

The Influence of Sex Steroid Hormones on Gingiva of Women

Eleni Markou^{1,*}, Boura Eleana², Tsalikis Lazaros³ and Konstantinides Antonios³

¹Department of Periodontology and Implant Biology, School of Dentistry, Aristotle University of Thessaloniki; ²Agathoupoleos 19, 54636, Thessaloniki, Greece; ³Department of Preventive Dentistry, Periodontology and Implant Biology, School of Dentistry, Aristotle University of Thessaloniki, Greece

Abstract: Steroid sex hormones have a significant effect on different organ systems. As far as gingiva are concerned, they can influence the cellular proliferation, differentiation and growth of keratinocytes and fibroblasts. Estrogen is mainly responsible for alterations in blood vessels and progesterone stimulates the production of inflammatory mediators. In addition, some micro-organisms found in the human mouth synthesize enzymes needed for steroid synthesis and catabolism. In women, during puberty, ovulation and pregnancy, there is an increase in the production of sex steroid hormones which results in increased gingival inflammation, characterized by gingival enlargement, increased gingival bleeding and crevicular fluid flow and microbial changes.

Key Words: Sex steroid hormones, gingiva, puberty, oral contraceptives, pregnancy, menopause.

INTRODUCTION

The main sex hormones exerting influence on the periodontium are estrogen and progesterone. Estrogen and progesterone can significantly influence different organ systems [1, 2]. For example, estrogens can influence the cytodifferentiation of stratified squamous epithelium, and the synthesis and maintenance of fibrous collagen [3]. Additionally, estrogen receptors in osteoblast-like cells provide a mechanism for direct action on bone while estrogen receptors in periosteal fibroblasts and periodontal ligament fibroblasts provide a mechanism for direct action on different periodontal tissues [1]. Estrogen, progesterone and chorionic gonadotropin, during pregnancy, affect the microcircularity system by producing the following changes: swelling of endothelial cells and pericytes of the venules, adherence of granulocytes and platelets to vessel walls, formation of microthrombi, disruption of the perivascular mast cells, increased vascular permeability and vascular proliferation [4-7]. Consequently, systemic endocrine imbalances may have an important impact on periodontal pathogenesis, and, *vis versa*, changes in periodontal conditions might be associated with variations in sex hormone levels. This association is evident in the recent periodontal disease classification which includes the following hormone related disease categories: puberty-associated gingivitis, menstrual cycle-associated gingivitis and pregnancy-associated gingivitis [1, 8].

MECHANISMS OF ACTION OF SEX STEROID HORMONES ON GINGIVA OF WOMEN

Sex steroid hormones have been shown to directly and indirectly exert influence on cellular proliferation, differentiation and growth in target tissues, including keratinocytes

and fibroblasts in the gingiva [2, 9]. There are two theories for the actions of the hormones on these cells: a) change of the effectiveness of the epithelial barrier to bacterial insult and b) effect on collagen maintenance and repair.

Estradiol can induce cellular proliferation while depressing protein production in cultures of human pre-menopausal gingival fibroblasts. This cellular proliferation appears to be the result of a specific population of cells within the parent culture that responds to physiologic concentrations of estradiol [10].

In contrast to the stimulatory effects of estrogen on gingival fibroblast proliferation, both collagen and non-collagen protein production were reduced when physiological concentrations of estradiol were introduced to fibroblasts in culture. The reductions of collagen and non-collagen protein production by fibroblast strains were similar (approximately 30% reduction in comparison to controls); therefore, there was no effect of estrogen on the relative amount of collagen synthesized by gingival fibroblasts. Similar effects of estrogen on protein synthesis have also been reported in other tissues. In human periodontal ligament cells, estrogen triggered an *in vitro* reduction in fibroblast collagen synthesis [11]. Furthermore, fibroblasts derived from human anterior cruciate ligament have also exhibited a reduction of collagen synthesis by more than 40% of controls at physiological concentrations of estrogen [12]. More specifically, estrogen induced a dose dependent decrease in the production of pro-collagen I from anterior cruciate ligament fibroblasts [13] of young adult women.

