Original Article

Effect of Various COVID-19 Vaccines on Dental and Palatal Development of Albino Rats Offspring

Rabab Hassan[1] and Basma Abdelrahman Ahmed[2]

[1]Department of Oral Biology, [2]Department of Oral Pathology, Faculty of Dentistry, Ain Shams University, Cairo, Egypt

ABSTRACT

Introduction: Many vaccines have been developed to alleviate the risk of infection against the pandemic corona virus disease 2019 (COVID-19). Tooth and palatal development are influenced by acquired or inherited variables that may affect the general health. The effect of vaccines on organ development is an essential step for vaccine safety insurance**.**

Aim of the study: To detect the effect of different types of COVID-19 vaccines on tooth and palatal development..

Materials and Methods: Sixty female rats were arranged into four groups. Each group received either intramuscular injection of saline (Group I), mRNA vaccine (Group II), adenoviral vector vaccine (Group III), or inactivated vaccine (Group IV) at 21 and 14 days before mating and on the ninth day of gestation. The heads of the offspring "one day old" were collected, processed and stained by hematoxylin and eosin (H&E), Beta-catenin (β-catenin) and transforming growth factor–Beta2 (TGF-β2). The expressions of β-catenin and TGF-β2 in the developing tooth germ and palate were analyzed statistically.

Results: No histological or morphological changes in the developing teeth and palate were recorded in all studied groups except for the developing tooth germs in groups III and IV. All groups showed positive β-catenin and TGF-β2 immunoreactivity in the developing tooth germ and palate. Statistically, the vaccinated groups showed a significant difference in immunopositive area% to Group I, except β-catenin in Group II, and TGF-β2 of the developing palate in groups II and IV.

Conclusion: mRNA COVID-19 vaccine is safer than adenoviral vector and inactivated vaccines regarding tooth or/and palatal development.

Received: 14 March 2024, **Accepted:** 31 March 2024

Key Words: β-catenin; COVID-19 vaccines; palate; TGF-β2 ; tooth germ.

Corresponding Author: Rabab Hassan, PhD, Department of Oral Biology, Faculty of Dentistry, Ain-Shams University, Cairo, Egypt, Organization of African Unity St., Cairo, Egypt. **Tel.:** +20 012 2410 1540, **E-mail:** rabab.hassan@dent.asu.edu.eg

ISSN: 1110-0559, Vol. 47, No. 4

INTRODUCTION

Corona virus disease 2019 (COVID-19) has affected millions of people worldwide since being formally announced a pandemic by the World Health Organization (WHO) on March 11, 2020[1]. Several types of COVID-19 vaccines have been developed within a year of the first reports of COVID-19[2], such as nucleic acid-based messenger RNA (mRNA) vaccine[3], vaccines using adenoviral vectors[4], and inactivated vaccine[5].

The lipid nanoparticle formulated nucleoside-modified mRNA vaccine demonstrated 95% efficacy and ≥90 % effectiveness in clinical trials and direct use studies respectively. It proved great success in preventing COVID-19 in adults[3, 6,7].

Adenovirus-vector protein vaccine successfully brought about immunogenicity with 81% efficacy[4,8]. Additionally, its production needs limited resources, as it does not require the same cold-chain management and is more affordable than the mRNA-based COVID-19 vaccines[9].

Inactivated or dead virus based vaccine has shown good immunogenicity in experimental animals[10], besides demonstrating 43.7% to 70.2% efficacy against symptomatic COVID-19[11].

Data have shown that pregnant women might be at higher risk related to COVID-19 infection, nevertheless with the development of COVID-19 vaccines, there was an increasing potential to alleviate this risk[12,13].

Some animal studies used mRNA and adenoviral vector vaccines without recording any risk on pregnant animals or developmental deformities on their neonates[14,15]. Furthermore, studies performed on pregnant women revealed the safety of different Covid-19 vaccines administration during pregnancy, as there was no detection of maternal or neonatal death[16,17].

Although WHO permits the use of different types of vaccines during pregnancy, the Royal College of Obstetricians and Gynaecologists recommended mRNA vaccines in pregnancy as most of the present studies were related to this type of vaccines without detection of any safety concerns. Yet, a number of countries put limitations

DOI: 10.21608/ejh.2024.276902.2041

on vaccination during pregnancy; some of them do not recommend vaccination during pregnancy, while others prevented vaccination in the first trimester due to very limited studies on vaccination in early pregnancy and long term follow up of infant development and growth[18].

Developmental abnormalities caused by genetic changes, either inherited, or acquired from physical, chemical and biological sources, are able to cause alteration in number, shape, size and structure of teeth[19,20]. Additionally, they affect the growth, elevation, or fusion of the palatal shelves[21].

Wingless - integrated / Beta-catenin (Wnt/β-catenin) canonical pathway is one of the pathways essential for the development and function of many cells and tissues, including craniofacial skeleton, muscles and teeth[22,23]. β-catenin is a key mediator for the canonical pathway, it also functions as an adhesive junctional protein through its complex with E-cadherin[24].

Tooth development is initiated by Wnt/β-catenin signaling which is considered upstream to other signalling pathways related to tooth formation[25]. Excessive activation or inactivation of β-Catenin in the formed tooth epithelium cause developmental abnormalities in the form of supernumerary teeth[22,26] or arrest at bud stage[27] respectively. During palatogenesis, β-catenin epithelial-mesenchymal interactions act a pivotal role in palatal elevation and fusion[27, 28].

On the other hand, transforming growth factor-Beta (TGF-β) is associated with multiple biological actions, including cell growth, apoptosis, synthesis and degradation of extracellular matrix, along with the development of multiple craniofacial structures[29]. TGF-β2 isoform is expressed in all stages of odontogenesis and is related specifically to cellular differentiation[30], besides being expressed during adherence of the palatal shelves[31,32].

From the previous data, this study aimed to evaluate the effect of differently synthesized COVID-19 vaccines on dental and palatal development in albino rat's offspring and to assess the expression of β-catenin and TGF-β2 in these tissues.

MATERIALS AND METHODS

Ethical Statement

The current research followed the ARRIVE guidelines 2.0 for reporting animal research and was approved by the Research Ethics Committee of the Faculty of Dentistry, Ain Shams University, Cairo, Egypt (FDASU-Rec IR102208).

Animals

Sixty fertile female Wistar albino rats weighing around 220g were used and housed in cages (five rats/ cage), until the time of mating when each three female rats were housed with one healthy fertile male rat in one cage at the animal house of "Medical Research Centre" of Ain-Shams University. Rats were maintained in a room with good ventilation and received regular diet composed of carbohydrates, protein and fresh vegetables till labour.

