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Original Article
Livestock Diseases

Monitoring periodontal lesions and their effects during pregnancy: microbiological aspects of the oral cavity and amniotic fluid in pregnant ewes[1]

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ABSTRACT.- Souza N.C., Ramos T.N.M., Borsanelli A.C., Saraiva J.R., Ferreira E.M., Schweitzer C.M., Gaetti-Jardim Jr. E. & Dutra I.S. 2023. **Monitoring periodontal lesions and their effects during pregnancy: microbiological aspects of the oral cavity and amniotic fluid in pregnant ewes**. *Pesquisa Veterinária Brasileira 43:e07160, 2023*. Departamento de Produção e Saúde Animal, Faculdade de Medicina Veterinária de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rua Clóvis Pestana 793, Cx. Postal 533, Araçatuba, SP 16050-680, Brasil. E-mail: iveraldo.dutra@unesp.br

Periodontitis affects the teeth supporting tissues, leading to tooth loss and damage to animal health. Evidence in humans suggests that oral microorganisms spread systemically, increasing the risk of pregnancy disorders such as miscarriage, prematurity, and low birth weight. This study aimed to verify whether periodontopathogenic microorganisms reach the transplacental unit, culminating in problems in pregnant ewes. After analyzing the oral cavity, 10 clinically healthy pregnant ewes (OGCH group) and 10 pregnant ewes with periodontitis (OGP group) were selected. The subgingival biofilm was collected for the polymerase chain reaction (PCR) test and amniotic fluid for both the PCR and interleukin (IL) analysis. Peripheral blood was collected for complete blood count, and analyses of IL-6, IL1-β, and tumor necrosis factor-α were performed. Placental fragments were collected to assess the inflammatory changes using optical microscopy. After giving birth, both the ewes and their lambs were weighed. On clinical examination, a positive correlation between bleeding and suppuration (correlation index – CI=0.54), suppuration and marginal gingivitis (CI=0.34), and marginal gingivitis and edema (CI=0.54) was observed. The weights of the ewes (p=0.013) and their respective lambs (p=0.04) in the OGP group were lower than those of their OGCH group counterparts. The hematological analysis revealed that the OGP group ewes showed a slight increase in the mean corpuscular volume (p=0.2447), segmented cells (p=0.3375), and eosinophils (p=0.3823) when compared with the OGCH group ewes, without a statistical difference. Regarding the microorganisms detected in the oral cavity, there was a significant difference between the occurrence of periodontal pockets and the presence of *Fusobacterium necrophorum* (p=0.0328), *Porphyromonas asaccharolytica* (p=0.0392), and the Mollicutes

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class (p=0.0352). *Staphylococcus* genus (p=0.9107) and *Archaea* domain (p=0.7245) were detected in the amniotic samples of both groups, without a significant difference, whereas *P. asaccharolytica* (p=0.2685) was only detected in one sample in the OGCH group. The expression of cytokine IL-6 in the OGP group differed significantly between the prepartum and postpartum periods (p=0.0039); moreover, it differed significantly in the postpartum period between the OGCH and OGP groups (p=0.0198). Histological examination showed a higher percentage of placental changes in the OGP group (70%) than in the OGCH group, such as the presence of macrophages, neutrophils, plasma cells, and multifocal areas of calcification. These results do not corroborate the hypothesis of dissemination of oral microorganisms to the placental unit, suggesting that it constitutes placental isolation in sheep.

INDEX TERMS: Periodontitis, sheep, gestational complications, premature lambs, amniotic fluid, ewes, oral cavity.

RESUMO.- [**Monitoramento de lesões periodontais e seus efeitos na gestação: aspectos microbiológicos da cavidade bucal e líquido amniótico de ovelhas gestantes.**] A periodontite afeta os tecidos de suporte dos dentes levando à perda dentária e danos à saúde do animal. Evidências em humanos sugerem que os microrganismos orais se espalham sistemicamente, aumentando o risco de distúrbios da gravidez, como aborto espontâneo, prematuridade e baixo peso ao nascer. Este estudo teve como objetivo verificar se microrganismos periodontopatogênicos atingem a unidade transplacentária, culminando em problemas em ovelhas gestantes. Após análise da cavidade oral, foram selecionadas 10 ovelhas gestantes clinicamente saudáveis (grupo OGCH) e 10 ovelhas gestantes com periodontite (grupo OGP). O biofilme subgengival foi coletado para o teste de reação em cadeia da polimerase (PCR) e o líquido amniótico para teste de PCR e análise de interleucina (IL). Sangue periférico foi coletado para hemograma completo e análises de IL-6, IL1-β e fator de necrose tumoral-α foram realizadas. Fragmentos de placenta foram coletados para avaliação das alterações inflamatórias por meio de microscopia óptica. Após o parto, as ovelhas e seus cordeiros foram pesados. Ao exame clínico, observou-se correlação positiva entre sangramento e supuração (índice de correlação − IC=0,54), supuração e gengivite marginal (IC=0,34) e gengivite marginal e edema (IC=0,54). Os pesos das ovelhas (p=0,013) e de seus respectivos cordeiros (p=0,04) do grupo OGP foram inferiores aos do grupo OGCH. A análise hematológica revelou que as ovelhas do grupo OGP apresentaram discreto aumento no volume corpuscular médio (p=0,2447), células segmentadas (p=0,3375) e eosinófilos (p=0,3823) quando comparadas com as ovelhas do grupo OGCH, sem diferença estatística diferença. Em relação aos microrganismos detectados na cavidade oral, houve diferença significativa entre a ocorrência de bolsas periodontais e a presença de *Fusobacterium necrophorum* (p=0,0328), *Porphyromonas asaccharolytica* (p=0,0392) e da classe Mollicutes (p=0,0352). O gênero *Staphylococcus* (p=0,9107) e o domínio *Archaea* (p=0,7245) foram detectados nas amostras amnióticas de ambos os grupos, sem diferença significativa, enquanto *P. asaccharolytica* (p=0,2685) foi detectado apenas em uma amostra do grupo OGCH. A expressão da citocina IL-6 no grupo OGP diferiu significativamente entre os períodos pré e pós-parto (p=0,0039); além disso, diferiu significativamente no período pós-parto entre os grupos OGCH e OGP (p=0,0198). O exame histológico mostrou maior porcentagem de alterações placentárias no grupo OGP (70%) do que no grupo OGCH, como a presença de macrófagos, neutrófilos, plasmócitos e áreas multifocais de calcificação. Esses resultados não corroboram a hipótese de disseminação de microrganismos orais para a unidade placentária, sugerindo que se trata de um isolamento placentário em ovinos.

TERMOS DE INDEXAÇÃO: Periodontite, ovinos, complicações gestacionais, cordeiros prematuros, líquido amniótico, ovelhas, cavidade bucal.

INTRODUCTION

Periodontal diseases encompass a multifactorial etiology set of diseases that can affect domestic ruminants and directly affect productivity and animal welfare (Silva et al. 2016, Ramos et al. 2019, Borsanelli et al. 2021). In sheep, these diseases are responsible for early culling and the increased cost of replacing animals before the end of their reproductive life (West & Spence 2000), jeopardizing the achievement of satisfactory zootechnical indexes and increasing the cost of sheep farming.

Associations between oral diseases and systemic manifestations, such as cardiorespiratory and renal disorders, osteoarticular disorders, and gestational disorders, are well-established in humans (Kornman 2008, Kuo et al. 2008). Furthermore, studies have shown that pregnant women are susceptible to the risks of systemic disorders, such as premature delivery, miscarriages, and low birth weight, associated with the pathological processes of the oral cavity (Cetin et al. 2012, Hajishengallis 2015, Takii et al. 2018). Gestational complications in ewes may lead to reduced herd productivity, especially in sheep reared for meat (Ridler et al. 2015); however, data regarding the association between oral health and reproductive performance in this species are limited.

Dental biofilm dysbiosis seems to be the primary factor triggering periodontal diseases (Hajishengallis 2015), which initially manifests as gingivitis and could progress to periodontitis (Lyon 2005, Ramos et al. 2019). Furthermore, dietary factors seem to be involved in the etiology of periodontopathies in ruminants kept under different management conditions (Dutra et al. 1993, Silva et al. 2016, Ramos et al. 2019).

