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Fusobacterium nucleatum and alteration of the oral microbiome: from pregnancy to SARS-COV-2 infection

A. DESSÌ[1], A. BOSCO[2], R. PINTUS[1], G. ORRÙ[3], V. FANOS[1]

[1]Neonatal Intensive Care Unit, University Hospital of Cagliari, Monserrato, Cagliari, Italy
[2]Medical Lab, Sports Medicine and Physiotherapy Center, Vinovo, Turin, Italy
[3]Department of Surgical Sciences, Molecular Biology Service (MBS), University of Cagliari, Cagliari, Italy

Angelica Dessì and Alice Bosco contributed equally to this work

Abstract. – OBJECTIVE: The human being has evolved in close symbiosis with its own ecological community of commensal, symbiotic and pathogenic bacteria. After the intestinal microbiome, that of the oral cavity is the largest and most diversified. Its importance is reflected not only in local and systemic diseases, but also in pregnancy since it would seem to influence the placental microbiome.

MATERIALS AND METHODS: This is a literature review of articles published in PubMed about *Fusobacterium Nucleatum* and both its implications with systemic and oral health, adverse pregnancy outcomes, flavors perception and its interference in the oral-nasal mucosal immunity.

RESULTS: It is in maintaining the microbiome's homeostasis that the *Fusobacterium nucleatum*, an opportunistic periodontal pathogen of the oral cavity, plays a crucial role both as a bridge microorganism of the tongue biofilm, and in maintaining the balance between the different species in the oral-nasal mucosal immunity also by taste receptors interaction. It is also involved in the flavor perception and its detection in the oral microbiome of children from the first days of life suggests a possible physiological role. However, the dysbiosis can determine its pathogenicity with local and systemic consequences, including the pathogenesis of respiratory infections.

CONCLUSIONS: It is interesting to evaluate its possible correlation with Sars-CoV-2 and the consequences on the microflora of the oral cavity, both to promote a possible broad-spectrum preventive action, in favor of all subjects for whom, by promoting the eubiosis of the oral microbiome, a defensive action could be envisaged by the commensals themselves but, above all, for patients with specific comorbidities and therefore already prone to oral dysbiosis.

Key Words:
Fusobacterium Nucleatum, Sars-Cov-2 infection, Microbiome, Oral dysbiosis.

Introduction

The human beings are inseparable from their own microbial community, with whom they have evolved over millions of years, establishing a perfect symbiotic relationship, essential for maintaining a good state of health. Indeed, together they form a real super-organism, where this symbiosis is maintained thanks to a dynamic balance. This cooperation brings various benefits to the host organism. It confers resistance to colonization by pathogens, supporting both the defensive systems and the antioxidant activity. Moreover, it favors the correct functionality of the cardiovascular and digestive systems, without forgetting the important contribution to numerous metabolic processes[1].

This microbial community was called microbiome by Nobel laureate Joshua Lederberg with the intention of defining a real ecological community of commensal, symbiotics and pathogenic bacteria that literally share the body space with the human being. Futhermore, nowdays, it is also known that the various microorganisms, the microbiota, which make up the microbiome are not individual single-cell organisms present in free form, but they aggregate in an organized structure firmly attached to the surfaces. This structure is the biofilm, inside which there is both a close cooperation and a healthy antagonism between different species. Within the biofilm there is also a fruitful

Corresponding Author: Angelica Dessì, MD; e-mail: angelicadessi@hotmail.it

communication between the various components through quorum-sensing. The presence of the microbiome is essential for the human organism in the same way as the set of cells that compose it. In fact, it is now known how numerous factors can influence this precious ecosystem which must therefore be protected and considered as a whole with the host organism[2]. Pregnancy, as well as numerous systemic pathologies, is in fact closely related to the health of the oral microbiome as its alteration can cause adverse effects. Similarly, respiratory tract infections seem to be associated with oral health. Thus, it is questionable whether, in the midst of the Covid-19 pandemic, the prevention of oral dysbiosis can offer any advantages. These hypotheses emerge from the analysis of the literature concerning the *Fusobacterium nucleatum*, an opportunistic bacterium of the oral cavity, a pathogen, essential in the creation of the biofilm at the level of the buccal cavity, tongue and subgingival plaque[3]. It plays a key role in the onset of periodontal pathology and is related both to various systemic pathologies and to some adverse effects in pregnancy. Therefore, it may be interesting to evaluate its presence both during gestation, a non-pathological clinical condition associated with an alteration of the microbiome, and in children from birth in order to investigate its possible function. Moreover, the same alteration of the microbiome can be decisive in infectious diseases of the respiratory tract, such as Sars-Cov-2, both at the pathogenetic level and as a possible complication. A further correlation between the two microorganisms to be analyzed, is associated with the possible repercussions on the olfactory system.

Oral Microbiome Before and After Pregnancy

After the intestinal microbiome, that of the oral cavity is the largest and most diverse in the human body. It hosts more than 700 species of microorganisms that find an excellent habitat inside the mouth where they can colonize different surfaces including teeth, gums, the gingival sulcus, the palate and the lips[4]. Among these locations, the teeth represent a very particular area as, unlike the mucous membranes, they do not fall apart and can therefore represent a perfect district for a firm bacterial adhesion. It is precisely this heterogeneity of surfaces within the oral cavity that justifies a well-varied microbiome. In fact, very different microbial species can be found, belonging to as many as 12 *phyla*: Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Firmicutes, Fusobacteria, Gracilibacteria (GN02), Proteobacteria, Spirochaetes, SR1, Synergistetes, and Saccharibacteria (TM7)[5].