COVID-19 vaccines

Nine vials of mRNA (1.8 ml), six vials of adenoviral vector (5 ml) and six vials of inactivated (5 ml) COVID-19 vaccines were obtained from the Egyptian Ministry of Health and Population.

Animal grouping

Female rats were arranged into four groups (15 rats each), the rats received either intramuscular injection of saline (Group I Control), 30 µg/0.3 ml/dose of mRNA vaccine (Group II)[14], 0.5 ml/dose of adenoviral vector vaccine (Group III)[33], or 0.5 ml/dose of inactivated vaccine (Group IV)[34].

The rats were administered a total three doses of either saline or vaccines 21 and 14 days before mating and on the ninth day of gestation, which was confirmed by the collected vaginal smears[14].

Sample collection and preparation

After birth, one newborn (one day old) of each female rat was sacrificed by overdose of anaesthesia (Ketamine) and was considered the sample of the present study, (n=15) from each group.

The heads of the newborns were dissected, divided coronally in front of external ear into two halves, fixed in 10% phosphate buffered formalin solution for five days, immersed in ethylene diamine tetra-acetic acid for 1 week, dehydrated, and embedded in paraffin wax[35]. Afterwards, sections were cut into 4 µm thickness and stained by hematoxylin and eosin (H&E), in addition to β-catenin and TGF-β2 immunohistochemical markers.

H&E staining

After mounting the sections, on regular microscopic slides, deparaffinizing in xylene, and rehydrating in descending concentrations of ethanol, they were finally stained by H&E stain[35].

Immunohistochemical staining

Sections were mounted on positively charged microscopic slides and microwaved for 20 min in 0.01mol/L citrate buffer (pH 6) to retrieve the antigen. Blocking of endogenous peroxidase was performed using 3% of H2O2, while blocking of the non-specific reactions was done by incubating the sections in goat serum for 30 min. Next, β-catenin (14) mouse monoclonal primary antibody, (Cell Marque Corporation, USA, Catalog# 224M) and TGF-β2 polyclonal primary antibody, (Boster Biological Technology, Pleasanton CA, USA, Catalog # A00892) were used with dilution 1:200 each at 4°C for 24 h. Following that, sections were incubated in biotinylated secondary antibody with streptavidin-horseradish peroxidase conjugate. For developing brown colour, diaminobenzidine was added to the sections for 10

min. Lastly, sections were counterstained by using Mayer hematoxylin[36,37]. The positive brown immunoreactivity appeared cytoplasmic and membranous in β-catenin[38] and cytoplasmic in TGF-β2[30].

All slides were captured at 40× and 400× magnifications by light microscope (Olympus® BX 60, Tokyo, Japan).

Histomorphometric analysis

Non overlapping six fields of the developing tooth germs and three fields of the developing palate from one section of each rat in all four groups, stained with β-catenin and TGF-β2 antibodies were captured at 400×. The positive immunoreactivity area% of these fields was calculated using Image analysis software (Image J, 1.41a, NIH, USA) after conversion of the captured images into 8-bit monochrome and performing colour thresholding.

Statistical analysis

Statistical Package for the Social Sciences 20 (SPSS® Inc., Chicago, IL, USA) was used to analyze the recorded area% from the histomorphometric analysis. Data were presented as mean values ± standard deviation (±SD). One-way ANOVA test for comparing between all studied groups was used followed by Tukey's post hoc test for pairwise comparison. *P-value* < 0.001 or ≤ 0.05 or >0.05 indicates high significant, significant or insignificant results respectively.

RESULTS

H&E results

Tooth development

All examined upper and lower first molar tooth germs of Group I appeared in the early bell stage and were connected to the oral epithelium by lateral dental lamina. Dental organ enclosed dense dental papilla and both were surrounded by a less dense dental sac (Figure 1a). Dental organ showed tall columnar inner dental epithelium resting on a thin basement membrane. Two to three rows of flatten stratum intermedium cells, network of stellate reticulum cells and cuboidal outer dental epithelium cells were noticed. Dental papilla appeared dense with numerous cells. Dental sac appeared loose and was formed of spindle shaped fibroblasts connected to each other by cytoplasmic processes (Figure 1b).

In Group II, all upper and lower first molar tooth germs represented early bell stage and were connected by lateral dental lamina to the oral epithelium (Figure 1c). Dental organ, dental papilla and dental sac showed almost the same histological picture as in Group I except for the outer dental epithelium cells that appeared more flattened (Figure 1d**)**.

All first molar tooth germs of Group III demonstrated early bell stage with changed morphology in the upper tooth germ, while intact lateral dental lamina connecting both tooth germs to oral epithelium was noticed (Figure 1e). Tall columnar inner dental epithelium, flatten stratum intermedium cells formed of two to three rows, and cuboidal outer dental epithelium cells were detected. Some areas of cellular degeneration were observed in-between stellate reticulum cells. Dense dental papilla and loose dental sac were seen (Figure 1f).

Group IV first molar tooth germs appeared in the early bell stage, showed morphological changes, and were connected to the oral epithelium by ordinary lateral dental lamina (Figure 1g). Inner dental epithelium showed cellular degeneration and were detached from basement membrane at some regions. Several rows of stratum intermedium cells and network of stellate reticulum cells were detected. A flat and discontinued layer of outer dental epithelium cells was noticed. Dental papilla showed thin layer of predentin, single layer of columnar shaped odontoblasts with apically located nucleus, and numerous spindle shaped cells. Dental sac with spindle shaped cells was observed (Figure 1h).

Palatal development

All examined specimens in all groups revealed fusion of bilateral palatine shelves with remnant of midline epithelial seam in-between **(**Figures 2 a,c,e,g**)**. The midline epithelial seam was obviously detected in Group III (Figure 2e). With a higher magnification, the developing secondary palate was covered from the oral side by keratinized stratified squamous epithelium. The underlining connective tissue showed spindle shaped fibroblasts connected by cytoplasmic processes, some blood vessels and traces of midline epithelial seam. Intact connective tissue in all groups was noticed, it appeared dense in GroupI (Figure 2b), and Group IV (Figure 2h), and loose in Group II (Figure 2d) and Group III (Figure 2f).

