Influência do tipo de substrato e tratamentos de superfície na alteração da cor e rugosidade do esmalte clareado

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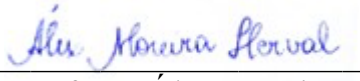

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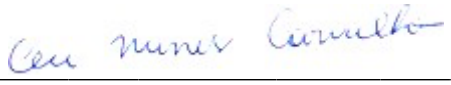

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DEDICATÓRIA

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“Se enxerguei mais longe, foi porque
estava sobre os ombros de gigantes.”

(Isaac Newton)

SUMÁRIO

RESUMO

A cor dos dentes é uma das preocupações estéticas mais importantes dos pacientes na sociedade contemporânea e os dentes brancos são considerados um padrão ideal de beleza. Uma complexa interação química e física entre os dentes e os agentes de coloração resulta em alterações de cor evidenciadas pela conjunção da cor intrínseca dos dentes e das manchas extrínsecas. As alterações extrínsecas da cor devem-se à ação de alguns medicamentos, hábitos de fumo, formação de biofilme e corantes alimentares ou de bebidas. Podem ser tratadas com profilaxia dentária e uso de protocolos de clareamento. Assim, este estudo foi dividido em dois capítulos. No capítulo 1, o objetivo do artigo foi avaliar a eficácia do clareamento em dentes bovinos em diferentes faixas etárias (24 a 36 meses e acima de 48 meses) analisando as alterações de cor após protocolos de clareamento com peróxido de hidrogênio 7,5% (técnica caseira) e com peróxido de hidrogênio 35% (técnica de consultório). Materiais e métodos: 40 coroas bovinas foram alocadas aleatoriamente em quatro grupos (n=10), de acordo com os protocolos de clareamento: caseiro - protocolo padrão com peróxido de hidrogênio (HP) 7,5% ou de consultório - protocolo padrão com HP 35%; e a idade do dente bovino: 1- Mais jovem: extraídos de animais com idade de 24 a 36 meses ou 2- Mais velhos: extraídos de animais com idade superior a 48 meses. O índice de brancura (WI) foi medido antes e após o clareamento, e a variação de cor foi avaliada usando ΔEab e ΔE00. ANOVA de duas vias foi usada para avaliar ΔEab e ΔE00, enquanto ANOVA de medidas repetidas de duas vias foi usada para analisar WI, e ambos os testes foram seguidos pelo teste de Tukey (=0,05). Resultados: A técnica de clareamento caseiro foi mais eficaz do que a técnica de consultório (ΔEab: P = 0,036/ ΔE00: P = 0,025/ WI: P<0,001) e os dentes mais velhos apresentaram mais alterações de cor do que os mais jovens (ΔEab: P=0,014/ ΔE00: P=0,010). Conclusões: A variação de cor dos tratamentos clareadores de dentes bovinos foi determinada pela idade. Dentes bovinos mais velhos clarearam de forma mais eficaz do que dentes mais jovens, e os procedimentos de clareamento caseiro produzem melhores resultados de clareamento em dentes bovinos mais velhos. No capítulo 2, o objetivo do artigo foi avaliar o efeito do polimento na rugosidade e estabilidade da cor de dentes clareados após imersão em café. Materiais e métodos: Noventa coroas bovinas foram alocadas aleatoriamente em seis grupos (n =15), de acordo com os protocolos de clareamento: caseiro - protocolo padrão com peróxido de hidrogênio (HP) 10%, ou de consultório - protocolo padrão com HP 35%; e com protocolos de polimento: (1) sem polimento, (2) esmalte clareado polido com pasta de polimento de granulação #0,5 μm ou (3) #2–4 μm. As amostras foram imersas diariamente na solução de café por 45 min seguido de simulação de escovação mecânica (30 s) por

30 dias. A rugosidade da superfície (Ra) e alteração de cor, expressa por ΔEab, ΔE00, e índice de brancura (WI) foram analisados inicialmente, após protocolos de clareamento/polimento e após pigmentação com solução de café. A superfície de cada grupo foi examinada usando um microscópio eletrônico de varredura. Os dados foram analisados por análise de variância de medidas repetidas de duas vias seguida do teste de Tukey (α = 0,05). Resultados: A imersão no café aumentou a Ra, ΔEab, ΔE00, e diminui os valores de WI. O polimento após o clareamento não evitou a alteração da cor, no entanto, o dente polido com pasta de polimento de granulação 0,5μm apresentou melhor desempenho do que a granulação 2–4 μ (ΔEab:p =0,001/ΔE00: p =0,003). O microscópio eletrônico de varredura revelou uma superfície mais irregular após a coloração com café para todos os grupos, independentemente dos protocolos de clareamento/polimento. Conclusões: O uso de pasta diamantada de granulação 0,5 μ para polir o esmalte clareado na técnica de consultório com 35% HP reduz a rugosidade e a pigmentação do dente. No entanto, o polimento após o esmalte clareado pela técnica caseira, com 10%HP, não afeta a rugosidade nem melhora a estabilidade da cor do dente após a exposição ao café.

Palavras-chave: Clareamento Dental; dente bovino; polimento; rugosidade.

ABSTRACT

Tooth color is one of the most important esthetic-related patients concerns in contemporary society and white teeth are regarded as an ideal pattern of esthetic beauty. A complex chemical and physical interaction between teeth and staining agents result in color changes evidenced by the conjunction of the intrinsic color of teeth and extrinsic stains. The extrinsic alterations of color are due to the action of some medicines, smoking habits, biofilm buildup, and food or drink dyes. It can be treated by dental prophylaxis and the use of whitening protocols. Thus, this study was divided into two chapters. In chapter 1, the purpose of the article was evaluate the efficacy of whitening on bovine teeth at different age ranges (24 - 36 months and older than 48 months) by analyzing color changes after protocols with 7.5% hydrogen peroxide (at-home technique) and with 35% hydrogen peroxide (in-office technique). Materials and methods: 40 bovine crowns were randomly allocated to four groups (n=10), according to bleaching protocols: At home: standard protocol using 7.5% hydrogen peroxide (HP) or In-office: standard protocol using 35% HP; and with tooth bovine age: 1- Younger: from animals aged 24 to 36 months or 2- Older: from cattle aged over 48 months. At baseline and after bleaching, the whitening index (WI) was measured, and also the global color change was assessed using ΔEab and ΔE00. Two-way ANOVA was used to evaluate ΔEab and ΔE00, while two-way repeated measure ANOVA was used to analyze WI, and both tests were followed by the Tukey test (=0.05). Results: The at-home bleaching technique was more effective than in-office (ΔEab: P = 0.036/ ΔE00: P = 0.025/ WI: P<0.001) and older teeth presented more color changes than younger ones (ΔEab: P=0.014/ ΔE00: P=0.010). Conclusions: The color variation of bovine teeth bleaching treatments was determined by age. Older teeth whiten more effectively than younger teeth, and at-home bleaching procedures produce better whitening outcomes in older bovine teeth. In chapter 2, the purpose of the article was evaluate the effect of polishing on roughness and color stability of bleached teeth after coffee immersion. Materials and Methods: Ninety bovine crowns were randomly allocated to six groups (n = 15), according to bleaching protocols: At-home: standard protocol using 10% hydrogen peroxide (HP) or In-office: standard protocol using 35% HP; and with polishing protocols: (1) no polishing, (2) bleached enamel polished with #0.5 µm or (3) #2–4 µm diamond particles grit pastes. Samples were daily immersed into coffee solution for 45 min followed by mechanical brushing simulation (30 s) for 30 days. The surface roughness (Ra) and color alteration, expressed by ΔE_{ab}, ΔE_{00}, and whitening index (WI) were analyzed at baseline, after bleaching/polishing protocols and after coffee solution staining. The

surface from each group was examined using a scanning electron microscope. Data were analyzed by two-way repeated measure analysis of variance followed by the Tukey test (α = 0.05). Results: Staining increases Ra, ΔE_{ab}, ΔE_{00}, and decreases WI values. Polishing after bleaching did not prevent staining, however, tooth polished with #0.5 µ-grit polishing paste showed better performance than #2–4 µ-grit (ΔE_{ab}: p = 0.001/ΔE_{00}: p = 0.003). Scanning electron microscope revealed a more irregular surface after coffee staining for all groups regardless bleaching/polishing protocols. Conclusions: Using #0.5 µ-grit diamond paste to polish 35%HP in-office bleached enamel reduces the roughness and tooth staining. However, polishing after 10%HP at-home bleached enamel neither affects roughness nor improves tooth color stability after exposure to coffee.

Keywords: Tooth Whitening; bovine tooth; polishing; roughness.

1.INTRODUÇÃO

1. INTRODUÇÃO

O sorriso harmônico e com dentes mais claros constitui um dos pilares da estética facial na atualidade (KOTHARI et al., 2019; CARVALHO et al., 2022). O clareamento dental, utilizando géis à base de peróxido de hidrogênio ou carbamida em diferentes concentrações é um procedimento conservador, não invasivo e de alta efetividade (CORDEIRO et al., 2019; KOTHARI et al., 2019). Ele tem sido associado a melhorias na qualidade de vida relacionada à saúde bucal dos indivíduos tratados, principalmente facilitando as relações interpessoais, no que tange os fatores psicossociais associados à estética e autoestima (KOTHARI et al., 2019).

Basicamente, o clareamento pode ser realizado pela técnica de consultório (DE GEUS et al., 2016; RODRÍGUEZ-MARTÍNEZ; VALIENTE; SÁNCHEZ-MARTÍN, 2019), ou pela técnica caseira (DE GEUS et al., 2016; RODRÍGUEZ-MARTÍNEZ; VALIENTE; SÁNCHEZ-MARTÍN, 2019). A técnica em consultório caracteriza-se pela aplicação de géis clareadores de concentrações mais altas do peróxido, realizada pelo cirurgião-dentista, gerando o efeito clareador mais rápido, em um curto espaço de tempo (DE GEUS et al., 2016; RODRÍGUEZ-MARTÍNEZ; VALIENTE; SÁNCHEZ-MARTÍN, 2019). Já a técnica caseira utiliza produtos com concentrações mais baixas, que são aplicados diariamente pelo próprio paciente, sendo recomendado acompanhamento profissional durante o tratamento, e resultados satisfatórios podem ser alcançados após cerca de duas semanas de aplicação (DE GEUS et al., 2016; RODRÍGUEZ-MARTÍNEZ; VALIENTE; SÁNCHEZ-MARTÍN, 2019).

