from drinking alcohol on the previous day, and were instructed to abstain from eating, drinking, and brushing their teeth before the collection of saliva samples after waking up. Unstimulated whole saliva (UWS) was collected for 10 min using the spitting method. Stimulated whole saliva (SWS) was collected for the next 5 min with habitual chewing of 1 g of gum base after a 2 min pre-stimulation period to remove saliva retained in the ducts. The flow rate of saliva was expressed as mL/min. Two millimeters of SWS samples were transferred to Oragene RNA RE-100 (DNA Genotek Inc., Ottawa, ON, Canada) for RNA isolation. UWS and the remaining portion of SWS were centrifuged at 10,000 xg for 15 min at 4°C to remove cellular debris. Aliquots of clarified supernatant were

stored at -70°C for the analyses.

5. Measurement of MUC1 expression by real-time PCR

MUC1 mRNA expression levels, relative to β-actin and GAPDH levels, were quantified using real-time PCR. Total RNA was extracted from each SWS sample using an RNeasy Micro kit (Qiagen, Hilden, Germany) and RNA concentration was measured at 260 nm using a spectrophotometer (Nanodrop 1000, Thermo Fisher Scientific, Wilmington, DE, USA). The absorbance ratio (A260/A280) of each sample was measured and the RNA samples were stored at -70°C. cDNA was synthesized by reverse transcription using a High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA) in a total reaction volume of 20 μL for 1 μg total RNA. Incubation was at 37°C for 60 min, followed by at 95°C for 5 min. Quantitative PCR reactions were performed with an ABI 7500 system (Applied Biosystems) using TaqMan PCR Master Mix and TaqMan probes (Applied Biosystems) for β-actin (Assay ID Hs99999903_m1), GAPDH (Assay ID Hs99999905_m1), and MUC1 (Assay ID Hs00159357_m1). The 20-μL reaction contained template cDNA (1 μL, 50 ng), TaqMan Gene Expression Master Mix (10 μL), TaqMan Gene Expression Assay (1 μL), and nuclease free water (8 μL). The reaction components were assembled in a strip tube with an optical strip cap. The reaction mixture was incubated at 50°C for 2 min and at 95°C for 10 min prior to the PCR step, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Validation experiments were performed to confirm equivalent PCR efficiencies for the target and housekeeping genes. The crossing point of MUC1 with both β-actin and GAPDH was applied to the formula, $2^{-(\text{MUC1-β-actin(or GAPDH)})}$, to quantify the relative mRNA amount of MUC1 to both housekeeping genes. In this study, the fold ratio was calculated using the follicular phase

as a reference. Finally, fold ratios from both housekeeping genes were averaged and used as the relative MUC1 mRNA level. All experiments were performed in duplicate. Two negative control reactions (one with no template and one with no reverse transcriptase) were also performed.

6. Analysis of salivary 17β-estradiol, progesterone, DHEA, cortisol, chromogranin A (CgA), and blood contamination levels

The levels of 17β-estradiol, progesterone, DHEA, cortisol, and blood contamination were determined from UWS and SWS using immunoassay kits (Salimetrics, State College, PA, USA). CgA levels were also analyzed using an immunoassay kit (Yanaihara institute Inc., Shizuoka, Japan). The levels of transferrin in saliva samples were measured to determine blood contamination levels in saliva (Salimetrics). All assays were performed in duplicate.

Intra-assay and inter-assay coefficients of variations were 8.1% and 8.9%, respectively, for the 17β-estradiol assay (sensitivity, 0.1 pg/mL), 6.2% and 7.6%, respectively, for the progesterone assay (sensitivity, 5.0 pg/mL), 5.6% and 8.2%, respectively, for the DHEA assay (sensitivity, 5.0 pg/mL), 3.0% and 3.0%, respectively, for the cortisol assay (sensitivity, 0.007 μg/dL), 10.5% and 13.3%, respectively, for the CgA assay (sensitivity, 0.14 pmol/mL), and 4.9% and 7.1%, respectively, for the blood contamination assay (sensitivity, 0.08 mg/dL). The cross-reactivity of the cortisol assay with cortisone was 0.13%.

7. Statistics

The Kolmogorov-Smirnov normality test was applied to our data. Because the data were normally distributed, parametric tests were used. The repeated-measures analysis of variance (ANOVA) with Tukey's post-hoc test was used for the comparison of variables between the phases throughout the menstrual cycle. The Pearson correlation analysis was used to determine relationships between the variables. All tests were two-sided and the significance level was set at $P < 0.05$.

IV. RESULTS

1. Oral examination

All participants presented with satisfactory oral hygiene without apparent plaque and calculus formation. The average and sum of GI scores were 0.64 ± 0.58 (range: 0 to 1.79) and 17.7 ± 15.8 (range: 0 to 50), respectively. The average and sum of PPDs were 2.8 ± 0.5 mm (range: 2.0 to 3.8 mm) and 78.5 ± 13.1 mm (range: 56 to 106 mm), respectively. The mean number of teeth was 28.0 ± 1.2 (range: 24 to 32).

2. Salivary flow rate

Changes in salivary flow rates are shown in Table 1. The mean flow rate of UWS was 0.50 ± 0.28 mL/min and that of SWS was 1.55 ± 0.50 mL/min. The flow rates of both UWS and SWS did not show any significant changes throughout the menstrual cycle ($P > 0.05$).

3. Oral mucosal epithelial MUC1 expression

There were no significant differences in MUC1 expression levels throughout the menstrual cycle (P = 0.350) (Table 1). In correlation analyses using data from all four phases together, MUC1 expression showed significant correlations with DHEA in SWS (P = 0.014, r = 0.224) and with cortisol/DHEA in UWS (P = 0.002, r = -0.285) and SWS (P = 0.002, r = -0.285) (Tables 2 and 3).

When each phase was analyzed, the luteal phase was distinctly different from the other phases with respect to the relationship of MUC1 expression with salivary analytes (Appendix Tables 1-8). In the mid-luteal phase, MUC1 expression levels showed significant correlations with cortisol (P = 0.037, r = -0.382), progesterone (P = 0.008, r = -0.475), and cortisol/DHEA (P = 0.006, r = -0.489) in UWS and with progesterone (P = 0.020, r = -0.421) and cortisol/DHEA (P = 0.049, r = -0.362) in SWS. In the late-luteal phase, MUC1 expression levels was significantly correlated with cortisol/DHEA (P = 0.026, r = -0.406) in UWS.

4. Salivary analytes

Changes in 17β-estradiol, progesterone, DHEA, cortisol, CgA, and blood contamination levels in UWS and SWS throughout the menstrual cycle are shown in Table 1. The mean concentrations of all salivary analytes were higher in UWS than in SWS. Salivary concentrations of DHEA, cortisol, CgA, and blood contamination were not significantly different throughout the menstrual cycle (P > 0.05). 17β-Estradiol levels in UWS and SWS were elevated at the ovulatory and mid-luteal phases compared to the other phases with no significant differences (P > 0.05). Progesterone levels in UWS (P = 0.001) and SWS (P <

0.001) showed significant differences across the cycle. Progesterone levels in both UWS and SWS in the mid-luteal phase were significantly higher compared to those in the follicular ($P < 0.01$) and ovulatory phases ($P < 0.05$).

5. Correlations between variables

Tables 2 and 3 show correlation results between all variables when all phases were analyzed together in UWS and SWS, respectively. When data were analyzed in each individual phase, the correlation results from the follicular and late-luteal phases were almost the same as those derived from the analysis of all phases together. The correlation results from the ovulatory and mid-luteal phases were mainly affected by changes in 17β-estradiol and progesterone in these phases (Appendix Tables 1-8).

In analyses using all data from the four phases combined, the mean concentrations of each analyte in UWS had significant positive correlations with those of SWS (r = 0.355 - 0.935). A significant positive correlation was also found between the salivary flow rates of UWS and SWS ($P < 0.001$, r = 0.438). There were significant positive correlations between two female gonadal hormones, 17β-estradiol and progesterone, in UWS ($P < 0.001$, r = 0.381) and SWS ($P < 0.001$, r = 0.321). There were also significant positive correlations between 17β-estradiol and DHEA ($P = 0.041$, r = 0.187), and between cortisol and CgA ($P = 0.012$, r = 0.229) only in UWS. In addition, there were significant positive correlations among progesterone, cortisol, and DHEA in both UWS and SWS (Tables 2 and 3).

When the data were analyzed in the follicular phase with the inclusion of clinical periodontal parameters, there were significant positive correlations among the sum of GI

scores, the sum of PPDs, and the blood contamination levels in SWS, but not in UWS. No significant correlations were found between MUC1 expression levels and either periodontal parameter ($P > 0.05$). The sum of PPDs showed significant positive correlations with cortisol and DHEA in both UWS and SWS ($P < 0.05$), and CgA in SWS ($P < 0.01$) (Table 4).

V. DISCUSSION

The present study is the first report to investigate relationships among salivary stress markers and female gonadal hormones and between oral mucosal MUC1 expression and these components throughout the menstrual cycle.

Among these relationships, the positive correlation between cortisol and DHEA was the most prominent. This relationship occurs because both cortisol and DHEA are produced in the adrenal glands under the stimulation of adrenocorticotropic hormone (ACTH). Although the change in DHEA levels varies according to the type of stress and stress-related history, cortisol/DHEA ratios usually increase under stressful conditions (Jeckel et al., 2010; Lennartsson et al., 2012; Warnock et al., 2010). An enhanced response to stress during the progesterone-dominated luteal phase in women (Kirschbaum et al., 1999) and an increase in progesterone with acute stress in an animal study (Krause et al., 2014) may explain the positive correlations among progesterone, cortisol, and DHEA.

The positive correlation between 17β-estradiol and DHEA could be explained by the fact that estradiol has been proposed to increase DHEA in women, as well as in human adult adrenal cells (Lobo et al., 1982; Winter and Smail, 1983). Inhibition of 3β-hydroxysteroid

dehydrogenase activity in adrenal cells by estradiol has been suggested as a mechanism for DHEA increase (Gell et al., 1998). DHEA could also act as a precursor for enzymatic conversion to estrogen. CgA, which has been suggested to be an indicator of sympatho-adrenal activity, had a significant positive relationship with cortisol, the indicator of the HPA activity. However, previous studies showed that the positive relationship is not consistently observed and that different types of stress and various stress-related factors affect these two molecules differently (Filaire et al., 2009; Fukui et al., 2010; Toda et al., 2005). Therefore, more information is needed before salivary CgA can be used as a stress biomarker.

The amount of blood contamination in saliva is very important for the diagnostic reliability of saliva samples because concentrations of analytes in blood are usually very high compared to those in saliva. Salivary transferrin has been known as a useful biomarker measuring blood contamination levels (Kivlighan et al., 2004; Kivlighan et al., 2005; Schwartz and Granger, 2004). In the present study, the levels of transferrin in SWS, but not UWS, showed significant positive relationships with two indices related to gingival inflammation. Because all subjects had intact oral mucosal integrity, the only blood source in saliva would be from the gingival crevice and the level would be affected by the degree of gingival inflammation. The leakage of serum-originated gingival crevicular fluid could be increased by the chewing used as a mechanical stimulant for SWS collection. Therefore, research data from saliva samples, especially SWS, should be carefully interpreted with respect to possible blood contamination. Although transferrin levels in UWS also showed positive relationships with some salivary analytes, a previous study reported that blood might be not the only source of salivary transferrin (Nashida et al., 2009). Salivary flow rates could also affect transferrin levels. Therefore, examination of gingival inflammations, as well as evaluation of blood contamination, should be considered for accurate evaluation of salivary data.

It was noteworthy that oral mucosal MUC1 expression levels had significant positive correlations with DHEA, an anabolic hormone with protective effects against stress, and had significant negative correlations with cortisol, a catabolic stress hormone, and the cortisol/DHEA ratio. The increased HPA response to stress in the progesterone-dominated luteal phase (Kajantie and Phillips, 2006; Kirschbaum et al., 1999) may explain the significant negative relationship between MUC1 expression and progesterone levels. In contrast, expression of uterine MUC1, which is involved in embryo attachment, is mainly regulated by gonadal hormones (Brayman et al., 2006; Hey et al., 1994; Meseguer et al., 2001). Progesterone combined with estradiol priming regulates MUC1 expression at the receptive endometrium (Meseguer et al., 2001). The progesterone receptor (PR) isoforms, PR-A and PR-B, differentially regulate MUC1 expression in uterine epithelial cells; liganded PR-B stimulates MUC1 expression, whereas liganded PR-A represses MUC1 expression (Brayman et al., 2006). Although there have been reports of the presence of PR in the overlying epithelium of oral pyogenic granulomas during pregnancy (Whitaker and Bouquot, 1994) and in cultured gingival fibroblasts (Kawahara and Shimazu, 2003), there is no information about whether PR is present on healthy oral epithelium and the possible influences of progesterone on oral mucosal membrane.

The results of the present study suggest that stress-related psychoendocrinological interactions may deteriorate oral mucosal defense. In fact, psychological stress may act as a triggering and modifying factor for provoking oral ulcerations (Preeti et al., 2011). It has been reported that oral ulcerations are associated with the onset of menstruation or the luteal phase of the menstrual cycle (Oh et al., 2009; Preeti et al., 2011), i.e. the period of decreased MUC1 expression in the present study. Previous studies also showed that oral mucosal MUC1 expression decreased in aged population (Chang et al., 2011) and increased in patients with

burning mouth syndrome (Kho et al., 2013). Thus, the accumulated information suggests that oral mucosal MUC1 expression is influenced by psychoendocrinological changes, aging, and diseases. The findings in the present study suggest that psychoendocrinological interactions across the menstrual cycle could influence oral mucosal defense and possibly the occurrence and aggravation of oral mucosal diseases.

VI. CONCLUSIONS

The relationships among oral mucosal MUC1 expression and salivary stress and female gonadal hormones throughout the menstrual cycle were investigated in the present study. Young healthy women with normal menstrual cycle participated and were evaluated during the follicular, ovulatory, mid-luteal, and late-luteal phases. The expression level of oral mucosal MUC1 was analyzed by real-time PCR. The supernatants from unstimulated (UWS) and stimulated whole saliva (SWS) were used to measure 17β-estradiol, progesterone, dehydroepiandrosterone (DHEA), cortisol, and chromogranin A (CgA) levels. MUC1 expression showed significant negative correlations with cortisol, progesterone, and cortisol/DHEA ratios in UWS and with progesterone and cortisol/DHEA ratios in SWS during the mid-luteal phase. A significant negative correlation was also observed between MUC1 expression and cortisol/DHEA ratios in UWS during the late-luteal phase. In conclusion, oral mucosal MUC1 expression correlated with changes in salivary stress and gonadal hormone levels throughout the menstrual cycle. Stress-related psychoendocrinological interactions resulted in a decrease in oral MUC1 expression, which

can be associated with the occurrence and aggravation of oral mucosal defense. Further studies will be needed to confirm the possible relationships among the oral MUC1 expression, salivary stress and gonadal hormones across the phases of the menstrual cycle.

REFERENCES

Altemus M, Redwine LS, Leong YM, Frye CA, Porges SW, Carter CS (2001). Responses to laboratory psychosocial stress in postpartum women. *Psychosom Med* 63: 814-821.

American Academy of Pediatrics, Committee on Adolescence, American College of Obstericians and Gynecologists, Committee on Adolescent Health Care (2006). Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. *Pediatrics* 118: 2245-2250.

Attardi BJ, Zeleznik A, Simhan H, Chiao JP, Mattison DR, Caritis SN *et al* (2007). Comparison of progesterone and glucocorticoid receptor binding and stimulation of gene expression by progesterone, 17-alpha hydroxyprogesterone caproate, and related progestins. *Am J Obstet Gynecol* 197: 591-597.

Barbieri RL (2014). The endocrinology of the menstrual cycle. *Methods Mol Biol* 1154: 145-169.

Baulieu E, Schumacher M (2000). Progesterone as a neuroactive neurosteroid, with special reference to the effect of progesterone on myelination. *Steroids* 65: 605-612.

Berg DK, Thompson JG, Peterson AJ, Asher GW (2008). The temporal relationship between oocyte maturation and early fertilisation events in relation to the pre-ovulatory LH peak and preimplantation embryo development in red deer. *Anim Reprod Sci* 105: 332-343.

Blauer KL, Poth M, Rogers WM, Bernton EW (1991). Dehydroepiandrosterone antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. *Endocrinology* 129: 3174-3179.

Brayman M, Thathiah A, Carson DD (2004). MUC1: a multifunctional cell surface component of reproductive tissue epithelia. *Reprod Biol Endocrinol* 2:4.

Brayman MJ, Julian J, Mulac-Jericevic B, Conneely OM, Edwards DP, Carson DD (2006). Progesterone receptor isoforms A and B differentially regulate MUC1 expression in uterine epithelial cells. *Mol Endocrinol* 20: 2278-2291.

Bredemann TM, McMahon LL (2014). 17beta Estradiol increases resilience and improves hippocampal synaptic function in helpless ovariectomized rats. *Psychoneuroendocrinology* 42: 77-88.

Brooke AM, Kalingag LA, Miraki-Moud F, Camacho-Hubner C, Maher KT, Walker DM *et al* (2006). Dehydroepiandrosterone improves psychological well-being in male and female hypopituitary patients on maintenance growth hormone replacement. *J Clin Endocrinol Metab* 91: 3773-3779.

Campbell J, Ehlert U (2012). Acute psychosocial stress: does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology* 37: 1111-1134.

Cardoso EM, Arregger AL, Tumilasci OR, Contreras LN (2009). Diagnostic value of salivary cortisol in Cushing's syndrome. *Clin Endocrinol* 70: 516-521.

Chang DM, Lan JL, Lin HY, Luo SF (2002). Dehydroepiandrosterone treatment of women with mild-to-moderate systemic lupus erythematosus: a multicenter randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 46: 2924-2927.

Chang WI, Chang JY, Kim YY, Lee G, Kho HS (2011). MUC1 expression in the oral mucosal epithelial cells of the elderly. *Arch Oral Biol* 56: 885-890.

Chen F, Knecht K, Birzin E, Fisher J, Wilkinson H, Mojena M *et al* (2005). Direct agonist/antagonist functions of dehydroepiandrosterone. *Endocrinology* 146: 4568-4576.

