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IMPACT OF OXFORD VACCINE DURING PREGNANCY ON TOOTH GERM DEVELOPMENT AND HARD TISSUE THICKNESS IN THE ALBINO RATS OFFSPRING

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KEYWORDS

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ABSTRACT

Introduction: Teeth development and eruption are complicated events embracing plenty of structures and signaling pathways. Severe acute respiratory syndrome coronavirus-2 is the causative agent of coronavirus infectious disease-2019 (COVID-19). Pregnant females infected with COVID-19 have disturbances in the inflammatory reaction that would lead to a severe disease; therefore, their vaccination is obligatory. **Materials and Methods:** Pregnant female rats were equally distributed into two groups: Control group received saline and treated group received COVID-19 vaccine. At the proposed time, pups were euthanized, and the mandible of each pup was dissected for histological examination. Histomorphometric examination was performed for the thickness of enamel and dentin of the lower first molar on the 9th and 14th postnatal days. **Results:** Developmental stages of the lower first molar tooth germ in both groups were nearly comparable with no histological differences. Enamel thickness increased in a non-statistically significant manner in the treated group (P-value ≥ 0.05) while dentin thickness increased in a statistically significant manner in the treated group at the same time points (P-value ≤ 0.05). **Conclusion:** Vaccination of pregnant mothers did not influence the development of lower first molar tooth germ in the offspring. However, hard tissue thickness may be affected by vaccination.

INTRODUCTION

Teeth development and eruption are complicated events embracing plenty of structures, cells, and signaling pathways. In humans, the beginning of enamel organ appearance starts at 7 weeks of the intrauterine life for the incisors, 8 weeks for the canines and first molars and 9 weeks for the second molars. This sensitive time in the pregnancy course must be carefully monitored in respect of drugs and vaccines the mother takes in order to avoid any possible malformations or diseases in the fetus [(1)].

Disturbances in tooth eruption include premature tooth eruption, delayed tooth eruption (due to systemic, genetic, or localized factors), ankylosis, ectopic eruption, eruption cyst, etc [(2)].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus infectious disease-2019 (COVID-19)

which has unfortunately caused more than 6.4 million deaths across the world by August 2022. SARS-CoV-2 virus was first reported in swaps of bronchoalveolar lavage fluid from patients in Wuhan, China and was proved as the reason of COVID-19 outbreak on January 24, 2020 [3].

In SARS-CoV-2 severely infected patients, severe inflammatory reactions occur due to dysregulated immune response, alteration of the protective immunity, enormous cytokine release, and multisystem inflammatory syndrome [3].

Laboratory and clinical trials have been conducted to prove the safety and efficacy of different Coronavirus vaccines. Several platforms and companies have developed different vaccines of diverse composition among them inactivated, live attenuated, recombinant protein, vectored, and mRNA-based vaccines [4]. These vaccines promote the secretion of neutralizing antibodies (NAbs) against the SARS-CoV-2 spike (S) protein. These Nabs clear viruses by joining viral receptors and obstructing viral particles from binding host cells. Hence, they hinder the binding of viral particles with angiotensin-converting enzyme 2 (ACE2) receptors present on the cell membrane thereby inhibiting viral entry [5].

Various types of COVID-19 vaccines have been developed. They could be categorized into 4 main categories [6]:

A- Nucleic acid (mRNA vaccines) (BNT162b2/ Pfizer, mRNA 1273/Moderna).

B- Nonreplicating viral vector (ChAdOx1 nCoV019/AstraZeneca, Ad26.COV2. S/ Janssen, Sputnik V).

C- Inactivated whole virus (BBIBP-CorV/Sinopharm, CoronaVac/Sinovac, BBV152/Covaxin).

D- Protein subunit (NVX-CoV2373/Novavax).