Os peróxidos são considerados padrão-ouro para realização do clareamento dental, por gerar efeito oxidante, produzindo radicais livres reativos aos tecidos dentários e às manchas presentes em esmalte e dentina, assim gerando alteração de cor, tornando os dentes visivelmente mais claros. (MINOUX e SERFATY, 2008; FÉLIZ-MATOS; HERNÁNDEZ; ABREU, 2015; BARCESSAT; GURGEL-JUAREZ; WETTER, 2018).

Apesar do desenvolvimento e melhorias realizadas nas formulações dos géis clareadores, a literatura descreve alterações na morfologia da superfície do esmalte dentário após o clareamento. Essas alterações incluem a susceptibilidade ao desgaste (CARVALHO et al., 2022), aumento da rugosidade (CARVALHO et al., 2022; DA ROSA et al., 2020), perda do conteúdo mineral (CARVALHO et al., 2022; DE GEUS et al., 2016) e redução da microdureza (CARVALHO et al., 2022). Essas alterações podem estar relacionadas a fatores como a composição do agente clareador (DA ROSA et al., 2020), concentração do peróxido (DA ROSA et al., 2020; DUTRA et al., 2018), pH (DA ROSA et al., 2020; FÉLIZ-MATOS et al., 2014), tempo de aplicação (CARVALHO et al., 2022) ou protocolos de uso (DE GEUS et al., 2016). Essas modificações tornam-se indesejáveis por resultar em maior adesão do biofilme (LOGUERCIO et al., 2017; LUQUE-MARTINEZ

et al., 2016) ou de pigmentos contidos em alimentos ou bebidas corantes como o café, tornando o dente clareado mais susceptível a descoloração extrínseca (BLANCHARD et al., 2020), comprometendo a efetividade do tratamento (BLANCHARD et al., 2020). Para obter uma superfície de esmalte mais lisa, alguns fabricantes recomendam o polimento após o clareamento (RIBEIRO et al., 2020), que pode ser feito com discos abrasivos e pastas diamantadas (CORDEIRO et al., 2019; NATHOO et al., 1997). No entanto, a literatura é escassa quanto ao efeito do polimento na rugosidade da superfície do esmalte clareado, e evidências sobre os efeitos do polimento na alteração de cor dos dentes (RODRÍGUEZ-MARTÍNEZ et al., 2019) após tratamentos clareadores, com diferentes concentrações de peróxido de hidrogênio, são insuficientes e podem desempenhar um papel importante nos resultados de estabilidade de cor do clareamento.

Ademais, sabe-se que a realização de pesquisas laboratoriais prévias à pesquisa clínica é importante para criar protocolos e prever a resposta biológica dos fatores estudados. O estudo com dentes humanos tem sido cada vez mais restrito, pois muitas vezes os dentes humanos não estão disponíveis em número suficiente para fins de pesquisa, assim, dentes bovinos têm sido utilizados como substitutos para testes in vitro em diversos estudos (SEIXAS et al., 2007). Em relação à eficácia do clareamento, a idade humana correlaciona-se negativamente com o efeito clareador (REZENDE M et al., 2016), os pacientes adultos não responderam ao clareamento como os pacientes mais jovens, o que pôde ser atribuído às alterações fisiológicas, como o aumento da espessura da dentina e a redução do diâmetro dos túbulos dentinário que ocorrem nos tecidos dentais ao longo do tempo, reduzindo a permeabilidade do dente ao peróxido de hidrogênio (REZENDE M, et al, 2016). No entanto, pouco se sabe se o mesmo efeito ocorre nos dentes bovinos, extraídos de animais de diferentes idades.

Existem várias pesquisas envolvendo o clareamento dentário (CARVALHO et al., 2022; CARLOS et al., 2021; MENEZES et al., 2021; TAVARES et al., 2021; WANG et al., 2021), no entanto uma diversidade de técnica e materiais aplicados laboratorialmente nestas análises o que confere baixa qualidade de evidência científica por influência de vieses e falta de padronização, sendo ainda imperativo que se realizem estudos com protocolos bem elaborados, conduzidos e descritos (LOGUERCIO et al., 2017). Este trabalho avaliou se o tipo de substrato bovino ou o polimento realizado após o clareamento são fatores que podem modificar a eficácia dos resultados do clareamento dental caseiro e de consultório. Duas hipóteses foram avaliadas: 1- Dentes bovinos extraídos de animais acima de 48 meses de idade clareiam menos do que aqueles extraídos de animais com idade de 20 a 36 meses, quando expostos ao gel clareador de uso caseiro (peróxido de hidrogênio à 7.5%) ou de uso em consultório (peróxido de hidrogênio à 35%). 2- O polimento realizado com pastas diamantadas de diferentes

granulações, sobre o esmalte clareado pela técnica caseira ou de consultório, melhora a estabilidade de cor dos dentes após a exposição ao café.

CAPÍTULO 1

2. Capítulo 1: Does the tooth bovine age influence the bleaching effectiveness?

Este artigo será submetido na revista Journal of Esthetic and Restorative Dentistry (JERD)

Abstract

Objectives: Dental bleaching protocols are worldwide techniques used to make teeth look whiter. This study aimed to evaluate the efficacy of whitening on bovine teeth at different age ranges (24 - 36 months and older than 48 months) by analyzing color changes after protocols with 7.5% hydrogen peroxide (at-home technique) and with 35% hydrogen peroxide (in-office technique). Materials and methods: 40 bovine crowns were randomly allocated to four groups (n=10), according to bleaching protocols: At- home: standard protocol using 7.5% hydrogen peroxide (HP) or In-office: standard protocol using 35% HP; and with tooth bovine age: 1- Younger: from animals aged 24 to 36 months or 2- Older: from cattle aged over 48 months. At baseline and after bleaching, the whitening index (WI) was measured, and also the global color change was assessed using ΔEab and ΔE00. Two-way ANOVA was used to evaluate ΔEab and ΔE00, while two-way repeated measure ANOVA was used to analyze WI, and both tests were followed by the Tukey test (=0.05). Results: The at-home bleaching technique was more effective than in-office (ΔEab: P = 0.036/ ΔE00: P = 0.025/ WI: P<0.001) and older teeth presented more color changes than younger ones (ΔEab: P=0.014/ ΔE00: P=0.010). Conclusions: The color variation of bovine teeth bleaching treatments was determined by age. Older teeth whiten more effectively than younger teeth, and at-home bleaching procedures produce better whitening outcomes in older bovine teeth.

Clinical significance: To avoid bias in scientific studies, bovine teeth used in color analysis research must be age-standardized.

Keywords: Tooth Whitening; Hydrogen Peroxide; Color change; bovine tooth.

INTRODUCTION

The perfect smile is comprised of the harmonic, lined up, evident, and white teeth arrangement. Therefore, patients looking for aesthetic procedures, wish for constant dentistry development of techniques, such as bleaching protocols.[1,2] A complex chemical and physical interaction between teeth and staining agents result in color changes [3,4], evidenced by the conjunction of the intrinsic color of teeth and extrinsic stains. The extrinsic alterations of color are due to the action of some medicines, smoking habits, biofilm buildup, and food or drink dyes[3]. It can be treated by dental prophylaxis and the use of whitening protocols[5,6].

The clinical performing of bleaching protocols consists of the use of a whitening gel over the dental surface[7,8]. The product is based on different hydrogen peroxide (H2O2) or

carbamide peroxide ($CH_6N_2O_3$)[9,10] concentrations. Two common techniques are used: at-home technique and in-office. Depending on the chosen procedure, the concentration parameters of the bleaching gel and the time of use may vary. At-home bleaching is a low-cost and conservative protocol executed by the patient use of individual dental trays filled with whitening gel, under the guidance of the dentist professional. Bleaching agents such as carbamide peroxide[10] in concentrations of 10 to 22% or hydrogen peroxide of 2 to 7% are frequently used[1]. On the other hand, In-office bleaching is an alternative procedure for patients looking for faster results[11]. This technique also used carbamide and hydrogen peroxide-based gel, although in higher concentrations levels.

It is known that conducting laboratory research before clinical is important for creating protocols and predicting the biological response of the factors studied. Study with human teeth has been increasingly restricted, as human teeth are often not available in sufficient numbers for research purposes, thus, bovine teeth have been used as a substitute[12–14] for *in vitro* tests in several bleaching studies[3,5,15]. However, few studies describe whether there was a standardization of the age of the bovine teeth used. Regarding bleaching efficacy, human age is negatively correlated with the bleaching effect[16] to adult patients that did not respond to bleaching like younger patients[16], which could be attributed to physiological changes such as increased dentin thickness and reduction in the diameter of dentinal tubules that occur in dental tissues over time, reducing the permeability of the tooth to hydrogen peroxide[16]. However, little is known if the same effect occurs in bovine teeth, extracted from animals of different ages. Therefore, this study aimed to evaluate the bleaching effect using hydrogen peroxide (HP7.5%: at-home or HP 35%: in-office) to bovine younger teeth (zebu cattle aged 24–36 months) or bovine older teeth (zebu cattle aged over 48 months) on color change. The hypotheses tested were that 1) the younger teeth whitening more than older ones 2) Both at-home or in-office bleaching techniques result in similar whitening effectiveness to younger or older teeth.

MATERIALS AND METHODS

Sample Size

The Sample size calculation was based on color difference thresholds in dentistry de 0.80[17]. Ten teeth per group were required to have an 95% chance of detecting as significant at the 5% level. The calculation was performed using the statistical software package SigmaStat version 12.5 (Systat Software Inc., San Jose, CA, USA).

Specimens Preparation

Approximately 200 central incisors from zebu cattle aged 24–36 months or over 48 months age[18] were initially obtained from a local abattoir (Real, Uberlândia, MG, Brazil). The teeth were cleaned with periodontal curettes and prophylaxis with pumice and rubber cup. Forty teeth were selected to this research and were stored in distilled water at 4℃ until the experimental step, but no longer than one month. The age was estimated using a guideline.[19] The younger bovine teeth (24–36 months) were extracted from animals which central pair of temporary incisor teeth or pinchers had replaced by the permanent pinchers and the central permanent incisors attain full development. To older teeth (over 48 months age), was observed if the corner teeth had been replaced and if the animal usually had the full complement of incisors with the corners fully developed[20].