Sex steroid hormones have also been shown to increase the rate of folate metabolism in oral mucosa [14]. Since folate is required for tissue maintenance, increased metabolism can deplete folate stores and inhibit tissue repair [9].

Estrogen is the main sex steroid hormone responsible for alterations in blood vessels of target tissues in females,

*Address correspondence to this author at the 16 Kidonion Str, Thessaloniki, Greece; Tel: +30 6947128160; Fax: +30 2310420273; E-mail: markouelena@yahoo.gr

stimulating endometrial blood flow during the estrogen plasma rise seen in the follicular phase. Subsequently, endometrial blood flow decreased during the luteal phase of the cycle with waning estrogen levels [15]. In contrast, progesterone has been shown to have little effect on the vasculature of systemic target tissues [9]. On the other hand, in gingiva and other non-periodontal intraoral tissues, more evidence has accumulated for progesterone affecting the local vasculature than for estrogen. In addition, progesterone has been shown to reduce corpuscular flow rate, allowing for accumulation of inflammatory cells, increased vascular permeability and proliferation [16-19]. Human PDL cells possessed immunoreactivity for (towards) estrogen receptors. More specifically estrogenic effects in PDL cells are mediated *via* estrogen receptors beta (ERbeta), whereas no immunoreactivity was expressed in these cells for progesterone receptors, which implies that progesterone does not have a direct effect on PDL cell function [20].

These hormones may alter immunologic factors and responses, including antigen expression and presentation, and cytokine production, as well as the expression of apoptotic factors and cell death [21]. Several studies have focused on the observation that immune system components have been identified as possessing sex steroid receptors [9]. In mice, the presence of oestrogen receptors on various immune cells has been demonstrated, as well as the presence of androgen receptors on T and B lymphocytes [22]. Progesterone in particular has been shown to stimulate the production of the inflammatory mediator, prostaglandin E2 and to enhance the accumulation of polymorphonuclear leukocytes in the gingival sulcus [23]. Progesterone has also been found to enhance the chemotaxis of polymorphonuclear leukocytes, while low concentrations of estradiol have been demonstrated to reduce polymorphonuclear leukocytes chemotaxis [24]. In addition, sex steroid hormones seem to modulate the production of cytokines [25], and progesterone has been shown to down-regulate Il-6 production by human gingival fibroblasts to 50% of that of control values [26, 27].

According to a radically new insight into the diversity of human oral microflora, the human mouth consists of an estimated number of 19,000 phylotypes, which is considerably higher than previously reported [28]. Although the hypothesis that hormonal changes in the menstrual cycle cause changes in the oral microbiota could not be confirmed [29]. Some micro-organisms, as *Aggregatibacter. actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*, are known to synthesize steroid metabolizing enzymes needed for steroid synthesis and catabolism [30]. The steroid metabolites may also contribute to nutritional requirements of the pathogens, or enable synthesis of matrices associated with host evasion mechanisms [31]. The need for an androgen metabolic pathway in pathogens may be an adaptation to a parasitic presence in the host. Culture supernatants of these micro-organisms have been shown to enhance the expression of 5a-reductase activity in human gingiva and in cultured gingival fibroblasts, resulting in the formation of 5a-dihydrotestosterone (DHT) from androgen substrate [32]. The DHT can influence protein synthetic activity in these pathogens, for which there is a variety of applications. Some of these functions are: a) the formation of surface capsular protein contributing to their evasion of host elimination mechanisms, such as phagocytosis, by preventing opsonisa-

tion b) persistence and dissemination with the host and c) interspecies aggregation and energy generation as a result of the electron transfers involved in these enzyme activities [31]. 5a-reductase activity can be activated in a phospholipid environment [33]. Increased amounts of phospholipases A2 and C are synthesized by periodontal pathogens (*e.g.* spirochaetes) during inflammatory episodes in the periodontium. Phospholipase C is also released from leukocytes during cell lysis and in addition to degrading gingival crevicular epithelium it is also known to stimulate 5a-reductase activity [32].

INFLUENCE ON PERIODONTIUM DURING PUBERTY

Puberty is a complex process of sexual maturation and it is responsible for changes in physical appearance and behavior that are related to increased levels of the steroid sex hormones, testosterone in males and estradiol in females [34].

