Role of sex hormones in periodontium during pregnancy: A review

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Abstract
There exists a connection between increased plasma levels of pregnancy hormones and a decline in periodontal health status. It has been noted that the influence of sex hormones can be minimized with good plaque control during pregnancy. This review article highlights the effects of female sex hormones on the periodontium during pregnancy.

Keywords: pregnancy gingivitis, pyogenic granuloma, female sex hormones, pregnancy

1. Introduction
The earliest study of the relationship between pregnancy and periodontal disease dates back to 1933 [1]. Since then, many authors have said that hormonal changes together with plaque were responsible for the alterations produced in periodontal tissues during pregnancy [2]. Though the etiology is not fully known, it is believed that during pregnancy, increasing levels of plasma sex steroid hormones have a dramatic effect on the periodontium. Current works of research have shown that the increasing levels of estrogen & progesterone during pregnancy are supposed to be responsible for an exaggerated inflammatory response to plaque & progression of gingivitis.

2. Female sex steroid hormones
Estrogens are responsible for essential reproductive functions such as development, growth, and maintenance of secondary female sex characteristics in females. Estrogens appear naturally in three forms; estrone, estradiol & estriol. Estradiol is the most potent & abundant estrogen in fertile women [3]. Synergistically acting with estrogens, progesterone controls the menstrual cycle by regulating the gonadotropin secretion from the anterior pituitary gland. During pregnancy, its main function is to maintain the endometrial effect and the uterine quiescence. Both estrogen and progesterone are found in very low concentrations (10^-9-10^-12 mol/l) in the blood circulation, and the vast majority is bound to plasma proteins, i.e. albumin and globulins, such as the sex-hormone-binding globulin (SHBG) and corticosteroid-binding globulin (CBG). Only the unbound (free) hormones bind to the specific hormone receptors and then, initiate the hormone actions and effects in the involved tissue as they are biologically active & can enter the target cell by diffusion. Furthermore, unconjugated steroid hormones are able to enter the saliva by diffusing through the acinar and ductal cells of the salivary glands independent of the salivary flow rate. Therefore, the unconjugated salivary estrogens and progesterone concentrations closely reflect their unbound concentrations in plasma [4]. During pregnancy, plasma levels of estradiol may be increased up to 1–30 ng/ml and progesterone upto 10–300 ng/ml [5]. These concentrations are approximately 100–1000 times higher than observed during the luteal phase of the reproductive cycle [6]. When the placenta is disengaged at parturition, these female sex hormone levels remarkably decrease, reaching their non-pregnant concentrations within 2-3 days after delivery.

3. Female sex steroid hormones and periodontium
Localization of estrogen receptor (ER) and progesterone receptor (PgR) has been reported in the human periodontium, demonstrating that the periodontium is one of the target tissues for these hormones [7]. Later, two distinct subtypes of receptors, ERα and ERβ have been found to
mediate the direct effects of estrogens \[8\] Classical estrogen-target tissues, such as the endometrium, ovaries and mammary glands, contain predominantly ER\(\alpha\), whereas ER\(\beta\) is also found in non-reproductive tissues, including periodontal ligament. \[9\] gingival epithelium \[10\] and salivary glands. \[11\] This explains why periodontal tissues are sensitive to changes in circulating steroid hormone levels. Kawahara and Shimazu have reported that human GFs (Gingival Fibroblasts) expressed poor ER-\(\alpha\) signal but chiefly expressed ER-\(\beta\). This was speculated to be the first description of the ER subtype in gingival component cells \[7\]. ER-\(\beta\) immunoreactivity was observed in the nuclei of about 40% of cultured human periodontal ligament cells, while no ER-\(\alpha\) immunoreactivity was detected. This suggested that estrogen influences the functional properties of periodontal ligament cells preferentially through ER-\(\beta\). According to the authors, this was the first report revealing that ER-\(\beta\) is expressed in human PDLCs \[12\]. Recently, it was further suggested that ER-\(\beta\) localizes in mitochondria of human PDLCs and this could demonstrate that estrogen, probably via ER-\(\beta\), influences mitochondrial function and energy metabolism in human PDLCs \[13\]. Jonsson et al. found that no PgR was expressed in human PDLC \[14\]. Kawahara and Shimazu et al. concluded that human Gingival Fibroblasts expressed low Progesterone Receptor expression. In a recent study in China, the authors detected the expression of PgR in human PDLCs by RT-PCR and immunocytochemistry, demonstrating that the PgR was expressed in human PDLCs at the gene and protein levels \[15\]. The staining ways and procedures, menstrual cycle stage, cell supply and age of donors may make a case for the discrepancies between the results. Thus it is evident that the periodontium is a potential target tissue for estrogen and progesterone, although the presence of PgR has not been conclusively demonstrated in these tissues.