The colonization by such a large number of species is also favored by other factors such as an optimal temperature (about 37°C), adequate hydration (saliva and crevicular fluid), a favorable pH (about 6.5-7) and a richness of nutrients including proteins present in saliva, glycoproteins and the crevicular fluid. The maintenance of homeostasis despite the presence of a high microbial load, is normally guaranteed by the close collaboration between the host's immune system and the set of resident microorganisms. Indeed, there is the pro and anti-inflammatory activity of the various bacterial species and the presence in the saliva and in the crevicular fluid of both nutrients for the microbiota itself and of molecules with antimicrobial action including type A immunoglobulins, lactoferrin and nitrates[1].

Furthermore, both the cells of the epithelial mucosa of the oral cavity and those of the immune system act directly and indirectly in maintaining the balance within the microbiome[6]. In fact, there is a close collaboration: the mucosal cells express a series of antimicrobial peptides, such as ß-defensins, also capable of stimulating APCs[7], meaning the Antigen Presenting Cells. These cells, in turn, activate the specific immune response. The mucosal cells also express chemokines, necessary for the recruitment of monocytes and neutrophils, and cytokines, which are also essential for the specific immune response[8].

In the study by Krisanaprakornkit et al[9] it has been shown that the production of these molecules by the cells of the gingival epithelial mucosa occurs both in response to an inflammatory stimulus and thanks to the continuous stimulation operated by the microbiota. Indeed, purely periodontopathogenic microorganisms such as *Porphyronomas (P. gingivalis)* do not show the aforementioned properties, as the attempt to escape the human immune system is one of the determining factors of virulence. In fact, elevated numbers of certain oral bacteria, especially *P. gingivalis*, has been correlated with higher incidence of major fatals diseases like pancreatic cancer and liver cirrhosis [10]. With regard to this, in recent years, the correlation between oral microbiota and systemic diseases has been studied in greater depth. Even if further investigation is needed[11], with the contribution of new analytical techniques like, for example metagenomics and culturomics, is now

possible to identify a large number of bacterial species that was hardly detectable before[12-14].

This complex balance between the immune system and the microbiome is fundamental for the well-being of every humans. While its alteration, the so-called dysbiosis, can determine systemic and local pathological situations. It can cause mainly periodontal pathology, meaning a chronic inflammation of the periodontium, gum, periodontal ligament and alveolar bone[15,16]. The importance of this balance is demonstrated by the fact that a possible therapeutic intervention is the total removal of the resident altered flora rather than the insertion of specific predatory bacteria of pathogenic species[17].

Indeed, the composition of a healthy oral microbiota is quite stable after its complete maturation in childhood, albeit with numerous inter-individual variables. Nevertheless, there are several factors that can perturb its composition, including the important variation of the hormonal structure that occurs during pregnancy, some metabolic disease such as diabetes mellitus, the use of antibiotics, stress, diet, smoking, oral hygiene, the different composition of saliva and particular genetic characteristics of each individual.

During pregnancy, a woman's high hormone levels can cause important changes that can lead to an immune-compromised state[18]. This could be responsible for both the higher susceptibility to infections and for an alteration of the oral microbiota, with a subsequent higher gum inflammability and bleeding[19] to which is added a decrease in the amount of saliva[1,2]. The variation observed in the composition of the microbiome appears to be constant throughout gestation, as shown by DiGiulio et al[20], then subsequently by Balan et al[21] since this change seems to be caused mainly by the innate peculiar characteristics of each woman. In addition, studies aimed at analyzing the composition of the oral microbiome during gestation have shown that in pregnant women, both in conditions of good oral health and in pathological situations, there is an increase in pathogenic species. This highlights how the modification of the microbiota is the necessary starting condition for the onset of an eventual pathology[21]. In fact, some bacteria of the microbiota itself would seem to initiate and promote progression towards periodontal pathology by exacerbating the host's immune response[22,23]. To detect this, Balan et al[21], found that during pregnancy there is an increase in pathogenic taxa in the genus *Prevotella*, *Streptococcus* and *Veillonella*, contrary to a prevalence of the genus *Haemophilus*, *Neisseria*, *Rothia* and *Streptococcus* found by various studies in non-pregnant women[24,25]. The above studies indicates the results of similar investigations carried out previously[26]. Similarly, an increase in pathogenic *taxa* has also been observed at level of species in pregnant women, especially of *Prevotella species, Porphyromonas gingivalis* and *Fusobacterium nucleatum*[21]. However, if the abundance of periodontal-pathogenic species is associated with the high frequency of gingivitis during pregnancy[21], the species most closely associated with gingival bleeding were poorly represented. This could be explained by the fact that, given the polymicrobial nature of periodontal pathologies and the high complexity of the microbiome also due to the identification of new species, as confirmed by Patini et al[27], it is not only the most present species that determine the balance of the ecosystem but the complex of interactions between the various components[28,29].

In addition to this, it would seem that for the progression from gingivitis, an initial and reversible inflammatory state of the soft tissues surrounding the tooth, to a significant pathological stage such as periodontitis, certain environmental factors are also decisive, including diet and poor oral hygiene. Indeed, it is common for women to change their diet during pregnancy, especially initially, with a strong carbohydrates' imbalance and that, given the gingival sensitivity and possible nausea, oral hygiene is neglected[2]. All these changes can favor the increase of plaque, within which specific niches of anaerobiosis are favored where some pathogenic species proliferate more easily. This progression towards a pathological condition must be prevented not only to preserve the health of the oral cavity but also to avoid adverse outcomes in pregnancy[30-33]. In this regard, a significant species is represented by the *Fusobacterium nucleatum*, a commensal bacterium of the oral cavity. It is one of the most abundant species in the gingival sulcus, gram-negative, anaerobe, with a possible role as a modulator of the taste and the odor of some foods[34,35] that, however, could have a pathological connotation. In reality, *F. nucleatum* is frequently involved in several forms of periodontal problems[2,36]. Indeed, the increase of severity of the periodontal pathology and the inflammation corresponds to an increase of the presence of the bacterium[16]. Furthermore, this bacterium can also determine systemic effects, especially adverse effects in pregnancy[21].