Childs E, Van Dam NT, de Wit H (2010). Effects of acute progesterone administration upon responses to acute psychosocial stress in men. *Exp Clin Psychopharmacol* 18: 78-86.

Clow A, Thorn L, Evans P, Hucklebridge F (2004). The awakening cortisol response: methodological issues and significance. *Stress* 7: 29-37.

Corona G, Rastrelli G, Giagulli VA, Sila A, Sforza A, Forti G *et al* (2013). Dehydroepiandrosterone supplementation in elderly men: a meta-analysis study of placebo-controlled trials. *J Clin Endocrinol Metab* 98: 3615-3626.

Curry WJ, Barkatullah SC, Johansson AN, Quinn JG, Norlen P, Connolly CK *et al* (2002). WE-14, a chromogranin a-derived neuropeptide. *Ann N Y Acad Sci* 971: 311-316.

Daniel AR, Gaviglio AL, Czaplicki LM, Hillard CJ, Housa D, Lange CA (2010). The progesterone receptor hinge region regulates the kinetics of transcriptional responses through acetylation, phosphorylation, and nuclear retention. *Mol Endocrinol* 24: 2126-2138.

Den R, Toda M, Nagasawa S, Kitamura K, Morimoto K (2007). Circadian rhythm of human salivary chromogranin A. *Biomed Res* 28: 57-60.

Dickerson SS, Kemeny ME (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull* 130: 355-391.

Felmingham KL, Fong WC, Bryant RA (2012). The impact of progesterone on memory consolidation of threatening images in women. *Psychoneuroendocrinology* 37: 1896-1900.

Ferrari E, Cravello L, Muzzoni B, Casarotti D, Paltro M, Solerte SB *et al* (2001). Age-related changes of the hypothalamic-pituitary-adrenal axis: pathophysiological correlates. *Eur J Endocrinol* 144: 319-329.

Filaire E, Dreux B, Massart A, Nourrit B, Rama LM, Teixeira A (2009). Salivary alpha-amylase, cortisol and chromogranin A responses to a lecture: impact of sex. *Eur J Appl Physiol* 106: 71-77.

Fukui M, Hinode D, Yokoyama M, Yoshioka M, Kataoka K, Ito HO (2010). Levels of salivary stress markers in patients with anxiety about halitosis. *Arch Oral Biol* 55: 842-847.

Ganong WF (2005). *Review of Medical Physiology,* 22nd edn. McGraw Hill Education: New York (NY), pp. 362.

Gao W, Bohl CE, Dalton JT (2005). Chemistry and structural biology of androgen receptor. *Chem Rev* 105: 3352-3370.

Gell JS, Oh J, Rainey WE, Carr BR (1998). Effect of estradiol on DHEAS production in the human adrenocortical cell line, H295R. *J Soc Gynecol Investig* 5: 144-148.

Gendler SJ (2001). MUC1, the renaissance molecule. *J Mammary Gland Biol Neoplasia* 6: 339-353.

Goldstein JM, Jerram M, Poldrack R, Ahern T, Kennedy DN, Seidman LJ *et al* (2005). Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging. *J Neurosci* 25: 9309-9316.

Goodyer IM, Herbert J, Tamplin A (2003). Psychoendocrine antecedents of persistent first-episode major depression in adolescents: a community-based longitudinal enquiry. *Psychol Med* 33: 601-610.

Gooren LJ, van der Veen EA, van Kessel H, Harmsen-Louman W (1984). Estrogens in the feedback regulation of gonadotropin secretion in men: effects of administration of estrogen to

agonadal subjects and the antiestrogen tamoxifen and the aromatase inhibitor delta'-testolactone to eugonadal subjects. *Andrologia* 16: 568-577.

Hansen AM, Garde AH, Persson R (2008). Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: a review. *Scand J Clin Lab Invest* 68: 448-458.

Hattrup CL, Gendler SJ (2008). Structure and function of the cell surface (tethered) mucins. *Annu Rev Physiol* 70: 431-457.

Hellhammer DH, Wust S, Kudielka BM (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 34: 163-171.

Helman LJ, Ahn TG, Levine MA, Allison A, Cohen PS, Cooper MJ *et al* (1988). Molecular cloning and primary structure of human chromogranin A (secretory protein I) cDNA. *J Biol Chem* 263: 11559-11563.

Hey NA, Graham RA, Seif MW, Aplin JD (1994). The polymorphic epithelial mucin MUC1 in human endometrium is regulated with maximal expression in the implantation phase. *J Clin Endocrinol Metab* 78: 337-342.

Hofman LF (2001). Human saliva as a diagnostic specimen. *J Nutr* 131: 1621S-1625S.

Hollingsworth MA, Swanson BJ (2004). Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 4: 45-60.

Hucklebridge F, Clow A, Evans P (1998). The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. *Int J Psychophysiol* 31: 69-76.

Izawa S, Sugaya N, Shirotsuki K, Yamada KC, Ogawa N, Ouchi Y *et al.* (2008). Salivary dehydroepiandrosterone secretion in response to acute psychosocial stress and its correlations with biological and psychological changes. *Biol Psychol* 79: 294-298.

Jeckel CM, Lopes RP, Berleze MC, Luz C, Feix L, Argimon, II *et al* (2010). Neuroendocrine and immunological correlates of chronic stress in 'strictly healthy' populations. *Neuroimmunomodulation* 17: 9-18.

Kajantie E, Phillips DI (2006). The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology* 31: 151-178.

Kalimi M, Shafagoj Y, Loria R, Padgett D, Regelson W (1994). Anti-glucocorticoid effects of dehydroepiandrosterone (DHEA). *Mol Cell Biochem* 131: 99-104.

Kanaley JA, Weltman JY, Pieper KS, Weltman A, Hartman ML (2001). Cortisol and growth hormone responses to exercise at different times of day. *J Clin Endocrinol Metab* 86: 2881-2889.

Kanno T, Asada N, Yanase H, Iwanaga T, Ozaki T, Nishikawa Y *et al* (1999). Salivary secretion of highly concentrated chromogranin a in response to noradrenaline and acetylcholine in isolated and perfused rat submandibular glands. *Exp Physiol* 84: 1073-1083.

Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H *et al* (1990). Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J* 9: 1603-1614.

Kawahara K, Shimazu A (2003). Expression and intracellular localization of progesterone receptors in cultured human gingival fibroblasts. *J Periodontal Res* 38: 242-246.

Kho HS, Chang JY, Kim YY, Kim Y (2013). MUC1 and Toll-like receptor-2 expression in burning mouth syndrome and oral lichen planus. *Arch Oral Biol* 58: 837-842.

Kibaly C, Meyer L, Patte-Mensah C, Mensah-Nyagan AG (2008). Biochemical and functional evidence for the control of pain mechanisms by dehydroepiandrosterone endogenously synthesized in the spinal cord. *FASEB J* 22: 93-104.

Kirschbaum C, Wust S, Hellhammer D (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosom Med* 54: 648-657.

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med* 61: 154-162.

Kivlighan KT, Granger DA, Schwartz EB, Nelson V, Curran M, Shirtcliff EA (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Horm Behav* 46: 39-46.

Kivlighan KT, Granger DA, Schwartz EB (2005). Blood contamination and the measurement of salivary progesterone and estradiol. *Horm Behav* 47: 367-370.

Krause JS, Dorsa D, Wingfield JC (2014). Changes in plasma concentrations of progesterone, dehydroepiandrosterone and corticosterone in response to acute stress of capture, handling and restraint in two subspecies of white-crowned sparrows. *Comp Biochem Physiol A Mol Integr Physiol* 177: 35-40.

Kudielka BM, Kirschbaum C (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology* 28: 35-47.

Kudielka BM, Hellhammer DH, Wust S (2009). Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology* 34: 2-18.

Kumari V, Konstantinou J, Papadopoulos A, Aasen I, Poon L, Halari R *et al* (2010). Evidence for a role of progesterone in menstrual cycle-related variability in prepulse inhibition in healthy young women. *Neuropsychopharmacology* 35: 929-937.

Kunz-Ebrecht SR, Kirschbaum C, Marmot M, Steptoe A (2004). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology* 29: 516-528.

Lee T, Shimizu T, Iijima M, Obinata K, Yamashiro Y, Nagasawa S (2006). Evaluation of psychosomatic stress in children by measuring salivary chromogranin A. *Acta Paediatr* 95: 935-939.

Lennartsson AK, Kushnir MM, Bergquist J, Jonsdottir IH (2012). DHEA and DHEA-S response to acute psychosocial stress in healthy men and women. *Biol Psychol* 90: 143-149.

Li X, Wang L, Nunes DP, Troxler RF, Offner GD (2003). Pro-inflammatory cytokines up-regulate MUC1 gene expression in oral epithelial cells. *J Dent Res* 82: 883-887.

Little JW. Falace DA, Miller CS, Rhodus NL (2013). *Dental management of the medically compromised patient,* 8th edn. Elsevier: St. Louis (MO), pp. 534-536.

Lobo RA, Goebelsmann U, Brenner PF, Mishell DR Jr (1982). The effects of estrogen on adrenal androgens in oophorectomized women. *Am J Obstet Gynecol* 142: 471-478.

Löe H (1967). The gingival index, the plaque index and the retention index systems. *J Periodontol* 38: 610-616.

Marti-Alamo S, Mancheno-Franch A, Marzal-Gamarra C, Carlos-Fabuel L (2012). Saliva as a

diagnostic fluid. Literature review. *J Clin Exp Dent* 4: 237-243.

Matsumoto T, Asakura H, Hayashi T (2014). Effects of olfactory stimulation from the fragrance of the Japanese citrus fruit yuzu on mood states and salivary chromogranin A as an endocrinologic stress marker. *J Altern Complement Med* 20: 500-506.

Maurice T, Urani A, Phan VL, Romieu P (2001). The interaction between neuroactive steroids and the sigma 1 receptor function: behavioral consequences and therapeutic opportunities. *Brain Res Brain Res Rev* 37: 116-132.

McAuley JL, Linden SK, Png CW, King RM, Pennington HL, Gendler SJ *et al* (2007). MUC1 cell surface mucin is a critical element of the mucosal barrier to infection. *J Clin Invest* 117: 2313-2324.

McEwen BS (2000). Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology* 22: 108-124.

Merz CJ, Tabbert K, Schweckendiek J, Klucken T, Vaitl D, Stark R *et al* (2012). Neuronal correlates of extinction learning are modulated by sex hormones. *Soc Cogn Affect Neurosci* 7: 819-830.

Meseguer M, Aplin JD, Caballero-Campo P, O'Connor JE, Martin JC, Remohi J *et al* (2001). Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. *Biol Reprod* 64: 590-601.

Meyer C, Schmid R, Schmieding K, Falkenstein E, Wehling M (1998). Characterization of high affinity progesterone-binding membrane proteins by anti-peptide antiserum. *Steroids* 63: 111-116.

Miller E (1995). Long-term monitoring of the diabetic dog and cat. Clinical signs, serial blood glucose determinations, urine glucose, and glycated blood proteins. *Vet Clin North Am Small Anim Pract* 25: 571-584.

Mo Q, Lu SF, Simon NG (2006). Dehydroepiandrosterone and its metabolites: differential effects on androgen receptor trafficking and transcriptional activity. *J Steroid Biochem Mol Biol* 99: 50-58.

Moncada DM, Kammanadiminti SJ, Chadee K (2003). Mucin and Toll-like receptors in host defense against intestinal parasites. *Trends Parasitol* 19: 305-311.

Morgan CA 3rd, Southwick S, Hazlett G, Rasmusson A, Hoyt G, Zimolo Z *et al* (2004). Relationships among plasma dehydroepiandrosterone sulfate and cortisol levels, symptoms of dissociation, and objective performance in humans exposed to acute stress. *Arch Gen Psychiatry* 61: 819-825.

Nagar R, Msalati AA (2013). Changes in serum PSA during normal menstrual cycle. *Indian J Clin Biochem* 28: 84-89.

Nakane H, Asami O, Yamada Y, Ohira H (2002). Effect of negative air ions on computer operation, anxiety and salivary chromogranin A-like immunoreactivity. *Int J Psychophysiol* 46: 85-89.

Nashida T, Yoshie S, Imai A, Shimomura H (2009). Transferrin secretory pathways in rat parotid acinar cells. *Arch Biochem Biophys* 487: 131-138.

Negrao AB, Deuster PA, Gold PW, Singh A, Chrousos GP (2000). Individual reactivity and physiology of the stress response. *Biomed Pharmacother* 54: 122-128.

Nepomnaschy PA, Sheiner E, Mastorakos G, Arck PC (2007). Stress, immune function, and women's reproduction. *Ann N Y Acad Sci* 1113:350-364.

Nepomnaschy PA, Altman RM, Watterson R, Co C, McConnell DS, England BG (2011). Is cortisol excretion independent of menstrual cycle day? A longitudinal evaluation of first morning urinary specimens. *PloS one* 6: e18242.

Newman MG, Takei HH, Carranza FA (2002). *Clinical periodontology*, 9th edn. WB Saunders Company: Philadelphia (PA), pp. 80.

Nieschlag E, Loriaux DL, Ruder HJ, Zucker IR, Kirschner MA, Lipsett MB (1973). The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man. *J Endocrinol* 57: 123-134.

Oberbeck R, Benschop RJ, Jacobs R, Hosch W, Jetschmann JU, Schurmeyer TH *et al* (1998). Endocrine mechanisms of stress-induced DHEA-secretion. *J Endocrinol Invest* 21: 148-153.

Ockenfels MC, Porter L, Smyth J, Kirschbaum C, Hellhammer DH, Stone AA (1995). Effect of chronic stress associated with unemployment on salivary cortisol: overall cortisol levels, diurnal rhythm, and acute stress reactivity. *Psychosom Med* 57: 460-467.

Oh SH, Han EC, Lee JH, Bang D (2009). Comparison of the clinical features of recurrent

aphthous stomatitis and Behcet's disease. *Clin Exp Dermatol* 34: e208-212.

Padgett DA, Glaser R (2003). How stress influences the immune response. *Trends Immunol* 24: 444-448.

Paul SM, Purdy RH (1992). Neuroactive steroids. *FASEB J* 6: 2311-2322.

Pinto A, Malacrida B, Oieni J, Serafini MM, Davin A, Galbiati V *et al* (2015). DHEA modulates the effect of cortisol on RACK1 expression via interference with the splicing of the glucocorticoid receptor. *Br J Pharmacol* 172: 2918-2927.

Preeti L, Magesh K, Rajkumar K, Karthik R (2011). Recurrent aphthous stomatitis. *J Oral Maxillofac Pathol* 15: 252-256.

Rabkin JG, McElhiney MC, Rabkin R, McGrath PJ, Ferrando SJ (2006). Placebo-controlled trial of dehydroepiandrosterone (DHEA) for treatment of nonmajor depression in patients with HIV/AIDS. *Am J Psychiatry* 163: 59-66.

Roca CA, Schmidt PJ, Deuster PA, Danaceau MA, Altemus M, Putnam K *et al* (2005). Sex-related differences in stimulated hypothalamic-pituitary-adrenal axis during induced gonadal suppression. *J Clin Endocrinol Metab* 90: 4224-4231.

Rosenfeld RS, Hellman L, Roffwarg H, Weitzman ED, Fukushima DK, Gallagher TF (1971). Dehydroisoandrosterone is secreted episodically and synchronously with cortisol by normal man. *J Clin Endocrinol Metab* 33: 87-92.

Rupprecht R, Reul JM, van Steensel B, Spengler D, Söder M, Berning B *et al* (1993). Pharmacological and functional characterization of human mineralocorticoid and glucocorticoid receptor ligands. *Eur J Pharmacol* 247: 145-154.

Ryan KJ (1982). Biochemistry of aromatase: significance to female reproductive physiology. *Cancer Res* 42: 3342s-3344s.

Saldanha CJ, Remage-Healey L, Schlinger BA (2011). Synaptocrine signaling: steroid synthesis and action at the synapse. *Endocr Rev* 32: 532-549.

Saussez S, Marchant H, Nagy N, Decaestecker C, Hassid S, Jortay A *et al* (1998). Quantitative glycohistochemistry defines new prognostic markers for cancers of the oral cavity. *Cancer* 82: 252-260.

Sawczuk B, Golebiewska M, Mazurek A, Chyczewski L (2014). Evaluation of estrogen receptor-beta expression in the epithelium of the oral mucosa in menopausal women under hormone replacement therapy. *Adv Med Sci* 59: 85-89.

Schwartz EB, Granger DA (2004). Transferrin enzyme immunoassay for quantitative monitoring of blood contamination in saliva. *Clin Chem* 50: 654-656.

Shansky RM, Glavis-Bloom C, Lerman D, McRae P, Benson C, Miller K *et al* (2004). Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. *Mol Psychiatry* 9: 531-538.

Shirotsuki K, Izawa S, Sugaya N, Yamada KC, Ogawa N, Ouchi Y *et al* (2009). Salivary cortisol and DHEA reactivity to psychosocial stress in socially anxious males. *Int J Psychophysiol* 72: 198-203.

Shirtcliff E, Zahn-Waxler C, Klimes-Dougan B, Slattery M (2007). Salivary dehydroepiandrosterone responsiveness to social challenge in adolescents with internalizing problems. *J Child Psychol Psychiatry* 48: 580-591.

Shors TJ, Leuner B (2003). Estrogen-mediated effects on depression and memory formation in females. *J Affect Disord* 74: 85-96.

Singh PK, Hollingsworth MA (2006). Cell surface-associated mucins in signal transduction. *Trends Cell Biol* 16: 467-476.

Smith CC, Vedder LC, Nelson AR, Bredemann TM, McMahon LL (2010). Duration of estrogen deprivation, not chronological age, prevents estrogen's ability to enhance hippocampal synaptic physiology. *Proc Natl Acad Sci U S A* 107: 19543-19548.

Somer E, Ben-Aryeh H, Laufer D (1993). Salivary composition, gender and psychosocial stress. *Int J Psychosom* 40: 17-21.

Steptoe A, Cropley M, Griffith J, Kirschbaum C (2000). Job strain and anger expression predict early morning elevations in salivary cortisol. *Psychosom Med* 62: 286-292.