Considering the relevance of periodontal diseases in systemic health and their influence on the economic aspects of sheep farming, this study aimed to evaluate the impact of periodontal conditions in pregnant ewes. We assessed the effects on the microbiota of the buccal biofilm and amniotic fluid and their possible contaminants. We aimed to detect

inflammatory mediators in the blood serum and amniotic fluid, correlate the birth weight of lambs with the mother's oral health and verify hematological changes in the mothers and placental tissue.

MATERIALS AND METHODS

Animals. This study included 20 crossbred pregnant ewes from the Dorper/Santa Inês breed aged 2-4 years. The animals remained in farrowing stalls under an intensive rearing system throughout the study. The ewes were subjected to the standard protocol of fixed-time artificial insemination, and pregnancy was confirmed by ultrasonographic examination at 35 and 90 days after the insemination date. Initially, the animals were examined and divided according to the presence or absence of clinical periodontal alterations in their incisor teeth into clinically healthy pregnant ewes (OGCH group; n=10) and pregnant ewes with periodontal disease (OGP group; n=10).

Food management. All animals received the same diet twice a day consisting of sugarcane bagasse, soybean meal, corn, salt, and urea.

Periodontal clinical evaluation of pregnant ewes. The clinical periodontal status of the 20 pregnant ewes was established before parturition after careful inspection of the oral cavity and probing of the gingival margin of the incisors using a Williams periodontal probe. Probing was performed as described by Borsanelli et al. (2021). The ewes presenting gingival recession, inflammatory alterations at the gingival borders, bleeding (spontaneous or on probing), necrotizing gingivitis, mobility, loss of conjunctival attachment, pigmented supragingival biofilm, suppuration, or periodontal pockets of depth >5mm were allocated to the OGP group (n=10). The OGCH group comprised healthy ewes who showed no signs of alteration of the gingival borders or insertion periodontium (n=10).

Collection of samples for polymerase chain reaction. Samples of dental biofilms or from periodontal pockets were collected from all animals using gauze and sterile curette. The clinical specimens were then placed in microtubes containing 1mL of sterilized ultrapure water and stored in a freezer at -80°C for DNA extraction. Samples from OGCH group were collected from the gingival sulcus on the labial surfaces of teeth 301 and 401 using left and right tweezers, respectively. Samples from the OGP group were collected from periodontal pockets of depth >5mm and with suppuration and/or bleeding following the criteria established by Gaetti-Jardim Jr. et al. (2012) and Borsanelli et al. (2021).

Blood collection and hematological profile analysis. The hematological profile of all ewes was evaluated 20 days before parturition. The jugular vein was punctured using a Vacutainer needle to collect 2mL of the blood sample. The hemogram was generated using an automatic counter (Nihon Kohden MEK-6500); subsequently, blood smears stained by the rapid panoptic method (Weiss & Tvedten 2004) were analyzed using optical microscopy (1000x magnification).

Analysis of interleukins and tumor necrosis factor-α. For analysis of the inflammatory profile, approximately 10mL of blood was collected to obtain blood serum 20 days before parturition (prepartum) and immediately after lamb birth (postpartum). Concentrations of interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α in the supernatant were determined using Human DuoSet ELISA kit (R&D Systems, USA), as described by Melo et al. (2019).

Delivery monitoring and amniotic fluid collection. All animals were evaluated for the entire period until delivery. Amniotic fluid was collected according to the methodology described by Souza et al. (2020). Sample collection was performed using a 40 × 12 gauge needle attached to a 20-mL syringe during the expulsion phase of the product. In cases of placental rupture, amniotic fluid was obtained directly from the vulva using a 50-mL conical tube. Samples were separated into 15-mL aliquots and frozen at −80°C for microbiological examination later. The ewes and their respective lambs were weighed immediately after delivery.

Collection of the postpartum placenta. Placental fragments measuring approximately 10cm were collected using tweezers and a scalpel and subsequently placed in a sterile container with 10% buffered formalin solution, followed by immersion in paraffin and processing for histopathological analysis (Machado et al. 2012). Following this, all 20 slides were evaluated using optical microscopy at magnifications from 10x to 40x.

Detection of target microorganisms using PCR. Total microbial DNA was extracted from the buccal biofilm and amniotic fluid samples placed in ultrapure water using GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, Brazil). Specific primers and amplification conditions were used for the following main microbial groups associated with periodontitis: *Fusobacterium nucleatum, Fusobacterium necrophorum*, *Porphyromonas asaccharolytica*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Prevotella buccae, Prevotella intermedia*, *Prevotella melaninogenica*, *Prevotella nigrescens*, *Tannerella forsythia*, *Treponema amylovorum*, *Treponema denticola*, class Mollicutes, and genus *Staphylococcaebacteria* (Ashimoto et al. 1996, Tran et al. 1997, Fouad et al. 2002, Mayanagi et al. 2004, Nadkarni et al. 2012, Antiabong et al. 2013). Amplifications were performed for 25µL volume containing 1XPCR/Mg^{2+} buffer (Boehring Mannheim, Indianapolis/IN, USA), 0.2µL of each deoxynucleoside triphosphate (Pharmacia Biotech, Piscataway/NJ, USA), 0.1µL Taq DNA polymerase (Invitrogen do Brasil, São Paulo/SP, Brazil), 1.0µl of each primer pair (Invitrogen), and 10ng of template. Amplification was performed in a thermocycler (Gene Amp PCR System 9700, Thermo Fisher, USA) programmed for one cycle at 94°C (5 min), 30-36 cycles at 94°C (1 min), the annealing temperature of 72°C for each primer ranging from 30 s to 2 min, and one cycle at 72°C (5 min) for extension of the DNA chain. PCR amplification products were subjected to electrophoresis in 1% agarose gel stained with ethidium bromide (0.5mg/mL) and photographed using an ultraviolet light transilluminator (UV Light Transilluminator, Eastman Kodak Co., NY, USA). As a control for the detection procedures, DNA samples of the standard strains of the studied microorganisms as well as the positive clinical samples for the target microorganisms were used (Gaetti-Jardim Jr. et al. 2012). Ultrapure water free of DNAse and RNAse (CAS - 7732-18-5; Sigma-Aldrich Co., ML) was used as the negative control.

Statistical analysis. The samples were subjected to punctual statistical tests and confidence intervals based on the clinical criteria established at the beginning of the experiment. The data were tabulated and analyzed in spreadsheets of software Statistica version 7 (Stat Soft Ltd.) and SISVAR 5.0. Dichotomous comparisons were evaluated using the student's t-test, while variables with three or more categories were subjected to the Pearson chi-square test for analyzing proportions. Differences in the distribution of microorganisms, clinical parameters, blood counts, and placental data were analyzed using the Spearman correlation test. The significance level adopted in the tests was 5%. In the IL analyses, all statistical variables were tested for normality using the Shapiro-Wilk test. The Wilcoxon test was used for intragroup comparison of the concentrations of IL-6, IL1-β, and TNF-α. The Mann-Whitney U test was used to compare the results between the groups. All *p*-values <0.05 were considered statistically significant.

Ethics Committee approval. The experiment was approved by the Committee on Ethics in the Use of Animals of the School of Dentistry FOA-Unesp, Campus Araçatuba, SP, Brazil (Process No. 00296-2018).

RESULTS

Periodontal clinical condition of the assessed groups and maternal and neonatal weights

The results of the Spearman correlation analysis indicated positive correlations between the occurrence of bleeding and suppuration (correlation index – CI=0.54), suppuration and marginal gingivitis (CI=0.34), edema and gingivitis marginal (CI=0.54), and maternal postpartum weight and neonatal weight (CI=0.49). Negative correlations were observed between postpartum maternal weight and gingivitis (CI=-0.47), maternal postpartum weight and periodontal bleeding (CI=-0.52), postpartum maternal weight and suppuration (CI=-0.41), neonatal weight and maternal gingivitis (CI=-0.56), neonatal weight and maternal gingival bleeding (CI=-0.38), and neonatal weight and maternal gingival edema (CI=-0.57). The weight of the ewes in the immediate postpartum period was higher in the OGCH group than in the OGP group (p=0.046). In contrast, the birth weight of the neonates of the OGCH ewes was higher than that of the neonates of the OGP ewes (p=0.011). Other clinical alterations, such as gingival necrosis, did not correlate with other alterations and with the maternal weight in the postpartum period or the neonatal weight.