The Placental Microbiome

For years the fetal environment has been considered sterile but to date, despite numerous debates[37], there are several scientific evidence that have demonstrated the presence of a placental microbiome[38-42]. It is less abundant than the gut microbiome but very metabolically active, composed mainly of non-pathogenic commensal *phila* including: *Firmicutes, Tenericutes, Proteobacteria, Bacterioides* e *Fusobacteria.* This microbiome appears to be very similar to the oral microbiome[30] given the conspicuous presence of microorganisms such as *Fusobacterium, Streptococcus, Prevotella, Neisseria e Porphyromonas.* At the basis of this similarity there would be the hypothesis that commensal bacteria of the oral cavity reach the placenta by hematogenous route (low-grade bacteremia). This condition is exacerbated in the presence of pathologies, mainly on an inflammatory basis, of the oral cavity. Furthermore, this finding would be consistent with the association between some pathogenic species of the oral cavity and adverse effects in pregnancy such as preterm birth, chorioamnionitis, neonatal sepsis, preeclampsia and newborn mortality[30-33]. A different composition of the placental microbiome has also emerged in preterm *vs.* full-term pregnancies, further confirming the correlation between the microbiome and fetal health[43-45]. Furthermore, Prince et al[43] confirmed both the different composition of the placental microbiome between at term and preterm pregnancies and the similarity with the oral microbiome and its possible correlation with adverse effects. Moreover, they detect a different bacterial metabolism in presence of chorioamnionites. Indeed, in full-term pregnancies with chorioamnionitis a decrease in the metabolism of butyrate and riboflavin has been observed. Butyrate has been shown to suppress inflammation in the gut[46-48] and a decrease in riboflavin has been associated with an increase in inflammation. Thus, these changes in placental bacterial metabolism of full-term pregnancies can cause the histological inflammation observed. While, in preterm pregnancies, the pentose phosphate pathway and the metabolism of the glycerophospholipids were significantly decreased in association with chorioamnionitis. It should be noted that glucose is necessary for the pentose phosphate pathway and in preterm pregnancies with chorioamnionitis there is a decrease in glucose in the amniotic fluid[49]. Moreover, a decrease in the metabolism of the glycerophospholipids can cause an increase in arachidonic acid, which in turn promotes inflammation and the synthesis of prostanoids, potentially responsible for the induction of labor. Therefore, not only the alterations in the microbiome lead to inflammation, but these alterations can also stimulate the prostanoids to induce preterm labor. The different metabolic pathways observed in the microbiomes of at term and preterm pregnancies may be due to the different bacterial taxa associated with the two conditions. In fact, the results show that at term subjects with chorioamnionitis can have more homogeneous alterations in the microbiome of the placental membrane in association with inflammation. Contrary to what is observed in preterm, in particular those with severe chorioamnionitis, where a high variability in taxa related to severe inflammation was found. Nevertheless, it was not possible to correlate these altered metabolisms with the individual bacterial taxa detected. In any case, the result is completely new compared to what emerged previously. In fact, it would seem to highlight that, probably, the tissue alterations found at the placental level are mainly attributable to an altered bacterial metabolism rather than the presence of specific taxa[43].

The Oral Microbiome of the Newborn

As mentioned in the previous paragraph, the microbial colonization would already begin in the fetal period. However, immediately after birth, the infant's oral microbiome is strongly influenced by the surrounding environment. In this scenario, the contribution of the different microbiomes of the mother (vaginal, intestinal and cutaneous), the mother's milk or formula and the infant's immune system may be primarily determinant. While, the first foods offered to the baby may be subsequently determinant[50,51]. At birth, the first influence on the oral microbiome of the newborn is due to the type of birth[52-54]. Indeed, if vaginal birth is responsible for a more conspicuous enrichment of the nascent microbiome with different taxa among which *Prevotella, Lactobacillus, Sneathia, Bacterioides* and *TM7* prevail, the C-section determines a smaller colonization, among which *Propionibacterium, Corynebacterium, Staphilococcus, Slakia* e *Veillonella* predominate[55,56].

However, although there is a significant exposure to a large number of microorganisms, the colonization of the infant's oral microbiome will follow specific evolutionary stages during which only certain species will become resident. In any case, the effects of the first colonizations will influence the formation of a complex ma-

ture ecosystem in adulthood[54]. This necessary maturation process provides in the first months (0-3 months) a rapid colonization, immediately after birth, by the so-called "pioneer colonizers". These are normally aerobic or facultative anaerobic bacteria, including the genus *Streptococcus* spp., *Staphylococcus spp.* and *Actinomyces*. They facilitate the colonization by other species[57], such as *Fusobacterium* to which *E. coli, Pseudomonas, Lactobacillus crispatus* and *Lactobacillus gasseri* are added, through the production and excretion of by-products of their metabolism[58-60]. The predominance of the genus *Streptococcus,* especially of *S. epidermidis* and *S. salivarius,* is due to several factors. One is the abundance of an optimal substrate for the genus such as the oligosaccharides present in milk togheter with the cleavage capacity of immunoglobulins A1 (IgA), abundant in the oral cavity but especially in breast milk[61,62]. They also have the ability to adhere to the mucous membranes to which we must add the fact that streptococci are the most abundant microorganisms in breast milk[60]. Regarding this, Timby et al[63] showed that up to four months, breastfed babies have a more varied and richer microbiome even if at 12 months this difference is canceled. However, in breastfed infants, specific microbial communities are present even at 12 months, which are totally absent in formula-fed babies, supporting the possible long-term contribution of breastfeeding[63].