The roots were removed from the coronary portion with double-faced diamond discs (KG Sorensen, Barueri, Brazil). The crowns were parcially included in polyether resin (Maxi Rubber, Campinas, Brazil) and the buccal surface was ground using #600-grit (AROPOL-VV, Arotec, Cotia, São Paulo, Brazil) by wet grinding, sequentially, with #1200-, #1500-, #2000-grit silicon carbide papers, and alumina suspension at 0.3 μm (Buehler, Lake Bluff, IL, USA) and a felt cloth (Buehler) to obtain a polished and standardized surface[3]. The specimens were then allocated and submitted to in-office or at-home bleaching protocols (n=10) (Table 1). Figure 1 shows a schematic illustration of the experimental design. To at-home bleaching, 20 individual plastic trays were made in the dental vacuum forming molding machine. After bleaching, the specimens were ultrasonically washed for 10 min and were stored in artificial saliva, handled at the Manipulation Pharmacy (KIROPHARMA FARMÁCIA, UBERLÂNDIA, BRAZIL).

The color measurement was made using a spectrophotometer (Easyshade Compact Advance 4.0, Vita-Zahnfabrik, Bad Säckingen, Germany) at baseline and 30 days after the final bleaching session[16]. For spectrophotometer analysis, an index of polyvinylsiloxane impression material (Coltoflax and Perfil Cub; Vigodent, Rio de Janeiro, Brazil) was generated by obtaining to standardize the area of tooth color measurement during the entire experiment. A 6-mm-diameter rounded window was generated on the index corresponding to the buccal surface of the tooth to allow the position of the spectrophotometer tip. The CIELAB color difference (ΔEab) equation was calculated as follows: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$ where ΔL*, Δa*, and Δb* refers to lightness, green-red, and blue-yellow differences between baseline and other assessment times. The CIEDE2000 color difference (ΔE00) was calculated as follows: $\Delta E00 = [(\Delta L/KLSL)^2 + (\Delta C/KCSC)^2 + (\Delta H/KHSH)^2 + RT\ (\Delta C/KCSC)\ (\Delta H/KHSH)]^{1/2}$ where ΔL, ΔC and ΔH are considered lightness, chroma, and hue differences between color measurements. KL, KC, and KH are the parametric factors for viewing conditions and illuminating conditions influence. RT is the function for hue and chroma differences interaction in the blue region.

SL, SC, and SH are the weighting functions for color difference adjustment considering the location variation of L*, a*, and b* coordinates.[21] The whitening indexes (WI) were calculated at baseline and after bleaching/polishing protocols using the following formula: WI= 0.551L* - 2.324a* - 1.1b* [22,23].

Statistical analysis

Two-way ANOVA and Tukey's tests were used to compare the color differences (ΔEab and ΔE00). Two-way repeated-measures ANOVA and Tukey's tests were used to compare the WI and L*, a* and b* axis where the 'assessment time' was considered as a repetition factor. Statistical analysis was performed using Jamovi 2.0 statistical software package (dev.jamovi.org). The significance level was set at $\alpha = 0.05$ for all data analyses.

RESULTS

Color changes according to bleaching protocols and tooth bovine age are shown in Table 2. Two-way ANOVA showed influence on "bleaching technique" (ΔEab: P = 0.036/ ΔE00: P = 0.025) and to "tooth bovine age" (ΔEab: P=0.014/ ΔE00: P=0.010), but not to the interaction between factors (ΔEab: P = 0.426/ ΔE00: P = 0.365). The at-home bleaching technique was more effective than in-office. Older teeth presented more color changes than younger ones.

Whitening indexes (WI) means and standard deviations are presented in Table 3. Two-way repeated measure ANOVA showed significance for "assessment time" factor (P=0.027) and for "assessment time*bleaching technique" (P<0.001). Only the at-home technique resulted in a greater WI than the one observed at baseline regardless of the tooth bovine age. WI presented after at-home bleaching was greater than after in-office bleaching.

Figure 2 shows the CIELAB parameters. L* axis represents the degree of lightness within a specimen and ranges from 0 (black) to 100 (white). Two-way repeated measure ANOVA showed significance for "assessment time" factor (P<0.001), for "assessment time*bleaching technique" (P=0.002) and for "assessment time*tooth bovine age". Both bleaching techniques increase L* but only older teeth presented a significant increase to this parameter.

In relation to the a* parameter (red-green gradient) and the b* parameter (blue-yellow gradient), two-way repeated measure ANOVA showed significance only for "assessment time*bleaching technique" (a*: P=0.009/ b*: P < 0.001). Only the at-home technique decreases a* and b*. Tooth bleached with in-office technique revealed little increase in the mean values of b* significant differs to teeth bleached at-home.

DISCUSSION

The hypotheses of this study were rejected. The teeth of older cattle, over 48 months old, showed greater color changes than those of 24-36 months old. Moreover, at-home bleaching protocol showed better effectiveness to color change comparing to in-office bleaching technique.

Because of its low molecular weight, hydrogen peroxide can penetrate dentin and liberate oxygen and it whitens teeth by oxidizing their transparent organic matrix into an opaque whiter substance, indicating that hydrogen peroxide tooth whitening is influenced by the organic content of the teeth[4]. As a result, one of the reasons for the large range in the results produced among persons subjected to dental bleaching could be the substantial heterogeneity in organic content of teeth among people[16].

Some physiological changes in dental tissues have been described over time[24,25] as it is well known that the organic component present in teeth decreases with age due to the continuous deposition of secondary dentin and the demineralization process[25], resulting in difficult color changes in human bleaching protocols. [16] The dentin, which is rich in organic material, is primarily responsible for the color of the teeth[25]. It's possible that peritubular dentin deposition causes a rise in dentin density and a decrease in dentinal tubule diameter with time[25], resulting in teeth darkening with age[16]. It has also been noted that as people become older, the amount of enamel and crystals in their teeth rises, lowering the organic content and decreasing the permeability of the enamel to hydrogen peroxide activity[16,25]. As a result, hydrogen peroxide can't reach the organic material of the tooth structure, which is protected in older teeth due to their higher mineral content. However, because cattle do not live as long as humans, it appears that bovine teeth do not have the same amount of time to biologically mature and alter form as human teeth.

Teeth of older cattle are also darker than those of younger ones, indicating a better whitening reaction[8,16]. As a result, the whitening response of bovine teeth appears to be more dependent on the baseline color (darker in older animals) than structural changes throughout time[16] in the current investigation. Because tooth color is primarily determined by the color of dentin[26], which is an organic-rich substrate, it's reasonable to assume that better bleaching efficacy in bovine teeth with yellower reflects a higher organic content and greater availability of organic substrate for the hydrogen peroxide's oxidizing action.

In terms of bleaching efficacy, some authors claim that at-home bleaching provides better and more consistent whitening than in-office bleaching and others found that both strategies yielded similar short- and long-term results[1]. Based on the present

findings, at-home bleaching was more successful than in-office bleaching, considering the global color change. Moreover, considering *L, which is the metric that usually indicates the main aesthetic concern (darkness to lightness), the older teeth showed a considerable increase in this parameter, particularly when home whitening is used. The color change results are regulated by a time- and concentration-dependent technique. Perhaps the longer time spent with a low concentration hydrogen peroxide allowed for a higher whitening degree than a higher concentration product utilized for a shorter time to bovine teeth. However, in terms of changes compared to established visual thresholds[27], both bleaching processes tested had color change values that were higher than the acceptable threshold (Eab = 2.66 and E00 = 1.77, respectively)[27].

The whitening index (WI), a simple linear formulation obtained using the values of the three CIELab chromatic coordinates, was utilized in conjunction with the global color change.[22,23] Because it correlates with the perception of tooth whiteness, this measure is an important step in assessing color change. The outcomes of this method are more therapeutically relevant and easier to interpret: high positive WI values suggest higher whitening levels. Although tooth yellowness isn't a precise antonym for tooth whiteness, WI can be used to represent perceptual yellowness.[22,23] Similar to ΔE00 and ΔEab and regardless of the tooth bovine age, only the at-home approach resulted in a higher WI than the baseline. WI was higher after at-home bleaching than in-office. However, it is important to note that all of the techniques tested had values higher than the acceptable threshold (2.20 for dentists and 2.95 for laypeople)[23], and in terms of visual discrimination of the whiteness difference on a scale of 0 to 3, the results after bleaching can be considered hardly acceptable[23], regardless of the protocols used or the age of the cattle teeth.

Finally, laboratory research can be utilized to forecast how well dental materials and treatments will function. Even if research lab cannot replicate all aspects of the oral environment, it can be conducted more rapidly and at a lower cost than randomized clinical trials. However, in light of the findings of this study, consideration should be made to the usage of bovine teeth. Standardizing bovine age for color change analysis in dental research is highly recommended to reduce study bias.

CONCLUSION

The age of the bovine tooth on bleaching technique was a determining factor in color variance. Older teeth whitening is more effective than younger teeth, and older bovine teeth submitted by at-home bleaching technique have better effect in terms of whitening results. Thus, researchers should use cattle teeth of similar age for *in-vitro color changes analysis to avoid bias in* scientific studies.

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TABELAS

Table 1. Components and protocols of products used

Protocols	Product	Manufacturer	Composition	Protocols of Use
At-home	White class 7.5%	FGM Odontology Products, Joinville, SC, Brazil	7.5% hydrogen peroxide, neutralized carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water and surfactant.	14 applications for 1 hour
In-office	Whiteness HP AutoMixx		After mixing the phases: 35% hydrogen peroxide, thickeners, neutralizer, calcium gluconate, glycol, green dye, deionized water.	2 applications for 45 minutes

Table 2- Mean and standard deviation of color changes parameters (ΔEab and ΔE00) for two bleaching techniques, according to bovine tooth age.

Bleaching techniques	**Bovine tooth age**			
	Younger (24-36 months)		*Older (≥48 mounths)*	
	ΔEab	*ΔE00*	*ΔEab*	*ΔE00*
At-home	6.3 ± 3.2 ^{Ba}	3.63 ± 1.99 ^{Ba}	10.3 ± 5.8 ^{Aa}	6.16 ± 3.24 ^{Aa}

Different letters (uppercase for comparing the tooth bovine age - in lines for each color parameter; lowercase for comparing bleaching techniques – in columns) indicate significant difference at Tukey`stest (P<0.05).

Table 3- Mean and standard deviation of Whiteness Index (WI) according to bovine tooth age, bleaching techniques and assessment time.