Streckfus CF, Bigler LR (2002). Saliva as a diagnostic fluid. *Oral Dis* 8: 69-76.

Thomas P, Pang Y (2012). Membrane progesterone receptors: evidence for neuroprotective, neurosteroid signaling and neuroendocrine functions in neuronal cells. *Neuroendocrinology* 96: 162-171.

Toda M, Den R, Nagasawa S, Kitamura K, Morimoto K (2005). Relationship between lifestyle scores and salivary stress markers cortisol and chromogranin A. *Arch Environ Occup Health* 60: 266-269.

Tsigos C, Chrousos GP (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* 53: 865-871.

van Niekerk JK, Huppert FA, Herbert J (2001). Salivary cortisol and DHEA: association with measures of cognition and well-being in normal older men, and effects of three months of DHEA supplementation. *Psychoneuroendocrinology* 26: 591-612.

Vedtofte P, Dabelsteen E, Hakomori S, Young WW (1984). Regional variations of cell surface carbohydrates in human oral stratified epithelium. *Differentiation* 25: 221-228.

Villareal DT, Holloszy JO (2006). DHEA enhances effects of weight training on muscle mass and strength in elderly women and men. *Am J Physiol Endocrinol Metab* 291: E1003-1008.

Warnock F, McElwee K, Seo RJ, McIsaac S, Seim D, Ramirez-Aponte T *et al* (2010). Measuring cortisol and DHEA in fingernails: a pilot study. *Neuropsychiatr Dis Treat* 6: 1-7.

Webb SJ, Geoghegan TE, Prough RA, Michael Miller KK (2006). The biological actions of dehydroepiandrosterone involves multiple receptors. *Drug Metab Rev* 38: 89-116.

Weiss EL, Longhurst JG, Mazure CM (1999). Childhood sexual abuse as a risk factor for depression in women: psychosocial and neurobiological correlates. *Am J Psychiatry* 156: 816-828.

Whitaker SB, Bouquot JE (1994). Estrogen and progesterone receptor status of central giant cell lesions of the jaws. *Oral Surg Oral Med Oral Pathol* 77: 641-644.

Winkler H, Fischer-Colbrie R (1992). The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience* 49: 497-528.

Winter JS, Smail PJ (1983). Effects of ACTH and estradiol on steroid production by cultured adrenal cells from an anencephalic fetus and from normal adults. *Steroids* 42: 677-685.

Wolf OT, Kirschbaum C (1999). Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. *Brain Res Brain Res Rev* 30: 264-288.

Wolfram M, Bellingrath S, Kudielka BM (2011). The cortisol awakening response (CAR) across the female menstrual cycle. *Psychoneuroendocrinology* 36: 905-912.

Yamakoshi T, Park SB, Jang WC, Kim K, Yamakoshi Y, Hirose H (2009). Relationship between salivary Chromogranin-A and stress induced by simulated monotonous driving. *Med Biol Eng Comput* 47: 449-456.

Yamamoto K, Okazaki A, Sakamoto Y, Funatsu M (2009). The relationship between premenstrual symptoms, menstrual pain, irregular menstrual cycles, and psychosocial stress among Japanese college students. *J Physiol Anthropol* 28: 129-136.

Young AH, Gallagher P, Porter RJ (2002). Elevation of the cortisol-dehydroepiandrosterone ratio in drug-free depressed patients. *Am J Psychiatry* 159: 1237-1239.

Young E, Korszun A (2010). Sex, trauma, stress hormones and depression. *Mol Psychiatry* 15: 23-28.

Table 1. The means and standard deviations of variables throughout the menstrual cycle.

		Follicular	Ovulatory	Mid-Luteal	Late-Luteal	*P*-value	Total
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD
MUC1 (Fold ratio)		1.000 ± 0.699	1.351 ± 1.081	1.094 ± 0.608	1.093 ± 0.669	.350	1.134 ± 0.788
Flow rate (mL/min)	U	0.46 ± 0.27	0.50 ± 0.27	0.50 ± 0.28	0.54 ± 0.31	.753	0.50 ± 0.28
	S	1.54 ± 0.48	1.39 ± 0.44	1.64 ± 0.52	1.64 ± 0.53	.149	1.55 ± 0.50
Cortisol (μg/dL)	U	0.479 ± 0.233	0.513 ± 0.286	0.500 ± 0.302	0.536 ± 0.303	.887	0.507 ± 0.279
	S	0.384 ± 0.204	0.426 ± 0.256	0.433 ± 0.267	0.440 ± 0.242	.809	0.420 ± 0.241
DHEA (pg/mL)	U	266.8 ± 129.7	286.3 ± 127.0	291.4 ± 166.8	291.6 ± 168.4	.905	284.0 ± 147.7
	S	162.8 ± 74.2	185.3 ± 85.6	169.3 ± 103.0	175.9 ± 94.0	.793	173.3 ± 89.1
C/D ratio	U	18.9 ± 7.3	17.8 ± 6.3	18.8 ± 8.6	20.5 ± 11.4	.682	19.0 ± 8.5
	S	24.5 ± 12.6	23.4 ± 9.5	30.3 ± 19.3	27.6 ± 15.0	.250	26.5 ± 14.6
17β-Estradiol (pg/mL)	U	2.107 ± 0.704	2.347 ± 0.847	2.369 ± 0.692	2.141 ± 0.696	.385	2.241 ± 0.738
	S	1.639 ± 0.584	1.886 ± 0.748	1.937 ± 0.941	1.722 ± 0.495	.333	1.796 ± 0.714
Progesterone (pg/mL)	U	149.3 ± 93.5[a]	182.3 ± 118.2[b]	292.6 ± 178.7[a,b]	224.6 ± 134.2	.001[**]	212.2 ± 143.5
	S	77.5 ± 49.4[a]	105.0 ± 78.1[b]	173.1 ± 118.1[a,b]	136.5 ± 93.2	< .001[***]	123.0 ± 94.2
CgA (pmol/mL)	U	91.5 ± 48.2	90.2 ± 47.0	98.5 ± 56.3	81.8 ± 45.7	.634	90.5 ± 49.2
	S	39.2 ± 19.0	44.5 ± 30.7	40.5 ± 23.1	40.1 ± 17.2	.819	41.1 ± 22.9
Blood contamination (mg/dL)	U	1.164 ± 0.959	1.389 ± 0.932	1.097 ± 0.792	1.168 ± 0.879	.611	1.204 ± 0.888
	S	0.490 ± 0.382	0.578 ± 0.403	0.578 ± 0.449	0.518 ± 0.410	.791	0.541 ± 0.408

SD, standard deviation; MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A; U, unstimulated whole saliva; S, stimulated whole saliva

[a] The mean of the mid-luteal phase is higher than that of the follicular phase ($P < 0.01$, by the Tukey's post -hoc test)

[b] The mean of the mid-luteal phase is higher than that of the ovulatory phase ($P < 0.05$, by the Tukey's post -hoc test).

[**] $P < 0.01$; [***] $P < 0.001$ by the repeated-measures ANOVA

Table 2. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.119							
Cortisol	-.154	-.050						
DHEA	.094	-.230*	.637***					
C/D ratio	-.285**	.212*	.491***	-.263**				
17β-Estradiol	.138	.040	.079	.187*	-.123			
Progesterone	-.071	-.100	.262**	.207*	.088	.381***		
CgA	-.040	-.236**	.229*	.166	.033	.028	.339***	
Blood contamination	.006	-.315***	.003	.034	-.085	.342***	.240**	.260**

MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Table 3. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.111							
Cortisol	-.093	-.118						
DHEA	.224*	-.146	.597***					
C/D ratio	-.285**	.039	.508***	-.297**				
17β-Estradiol	.114	-.009	.099	.126	.075			
Progesterone	-.047	.052	.213*	.207*	.114	.321***		
CgA	-.058	-.269**	.107	.197*	-.084	.332***	.146	
Blood contamination	-.067	-.366***	.187*	.193*	.002	.171	.191*	.370***

MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Table 4. Correlation coefficients (r) between the periodontal parameters and other variables in the follicular phase.

r		Sum of GI scores	Sum of PPDs
MUC1		-.311	-.292
Flow rate	U	-.246	-.212
	S	-.341	-.043
Cortisol	U	.267	.408*
	S	.283	.460*
DHEA	U	.358	.391*
	S	.291	.396*
C/D ratio	U	-.109	.089
	S	-.018	.202
17β-Estradiol	U	-.063	.143
	S	-.224	.129
Progesterone	U	.081	.267
	S	-.061	.324
CgA	U	.069	.114
	S	.230	.543**
Blood contamination	U	.109	-.193
	S	.462*	.451*
Sum of PPDs		.441*	

MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A; GI, Gingival index; PPD, Periodontal probing depth; U, unstimulated whole saliva; S, stimulated whole saliva

* $P < 0.05$; ** $P < 0.01$ by the Pearson correlation analysis

Appendix Table 1. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva in the follicular phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.017							
Cortisol	-.136	-.039						
DHEA	-.121	-.103	.669***					
C/D ratio	.060	.189	.476**	-.273				
17β-Estradiol	-.025	-.020	.148	.294	-.228			
Progesterone	-.051	-.234	.529**	.462*	-.001	.671***		
CgA	.181	-.354	.324	.090	.126	.075	.561**	
Blood contamination	.024	-.455*	.019	.010	-.133	.382*	.509**	.396*

MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 2. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva in the follicular phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.202							
Cortisol	-.135	-.224						
DHEA	.150	-.214	.546**					
C/D ratio	-.168	-.006	.676***	-.176				
17β-Estradiol	.038	.013	.275	.264	.172			
Progesterone	-.040	-.142	.509**	.564**	.108	.726***		
CgA	.060	-.364*	.349	.416*	.048	.325	.433*	
Blood contamination	-.097	-.506**	.369*	.446*	-.001	.352	.420*	.496**

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 3. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva in the ovulatory phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.217							
Cortisol	-.042	-.006						
DHEA	.261	-.135	.792***					
C/D ratio	-.328	.241	.569**	.033				
17β-Estradiol	.299	.137	.074	.360	-.250			
Progesterone	.125	.155	.130	.260	-.009	.211		
CgA	-.148	-.259	-.053	-.099	-.015	-.236	.160	
Blood contamination	.073	-.259	.122	.143	.051	.375*	.174	.184

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 4. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva in the ovulatory phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	.014							
Cortisol	.035	-.107						
DHEA	.394*	-.088	.733***					
C/D ratio	-.350	-.019	.534**	-.104				
17β-Estradiol	.108	.211	-.054	.166	-.199			
Progesterone	.064	.005	.228	.267	.087	.125		
CgA	-.202	-.249	-.145	.025	-.237	.268	.024	
Blood contamination	-.257	-.162	.092	.199	-.041	.143	.178	.311

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 5. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva in the mid-luteal phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	.153							
Cortisol	-.382*	-.076						
DHEA	-.028	-.216	.582**					
C/D ratio	-.489**	.139	.458*	-.352				
17β-Estradiol	.139	.301	.097	.009	.063			
Progesterone	-.475**	-.261	.348	.167	.221	.232		
CgA	-.173	-.273	.390*	.336	.089	.120	.400*	
Blood contamination	-.235	-.277	.034	.019	.020	.038	.266	.337

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 6. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva in the mid-luteal phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.039							
Cortisol	-.306	.005						
DHEA	.011	-.067	.506**					
C/D ratio	-.362*	.125	.495**	-.420*				
17β-Estradiol	.232	-.094	.137	.113	.119			
Progesterone	-.421*	.110	.150	.233	.037	.241		
CgA	-.089	-.415*	.059	.125	-.059	.491**	.134	
Blood contamination	-.008	-.369*	-.037	-.013	-.078	.169	.144	.226

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 7. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva in the late-luteal phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.345							
Cortisol	-.186	-.096						
DHEA	.181	-.418*	.570**					
C/D ratio	-.406*	.257	.509**	-.334				
17β-Estradiol	-.070	-.247	.017	.120	-.094			
Progesterone	.084	-.139	.173	.077	.040	.606***		
CgA	.037	-.056	.267	.242	-.022	.145	.305	
Blood contamination	-.019	-.288	-.164	-.018	-.188	.541**	.300	.149

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 8. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva in the late-luteal phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.177							
Cortisol	-.113	-.218						
DHEA	.244	-.202	.614***					
C/D ratio	-.349	-.128	.452*	-.337				
17β-Estradiol	-.039	-.124	.050	-.046	.097			
Progesterone	.194	-.068	.129	.039	.045	.467**		
CgA	.154	-.027	.424*	.393*	-.081	.147	.297	
Blood contamination	.135	-.432*	.398*	.238	.127	-.037	.150	.587**

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

국문초록

여성 생리주기에 따른 구강점막 MUC1 발현 및 타액 호르몬의 변화

이 연 희

서울대학교 대학원 치의과학과 구강내과·진단학 전공

(지도교수 **고 홍 섭**)

시상하부 뇌하수체 부신피질 축은 스트레스 관련 연구에서 가장 중점적으로 다뤄지는 주제이며, 주로 cortisol, dehydroepiandrosterone (DHEA), cortisol/DHEA ratio의 변화를 통해 연구되어 왔다. 여성 성호르몬 역시 스트레스에 반응한 내분비계의 작용과 상호작용하여 분비된다. 따라서 여성이 남성과 다른 스트레스 반응을 보이는 것은 생물학적 성과 생리주기에 따른 스트레스 반응의 차이로 설명될 수 있다. 이러한 스트레스 호르몬과 성호르몬의 변화는 구강점막 방어에 영향을 미치고, 여성의 생리주기는 구강점막 질환의 발생과 악화에 영향을 미친다는 결과가 보고되어 왔다. 이 연구의 목적은 구강점막 상피세포의 MUC1 발현과 타액 내의 스트레스 및 성호르몬의 관계를 여성의 생리주기에 따라 분석·탐구하는 것이다.

연구는 정상적인 생리주기를 가진 30명의 건강한 여성(25.9 ± 2.1세)을 대상으로 하였다. 모든 참여자를 대상으로 연구과정 시작 전에, 구강점막과 치주 건강에 대한 검사를 실시하였다. 참여자들은 한 번의 생리 주기 동안 여포기, 배란기, 황체중기 및 황체후기에 해당하는 날, 총 4회에 걸쳐 연구에 참여하였고, 모든 연구대상으로부터 참여시 마다 비자극성 전타액과 자극성 전타액이 채취되었다. 구강점막 MUC1의 발현은 타액으로부터 추출한 RNA를 활용하여 real-time PCR로 분석하였고, 비자극성 및 자극성 전타액의 상층액을 활용하여 17β-estradiol, progesterone, DHEA, cortisol 및 chromogranin A (CgA)의 농도를 측정하였다. 이와 함

께 타액검체의 혈액오염 정도를 transferrin 농도 측정으로 살펴보았다.

전체 결과를 모두 함께 분석하였을 때, 비자극성 전타액과 자극성 전타액 모두에서 cortisol, progesterone과 DHEA, 세 가지 요소들 간의 양의 상관관계가 관찰되었다. 또, 비자극성 전타액에서 17β-estradiol과 DHEA간, 비자극성 전타액에서 cortisol과 CgA간, MUC1 발현과 자극성 전타액에서의 DHEA간에 유의한 양의 상관관계가 있었다. 이와 함께, MUC1 발현과 cortisol/DHEA ratio사이에 유의한 음의 상관관계가 비자극성 전타액과 자극성 전타액 모두에서 관찰되었다. 각 시기로 나누어 연구결과를 분석해 보았을 때, 황체중기에서 MUC1 발현은 비자극성 전타액의 cortisol, progesterone, 그리고 cortisol/DHEA ratio 각각의 요소와 유의한 음의 상관관계를 보였고, 자극성 전타액의 progesterone과 cortisol/DHEA ratio와는 유의한 음의 상관관계를 보였다. 황체후기에서는 MUC1의 발현과 비자극성 전타액의 cortisol/DHEA ratio간의 유의한 음의 상관관계가 관찰되었다.

결론적으로 본 연구의 결과는 구강점막 MUC1 발현이 생리 주기에 따른 타액 스트레스 호르몬과 성호르몬의 변화에 영향을 받는다는 것을 제시한다. 즉, 스트레스와 관련된 심리-내분비학적인 상호작용은 구강점막 MUC1 발현, 나아가 구강점막 방어기전에 영향을 미칠 수 있음을 알 수 있다.

주요어 : MUC1, 스트레스, 스테로이드 호르몬, 생리주기

학 번 : 2012-31183

치의과학박사 학위논문

Changes in Oral Mucosal MUC1 Expression and Salivary Hormones throughout the Menstrual Cycle

여성 생리주기에 따른 구강점막 MUC1 발현 및 타액 호르몬의 변화

2016년 2월

서울대학교 대학원

치의과학과 구강내과·진단학 전공

이 연 희

Changes in Oral Mucosal MUC1 Expression and Salivary Hormones throughout the Menstrual Cycle

여성 생리주기에 따른 구강점막 MUC1 발현 및 타액 호르몬의 변화

지도교수 고 홍 섭

이 논문을 치의과학박사 학위논문으로 제출함

2015년 10월

서울대학교 대학원

치의과학과 구강내과·진단학 전공

이 연 희

이연희의 치의과학박사 학위논문을 인준함

2015년 12월

위 원 장 _____

부위원장 _____

위 원 _____

위 원 _____

위 원 _____

ABSTRACT

Changes in Oral Mucosal MUC1 Expression and Salivary Hormones throughout the Menstrual Cycle

Yeon-Hee Lee, D.D.S., M.S.D.

Program in Oral Medicine and Oral Diagnosis, Dept. of Dental Science, Graduate School, Seoul National University

(Directed by Professor **Hong-Seop Kho**, D.D.S., M.S.D., Ph.D.)

The purpose of this study was to investigate relationships among oral mucosal epithelial MUC1 expression, salivary stress markers, and female gonadal hormones throughout the menstrual cycle.

Thirty healthy women (25.9 ± 2.1 years) with regular menstrual cycle were included. Unstimulated (UWS) and stimulated whole saliva (SWS) were collected during the menstrual cycle. The expression level of oral mucosal MUC1 was analyzed. 17β-Estradiol, progesterone, dehydroepiandrosterone (DHEA), cortisol, chromogranin A (CgA), and blood contamination levels were measured from unstimulated (UWS) and stimulated whole saliva (SWS).