Immunohistochemical and statistical results

β-catenin result

Tooth development

All groups showed positive immunoreactivity that was more detectable in all dental organs and odontoblasts more than other cells in dental papilla and dental sac. The highest mean area% was recorded in Group I (Figure 3a**)**, followed by Group II (Figure 3b**)** that showed no statistically significant difference to Group I, then Group III (Figure 3c). The least mean area% was recorded in Group IV (Figure 3d) that showed no statistically significant difference to Group III (Table 1, Figure 3i).

Palatal development

Positive immunoreactivity was apparently observed in the oral epithelium more than the connective tissue cells in all studied groups. The midline epithelial seam showed apparently few positive immunoreactivity in groups I and II, and negative immunoreactivity in the other two groups. Group I (Figure 3e**)** represented the highest mean area%, followed by Group II (Figure 3f) with no statistically significant difference between both groups, then followed by Group IV (Figure 3h). Group III (Figure 3g) showed the least mean area% (Table 1, Figure 3j**).**

TGF-β2 result

Tooth development

All groups showed more obvious positive immunoreactivity in the inner dental epithelium cells and odontoblasts than other cells in the dental organ, dental papilla and dental sac. Moreover, Group I showed detectable positive immunoreactivity in outer dental epithelium cells. Group I (Figure 4a) recorded the highest mean area%, then Group III (Figure 4c), followed by Group II (Figure 4b) with no statistically significant difference between it and Group III. Group IV (Figure 4d) represented the least mean area% (Table 1, Figure 4i).

Palatal development

Positive immunoreactivity was observed in the oral epithelium and connective tissue cells of all groups. Only Group III showed positive immunoreactivity in the midline epithelial seam. Group III (Figure 4g) revealed the highest mean area%, followed by Group IV (Figure 4h), then Group II (Figure 4f), and the least mean area% was recorded in Group I (Figure 4e). There was no statistically significant difference between Group I, II and IV (Table 1, Figure 4j).

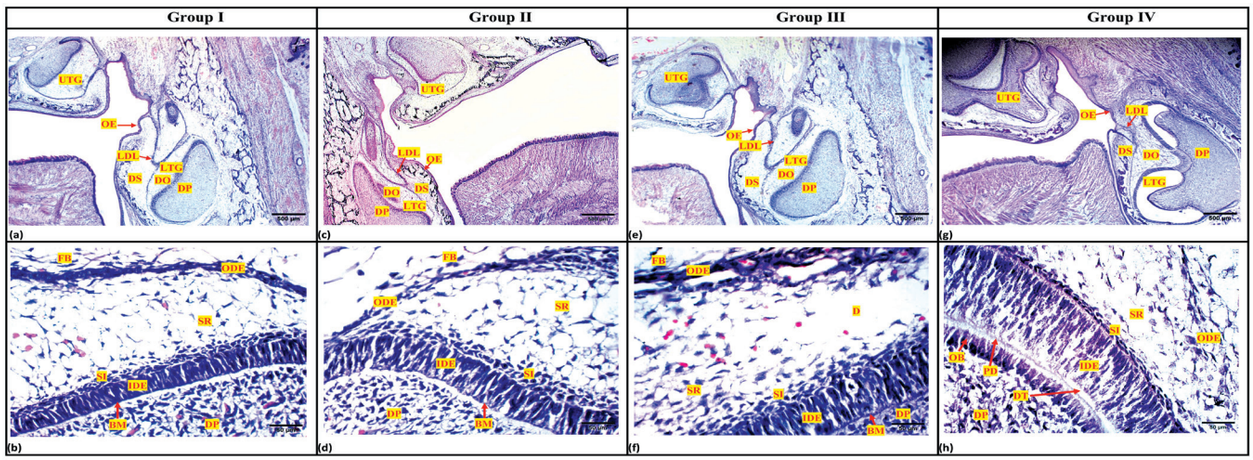

Fig. 1.Photomicrographs of the developing first molars tooth germ in (a & b)- Group I, (c & d)- Group II, (e & f)- Group III, and (g & h)- Group IV showing: lateral dental lamina connecting tooth germ to oral epithelium. Dental organ formed of inner dental epithelium resting on basement membrane, stratum intermedium, stellate reticulum and outer dental epithelium cells. Dense dental papilla and loose dental sac with spindle shaped fibroblasts. Group III revealed some degenerated stellate reticulum cells. Group IV showed degenerated inner dental epithelium with detachment from basement membrane at some regions, predentin and odontoblasts. UTG, upper tooth germ; LTG, lower tooth germ; OE, oral epithelium; LDL, lateral dental lamina; DO, dental organ; DP, dental papilla; DS, dental sac; IDE, inner dental epithelium; ODE, outer dental epithelium, SR, stellate reticulum; SI, stratum intermedium; BM, basement membrane; FB, fibroblasts; OB, odontoblasts, PD, predentin; D, degeneration; DT, detachment (H&E, original magnification (a, c, e, g) 40×, (b, d, f, h) 400×).