In the following months (3-6 months) the "second colonizers" appear, such as *Granulicatella, Rothia* and *Haemophilus.* There is a further increase in biodiversity with the eruption of the first teeth which shows the predominance of different species, such as *Streptococcus mutans, Fusobacterium, TM7, SR1, Tenericutes* and *Synergistetes*, more closely related to potentially cariogenic microbiomes[64,65]. This variation is most likely favored by both the appearance of not flaking new tissues, the teeth, responsible for the formation of a new specific microbial ecosystem of dental plaque, and by the influence of the external environment[66-71]. However, this evolution represents a fundamental condition for achieving a good biodiversity of the oral microbiome, which thus becomes more and more varied to be stabilized definitively in the transition to adulthood. Nevertheless, further investigations are needed to better understand the influence of the external environment on the oral microbiome. Indeed, scientific evidence regarding how the oral microbiome develops during early childhood and how the external environment influences this complex ecological system is scarce[57]. Just as much as the contribution of genetic heritage should be further investigated, on which studies are conflicting. On the one hand, some research on twins (high-throughput sequencing and fingerprinting methods)[72,73] seem to attribute no role to the genetic component. On the other hand, other studies (twin cohort studies)[74,75] have highlighted the possibility of inheriting specific taxa, potentially associated with a greater onset of oral cavity diseases.

The Role of Fusobacterium Nucleatum

The *Fusobacterium nucleatum* is a Gram-negative anaerobic bacterium, belonging to the Fusobacteriaceae family, phylum Fusobacteria. It is one of the most abundant species in the oral cavity of many human beings[76] since birth, suggesting not only a relevant biological role in the oral microbiome but also possible broader spectrum effects. In fact, research on the first colonizers of the newborn's buccal cavity reported the presence of the *F. nucleatum* already in the first months (0-3) with a progressive increase associated with growth[56]. Furtermore, Angius et al[77] aimed at analyzing the presence and the role of anaerobic bacteria associated to the periodontal pathology in mothers and their offspring, highlighted how 92.5% of the mothers resulted to be positive to *F. nucleatum*. Research on biofilm formation has also highlighted how *Fusobacterium nucleatum* evolved in close association not only with human cells and tissues but also in relation to the oral microbiota. In fact, it would seem to play a key role both in health and in the pathology of the buccal cavity[78].

Mutualistic Symbiosis in the Oral Cavity

The direct interactions of *Fusobacterium nucleatum* with the tissues of the human body can vary from a neutral or positive effect, as a symbiont of the human oral cavity, to a pathological one, as an opportunist[78]. The positive aspects of the presence of *F. nucleatum* within the human oral cavity are inseparable from its role within the biofilm. Actually, it showed to have a mutualistic relationship with the other members of the oral microbiome[78]. Indeed, *F. nucleatum* plays a fundamental role, acting both as an essential structural support in the formation of the biofilm and as a mediator of interactions both with the microbiota itself and with the host tissues. It therefore represents a bridge microorganism

which, thanks to the particular elongated bacillus structure, allows the connection between the first colonizers of dental plaque, mainly aerobi-facultative anaerobes, such as *Streptococcus spp.* and the "second colonizers", purely anaerobic, including *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*[79] in the formation of dental plaque[80], a clear example of a polymicrobial biofilm. In fact, where there is an absence of *Fusobacterium nucleatum*, the lack of "second colonizers" is reported[81].

Having said that, if on the one hand the elongated shape of *Fusobacterium nucleatum* is crucial in its function as a "bridge" between the different colonizers of the plaque, the presence of particular adhesion molecules, adhesins, is necessary to mediate and directly organize the interactions with both the tissues of the host and those among the various components of the microbiota such as, for example, *Streptococcus mutans* and *Candida albicans*[82-84], favoring their permanence inside the oral cavity. However, it should be emphasized that, within the biofilm, there are not only the aforementioned physical interactions but also metabolic interactions. Unfortunately, data on these phenomena are scarce due to the considerable difficulty of reproducing this complex communication network *in vitro* and the few studies on *F. nucleatum*[78].

A further implication of the presence of *F. Nucleatum* within the microflora of the oral cavity is the modulation of the perception of taste of some compounds, the cysteine-S-conjugates, present in many vegetables. According to Starkenmann et al[34], it would be the anaerobic component of the microbiota, especially *Fusobacterium nucleatum*, the main architect of the transformation of these compounds into volatile thiol derivatives. This transformation would therefore be responsible for a persistent sulfhydryl odor-taste following the ingestion of certain foods. It provides another dimension to the taste perception of food[34] closely related to the health of the oral microbiome, in turn associated with diet. The study by Angius et al[77] highlighted how in the mouth of children the growth-related increase in *F. nucleatum* and in sulfur compounds could suggest a possible physiological role of the bacterium in infants from the first days of life, probably associated with taste perception.

Furthermore, there are various scientific evidence that have highlighted how the presence of *Fusobacterium nucleatum* is influenced by numerous external factors. Smoking is in fact responsible for an increase in the bacterium both in physiological and pathological conditions[85,86]. While, subjects suffering from severe forms of periodontitis and those with uncontrolled type 2 diabetes show a more conspicuous presence of *F. nucleatum*[87].