In analyses using all data from the four phases combined, significant positive correlations

were observed between 17β-estradiol and DHEA in UWS, cortisol and CgA in UWS, MUC1 expression and DHEA in SWS, and among cortisol, progesterone, and DHEA in UWS and SWS. Significant negative correlations were observed between MUC1 and cortisol/DHEA ratio in UWS and SWS. When each phase was analyzed individually, MUC1 expression showed significant negative correlations with cortisol, progesterone, and cortisol/DHEA ratio in UWS and with progesterone and cortisol/DHEA ratio in SWS during the mid-luteal phase. A significant negative correlation was also observed between MUC1 and cortisol/DHEA ratio in UWS during the late-luteal phase.

In conclusions, stress-related psychoendocrinological interactions throughout the menstrual cycle resulted in a decrease in oral mucosal epithelial MUC1 expression and a weakening of oral mucosal defense.

Key words: MUC1, Stress, Steroid hormone, Saliva, Menstrual cycle

Student Number: 2012-31183

Changes in Oral Mucosal MUC1 Expression and Salivary Hormones throughout the Menstrual Cycle

여성 생리주기에 따른 구강점막 MUC1 발현 및 타액 호르몬의 변화

Yeon-Hee Lee, D.D.S., M.S.D.

Program in Oral Medicine and Oral Diagnosis, Dept. of Dental Science, Graduate School, Seoul National University

(Directed by Professor **Hong-Seop Kho**, D.D.S., M.S.D., Ph.D.)

이 연 희

서울대학교 대학원 치의과학과 구강내과·진단학 전공

(지도교수 고 홍 섭)

-CONTENTS-

I. INTRODUCTION

II. REVIEW OF LITERATURE

III. MATERIALS AND METHODS

IV. RESULTS

V. DISCUSSION

VI. CONCLUSIONS

REFERENCES

TABLES

KOREAN ABSTRACT

I. INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis has been the main focus of stress-related studies through the measurement of cortisol, dehydroepiandrosterone (DHEA), and cortisol/DHEA ratio (Hellhammer et al., 2009; Jeckel et al., 2010; Lennartsson et al., 2012; Tsigos and Chrousos, 2002). Female gonadal hormones also affect stress-related responses, thereby explaining differences in stress responses depending on sex and menstrual cycle (Childs et al., 2010; Kajantie and Phillips, 2006; Wolfram et al., 2011). It has been reported that the HPA axis response to acute stress was greater in the progesterone-dominated luteal phase (Kajantie and Phillips, 2006), and that cortisol awakening response was elevated in the ovulatory phase (Wolfram et al., 2011). Progesterone administration also affected the psychological responses to acute stress (Childs et al., 2010).

Stress and gonadal hormones can affect oral mucosal defense. Chronic stress decreases the salivary flow rate and sIgA levels (Hucklebridge et al., 1998; Somer et al., 1993). Many oral mucosal diseases have etiopathophysiological relationships with stress (Little et al, 2013). The estrogen receptor has been reported to be present in oral mucosal epithelium (Sawczuk et al., 2014). Additionally, the menstrual cycle affects the occurrence or exacerbation of recurrent aphthous stomatitis (Oh et al., 2009).

MUC1, a large transmembrane glycoprotein, expressed in epithelial cell linings provides mucosal defense in the respiratory, reproductive, and gastrointestinal tracts (Gendler, 2001). Although the role of MUC1 may be somewhat different depending on anatomical location, epithelial MUC1 expression in both the oral cavity and female reproductive tract is up-regulated by the presence of microbial infections and pro-inflammatory cytokines (McAuley et al., 2007; Meseguer et al., 2001). Human endometrial MUC1 expression is also affected by

the menstrual cycle and is up-regulated during the progesterone-dominated luteal phase (Brayman et al., 2004; Hey et al., 1994; Meseguer et al., 2001). However, it is currently unknown whether oral mucosal epithelial MUC1 expression is affected by changes in female gonadal hormones throughout the menstrual cycle.

The hypothesis in the present study was oral mucosal epithelial MUC1 expression related to oral mucosal defense could be affected by stress and female gonadal hormonal changes throughout the menstrual cycle. Saliva samples were used because steroid hormone levels in saliva are well correlated with the free protein-unbound active hormone levels in blood (Streckfus and Bigler, 2002). The non-invasive nature of saliva collection provides an additional advantage for studies requiring repetitive sampling. The aim of the present study was to investigate whether changes in salivary stress markers and female gonadal hormones throughout the menstrual cycle affect oral mucosal epithelial MUC1 expression. The relationships among salivary stress markers and female gonadal hormones throughout the menstrual cycle were also investigated.

II. REVIEW OF LITERATURE

1. Stress markers and gonadal hormones

(1) Cortisol

Cortisol is a steroid hormone, in the glucocorticoid class of hormones, and is produced in humans by the zona fasciculatla of the adrenal cortex within the adrenal gland. It has been suggested that cortisol secretion is stimulated by psychological distress and social evaluative threats (Dickerson and Kemeny, 2004). Thus, glucocorticoid levels are frequently used to evaluate physiologic stress levels within and between individuals (Kanaley et al., 2001; Kunz-Ebrecht et al., 2004; Negrao et al., 2000). Many studies have also investigated the relationship between daily life stress and cortisol. For example, work stress (Steptoe et al., 2000), unemployment, and divorce (Ockenfels et al., 1995) were associated with increased morning or night-time cortisol levels. Recent studies also investigated the cortisol awakening response and the stress in daily life. Cortisol levels in human salivary and plasma fluid increased by 50–60% within 30 minutes after awakening. In addition, recent studies have shown that the cortisol awakening response is a useful indicator of HPA activity (Clow et al., 2004).

Cortisol is one of the most important end products of hypothalamic-pituitary-adrenal (HPA) axis activation (Kudielka and Kirschbaum, 2003). It is well known that physiologic response to stress is mediated by the HPA axis. HPA function is linked to critical metabolic tasks such as immune response, cardiovascular function, reproductive physiology, and general well-being. When stimulated by endogenous and exogenous challenges, the paraventricular

nucleus of the hypothalamus increases its production of corticotropin-releasing hormone (CRH), which in turn promotes the release of adrenocorticotropin (ACTH) by the anterior pituitary, leading to an increase in the secretion of glucocorticoids, including cortisol, by the adrenal cortex. Increases in cortisol levels trigger gluconeogenesis, resulting in higher levels of circulating glucose. Glucose provides energy to the tissues involved in responding to the challenges that trigger the activation of the HPA axis in the first place (Nepomnaschy et al., 2007). Thus, cortisol levels are frequently used to monitor HPA axis function and activation, and are interpreted as proxies of physiologic stress levels (Altemus et al., 2001; Kanaley et al., 2001; Padgett and Glaser, 2003). Furthermore, cortisol is considered as one of the endogenous hormones that modulate energy homeostasis. A major physiological role of cortisol is considered to be gluconeogenesis and maintenance of blood glucose (Miller, 1995). Hence it increases glucose in the bloodstream, increases the availability of substances that repair tissues (Kunz-Ebrecht et al., 2004). Cortisol also has inhibitory functions that would be nonessential or detrimental in a fight-or-flight situation. It alters immune system responses and suppresses the digestive system, the reproductive system and growth processes. This complex natural alarm system also communicates with regions of the brain that control mood, motivation and fear. Moreover, cortisol is associated with health-related variables such as psychoses and cardiovascular disease; therefore, cortisol is a mediator between psychosocial stress and health (McEwen, 2000).

(2) Dehydroepiandrosterone (DHEA)

DHEA, more correctly didehydroepiandrosterone, also known as androstenolone or prasterone, as well as 3β-hydroxyandrost-5-en-17-one or 5-androsten-3β-ol-17-one, is an

important endogenous steroid hormone (Mo et al., 2006). It is the most abundant circulating steroid hormone in human body (Ganong, 2005), in whom it is produced in the adrenal glands, the gonads, and the brain, where it functions predominantly as a metabolic intermediate in the biosynthesis of the androgen and estrogen sex steroids (Webb et al., 2006). DHEA and its sulfated form, DHEA-S, are circulating steroid hormones that are more abundant in humans, and despite not showing an intrinsic androgenic activity, both are precursors of potent androgens and estrogens (Nieschlag et al., 1973). DHEA also has a variety of potential biological effects in its own right, binding to an array of nuclear and cell surface receptors, and acting as a neurosteroid.

The associations between psychosocial stress and DHEA levels have also been recently investigated. The main secretagogue for DHEA is ACTH, and DHEA was reported to be secreted synchronously with cortisol during the night and day and had anti-glucocorticoid and anti-cortisol effects (Rosenfeld et al., 1971). DHEA may affect certain brain functions by modulating neurotransmitter receptors such as gamma-aminobutyric acid (GABA) or N-methyl-D-aspartic acid (NMDA) receptors, and be involved in the pathophysiology of cognitive decline and mood disorders (Wolf and Kirschbaum, 1999). Acute psychosocial stress was reported to increase DHEA concentration (Izawa et al., 2008; Shirtcliff et al., 2007). However, despite the well-documented effects of stress on HPA axis function, studies of stress-induced increases in DHEA and DHEA-S response have yielded mixed results. (Morgan et al., 2004; Oberbeck et al., 1998; Shirotsuki et al., 2009).

The mechanisms of action of DHEA in humans are not completely understood, although it is considered to be an important regulator of body fat, immune response, insulin sensitivity, and cognitive function (Corona et al., 2013). In addition, Kibaly et al. (2008) demonstrated

the involvement of this steroid in the modulation of sensory processes, especially neuropathic pain. Reductions in DHEA concentrations are found in some diseases, such as diabetes, schizophrenia, systemic lupus erythematosus, Alzheimer disease, depression, and other psychologic disturbances (Brooke et al., 2006; Chang et al., 2002; Rabkin et al., 2006; Villareal and Holloszy, 2006). DHEA, in addition to cortisol, is a major steroid produced by the zona reticularis of the adrenal cortex. Some studies have suggested that lower DHEA levels are associated with lower psychological well-being (van Niekerk et al., 2001).

Salivary concentrations of DHEA reflect those in serum. In saliva, the concentration of this steroid is independent of salivary flow rate and represents accurately the fraction of the free and biologically active hormone in the blood circulation (Chen et al., 2005; Gao et al., 2005). It is meaningful to examine the salivary DHEA response to situations causing social anxiety.

(3) Cortisol/DHEA ratio

Cortisol and DHEA are steroid hormones, both synthesized from pregnenelone, the master steroid hormone, which is derived from cholesterol. DHEA can counteract several adverse effects of glucocorticoid in different tissues (Ferrari et al., 2001; Kalimi et al., 1994). Its mechanism of action and physiological implications are still not fully understood. In the context of the immune system, DHEA could exert its anti-glucocorticoid activity by modulating glucocorticoid receptor (GR) beta expression and antagonizing the function of GR alpha (Pinto et al., 2015).

The effects of DHEA on brain functions are believed to be opposite to those of cortisol. For example, it was reported that the cortisol/DHEA ratio was elevated in depressive patients

(Young et al., 2002), and that a high cortisol/DHEA ratio in adolescents was predictive of persistent major depression (Goodyer et al., 2003). Moreover, under acutely stressful situations, the cortisol/DHEA ratio was reported to be correlated with a negative mood level (Izawa et al., 2008). Although the association between cortisol/DHEA ratio and mood under prolonged stress has not been investigated, DHEA could antagonize cortisol activity indicated that (Blauer et al., 1991). Therefore, it is considered that the cortisol to DHEA ratio may be a marker of endocrine imbalance. For example, persistently depressed individuals had a higher cortisol to DHEA ratio than non-depressed and remitted individuals (Goodyer et al., 2003). Goodyer et al. (2003) suggested that the high ratio might be a marker of persistent psychiatric disorders. Other studies have found that a higher morning cortisol to DHEA ratio is associated with higher anxiety (van Niekerk et al., 2001). Young et al. (2002) showed that the cortisol to DHEA ratio from saliva samples correlated with the length of the current depressive episode and suggested that the cortisol to DHEA ratio could be a marker of depressive states. Thus, it has been speculated that the cortisol to DHEA ratio would represent an endocrine imbalance of the HPA axis function and may be a marker of the state of other psychiatric disorders.

(4) Chromogranin A

Chromogranin A (CgA), an acidic glycoprotein initially isolated as the major soluble protein of adrenal medullary chromaffin granules, has been reported to localize in secretory granules of a wide variety of endocrine cells and neurons (Winkler and Fischer-Colbrie, 1992). In humans, CgA protein is encoded by the parathyroid secretory protein gene (Helman et al., 1988). CgA are produced from chromaffin cells of the adrenal medulla, paraganglia,

enterochromaffin-like cells, and beta cells of the pancreas, and it induces and promotes the generation of secretory granules such as those containing insulin in pancreatic islet beta cells. CgA is the precursor to several functional peptides including vasostatin-1, vasostatin-2, pancreastatin, catestatin, and parastatin. These peptides negatively modulate the neuroendocrine function of the releasing cell or nearby cells (Curry et al., 2002). Recently, Den et al. (2007) reported that the human salivary CgA concentration peaked upon awakening, quickly decreased to a nadir after 1 hour, then remained at a low level throughout the day.

CgA is also produced by human submandibular glands and, in response to activation of the autonomic nervous system innervating the submandibular gland, is released directly from the exocrine cells of the granular convoluted tubules into the saliva. In addition, the highly concentrated salivary CgA is released in response to noradrenaline and acetylcholine in isolated and perfused rat submandibular glands (Kanno et al., 1999). Thus, salivary CgA has been proposed as a marker of sympathetic nervous activity involving the sympathetic-adrenomedullary system (Nakane et al., 2002) and might be used as an alternative to the measurement of plasma catecholamine concentration (Kanno et al., 1999).

Responding to stress, CgA and catecholamines are co-released into the extra-cellular environment. A prompt elevation in salivary CgA levels and a delayed increase in salivary cortisol levels when psychosomatic stress was induced by a test involving an oral presentation in front of an audience or a driving situation (Lee et al., 2006; Nakane et al., 2002). Lee et al. (2006) also found that salivary CgA concentration depicted an increase during the mental stress tasks and decrease during the intermissions, demonstrating the possible candidacy of CgA as a biomarker for a short-term mental workload. These studies

might suggest that salivary CgA may be a sensitive and promising index for psychosomatic stress.

Since researchers first applied noninvasive technology to measure CgA in saliva to the field of human science, substantial evidence of the psychological properties of salivary CgA has accumulated. In short-term stressful situations, such as public speaking, salivary CgA levels elevate and peak immediately before and decrease immediately after the event (Lee et al., 2006). In contrast, stress-reduction intervention, such as aromatherapy with lavender essential oil and exposure to negative air ions, has decreased elevated salivary CgA levels after stressful mental tasks (Matsumoto et al., 2014). Although CgA has been reported as a possible marker of stress, there were also inconsistent results showed that CgA levels were not increased in the stressful situation (Yamakoshi et al., 2009). Although CgA could serve as a potential neuropsychophysiological index for objectively assessing periodic emotional fluctuations, including late-luteal-specific emotional disturbances observed in women suffering from premenstrual syndrome, the scientific literature has failed to report on this aspect of the use of this biochemical stress marker.

(5) 17 beta-estradiol

Estradiol, or more precisely, 17β-estradiol, is a steroid and estrogen sex hormone, and the primary female sex hormone. It is named for and is important in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is essential for the development and maintenance of female reproductive tissues (Ryan, 1982), but it also has important effects in many other tissues including bone. While estrogen levels in men are lower compared to

women, estrogens have essential functions in men as well (Gooren et al., 1984). Estradiol is produced especially within the follicles of the female ovaries, but also in other endocrine and non-endocrine tissues including fat, liver, adrenal, breast, and neural tissues. Estradiol is biosynthesized from progesterone and arrived at in two steps from cholesterol, via intermediate pregnenolone (Saldanha et al., 2011). One principle pathway then converts progesterone to its 17-hydroxy derivative, 17-hydroxyprogesterone, and then to Δ4-androstenedione via sequential cytochrome P450-catalyzed oxidations. Alternatively, Δ4-androstenedione can be converted into the androgen, testosterone, which in turn can be converted directly into 17β-estradiol. In the female, estradiol acts as a growth hormone for tissue of the reproductive organs, supporting the lining of the vagina, the cervical glands, the endometrium, and the lining of the fallopian tubes. It enhances growth of the myometrium. Estradiol appears necessary to maintain oocytes in the ovary.

Women are more likely than men to show variations in HPA function in response to stressors (Weiss et al., 1999) and during depressive episodes (Young and Korszun, 2010). These differences in stress system response likely contribute to mood disorder risk in women (Weiss et al., 1999), and may be modulated by ovarian hormone fluctuations across the menstrual cycle (Kajantie and Phillips, 2006; Roca et al., 2005). While the role of corticosteroids in stress response and regulation is well known, the effects of estradiol along the hypothalamic-pituitary-gonadal (HPG) axis which can be stimulated by various stressors, including anxiety and depression are less well characterized. Brain activity related to processing negative emotional information is also modulated by changing estradiol levels across the menstrual cycle (Goldstein et al., 2005; Merz et al., 2012), suggesting that estrogen may alter the mood response to negative information, making this information more or less salient to cognitive processes and subsequently mood states.

Animal models suggest that female rodents do not gain the same beneficial effect of acute stress on hippocampally-mediated or prefrontal tasks as male animals, and that high estrogen levels enhance the negative effects of stress (Shansky et al., 2004; Shors and Leuner, 2003). However, estrogen replacement therapy increases resilience to stress in the learned helplessness model using ovariectomized rats, which might be related to hippocampal synaptic plasticity (Bredemann and McMahon, 2014; Smith et al., 2010). The modulation of stress effects on brain activity and function remain unclear and likely depend on the type of stress and measure of function (Shansky et al., 2004; Shors and Leuner, 2003). There is a sex difference in the endocrine response to psychosocial stress, and that cycling ovarian hormones may modulate this response in women (Kirschbaum et al., 1992; Kirschbaum et al., 1999).