SARS-CoV-2 through pregnancy has been reported to provoke unique maternal (and fetal) immune responses. It appears that infected pregnant mothers have increased levels of IL-8, IL-6, IL-10, IL15 in the circulation of which IL-6 may aid in the prediction of severity and prognosis. Increased levels of IL-6 mean rigorous pathology and mortality. In consequence, pregnant females would then have disturbances in the inflammatory reaction that would lead to a severe disease [7].

Symptoms of SARS-CoV-2 in pregnant females are not different from the general population. Major symptoms are fever, muscle pain, cough, difficulty of breathing, headache, nausea and vomiting [8].

In a study conducted on rats as experimental animals, there was lacking evidence that Oxford vaccine has any related adverse effects on female fertility, duration of pregnancy, fetal growth, postnatal events, involving pup survival and development. Following vaccination, antibodies were detected in the umbilical cord blood and breast milk. Hence, passive protection to neonates, that have still incomplete immune systems, is worthy to defend them against infection or to lessen the severity of disease [9].

Other studies on experimental animals (hACE2 mice) confirmed that vaccination with CoronaVac/ Sinovac was harmless and did not have any noteworthy side effects on the course of pregnancy, lactation, teeth eruption or the overall development and growth of offspring [10]. Moreover, human studies reported safety of mRNA vaccines during pregnancy [11].

Therefore, this research focuses on the effect of Oxford vaccine administration during pregnancy on the teeth development and the thickness of hard tissue in their lower first molar in the offspring.

MATERIALS AND METHODS

Ethical approval:

The present research has been performed following approval of the Research Ethics Committee (REC) of the Faculty of Dentistry, Suez Canal University (510/2022). Animals were kept in the animal house of Faculty of Medicine, Suez Canal university.

Study design:

Ten adult female albino rats, having body weight 160-180 gm were paired for breeding 1:1 with an untreated male until the identification of male sperms in vaginal smears in 1-3 days [12]. The first gestational day is the day of confirmation of pregnancy. Females were then separately accommodated during the gestation and lactation periods in well-ventilated animal house. All animals were fed a suitable diet involving fresh vegetables, dried bread and drinking water *ad libitum*. They also were kept under good conditions of temperature and a 12-h light /12-h dark cycle.

Animal grouping and treatment protocol:

Pregnant female rats were then equally distributed into two groups:

1. **Group I (Control group):** treated with saline solution.
2. **Group II (treated group):** treated with COVID-19 vaccine of the Oxford type (batch PW40179) as the test agent.

Pregnant mother rats were injected by 0.035 mL of either saline or vaccine through the intramuscular route in the thigh of each hindlimb (total dose per session: 0.07 mL/session). Injection was performed on gestational days 6 and 15. Rats delivered normally after nearly 21-23 days with regular inspection to examine any abnormalities during giving birth. The average number of offspring born for each rat was 5-7 pups. Male albino rats were excluded from the study directly after confirmation of pregnancy [9].

Methods of examination of pups:

I- Histological Examination:

At the proposed time (day of birth, 9th, 14th , and 30 days postnatal), pups were euthanized, and the mandible of each pup was dissected out. Then the samples were fixed instantly in a 10% buffered formalin solution for 2 days. Decalcification of mandibles was carried out using 10% EDTA solution (PH 7.4) until properly softened. Specimens were stained with Hematoxylin and Eosin stains to evaluate the lower first molar tooth germ developmental stage on zero,14th and 30th postnatal days.

II- Histo-morphometric Examination:

Six high power fields (x 200) were selected in each serial section of the examined groups. Area percentage was determined via Leica QWin 500 image analyzer computer system (England). Records of each parameter were statistically labelled in terms of mean and standard deviation (mean ± SD) for area percentage. Area percentage was measured for the thickness of enamel and dentin formed at the cusp tip of the lower first molar on the 9th and 14th postnatal days [13].