Puberty gingivitis is characterized clinically by the onset of exuberant inflammation of the marginal and, by direct extension, adjacent attached gingiva, especially in the interdental papillae [35, 36], with increased gingival bleeding during puberty [35]. This gingival enlargement, is found primarily on the facial surfaces, with the lingual surfaces remaining relatively unaltered [9].

Several reports [37-39] have indicated that there is a significant increase in gingivitis in children entering puberty and during the pubertal period. A peak prevalence of gingivitis has been determined at 12 years, 10 months in females and 13 years, 7 months in males, which is consistent with the onset of puberty [40]. This increase is believed to be related, at least in part, to an alteration in the subgingival microflora. [41, 42] including the presence of *Prevotella intermedia*, which can substitute estrogen and progesterone for vitamin K, an essential bacterial growth factor [43, 44]. There also is an increase in the quantity of plaque in general [41] and other species in particular, including spirochetes, *Capnocytophaga sp.*, *Actinomycetes sp.*, and *Eikenella corrodens* [38, 42, 45]. *Capnocytophaga* species have been associated with a tendency towards increased bleeding [45] Tiainen *et al.* [41] showed that the severity of puberty gingivitis was related more closely to plaque build up than to hormones.

The removal of local factors by oral hygiene techniques was the key to management of hormone-related gingivitis, as it was pointed by Oh *et al.* [46]. However, other studies have not confirmed these relationships [47, 48]. In a longitudinal study, Yanover and Ellen [48] were unable to detect any changes in the oral microbiota during puberty and found no correlation between plasma estradiol levels and levels of black pigmented anaerobic bacteria.

INFLUENCE ON PERIODONTIUM DURING THE MENSTRUAL CYCLE

The menstrual cycle is a 25-30-day period, controlled by the secretion of sex hormones, which is responsible for continued ovulation until menopause [1, 32]. It can be divided into two phases: a proliferative and a secretory phase, corresponding to pre- and post-ovulatory events in the ovaries. The proliferative phase is characterized by a gradual increase in production of gonadotropin (FSH) and of estrogens and, to

a lesser degree, progesterone. At ovulation there is a sudden and marked increase in production of gonadotropin and of estrogens [50]. Lindhe and Attström [50] have demonstrated that a small gradual increase of the gingival exudation is observed in all females on the day of ovulation, while the secretory phase is characterized by a gradual decrease in gingival exudation. In a longitudinal study, Hugoson [51] discovered that gingival exudate increased by at least 20% during ovulation in more than 75% of the females examined. Lindhe and Attström [50] noted that during their menstrual cycles, women without clinical gingivitis showed no increase in gingival fluid, whereas those with gingivitis showed increases in gingival fluid. It is generally accepted that increased sex hormones during the menstrual cycle modulate the development of localized gingival inflammation, although this has not been fully experimentally proven [26, 49, 52-54]. More specifically, Holm-Pedersen and Loe in 1967 [55] showed that no correlation existed between the condition of the gingiva and the different phases of the menstrual cycle in clinically healthy gingiva, whereas a significant deterioration of pre-existing gingivitis was observed during the day of menstruation. In contrast, Klein (1934) reported a "gingivitis menstrualis" and Muhlemann (1948) presented a "gingivitis intermenstrualis". Lindhe and Attstrom (1967) studied the variations in gingival fluid flow during the menstrual cycle and found a positive correlation between GCF and FSH and estrogens and a negative correlation between GCF and progesterone. More recently, Machtei *et al.* (2004) [52] in a longitudinal study of 18 premenopausal women reported no statistically significant differences in plaque index, but an increase in gingival index during ovulation and premenstruation. The cellular pathway for this hormonal-tissue interaction has been studied only in the peripheral blood. Brannstrom *et al.* (1999) [53] reported that TNF- α showed significant fluctuation during the whole cycle, with surges just before OV and PM and implicated TNF- α as a mediator through which gingival inflammation is mediated. Gornstein *et al.* (1999) [54] and Lap *et al.* (1995) [26] found that IL-6 production is decreased by progesterone. They concluded that this lends support to the hypothesis that increased sex hormones modulate the development of localized gingival inflammation. Miyagi and coworkers [56] demonstrated that both estradiol (at 20ng/ml) and progesterone (in various concentrations) resulted in an elevated PGE2 production.