Estrogen effects on periodontium
A. Vascular Effects
* Increase cellular proliferation in blood vessels

B. Cellular Effects
* Decrease epithelial keratinization while increasing epithelial glycogen
* Alteration in fibroblast proliferation

C. Immunological Effects
* Decrease PMN chemotaxis & phagocytosis
* Decrease T-cell response
* Decrease antibody response
* Decrease leukocyte production from the bone marrow
* Modulation of proinflammatory cytokine expression

Progesterone Effects on Periodontium
A. Vascular Effects
* Increase vascular dilatation & permeability
* Increases proliferation of newly formed capillaries in gingival tissues.

B. Cellular Effects
* Increase glycogen
* Alteration on collagen synthesis
* Decrease tissue repair
* Increase folate metabolism
* Alteration in fibroblast proliferation

C. Immunological Effects
* Decrease PMN chemotaxis & phagocytosis
* Decrease antibody response
* Increase prostaglandin production by macrophages

4. Pregnancy Related periodontal Changes
During pregnancy there are specific gingival alterations such as pregnancy gingivitis, characterized by hyper-vasculality and non-specific cellular inflammatory infiltration. Another alteration is the pyogenic granuloma which is an exaggerated proliferative fibrovascular inflammatory reaction located in the gingival.

4.1 Pregnancy gingivitis
The development of gingivitis is very frequent, with a reported prevalence of 35% to 100% in pregnancy \[16\]. Pregnancy gingivitis usually develops between the second and eighth month of pregnancy \[17\]. Pregnancy itself does not seemingly cause gingivitis, and a healthy gingival without bacterial challenge usually remains unaffected \[18\]. Periodontitis may be recognized in certain pregnant individuals as a predictive factor for increased risk of having pregnancy complications \[19\]. Alterations in the immune inflammatory process by hormonal imbalance suggest that estradiol and progesterone are potential determinants responsible for the development of pregnancy gingivitis \[20\]. The levels of serum estradiol and progesterone are elevated throughout the third trimesters of pregnancy, reaching the highest levels during that trimester \[16\]. Interestingly, increased levels of both hormones in saliva have been found associated with the severity of gingival inflammation during pregnancy \[21\]. Several biological mechanisms have been proposed to explain the pathobiology of the interaction between pregnancy and periodontal disease \[22\], including increased vascularity and vascular flow, cellular changes, alterations in oral biofilms and depression of the immune system \[21,23,24\]. It has been postulated that the particularly high susceptibility to develop gingival inflammation during pregnancy is associated with qualitative deficiency of maternal immunity such as decreased function of neutrophils, reduced levels of cytokine production and impaired function of maternal T lymphocytes. In vitro, several studies show that estradiol and progesterone can have immunosuppressive effects \[25\]. In addition, clinical studies have also demonstrated the biological link between changes in the local cytokine levels and gingival inflammation during pregnancy \[20\]. The ground breaking experiments by Loe and Silness suggested that pregnancy gingivitis is not identical to common plaque-associated gingivitis due to a significant shift in the correlation between observed bacterial plaque mass and the ensuing extent of gingival inflammation before and after pregnancy \[27\]. Subsequent evidence from in vitro studies and clinical trials confirmed and further corroborated the validity of this distinction \[17\]. The putative etiological explanation has been ascribed to the manifestation of various histological, serological and immunological changes within the gingival tissues during pregnancy. These result in a significant increase of the gingival crevicular fluid flow rate and a concomitant rise in the concentration of proinflammatory cytokines as well as pregnancy-associated sex hormones, favoring the overgrowth of gingivitis-inducing microorganisms \[28\]. Kornman and Loesche reported that in the second trimester of pregnancy, the increased recovery of Prevotella intermediary from inflamed gingival sites was correlated with increased plasma levels of estrogen and progesterone both providing a substitute for menadione, an essential P. intermedia growth factor. Nevertheless a clear
distinction between pregnancy-associated gingivitis and general gingivitis based on the composition of the oral microbiome has not been possible so far. Pregnancy-associated gingivitis is highly prevalent and disappears after delivery. The bleeding gum provides an entry point for the bacteria in the mouth to enter the blood circulation, a transient bacteremia. Under normal healthy conditions, our body can readily fend off such transient bacteremia. However, if the mother has other underlying conditions that compromise her immune system, the bacteria in the blood may escape her immune defense and invade into her womb. In clinical practice pregnancy gingivitis is usually controlled by professional tooth cleaning and the establishment of adequate oral hygiene measures. In the observed cohort of healthy pregnant women however the regular consumption of L.reuteri-containing lozenges proved to be a valuable adjunct in the control of pregnancy-associated gingivitis being effective even in the intentional absence of concomitant professional plaque removal and oral hygiene training.