(6) Progesterone

Progesterone is an endogenous steroid and sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species (Kumari et al., 2010). Progesterone is also a crucial metabolic intermediate in the production of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neurosteroid (Baulieu and Schumacher, 2000). Progesterone is produced in high amounts in the ovaries by the corpus luteum from the onset of puberty to menopause, and is also produced in smaller amounts by the adrenal glands after the onset of adrenarche in both males and females. During human pregnancy, progesterone is produced in increasingly high amounts by the ovaries and placenta by the presence of human chorionic gonadotropin. However, after the 8th week, production of progesterone shifts to the placenta. At term the

placenta produces about 250 mg progesterone per day. Whether a decrease in progesterone levels is critical for the initiation of labor has been argued and may be species-specific. After delivery of the placenta and during lactation, progesterone levels are very low. Adult males have levels similar to those in women during the follicular phase of the menstrual cycle.

Progesterone is the most important progestogen in the body, the result of its action as a potent agonist of the nuclear progesterone receptor (Daniel et al., 2010; Kumari et al., 2010). In addition, progesterone is an agonist of the more recently discovered membrane progesterone receptors (Thomas and Pang, 2012), as well as a ligand of the progesterone receptor membrane component 1, PGRMC1 (Meyer et al., 1998). Moreover, progesterone is also known to be an antagonist of the σ1 receptor (Maurice et al., 2001), and a negative allosteric modulator of the acetylcholine receptor (Baulieu and Schumacher, 2000), and a potent antagonist of the mineralocorticoid receptor (MR) (Rupprecht et al., 1993). Progesterone prevents MR activation by binding to this receptor with an affinity exceeding even those of aldosterone and glucocorticoids such as cortisol and corticosterone (Rupprecht et al., 1993), and produces anti-mineralocorticoid effects at physiological concentrations. In addition, progesterone binds to and behaves as a partial agonist of the glucocorticoid receptor (Attardi et al., 2007). Progesterone, through its neurosteroid active metabolites, acts indirectly as a positive allosteric modulator of the GABA receptor (Paul and Purdy, 1992) .

Progesterone has a number of physiological effects that are amplified in the presence of estrogens. Estrogens through estrogen receptors induce or up-regulate the expression of the progesterone receptor (Kastner et al., 1990). In addition, elevated levels of progesterone potently reduce the sodium-retaining activity of aldosterone, resulting in natriuresis and a reduction in extracellular fluid volume. Progesterone withdrawal, on the other hand, is

associated with a temporary increase in sodium retention due to the compensatory increase in aldosterone production, which combats the blockade of the mineralocorticoid receptor by the previously elevated level of progesterone.

2. Changes of gonadal hormones according to the menstrual cycle

The menstrual cycle is the regular natural changes that occurs in the uterus and ovaries that make pregnancy possible (Barbieri, 2014; Nagar and Msalati, 2013). The typical length of time between the first day of one period and the first day of the next is 21 to 45 days in young women and 21 to 31 days with an average of 28 days in adults. A woman's menstrual cycle typically follows a 28-day cycle and ends with the shedding of uterine lining leading to bleeding. The normal menstrual cycle indicates the proper functioning of hormones, having a normal menstrual cycle signifies a healthy HPA axis with a normal uterus. Menstruation stops occurring after menopause which usually occurs between 45 and 55 years of age. Bleeding usually lasts around 2 to 7 days (American Academy of Pediatrics et al., 2006).

The menstrual cycle is governed by hormonal changes. Each cycle can be divided into three phases based on events in the ovary or in the uterus. The ovarian cycle consists of the follicular phase, ovulation, and luteal phase whereas the uterine cycle is divided into menstruation, proliferative phase, and secretory phase. Stimulated by gradually increasing amounts of estrogen in the follicular phase, discharges of blood flow stop, and the lining of the uterus thickens. Approximately mid-cycle, 24–36 hours after the luteinizing hormone (LH) surges, the dominant follicle releases an ovocyte, in an event called ovulation. After ovulation, the ovocyte only lives for 24 hours or less without fertilization while the remains of the

dominant follicle in the ovary become a corpus luteum; this body has a primary function of producing large amounts of progesterone. Under the influence of progesterone, the uterine lining changes to prepare for potential implantation of an embryo to establish a pregnancy. If implantation does not occur within approximately two weeks, the corpus luteum will involute, causing a sharp drop in levels of both progesterone and estrogen. The hormone drop causes the uterus to shed its lining in a process termed menstruation.

Through the influence of a rise in follicle stimulating hormone (FSH) during the first days of the cycle, a few ovarian follicles are stimulated. These follicles, which were present at birth and have been developing. Ovulation is the second phase of the ovarian cycle in which a mature egg is released from the ovarian follicles into the oviduct. During the follicular phase, estradiol suppresses production of LH from the anterior pituitary gland. When the egg has nearly matured, levels of estradiol reach a threshold above which this effect is reversed and estrogen stimulates the production of a large amount of LH. This process, known as the LH surge, starts around day 12 of the average cycle and may last 48 hours. The exact mechanism of these opposite responses of LH levels to estradiol is not well understood. This may be enabled by the presence of two different estrogen receptors in the hypothalamus: estrogen receptor alpha, which is responsible for the negative feedback estradiol-LH loop, and estrogen receptor beta, which is responsible for the positive estradiol-LH relationship (Berg et al., 2008). However, in humans it has been shown that high levels of estradiol can provoke abrupt increases in LH suggesting that estrogen acts directly on the pituitary to provoke the LH surge.

The luteal phase is the final phase of the ovarian cycle and it corresponds to the secretory phase of the uterine cycle. During the luteal phase, the pituitary hormones FSH and LH cause

the remaining parts of the dominant follicle to transform into the corpus luteum, which produces progesterone. The increased progesterone in the adrenals starts to induce the production of estrogen. The hormones produced by the corpus luteum also suppress production of the FSH and LH that the corpus luteum needs to maintain itself. Consequently, the level of FSH and LH fall quickly over time, and the corpus luteum subsequently atrophies. Falling levels of progesterone trigger menstruation and the beginning of the next cycle. From the time of ovulation until progesterone withdrawal has caused menstruation to begin, the process typically takes about two weeks.

In the normal menstrual cycle, estradiol levels measure typically <50 pg/ml at menstruation, rise with follicular development (peak: 200 pg/ml), drop briefly at ovulation, and rise again during the luteal phase for a second peak. At the end of the luteal phase, estradiol levels drop to their menstrual levels unless there is a pregnancy. In women, progesterone levels are relatively low during the preovulatory phase of the menstrual cycle, rise after ovulation, and are elevated during the luteal phase. Progesterone levels tend to be < 2 ng/ml prior to ovulation, and > 5 ng/ml after ovulation (Barbieri, 2014).

3. Changes of stress hormone according to the menstrual cycle

Past research has successfully identified factors associated with the modulation of both the subjective and neuroendocrine stress responses in women (Kudielka et al., 2009). Menstrual cycle phases were examined in the association between subjective stress and physiological response (Kajantie and Phillips, 2006). Compared to the follicular phase, which is characterized by rising levels of circulating estrogens, many studies found the luteal phase,

which is characterized by high circulating levels of estrogens and progesterone, to be associated with increases in physiological responses to stress, although this is not consistently observed (Childs et al., 2010; Felmingham et al., 2012; Kirschbaum et al., 1999), Interestingly, most of the studies investigating the association between the subjective and neuroendocrine stress responses failed to control for possible effect of gender and/or cycle variations (Campbell and Ehlert, 2012).

It is often assumed that stress responsivity varies across the menstrual cycle. In addition, the psychosocial stress is associated with the experience of irregular menstrual cycles among college students. The students with high stress levels experienced irregular cycles more often than the ones with low stress levels (Yamamoto et al., 2009). Felmingham et al. (2012) reported that women in mid-luteal phase demonstrated a positive association between cortisol stress response and recall for threatening stimuli, which was not observed in other phases. According to the recent study, salivary CgA levels significantly increased in the late-luteal phase in women experiencing a cluster of severe negative emotional symptoms premenstrually (Matsumoto et al., 2014). Despite the importance of the assumption mentioned earlier to research on stress, however, the evidence suggesting that baseline cortisol levels vary across the menstrual cycle is contradictory.

It is obviously important to consider women's menstrual cycles when assessing variations in HPA axis function and physiologic stress levels. Nonetheless, our understanding of HPA axis function is quite incomplete as we still lack a proper characterization of the changes in HPA axis functioning across most reproductive transitions. Longitudinal changes in HPA axis functioning across women's menstrual cycles, for example, are yet to be properly characterized. Changes of stress hormones including DHEA and CgA in women across the

menstrual cycle did not have well-documented. Since the secretion of the stress hormone is affected by numerous factors its use as a biomarker of physiologic stress is not simple and should always be accompanied by proper controls.

4. Diagnostic use of saliva

Saliva is a clinically informative, biological fluid that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, and monitoring and management of patients with both oral and systemic diseases. Over the decade, the use of saliva as a vehicle for the determination of plasma steroid hormone levels has increased dramatically. Advantages of saliva sampling include the noninvasive nature of saliva collection; the convenience of multiple sampling; the ease of collection, storage, and shipment of individual samples for analysis; elimination of many ethical issued relating to studies directed at various groups including neonates, the elderly, the mentally and physically handicapped, etc. Over the past decade, new, highly sensitive analytical procedures have been developed for steroid determination. These newer procedures have permitted the automation of saliva analysis. In addition, it has been reported that saliva contains specific soluble biological markers. Most of the biomarkers present in blood and urine can also be detected in a sample of saliva (Hofman, 2001; Marti-Alamo et al., 2012).

Some hormones have specific protein carriers that have relatively high affinity for the respective hormone, whereas much of the nonspecific binding is to albumin. Therefore, the free concentration of a particular steroid hormone is dependent on the affinity and total binding capacity of the various binding proteins in plasma. The primary entry of the steroid

hormones into saliva is via passive diffusion through the salivary gland epithelium. The free concentration of the hormone in the plasma provides the concentration gradient of the steroid through the epithelial membrane, and the movement into the primary secretory fluid within the acinar intercalated duct complex. Because of the slow dissociation rate of the steroid or weakly bound steroid hormone that disassociates and passes through these various membrane structures as the blood passes through the salivary gland during saliva formation. The saliva levels will therefore reflect the free concentration of hormones in plasma (Cardoso et al., 2009; Hofman, 2001). However, if a particular steroid hormone does not have a high-affinity, high-capacity binding protein in the plasma, the saliva levels will correlate with the total plasma hormone concentration.

The steroid hormone can be metabolized by the salivary gland epithelial cells and during transcellular movement of the hormone. Metabolism of the hormone can also occur by the oral bacteria in the saliva at the time of collection and, therefore, appropriate sample collection and storage conditions may need to be developed. Despite the aforementioned complications, noninvasive saliva collection is an excellent medium for the monitoring of plasma steroid levels. For example, saliva has been successfully used in combination with experimental stress challenges to assess stress reactivity in different phases of the menstrual cycle (Nepomnaschy et al., 2011). The literature has reported cortisol in saliva as a useful marker for objectively assessing stress (Hansen et al., 2008). Most studies consider salivary cortisol levels a reliable measure of HPA axis adaptation to stress. However, the stress response of the HPA axis is rather complex and modulated by numerous factors. In addition, measuring salivary catecholamine proves rather difficult because of its low concentration and rapid degradation (Nakane et al., 2002). In saliva, the concentration of DHEA is independent of salivary flow rate and represents accurately the fraction of the free and biologically active

hormone in the blood circulation (Chen et al., 2005; Gao et al., 2005). In other words, salivary concentrations of DHEA reflect those in serum. In addition, the collection of saliva is less expensive without causing stress to the patient, which could in turn cause alterations in DHEA levels. Saliva sampling has permitted the evaluation and assessment of a multitude of endocrine studies that would have been extremely difficult.

5. MUC1

(1) Structure

MUC1 (Mucin1) is a member of the mucin family and encodes a membrane bound, glycosylated phosphoprotein. MUC1 has a core protein mass of 120-225 kDa which increases to 250-500 kDa with glycosylation. It extends 200-500 nm beyond the surface of the cell (Brayman et al., 2004). MUC1 is cleaved in the endoplasmic reticulum into two pieces, the cytoplasmic tail including the transmembrane domain and the extracellular domain. It anchored to the apical surface of many epithelia by a transmembrane domain. The cytoplasmic tail of MUC1 is 72 amino acids long and contains several phosphorylation sites (Singh and Hollingsworth, 2006). Beyond the transmembrane domain is a SEA domain that contains a cleavage site for release of the large extracellular domain. The release of mucins is performed by sheddases (Hattrup and Gendler, 2008). The extracellular domain includes a 20 amino acid variable number tandem repeat (VNTR) domain, with the number of repeats varying from 20 to 120 in different individuals. These repeats are rich in serine, threonine, and proline residues which permits heavy O-glycosylation (Brayman et al., 2004). Multiple alternatively spliced transcript variants that encode different isoforms of this gene have been

reported, but the full-length nature of only some has been determined.

(2) Functions

MUC1 is ubiquitously expressed and lines the apical surface of epithelial cells in the lungs, stomach, intestines, eyes and several other organs (Hollingsworth and Swanson, 2004). MUC1 is primarily involved in the protection of epithelial surfaces. It protects the body from infection by pathogen binding to oligosaccharides in the extracellular domain, preventing the pathogen from reaching the cell surface (Moncada et al., 2003). The cell-surface MUC1 is highly expressed on the mucosal surface and limits the density of *Helicobacter pylori* in a murine infection model (McAuley et al., 2007). Although cleavage by unknown MUC1 sheddase cannot be definitively excluded, the most likely scenario is disassociation of the non-covalent interaction between the transmembrane and extracellular domains at the SEA module, a site of cleavage during synthesis found in most cell surface mucins. Disassociation could occur due to conformational changes in MUC1 following binding or due to shear forces following binding to the highly motile bacteria.

MUC1 also functions in a cell signaling capacity (Singh and Hollingsworth, 2006). The cytoplasmic tail of MUC1 is involved in several signaling pathways, including those involving Ras, β-catenin, p120 catenin, p53 and estrogen receptor α. The cytoplasmic domain of MUC1 also forms complexes with transcription factors, and then translocates to the nucleus by an unknown mechanism, where it is believed to influence the transcription of their target genes. It has also been proposed to localize to mitochondrial membranes under conditions of genotoxic stress, where it attenuates the apoptotic pathway in response and

confers resistance to apoptosis-inducing drugs. Overexpression and changes in glycosylation of MUC1 are often associated with colon, breast, ovarian, lung, and pancreatic cancers (Gendler, 2001).

(3) MUC1 expression in the oral cavity

MUC1 is primarily involved in the protection of epithelial surfaces. The protective role of MUC1 can be important in oral mucosal defense because epithelial surfaces are constantly attacked by a variety of both pathogenic and commensal microbes (Li et al., 2003). In fact, up-regulation of MUC1 expression in oral epithelial cells has been reported to result from *Porphyromonas gingivalis* infection and increases of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. These results suggested the possibility that up-regulation of MUC1 could be a component of the host response to bacterial infection in the oral cavity. In addition, there is a possibility that MUC1 expression in the oral mucosal epithelial cells is affected by changes of pro-inflammatory cytokine levels in saliva. (Li et al., 2003; McAuley et al., 2007). In the elderly, there was decreased MUC1 expression in oral mucosal epithelial cells compared with young adults. This decrease of oral mucosal defense provided by MUC1 can be a predisposing factor for oral mucosal diseases in the aged population (Chang et al., 2011). There were increased oral mucosal MUC1 transcripts in BMS patients compared with oral lichen planus patients as well as healthy controls (Kho et al., 2013). Lectin-binding studies on oral lesions such as leukoplakias and carcinomas demonstrate that there is aberrant glycosylation associated with increasing dysplasia in the oral glycocalyx. These lectin-labeled carbohydrate residues may be from MUC1 in the oral epithelium (Saussez et al., 1998; Vedtofte et al., 1984)

(4) Perspectives in MUC1 research

Although much work is still needed to fully understand the relationship between MUC1 and oral mucosal defense, significant progress towards to clarify the relationship has been made in recent years. Better insights of MUC1 expression in oral cavity have arisen from human and also animal models. The molecular framework and biophysical properties of MUC1 might involve in the protection of oral cavity. Altered MUC1 glycosylation and phosphorylation, and changes of saliva rheological properties can affect protection against a variety of pathogens in the mouth. Alternative splicing may regulate MUC1 expression and possibly function. It might be explained with alternative splice variants of MUC1 including a form lacking the mucin-like repeat domain which have been identified in the field of cancer immunology. Further research should consider these concepts to comprehensively understand functions and properties of oral mucosal MUC1. Additionally, clarifying a molecular mechanism of MUC1 expression in oral epithelium can provide the crucial information about various diseases and conditions of oral cavity.

III. MATERIALS AND METHODS

1. Participants

Thirty healthy women (mean age ± SD, 25.9 ± 2.1 years, range: 22 to 32 years), with regular menstrual cycles of 28-32 days, were included. Women were recruited from the Medical, Dental, and Nursing campus of the Seoul National University and participated voluntarily. The average length and regularity of their menstrual cycles over the past 3 months were monitored. Inclusion criteria were; age of greater than 20 and less than 40 years,

no oral mucosal pain and diseases, no history of serious illness, and no history of taking medication known to affect the salivary flow rate for the past 3 months. Exclusion criteria were; smokers, wearing removable dentures or orthodontic appliances, having a treatment history for cancer, having a history of hormonal therapy, taking oral contraceptives, or being incapable of communication. The research protocol was approved by the IRB of the Seoul National University Dental Hospital (#CRI13012) on 8 Aug, 2013 and informed consent was obtained from all participants.

2. Overall procedures according to the menstrual cycle

All participants were evaluated 4 times during the menstrual cycle, (1) 5 to 7 days after the start of the cycle (follicular), (2) 13 to 15 days (ovulatory), (3) 21 to 23 days (mid-luteal), and (4) 26 to 28 days (late-luteal). These phases were determined on the basis of self-reported menstrual cycle day-counts.