Bleaching technique	Bovine tooth age	Baseline	After bleaching
At-home	20-36m	20.2 ± 8.6 Ab	28.2 ± 8.6 Aa*
	≥48m	15.6 ± 10.6 Ab	26.7 ± 12.2 Aa*
In-office	20-36m	21.6 ± 10.5 Aa	24.4 ± 6.8 Aa
	≥48m	13.8 ± 10.0 Aa	16.0 ± 13.3 Aa

*Different letters (uppercase for comparing the tooth bovine age – in columns); lowercase for comparing assessment time – in lines); *symbol to compare assessment time*bleaching technique indicating significant difference at Tukey`s test (P<0.05).*

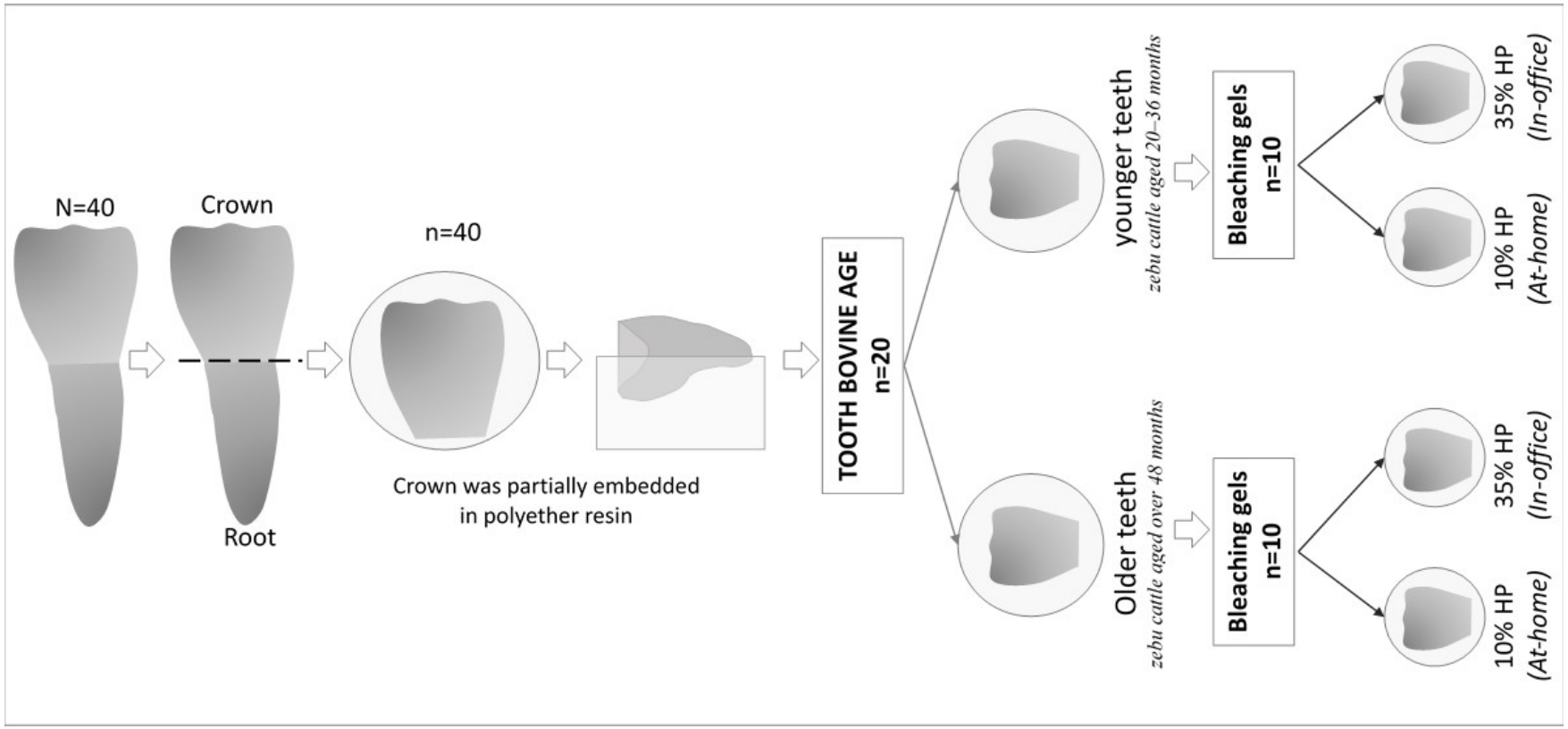

Figure 1- Schematic illustration of the experimental design.

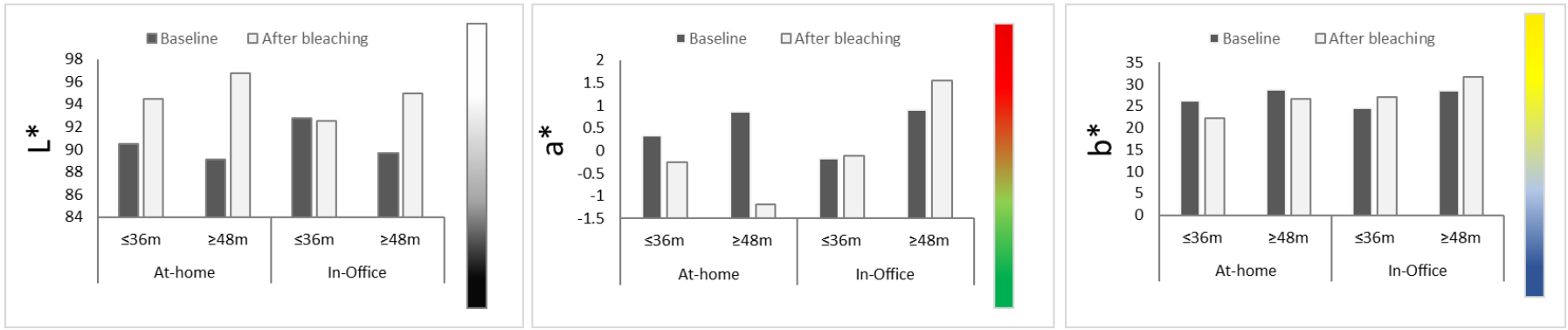

Figure 2- **I-** The L* axis represents the degree of lightness within a specimen and ranges from 0 (black) to 100 (white). *Different letters (uppercase for comparing the tooth bovine age*assessment time and lowercase for comparing assessment time*bleaching techniques) indicate significant difference.* **II**- a* represents the degree of green/red. *Different letters (lowercase for comparing assessment time*bleaching techniques) indicate significant difference.* **III**- b* represents the degree of blue/yellow. *(lowercase for comparing assessment time*bleaching techniques) indicate significant difference. Tukey`s test (P<0.05).*

3. CAPÍTULO 2

3. Capítulo 2: Does polishing of bleached enamel affect roughness and tooth color stability after exposure to coffee?

Artigo publicado na revista Journal of Esthetic and Restorative Dentistry (JERD).

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ABSTRACT

Objective: This laboratory randomized study was designed to evaluate the effect of polishing on roughness and color stability of bleached teeth after coffee immersion. Materials and Methods: Ninety bovine crowns were randomly allocated to six groups (n = 15), according to bleaching protocols: At-home: standard protocol using 10% hydrogen peroxide (HP) or In-office: standard protocol using 35% HP; and with polishing protocols: (1) no polishing, (2) bleached enamel polished with #0.5 μm or (3) #2–4 μm diamond particles grit pastes. Samples were daily immersed into coffee solution for 45 min followed by mechanical brushing simulation (30 s) for 30 days. The surface roughness (Ra) and color alteration, expressed by ΔEab, ΔE00, and whitening index (WI) were analyzed at baseline, after bleaching/polishing protocols and after coffee solution staining. The surface from each group was examined using a scanning electron microscope. Data were analyzed by two-way repeated measure analysis of variance followed by the Tukey test (α = 0.05). Results: Staining increases Ra, ΔEab, ΔE00, and decreases WI values. Polishing after bleaching did not prevent staining, however, tooth polished with #0.5 μ-grit polishing paste showed better performance than #2–4 μ-grit (ΔEab: p = 0.001/ΔE00: p = 0.003). Scanning electron microscope revealed a more irregular surface after coffee staining for all groups regardless bleaching/polishing protocols. Conclusions: Using #0.5 μ-grit diamond paste to polish 35%HP in-office bleached enamel reduces the roughness and tooth staining. However, polishing after 10%HP at-home bleached enamel neither affects roughness nor improves tooth color stability after exposure to coffee.

Clinical Significance: Polishing after at-home bleaching does not have benefits but after 35% hydrogen peroxide in-office bleaching, the polishing with #0.5 μ-grit polishing paste is indicated to reduce roughness and the tooth staining over time.

KEYWORDS: bleaching, color, enamel, polishing, roughness

INTRODUCTION

Tooth color is one of the most important esthetic-related patients' concerns in contemporary society and white teeth are regarded as an ideal pattern of esthetic beauty.[1] Bleaching treatment produces positive changes in young patients' quality of life,[1] and it leads to judgments that are more positive on personality traits such as social competence

and appeal, intellectual ability, and relationship satisfaction.[1] Bleaching is a conservative, effective, and non-invasive procedure that is performed using gels containing hydrogen peroxide or carbamide peroxide at different concentrations. Dental bleaching can be accomplished at the dental clinic (in-office bleaching) and at home by following professional instructions,[1–5] by also using prefabricated trays, whitening strips, and paint-on applications without the dentist's supervision.[2]

Despite all the developments in bleaching materials, there is still concern about enamel surface morphology alteration after bleaching procedures.[6,7] These alterations include the susceptibility of the enamel surface to wear,[8] increase in surface roughness,[6,9] loss of min- eral content,[6,10] reductions in surface microhardness,[6] or translu- cency.[11] Enamel alterations occur due to several factors including bleaching agents composition,[9] peroxide concentration,[9,12] pH,[9,13] application time,[6] a storage medium (water or saliva) that sample is maintained during in vitro test,[14] and bleaching protocols used.[10] These surface alterations may lead to greater adhesion of biofilms[15,16] and pigments by beverages[17] such as coffee[5,18] to bleached tooth sur- face making it susceptible to extrinsic discoloration, compromising treatment effectiveness.[5]

The application of remineralizing agents such as fluoride[19,20] and calcium-based formulations[19,20] or bioactive glass[20] was found to restore the morphological defects caused by bleaching reducing the surface irregularities and roughness,[19,20] although none of the agents were able to completely reverse the negative effect of high- concentration in-office HP on enamel.[19,20] To achieve a smoother enamel surface, some manufacturers recommend polishing after bleaching,[21] which can be done with abrasive disks and polishing pas- tes.[7,19] Commercially available polishing pastes are typically composed of a lubricant base, thickener, emulsifier, and micronized diamonds with different particle sizes.[7] However, the literature is scarce regarding the effect of polishing on surface roughness of bleached enamel, and evidence toward the effects of polishing on tooth color changes[22] after bleaching treatments with different hydrogen peroxide concentrations is insufficient and could play an important role on bleaching color stability outcomes.