Association between the maternal and neonatal weights and maternal periodontal condition

The student's t-test revealed that the association of the maternal postpartum weight and neonatal birth weight with the maternal periodontal condition was lower in the OGP group ewes (p=0.013) and their lambs (p=0.04) than in their OGCH counterparts. The signs showing a high correlation with low maternal weight included bone loss (p=0.0063), gingival bleeding (p=0.025), and low birth weight of the lambs (p=0.04). The chi-square test results verified that the breed of the pregnant ewes was not relevant to the development of periodontal symptoms, maternal weight in the postpartum period, or neonatal weight (p=0.5341).

Hematological profile of pregnant ewes

Table 1 shows the average values of the erythrocyte profile. Although the average values of the mean corpuscular volume increased in both the groups, this increase was slightly more pronounced in the OGP group than in the OGCH group (37.96±1.94 vs. 36.94±1.84), without a statistically significant difference. In the leukogram results (Table 2), the mean values of the different types of leukocytes were similar between the two groups. Within the evaluated criteria, the total leukocytes, segmented cells, eosinophils, and lymphocytes were slightly higher in the OGP group than in the OGCH group, without a statistically significant difference and with very homogeneous samples and low standard deviations. The Spearman correlation test revealed that regardless of the group, there was a positive correlation between the total number of leukocytes and the number of segmented leukocytes (CI=0.93) and/or eosinophils (CI=0.62).

Evaluation of the pre- and postpartum serum levels of IL-6, IL1-β, TNF-α and in the amniotic fluid

No significant difference was observed between both groups in the serum analysis of IL1-β. The serum levels remained <2pg/mL both pre- (p=0.2966) and postpartum (p=0.7829) (Fig.1). However, the IL1-β concentration in the amniotic fluid showed a slight increase in the OGCH group when compared with the OGP group (3.96±6.02pg/mL vs. 1.59±0.01pg/mL) (Fig.2), without a significant difference (p=0.5588). Furthermore, there were significant differences in the OGP group between the pre- and postpartum serum levels of IL-6 (p=0.0039) (Fig.3). A significant difference was also observed in the IL-6 levels in the amniotic fluid between the OGCH and OGP groups (p=0.0198) (Fig.4). The amniotic fluid analysis of the ILs revealed no significant differences between the groups (p=0.8055); the OGCH group showed slightly higher levels than those of the OGP group (4.04±4.11pg/mL vs. 3.69±3.93pg/mL).

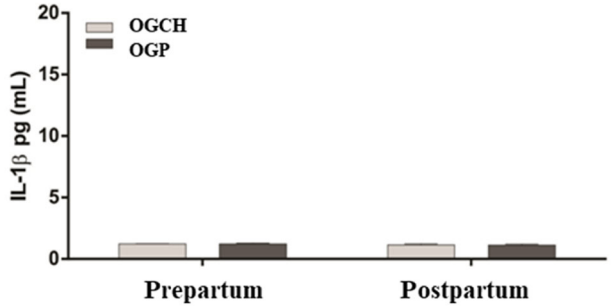
Table 1. Hematological profile of clinically healthy pregnant ewes (OGCH) and those with periodontitis (OGP)

Variables	OGCH group	OGP group	p-value
He (x10^6/μL)	8.65 ± 0.84	8.8 ± 0.74	p = 0.693
GV (%)	32 ± 3.56	33.4 ± 3.27	p =0.3719
Hb (g/dL)	10.58 ± 1.03	10.84 ± 0.96	p =0.5651
MCV (fL)	36.943 ± 1.84	37.96 ± 1.94	p =0.2447
MCHC (%)	33.281 ± 1.12	32.491 ± 0.85	p =0.0933
TPP (g/dL)	6.85 ± 0.43	7.03 ± 0.59	p =0.4452

Table 2. Leukocyte profile of clinically healthy pregnant ewes (OGCH) and pregnant ewes with periodontitis (OGP)

Variables (μL)	OGCH group	OGP group	p-value
Leukocytes	10260 ± 2031.53	11670 ± 3662.13	p = 0.3010
Segmented	7334.7 ± 1382.49	8490.7 ± 3443.13	p = 0.3375
Lymphocytes	1795.5 ± 555.302	2587.8 ± 2475.79	p = 0.3365
Eosinophils	933.6 ± 682.997	1234.5 ± 814.03	p = 0.3823
Basophils	0 ± 0	0 ± 0	p = 0.0933
Monocytes	196.20 ± 198.46	175.10 ± 144.67	p = 0.7889

The analysis of the cytokine TNF-α in the blood serum showed no significant difference between the groups. However, the serum level in the prepartum period increased considerably in the OGP group (11.68±26.3pg/mL) when compared with that in the prepartum OGCH group (2.57±4.98pg/mL) (Fig.5) and that of both the groups in the postpartum period. The TNF-α cytokine showed a marked increase in the amniotic fluid of the OGCH and OGP groups (18.07±2.79pg/mL and 17.26±0.06pg/mL, respectively), without a statistically significant difference between them (Fig.6).



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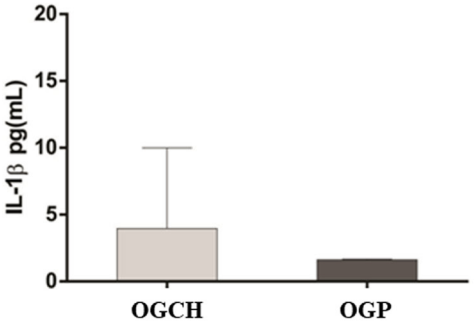

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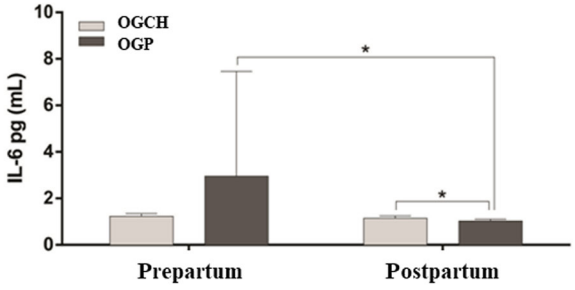
Fig.1-2. (**1**) Serum concentrations of IL-1β levels between the clinically healthy pregnant ewes (OGCH) and pregnant ewes with periodontitis (OGP) groups in pre- and postpartum moments. (**2**) IL-1β concentrations in the amniotic fluid of ewes belonging to the OGCH and OGP groups.



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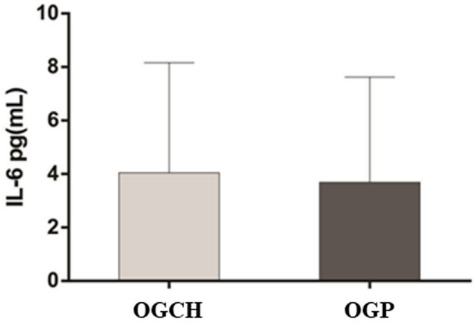

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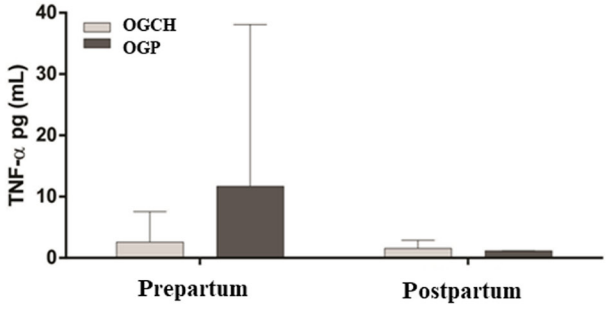
Fig.3-4. (**3**) Serum concentrations of IL-6 levels between clinically healthy pregnant ewes (OGCH) and pregnant ewes with periodontitis (OGP) groups in prepartum and postpartum moments. (**4**) IL-6 concentrations in the amniotic fluid of ewes belonging to the OGCH and OGP groups.