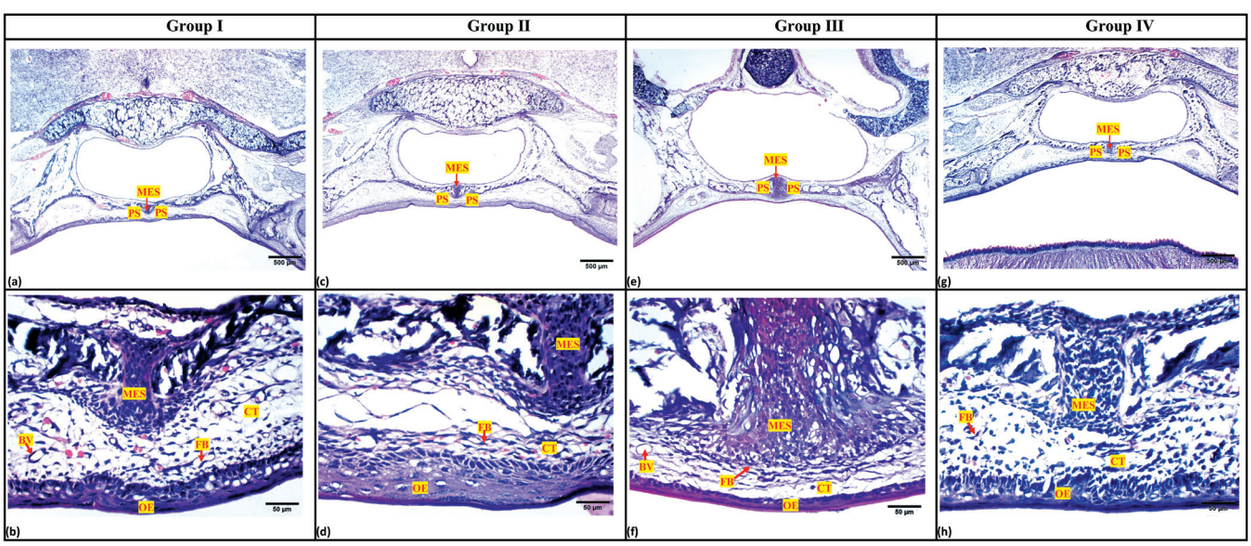

Fig. 2. Photomicrographs of the developing secondary palate in (a & b)- Group I, (c & d)- Group II, (e & f)- Group III, and (g & h)- Group IV showing: fusion of the two palatal shelves with remnant of midline epithelial seam and keratinized stratified squamous epithelium covering the secondary palate from the oral side. Connective tissue revealed spindle shaped fibroblasts, some blood vessels and traces of midline epithelial seam. PS, palatal shelf; MES, midline epithelial seam; OE, Oral epithelium; FB, fibroblasts; BV, blood vessels; CT, connective tissue (H&E, original magnification (a, c, e, g) 40×, (b, d, f, h) 400×).

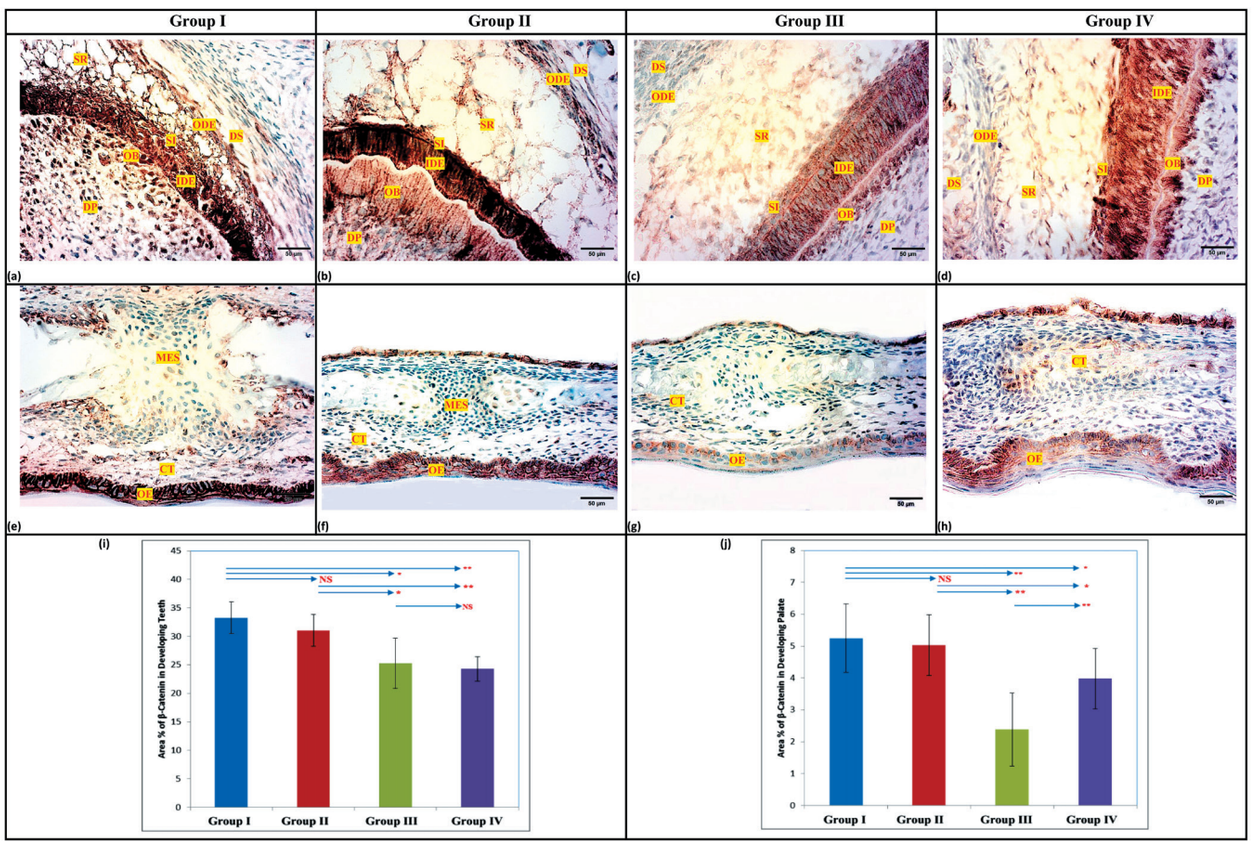

Fig.3 Photomicrographs of the developing tooth germ in (a)- Group I, (b)- Group II, (c)- Group III, and (d)- Group IV showing: positive immunoreactivity in inner dental epithelium, stratum intermedium, stellate reticulum, outer dental epithelium cells, odontoblasts and cells in dental papilla and dental sac. IDE, inner dental epithelium; ODE, outer dental epithelium, SR, stellate reticulum; SI, stratum intermedium; OB, odontoblasts, DP, dental papilla; DS, dental sac. Photomicrographs of the developing palate in (e)- Group I, (f)- Group II, (g)- Group III, and (h)- Group IV showing: positive immunoreactivity in oral epithelium and connective tissue cells, in addition to positive immunoreactivity of midline epithelial seam in Group I and II. OE, oral epithelium; CT, connective tissue; MES, midline epithelial seam. (Anti-β-catenin antibody, original magnification 400×). Bar charts representing mean and SD values of β-catenin immunopositive area% in (i)- developing teeth. (j)- developing palate. (* Significant; ** Highly significant; NS: non-significant).