4.2 Pyogenic Granuloma

The pregnancy-related pyogenic granuloma develops only in up to 5% of pregnant women. Localized gingival enlargement resembling pyogenic granulomas also known as pregnancy tumor / pregnancy epulis/ epulis gravidarum have been described as a painless, exophytic mass that has either a sessile or pedunculated predominantly from the gingival margin or, from the interproximal tissues in the maxillary anterior region. The pregnancy tumor bleeds easily & becomes hyperplastic & nodular and may range in color from purplish red to deep blue, although most commonly is red in color. The lesion classically occurs in an area of gingivitis & is associated with poor oral hygiene & calculus. Pyogenic granulomas seem most frequently throughout the second or third month of physiological condition. pyogenic granulomas of pregnancy is usually not associated with alveolar bone loss. Tooth mobility, pocket depth, and gingival fluid are also increased in pregnancy. The levels of sex steroid hormones in saliva increases during pregnancy resulting in alterations in the microbial populations which may contribute to these pathologic changes. When excited the lesions usually do not have a large defect. Afro-American women and women with poor socio-economic status have a significantly increased tendency to develop aggravated pregnancy-related periodontal changes.

5. Subgingival microbial diversity

Qualitative changes in the subgingival microbiota from aerobic or facultative gram-positive species towards anaerobic gram-negative species have been observed along with the elevated serum levels of estrogens and progesterone during pregnancy. Especially during the second trimester, when gingival bleeding reaches its highest levels, the proportion of P. intermedia in subgingival plaque has been reported to increase significantly. Two pigmented Prevotella species, P. melaninogenica and P. intermedia are actually able to use both estrogen and progesterone for their growth instead of vitamin K according to Kornman and Loesche. Currently the association of P. intermedia and P. nigrescens with pregnancy gingivitis remains to be individually clarified. Yokoyama and co-workers showed in their in vitro studies that estradiol is able to increase the growth of C. rectus, which is another potential periodontal pathogen. In their cross-sectional study, a positive correlation was found between the salivary estradiol concentrations and levels of C. rectus, P. gingivalis, and F. nucleatum in pregnant women. Additionally, the salivary C. rectus levels positively correlated with the percentages of sites with 4 mm pocket depth without any attachment loss. According to a pilot study, when the third molars were present, the efficacy of scaling and root planing during pregnancy proved to be limited to reduce the amount of periodontal pathogens measured from the mesiobuccal sites of first molars. Especially, increased counts of T. forsythia and P. nigrescens were significantly associated with the presence of third molars. Thus, third molars in pregnant subjects are suggested to act as a reservoir for periodontal pathogens by serving as suitable niches for their growth. However, there are discrepancies in the current literature about the correlation between increased hormone levels and subgingival microbiota during pregnancy. In a follow-up study, no significant changes in the proportions of subgingival P. intermedia were found between pregnant and non-pregnant subjects molars. In addition, no correlation was found between the microbiological or clinical parameters and hormonal status. In contrast, in a recent study by Carrillo-de-Albornoz et al pregnant women without periodontitis, harbouring P. gingivalis or P. intermedia in subgingival biofilms, presented a significantly increased gingival inflammation tendency during mid-pregnancy and the presence of P. intermedia and P. gingivalis positively correlated with the salivary female sex hormone levels. Based on these results, the effects of female sex hormone on subgingival microbiota remain generally conflicting, as the results from different studies are still inconclusive & thus, no clear etiology for pregnancy gingivitis can be given.