INFLUENCE ON PERIODONTIUM DURING PREGNANCY

Pregnancy is accompanied by remarkable endocrine alterations. During this period, both progesterone and estrogen are elevated due to continuous production of these hormones by the corpus luteum. By the end of the third trimester, progesterone and estrogen reach peak plasma levels of 100 and 6ng/ml respectively, which represent 10 and 30 times the levels observed during the menstrual cycle [2].

The levels of sex steroid hormones in saliva also increase during pregnancy [57]. Pregnant women present more inflammation and gingival bleeding than the general population and this effect is related to the dental biofilm, the microbial flora and the hormonal levels [58]. Susceptibility to infections (*e.g.* periodontal infection) increases during early gestation due to alterations in the immune system [59] and can be explained by the hormonal changes observed during

pregnancy [60], suppression of T-cell activity [61], decreased neutrophil chemotaxis and phagocytosis, altered lymphocyte response and depressed antibody production [62] and even chronic maternal stress [63]. Pregnancy gingivitis is extremely common and affects 30-100% of all pregnant women [9].

Pregnancy gingivitis and is clinically characterized by an increase in probing depth and bleeding on probing [64] increased gingival crevicular fluid flow [51] and microbial changes [9]. An *in vitro* study [65] showed that progesterone may control and reduce local production of matrix metalloproteinases, and thereby explain why pregnancy gingivitis is not necessarily characterized by progression to periodontitis. Rateitschak [66] reported a significant change in mobility during and after pregnancy, mainly because of an increase in the initial free intrasocket movement of the roots. Initial mobility is dependent on the degree of vascularisation and the vascular volume of the periodontal membrane. When acting at high concentrations over longer periods of time, female sex hormones may have a hyperemic- and permeability-increasing action on the periodontal vascular system. In respect to the periodontal membrane, slight edema has a tooth extruding effect, with this mechanism leading to increased horizontal mobility [66].

Other investigators [67] showed that experimentally developed gingivitis during pregnancy resulted in only limited microbial changes. Contradictory results have also been reported in that hormonal changes during pregnancy may result in >55 times higher levels of bacteroides species in periodontal samples compared to non-pregnant women [68]. In addition to the gingival changes seen during pregnancy, 0.5-9.6% of pregnant women also experience localized gingival enlargement consistent with pyogenic granulomas. The pregnancy-associated pyogenic granuloma, or pregnancy tumor, is not a neoplasm at all, and clinically and histologically cannot be distinguished from pyogenic granulomas occurring in women who are not pregnant [9]. These lesions have been described as a painless, exophytic mass that has either a sessile or pedunculated base extending from the gingival margin or, in most instances, from the interproximal tissues in the maxillary anterior [69]. The pregnancy tumor develops as a result of an exaggerated inflammatory response to an irritation (often calculus), enlarges rapidly, bleeds easily and may range in color from purplish red to deep blue, although most commonly is red in color with small fibrin spots [3, 9, 70]. It rarely reaches more than 2cm in size and has a tendency to recur if not completely removed after pregnancy [70]. Kornman and Loesche [71] reported that during the second trimester, although plaque levels remained constant, the ratio of subgingival bacterial anaerobes-to-aerobes increased, as well as proportions of *Bacteroides melaninogenicus* and *P.intermedia*. Subgingival plaque samples from these patients also demonstrated a significantly higher accumulation of estradiol and progesterone than plaque samples from the same patients at other time periods. As mentioned before, both estradiol and progesterone were shown to be selectively accumulated by P.i. as a substitute for vitamin K [44] and thus postulated to be acting as a growth factor for this micro-organism [72]. Jensen and coworkers [72] demonstrated a 55-fold increase over the control group in the population of *Bacteroides* species in pregnant women.