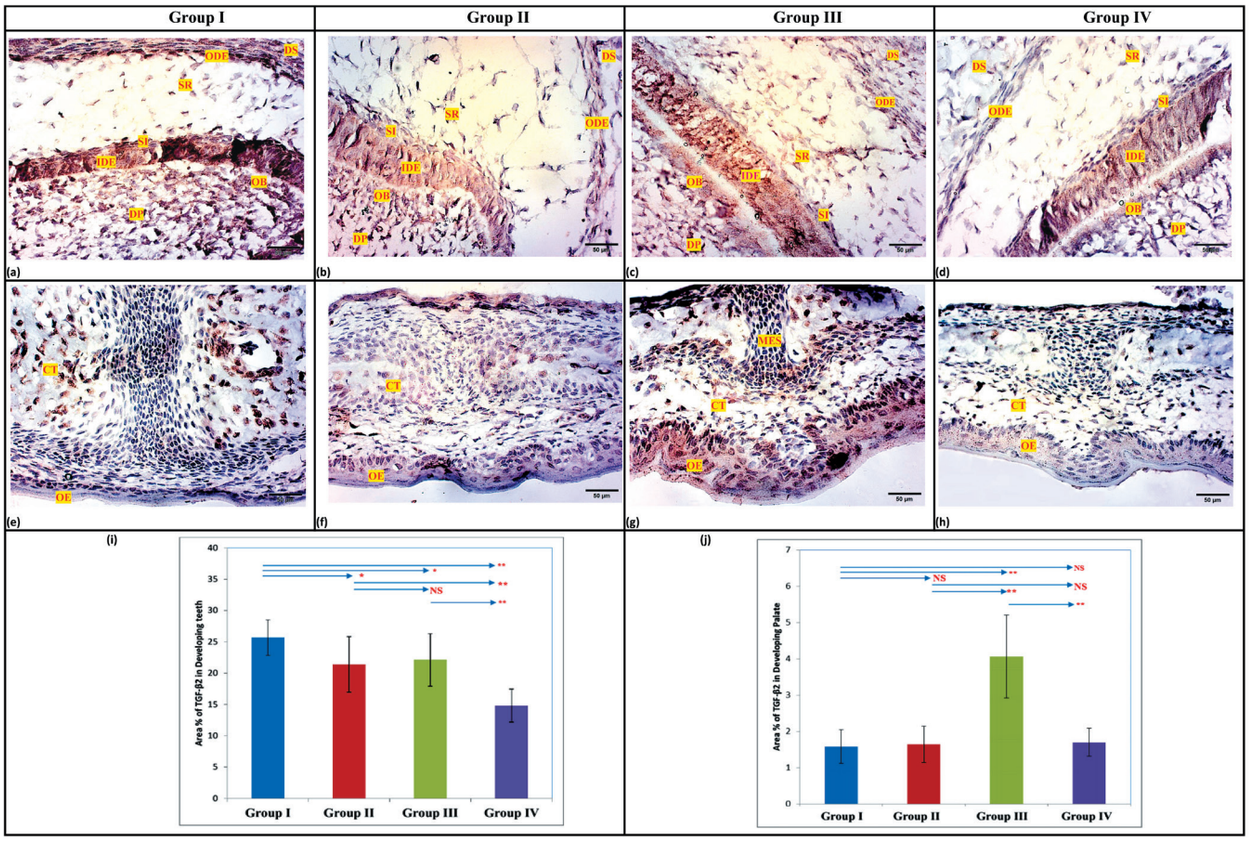

Fig. 4 Photomicrographs of the developing tooth germ in Group I, (b)- Group II, (c)- Group III, and (d)- Group IV showing: detectable positive immunoreactivity in inner dental epithelium and odontoblasts in comparison to stratum intermedium, stellate reticulum, outer dental epithelium cells, and cells in dental papilla and dental sac. IDE, inner dental epithelium; ODE, outer dental epithelium, SR, stellate reticulum; SI, stratum intermedium; OB, odontoblasts, DP, dental papilla; DS, dental sac. Photomicrographs of the developing palate in (e)- Group I, (f)- Group II, (g)- Group III, and (h)- Group IV showing: positive immunoreactivity in oral epithelium and connective tissue cells as well as positive immunoreactivity of midline epithelial seam in Group III. OE, oral epithelium; CT, connective tissue; MES, midline epithelial seam. (Anti-TGF-β2 antibody, original magnification 400×). Bar charts representing mean and SD values of TGF-β2 immunopositive area% in (i)- developing teeth. (j)- developing palate. (* Significant; ** Highly significant; NS: non-significant)

Table :1 Showing the mean ± SD values, results of ANOVA and post hoc tests for the comparison between different groups regarding immunopositive area%.

Immunopositive area%		Group I	Group II	Group III	Group IV	F-test	*p-value*
β-Catenin in Developing Teeth	Mean ±SD	33.27 ^{a} ± 2.82	31.01 ^{a} ± 2.78	25.26 ^{b} ± 4.40	24.28 ^{b} ± 2.18	28.854	0.000**
β-Catenin in Developing Palate	Mean ±SD	5.25 ^{a} ± 1.07	5.03 ^{a} ± 0.96	2.38 ^{c} ± 1.15	3.98 ^{b} ± 0.95	23.942	0.000**
TGF-β2in Developing Teeth	Mean ±SD	25.67 ^{a} ± 2.81	21.41 ^{b} ± 4.45	22.10 ^{b} ± 4.18	14.81 ^{c} ± 2.64	23.587	0.000**
TGF-β2in Developing Palate	Mean ±SD	1.58 ^{b} ± 0.46	1.65 ^{b} ± 0.50	4.06 ^{a} ± 1.14	1.70 ^{b} ± 0.38	45.890	0.000**

Significance level $p \leq 0.05$, *significant Tukey's post hoc: Means sharing the same superscript letter are not significantly different

DISCUSSION

Since the pandemic of COVID-19 was declared, no effort has been spared to deal with its catastrophic health effects. Various types of vaccines were introduced in an attempt to control and prevent the infection; however, proper insurance of their safety was an inevitable step[39]. Pregnant women were excluded from the primary clinical trials of COVID-19 vaccines, thus, developmental and reproductive toxicity studies based on animal models were considered a safe alternative[14].

Messenger RNA, adenovirus-vector protein, and inactivated vaccines were the vaccines of choice in this study as they include almost all types of vaccines, which proved effective to protect against Covid-19 pandemic[5, 40].

In our study, the doses and frequency of vaccine administration were guided by the recommendations of 2006 FDA Guidance Considerations for Developmental Toxicity Studies for Preventative and Therapeutic Vaccines for Infectious Disease Indications and ICH S5 (R3) guidelines[41, 42]. According to the guidelines, we used a full

human dose for the three used vaccines and applied two priming doses before mating, to bring out a peak antibody titre during the critical phases of pregnancy, in addition to one dose during organogenesis.