Therefore, this study aimed to evaluate polishing effect, using pastes with different grit sizes, on roughness and color stability of bleached tooth (at-home or in-office) submitted to staining in coffee solution. The hypotheses tested were that 1) the diamond polishing pastes (#0.5 μm-grit or #2–4 μm-grit) affect roughness and color stability of bleached enamel; and 2) the polishing of enamel (bleached with 10%HP or 35%HP) improves the tooth color stability after expo- sure to coffee.

MATERIALS AND METHODS

Sample size

The Sample size calculation was based on a previous study.[5] Fifteen teeth per group were required to have an 99% chance of detecting as significant at the 5% level (2-sided test), with a mini- mum detectable difference in means of 3.64 with an expected standard deviation of 2.18 with regards to the primary outcome (color discoloration—ΔE_{ab}), evaluated by a sphere spectrophotometer. The calculation was performed using the statistical software package SigmaStat version 12.5 (Systat Software Inc., San Jose, CA, United States).

Specimens preparation

Ninety freshly extracted bovine incisors were obtained, cleaned with periodontal curettes and prophylaxis with pumice and rubber cup, and stored in deionized water in a refrigerator at 4°C until the experimental step, but no longer than 1 month. The roots were removed from the coronary portion with double-faced diamond discs (KG Sorensen, Barueri, Brazil). The crowns were sectioned and were embedded in polyether resin (Maxi Rubber, Campinas, Brazil) and the buccal surface was ground using #600-grit (AROPOL-VV, Arotec, Cotia, S~ao Paulo, Brazil) by wet grinding, sequentially, with #1200-, #1500-, #2000-grit silicon carbide papers, and alumina suspension at 0.3 μm (Buehler, Lake Bluff, IL, United States) and a felt cloth (Buehler) to obtain a polished and standardized surface. The specimens were then numbered, randomly allocated and submitted to different bleaching/ polishing protocols (n = 15) (Table 1). Figure 1 shows a schematic illustration of the experimental design. The randomization process (blocked random scheme) was carried out using the website www. sealedenvelope.com. The identification of the treatment to be applied on samples followed the sequentially numbered previously. Randomization and allocation were made by the same researcher (GRS), who was not involved in the implementation (RFC) and evaluation process (AMG). Because they did not know the coding method, those who assessed color measures (AMG) and statically evaluated the data (GRS) were blinded.

Roughness measurements

The surface roughness was analyzed using a roughness meter (Mitutoyo, Aurora, IL, United States) at baseline, after bleaching/ polishing protocols and after coffee staining. The mean roughness (Ra) was measured with a static load of 5 N and a speed of 0.05 mm/s. The cutoff value was 0.25 μm in a sequential mode, and the measurement distance was 1 mm. Three readings were made for each specimen from the center of the surface, and the arithmetical mean was calculated.

Color analysis

The colors of each specimen were measured using a visible/ultraviolet reflection spectrophotometer (Ci64UV, X-Rite, Grand Rapids, MI, United States) and rated according to the color system established by the Commission Internationale de L'Eclairage (CIE), which is based on the dimensions L^* (white to black), a^* (red to green), and b^* (yellow to blue). The color was measured at baseline, after bleaching/polishing protocols and after coffee staining by placing the specimens at the same position for both measurements. The specimens were positioned in focus on a clear acrylic stand, and measurements were performed with a standard illuminant D65, with wavelength ranging from 400 to 700 nm, and with the specular light included (SPIN mode). Due to the spectrophotometer spherical shape, the object was diffusely illuminated, and the detector received the reflected light at 88° with the enamel surface. The color measurements were performed in triplicate over a white background (L^*_{white} = 95.2, a^*_{white} = 21.2, b^*_{white} = 50.3), and the mean values were used for data analysis. The whitening indexes (WI) were calculated at baseline and after bleaching/polishing protocols using the following formula: WI = 0.551 L^* — 2.324a^* — 1.1b^*.[23,24] Overall color changes from baseline compared with other assessment times (after bleaching/ polishing or after coffee staining) were calculated using both ΔE_{ab} and ΔE_{00} formulas.[25] The CIELAB color difference (ΔE_{ab}) equation was calculated as follows: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$ where ΔL^*, Δa^*, and Δb^* refers to lightness, green-red, and blue-yellow differences between baseline and other assessment times. The CIEDE2000 color difference (ΔE_{00}) was calculated as follows: $\Delta E_{00} = [(\Delta L/K_L S_L)^2 + (\Delta C/K_C S_C)^2 + (\Delta H/K_H S_H)^2 + R_T (\Delta C/K_C S_C) (\Delta H/K_H S_H)]^{1/2}$ where ΔL, ΔC, and ΔH are considered lightness, chroma, and hue differences between color measurements. K_L, K_C, and K_H are the parametric factors for viewing conditions and illuminating conditions influence. RT is the function for hue and chroma differences interaction in the blue region. S_L, S_C, and S_H are the weighting functions for color difference adjustment considering the location variation of L^*, a^*, and b^* coordinates[25].

Bleaching/polishing protocols

The prepared teeth were submitted to bleaching/polishing sessions in a room under controlled temperature (25.0 ± 1.0°C). The bleaching agents were applied according to the manufacturer's instructions. Table 1 shows the materials used, their protocols, compositions, and manufacturers. Using a plastic pipette tip, the enamel surface (8 × 8 mm) received 0.02 ml of bleaching gel for each session and removal of the bleaching agents was done initially with gauze, followed by washing with distilled water for 10 s, and then the specimens were placed in an ultrasonic cleaner (Cristo´foli, Campo Mour~ao,

PR, Brazil) for 10 min to complete removal of the gel. In control groups, the polishings procedures were not performed.

For the experimental groups (Figure 1), after bleaching (in-home or in-office), the specimens were submitted to polishing using a standardized amount of diamond polishing paste #0.5 μm-grit (Ultradent) or #2–4 μm-grit (FGM) with a felt wheel (FGM) applied on the enamel surface with intermittent movements, maintaining the same pressure during 30 s. The same cleaning protocol used to remove bleaching gel was used to remove the polishing paste. Subsequently, the specimens were stored in deionized water at 37°C for 24 h until the next assessment time.

Staining protocol

After the initial readings of color and surface roughness, all samples were submitted to staining with coffee. The coffee manufacturer states that the average time for consumption of one cup of a drink is 15 min, and, among coffee drinkers, the average consumption of cof fee is 3.2 cups per day.[26] The coffee was prepared with 500 ml of hot water and 15 g of soluble coffee (Nescafe Original, Nestle, Araras, SP, Brazil).[27] The pH of coffee solution was measured with a pH meter (Adwa, Szeged, Hungary), which monitors the degree of acidity or alkalinity via an electrode coupled to a potentiometer (potential differ ence meter). The pH measurements were calibrated using a standard buffered potassium chloride solution. Three measurements were taken and the average was obtained. The pH electrode was calibrated with standard solutions before each measurement to ensure the sen sitivity of the pH meter. The pH mean of coffee solution was 4.9.

The samples were daily immersed individually in a 5 ml coffee solution for each sample for 45 min (3 immersions of 15 min) per 30 days. After each immersion, to simulate the oral hygiene, the specimens were washed in distilled water for 10 s and then were submitted to mechanical brushing cycles in a toothbrushing machine (Odeme Dental Research, Luzerna, SC, Brazil) which was performed with vertical loading of 300 g over the toothbrush heads, temperature-controlled (35 ± 1°C) executing a linear motion for 30 s. A toothpaste slurry (Colgate Total 12, Palmolive Company, New York, NY, United States) was prepared with distilled water at a 1:3 (wt/vol) pro portion and applied during the brushing cycles. Daily, after staining and mechanical brushing cycles, the specimens were ultrasonically washed for 10 min and were stored in distilled water at 37°C. At the end of 30 days, the specimens were dried and reevaluated for rough ness and color.

Surface morphology

The surface of one specimen from each group was examined for finished surface morphology. The specimens were subjected to vacuum in a sputter coater (SCD 050 Sputter Coater, Capovani Brothers Inc, New York, United States) to deposit a thin layer of gold before sub- mitting to SEM (Zeiss EVO MA10, Jena, Turingia, Germany).

Statistical analysis

Two-way repeated-measures analysis of variance (ANOVA) and Tukey's tests were used to compare the roughness and the color parameters (ΔE_{ab}, ΔE_{00}, and WI), the "assessment time" was considered as a repetition factor. Statistical analysis was performed using SigmaPlot 12.5 statistical software package (Systat Software). The significance level was set at $\alpha = 0.05$ for all data analyses.

RESULTS

Color change according to bleaching/polishing protocols after staining are shown in Table 2. Two-way repeated measure ANOVA showed no influence on "bleaching/polishing protocols" (ΔE_{ab}: $p = 0.102$/ ΔE_{00}: $p = 0.138$) but on "assessment time" (ΔE_{ab}: $p < 0.001$/ΔE_{00}: $p < 0.001$), and the interaction between both factors were significant (ΔE_{ab}: $p = 0.008$/ΔE_{00}: $p = 0.009$). Polishing after bleaching did not prevent the staining, but the use of #0.5 µ-grit polishing paste made teeth bleached in-office less stained than those polished with the #2-4-grit one (ΔE_{ab}: $p < 0.001$/ ΔE_{00}: $p < 0.001$).

WI means and standard deviations are presented in Table 3. Two- way repeated measure ANOVA showed significance only for "assessment time" factor ($p < 0.001$). The staining procedure resulted in a lower WI than the one observed at baseline or after bleaching/polishing. Alterations in color parameters $*L$, $*a$, and $*b$ are illustrated in Figure 2.

The mean and standard deviations of Ra are shown in Table 4. No significant difference was verified between the bleaching/ polishing protocols for surface roughness ($p = 0.852$); however, "assessment time" ($p < 0.001$), and the interaction between both factors were significant ($p = 0.002$). In-Office bleaching caused significant increasing on Ra values, however, the polishing procedures promoted the Ra return for the baseline values, reestablishing the enamel smoothness. Coffee solution significantly increased the Ra of bleached enamel mostly when 35%HP is used compared to 10%HP.

Figure 3 is an SEM images of enamel surface (1; bleaching with 10% HP (at-home), 2; bleaching with 35% HP (in-office), BS, before coffee staining or AS, after coffee staining. Photomicrographs of representative areas were taken at 500× magnification for all groups. SEM characterization of the enamel surfaces after different surface treatments revealed a

more irregular surface after coffee staining for all groups regardless bleaching/polishing protocols.