On the first examination day, the follicular phase, whole saliva samples were collected and salivary flow rates were measured. Then, an intra-oral examination, including measurements of gingival index (GI) and periodontal probing depth (PPD), was performed. During the 3 subsequent visits, collections of whole saliva samples and measurements of flow rates were done.

3. Determination of periodontal health

The extent of gingival inflammation was evaluated using the GI (Löe, 1967; Newman *et al*, 2002) and measurement of PPD by a single examiner (YHL). To determine the GI, four

gingival areas (facial, mesial, distal, and lingual) adjacent to each tooth were assessed and were given a score from 0 to 3. The scores from the four areas of each tooth were totaled and divided by four to give an average GI score for each tooth. By adding the average GI scores from all teeth, the sum of GI scores for each individual was obtained. The PPD was measured with the Michigan-O probe (Hu-Friedy Mfg. Co., Chicago, IL, USA) in six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) of each tooth. The average PPD of each tooth and the sum of PPDs for each individual were obtained as described for the GI score. The sum of GI scores and sum of PPDs were used as representatives of the overall status of periodontal inflammation in individuals.

4. Collection of whole saliva samples

Saliva samples were collected between 7:30 a.m. and 9:30 a.m., to minimize diurnal variability. The times of awakening reported by the participants varied between 5:30 a.m. and 7:30 a.m., and the mean time-difference between waking up and collection was 1.5 h. All participants refrained from drinking alcohol on the previous day, and were instructed to abstain from eating, drinking, and brushing their teeth before the collection of saliva samples after waking up. Unstimulated whole saliva (UWS) was collected for 10 min using the spitting method. Stimulated whole saliva (SWS) was collected for the next 5 min with habitual chewing of 1 g of gum base after a 2 min pre-stimulation period to remove saliva retained in the ducts. The flow rate of saliva was expressed as mL/min. Two millimeters of SWS samples were transferred to Oragene RNA RE-100 (DNA Genotek Inc., Ottawa, ON, Canada) for RNA isolation. UWS and the remaining portion of SWS were centrifuged at 10,000 xg for 15 min at 4°C to remove cellular debris. Aliquots of clarified supernatant were

stored at -70°C for the analyses.

5. Measurement of MUC1 expression by real-time PCR

MUC1 mRNA expression levels, relative to β-actin and GAPDH levels, were quantified using real-time PCR. Total RNA was extracted from each SWS sample using an RNeasy Micro kit (Qiagen, Hilden, Germany) and RNA concentration was measured at 260 nm using a spectrophotometer (Nanodrop 1000, Thermo Fisher Scientific, Wilmington, DE, USA). The absorbance ratio (A260/A280) of each sample was measured and the RNA samples were stored at -70°C. cDNA was synthesized by reverse transcription using a High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA) in a total reaction volume of 20 μL for 1 μg total RNA. Incubation was at 37°C for 60 min, followed by at 95°C for 5 min. Quantitative PCR reactions were performed with an ABI 7500 system (Applied Biosystems) using TaqMan PCR Master Mix and TaqMan probes (Applied Biosystems) for β-actin (Assay ID Hs99999903_m1), GAPDH (Assay ID Hs99999905_m1), and MUC1 (Assay ID Hs00159357_m1). The 20-μL reaction contained template cDNA (1 μL, 50 ng), TaqMan Gene Expression Master Mix (10 μL), TaqMan Gene Expression Assay (1 μL), and nuclease free water (8 μL). The reaction components were assembled in a strip tube with an optical strip cap. The reaction mixture was incubated at 50°C for 2 min and at 95°C for 10 min prior to the PCR step, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Validation experiments were performed to confirm equivalent PCR efficiencies for the target and housekeeping genes. The crossing point of MUC1 with both β-actin and GAPDH was applied to the formula, $2^{-(\text{MUC1-β-actin(or GAPDH)})}$, to quantify the relative mRNA amount of MUC1 to both housekeeping genes. In this study, the fold ratio was calculated using the follicular phase

as a reference. Finally, fold ratios from both housekeeping genes were averaged and used as the relative MUC1 mRNA level. All experiments were performed in duplicate. Two negative control reactions (one with no template and one with no reverse transcriptase) were also performed.

6. Analysis of salivary 17β-estradiol, progesterone, DHEA, cortisol, chromogranin A (CgA), and blood contamination levels

The levels of 17β-estradiol, progesterone, DHEA, cortisol, and blood contamination were determined from UWS and SWS using immunoassay kits (Salimetrics, State College, PA, USA). CgA levels were also analyzed using an immunoassay kit (Yanaihara institute Inc., Shizuoka, Japan). The levels of transferrin in saliva samples were measured to determine blood contamination levels in saliva (Salimetrics). All assays were performed in duplicate.

Intra-assay and inter-assay coefficients of variations were 8.1% and 8.9%, respectively, for the 17β-estradiol assay (sensitivity, 0.1 pg/mL), 6.2% and 7.6%, respectively, for the progesterone assay (sensitivity, 5.0 pg/mL), 5.6% and 8.2%, respectively, for the DHEA assay (sensitivity, 5.0 pg/mL), 3.0% and 3.0%, respectively, for the cortisol assay (sensitivity, 0.007 μg/dL), 10.5% and 13.3%, respectively, for the CgA assay (sensitivity, 0.14 pmol/mL), and 4.9% and 7.1%, respectively, for the blood contamination assay (sensitivity, 0.08 mg/dL). The cross-reactivity of the cortisol assay with cortisone was 0.13%.

7. Statistics

The Kolmogorov-Smirnov normality test was applied to our data. Because the data were normally distributed, parametric tests were used. The repeated-measures analysis of variance (ANOVA) with Tukey's post-hoc test was used for the comparison of variables between the phases throughout the menstrual cycle. The Pearson correlation analysis was used to determine relationships between the variables. All tests were two-sided and the significance level was set at $P < 0.05$.

IV. RESULTS

1. Oral examination

All participants presented with satisfactory oral hygiene without apparent plaque and calculus formation. The average and sum of GI scores were 0.64 ± 0.58 (range: 0 to 1.79) and 17.7 ± 15.8 (range: 0 to 50), respectively. The average and sum of PPDs were 2.8 ± 0.5 mm (range: 2.0 to 3.8 mm) and 78.5 ± 13.1 mm (range: 56 to 106 mm), respectively. The mean number of teeth was 28.0 ± 1.2 (range: 24 to 32).

2. Salivary flow rate

Changes in salivary flow rates are shown in Table 1. The mean flow rate of UWS was 0.50 ± 0.28 mL/min and that of SWS was 1.55 ± 0.50 mL/min. The flow rates of both UWS and SWS did not show any significant changes throughout the menstrual cycle ($P > 0.05$).

3. Oral mucosal epithelial MUC1 expression

There were no significant differences in MUC1 expression levels throughout the menstrual cycle (P = 0.350) (Table 1). In correlation analyses using data from all four phases together, MUC1 expression showed significant correlations with DHEA in SWS (P = 0.014, r = 0.224) and with cortisol/DHEA in UWS (P = 0.002, r = -0.285) and SWS (P = 0.002, r = -0.285) (Tables 2 and 3).

When each phase was analyzed, the luteal phase was distinctly different from the other phases with respect to the relationship of MUC1 expression with salivary analytes (Appendix Tables 1-8). In the mid-luteal phase, MUC1 expression levels showed significant correlations with cortisol (P = 0.037, r = -0.382), progesterone (P = 0.008, r = -0.475), and cortisol/DHEA (P = 0.006, r = -0.489) in UWS and with progesterone (P = 0.020, r = -0.421) and cortisol/DHEA (P = 0.049, r = -0.362) in SWS. In the late-luteal phase, MUC1 expression levels was significantly correlated with cortisol/DHEA (P = 0.026, r = -0.406) in UWS.

4. Salivary analytes

Changes in 17β-estradiol, progesterone, DHEA, cortisol, CgA, and blood contamination levels in UWS and SWS throughout the menstrual cycle are shown in Table 1. The mean concentrations of all salivary analytes were higher in UWS than in SWS. Salivary concentrations of DHEA, cortisol, CgA, and blood contamination were not significantly different throughout the menstrual cycle (P > 0.05). 17β-Estradiol levels in UWS and SWS were elevated at the ovulatory and mid-luteal phases compared to the other phases with no significant differences (P > 0.05). Progesterone levels in UWS (P = 0.001) and SWS (P <

0.001) showed significant differences across the cycle. Progesterone levels in both UWS and SWS in the mid-luteal phase were significantly higher compared to those in the follicular ($P < 0.01$) and ovulatory phases ($P < 0.05$).

5. Correlations between variables

Tables 2 and 3 show correlation results between all variables when all phases were analyzed together in UWS and SWS, respectively. When data were analyzed in each individual phase, the correlation results from the follicular and late-luteal phases were almost the same as those derived from the analysis of all phases together. The correlation results from the ovulatory and mid-luteal phases were mainly affected by changes in 17β-estradiol and progesterone in these phases (Appendix Tables 1-8).

In analyses using all data from the four phases combined, the mean concentrations of each analyte in UWS had significant positive correlations with those of SWS (r = 0.355 - 0.935). A significant positive correlation was also found between the salivary flow rates of UWS and SWS ($P < 0.001$, r = 0.438). There were significant positive correlations between two female gonadal hormones, 17β-estradiol and progesterone, in UWS ($P < 0.001$, r = 0.381) and SWS ($P < 0.001$, r = 0.321). There were also significant positive correlations between 17β-estradiol and DHEA ($P = 0.041$, r = 0.187), and between cortisol and CgA ($P = 0.012$, r = 0.229) only in UWS. In addition, there were significant positive correlations among progesterone, cortisol, and DHEA in both UWS and SWS (Tables 2 and 3).

When the data were analyzed in the follicular phase with the inclusion of clinical periodontal parameters, there were significant positive correlations among the sum of GI

scores, the sum of PPDs, and the blood contamination levels in SWS, but not in UWS. No significant correlations were found between MUC1 expression levels and either periodontal parameter ($P > 0.05$). The sum of PPDs showed significant positive correlations with cortisol and DHEA in both UWS and SWS ($P < 0.05$), and CgA in SWS ($P < 0.01$) (Table 4).

V. DISCUSSION

The present study is the first report to investigate relationships among salivary stress markers and female gonadal hormones and between oral mucosal MUC1 expression and these components throughout the menstrual cycle.

Among these relationships, the positive correlation between cortisol and DHEA was the most prominent. This relationship occurs because both cortisol and DHEA are produced in the adrenal glands under the stimulation of adrenocorticotropic hormone (ACTH). Although the change in DHEA levels varies according to the type of stress and stress-related history, cortisol/DHEA ratios usually increase under stressful conditions (Jeckel et al., 2010; Lennartsson et al., 2012; Warnock et al., 2010). An enhanced response to stress during the progesterone-dominated luteal phase in women (Kirschbaum et al., 1999) and an increase in progesterone with acute stress in an animal study (Krause et al., 2014) may explain the positive correlations among progesterone, cortisol, and DHEA.

The positive correlation between 17β-estradiol and DHEA could be explained by the fact that estradiol has been proposed to increase DHEA in women, as well as in human adult adrenal cells (Lobo et al., 1982; Winter and Smail, 1983). Inhibition of 3β-hydroxysteroid

dehydrogenase activity in adrenal cells by estradiol has been suggested as a mechanism for DHEA increase (Gell et al., 1998). DHEA could also act as a precursor for enzymatic conversion to estrogen. CgA, which has been suggested to be an indicator of sympatho-adrenal activity, had a significant positive relationship with cortisol, the indicator of the HPA activity. However, previous studies showed that the positive relationship is not consistently observed and that different types of stress and various stress-related factors affect these two molecules differently (Filaire et al., 2009; Fukui et al., 2010; Toda et al., 2005). Therefore, more information is needed before salivary CgA can be used as a stress biomarker.

The amount of blood contamination in saliva is very important for the diagnostic reliability of saliva samples because concentrations of analytes in blood are usually very high compared to those in saliva. Salivary transferrin has been known as a useful biomarker measuring blood contamination levels (Kivlighan et al., 2004; Kivlighan et al., 2005; Schwartz and Granger, 2004). In the present study, the levels of transferrin in SWS, but not UWS, showed significant positive relationships with two indices related to gingival inflammation. Because all subjects had intact oral mucosal integrity, the only blood source in saliva would be from the gingival crevice and the level would be affected by the degree of gingival inflammation. The leakage of serum-originated gingival crevicular fluid could be increased by the chewing used as a mechanical stimulant for SWS collection. Therefore, research data from saliva samples, especially SWS, should be carefully interpreted with respect to possible blood contamination. Although transferrin levels in UWS also showed positive relationships with some salivary analytes, a previous study reported that blood might be not the only source of salivary transferrin (Nashida et al., 2009). Salivary flow rates could also affect transferrin levels. Therefore, examination of gingival inflammations, as well as evaluation of blood contamination, should be considered for accurate evaluation of salivary data.

It was noteworthy that oral mucosal MUC1 expression levels had significant positive correlations with DHEA, an anabolic hormone with protective effects against stress, and had significant negative correlations with cortisol, a catabolic stress hormone, and the cortisol/DHEA ratio. The increased HPA response to stress in the progesterone-dominated luteal phase (Kajantie and Phillips, 2006; Kirschbaum et al., 1999) may explain the significant negative relationship between MUC1 expression and progesterone levels. In contrast, expression of uterine MUC1, which is involved in embryo attachment, is mainly regulated by gonadal hormones (Brayman et al., 2006; Hey et al., 1994; Meseguer et al., 2001). Progesterone combined with estradiol priming regulates MUC1 expression at the receptive endometrium (Meseguer et al., 2001). The progesterone receptor (PR) isoforms, PR-A and PR-B, differentially regulate MUC1 expression in uterine epithelial cells; liganded PR-B stimulates MUC1 expression, whereas liganded PR-A represses MUC1 expression (Brayman et al., 2006). Although there have been reports of the presence of PR in the overlying epithelium of oral pyogenic granulomas during pregnancy (Whitaker and Bouquot, 1994) and in cultured gingival fibroblasts (Kawahara and Shimazu, 2003), there is no information about whether PR is present on healthy oral epithelium and the possible influences of progesterone on oral mucosal membrane.

The results of the present study suggest that stress-related psychoendocrinological interactions may deteriorate oral mucosal defense. In fact, psychological stress may act as a triggering and modifying factor for provoking oral ulcerations (Preeti et al., 2011). It has been reported that oral ulcerations are associated with the onset of menstruation or the luteal phase of the menstrual cycle (Oh et al., 2009; Preeti et al., 2011), i.e. the period of decreased MUC1 expression in the present study. Previous studies also showed that oral mucosal MUC1 expression decreased in aged population (Chang et al., 2011) and increased in patients with

burning mouth syndrome (Kho et al., 2013). Thus, the accumulated information suggests that oral mucosal MUC1 expression is influenced by psychoendocrinological changes, aging, and diseases. The findings in the present study suggest that psychoendocrinological interactions across the menstrual cycle could influence oral mucosal defense and possibly the occurrence and aggravation of oral mucosal diseases.

VI. CONCLUSIONS

The relationships among oral mucosal MUC1 expression and salivary stress and female gonadal hormones throughout the menstrual cycle were investigated in the present study. Young healthy women with normal menstrual cycle participated and were evaluated during the follicular, ovulatory, mid-luteal, and late-luteal phases. The expression level of oral mucosal MUC1 was analyzed by real-time PCR. The supernatants from unstimulated (UWS) and stimulated whole saliva (SWS) were used to measure 17β-estradiol, progesterone, dehydroepiandrosterone (DHEA), cortisol, and chromogranin A (CgA) levels. MUC1 expression showed significant negative correlations with cortisol, progesterone, and cortisol/DHEA ratios in UWS and with progesterone and cortisol/DHEA ratios in SWS during the mid-luteal phase. A significant negative correlation was also observed between MUC1 expression and cortisol/DHEA ratios in UWS during the late-luteal phase. In conclusion, oral mucosal MUC1 expression correlated with changes in salivary stress and gonadal hormone levels throughout the menstrual cycle. Stress-related psychoendocrinological interactions resulted in a decrease in oral MUC1 expression, which

can be associated with the occurrence and aggravation of oral mucosal defense. Further studies will be needed to confirm the possible relationships among the oral MUC1 expression, salivary stress and gonadal hormones across the phases of the menstrual cycle.

REFERENCES

Altemus M, Redwine LS, Leong YM, Frye CA, Porges SW, Carter CS (2001). Responses to laboratory psychosocial stress in postpartum women. *Psychosom Med* 63: 814-821.

American Academy of Pediatrics, Committee on Adolescence, American College of Obstericians and Gynecologists, Committee on Adolescent Health Care (2006). Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. *Pediatrics* 118: 2245-2250.

Attardi BJ, Zeleznik A, Simhan H, Chiao JP, Mattison DR, Caritis SN *et al* (2007). Comparison of progesterone and glucocorticoid receptor binding and stimulation of gene expression by progesterone, 17-alpha hydroxyprogesterone caproate, and related progestins. *Am J Obstet Gynecol* 197: 591-597.

Barbieri RL (2014). The endocrinology of the menstrual cycle. *Methods Mol Biol* 1154: 145-169.

Baulieu E, Schumacher M (2000). Progesterone as a neuroactive neurosteroid, with special reference to the effect of progesterone on myelination. *Steroids* 65: 605-612.

Berg DK, Thompson JG, Peterson AJ, Asher GW (2008). The temporal relationship between oocyte maturation and early fertilisation events in relation to the pre-ovulatory LH peak and preimplantation embryo development in red deer. *Anim Reprod Sci* 105: 332-343.

Blauer KL, Poth M, Rogers WM, Bernton EW (1991). Dehydroepiandrosterone antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. *Endocrinology* 129: 3174-3179.

Brayman M, Thathiah A, Carson DD (2004). MUC1: a multifunctional cell surface component of reproductive tissue epithelia. *Reprod Biol Endocrinol* 2:4.

Brayman MJ, Julian J, Mulac-Jericevic B, Conneely OM, Edwards DP, Carson DD (2006). Progesterone receptor isoforms A and B differentially regulate MUC1 expression in uterine epithelial cells. *Mol Endocrinol* 20: 2278-2291.