III- Statistical analysis:

- ***Sample Size Calculation:***

The sample size for this study was calculated according to **Charan and Biswas** [14] using the following equation:

$$N = \frac{(Z_\alpha)^2 * (S)^2}{(d)^2}$$

N = Total sample size

Z_α = Standard normal variation and its equal to 1.96 at P< 0.05

S = Standard deviation of variable

d = Absolute error or precision

Zα	SD	D
1.96	2.88	2

Total sample size $N = \frac{(1.96)^2 x (2.88)^2}{(2)^2} = 7.99 \approx 10$

(pregnant rats) equally divided into two groups.

Statistical tests

Data were examined for normality via Kolmogorov-Smirnov test of normality. The results of this test pointed out that some data were normally distributed (parametric data). According to descriptive analysis, One Way-Anova, Post Hock tests were operated for intergroup relationship and others undergo independent sample t test and Levene's Test. *P*-values fewer than 0.05 were represented as statistically significant. Statistical analysis was conducted using SPSS 26.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

RESULTS

I) Results of histological examination:

- ***Day Zero (Day of birth) (Fig.1 a,b), (Fig.2 a,b):***

Control group:

In pups of control mother rats on day zero, the lower first molar tooth germs were at the transition from cap to early bell stages of odontogenesis. Some teeth germs were totally in the early bell stage. The tooth germ is formed of four types of cells namely, the outer enamel epithelium, the inner enamel epithelium, the stellate reticulum, and stratum intermedium. The cervical loop is formed of inner and outer enamel epithelium without stellate reticulum or stratum intermedium. The epithelial diaphragm is not yet evident. The dental papilla appeared as dense connective tissue enclosed in the tooth germ. Odontoblasts are crowded and aligned at the periphery of the dental papilla with proximally situated oval shaped nuclei. The whole structure is surrounded by tooth follicle formed of dense connective tissue and separates the tooth germ from the bony crypt.

Treated group:

Similarly, in pups of treated mother rats, the teeth germs of the lower first molar were at the same stages of odontogenesis as the control pups teeth germs (transition from cap to early bell stages) with the same histological features.

- ***Fourteen days postnatal (14th day) (immediately prior to eruption) (Fig.1 c,d), (Fig.2 c,d):***

Control and treated groups:

The tooth germ of first lower molar of both control and treated pups on the 14th postnatal day showed advanced bell stage with no eruption yet. The presence of oral epithelium above the tooth germ is also evident. The whole tooth germ is enclosed in a bony crypt formed of thin bone trabeculae. Complete deposition of dental hard tissues was seen in the crown with thick dentin and thinner predentin. Due to the high proportion of mineral in the enamel, its organic matrix has disintegrated during demineralization, resulting in

an artifactual cleft covering the dentin. However, in some cervical areas, enamel matrix is still present below the ameloblastic layer. Ameloblasts were still present, in the form of a continuous layer; however, they demonstrated a reduction in size with time. The pulp appeared as moderately dense connective tissue. The odontoblasts appeared crowded and pseudostratified. Root formation has progressed to a considerable extent with proliferation of odontoblasts and dental papilla and formation of root dentin.

- ***One month (30th day) postnatal (after eruption of the lower first molar) (Fig.1 e,f), (Fig.2 e,f):***

Control and treated groups:

The lower first molar has erupted normally at both the control and treated groups and showed normal stages of development. Dentin, predentin and odontoblasts appeared at the normal stages of development. Pulp appeared normally with widely dilated blood vessels. Normal architecture of the periodontal ligament fibers and alveolar bone is evident.