Pathogen of the Oral Cavity

The interactions of the *Fusobacterium nucleatum* with the host tissues can have pathological characteristics, in fact it is one of the key bacteria in the onset of periodontal pathology. Specifically, understanding the pathogenetic role of *F. nucleatum* can be complex, also considering its role as a commensal in the oral cavity[88]. It can in fact mediate, through numerous adhesins[89,90], the colonization and the bacterial dissemination together with other pathogenic periodontium species. At the same time, adhesins can exacerbate the host response[91] favoring the establishment of a highly inflammatory environment by stimulating a massive release of inflammatory cytokines such as IL-6, IL-8 and TNF. Although the virulence factors associated with this bacterium are numerous, there are in fact endotoxins, such as LPS and some proteases necessary for the antagonism with other bacterial species[80], the adhesins seem to play a key role. The best knowns are FadA, RadD, Fap2 and aid1[91], responsible for mediating the adhesion both with the host tissues and between different bacteria, allowing the formation of the polymicrobial biofilm. In addition, in the study by Fardini et al[92], it emerged that FadA also behaves as invasion. Indeed, through the link with endothelial vascular cadherins, it can increase endothelial permeability favoring systemic dissemination. A further virulence factor associated with the presence of adhesins, is the induction of cell death in human lymphocytes by Fap2 and RadD[90]. It was also highlighted by Signat et al[88], that the *Fusobacterium nucleatum* can directly induce the release, by the gingival tissue, of particular antimicrobial peptides such as ß-defensins 2. At the same time, this bacterium be very sensitive to ß-defensins 3 thus showing a behavior in some ways different from others predominantly pathogenic periodontium species. Indeed, in conditions of eubiosis it shows a weak action on the immune system and a high sensitivity to numerous cytokines[93-95]. It seems to be part of the commensal bacteria's task to keep the host's defenses active without being excessively dangerous. Nevertheless, by acting as a structural "bridge" in the bacterial biofilm, *F. nucleatum*

can cause an excessive increase in the anaerobic flora (second colonizers) favoring the growth of a dysbiotic microflora[96]. In fact, from the study by Socransky et al[97], in the analysis of more than 13,000 samples of subgingival periodontal plaque, two main groups of bacteria emerged: red and orange. The red group, consisting of *P. gingivalis, Treponema denticola,* and *Tannerella forsythia*, it is characterized by a high pathogenic potential and correlated to clinical indices of periodontal pathology such as pocket depth index (PPD), probing bleeding index (PBI). While the orange one, of which *Fusobacterium Nucleatum* belongs, is decisive both in favoring the colonization by the red group and in determining the progression of periodontal pathology. It is probable that in this context of alteration of the microbiome, there is both an imbalance towards an inflammatory state presumably related to an exacerbation of the host response and to the co-aggregation between different microbial species. These are both determining factors in the progression towards periodontal pathology. For example, it has been shown that *Fusobacterium nucleatum* increases the invasiveness of *P. gingivalis*[98,99]. It could be due to a close cooperation between the two species, which leads to the escape from the human body's immune system, and the creation of a highly inflammatory environment. Studies on this synergy are various, however the factors involved seem to be numerous. On the one hand, a dysregulation of the inflamosome[100] could be the cause of the excessive reaction triggered by the host organism. However, the creation by *F. nucleatum* of lipid rafts through which it allows the entry of *P. gingivalis* into the cells of the human body[98] seems to be only one of the mechanisms through which *F. Nucleatum* mediates the host invasion by other pathogens. Despite the abundant literature, there are still numerous mechanisms to be clarified. Therefore, as emerged from the study by Tefiku et al[91], certainly *F. nucleatum* plays a key role in pathologies of the oral cavity, however it is not possible to state with certainty whether its role is dominant or secondary.

Correlation with Adverse Affects in Pregnancy and Systemic Pathologies

The correlation between *Fusobacterium nucleatum* and adverse affects in pregnancy is supported by numerous scientific evidence[16,19], likewise there are numerous researches that correlate poor oral health to abortions, neonatal mortality, preterm births, preeclampsia and chorioamnionitis[101-106]. As previously discussed, two factors are very likely to be decisive: the first concerns the hormonal changes typical of gestation that expose the future mother to an alteration of the oral microbiome[18] and the second concerns the strong similarity observed between the placental and the oral microbiome[30]. In 2012, with the aim of evaluating the epidemiological evidence on the impact of periodontal disease on adverse pregnancy outcomes and identifying its potential mechanisms, the first consensus document was drawn up in synergy between the EFP and the AAP[107]. To date, compared to the past, the evidence supporting the correlation between periodontal pathology and adverse affects in pregnancy has been strengthened. The scientific literature of the last century regarding the association between adverse effects in pregnancy and *F. nucleatum* was limited by the need to use culture media that sometimes do not allow the detection of some bacterial species[108-110]. Nevertheless, in recent years, the use of innovative techniques such as 16S-23S rRNA gene intergenic transcribed spacer region, has resulted in significant progress that has led to a more solid correlation between *F. nucleatum* and preterm birth[31,111]. It was also possible to highlight the presence, in the saliva samples of the mother with preterm birth, of the same strain of *F. nucleatum* found in the gastric aspirate of the newborn[112]. These findings also support previous studies on the different microbiome observed in term and preterm pregnancies[113], as discussed above.

A further contribution to understand the possible origin of the detected infections and the underlying mechanisms was provided by preclinical studies on murine specimens, thanks to which it was possible to highlight how adhesin-invasin FadA mediates the crossing of the placental barrier. Indeed, to date, years after the first consensus document of the EFP and the AAP from which two possible mechanisms underlying the placental infection emerged, the hypothesis of hematogenous dissemination of oral microorganisms and their metabolites seems to prevail with consequent immune and inflammatory reaction at the level of the fetal-placental unit[114]. Even stillbirths and the chorioamnionites[30,32,115,116] were related to the presence of *F. nucleatum.* In 2010, Han et al[30] have in fact described the first case of neonatal mortality in the presence of chorioamnionitis associated with *Fusobacterium nucleatum*. They ascertained the oral origin of the infection found at the level of the fetal-placental unit, correlating it with a weakening of the immune defenses as-

sociated with a respiratory infection contracted by the woman a few days earlier. The role of the weakening of the host's immune defenses, related to the fact that many infections are actually much more complex than previously thought due to the possible polymicrobial origin[28], suggests that the commensal bacteria as well as the interactions between various microorganisms can have a decisive impact on both the development of virulence and the outcome of the infection.