In the current study, the offspring were sacrificed at day one after birth for two reasons; first: because different peptides and enzymes responsible for epithelial-mesenchymal interaction in dental tissues development demonstrate maximum activity at early days after birth[43, 44]. Second: based on the number and durations of vaccination doses in this study, the potential of any developmental abnormalities related to vaccine and/or developed antibodies to vaccine is presented during the gestation period or within the first days after birth[41, 42].

In the present study, histological examination of the developing teeth and palate revealed almost the same picture in all studied groups except for some cellular degeneration and morphological changes in most of the tooth germs of Group III and IV. These findings are in parallel with Bowman *et al*., [14] who could not detect any adverse effects from mRNA vaccine on fertility of pregnant rats or growth and physical development of the offspring. In addition, a cohort human study evaluated by ultrasonography did not detect any increase in fetal structural malformation when mRNA and adenovirus vector COVID-19 vaccines were administered during early pregnancy[45].

In the herein study, β-catenin expression was more detectable in dental organ cells and odontoblasts in all groups. This could be explained by Liu & Miller,[23] who linked the activity of Wnt to its ability in maintaining and controlling the teeth shape. Furthermore, the expression of TGF-β2 in all groups of this study is in accordance with Sassá Benedete *et al*.,[30] and Heikinheimo *et al*.,[46] who reported its expression during the cap and early bell stages. Statistically, groups III and IV showed significant low mean area% of both β-catenin and TGF-β2 in comparison to Group I, in addition to morphological changes in most of the tooth germs that were recorded during histological examination of both groups. This change in tooth germ morphology could be explained by Sarkar & Sharpe,[47] who reported alternation of tooth germ shape with the treatment of Wnt inhibitor at early bell stage, and Sassá Benedete *et al*.,[30] who proved the role of TGF-β2 in regulating epithelial differentiation and determination of crown size.

In the present study, there was no effect of any of the vaccines on palatal fusion; a finding asserted by the diffuse epithelial expression of β-catenin and TGF-β2. In our study, the high β-catenin and low TGF-β2 mean area% recorded in all studied groups, except Group III, could be explained by He *et al*.,[28] who found that the expression pattern of β-catenin and other Wnt ligands and receptors in the developing palate coincides with the primary action of the pathway in the palatal epithelium particularly in the medial edge epithelium. Moreover, Gehris, *et al*.,[31] and Iwata *et al*.,[32] claimed lower expression of TGF-β2 in midline connective tissue after adherence of the palatal shelves. Conversely, the lowest β-catenin and highest TGF-β2 mean area% as well as the histological detection of noticeable midline epithelial seam in Group III may indicate that the developing palate is in active fusion stage.

Further studies are recommended to evaluate the effect of different types of COVID-19 vaccines administration on tooth germ calcification, besides ultrastructural investigations on various oral and dental tissues during pregnancy and lactation.

CONCLUSIONS

Regarding administration of COVID-19 vaccines during pregnancy, mRNA vaccine acts safely on the developing teeth and palate. In contrast, adenoviral vector and inactivated vaccines cause histological and morphological alternations in tooth germ; also, adenoviral vector vaccine may elongate the palatal fusion time.

CONFLICT OF INTERESTS

There are no conflicts of interest.

ABBREVIATIONS

(COVID-19): Coronavirus disease 2019, **(mRNA):** messenger RNA, **(Wnt/β-catenin):** Wingless-integrated/Beta-catenin, **(TGF-β):** transforming growth factor-Beta, **(H&E):** Hematoxylin and Eosin.

REFERENCES

1. Cucinotta D and Vanelli M. WHO Declares COVID-19 a Pandemic. Acta Biomed. 2020; 91(1):157-160. https://doi.org/10.23750/abm.v91i1.9397
2. Kaur SP and Gupta V. COVID-19 Vaccine: A comprehensive status report. Virus Res. 2020; 288:198114. https://doi.org/10.1016/j.virusres.2020.198114
3. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, *et al*. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020; 383(27):2603-2615. https://doi.org/10.1056/nejmoa2034577
4. Doroftei B, Ciobica A, Ilie OD, Maftei R and Ilea C. Mini-Review Discussing the Reliability and Efficiency of COVID-19 Vaccines. Diagnostics (Basel). 2021;11(4):579. https://doi.org/10.3390/diagnostics11040579
5. Jin L, Li Z, Zhang X, Li J and Zhu F. CoronaVac: A review of efficacy, safety, and immunogenicity of the inactivated vaccine against SARS-CoV-2. Hum Vaccin Immunother. 2022; 18(6): 2096970. https://doi.org/10.1080%2F21645515.2022.2096970
6. Dagan N, Barda N, Kepten E, Miron O, Perchik S, Katz MA, *et al*. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. N Engl J Med. 2021; 384(15):1412-1423. https://doi.org/10.1056/nejmoa2101765

7. Thompson MG, Burgess JL, Naleway AL, Tyner HL, Yoon SK, Meece J, *et al*. Interim Estimates of Vaccine Effectiveness of BNT162b2 and mRNA-1273 COVID-19 Vaccines in Preventing SARS-CoV-2 Infection Among Health Care Personnel, First Responders, and Other Essential and Frontline Workers - Eight U.S. Locations, December 2020-March 2021. MMWR Morb Mortal Wkly Rep. 2021; 70(13):495-500. https://doi.org/10.15585/mmwr.mm7013e3

8. Ramasamy MN, Minassian AM, Ewer KJ, Flaxman AL, Folegatti PM, Owens DR, *et al*. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet. 2020; 396(10249):467-478. https://doi.org/10.1016/s0140-6736(20)32466-1

9. Wouters OJ, Shadlen KC, Salcher-Konrad M, Pollard AJ, Larson HJ, Teerawattananon Y, *et al*. Challenges in ensuring global access to COVID-19 vaccines: production, affordability, allocation, and deployment. Lancet. 2021; 397(10278):1023-1034. https://doi.org/10.1016/s0140-6736(21)00306-8

10. Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, *et al*. Development of an inactivated vaccine candidate for SARS-CoV-2. Science. 2020; 369(6499): 77-81. https://doi.org/10.1126/science.abc1932

11. Ranzani OT, Hitchings MDT, Dorion M, D'Agostini TL, de Paula RC, de Paula OFP, *et al*. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. BMJ. 2021; 374:n2015. https://doi.org/10.1136/bmj.n2015

12. Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, *et al*. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status-United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020; 69(44):1641-1647. https://doi.org/10.15585/mmwr.mm6944e3

13. Wastnedge EAN, Reynolds RM, van Boeckel SR, Stock SJ, Denison FC, Maybin JA, *et al*. Pregnancy and COVID-19. Physiol Rev. 2021; 101(1):303-318. https://doi.org/10.1152/physrev.00024.2020