Bredemann TM, McMahon LL (2014). 17beta Estradiol increases resilience and improves hippocampal synaptic function in helpless ovariectomized rats. *Psychoneuroendocrinology* 42: 77-88.

Brooke AM, Kalingag LA, Miraki-Moud F, Camacho-Hubner C, Maher KT, Walker DM *et al* (2006). Dehydroepiandrosterone improves psychological well-being in male and female hypopituitary patients on maintenance growth hormone replacement. *J Clin Endocrinol Metab* 91: 3773-3779.

Campbell J, Ehlert U (2012). Acute psychosocial stress: does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology* 37: 1111-1134.

Cardoso EM, Arregger AL, Tumilasci OR, Contreras LN (2009). Diagnostic value of salivary cortisol in Cushing's syndrome. *Clin Endocrinol* 70: 516-521.

Chang DM, Lan JL, Lin HY, Luo SF (2002). Dehydroepiandrosterone treatment of women with mild-to-moderate systemic lupus erythematosus: a multicenter randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 46: 2924-2927.

Chang WI, Chang JY, Kim YY, Lee G, Kho HS (2011). MUC1 expression in the oral mucosal epithelial cells of the elderly. *Arch Oral Biol* 56: 885-890.

Chen F, Knecht K, Birzin E, Fisher J, Wilkinson H, Mojena M *et al* (2005). Direct agonist/antagonist functions of dehydroepiandrosterone. *Endocrinology* 146: 4568-4576.

Childs E, Van Dam NT, de Wit H (2010). Effects of acute progesterone administration upon responses to acute psychosocial stress in men. *Exp Clin Psychopharmacol* 18: 78-86.

Clow A, Thorn L, Evans P, Hucklebridge F (2004). The awakening cortisol response: methodological issues and significance. *Stress* 7: 29-37.

Corona G, Rastrelli G, Giagulli VA, Sila A, Sforza A, Forti G *et al* (2013). Dehydroepiandrosterone supplementation in elderly men: a meta-analysis study of placebo-controlled trials. *J Clin Endocrinol Metab* 98: 3615-3626.

Curry WJ, Barkatullah SC, Johansson AN, Quinn JG, Norlen P, Connolly CK *et al* (2002). WE-14, a chromogranin a-derived neuropeptide. *Ann N Y Acad Sci* 971: 311-316.

Daniel AR, Gaviglio AL, Czaplicki LM, Hillard CJ, Housa D, Lange CA (2010). The progesterone receptor hinge region regulates the kinetics of transcriptional responses through acetylation, phosphorylation, and nuclear retention. *Mol Endocrinol* 24: 2126-2138.

Den R, Toda M, Nagasawa S, Kitamura K, Morimoto K (2007). Circadian rhythm of human salivary chromogranin A. *Biomed Res* 28: 57-60.

Dickerson SS, Kemeny ME (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull* 130: 355-391.

Felmingham KL, Fong WC, Bryant RA (2012). The impact of progesterone on memory consolidation of threatening images in women. *Psychoneuroendocrinology* 37: 1896-1900.

Ferrari E, Cravello L, Muzzoni B, Casarotti D, Paltro M, Solerte SB *et al* (2001). Age-related changes of the hypothalamic-pituitary-adrenal axis: pathophysiological correlates. *Eur J Endocrinol* 144: 319-329.

Filaire E, Dreux B, Massart A, Nourrit B, Rama LM, Teixeira A (2009). Salivary alpha-amylase, cortisol and chromogranin A responses to a lecture: impact of sex. *Eur J Appl Physiol* 106: 71-77.

Fukui M, Hinode D, Yokoyama M, Yoshioka M, Kataoka K, Ito HO (2010). Levels of salivary stress markers in patients with anxiety about halitosis. *Arch Oral Biol* 55: 842-847.

Ganong WF (2005). *Review of Medical Physiology,* 22nd edn. McGraw Hill Education: New York (NY), pp. 362.

Gao W, Bohl CE, Dalton JT (2005). Chemistry and structural biology of androgen receptor. *Chem Rev* 105: 3352-3370.

Gell JS, Oh J, Rainey WE, Carr BR (1998). Effect of estradiol on DHEAS production in the human adrenocortical cell line, H295R. *J Soc Gynecol Investig* 5: 144-148.

Gendler SJ (2001). MUC1, the renaissance molecule. *J Mammary Gland Biol Neoplasia* 6: 339-353.

Goldstein JM, Jerram M, Poldrack R, Ahern T, Kennedy DN, Seidman LJ *et al* (2005). Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging. *J Neurosci* 25: 9309-9316.

Goodyer IM, Herbert J, Tamplin A (2003). Psychoendocrine antecedents of persistent first-episode major depression in adolescents: a community-based longitudinal enquiry. *Psychol Med* 33: 601-610.

Gooren LJ, van der Veen EA, van Kessel H, Harmsen-Louman W (1984). Estrogens in the feedback regulation of gonadotropin secretion in men: effects of administration of estrogen to

agonadal subjects and the antiestrogen tamoxifen and the aromatase inhibitor delta'-testolactone to eugonadal subjects. *Andrologia* 16: 568-577.

Hansen AM, Garde AH, Persson R (2008). Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: a review. *Scand J Clin Lab Invest* 68: 448-458.

Hattrup CL, Gendler SJ (2008). Structure and function of the cell surface (tethered) mucins. *Annu Rev Physiol* 70: 431-457.

Hellhammer DH, Wust S, Kudielka BM (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 34: 163-171.

Helman LJ, Ahn TG, Levine MA, Allison A, Cohen PS, Cooper MJ *et al* (1988). Molecular cloning and primary structure of human chromogranin A (secretory protein I) cDNA. *J Biol Chem* 263: 11559-11563.

Hey NA, Graham RA, Seif MW, Aplin JD (1994). The polymorphic epithelial mucin MUC1 in human endometrium is regulated with maximal expression in the implantation phase. *J Clin Endocrinol Metab* 78: 337-342.

Hofman LF (2001). Human saliva as a diagnostic specimen. *J Nutr* 131: 1621S-1625S.

Hollingsworth MA, Swanson BJ (2004). Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 4: 45-60.

Hucklebridge F, Clow A, Evans P (1998). The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. *Int J Psychophysiol* 31: 69-76.

Izawa S, Sugaya N, Shirotsuki K, Yamada KC, Ogawa N, Ouchi Y *et al.* (2008). Salivary dehydroepiandrosterone secretion in response to acute psychosocial stress and its correlations with biological and psychological changes. *Biol Psychol* 79: 294-298.

Jeckel CM, Lopes RP, Berleze MC, Luz C, Feix L, Argimon, II *et al* (2010). Neuroendocrine and immunological correlates of chronic stress in 'strictly healthy' populations. *Neuroimmunomodulation* 17: 9-18.

Kajantie E, Phillips DI (2006). The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology* 31: 151-178.

Kalimi M, Shafagoj Y, Loria R, Padgett D, Regelson W (1994). Anti-glucocorticoid effects of dehydroepiandrosterone (DHEA). *Mol Cell Biochem* 131: 99-104.

Kanaley JA, Weltman JY, Pieper KS, Weltman A, Hartman ML (2001). Cortisol and growth hormone responses to exercise at different times of day. *J Clin Endocrinol Metab* 86: 2881-2889.

Kanno T, Asada N, Yanase H, Iwanaga T, Ozaki T, Nishikawa Y *et al* (1999). Salivary secretion of highly concentrated chromogranin a in response to noradrenaline and acetylcholine in isolated and perfused rat submandibular glands. *Exp Physiol* 84: 1073-1083.

Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H *et al* (1990). Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J* 9: 1603-1614.

Kawahara K, Shimazu A (2003). Expression and intracellular localization of progesterone receptors in cultured human gingival fibroblasts. *J Periodontal Res* 38: 242-246.

Kho HS, Chang JY, Kim YY, Kim Y (2013). MUC1 and Toll-like receptor-2 expression in burning mouth syndrome and oral lichen planus. *Arch Oral Biol* 58: 837-842.

Kibaly C, Meyer L, Patte-Mensah C, Mensah-Nyagan AG (2008). Biochemical and functional evidence for the control of pain mechanisms by dehydroepiandrosterone endogenously synthesized in the spinal cord. *FASEB J* 22: 93-104.

Kirschbaum C, Wust S, Hellhammer D (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosom Med* 54: 648-657.

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med* 61: 154-162.

Kivlighan KT, Granger DA, Schwartz EB, Nelson V, Curran M, Shirtcliff EA (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Horm Behav* 46: 39-46.

Kivlighan KT, Granger DA, Schwartz EB (2005). Blood contamination and the measurement of salivary progesterone and estradiol. *Horm Behav* 47: 367-370.

Krause JS, Dorsa D, Wingfield JC (2014). Changes in plasma concentrations of progesterone, dehydroepiandrosterone and corticosterone in response to acute stress of capture, handling and restraint in two subspecies of white-crowned sparrows. *Comp Biochem Physiol A Mol Integr Physiol* 177: 35-40.

Kudielka BM, Kirschbaum C (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology* 28: 35-47.

Kudielka BM, Hellhammer DH, Wust S (2009). Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology* 34: 2-18.

Kumari V, Konstantinou J, Papadopoulos A, Aasen I, Poon L, Halari R *et al* (2010). Evidence for a role of progesterone in menstrual cycle-related variability in prepulse inhibition in healthy young women. *Neuropsychopharmacology* 35: 929-937.

Kunz-Ebrecht SR, Kirschbaum C, Marmot M, Steptoe A (2004). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology* 29: 516-528.

Lee T, Shimizu T, Iijima M, Obinata K, Yamashiro Y, Nagasawa S (2006). Evaluation of psychosomatic stress in children by measuring salivary chromogranin A. *Acta Paediatr* 95: 935-939.

Lennartsson AK, Kushnir MM, Bergquist J, Jonsdottir IH (2012). DHEA and DHEA-S response to acute psychosocial stress in healthy men and women. *Biol Psychol* 90: 143-149.

Li X, Wang L, Nunes DP, Troxler RF, Offner GD (2003). Pro-inflammatory cytokines up-regulate MUC1 gene expression in oral epithelial cells. *J Dent Res* 82: 883-887.

Little JW. Falace DA, Miller CS, Rhodus NL (2013). *Dental management of the medically compromised patient,* 8th edn. Elsevier: St. Louis (MO), pp. 534-536.

Lobo RA, Goebelsmann U, Brenner PF, Mishell DR Jr (1982). The effects of estrogen on adrenal androgens in oophorectomized women. *Am J Obstet Gynecol* 142: 471-478.

Löe H (1967). The gingival index, the plaque index and the retention index systems. *J Periodontol* 38: 610-616.

Marti-Alamo S, Mancheno-Franch A, Marzal-Gamarra C, Carlos-Fabuel L (2012). Saliva as a

diagnostic fluid. Literature review. *J Clin Exp Dent* 4: 237-243.

Matsumoto T, Asakura H, Hayashi T (2014). Effects of olfactory stimulation from the fragrance of the Japanese citrus fruit yuzu on mood states and salivary chromogranin A as an endocrinologic stress marker. *J Altern Complement Med* 20: 500-506.

Maurice T, Urani A, Phan VL, Romieu P (2001). The interaction between neuroactive steroids and the sigma 1 receptor function: behavioral consequences and therapeutic opportunities. *Brain Res Brain Res Rev* 37: 116-132.

McAuley JL, Linden SK, Png CW, King RM, Pennington HL, Gendler SJ *et al* (2007). MUC1 cell surface mucin is a critical element of the mucosal barrier to infection. *J Clin Invest* 117: 2313-2324.

McEwen BS (2000). Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology* 22: 108-124.

Merz CJ, Tabbert K, Schweckendiek J, Klucken T, Vaitl D, Stark R *et al* (2012). Neuronal correlates of extinction learning are modulated by sex hormones. *Soc Cogn Affect Neurosci* 7: 819-830.

Meseguer M, Aplin JD, Caballero-Campo P, O'Connor JE, Martin JC, Remohi J *et al* (2001). Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. *Biol Reprod* 64: 590-601.

Meyer C, Schmid R, Schmieding K, Falkenstein E, Wehling M (1998). Characterization of high affinity progesterone-binding membrane proteins by anti-peptide antiserum. *Steroids* 63: 111-116.

Miller E (1995). Long-term monitoring of the diabetic dog and cat. Clinical signs, serial blood glucose determinations, urine glucose, and glycated blood proteins. *Vet Clin North Am Small Anim Pract* 25: 571-584.

Mo Q, Lu SF, Simon NG (2006). Dehydroepiandrosterone and its metabolites: differential effects on androgen receptor trafficking and transcriptional activity. *J Steroid Biochem Mol Biol* 99: 50-58.

Moncada DM, Kammanadiminti SJ, Chadee K (2003). Mucin and Toll-like receptors in host defense against intestinal parasites. *Trends Parasitol* 19: 305-311.

Morgan CA 3rd, Southwick S, Hazlett G, Rasmusson A, Hoyt G, Zimolo Z *et al* (2004). Relationships among plasma dehydroepiandrosterone sulfate and cortisol levels, symptoms of dissociation, and objective performance in humans exposed to acute stress. *Arch Gen Psychiatry* 61: 819-825.

Nagar R, Msalati AA (2013). Changes in serum PSA during normal menstrual cycle. *Indian J Clin Biochem* 28: 84-89.

Nakane H, Asami O, Yamada Y, Ohira H (2002). Effect of negative air ions on computer operation, anxiety and salivary chromogranin A-like immunoreactivity. *Int J Psychophysiol* 46: 85-89.

Nashida T, Yoshie S, Imai A, Shimomura H (2009). Transferrin secretory pathways in rat parotid acinar cells. *Arch Biochem Biophys* 487: 131-138.

Negrao AB, Deuster PA, Gold PW, Singh A, Chrousos GP (2000). Individual reactivity and physiology of the stress response. *Biomed Pharmacother* 54: 122-128.

Nepomnaschy PA, Sheiner E, Mastorakos G, Arck PC (2007). Stress, immune function, and women's reproduction. *Ann N Y Acad Sci* 1113:350-364.

Nepomnaschy PA, Altman RM, Watterson R, Co C, McConnell DS, England BG (2011). Is cortisol excretion independent of menstrual cycle day? A longitudinal evaluation of first morning urinary specimens. *PloS one* 6: e18242.

Newman MG, Takei HH, Carranza FA (2002). *Clinical periodontology*, 9th edn. WB Saunders Company: Philadelphia (PA), pp. 80.

Nieschlag E, Loriaux DL, Ruder HJ, Zucker IR, Kirschner MA, Lipsett MB (1973). The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man. *J Endocrinol* 57: 123-134.

Oberbeck R, Benschop RJ, Jacobs R, Hosch W, Jetschmann JU, Schurmeyer TH *et al* (1998). Endocrine mechanisms of stress-induced DHEA-secretion. *J Endocrinol Invest* 21: 148-153.

Ockenfels MC, Porter L, Smyth J, Kirschbaum C, Hellhammer DH, Stone AA (1995). Effect of chronic stress associated with unemployment on salivary cortisol: overall cortisol levels, diurnal rhythm, and acute stress reactivity. *Psychosom Med* 57: 460-467.

Oh SH, Han EC, Lee JH, Bang D (2009). Comparison of the clinical features of recurrent

aphthous stomatitis and Behcet's disease. *Clin Exp Dermatol* 34: e208-212.

Padgett DA, Glaser R (2003). How stress influences the immune response. *Trends Immunol* 24: 444-448.

Paul SM, Purdy RH (1992). Neuroactive steroids. *FASEB J* 6: 2311-2322.

Pinto A, Malacrida B, Oieni J, Serafini MM, Davin A, Galbiati V *et al* (2015). DHEA modulates the effect of cortisol on RACK1 expression via interference with the splicing of the glucocorticoid receptor. *Br J Pharmacol* 172: 2918-2927.

Preeti L, Magesh K, Rajkumar K, Karthik R (2011). Recurrent aphthous stomatitis. *J Oral Maxillofac Pathol* 15: 252-256.

Rabkin JG, McElhiney MC, Rabkin R, McGrath PJ, Ferrando SJ (2006). Placebo-controlled trial of dehydroepiandrosterone (DHEA) for treatment of nonmajor depression in patients with HIV/AIDS. *Am J Psychiatry* 163: 59-66.

Roca CA, Schmidt PJ, Deuster PA, Danaceau MA, Altemus M, Putnam K *et al* (2005). Sex-related differences in stimulated hypothalamic-pituitary-adrenal axis during induced gonadal suppression. *J Clin Endocrinol Metab* 90: 4224-4231.

Rosenfeld RS, Hellman L, Roffwarg H, Weitzman ED, Fukushima DK, Gallagher TF (1971). Dehydroisoandrosterone is secreted episodically and synchronously with cortisol by normal man. *J Clin Endocrinol Metab* 33: 87-92.

Rupprecht R, Reul JM, van Steensel B, Spengler D, Söder M, Berning B *et al* (1993). Pharmacological and functional characterization of human mineralocorticoid and glucocorticoid receptor ligands. *Eur J Pharmacol* 247: 145-154.

Ryan KJ (1982). Biochemistry of aromatase: significance to female reproductive physiology. *Cancer Res* 42: 3342s-3344s.

Saldanha CJ, Remage-Healey L, Schlinger BA (2011). Synaptocrine signaling: steroid synthesis and action at the synapse. *Endocr Rev* 32: 532-549.

Saussez S, Marchant H, Nagy N, Decaestecker C, Hassid S, Jortay A *et al* (1998). Quantitative glycohistochemistry defines new prognostic markers for cancers of the oral cavity. *Cancer* 82: 252-260.

Sawczuk B, Golebiewska M, Mazurek A, Chyczewski L (2014). Evaluation of estrogen receptor-beta expression in the epithelium of the oral mucosa in menopausal women under hormone replacement therapy. *Adv Med Sci* 59: 85-89.

Schwartz EB, Granger DA (2004). Transferrin enzyme immunoassay for quantitative monitoring of blood contamination in saliva. *Clin Chem* 50: 654-656.

Shansky RM, Glavis-Bloom C, Lerman D, McRae P, Benson C, Miller K *et al* (2004). Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. *Mol Psychiatry* 9: 531-538.