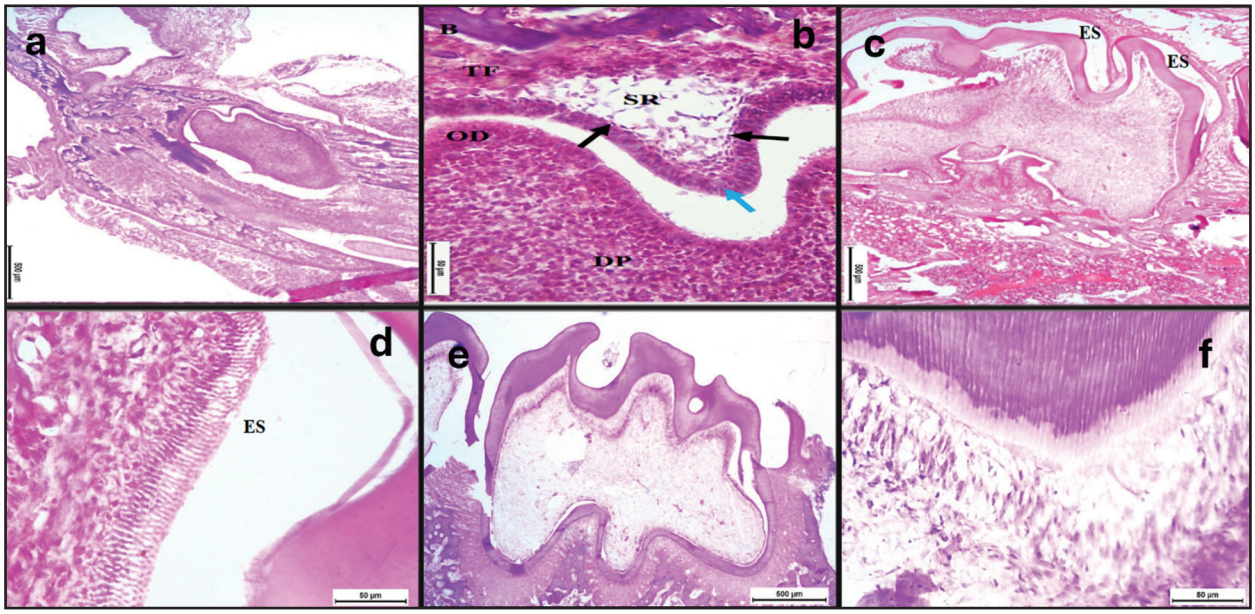

Fig. (1) Photomicrographs of developing lower first molar tooth germ. a: A photomicrograph showing the developing lower first molar tooth germ of control group pups on day zero at the transition from cap to early bell stages of odontogenesis. b: A photomicrograph showing the enamel organ of developing lower first molar of control group on day zero (day of birth). Note the stellate reticulum cells (SR) maintaining intercellular desmosomal junctions giving a star-like configuration. Tooth follicle (TF) is evident and stratum intermedium cells (black arrows) are present in the form of flat cells on top of inner enamel epithelium (blue arrows). Odontoblasts (OD) are seen lining the dental papilla (DP). Bone islands forming the bony crypt (B) are surrounding the tooth germ. c: A photomicrograph showing the developing tooth germ of lower first molar of control pups on the 14th postnatal day. Enamel space (ES) is evident due to enamel maturation and subsequent demineralization by EDTA solution. d: A photomicrograph showing ameloblastic layer of the developing lower first molar enamel organ of control pups on the 14th postnatal day. Note the beginning of shortening of ameloblasts and the wide enamel space (ES) due to enamel mineralization. e: A photomicrograph showing the erupted lower first molar of control pups on the 30th postnatal day. Note the advanced root formation. f: A photomicrograph showing the odontoblastic layer attached to predentin in the lower first molar of control pups on the 30th postnatal day. Odontoblasts show marked pseudostratification with their nuclei situated proximally and the odontoblastic processes are penetrating the predentin which is pale stained compared to dentin (H&E, Orig. mag. 40,400).