14. Bowman CJ, Bouressam M, Campion SN, Cappon GD, Catlin NR, Cutler MW, *et al*. Lack of effects on female fertility and prenatal and postnatal offspring development in rats with BNT162b2, a mRNA-based COVID-19 vaccine. Reprod Toxicol. 2021; 103:28-35. https://doi.org/10.1016/j.reprotox.2021.05.007

15. Stebbings R, Maguire S, Armour G, Jones C, Goodman J, Maguire AK, Tang CM, Skellett V and Harris J. Developmental and reproductive safety of AZD1222 (ChAdOx1 nCoV-19) in mice. Reprod Toxicol. 2021;104, 134-142. https://www.sciencedirect.com/science/article/pii/S0890623821001180

16. Trostle, ME, Limaye, MA, Avtushka V, Lighter, JL, Penfield CA and Roman AS. COVID-19 vaccination in pregnancy: early experience from a single institution. Am J Obstet Gynecol MFM. 2021; 3, 100464. https://doi.org/10.1016/j.ajogmf.2021.100464

17. Zauche LH, Wallace B, Smoots, AN, Olson CK, Oduyebo T, Kim SY, Petersen EE, Ju J, Beauregard J, Wilcox AJ, Rose CE, Meaney-Delman DM and Ellington SR. CDC v-safe Covid-19 Pregnancy Registry Team. Receipt of mRNA Covid-19 Vaccines and Risk of Spontaneous Abortion. N Engl J Med. 2021; 385, 1533-1535. https://doi.org/10.1056/nejmc2113891

18. Badell ML, Dude CM, Rasmussen SA and Jamieson DJ. Covid-19 vaccination in pregnancy. bmj. 2022; 10, 378. https://www.bmj.com/content/378/bmj-2021-069741.

19. Arte S, Nieminen P, Apajalahti S, Haavikko K, Thesleff I and Pirinen S. Characteristics of incisor-premolar hypodontia in families. J Dent Res. 2001; 80(5):1445-1450. https://doi.org/10.1177/00220345010800051201

20. White SC and Pharoah MJ. Oral Radiology : Principles and Interpretation. 5th ed. St. Louis Mo: Mosby; 2004. https://www.worldcat.org/title/52895277

21. Bush JO and Jiang R. Palatogenesis: morphogenetic and molecular mechanisms of secondary palate development. Development. 2012; 139(2):231-243. https://doi.org/10.1242/dev.067082

22. Liu F, Chu EY, Watt B, Zhang Y, Gallant NM, Andl T, *et al*. Wnt/beta-catenin signaling directs multiple stages of tooth morphogenesis. Dev Biol. 2008; 313(1):210-224. https://doi.org/10.1016/j.ydbio.2007.10.016

23. Liu F, Millar SE. Wnt/beta-catenin signaling in oral tissue development and disease. J Dent Res. 2010; 89(4):318-330. https://doi.org/10.1177%2F0022034510363373

24. Sorrenson B, Cognard E, Lee KL, Dissanayake WC, Fu Y, Han W, *et al*. A Critical Role for β-Catenin in Modulating Levels of Insulin Secretion from β-Cells by Regulating Actin Cytoskeleton and Insulin Vesicle Localization. J Biol Chem. 2016; 291(50):25888-25900. https://doi.org/10.1074/jbc.m116.758516

25. Yang J, Cai W, Lu X, Liu S and Zhao S. RNA-Sequencing Analyses Demonstrate the Involvement of Canonical Transient Receptor Potential Channels in Rat Tooth Germ Development. Front Physiol. 2017; 8:455. https://doi.org/10.3389%2Ffphys.2017.00455

26. Järvinen E, Salazar-Ciudad I, Birchmeier W, Taketo MM, Jernvall J and Thesleff I. Continuous tooth generation in mouse is induced by activated epithelial Wnt/beta-catenin signaling. Proc Natl Acad Sci U S A. 2006; 103(49):18627-18632. https://doi.org/10.1073/pnas.0607289103

27. Chen J, Lan Y, Baek JA, Gao Y and Jiang R. Wnt/beta-catenin signaling plays an essential role in activation of odontogenic mesenchyme during early tooth development. Dev Biol. 2009; 334(1):174-185. https://doi.org/10.1016/j.ydbio.2009.07.015

28. He F, Xiong W, Wang Y, Li L, Liu C, Yamagami T, *et al*. Epithelial Wnt/β-catenin signaling regulates palatal shelf fusion through regulation of Tgfβ3 expression. Dev Biol. 2011; 350(2):511-519. https://doi.org/10.1016%2Fj.ydbio.2010.12.021

29. Miyazono K. Positive and negative regulation of TGF-beta signaling. J Cell Sci. 2000; 113(Pt7):1101-1109. https://doi.org/10.1242/jcs.113.7.1101

30. Sassá Benedete AP, Sobral AP, Lima DM, Kamibeppu L, Soares FA and Lourenço SV. Expression of transforming growth factor-beta 1, -beta 2, and -beta 3 in human developing teeth: immunolocalization according to the odontogenesis phases. Pediatr Dev Pathol. 2008; 11(3):206-212. https://doi.org/10.2350/07-09-0333.1

31. Gehris AL, D'Angelo M and Greene RM. Immunodetection of the transforming growth factors beta 1 and beta 2 in the developing murine palate. Int J Dev Biol. 1991; 35(1):17-24. https://pubmed.ncbi.nlm.nih.gov/1714290/

32. Iwata J, Parada C and Chai Y. The mechanism of TGF-β signaling during palate development. Oral Dis. 2011; 17(8):733-744. https://doi.org/10.1111%2Fj.1601-0825.2011.01806.x

33. Baldrick P. Development of COVID-19 therapies: Nonclinical testing considerations. Regul Toxicol Pharmacol. 2022; 132:105189. https://doi.org/10.1016/j.yrtph.2022.105189

34. Kozlovskaya LI, Piniaeva AN, Ignatyev GM, Gordeychuk IV, Volok VP, Rogova YV, *et al*. Long-term humoral immunogenicity, safety and protective efficacy of inactivated vaccine against COVID-19 (CoviVac) in preclinical studies. Emerg Microbes Infect. 2021; 10(1):1790-1806. https://doi.org/10.1080/22221751.2021.1971569