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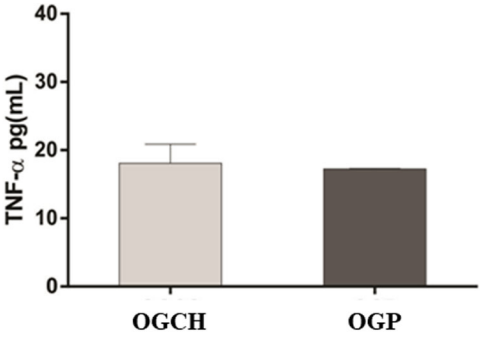

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Fig.5-6. (**5**) Serum concentrations of TNF-α levels between the clinically healthy pregnant ewes (OGCH) and pregnant ewes with periodontitis (OGP) groups in prepartum and postpartum moments. (**6**) TNF-α concentrations in the amniotic fluid of ewes belonging to the OGCH and OGP groups.

Microorganisms in the subgingival biofilm

Using the Pearson chi-square test, a statistically significant association was observed between the occurrence of periodontal pockets and the presence of *F. necrophorum* (p=0.0328), and the adjustment to obtain maximum likelihood revealed a similar relationship between the distribution of *P. asaccharolytica* (p=0.0392) and Mollicutes class (p=0.0352). Although distributed in most sites from where the samples were collected, the same correlation was not identified for *F. nucleatum* (p=0.8979), *Staphylococcus* genus (p=0.2536), and *Archaea* domain (p=0.1965). From the microbiological point of view, the Pearson correlation test revealed that *P. asaccharolytica* presented a negative correlation (p<0.05) with the mature biofilm, indicating that when the biofilm was evident in the sheep, this microorganism was not present. Gingival recession showed a negative correlation with the genus *Staphylococcus* (p<0.05), suggesting that this microorganism occurs more frequently in sites that do not present gingival recession. The t-test revealed significant differences between the means of the two groups with respect to the presence of *F. necrophorum*. This was reinforced by the Pearson correlation test, where this pathogen showed a significant positive correlation with the loss of conjunctival attachment and periodontitis. In contrast, *Staphylococcus* genus showed a negative correlation with bone loss (p=0.04). In tests detecting target microorganisms, *P. gingivalis*, *P. intermedia*, *P. melaninogenica*, *P. nigrescens*, and *T. amylovorum* were not observed in any of the evaluated samples.

Detection of microorganisms in the amniotic fluid

In all the amniotic fluid samples, microbial DNA related to the 16S ribosomal RNA gene amplified using universal primers was observed. There was no significant difference in the distribution of the target microorganisms in the amniotic fluid between the two groups. The samples of both the groups revealed the presence of genus *Staphylococcus* (63.3% and 61.5% incidence in OGCH and OGP groups, respectively; p=0.9107) and domain *Archaea* (45.5% and 38.5% incidence in OGCH and OGP groups, respectively; p=0.7245). Of the 16 other target microorganisms, only *P. asaccharolytica* was detected in a single amniotic fluid sample from the OGCH group (9.1% incidence; p=0.2685).

Histological analysis of the placentas

Few alterations indicative of an inflammatory process were observed in the placental slides; however, it was observed that among the 20 evaluated animals, eight presented placental alterations, of which five belonged to the OGP group and three to the OGCH group (Table 3). The incidence of alterations was noted, as more than one alteration occurred in some animals. The main histopathological findings were as follows: slight amount of macrophages in the lamina propria, moderate congestion, multifocal areas of calcification, autolysis, slight presence of polymorphonuclear cells (neutrophils) in the lamina propria, and slight to moderate presence of neutrophils and rare neutrophils around the vessels (Fig.7 and Fig.8). When comparing the groups (Table 3), the rate of placental alterations was higher in the OGP group than in the OGCH group (0.7% vs. 0.3%).

DISCUSSION

The presence of oral anaerobic microorganisms in the placental tissue or amniotic fluid has been continuously recorded over time in pregnant women (DiGiulio et al. 2010, Bohrer et al. 2012, McCuaig et al. 2018, Ye et al. 2020). These studies investigated whether anaerobic gram-negative rods, such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (DiGiulio et al. 2010, McCuaig et al. 2018, Ye et al. 2020), and the Mollicutes class (Sweeney et al. 2016, 2017) are associated with infectious and inflammatory periodontal conditions (Ercan et al. 2013, Calixto et al. 2019). In some cases, these pathogens can reach the placental membranes and induce abortion through acute chorioamnionitis (Bohrer et al. 2012). Considering that sheep born before 138 days of gestation are classified as premature (Radostits et al. 2002, Souza et al. 2020), signs of prematurity were not observed in any of the groups in this study. Furthermore, although the neonates in the OGP group had lower birth weights than those of the OGCH group (Table 4), they were all within the normal range (>2kg).

The diversity of microorganisms observed in cases of amniotic fluid infection is significant, and bacteriological culture and traditional PCR methods help narrow down the diversity detected (DiGiulio et al. 2010). The results of this study showed the presence of microbial DNA in all samples of amniotic fluid; however, due to the specificity of DNA amplification using PCR with specific primers, the identification of this septic content was limited, identifying only genus *Staphylococcus* and *Archaea* domain present in samples from the OGP and OGCH groups. The presence of the genus *Staphylococcus* in the amniotic fluid from the OGCH and OGP groups must be carefully discussed since these microorganisms are normal constituents of the cervicovaginal microbiota of sheep and have been reported both in normal

Table 3. Main placental alterations identified in clinically healthy pregnant ewes (OGCH) and those with periodontitis (OGP)

Placental changes	OGCH group N (%)	OGP group N (%)
Macrophages in lamina propria	1 (10)	1 (10)
Moderate vascular congestion	0 (0)	1 (10)
Multifocal areas of calcification	0 (0)	2 (20)
Presence of neutrophils, plasma cells and macrophages	0 (0)	1(10)
Mild to moderate presence of neutrophils	1 (10)	0 (0)
Perivascular neutrophils	1 (10)	0 (0)
Autolysis (artifact)	0 (0)	2 (20)
TOTAL	3 (30)	7 (70)

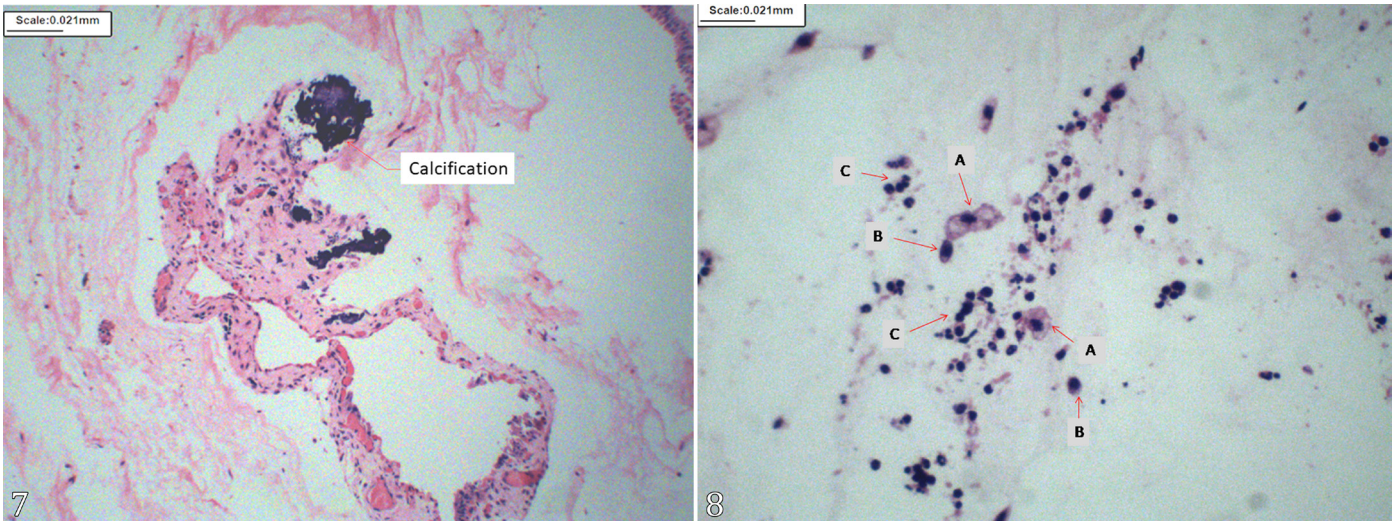

Fig.7-8. Photomicroscopy of histological examination in a sample of the placenta from ewe belonging to the group of pregnant ewes with periodontitis (OGP) showing (**7**) multifocal areas of calcification and (**8**) macrophages (A), plasma cells (B) and neutrophils (C). Magnification of 400x.