DISCUSSION

Based on the present findings, the hypothesis that polishing pastes affect enamel roughness and color changes of the bleached tooth was accepted. Polishing after bleaching did not prevent staining but it was able to reduce the roughness generated by 35% HP reestablishing the baseline enamel smoothness. However, the use of #0.5 μ-grit diamond polishing paste showed to be more suitable after in-office with 35% HP bleaching for reducing the coffee staining. The polishing procedure had no significant effect after 10% HP at-home bleaching regarding roughness and color changes rejecting the second hypothesis. Particularly in the present study, the sample size calculation was based on color outcomes from a previous study[5] assuming a minimum difference (3.64) and standard deviation (2.18) between bleached and stained teeth with coffee compared to teeth with not submitted to any staining solution. The coffee was used as a staining solution in this study and then, the mean assumed difference was above the acceptability threshold (ΔE_{ab} = 2.66).[22] The differences in the mean values among the treatment groups were greater than would be expected by chance and a statistically significant difference was found.

For measuring color in dentistry, the CIELAB color difference formula has been extensively used, allowing the comparison with previous similar studies.[2,13] It assumes the uniformity of CIELAB color space and the equal importance of CIELAB individual parameters (*L**, *a**, and *b**). However, a sensitivity discrepancy on the *L**, *a**, and *b** parameters has been demonstrated concerning visual perceptibility and acceptability thresholds.[22,24] The CIEDE2000 metrics have also been proposed due to better indicative of human visual thresholds, even closer with the adjustment of parametric factors KL, KC, and KH set (2:1:1).[25] Similar results were observed for both color difference parameters in this study. Some increase in lightness after bleaching/ polishing protocols and decreasing after coffee staining were observed. In general, the immersion in coffee solution increased tooth redness and yellowness (Figure 2). Regarding the differences relative to the established visual thresholds, all bleaching/polishing techniques assessed had color change values higher than what is considered the acceptable threshold (ΔE_{ab} = 2.66 and ΔE_{00} = 1.77).[22]

Associated with the global color change, the WI, a simple linear formulation obtained using the values of the three CIELab chromatic coordinates, was used.[23] This parameter represents a significant step for color change assessment because it correlates with the perception of tooth whiteness. The results of this method are more clinically

relevant and provide a clearer interpretation: high positive values of the WI indicate higher whitening values. Tooth yellowness may not be a perfect antonym of tooth whiteness, but WI could be used to reflect perceptual yellowness.[23] Despite not having shown statistical difference after coffee staining, greater numerical whiteness changes were seen after staining in “In-office/2–4 μm” group compared to “In-office/0.5 μm,” tending WI reduction. All studied groups showed an increasing WI after bleaching and decreasing after staining. It is important to note that all techniques tested had values higher than what is considered the acceptable threshold (2.20 to dentist and 2.95 to Lay- person)[24] and regarding the visual discrimination of the whiteness dif ference on a scale from 0 to 3, the outcomes can be considered hardly acceptable after both assessment time (bleaching and staining) regardness the protocols tested.

Coffee is a popular beverage worldwide, it is rich in pigments and is reported to have the greatest potential to cause tooth discoloration,[5,17] possibly due to the high concentrations of metals that coffee contains.[18] Moreover, coffee promotes loss in teeth mineral phase and demineralizes areas causing open spaces among the enamel prisms and dentinal tubules.[18] This beverage possesses low pH (4.9),[17] which could lead to a reduction in Ca and P concentrations. The sequestering of these elements causes not only stains on the external enamel surface but also affects the internal structure integrity.[18] Besides, coffee causes a decrease in inorganic elements crucial in dental structure, it increases other elements such as Mg and K which most likely facilitate enamel erosion.[18] The low pH of coffee might induce enamel dissolution and increase surface porosity, increasing roughness, and thus promoting tooth discoloration. According to results, coffee immersion increases about four times tooth staining and 50% roughness average after bleaching. Note that in this study, beyond coffee immersion, enamel surface was wear by brushing cycles, which could have turned the enamel surface more susceptible to morphological changes.[8,9] Nevertheless, in the oral cavity, the saliva is a key element protecting dental tissues or dissolving the enamel to induce erosion, according to the Ca/P concentrations.[18] In the present study, the salivary conditions were not included since the aim was to simulate the worst-case scenarios. However, saliva plays a buffer role to regulate acidity and it should be considered for further studies because it is possible that enamel remineralization produced by saliva could be effective in preventing tooth porosity and staining after bleaching treatment.[18]

Bleaching products have also been associated with enamel demineralization process through the oxidation mechanism caused not only by the pH of the peroxides but also some components of the whitening agents.[9,12] The free radicals are formed during peroxide oxidation and act nonspecifically being able to degrade both the organic and inorganic substrate matrix, causing enamel loss and affecting the ionic balance that would

enable calcium deposition on the enamel surface.[9] The enamel roughness values increased only after bleaching with 35% HP. Previous studies have indicated that 30%–35% hydrogen peroxide causes superficial alterations, increasing on average the surface roughness[12] and reduces calcium-phosphorus ratio[8] although the literature has reported that products currently available for dental bleaching have fewer detrimental effects due to the modernizing changes[9] made in their composition over time and 35% HP (pH 8.1) has been considered a neutral/ alkaline bleaching gel.[9] Polishing with diamond pastes reduced enamel roughness, especially the ones bleached with 35% HP, equalizing Ra values with the ones obtained at baseline. Some structural enamel changes following in-office bleaching such as susceptibility to wear,[8] reductions in sur- face microhardness,[6] and roughness increasing[6,9] as possible causes of both better-polished surface immediate after polishing as well as more stained, that is, more susceptible to the acidic effect of coffee. However, after coffee immersion, all protocols resulted in similar enamel Ra values, regardless the bleaching/ polishing protocol used.

When the average roughness is considered, the 35% HP caused less change in the enamel surface roughness than the one caused after staining protocol. Regardless the bleaching/polishing protocol, coffee doubled the roughness values in all groups and that leads us to think that the superficial change could not have had great clinical relevance when peroxides are utilized or that coffee should be used with caution because it causes considerable roughness and staining. SEM revealed a more irregular surface after coffee staining for all groups regardless bleaching/polishing protocols. However, it is known that saliva could minimize this deleterious effect by remineralization process. However, it was observed that polishing after bleaching with 35% HP regardless the diamond paste used, was able to return the enamel roughness to values found initially before bleaching. These diamond pastes have a Knoop hardness 7000–10,000 kg/mm^2, significantly higher than the Knoop hardness of human enamel which is 350–380 kg/mm^2, being able to soften the depressions and erosions produced by bleaching procedures. The roughness of intraoral hard tissues and restorative materials can influence plaque retentions[16] and discoloration,[5] and a smaller size of diamond particles in the polishing paste seem to be more favorable after 35% HP bleaching. The polishing does not minimize the deleterious effect that has occurred, but the use of #0.5 μ-grit polishing paste after in-office bleaching possibly reduces the staining over time due to enamel smoothness restablishment.

Although comparisons among bleached enamel polishing were not the main objective of some randomized clinical trials,[3,4] some conflicting long-term outcomes have been shown. Some studies that enamel polishing was performed have reported color stability[3] or color rebound.[4] On the other hand, when the teeth are not polished both outcomes have been shown, no color change over time,[13] or dental darkening even after

at-home bleaching.[4] However, this study is justified. Laboratory studies offer a predictive performance of mate- rials and techniques in dentistry. Despite not simulating all oral environment conditions, laboratory research can be executed more quickly and with lower costs compared to randomized clinical trials.

CONCLUSIONS

Based on the results of this study it was concluded that after in-office bleaching using 35% HP, the use of diamond polishing paste with #0.5 μ-grit reduces the roughness and enamel staining. However, polishing after 10% HP at-home bleached enamel neither affects roughness nor improves tooth color stability after exposure to coffee.

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Protocols		Product	Manufacturer	Composition	Protocols of use
Bleaching	At-home	White Class 10%	FGM Odontology Products, Joinville, SC, Brazil	10% hydrogen peroxide, neutralized carbopol, potassium nitrate, sodium, fluoride, calcium gluconate, stabilizer, deionized water and surfactant.	Fifteen applications for 30 min
	In-office	Whiteness HP Blue Calcium		After mixing the phases: 35% hydrogen peroxide, thickeners, neutralizer, calcium gluconate, glycol, inert blue or violet dye, deionized water.	Two applications for 40 min
Polishing	# 2–4 µm-grit diamond paste	Diamond Excel		Mixture of lubricant base, thickener and emulsifier using micronized diamond (#2–4 µ-grit).	After bleaching (at home or in- office), a small amount of diamond polish with a felt wheel was applied on the enamel surface with intermittent movements for 30 s
	# 0.5 µm-grit diamond paste	Diamond Polish Mint	Ultradent Products Inc., South Jordan, UT, United States	Mixture of diamond Powder (#0.5 µm-grit), Sucralose, Oils, Peppermint, water.	

TABLE 1: Components and protocols of products used.

TABLE 2: Means (standard deviation) of the color difference (ΔE_{ab} and ΔE_{00}) after bleaching/polishing protocols and staining (n = 15).

	Color parameters			
	Baseline		Stained	
Bleaching/polishing protocols	ΔE_{ab}	ΔE_{00}	ΔE_{ab}	ΔE_{00}
At-home	4.6 ± 2.3^{Aa}	3.2 ± 1.4^{Aa}	16.0 ± 8.9^{Bab}	11.6 ± 6.8^{Bab}
In-office	5.5 ± 2.5^{Aa}	3.7 ± 1.7^{Aa}	16.7 ± 9.0^{Bab}	12.3 ± 7.5^{Bab}
At-home/0.5 μ	5.5 ± 2.2^{Aa}	3.7 ± 1.4^{Aa}	14.2 ± 4.7^{Bab}	10.0 ± 3.5^{Bab}
At-home/2–4 μ	4.4 ± 1.8^{Aa}	3.0 ± 1.2^{Aa}	13.6 ± 4.6^{Bab}	9.6 ± 3.5^{Bab}
In-office/0.5 μ	4.7 ± 3.0^{Aa}	3.2 ± 2.1^{Aa}	10.6 ± 5.2^{Ba}	7.6 ± 3.7^{Ba}
In-office/2–4 μ	4.0 ± 2.1^{Aa}	2.7 ± 1.5^{Aa}	18.9 ± 6.0^{Bb}	13.6 ± 4.6^{Bb}

TABLE 3: Means and SD of whitening indexes of bleached/polished teeth measured in each assessment time (n = 15)

	Whiteness index parameter				
		Bleached/polished		Stained	
Bleaching/polishing protocols	Baseline (T0)	(T1)	ΔWI_{T1-T0}	(T2)	ΔWI_{T2-T1}
At-home	30.7 ± 3.8^{Ba}	37.5 ± 2.9^{Aa}	6.8 ± 3.8	17.0 ± 10.3^{Ca}	20.4 ± 12.4
In-office	27.9 ± 3.5^{Ba}	34.5 ± 3.2^{Aa}	6.6 ± 4.1	15.3 ± 8.8^{Ca}	19.3 ± 9.7
At-home/0.5 μ	28.4 ± 4.4^{Ba}	36.1 ± 2.8^{Aa}	7.7 ± 3.8	20.0 ± 5.9^{Ca}	16.1 ± 6.9
At-home/2–4 μ	30.3 ± 5.6^{Ba}	35.3 ± 2.8^{Aa}	5.0 ± 4.4	20.8 ± 6.5^{Ca}	14.5 ± 7.1
In-office/0.5 μ	29.8 ± 6.2^{Ba}	33.4 ± 4.9^{Aa}	3.6 ± 4.8	20.3 ± 5.0^{Ca}	13.1 ± 8.3
In-office/2–4 μ	31.8 ± 3.5^{Ba}	36.2 ± 3.2^{Aa}	4.5 ± 4.2	14.4 ± 8.2^{Ca}	21.9 ± 8.7

Note: Different letters (uppercase for comparing the assessment times—in lines; lowercase for comparing bleaching protocols—in columns) indicate significant difference at Tukey's test ($p < 0.05$).