35. Bancroft JD, Suvarna K and Layton C. Bancroft's Theory and Practice of Histological Techniques, 7th ed. Elsevier, Churchill Livingstone, 2013. https://doi.org/10.1016/B978-0-7020-4226-3.00010-X

36. Kale AD, Mane DR and Shukla D. Expression of transforming growth factor β and its correlation with lipodystrophy in oral submucous fibrosis: an immunohistochemical study. Med Oral Patol Oral Cir Bucal. 2013; 18(1):e12-e18. https://doi.org/10.4317/medoral.18226

37. Liu X, Zhang Z, Pan S, Shang S and Li C. Interaction between the Wnt/β-catenin signaling pathway and the EMMPRIN/MMP-2, 9 route in periodontitis. J Periodontal Res. 2018; 53(5):842-852. https://doi.org/10.1111/jre.12574

38. Kumamoto H and Ooya K. Immunohistochemical detection of beta-catenin and adenomatous polyposis coli in ameloblastomas. J Oral Pathol Med. 2005; 34(7):401-406. https://doi.org/10.1111/j.1600-0714.2005.00328.x

39. Haynes BF, Corey L, Fernandes P, Gilbert PB, Hotez PJ, Rao S, *et al*. Prospects for a safe COVID-19 vaccine. Sci Transl Med. 2020; 12(568):eabe0948. https://doi.org/10.1126/scitranslmed.abe0948

40. Meggiolaro A, Sane Schepisi M, Nikolaidis GF Mipatrini D, Siddu A and Rezza G. Effectiveness of Vaccination against SARS-CoV-2 Infection in the Pre-Delta Era: A Systematic Review and Meta-Analysis. Vaccines (Basel). 2022; 10(2),157. https://doi.org/10.3390/vaccines10020157

41. FDA. Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventative and Therapeutic Vaccines for Infectious Disease Indications. 2006. https://www.fda.gov/files/vaccines,%20blood%20&%20biologics/published/Guidance-for-Industry--Considerations-for-Developmental-Toxicity-Studies-for-Preventive-and-Therapeutic-Vaccines-for-Infectious-Disease-Indications.pdf

42. ICH I. S5 (R3) Guideline on Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals step 5. 2020. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s5-r3-guideline-detection-reproductive-developmental-toxicity-human-pharmaceuticals-step-5_en.pdf

43. Cotrim P, de Andrade CR, Line S, de Almeida OP and Coletta RD. Expression and activity of matrix metalloproteinase-2 (MMP-2) in the development of rat first molar tooth germ. Brazilian Dental Journal. 2002; 13(2), 97-102. https://doi.org/10.1590/s0103-64402002000200004

44. Gritli-Linde A, Bei M, Maas R, Zhang XM, Linde A and McMahon AP Shh signaling within the dental epithelium is necessary for cell proliferation, growth and polarization. Development. 2002; 129(23), 5323-5337. https://doi.org/10.1242/dev.00100

45. Ruderman RS, Mormol J, Trawick E, Perry MF, Allen EC, Millan D, *et al*. Association of COVID-19 Vaccination During Early Pregnancy With Risk of Congenital Fetal Anomalies. JAMA Pediatr. 2022; 176(7):717-719. https://doi.org/10.1001/jamapediatrics.2022.0164

46. Heikinheimo K, Happonen RP, Miettinen PJ and Ritvos O. Transforming growth factor beta 2 in epithelial differentiation of developing teeth and odontogenic tumors. J Clin Invest. 1993; 91(3):1019-1027. https://doi.org/10.1172/jci116258

47. Sarkar L and Sharpe PT. Inhibition of Wnt signaling by exogenous Mfrzb1 protein affects molar tooth size. J Dent Res. 2000; 79(4):920-925. https://doi.org/10.1177/00220345000790040601

الملخص العربى

تأثير لقاحات كوفيد-١٩ المختلفة على نمو الأسنان والحلق على نسل الجرذان المهق

رباب حسن[١]، بسمة عبدالرحمن أحمد[٢]

[١]قسم بيولوجيا الفم، [٢]قسم باثولوجيا الفم، كلية طب الاسنان، جامعة عين شمس

المقدمة: تم تطوير العديد من اللقاحات للحد من مخاطر الإصابة بمرض فيروس كورونا الجائح كوفيد-٢٠١٩. يتأثر نمو الأسنان والحلق بالمتغيرات المكتسبة أو الموروثة التي قد تؤثر على الصحة العامة. يعد تأثير اللقاحات على نمو الأعضاء خطوة أساسية لتأمين سلامة اللقاحات.

الهدف من العمل: الكشف عن تأثير الأنواع المختلفة من لقاحات كوفيد-١٩ على نمو الأسنان والحلق.

المواد والطرق: تم تقسيم ستين جرذ إلى أربع مجموعات. تم الحقن العضلى لكل مجموعة إما بمحلول ملحي (المجموعة الأولى)، أو بلقاح مرسال حامض النووى الريبى (المجموعة الثانية)، أو لقاح ناقلات الفيروسات الغدانية (المجموعة الثالثة)، أو لقاح معطل (المجموعة الرابعة) في ٢١ و ١٤ يومًا قبل التزاوج وفي اليوم التاسع من الحمل. تم جمع رؤوس نسل الجرذان " عندعمر يوم واحد" ومعالجتها وصبغها بواسطة الهيماتوكسيلين والايوسين وبيتا كاتينين وعامل النمو المتحول بيتا ٢ و تم تحليل تعبيراتهم في براعم الأسنان والحلق النامي إحصائيًا.

النتائج: لم يتم تسجيل أي تغيرات نسيجية او شكلية في براعم الاسنان والحلق النامى في جميع المجموعات باستثناء براعم الأسنان النامية في المجموعتين الثالثة والرابعة. أظهرت جميع المجموعات نشاطًا مناعيًا إيجابيًا لبيتا كاتينين وعامل النمو المتحول بيتا ٢ في براعم الأسنان والحلق النامية. إحصائيًا، أظهرت المجموعات الملقحة فرقًا كبيرًا في النسبة المئوية للمنطقة الإيجابية المناعية فى المجموعة الأولى، باستثناء بيتا كاتينين في المجموعة الثانية، و عامل النمو المتحول بيتا ٢ في الحلق النامي في المجموعتين الثانية والرابعة.

الاستنتاج: لقاح كوفيد-١٩ مرسال حامض النووى الريبى أكثر أمانًا من لقاحات ناقلات الفيروسات الغدانية واللقاحات المعطلة فيما يتعلق بنمو الأسنان و/أو الحلق.