Table 4. Weighing of ewes and their respective lambs immediately after parturition

OGCH group	Breed (pregnant)	Postpartum maternal weight (kg)	Lamb identification	Neonate weight (kg)
SP 15	½ Dorper	81.1	C1	4.6
3664	½ Dorper	80	C1	4.5
3937	¾ Dorper	92	C1	4.6
3952	½ Dorper	75.9	C1	4.2
3966	Santa Inês	64.7	C1	4.7
4010	½ Dorper	67.3	C1	4.3
4011	½ Dorper	75.2	C1	4.6
4042	½ Dorper	74.3	C1	4.8
4042	½ Dorper	twin birth	C2	3.5
4043	½ Dorper	70	C1	4
4043	½ Dorper	twin birth	C2	4.5
4083	Santa Inês	47.7	C1	4.1
Mean ± SD	-	72.6 ± 12.4[A]	-	4.3 ± 0.38[B]
OGP group	Breed (pregnant)	Postpartum maternal weight (kg)	Lamb identification	Neonate weight (kg)
3685	½ Dorper	77.4	C1	3.3
3685	½ Dorper	twin birth	C2	4.0
3939	¾ Dorper	85.6	C1	3.8
3939	¾ Dorper	twin birth	C2	3.9
3953	Santa Inês	53.6	C1	4.5
3993	Santa Inês	61.5	C1	4.7
4028	Santa Inês	51	C2	2.4
4082	⅞ Dorper	55.2	C1	3.5
4084	Santa Inês	61.5	C1	3.7
4084	Santa Inês	twin birth	C2	4.4
4246	Santa Inês	49.3	C1	2.6
4265	Santa Inês	41.2	C1	4.0
4294	Santa Inês	45.9	C1	3.4
Mean ± SD	-	58.7 ± 14.8[A]	-	3.7 ± 0.68[B]

[A] Clinically healthy ewes had higher birth weight than those with periodontopathies (p=0.046), [B] lambs from clinically healthy ewes had higher birth weight than those from dams with periodontopathies (p=0.011).

gestational conditions and in chorioamnionitis cases (Walker et al. 2017), evidencing the pathogenic and opportunistic potential of these microorganisms.

The presence of the Mollicutes class may be implicated in the pathogenesis of premature births, abortions, and genital infections (Sweeney et al. 2016, 2017, Pavlidis et al. 2020). Through toll-like receptors, a significant increase in the expression of interleukins and proinflammatory cytokines capable of initiating cell signaling is induced, which causes relaxation of the uterine cervix and contraction of the uterine musculature, in addition to affecting the development of the central nervous system and producing bronchopulmonary dysplasia of the fetus (Pavlidis et al. 2020). In this study, these mycoplasmas were not detected in the amniotic fluid but were mainly present in the subgingival biofilm of the ewes of the OGP group (p=0.03527), with an increased occurrence of *Porphyromonas asaccharolytica* (0.0392) and *Fusobacterium necrophorum* (p=0.0328).

This study aimed to determine the occurrence of the main anaerobic periodontopathogenic microorganisms because they have been implicated in the etiology of periodontal diseases in ruminants (Borsanelli et al. 2015a, 2015b, Ramos et al. 2019), especially sheep (Borsanelli et al. 2016, 2017, Silva et al. 2019). Moreover, these microorganisms have been detected in the amniotic fluid samples obtained from premature and low birth weight neonates (Ercan et al. 2013).

Although the literature on the relationship between periodontitis and prematurity and low birth weight in humans shows evidence of the presence of *P. gingivalis, Treponema denticola, F. nucleatum, F. necrophorum,* and *Treponema intermedia* in the amniotic fluid along with other microorganisms (Doyle et al. 2017, Ye et al. 2020), this association is not universal. These pathogens were not detected in the amniotic fluid samples in this study, and it is not well known how the interspecific interactions between the members of the oral microbiota and their amphibiotic interactions with the host could modify these patterns in other animal species, such as sheep. The low maternal weight of the ewes in the OGP group was associated with the weight of their lambs in this study, and the occurrence of *F. necrophorum*, Mollicutes class, and *P. asaccharolytica* was shown more in this group than in the OGCH group. However, their absence in the amniotic fluid samples may suggest that microbiota-induced proinflammatory effects may occur in the periodontium and do not require hematogenous spread.

Recent studies have used methods of evaluating the placental constituents to verify whether the uterus can be considered a sterile structure and whether the human fetus may be exposed to bacteria before birth. Stinson et al. (2019) analyzed the meconium of human fetuses. They reported the presence of bacterial DNA in all samples with mapped readings for *Staphylococcus* spp., particularly *S. haemolyticus* and *S. epidermidis*, both commensals of the skin, in addition to *Streptococcus* spp., such as *S. infantile*, which is a nasal and nasopharyngeal commensal. Although there are few studies on sheep, this hypothesis must be considered because a related study reported meconium contamination in approximately 40% of the samples, indicating fetal maturity, with an even higher incidence of genus *Staphylococcus* (OGCH group, 63.3%; OGP group, 61.5%) in the amniotic fluid.

Despite the increased occurrence of *F. necrophorum*, Mollicutes class, and *P. asaccharolytica* in the subgingival sites (Table 5), it was found that these microorganisms remained only in the oral cavity and were not detected in the amniotic fluid, probably because the mouth is a diverse environment and rich in microbiomes. This demonstrates the probable placental isolation in sheep, which forms a barrier inhibiting the dissemination of the microbiota to the fetal tissues. This characteristic is relevant because *F. necrophorum* has a great invasive capacity, and it has been considered an important factor in the occurrence of prematurity and low birth weight in neonates as well as abortions in humans (Van der Windt et al. 2018). Notably, other species of the genus *Fusobacterium,* such as *F. naviforme,* have already been described in sheep with periodontitis (McCourtie et al. 1989). Although they were not investigated in this study, it does not rule out their possible presence in the ovine placenta.

Despite the hypothesis of efficient placental isolation in sheep, due to the lack of observation of typical periodontal microorganisms in the amniotic fluid, we can assume that the presence of antigens of these microorganisms as well as components of the inflammatory and immunological responses elicited, could act on the uterine wall or even affect the amniotic fluid of these animals. The increased levels of inflammatory mediators, especially TNF-α, IL-1β, IL-6, prostaglandin E2 (PGE2), fibronectin, and α-photoproteins in

Table 5. Microbial species detected by means of the polymerase chain reaction (PCR) in the gingival sulcus and in the periodontal pocket of clinically healthy pregnant ewes (OGCH) and those with periodontitis (OGP), respectively

Microorganism	OGCH group N(%)	OGP group N(%)	p-value
F. necrophorum	0 (0.0)	4 (21.1)	p = 0.0328*
F. nucleatum	11 (55.0)	12 (63.2)	p = 0.8979
P. asaccharolytica	0 (0.0)	3 (15.8)	p = 0.0392*
P. endodontalis	0 (0.0)	1 (5.3)	p = 0. 9214
P. buccae	0 (0.0)	1 (5.3)	p = 0.9214
T. forsythia	4 (20)	6 (31.6)	p = 0.5218
T. denticola	0 (0.0)	2 (10.5)	p = 0.1569
Mollicutes class	0 (0.0)	4 (21.1)	p = 0.0352*
Staphylococcus genus	8 (40.0)	12 (63.2)	p = 0.2536
Enterobacterales	3 (15.0)	3 (15.8)	p = 0.9021
Archaea domain	4 (20.0)	8 (42.1)	p = 0.1965

* Values with significant difference.

the amniotic fluid, are associated with prematurity, low birth weight, and abortions induced by infectious and inflammatory periodontal conditions in humans (Madianos et al. 2013, Ao et al. 2015, Hajishengallis 2015, Liang et al. 2018, Uriza et al. 2018, Yarkac et al. 2018).