6. Immunoologic Changes

6.1 Cytokines

The immunologic changes might also be responsible for periodontal pathologic conditions observed during pregnancy. An increased susceptibility to gingival inflammation during pregnancy might, at least partly, be explained by the sex-hormone-related immunosuppression. IL-6 is of major importance in the periodontal disease pathogenesis, as it stimulates the B and T-lymphocyte differentiation, activates macrophages, and regulates osteoclastogenesis. Therefore, its up or down-regulation by estrogen modulates the disease and healing processes of periodontal tissues. Recently it was found that stimulation with both estrogen and progesterone at high concentrations comparable to those found in plasma of pregnant women enhanced the production of IL-6 and IL-8 by human GFs. This suggested the capacity of female sex hormones to enhance cytokines production by human GFs that has the potential to contribute to periodontal disease progression during pregnancy. Monocytes contribute to the direct immune response by producing cytokines, such as IL-1β, TNF-α, IL-6, IL-12, and IL-18. In a clinical follow-up study, where GCF samples were analysed and compared between pregnancy and post-partum, no changes in IL-1β and TNF-α levels were found. In contrast, according to Luppi et al monocytes from pregnant women produce more IL-1β and IL-12, and less TNF-α, in comparison with the non-pregnant stage. The suppression of cell-mediated immune response was explained by a shift from the Type-1 (Th1-mediated) to Type-2 (Th2-mediated) reactivity. Interestingly, the function and antibody production of B-lymphocytes are unaffected in pregnancy. These findings on systemic response do not correlate with an experimental gingivitis model during pregnancy; in that model, the number of B-cells decreased during pregnancy, but the number of Th1
cells increased. Overall, the role of T and B-cells and their inner shifts in the pathogenesis of pregnancy-related periodontitis need to be studied further. In response to the challenge by pathogenic periodontal bacteria, gingival tissues respond to inflammation by up-regulating endothelial adhesion molecules, increasing the secretion of chemotactic agents, and aggravating leukocyte chemotaxis. Estrogen attenuates these critical steps of inflammation by inhibiting the secretion of adhesion molecules and chemokines, i.e. MCP-1 and IL-8, respectively. It has been suggested that changes in the plasma concentrations of estradiol and progesterone not only affect the chemotaxis and phagocytosis of PMNs, but also their migration, motility, and deformability. Consequently, in vitro conditions, the chemotactic ability and migration of PMNs were enhanced by progesterone and reduced by estradiol, whereas no effects on monocytes were observed.

6.2 MMPs
Collagen-degrading MMP produced by fibroblasts have been studied in vitro conditions. When incubated with progesterone, IL-1β stimulated MMP-1,-3,-8,and-9 secretions were down-regulated. Overexpression of MMPs is of interest, since the main organic content of connective tissue and bone is collagen, which MMPs can degrade. In 2010, Gursoy and coworkers first demonstrated the relationship between the changes of neutrophilic enzymes in saliva and GCF and periodontal status during pregnancy and postpartum in their longitudinal study series. Results showed that despite increased inflammation and microbial shift towards anaerobes there was a significant reduction of paraffin-stimulated salivary MMPs and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) expression. Examination of enzymes in GCF did not reflect the increase in gingival inflammation. The levels of MMP-8 and PMN elastase in GCF remained at low levels during pregnancy inspite of the increase in BOP and PD scores. This was supported by supported by in vitro studies conducted by Lapp et al who showed that in response to IL-1, progesterone has the ability to control and reduce local production of MMPs by cultured human GFs. Impairment of neutrophil functions during pregnancy due to reduction of protease concentrations in saliva and GCF may partially explain induced or enhanced susceptibility to gingivitis during pregnancy. These findings explain the reason that pregnancy gingivitis itself can neither predispose nor proceed to periodontitis. However, our knowledge of the role of female sex hormones on MMPs and their regulators in periodontal tissues during pregnancy is limited, and especially neutrophilic enzymes, including MMPs, need to be studied thoroughly.

6.3 Prostaglandins
High concentration of estrogen and progesterone during pregnancy increases the synthesis of PGE2 which may also contribute to the pathological changes.

7. Conclusion
Female sex hormones are implicated in the changes in periodontium during pregnancy. The gingival inflammation is exacerbated during pregnancy. In order to explain the etiology of pregnancy-related gingivitis, several potential mechanisms have been proposed, including effects of hormones, subgingival microbiota and/or immune- inflammatory host response on periodontal tissues. However, so far the underlying mechanisms have not been completely clarified.

Strict oral hygiene maintenance is of prime importance for the patient because it is the dental plaque that leads to incidence and prevalence of disease while the level of hormone modifies the response of periodontium to the dental plaque. Dental consultation may be in the best interest of a female patient prior to planned pregnancy.

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