Finally, other adverse effects in pregnancy potentially related to the presence of *F. nucleatum* such as neonatal sepsis[117] and preeclampsia[118] have been found but they need further studies[19].

Table I summarizes the main studies regarding the association between *Fusobacterium nucleatum* and its possible adverse effects during pregnancy.

As reported in a recent review[16] the *Fusobacterium nucleatum* is also associated with numerous systemic pathologies such as some pathologies of the gastro-intestinal tract including inflammatory bowel disease[119,120], IBD, colorectal cancer[121-126], CRC, and appendicitis[127-129]. It has also been found in atherosclerotic plaques[130-132]. It has been related also to rheumatoid arthritis[133], to Alzheimer's disease[134], to Lemierre's syndrome[135], to brain aneurysms[136] and to some respiratory tract infections[135].

It is therefore clear how this pathogen can systematically spread in numerous districts of the organism. It could be due to the high adhesive capacity and to the different virulence mechanisms, however it is clear how its presence and its dissemination are closely associated with the state of health of the individual.

F. Nucleatum and Bitter Taste Receptors

The role of the gut microbiome as a modulator of human food preferences has been suggested by different authors, but the evidence with oral microbiota remains poorly defined, even if is known that the oral microbe community is strongly affected by human dietary habits. In this context, recent studies on dental calculi, indicate a significant difference in the periodontal bacteria titer between samples from preindustrial-era and modern ones[137]. The data suggest that socioeconomic conditions and different alimentary habits have determined a significant increase of periodontal pathogens in recent tooth biofilm. This discrepancy could be linked to the noticeable increase in degenerative diseases in the modern age. Recently, the taste receptor system has been related to oral-nasal microbiota in a dual function. In the first instance the taste perception and consequently food choices, nutrition, and eating behavior. But, in another perspective, this interaction acts as the mediation of infective respiratory and oral diseases; in particular, significant evidence is recently reported for the bitter taste receptors T2R. These receptors are commonly present in the oral cavity where they signal in the presence of toxic substances. For example, T2R38 regulates innate-immune responses in the oral and nasal mucosa due to microbial products. These molecular structures are induced in the release of antimicrobial peptides and cytokines in response to different oral bacteria metabolites. In the gingival epithelia Fn-T2R38 interaction causes the release of high levels of beta-defensin-2 (hBD-2)[138]. In addition, the secretion of AMPs has been evaluated, to prevent overgrowth of oral bacteria and regulate the microbial composition, avoiding a dysbiotic profile in the tissues. For this reason, Sandell et al[139] reported that genetic variation in the bitter taste receptor T2R38 taste genotype reflected in the microbial composition of oral mucosa in subjects from different geographical areas. Douglas et al[140], showed an interesting role of oral Gram-negative bacteria in T2Rs activation by bitter bacterial byproducts in the upper airway. Gram-negative bacteria produce acyl-homoserine lactones (AHLs), which bind to and activate T2Rs located in solitary chemosensory cells or in ciliate epithelial cells. This activates a biochemistry pathway that increases the nitric oxide (NO) production by the nitric oxide synthase (NOS), which both directly kills bacteria and enhances ciliary beating. Furthermore, the taste receptor intracellular signaling yields increased of Ca2+, via gap junctions. This cation diffuses into adjacent ciliated cells with a consequent increase of antimicrobial AMP secretion (Figure 1).

In this context, *F. nucleatum* could be admitted as an early oral colonizer on the first day of life, because it could stimulate the innate immune response of the newborns against oral as well as respiratory pathogens.

The Bacteria of The Oral Cavity: Possible Implications in Infection by SARS-COV-2

The oral cavity certainly represents a strategic location as an excellent entrance and exit gates for all the pathogenic species responsible for respiratory tract infections and therefore also for Sars-Cov-2 infection, especially considering its detection in saliva samples and the abundance of ACE2 receptors in the epithelium of the buccal

Table I. *Fusobacterium Nucleatum* and adverse pregnancy outcomes.

Results	Technique	Sample	Author	Year
First associations between *F. Nucleatum* and preterm birth	Culture	Amniotic fluid from 45 selected patients 33 patients with singleton pregnancies 773 transabdominal amniocenteses from women presenting with preterm labor and intact membranes	Miller et al[110] Wahbeh et al[109] Chaim et al[108]	1980 1984 1992
F. Nucleatum has been observed in preterm birth and preterm premature rupture of membranes (PPROM) Associations between *F. Nucleatum* and preterm birth was observed	16SrRNA-based culture indipendent	Samples of fetal membranes from 37 preterm infants, and 6 normal term controls delivered by caesarean section Amniotic fluid specimens from 46 pregnancies complicated by PTB and 16 asymptomatic	Cahill et al[111] Han et al[31]	2005 2009
Associations between *F. Nucleatum* and preterm birth was confirmed and suggestion that intra-amniotic *F. nucleatum* could originate from the patient's or the partner's oral microflora	PCR	3 women in preterm labor with intact membranes	Gauthier et al[106]	2011
Association between *F. Nucleatum* and preterm birth has beem condirmed and a novel association between *F. Nucleatum* and neonatal sepsis has been observed	16SrRNA-based culture indipendent + culture	44 patients with singleton pregnancies	Wang et al[117]	2013
Association between *F. Nucleatum* and preterm birth and choriomamnionitis that have been probably due to an ascending infection after orogenital contact.	Culture	Case reports of 1 pregnant woman	Dixon et a[1115]	1994
Association between *F. Nucleatum* and choriomamnionitis at term with potential adverse maternal and neonatal outcome	Culture	Case reports of 1 pregnant woman	Boher et al[32]	2012
Association between *F. Nucleatum* and stillbirth with choriomamnionitis Association between high serum antibody levels to F. Nucleatum and stillbirth	16SrRNA-based culture indipendent Enzyme-linked immunosorbent assay	Case study of a pregnant with associated gingivitis 786 serum samples at baseline; this was reduced to 635 by the 29- to 32-week visit; 620 matched samples were available for within- and between-patient comparisons of changes between baseline and 29 to 32 weeks	Han et al[30] Ebersole et al[116]	2010 2009
First association between *F. Nucleatum* and hypertensive disorders that may suggest a possible contribution of periopathogenic	PCR	16 placentas' samples obtained from cesarean sections of women with preeclampsia and from 14 age-matched healthy pregnant women.	Barak et al[118]	2007