Furthermore, although the PCR used in this study is a valuable technique for detecting periodontopathogenic microorganisms, modern studies using high-throughput sequencing have made it possible to observe important interactions of some key pathogens in the dysbiotic microbiome of cattle and sheep with periodontitis (Borsanelli et al. 2018, 2021). This could enable future investigations regarding the possible associations between the oral microbiome and cervicogenital and amniotic fluid microbiomes of ruminants with periodontal diseases.

In humans, several studies have shown an association between periodontitis and increased levels of inflammatory mediators, both in the blood serum and amniotic fluid, as well as between high levels of IL-1b, IL-6, TNF-α, PGE2, fibronectin, and α-photoprotein in the amniotic fluid and prematurity (Murtha et al. 1998, Shobokshi & Shaarawy 2002, Madianos et al. 2013, Hajishengallis 2015). Within the limits of our investigations, there is limited knowledge about the association between cytokine levels in the amniotic fluid and periodontitis, especially in sheep.

Regarding the sheep species, this pertinent study showed that IL-6 and IL-1β remained <5pg/mL, and only TNF-α significantly increased. Moreover, because there were no reports of prematurity and miscarriage in this study, the hypothesis that this cytokine is associated with the physiological triggering of labor cannot be ruled out. Although the prepartum and postpartum IL-6 levels significantly differed in the OGP group, the levels remained <5pg/mL. However, higher TNF-α serum concentrations were observed in prepartum ewes (11.68pg/mL), who also showed a significant increase in the IL-6 levels in the amniotic fluid, reaching 18pg/mL. Although the increase in TNF-α concentration is associated with gestational problems, some studies indicate that this cytokine participates in triggering normal delivery in humans (Moya-Araújo et al. 2009).

Placental inflammation is an important pathological process that leads to fetal and neonatal mortality (Machado et al. 2012). The objective of placental analysis in this study was to investigate any placental alterations, including inflammatory processes. Among the most relevant histological findings, the incidence of leukocytes and macrophages at the maternal-fetal interface (Fig.3-4) may be associated with labor onset (Alves et al. 2009). However, although neutrophils are physiologically present during labor, an exacerbated increase in the number of leukocytes in the placenta may indicate a placental infection called placentitis or chorioamnionitis (Machado et al. 2012).

Regarding the presence of placental calcification (Fig.1-2) observed in this study, some authors have demonstrated that this alteration comprises aged cells in the form of knots. According to Artico et al. (2009), syncytial nodes are associated with villous necrosis, fibrin deposits, and foci of calcification, leading to placental physiological aging. Wallingford et al. (2018) reported that clinical research on placental calcification is limited and discordant, as there are studies associating the degree of calcification with gestational complications, low birth weight, and fetal distress.

The racial characteristics of the animals could be relevant to the significant difference in the maternal and fetal weights between the two groups ($p<0.05$), since most of the OGP group ewes were of the Santa Inês breed. The racial differences in this study are relevant because of their individual characteristics (Table 4). The Santa Inês breed is native to northeastern Brazil, and adaptation to adverse environmental conditions is its main characteristic. However, regarding productive aspects, this breed has higher growth speed, carcass yield, sexual precocity, and prolificacy than the Dorper breed (Silva & Araújo 2000, Souza & Leite 2000, Barros et al. 2005).

Nutrition is another relevant factor that triggers heavier and/or lighter offspring; however, both breeds in this experiment received the same diet, and despite the differences between weights, all exhibited good body condition. A Santa Inês ewe of the OCGS group being the heaviest compared to the Santa Inês ewes of the OGP group (Table 4) raises the question of why the ewes of the same breed have different weights. These findings require further investigation regarding the racial predisposition to periodontal diseases, as the Santa Inês breed is usually lighter in weight and consequently has lighter neonates when compared to the Dorper breed.

Despite growing concerns about the health and well-being of animals, only a few studies have investigated the relationship between periodontal lesions and possible systemic disorders in sheep and their possible effects on the health of sheep.

CONCLUSION

The microbiota of the oral cavity of pregnant ewes with periodontitis is mainly associated with the presence of *Fusobacterium necrophorum.* In this study, the lambs born to ewes with periodontitis had lower birth weights than those born to ewes with healthy periodontium, suggesting that oral diseases can interfere with the productive performance of sheep. The absence of hematological changes and mild or moderate inflammatory changes in the placental tissues is associated with a reduced incidence of amniotic fluid infections. Furthermore, low concentrations of inflammatory mediators, such as IL-6 and IL1-β, suggest that in the ovine species, the placental barrier can effectively prevent infection by microorganisms associated with periodontal infections.

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REFERENCES

Alves J.A.G., Câmara L.M.C., Costa F.S., Rocha R.S. & Araújo M.N.T. 2009. Os macrófagos na placenta durante o trabalho de parto. Revta Bras. Ginecol. Obstet. 31(2):90-93. <https://dx.doi.org/10.1590/S0100-72032009000200007>

Antiabong J.F., Boardman W., Smith I., Brown M.H., Ball A.S. & Goodman A.E. 2013. "Cycliplex PCR" confirmation of *Fusobacterium necrophorum* isolates

from captives wallabies: a rapid and accurated approach. Anaerobe 19:44-49. <https://dx.doi.org/10.1016/j.anaerobe.2012.12.003> <PMid: 23246462>

Ao M., Miyauchi M., Furusho H., Inubushi T., Kitagawa M., Nagasaki A., Sakamoto S., Kozai K. & Takata T. 2015. Dental infection of *Porphyromonas gingivalis* induces preterm birth in mice. Plos One 10(8):e0137249. <https://dx.doi.org/10.1371/journal.pone.0137249> <PMid:26322971>

Artico L.G., Madi J.M., Godoy A.E.G., Coelho C.P., Rombaldi R.L. & Artico G.R. 2009. Alterações histopatológicas em placentas humanas relacionadas às síndromes hipertensivas. Revta Bras. Ginecol. 31(1):10-16. <https://dx.doi.org/10.1590/S0100-72032009000100003>

Ashimoto A., Chen C., Bakker I. & Slots J. 1996. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol. Immun. 11(4):266-173. <https://dx.doi.org/10.1111/j.1399-302x.1996.tb00180.x> <PMid:9002880>

Barros N.N., Vasconcelos V.R., Wander A.E. & Araújo M.R.A. 2005. Eficiência bioeconômica de cordeiros F1 Dorper x Santa Inês para a produção de carne. Pesq. Agropec. Bras. 40(8):825-831. <https://dx.doi.org/10.1590/S0100-204X2005000800014>

Bohrer J.C., Kanemoto L.E., Almeida P.G. & Ogasawara K.K. 2012. Acute chorioamnionitis at term caused by the oral pathogen *Fusobacterium nucleatum.* Hawaii J. Med. Publ. Health 71(10):280-281. <PMid:23115747>

Borsanelli A.C., Gaetti-Jardim Júnior E., Döbereiner J. & Dutra I.S. 2015a. *Treponema denticola* in microflora of bovine periodontitis. Pesq. Vet. Bras. 35(3):237-240. <https://dx.doi.org/10.1590/S0100-736X2015000300005>

Borsanelli A.C., Athayde F.R.F., Agostinho S.D., Riggio M.P. & Dutra I.S. 2021. Dental biofilm and its ecological interrelationships in ovine periodontitis. J. Med. Microbiol. 70(7):001396. <https://dx.doi.org/10.1099/jmm.0.001396> <PMid:34313584>

Borsanelli A.C., Gaetti-Jardim Jr. E., Schweitzer C.M., Viora L., Busin V., Riggio M.P. & Dutra I.S. 2017. Black-pigmented anaerobic bacteria associated with ovine periodontitis. Vet. Microbiol. 203:271-274. <https://dx.doi.org/10.1016/j.vetmic.2017.03.032> <PMid:28619155>

Borsanelli A.C., Gaetti-Jardim Júnior E., Schweitzer C.M., Döbereiner J. & Dutra I.S. 2015b. Presence of *Porphyromonas* and *Prevotella* species in the oral microflora of cattle with periodontitis. Pesq. Vet. Bras. 35(10):829-834. <https://dx.doi.org/10.1590/S0100-736X2015001000002>