Shirotsuki K, Izawa S, Sugaya N, Yamada KC, Ogawa N, Ouchi Y *et al* (2009). Salivary cortisol and DHEA reactivity to psychosocial stress in socially anxious males. *Int J Psychophysiol* 72: 198-203.

Shirtcliff E, Zahn-Waxler C, Klimes-Dougan B, Slattery M (2007). Salivary dehydroepiandrosterone responsiveness to social challenge in adolescents with internalizing problems. *J Child Psychol Psychiatry* 48: 580-591.

Shors TJ, Leuner B (2003). Estrogen-mediated effects on depression and memory formation in females. *J Affect Disord* 74: 85-96.

Singh PK, Hollingsworth MA (2006). Cell surface-associated mucins in signal transduction. *Trends Cell Biol* 16: 467-476.

Smith CC, Vedder LC, Nelson AR, Bredemann TM, McMahon LL (2010). Duration of estrogen deprivation, not chronological age, prevents estrogen's ability to enhance hippocampal synaptic physiology. *Proc Natl Acad Sci U S A* 107: 19543-19548.

Somer E, Ben-Aryeh H, Laufer D (1993). Salivary composition, gender and psychosocial stress. *Int J Psychosom* 40: 17-21.

Steptoe A, Cropley M, Griffith J, Kirschbaum C (2000). Job strain and anger expression predict early morning elevations in salivary cortisol. *Psychosom Med* 62: 286-292.

Streckfus CF, Bigler LR (2002). Saliva as a diagnostic fluid. *Oral Dis* 8: 69-76.

Thomas P, Pang Y (2012). Membrane progesterone receptors: evidence for neuroprotective, neurosteroid signaling and neuroendocrine functions in neuronal cells. *Neuroendocrinology* 96: 162-171.

Toda M, Den R, Nagasawa S, Kitamura K, Morimoto K (2005). Relationship between lifestyle scores and salivary stress markers cortisol and chromogranin A. *Arch Environ Occup Health* 60: 266-269.

Tsigos C, Chrousos GP (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* 53: 865-871.

van Niekerk JK, Huppert FA, Herbert J (2001). Salivary cortisol and DHEA: association with measures of cognition and well-being in normal older men, and effects of three months of DHEA supplementation. *Psychoneuroendocrinology* 26: 591-612.

Vedtofte P, Dabelsteen E, Hakomori S, Young WW (1984). Regional variations of cell surface carbohydrates in human oral stratified epithelium. *Differentiation* 25: 221-228.

Villareal DT, Holloszy JO (2006). DHEA enhances effects of weight training on muscle mass and strength in elderly women and men. *Am J Physiol Endocrinol Metab* 291: E1003-1008.

Warnock F, McElwee K, Seo RJ, McIsaac S, Seim D, Ramirez-Aponte T *et al* (2010). Measuring cortisol and DHEA in fingernails: a pilot study. *Neuropsychiatr Dis Treat* 6: 1-7.

Webb SJ, Geoghegan TE, Prough RA, Michael Miller KK (2006). The biological actions of dehydroepiandrosterone involves multiple receptors. *Drug Metab Rev* 38: 89-116.

Weiss EL, Longhurst JG, Mazure CM (1999). Childhood sexual abuse as a risk factor for depression in women: psychosocial and neurobiological correlates. *Am J Psychiatry* 156: 816-828.

Whitaker SB, Bouquot JE (1994). Estrogen and progesterone receptor status of central giant cell lesions of the jaws. *Oral Surg Oral Med Oral Pathol* 77: 641-644.

Winkler H, Fischer-Colbrie R (1992). The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience* 49: 497-528.

Winter JS, Smail PJ (1983). Effects of ACTH and estradiol on steroid production by cultured adrenal cells from an anencephalic fetus and from normal adults. *Steroids* 42: 677-685.

Wolf OT, Kirschbaum C (1999). Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. *Brain Res Brain Res Rev* 30: 264-288.

Wolfram M, Bellingrath S, Kudielka BM (2011). The cortisol awakening response (CAR) across the female menstrual cycle. *Psychoneuroendocrinology* 36: 905-912.

Yamakoshi T, Park SB, Jang WC, Kim K, Yamakoshi Y, Hirose H (2009). Relationship between salivary Chromogranin-A and stress induced by simulated monotonous driving. *Med Biol Eng Comput* 47: 449-456.

Yamamoto K, Okazaki A, Sakamoto Y, Funatsu M (2009). The relationship between premenstrual symptoms, menstrual pain, irregular menstrual cycles, and psychosocial stress among Japanese college students. *J Physiol Anthropol* 28: 129-136.

Young AH, Gallagher P, Porter RJ (2002). Elevation of the cortisol-dehydroepiandrosterone ratio in drug-free depressed patients. *Am J Psychiatry* 159: 1237-1239.

Young E, Korszun A (2010). Sex, trauma, stress hormones and depression. *Mol Psychiatry* 15: 23-28.

Table 1. The means and standard deviations of variables throughout the menstrual cycle.

		Follicular	Ovulatory	Mid-Luteal	Late-Luteal	*P*-value	Total
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD
MUC1 (Fold ratio)		1.000 ± 0.699	1.351 ± 1.081	1.094 ± 0.608	1.093 ± 0.669	.350	1.134 ± 0.788
Flow rate (mL/min)	U	0.46 ± 0.27	0.50 ± 0.27	0.50 ± 0.28	0.54 ± 0.31	.753	0.50 ± 0.28
	S	1.54 ± 0.48	1.39 ± 0.44	1.64 ± 0.52	1.64 ± 0.53	.149	1.55 ± 0.50
Cortisol (μg/dL)	U	0.479 ± 0.233	0.513 ± 0.286	0.500 ± 0.302	0.536 ± 0.303	.887	0.507 ± 0.279
	S	0.384 ± 0.204	0.426 ± 0.256	0.433 ± 0.267	0.440 ± 0.242	.809	0.420 ± 0.241
DHEA (pg/mL)	U	266.8 ± 129.7	286.3 ± 127.0	291.4 ± 166.8	291.6 ± 168.4	.905	284.0 ± 147.7
	S	162.8 ± 74.2	185.3 ± 85.6	169.3 ± 103.0	175.9 ± 94.0	.793	173.3 ± 89.1
C/D ratio	U	18.9 ± 7.3	17.8 ± 6.3	18.8 ± 8.6	20.5 ± 11.4	.682	19.0 ± 8.5
	S	24.5 ± 12.6	23.4 ± 9.5	30.3 ± 19.3	27.6 ± 15.0	.250	26.5 ± 14.6
17β-Estradiol (pg/mL)	U	2.107 ± 0.704	2.347 ± 0.847	2.369 ± 0.692	2.141 ± 0.696	.385	2.241 ± 0.738
	S	1.639 ± 0.584	1.886 ± 0.748	1.937 ± 0.941	1.722 ± 0.495	.333	1.796 ± 0.714
Progesterone (pg/mL)	U	149.3 ± 93.5[a]	182.3 ± 118.2[b]	292.6 ± 178.7[a,b]	224.6 ± 134.2	.001[**]	212.2 ± 143.5
	S	77.5 ± 49.4[a]	105.0 ± 78.1[b]	173.1 ± 118.1[a,b]	136.5 ± 93.2	< .001[***]	123.0 ± 94.2
CgA (pmol/mL)	U	91.5 ± 48.2	90.2 ± 47.0	98.5 ± 56.3	81.8 ± 45.7	.634	90.5 ± 49.2
	S	39.2 ± 19.0	44.5 ± 30.7	40.5 ± 23.1	40.1 ± 17.2	.819	41.1 ± 22.9
Blood contamination (mg/dL)	U	1.164 ± 0.959	1.389 ± 0.932	1.097 ± 0.792	1.168 ± 0.879	.611	1.204 ± 0.888
	S	0.490 ± 0.382	0.578 ± 0.403	0.578 ± 0.449	0.518 ± 0.410	.791	0.541 ± 0.408

SD, standard deviation; MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A; U, unstimulated whole saliva; S, stimulated whole saliva

[a] The mean of the mid-luteal phase is higher than that of the follicular phase ($P < 0.01$, by the Tukey's post -hoc test)

[b] The mean of the mid-luteal phase is higher than that of the ovulatory phase ($P < 0.05$, by the Tukey's post -hoc test).

[**] $P < 0.01$; [***] $P < 0.001$ by the repeated-measures ANOVA

Table 2. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.119							
Cortisol	-.154	-.050						
DHEA	.094	-.230*	.637***					
C/D ratio	-.285**	.212*	.491***	-.263**				
17β-Estradiol	.138	.040	.079	.187*	-.123			
Progesterone	-.071	-.100	.262**	.207*	.088	.381***		
CgA	-.040	-.236**	.229*	.166	.033	.028	.339***	
Blood contamination	.006	-.315***	.003	.034	-.085	.342***	.240**	.260**

MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Table 3. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.111							
Cortisol	-.093	-.118						
DHEA	.224*	-.146	.597***					
C/D ratio	-.285**	.039	.508***	-.297**				
17β-Estradiol	.114	-.009	.099	.126	.075			
Progesterone	-.047	.052	.213*	.207*	.114	.321***		
CgA	-.058	-.269**	.107	.197*	-.084	.332***	.146	
Blood contamination	-.067	-.366***	.187*	.193*	.002	.171	.191*	.370***

MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Table 4. Correlation coefficients (r) between the periodontal parameters and other variables in the follicular phase.

r		Sum of GI scores	Sum of PPDs
MUC1		-.311	-.292
Flow rate	U	-.246	-.212
	S	-.341	-.043
Cortisol	U	.267	.408*
	S	.283	.460*
DHEA	U	.358	.391*
	S	.291	.396*
C/D ratio	U	-.109	.089
	S	-.018	.202
17β-Estradiol	U	-.063	.143
	S	-.224	.129
Progesterone	U	.081	.267
	S	-.061	.324
CgA	U	.069	.114
	S	.230	.543**
Blood contamination	U	.109	-.193
	S	.462*	.451*
Sum of PPDs		.441*	

MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A; GI, Gingival index; PPD, Periodontal probing depth; U, unstimulated whole saliva; S, stimulated whole saliva

* $P < 0.05$; ** $P < 0.01$ by the Pearson correlation analysis

Appendix Table 1. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva in the follicular phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.017							
Cortisol	-.136	-.039						
DHEA	-.121	-.103	.669***					
C/D ratio	.060	.189	.476**	-.273				
17β-Estradiol	-.025	-.020	.148	.294	-.228			
Progesterone	-.051	-.234	.529**	.462*	-.001	.671***		
CgA	.181	-.354	.324	.090	.126	.075	.561**	
Blood contamination	.024	-.455*	.019	.010	-.133	.382*	.509**	.396*

MUC1, level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 2. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva in the follicular phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.202							
Cortisol	-.135	-.224						
DHEA	.150	-.214	.546**					
C/D ratio	-.168	-.006	.676***	-.176				
17β-Estradiol	.038	.013	.275	.264	.172			
Progesterone	-.040	-.142	.509**	.564**	.108	.726***		
CgA	.060	-.364*	.349	.416*	.048	.325	.433*	
Blood contamination	-.097	-.506**	.369*	.446*	-.001	.352	.420*	.496**

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 3. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva in the ovulatory phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.217							
Cortisol	-.042	-.006						
DHEA	.261	-.135	.792***					
C/D ratio	-.328	.241	.569**	.033				
17β-Estradiol	.299	.137	.074	.360	-.250			
Progesterone	.125	.155	.130	.260	-.009	.211		
CgA	-.148	-.259	-.053	-.099	-.015	-.236	.160	
Blood contamination	.073	-.259	.122	.143	.051	.375*	.174	.184

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 4. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva in the ovulatory phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	.014							
Cortisol	.035	-.107						
DHEA	.394*	-.088	.733***					
C/D ratio	-.350	-.019	.534**	-.104				
17β-Estradiol	.108	.211	-.054	.166	-.199			
Progesterone	.064	.005	.228	.267	.087	.125		
CgA	-.202	-.249	-.145	.025	-.237	.268	.024	
Blood contamination	-.257	-.162	.092	.199	-.041	.143	.178	.311

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 5. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva in the mid-luteal phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	.153							
Cortisol	-.382*	-.076						
DHEA	-.028	-.216	.582**					
C/D ratio	-.489**	.139	.458*	-.352				
17β-Estradiol	.139	.301	.097	.009	.063			
Progesterone	-.475**	-.261	.348	.167	.221	.232		
CgA	-.173	-.273	.390*	.336	.089	.120	.400*	
Blood contamination	-.235	-.277	.034	.019	.020	.038	.266	.337

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 6. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva in the mid-luteal phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.039							
Cortisol	-.306	.005						
DHEA	.011	-.067	.506**					
C/D ratio	-.362*	.125	.495**	-.420*				
17β-Estradiol	.232	-.094	.137	.113	.119			
Progesterone	-.421*	.110	.150	.233	.037	.241		
CgA	-.089	-.415*	.059	.125	-.059	.491**	.134	
Blood contamination	-.008	-.369*	-.037	-.013	-.078	.169	.144	.226

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 7. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of unstimulated whole saliva in the late-luteal phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.345							
Cortisol	-.186	-.096						
DHEA	.181	-.418*	.570**					
C/D ratio	-.406*	.257	.509**	-.334				
17β-Estradiol	-.070	-.247	.017	.120	-.094			
Progesterone	.084	-.139	.173	.077	.040	.606***		
CgA	.037	-.056	.267	.242	-.022	.145	.305	
Blood contamination	-.019	-.288	-.164	-.018	-.188	.541**	.300	.149

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

Appendix Table 8. Correlation coefficients (r) among MUC1 expression, flow rate, and analytes of stimulated whole saliva in the late-luteal phase.

r	MUC1	Flow rate	Cortisol	DHEA	C/D ratio	17β-Estradiol	Progesterone	CgA
Flow rate	-.177							
Cortisol	-.113	-.218						
DHEA	.244	-.202	.614***					
C/D ratio	-.349	-.128	.452*	-.337				
17β-Estradiol	-.039	-.124	.050	-.046	.097			
Progesterone	.194	-.068	.129	.039	.045	.467**		
CgA	.154	-.027	.424*	.393*	-.081	.147	.297	
Blood contamination	.135	-.432*	.398*	.238	.127	-.037	.150	.587**

MUC1, the level of MUC1 expression; C/D ratio, Cortisol/DHEA ratio; CgA, Chromogranin A

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by the Pearson correlation analysis

국문초록

여성 생리주기에 따른 구강점막 MUC1 발현 및 타액 호르몬의 변화

이 연 희

서울대학교 대학원 치의과학과 구강내과·진단학 전공

(지도교수 **고 홍 섭**)

시상하부 뇌하수체 부신피질 축은 스트레스 관련 연구에서 가장 중점적으로 다뤄지는 주제이며, 주로 cortisol, dehydroepiandrosterone (DHEA), cortisol/DHEA ratio의 변화를 통해 연구되어 왔다. 여성 성호르몬 역시 스트레스에 반응한 내분비계의 작용과 상호작용하여 분비된다. 따라서 여성이 남성과 다른 스트레스 반응을 보이는 것은 생물학적 성과 생리주기에 따른 스트레스 반응의 차이로 설명될 수 있다. 이러한 스트레스 호르몬과 성호르몬의 변화는 구강점막 방어에 영향을 미치고, 여성의 생리주기는 구강점막 질환의 발생과 악화에 영향을 미친다는 결과가 보고되어 왔다. 이 연구의 목적은 구강점막 상피세포의 MUC1 발현과 타액 내의 스트레스 및 성호르몬의 관계를 여성의 생리주기에 따라 분석·탐구하는 것이다.

연구는 정상적인 생리주기를 가진 30명의 건강한 여성(25.9 ± 2.1세)을 대상으로 하였다. 모든 참여자를 대상으로 연구과정 시작 전에, 구강점막과 치주 건강에 대한 검사를 실시하였다. 참여자들은 한 번의 생리 주기 동안 여포기, 배란기, 황체중기 및 황체후기에 해당하는 날, 총 4회에 걸쳐 연구에 참여하였고, 모든 연구대상으로부터 참여시 마다 비자극성 전타액과 자극성 전타액이 채취되었다. 구강점막 MUC1의 발현은 타액으로부터 추출한 RNA를 활용하여 real-time PCR로 분석하였고, 비자극성 및 자극성 전타액의 상층액을 활용하여 17β-estradiol, progesterone, DHEA, cortisol 및 chromogranin A (CgA)의 농도를 측정하였다. 이와 함

께 타액검체의 혈액오염 정도를 transferrin 농도 측정으로 살펴보았다.

전체 결과를 모두 함께 분석하였을 때, 비자극성 전타액과 자극성 전타액 모두에서 cortisol, progesterone과 DHEA, 세 가지 요소들 간의 양의 상관관계가 관찰되었다. 또, 비자극성 전타액에서 17β-estradiol과 DHEA간, 비자극성 전타액에서 cortisol과 CgA간, MUC1 발현과 자극성 전타액에서의 DHEA간에 유의한 양의 상관관계가 있었다. 이와 함께, MUC1 발현과 cortisol/DHEA ratio사이에 유의한 음의 상관관계가 비자극성 전타액과 자극성 전타액 모두에서 관찰되었다. 각 시기로 나누어 연구결과를 분석해 보았을 때, 황체중기에서 MUC1 발현은 비자극성 전타액의 cortisol, progesterone, 그리고 cortisol/DHEA ratio 각각의 요소와 유의한 음의 상관관계를 보였고, 자극성 전타액의 progesterone과 cortisol/DHEA ratio와는 유의한 음의 상관관계를 보였다. 황체후기에서는 MUC1의 발현과 비자극성 전타액의 cortisol/DHEA ratio간의 유의한 음의 상관관계가 관찰되었다.

결론적으로 본 연구의 결과는 구강점막 MUC1 발현이 생리 주기에 따른 타액 스트레스 호르몬과 성호르몬의 변화에 영향을 받는다는 것을 제시한다. 즉, 스트레스와 관련된 심리-내분비학적인 상호작용은 구강점막 MUC1 발현, 나아가 구강점막 방어기전에 영향을 미칠 수 있음을 알 수 있다.

주요어 : MUC1, 스트레스, 스테로이드 호르몬, 생리주기

학 번 : 2012-31183