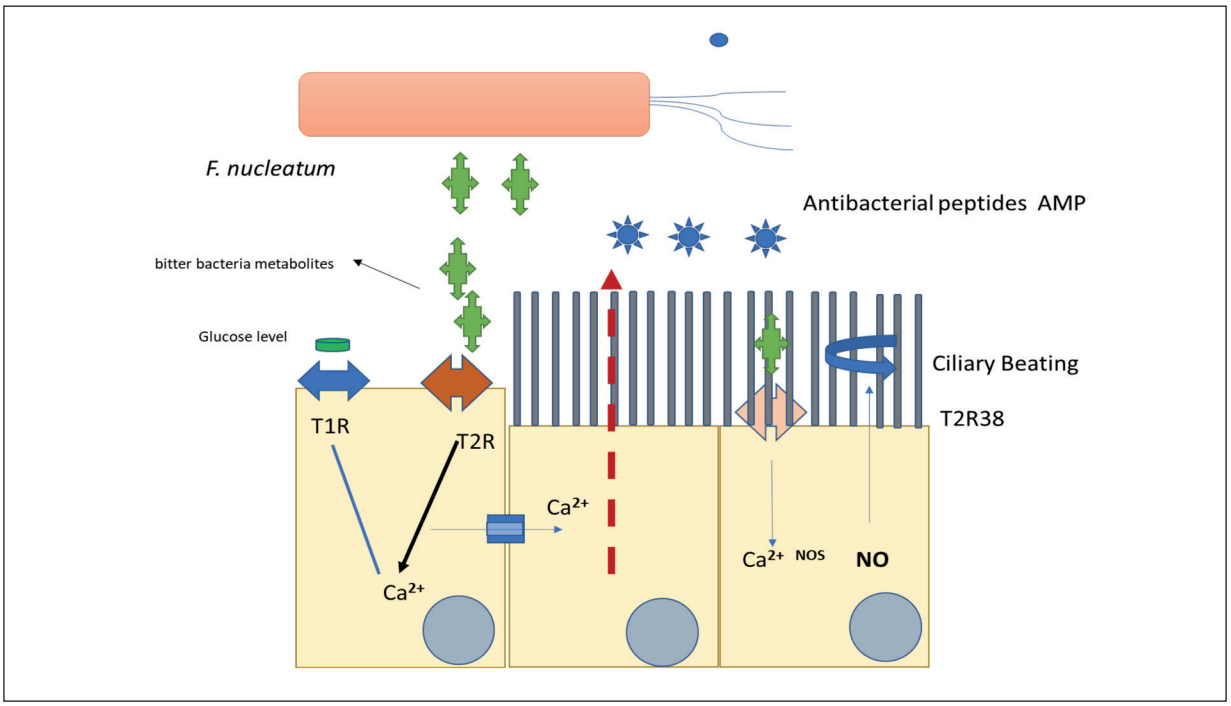

Figure 1. Schematic representation of innate antimicrobial activity by Gram negative bacteria stimulation of T2R receptors.

cavity[141]. Furthermore, the possible correlation between periodontal pathogenic microorganisms and the pathogenesis of respiratory infections has long been known[142]. In this regard, several studies have shown how the oral cavity can be crucial in the respiratory tract infection process and several possible mechanisms have in fact been proposed as the basis of the etiopathogenesis. The first concerns the aspiration into the lungs of typically pathogenic bacteria of the oral cavity such as *P. gingivalis* or *A. actinomycetemcomitans*, which are abundant in subjects suffering from periodontitis, resulting in lung infections. A second mechanism proposed, concerns the modification of the oral mucous membranes induced by inflammation. It results from the periodontal pathology, which would make them more susceptible to adhesion and colonization by pathogenic species of the respiratory tract. This would be followed by their possible aspiration into the lungs. These changes in the oral mucosa can also extend to the respiratory epithelium, making it more susceptible to infections, a possible further pathogenetic mechanism. Finally, it has also been hypothesized that the inflammatory state associated with periodontal pathology destroys the salivary film that covers the pathogenic bacteria, hindering their elimination from the mucous membranes[142]. Zheng et al[143] have highlighted how in the most serious patients with Covid-19 there was an increase in neutrophils and low levels of lymphocytes, an abnormal condition for a viral infection, compared to less severe patients. It was therefore assumed that the high level of neutrophils was to be associated with a bacterial co-infection. While the low level of lymphocytes, essential in the course of an immune response against viral species, was due to a functional exhaustion of the lymphocytes themselves or to the prevarication of bacterial co-infection[143]. As a confirmation of this, it would seem to be the study by Zhou et al[144], which found that 50% of those who died from Covid-19 had a secondary bacterial infection. To this must be added what emerged from the study by Cox et al[145] about the relevance at the level of clinical indices and mortality of the co-infections in patients affected by Covid-19. Probably in the light of these data, Patel and Sampson[146] hightlighted the possible impact of oral bacteria in Covid-19 co-infections. In fact, thanks to very recent metagenomic analyzes[147], the presence in patients suffering from severe acute respiratory syndrome 2 of a high number of cariogenic and periodontopathogenic bacteria, including the *Fusobacterium,* has frequently emerged, confirming the thesis that correlates