Borsanelli A.C., Lappinb D.F., Viora L., Bennett D., Dutra I.S., Brandt B.W. & Riggio M.P. 2018. Microbiomes associated with bovine periodontitis and oral health. Vet. Microbiol. 218:1-6. <https://dx.doi.org/10.1016/j.vetmic.2018.03.016> <PMid:29685214>

Borsanelli A.C., Ramos T.N.M., Gaetti-Jardim Jr. E., Schweitzer C.M. & Dutra I.S. 2016. *Treponema* species in the subgingival microflora of ovine periodontitis. Vet. Rec. 180(6):150. <https://dx.doi.org/10.1136/vr.103946> <PMid:27856942>

Calixto N.R.V., Alves C.M.C., Abreu L.M.G., Thomaz E.B.A.F., Vidal F.-C.B., Gomes Filho I.S. & Lopes F.F. 2019. Detection of periodontal pathogens in mothers of preterm birth and or low weight. Med. Oral Patol. Oral Cir. Bucal 24(6):e76-e81. <https://dx.doi.org/10.4317/medoral.23135> <PMid:31655839>

Cetin I., Pileri P., Villa A., Calabrese S., Ottolenghi L. & Abati S. 2012. Pathogenic mechanisms linking periodontal diseases with adverse pregnancy outcomes. Reprod. Sci. 19(6):633-641. <https://dx.doi.org/10.1177/1933719111432871> <PMid:22421445>

DiGiulio D.B., Romero R., Kusanovic J.P., Gómez R., Kim C.J., Seok K.S., Gotsch F., Mazaki-Tovi S., Vaisbuch E., Sanders K., Bik E.M., Chaiworapongsa T., Oyarzun E. & Relman D.A. 2010. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm prelabor rupture of membranes. Am. J. Reprod. Immunol. 64(1):38-57. <https://dx.doi.org/10.1111/j.1600-0897.2010.00830.x> <PMid:20331587>

Doyle R.M., Harris K., Kamiza S., Harjunmaa U., Ashorn U., Nkhoma M., Dewey K.G., Maleta K., Ashorn P. & Klein N. 2017. Bacterial communities found in placental tissues are associated with severe chorioamnionitis and adverse birth outcomes. Plos One 12(7):e0180167. <https://dx.doi.org/10.1371/journal.pone.0180167> <PMid:28700642>

Dutra I.S., Matsumoto T. & Döbereiner J. 1993. Surtos de periodontite em bezerros ("cara inchada") associados ao manejo do solo. Pesq. Vet. Bras. 13(1/2):1-4.

Ercan E., Eratalay K., Deren O., Gur D., Ozyuncu O., Altun B., Kanli C., Ozdemir P., Kanli C., Ozdemir P. & Akincibay H. 2013. Evaluation of periodontal pathogens in amniotic fluid and the role of periodontal disease in preterm birth and low birth weight. Acta Odontol. Scand. 71(3/4):553-559. <https://dx.doi.org/10.3109/00016357.2012.697576> <PMid:23638858>

Fouad A.F., Barry J., Caimano M., Clawson M., Zhu Q., Carver R., Hazlett K. & Radolf J.D. 2002. PCR-based identification of bacteria associated with endodontic infections. J. Clin. Microbiol 40(9):3223-3231. <https://dx.doi.org/10.1128/JCM.40.9.3223-3231.2002> <PMid:12202557>

Gaetti-Jardim Jr. E., Monti L.M., Ciesielski F.I.N., Gaetti-Jardim E.C., Okamoto A.C., Schweitzer C.M. & Avila-Campos M.J. 2012. Subgingival microbiota from *Cebus apella* (Capuchin monkey) with different periodontal conditions. Anaerobe 18(3):263-269. <https://dx.doi.org/10.1016/j.anaerobe.2012.02.002>

Hajishengallis G. 2015. Periodontitis: from microbial immune subversion to systemic inflammation. Nature 15(1):30-44. <https://dx.doi.org/10.1038/nri3785>

Kornman K.S. 2008. Mapping the pathogenesis of periodontitis: a new look. J. Periodontol. 79(8 Supl.):1560-1568. <https://dx.doi.org/10.1902/jop.2008.080213> <PMid:18673011>

Kuo L.-C., Polson A.M. & Kang T. 2008. Associations between periodontal diseases and systemic diseases: A review of the inter-relationships and interactions with diabetes, respiratory diseases, cardiovascular diseases and osteoporosis. Public Health 122(4):417-433. <https://dx.doi.org/10.1016/j.puhe.2007.07.004> <PMid:18028967>

Liang S., Ren H., Guo H., Xing W., Liu C., Ji Y., Jiang H., Zhang P. & Du M. 2018. Periodontal infection with *Porphyromonas gingivalis* induces preterm birth and lower birth weight in rats. Mol. Oral Microbiol. 33(4):312-321. <https://dx.doi.org/10.1111/omi.12227> <PMid:29754448>

Lyon K.F. 2005. Gingivostomatitis. Vet. Clin. N. Am., Small Anim. Pract. 35(4):891-911. <https://dx.doi.org/10.1016/j.cvsm.2005.02.001> <PMid:15979518>

Machado J.R., Rocha L.P., Barrilari S.E.G., Pucci K.R.M., Reis M.A., Castro E.C.C. & Corrêa R.R.M. 2012. Influência das intercorrências maternas e fetais nos diferentes graus de corioamnionite. Revta Bras. Ginecol. Obstet. 34(4):153-157. <https://dx.doi.org/10.1590/S0100-72032012000400003>

Madianos P.N., Bobetsis Y.A. & Offenbacher S. 2013. Adverse pregnancy outcomes (APOS) and periodontal disease: pathogenic mechanisms. J. Clin. Periodontol. 84(4 Supl.):S170-S180. <https://dx.doi.org/10.1902/jop.2013.1340015> <PMid:23631577>

Mayanagi G., Sato T., Shimauchi H. & Takahashi N. 2004. Detection frequency of periodontitis associated bacteria by polymerase chain reaction in subgingival and supragingival plaque of periodontitis and healthy subjects. Oral Microbiol. Immun. 19(6):379-385. <https://dx.doi.org/10.1111/j.1399-302x.2004.00172.x> <PMid:15491463>

McCourtie J., Poxton I.R., Spence J.A. & Aitchison G.U. 1989. Preliminary study of the anaerobic bacteria isolated from subgingival plaque from sheep. Vet. Microbiol. 21(2):139-146. <https://dx.doi.org/10.1016/0378-1135(89)90026-6> <PMid:2609499>

McCuaig R., Wong D., Gardiner F.W., Rawlinson W., Dahlstrom J.E. & Robson S. 2018. Periodontal pathogens in the placenta and membranes in term and preterm birth. Placenta 68:40-43. <https://dx.doi.org/10.1016/j.placenta.2018.06.310> <PMid:30055668>

Melo L.M., Bragato J.P., Venturin G.L., Rebech G.T., Costa S.F., Garcia L.E., Lopes F.L., Eugênio F.R., Santos P.S.P. & Lima V.M.F. Induction of miR 21 impairs the anti-*Leishmania* response through inhibition of IL-12 in canine splenic leukocytes. Plos One 14(12):e0226192. <https://dx.doi.org/10.1371/journal.pone.0226192> <PMid:31825987>

Moya-Araújo C.F., Prestes N.C., Piagentini M., Araújo G.H.M., Marconi C. & Silva M.G. 2009. Comparação da concentração de citocinas no líquido amnióticos de bezerros Nelore concebidos por meio de diferentes biotecnologias da reprodução. Ars Vet. 25(2):100-103.