INFLUENCE OF ORAL CONTRACEPTIVES ON PERIODONTIUM

Oral contraceptives act to establish hormonal levels of pregnancy and they have similar clinical incidence on tissues. Contrary to the 9-month duration of pregnancy, the effects of oral contraceptives may last much longer [70]. Lindhe and Björn, in a clinical study in 1967 [73], demonstrated that regular use of contraceptive pills for 12 months increases the amount of exudates obtainable from the gingival pockets of the anterior regions. Two years later, Kaufman and Gan [74] showed that a patient who received a weakly progestonic and strongly estrogenic contraceptive (1mg ethynodiol diacetate + 0.1 mg mestranol) presented with hyperplastic gingivitis and a pregnancy-tumor. Jensen and co-workers in 1981 [72] demonstrated a 16-fold increase over the control group in the population of *Bacteroides* species in women taking contraceptives, whereas Klinger and co-workers in 1998 [75], reported that *P.gingivalis* and *A.a.* were not detected and there was a 4.8% increase in *P.intermedia* in women receiving a contraceptive containing 0.02mg ethinyl estradiol and 0.15mg desogestrel after a 20-day use of this contraceptive. Current oral contraceptives consist of low doses of estrogens (50mg/d) and 1.5mg/d of progestins, in contrast to early formulations which contained higher concentrations of sex steroid hormones. As a result of the new combination in oral contraceptives, if low plaque levels are established and maintained during the period of hormonal contraceptive usage, their effects on the periodontium can be minimized [32].

MENOPAUSE

The menopause and the lack of ovarian steroids are known to promote important changes in connective tissue. [76] The mechanisms involved in this influence are not completely understood, but it is thought to be related to the action of estradiol on the connective tissue [77]. The menopause triggers a wide range of changes in women's bodies, and the oral cavity is also affected. Although elevated levels of ovarian hormones, as seen in pregnancy and oral contraceptive usage, can lead to an increase of gingival inflammation with an accompanying increase in gingival exudates, [78] conversely, the menopause – the absence of ovarian sex steroids – has been related to a worsening in gingival health, and hormonal replacement therapy seems to ameliorate this trend [79]. An increase in gingivitis, periodontal disease, tooth loss and dry mouth has been reported [80] and hormone replacement seems to be associated with decreased levels of several indicators of the severity of oral disease as compared with estrogen-insufficient women [81, 82].

During the menopause estrogen deficiency is one of the most frequent causes of osteoporosis in women and a possible cause of bone loss and insufficient skeletal development in men. Estrogen plays an important role in the growth and maturation of bone as well as in the regulation of bone turnover in adult bone. During bone growth estrogen is needed for proper closure of epiphyseal growth plates both in females and in males. Also in the young skeleton estrogen deficiency leads to increased osteoclast formation and enhanced bone resorption. In menopause estrogen deficiency induces cancellous as well as cortical bone loss. Highly increased bone resorption in cancellous bone leads to general

bone loss and destruction of local architecture because of penetrative resorption and microfractures. In cortical bone the first response of estrogen withdrawal is enhanced endocortical resorption. Later, also intracortical porosity increases. These lead to decreased bone mass, disturbed architecture and reduced bone strength [83].

The mechanism by which estrogen deficiency causes bone loss remains largely unknown. Estrogen deficiency leads to an increase in the immune function, which culminates in an increased production of TNF by activated T cells. TNF increases osteoclast formation and bone resorption both directly and by augmenting the sensitivity of maturing osteoclasts to the essential osteoclastogenic factor RANKL. Increased T cell production of TNF is induced by estrogen deficiency *via* a complex mechanism mediated by antigen-presenting cells and involving the cytokines IFN- γ , IL-7, and TGF- β . Experimental evidence suggests that estrogen prevents bone loss by regulating T cell function and immune cell bone interactions.

Remarkable progress has been made in elucidating the cross-talk between the immune system and bone, and in uncovering the mechanism by which sex steroids, infection, and inflammation lead to bone loss by disrupting the regulation of the T lymphocyte function in animal models. If the findings in experimental animals are confirmed in humans, it will, perhaps, be appropriate to classify osteoporosis as an inflammatory, or even an auto-immune condition and certainly new therapeutic “immune” targets will emerge [84].

CONCLUSION

Female sex hormones are neither necessary nor sufficient to produce gingival changes by themselves. However, they may alter periodontal tissue responses to microbial plaque and thus indirectly contribute to periodontal disease.

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