TABLE 4: Means (SD) of the roughness average (μm) after bleaching/ polishing procedures according to assessment times (n = 15)

Bleaching/polishing protocols	Assessment time		
	Baseline	Bleached/polished	Stained
At-home	0.14 ± 0.06^{Aa}	0.17 ± 0.07^{Aa}	0.28 ± 0.12^{Ba}
In-office	0.08 ± 0.03^{Aa}	0.20 ± 0.05^{Ba}	0.47 ± 0.31^{Ca}
At-home/0.5 μ	0.13 ± 0.09^{Aa}	0.13 ± 0.06^{Aa}	0.34 ± 0.14^{Ba}
At-home/2–4 μ	0.11 ± 0.06^{Aa}	0.13 ± 0.06^{Aa}	0.40 ± 0.15^{Ba}
In-office/0.5 μ	0.13 ± 0.10^{Aa}	0.18 ± 0.15^{Aa}	0.27 ± 0.12^{Ba}
In-office/2–4 μ	0.14 ± 0.07^{Aa}	0.20 ± 0.16^{Aa}	0.37 ± 0.16^{Ba}

Note: Different letters (uppercase for comparing the assessment times—in lines; lowercase for comparing bleaching protocols—in columns) indicate significant difference at Tukey's test (p < 0.05).

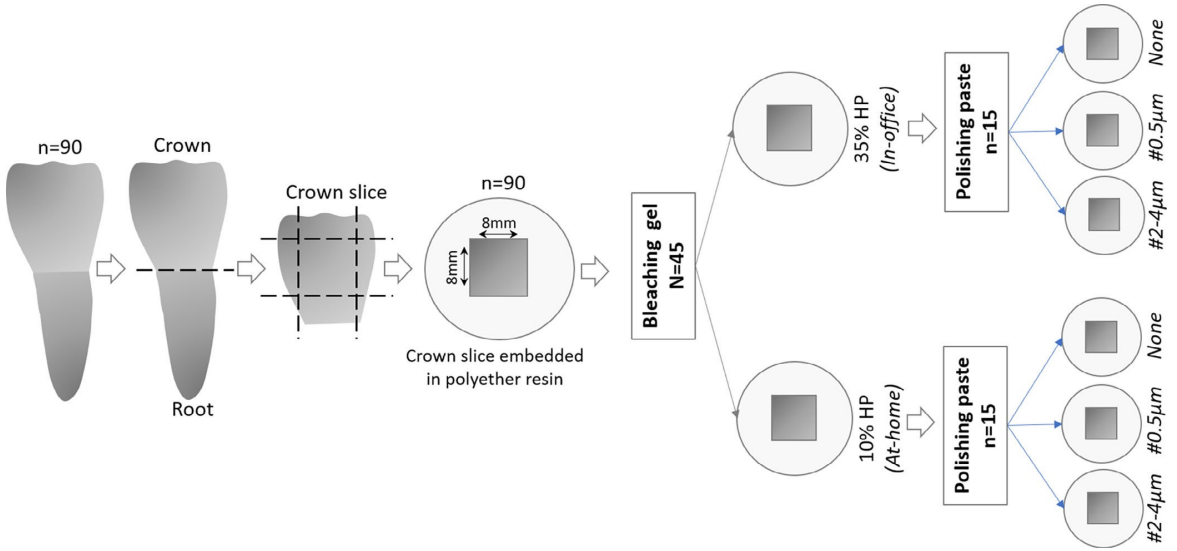

FIGURE 1: Schematic illustration of the experimental design.

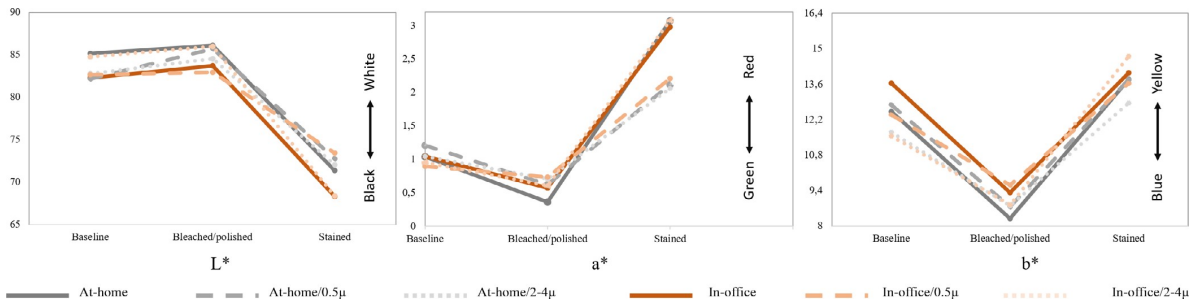

FIGURE 2: Mean *L**, *a**, and *b** values measured on the baseline, bleaching/polishing and staining. Color parameters according to CieLab.*L**, represents the lightness from 0 (black) to 100 (white). *a**, represents the degree of green/red measurement; *b**, represents the degree of blue/yellow.

4. CONSIDERAÇÕES FINAIS

A partir das hipóteses avaliadas, concluiu-se que:

1. A idade do dente bovino foi um fator determinante na variação de cor na técnica de clareamento. O clareamento em dentes mais velhos é mais eficaz do que em dentes mais jovens, e dentes bovinos mais velhos submetidos à técnica de clareamento caseiro têm melhor efeito em termos de resultados de clareamento. Assim, os pesquisadores devem usar dentes bovinos de idade semelhante para análise de mudanças de cor *in vitro* para evitar viés nos estudos científicos.

2- Após o clareamento de consultório com peróxido de hidrogênio a 35%, o uso de pasta de polimento diamantada de granulação # 0,5 μm reduz a rugosidade e a pigmentação do esmalte. No entanto, o polimento após clareamento caseiro com peróxido de hidrogênio a 10% não afeta a rugosidade nem melhora a estabilidade da cor do dente após a exposição ao café.

5. REFERÊNCIAS BIBLIOGRÁFICAS

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6. ANEXOS

Anexo 1: Normas da revista Journal of Esthetic and Restorative Dentistry

Journal of Esthetic and Restorative Dentistry now offers Free Format submission for a simplified and streamlined submission process.

Manuscripts can be uploaded either as a single document (containing the main text, tables and figures), or with figures and tables provided as separate files. Should your manuscript reach revision stage, figures and tables must be provided as separate files. The main manuscript file can be submitted in Microsoft Word (.doc or .docx).

Your main document file should include:

1. A short informative title containing the major key words. The title should not contain abbreviations
2. The full names of the authors with institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
3. Acknowledgments;
4. Abstract structured (intro/methods/results/conclusion) or unstructured
5. Up to seven keywords;
6. Main body: formatted as introduction, materials & methods, results, discussion, conclusion
7. References;
8. Tables (each table complete with title and footnotes);
9. Figures: Figure legends must be added beneath each individual image during upload AND as a complete list in the text.

Abstract

A structured abstract of no more than 200 words must be provided for each article. Footnotes, references, and abbreviations are not used in the abstract.

For original research articles, the abstract should include the following headings and sections: (1) Objective. This section includes a statement of the problem and the purpose of the study, (2) Materials and Methods. This section should include materials, methods and statistical analyses employed in the study. (3) Results. (4) Conclusions.

For clinical technique articles and case reports, the abstract should include the following headings and sections: (1) Objective. This section includes a statement of the problem and a general description of the topic or treatment to be addressed. (2) Clinical

Considerations. This section should include a brief description of the clinical materials and techniques employed. (3) Conclusions.

For systematic literature review articles, the abstract should include the following headings and sections: (1) Objective. This section should include a statement of the topic to be reviewed and a description of the search strategy of relevant literature (search terms and databases), (2) Materials and Methods. This section should contain inclusion criteria (language, type of studies i.e. randomized controlled trial or other, duration of studies and chosen endpoints). (3) Results. This section should include evaluation of papers and level of evidence. (4) Conclusions.

For general review articles the abstract should include the following headings and sections: (1) Objective. This section should include a statement of the topic to be reviewed. (2) Overview. This section should include a brief summary of the findings of the review. (3) Conclusions.

In addition to Abstracts, all papers should include the following:

Clinical Significance

In a few sentences, please indicate the clinical importance and implications of the research or clinical technique discussed, and if applicable, its relevance to esthetic dentistry.

Keywords

Add at least five keywords that reflect the primary content of the paper.

Main Text

Clinical and laboratory/fundamental research papers

Well written and properly structured research hypotheses are the central core of every section of a research manuscript. All research should be hypothesis-driven and clinical and laboratory research manuscripts must state proper research hypotheses, based on the pre-existing knowledge and scientific background supplied in the Introduction. The research hypothesis does not have to be assumed to be correct – it is perfectly acceptable if the research hypotheses are invalidated, as long as the authors provide substantive preliminary rationale for initiating the test, and subsequent information identifying factors that influenced the outcome. The null hypothesis should not be the framework of a paper based on the scientific method. Null hypotheses are applicable only when the collected data are structured for statistical analysis.