Murtha A.P., Greig P.C., Jimmerson C.E. & Herbert W.N.P. 1998. Maternal serum interleukin-6 concentrations as a marker for impeding preterm delivery. Obstet. Gynecol. 91(2):161-164. <https://dx.doi.org/10.1016/s0029-7844(97)00602-9> <PMid:9469268>

Nadkarni M.A., Browne G.V., Chhour K.-L., Byun R., Nguyen K.-A., Chapple C.C., Jacques N.A. & Hunter N. 2012. Pattern of distribution of *Prevotella* species/phylotypes associated with healthy gingiva and periodontal disease. Eur. J. Clin. Microbiol. Infect. 31(11):2989-2999. <https://dx.doi.org/10.1007/s10096-012-1651-5> <PMid:22684253>

Pavlidis I., Spiller O.B., Demarco G.S., MacPherson H., Howie S.E.M., Norman J.E. & Stock S.J. 2020. Cervical epithelial damage promotes *Ureaplasma parvum* ascending infection, intrauterine inflammation, and preterm birth induction in mice. Nat. Commun. 11(1):199. <https://dx.doi.org/10.1038/s41467-019-14089-y> <PMid:31924800>

Radostits O.M., Gay C.C., Blood D.C. & Hinchcliff K.W. 2002. Doenças do recém-nascido, p.102-136. In: Ibid. (Eds), Clínica Veterinária: um tratado de doenças dos bovinos, ovinos, suínos, caprinos e equinos. 9ª ed. Guanabara Koogan, Rio de Janeiro.

Ramos T.N.M., Borsanelli A.C., Saraiva J.R., Vaccari J., Schweitzer C.M., Gaetti-Jardim Jr. E. & Dutra I.S. 2019. Efficacy of virginiamycin for the control of periodontal disease in calves. Pesq. Vet. Bras. 39(2):112-122. <https://dx.doi.org/10.1590/1678-5150-PVB-5922>

Ridler A.L., Vallee E., Corner R.A., Kenyon P.R. & Heuer C. 2015. Factors associated with fetal losses in ewe lambs on a New Zealand sheep farm. N. Z. Vet. J. 63(6):330-334. <https://dx.doi.org/10.1080/00480169.2015.1037813> <PMid:25980526>

Shobokshi A. & Shaarawy M. 2002. Maternal serum and amniotic fluid cytokines in patients with preterm premature rupture of membranes with and without intrauterine infection. Int. J. Gynaecol. Obstet. 79(3)209-215. <https://dx.doi.org/10.1016/s0020-7292(02)00238-2> <PMid:12445984>

Silva F.L.R. & Araújo A.M. 2000. Características de reprodução e de crescimento de ovinos mestiços Santa Inês no Ceará. Revta Bras. Zootec. 29(6):1712-1720. <https://dx.doi.org/10.1590/S1516-35982000000600017>

Silva N.S., Borsanelli A.C., Gaetti-Jardim Júnior E., Schweitzer C.M., Silveira J.A.S., Bomjardim H.A., Dutra I.S. & Barbosa J.D. 2019. Subgingival bacterial microbiota associated with ovine periodontitis. Pesq. Vet. Bras. 39(7):454-459. <https://dx.doi.org/10.1590/1678-5150-PVB-5913>

Silva N.S., Silveira J.A.S., Lima D.H.S., Bomjardim H.A., Brito M.F., Borsanelli A.C., Dutra I.S. & Barbosa J.D. 2016. Epidemiological, clinical and pathological aspects an outbreak of periodontitis in sheep. Pesq. Vet. Bras. 36(11):1075-1080. <https://dx.doi.org/10.1590/S0100-736X2016001100003>

Souza N.C., Bovino F., Avilla L.G., Deschk M., Alcindo J.F., Fink M.F.C.B., Mendes L.N.M. & Feitosa F.L.F. 2020. Assessment of fetal lung maturity using analysis of amniotic fluid from ewes that gave birth prematurely and at term. Pesq. Vet. Bras. 40(12):1039-1047. <https://dx.doi.org/10.1590/1678-5150-PVB-6544>

Souza W.H. & Leite P.R. 2000. Ovinos de Corte: a raça Dorper. Empresa Estadual de Pesquisa Agropecuária da Paraíba (Emepa-PB), João Pessoa, p.75.

Stinson L.F., Boyce M.C., Payne M.S. & Keelan J.A. 2019. The not-so-sterile womb: evidence that the human fetus is exposed to bacteria prior to birth. Front. Microbiol. 10:1124. <https://dx.doi.org/10.3389/fmicb.2019.01124> <PMid:31231319>

Sweeney E.L., Dando S.J., Kallapur S.G. & Knox C.L. 2017. The human *Ureaplasma* species as causative agents of chorioamnionitis. Clin. Microbiol. Rev. 30(1):349-379. <https://dx.doi.org/10.1128/CMR.00091-16> <PMid:27974410>

Sweeney E.L., Kallapur S.G., Gisslen T., Lambers D.S., Chougnet C.A., Stephenson S.-A., Jobe A.H. & Knox C.L. 2016. Placental infection with *Ureaplasma* species is associated with histologic chorioamnionitis and adverse outcomes in moderately preterm and late-preterm infants. J. Infect. Dis. 213(8):1340-1347. <https://dx.doi.org/10.1093/infdis/jiv587> <PMid:26671889>

Takii R., Kadowaki T., Tsukuba T. & Yamamoto K. 2018. Inhibition of gingipains prevents *Porphyromonas gingivalis* induced preterm birth and fetal death in pregnant mice. Eur. J. Pharmacol. 824:48-56. <https://dx.doi.org/10.1016/j.ejphar.2018.01.028> <PMid:29409911>

Tran T., Flynn M.J., Chen C. & Slots J. 1997. Absence of *Porphyromonas asaccharolytica*, *Bacteroides fragilis* and *Chlamydia pneumoniae* in human subgingival plaque. Oral Microbiol. Immun. 12(6):377-378. <https://dx.doi.org/10.1111/j.1399-302x.1997.tb00742.x> <PMid:9573814>

Uriza C.L., Velosa-Porras J., Roa N.S., Margarita S., Lara Q., Silva J., Ruiz A.J. & Arregoces F.M.E. 2018. Periodontal disease, inflammatory cytokines, and PGE_2 in pregnant patients at risk of preterm delivery: a pilot study. Infect. Dis. Obstet. Gynecol. 2018:7027683. <https://dx.doi.org/10.1155/2018/7027683> <PMid:30154640>

Van der Windt D., Kornegoor R., Walhof R., Overbeek B.P. & Paarlberg K.M. 2018. Septicaemia with *Fusobacterium necrophorum* from periodontal disease in pregnancy resulting in immature birth: case report and review of literature. Obstet. Gynecol. Cases Rev. 5(1):116. <https://dx.doi.org/10.23937/2377-9004/1410116>

Walker R.W., Clemente J.C., Peter I. & Loos J.F. 2017. The prenatal gut microbiome: are we colonized with bacteria in utero? Pediatr. Obes. 12(Supl.1):3-17. <https://dx.doi.org/10.1111/ijpo.12217> <PMid:28447406>

Wallingford M.C., Benson C., Chavkin N.W., Chin M.T. & Frasch M.G. 2018. Placental vascular calcification and cardiovascular health: It is time to determine how much of maternal and offspring health is written in stone. Front. Physiol. 9:1044. <https://dx.doi.org/10.3389/fphys.2018.01044> <PMid:30131710>

Weiss D. & Tvedten H. 2004. The complete blood count and bone marrow examination: general comments and selective techniques, p.14-37. In: Willard M.D. & Tvedten H. (Eds), Small Animal Clinical Diagnosis by Laboratory Methods. 4th ed. Saunders, St. Louis. <https://dx.doi.org/10.1016/B0-72-168903-5/50006-9>

West D.M. & Spence J.A. 2000. Diseases of the oral cavity, p.125-131. In: Martin W.B. & Aitken I.D. (Eds), Diseases of Sheep. 3rd ed. Blackwell Science, London. 512p.

Yarkac F.U., Gokturk O. & Demir O. 2018. Effect non-surgical periodontal therapy on the degree of gingival inflammation and stress markers related to pregnancy. J. Appl. Oral Sci. 26:e20170630. <https://dx.doi.org/10.1590/1678-7757-2017-0630> <PMid:30043934>

Ye C., Katagiri S., Miyasaka N., Kobayashi H., Khemwong T., Nagasawa T. & Yzumi Y. 2020. The periodontopathic bacteria in placenta, saliva and subgingival plaque of threatened preterm labor and preterm low birth weight cases: a longitudinal study in Japanese pregnant women. Clin. Oral Investig. 24(12):4261-4270. <https://dx.doi.org/10.1007/s00784-020-03287-4> <PMid:32333174>