oral dysbiosis and the possible complications of Covid-19. However, the studies concerning the influence of Sars-CoV-2 on the microbiome are very limited, but it is curious to note, as reported by Bao et al[148] that other investigations on animal models of the swine epidemic diarrhea virus, belonging to the coronavirus family, have shown a high presence of the *Fusobacterium* in affected subjects. Indeed, as pointed out by several scientific investigations[146,148,149], there are several studies that suggest both the involvement of periodontopathogenic species in the pathogenesis of the respiratory infections, such as Sars-Cov2, and their correlation to various systemic diseases, including hypertension, cardiovascular diseases and diabetes. Furthermore, these pathologies have emerged as a frequent cause of comorbidities associated with an increased risk of serious complications and death from Covid-19. Given the numerous studies on the effectiveness of improving oral health on clinical indices and mortality in patients with pneumonia[150,151] as well as reducing the use of mechanical ventilation[152] but also the preventive role of a good oral health on pneumonia and respiratory tract infections in elderly hospitalized or nursing home patients[153] it could be assumed that the same benefits could be obtained in patients with Sars-Cov-2. In addition to this, on the one hand, the pulmonary hypoxia that some patients with Covid-19 undergo can favor a lung environment more prone to colonization by anaerobic bacteria. While, on the other hand, given the respiratory difficulties of some subjects, the mechanical ventilation is often associated with the onset of secondary pneumonia[148].

In this regard, promoting good oral health is essential for maintaining the eubiosis of the microbiome since a simple dysbiosis can affect the onset of co-infections. In fact, normal daily activities, including chewing and normal oral hygiene practices, cause micro-lesions inside the buccal cavity that can lead to bacteremia, through the hematogenous dissemination of oral bacteria and their inflammatory metabolites, with possible systemic inflammation in certain patients[146]. As a confirmation of the importance of the eubiosis of the oral microbiota there is the study of Wolff et al[154] which reports 4 cases of *F. nucleatum* bacteremia in patients with Covid-19 without the patients having known risk factors for *Fusobacterium nucleatum* infection. Therefore, the question arises whether the oral dysbiosis potentially caused by various antecedent factors, including stress and poor nutrition, has created a fertile ground for Covid-19 infection which destabilized the immune system and allowed the onset of bacteremia, or whether Sars-Cov-2 itself is responsible for the dysbiosis which, following the strong impact on the immune system, in some patients, can lead to bacteremia. Finally, it should be noted that both Sars-Cov-2 and *F. nucleatum* have direct and indirect repercussions on the olfactory-gustatory system, respectively. In the case of Covid-19 infection, a significant percentage of patients reported anosmia or hyposmia as a preliminary symptom[155]. In any case, this type of alterations seems to be transitory with a complete or partial recovery in a few weeks, however there are still no reliable data[156]. The origin of the dysfunction seems to be mainly attributed to an involvement at the central level of the olfactory bulb and only partially to peripheral damage at the level of the olfactory epithelium. The alteration of taste seems to be secondary to the olfactory dysfunction. Therefore, the role of *Fusobacterium nucleatum* in the modulation of gustatory perception, as well as its interference in the oral-nasal mucosal immunity by taste receptors interaction, must be strongly investigated in COVID-19 patients.

Discussion

From the analysis of the literature, it is clear that the homeostasis of the oral microbiome represents a key point for human health with local and systemic implications. In this context, the *Fusobacterium nucleatum* plays a crucial role thanks to its structural and organizational function within the microflora of the buccal cavity in order to maintain the homeostasis. In fact, as previously discussed, studies on the oral microbiome of children assume a possible physiological role of *F. nucleatum* from the first days of life. However, in particular conditions both pathological and non-pathological, its strong adhesive and invasive capacities result in an easy systemic dissemination in the body, also increasing very often the virulence of other pathogens through various mechanisms. In fact, it has been discussed how it can favor the escape from the host's immune defenses, facilitate the crossing of the epithelia and exacerbate the defensive response in the human being. These data suggest underlying polymicrobial pathologies. In fact, the most recent diagnostic investigation technologies have allowed us to ascertain that many infections

are actually much more complex than originally believed. Therefore, it appears clear that in an infection, despite often focusing on the dominant microbial species, other microorganisms, including commensals, can have an important impact both on the pathogenesis of the disease itself and on the outcomes. Furthermore, the examined data revealed that sometimes the outcomes of the presence of a microorganism are determined not uniquely by the specific characteristics of the single species but by the interaction of the various micro-organisms present. They can thus modify their own metabolism, virulence and cause an important alteration to the surrounding environment resulting in damage to the host's tissues.

It could be interesting to analyze the correlation between Sars-Cov-2 and *Fusobacterium nucleatum* both to evaluate a possible broad-spectrum preventive action, in favor of all subjects for whom, by promoting the eubiosis of the oral microbiome, a defensive action promoted by the commensal bacteria themselves, but, above all, for patients with specific comorbidities and therefore already prone to oral dysbiosis. In addition to this, after the infection, a possible intervention on the oral microbiome could represent an improvement in the prognosis, avoiding possible co-infections. In addition, as regards non-pathological clinical conditions that are still affected by an alteration of the microbiome such as pregnant women, a preventive intervention on the microflora of the oral cavity could boast even greater benefits, not only for what concerns the prevention of adverse effects typically associated with oral dysbiosis but also for respiratory tract infections, in this case by Sars-Cov-2. Furthermore, from the assessment of the correlation between Covid-19 and *F. nucleatum*, the role of other microorganisms could emerge that, through specific, synergistic, additive, or antagonistic actions, could prevent or favor Sars-Cov-2 infection or in any case affect its outcomes.

Conclusions

In summary, it may be assumed that the problems of the olfactory and gustatory system can be synergistic or additive and therefore in order to favor a complete recovery, given the long sequelae reported by some patients, the establishment of a new balance within the oral microbiome could be decisive.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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