Introduction: Provide sufficient background and listing of pre-existing knowledge (references) that support the anticipated outcome of the work. As a general rule, no new references should be introduced past this section. The only exception are references used

in supporting Materials and Methods. Do not use author names in the paper: instead of, e.g., Smith at al. reported…, use One study (or similar) reported that ___.34
(where 34 corresponds to the reference by Smith et al.)

State the purpose of your research. This portion should be presented as a paragraph on its own. Within this paragraph, describe the major experimental factors, parameters being measured, and experimental control.

Lastly, clearly state the research hypotheses, labeled as such, and provide a numerical listing of each hypothesis. This listing is key to the paper. The same sequence of hypothesis testing will be used to structure the Materials and Methods, Results, Discussion, and the Conclusions sections.

Materials and Methods: Follow the sequence of the listing of the research hypotheses in describing parameter testing. The detail level in this section should be such that someone experienced in the art and science of those methods could easily reproduce the same experiment in their laboratory.

Describe methods of statistical analysis and provide justification of sample size from pilot testing. The pre-set level of a Type 1 statistical error (the alpha) should be mentioned here as well. Usually, testing is performed at a pre-set alpha of 0.05, meaning that a significant difference exists with 95% confidence.

Note: Do not use Co., Corp. GmbH, Inc., ®, ©, ™, and similar in manuscripts.

Results: Present the results of the findings in the same sequence as the experimental parameters described in the Materials and Methods. If parametric statistical methods were used, provide the initial normality and equivalence of variation results. If those tests are not passed, indicate such and also provide what non-parametric analyses was used instead.

Present the data only once – in either tabular or graphical format. Using either method, provide an embedded coding system to identify groups that were identified as statistically not different if appropriate. Indicate the significance level of each major experimental factor, as well as any interaction terms (p-values).

Discussion: Without repeating the purpose of the research, start this section with addressing individual research hypotheses, ideally in separate, sequential paragraphs. Start with a sentence indicating if the experimental data upheld or invalidated the corresponding research hypothesis. After that, compare and contrast the current findings related to this hypothesis with work performed by others in the field (references from the Introduction). Provide insights as to why or why not similar information was found.

After addressing individual research hypotheses, put together the knowledge gained from these findings into one coherent theme. Discuss the clinical/research significance of the

findings or the significance of this new knowledge over that in the existing literature. This is where the author is allowed to speculate for the first and only time.

Provide a paragraph on the study limitations. Applying the research findings outside of the experimental design needs to be taken with caution. Lastly, provide insight as to what types of research need to be done as a consequence of the new knowledge found in the current project.

Conclusions should contain no speculative statements – only the facts as they are limited to what the data reveal about the tested research hypotheses, following their order. It is good to preclude the listing of conclusions with “Within the limitations of this current study, it was concluded that:”

1. Address Research Hypothesis #1
2. Address Research Hypothesis #2
3. and so on.

Do not use conditional/modal auxiliary verbs such as can, could, may, might, must, shall, should, will, would (It was concluded, not It can be concluded). Avoid interpretation and/or comparison of study results with literature findings and do not use abbreviations and acronyms in the conclusion section.

References

References should be numbered consecutively in the order in which they are first mentioned in the text, and listed at the end of the text in numeric, not alphabetic, order. Identify references in text, tables, and legends by Arabic numerals in superscript. References cited only in tables or figure legends should be numbered subsequent to the numbering of references cited in the text. Unpublished sources, such as manuscripts in preparation and personal communications, are not acceptable as references. Only sources cited in the text should appear in the reference list. List all authors when four or fewer; when more than four, list the first three and add "et al."

How to Format Citations

Journal Articles:

Donnelly PV, Miller C, Ciardullo T, et al. Occlusion and its role in esthetics. J Esthet Restor Dent., 1996; 8(2):111-118

Books:

Hickey JC, Zarb GA. Boucher's prosthodontic treatment for edentulous patients. 9th ed. St. Louis (MO): CV Mosby; 1985.

Tables

Type or print out each table with double spacing on a separate page. Ensure that each table is cited in the text, number tables consecutively in the order of their first citation in the text, and provide a brief title for each. Give each column a brief, descriptive heading. No table should contain data that could be included in the text in several sentences.

Figure Legends

Please include on a separate page all figure and/or illustration legends. This page should be clearly marked. Figure legends must be numbered to correspond with the figures and typed or printed on a separate page. Symbols, arrows, or letters used to identify parts of the illustration must be explained clearly in the legend. If a figure has been previously published, the legend must acknowledge the original source.

Figures and Illustrations

Images must be submitted as individual files, in either TIF or EPS format, as indicated below.

COLOR photographs should be saved as TIF files in CMYK at a minimum of 12.5 cm (5 in.) in width at 300 dpi.

BLACK AND WHITE photographs should be saved as TIF files in grayscale at a minimum of 12.5 cm (5 in.) in width at 300 dpi.

Line drawings should be prepared in Microsoft Word or PowerPoint, or in Adobe Illustrator without embedded images from other sources. Existing line drawings should be scanned at 1,200 dpi at a minimum of 12.5 cm (5 in.) in width and saved as EPS files.

All images must be labeled clearly in consecutive order with the figure number and part. Photomicrographs must feature internal scale markers. Symbols, arrows, or letters used in these should contrast with the background. Original magnification must be provided.

Figure reproduction cannot improve on the quality of the originals. It does not correct the exposure, sharpen the focus, or improve the contrast of the original print. Any special instructions about sizing, placement, or color should be clearly noted. Electronic submissions are not returned to the authors.

Guidelines for Cover Submissions

If you would like artwork related to your manuscript to be considered to appear on the cover of the journal, you will be able to indicate which image files should be considered in the system at the time of submission.

Miscellaneous Formatting Guidelines

Product trade names cited in the text must be accompanied by a generic term, and followed by the manufacturer, city, and state/country in parentheses.

References in the text and figure legends to teeth illustrated in a figure should be identified by name (eg, upper right central incisor), not by number.

The manuscripts submitted to the journal must be written in appropriate English. It is the author's responsibility to ensure this by either having sufficient English language skills or by obtaining the services of an English-as-second-language expert.

Please note that the term “esthetic” should be used in manuscripts as opposed to the alternative spelling “aesthetic.”

The same general headings and sections should be used in the articles as used in the abstract.

PERMISSIONS

Written permission must be obtained for material that has been published in copyrighted material; this includes tables, figures, and quoted text that exceeds 150 words.

Photographs of People

The Journal of Esthetic and Restorative Dentistry follows current HIPAA guidelines for the protection of patient/subject privacy. If an individual pictured in a digital image or photograph can be identified, his or her permission is required to publish the image. The journal may not collect consent forms under HIPPA guidelines, however authors are expected to be able to present a signed consent form if asked. Authors must have patient authorization for images, or else the image/photo must be altered such that the individual cannot be identified (black bars over eyes, etc).

Manipulation of Digital Photos

Authors should be aware that the Journal considers digital images to be data. Hence, digital images submitted should contain the same data as the original image captured. Any manipulation using graphical software should be identified in either the Disclosure and Acknowledgements section or the caption of the photo itself. Identification of manipulation should include both the name of the software and the techniques used to enhance or change the graphic in any way. Such a disclaimer ensures that the methods are repeatable and ensures the scientific integrity of the work.

No specific feature within an image may be enhanced, obscured, moved, removed, or

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The removal of artifacts or any non-integral data held in the image is not allowed. For instance, removal of papillae or "cleaning up" of saliva bubbles is not allowed.

Cases of deliberate misrepresentation of data will result in rejection of a manuscript, or if the misrepresentation is discovered after a manuscript's acceptance, revocation of acceptance, and the incident will be reported to the corresponding author's home institution or funding agency.

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All papers will be subject to examination with the iThenticate Professional Plagiarism Prevention program (**www.ithenticate.com**) prior to publication to look for plagiarism and unintentional duplication of wording from other sources. It is important to ensure that the papers are fully original in content to protect both the author and the journal. If the paper is found to include block segments of words or sentences that clearly come from another source or sources, the author will be asked to re-write the section and/or reference the material appropriately. If excessively large portions are found to have been copied verbatim, the paper may be subject to rejection.

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Anexo 2: Parecer da Comissão no Uso de Animais (CEUA UNICEUMA)

CEUMA – UNIVERSIDADE
Reitoria
Gerências de Graduação e Pós-Graduação
Comissão de Ética no Uso de Animais – CEUA UNICEUMA

DECISÃO DA CEUA – UNICEUMA SOBRE PROTOCOLO SUBMETIDO

DATA DO RECEBIMENTO: 03/07/2019

Nº DO PROTOCOLO: 126/19

Nº DO PARECER: 12/19

DATA DO PARECER: 05/11/2019

TÍTULO DO PROJETO/AULA: Influência do óleo no clareamento dentário caseiro

CARACTERÍSTICAS DA AMOSTRA: Dentes bovinos da raça Nelore, com cerca de 2/3 anos de idade e 300Kg de peso limpo

PESQUISADOR/PROFESSOR RESPONSÁVEL: Roberta Furtado Carvalho

COLABORADORES: Gisele Rodrigues da Silva

DECISÃO: (X) APROVADO ( ) PENDENTE ( ) EXCLUÍDO ( ) NÃO APROVADO

A CEUA-UNICEUMA, em sua função de examinar previamente os procedimentos de ensino e pesquisa a serem realizados na Instituição, para determinar sua compatibilidade com a legislação aplicável (Lei. 11794 e Resoluções do Conselho Nacional de Controle de Experimentação Animal – CONCEA). Reuniu-se no dia 05/11/2019, para apreciar a análise do relator da proposta de protocolo nº **126/19**, tendo chegado por votação da maioria dos membros presentes, as seguintes considerações:

Considerações: O objetivo deste estudo é o de avaliar a influência do óleo de ozônio na eficácia do clareamento dental, rugosidade do esmalte dentário e microdureza do esmalte dentário.

O pesquisador propõe usar dentes de bovinos de produção, ou seja, de animais abatidos para uso na alimentação humana e/ou animal, que seriam descartadas. A comissão entendendo suas atribuições, concluiu que não há objeção quanto a realização do projeto, por se tratar de matéria inerte oriunda de animais cujo abate só pode ser realizado sob fiscalização de órgãos publicos, que por princípio também devem zelar pelo bem estar do animal.

Conclusão: Aprovado

Com base nos dados fornecidos pelo proponente, a Comissão, autoriza o protocolo supracitado, devendo o presente documento ser apresentado a Coordenação do Biotério, para agendamento do início dos procedimentos.

Cópia do protocolo segue anexa.

São Luís 05/11/2019

Lidio Gonçalves Lima Neto
Coordenador CEUA-UNICEUMA