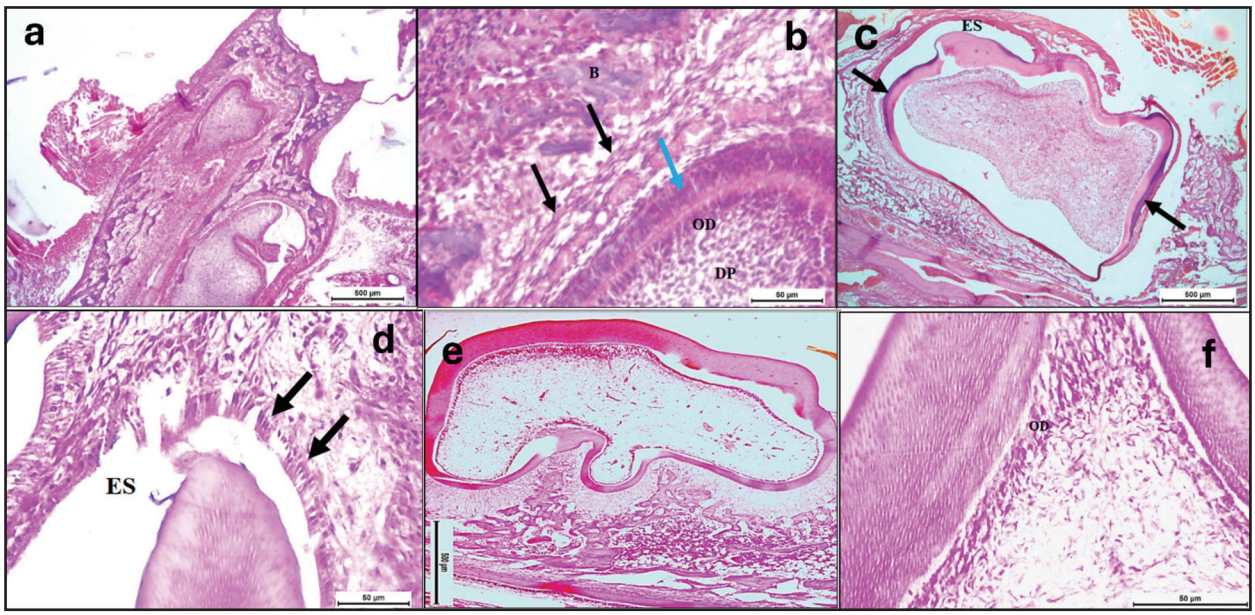

Fig. (2) Photomicrographs of developing lower first molar tooth germ. **a:** A photomicrograph showing the developing lower first molar enamel organ of treated group pups on day zero at the transition from cap to early bell stages of odontogenesis. **b:** A photomicrograph showing the tooth follicle (black arrows) surrounding the developing lower first molar enamel organ of treated group pups on day zero. Note the inner enamel epithelium (blue arrow), odontoblasts (OD) and dental papilla (DP). Bone islands (B) are surrounding the tooth germ to form the bony crypt. **c:** A photomicrograph showing the developing tooth germ of lower first molar of treated pups on the 14th postnatal day. Enamel space is evident due to enamel demineralization by EDTA solution. However, some enamel matrix is evident in certain areas (arrows) **d:** A photomicrograph showing ameloblastic layer of the developing lower first molar enamel organ of treated pups on the 14th postnatal day. Note the shortening of ameloblasts (arrows), the disruption in their continuity and the wide enamel space (ES) due to enamel mineralization. **e:** A photomicrograph showing the erupted lower first molar of treated pups at the 30th postnatal day. Note the ongoing root formation. **f:** A photomicrograph showing the odontoblastic layer (OD) attached to predentin in the lower first molar of treated pups on the 30th postnatal day. Odontoblasts show prominent pseudostratification (H&E, Orig. mag. 40,400).

II) Results of histomorphometric assessment of the thickness of enamel matrix and dentin:

- ***Enamel thickness:***

Data revealed that there is a significant difference between enamel thickness in control group pups on the 9th postnatal day and enamel space thickness in control group pups on the 14th postnatal day at p value ≤ 0.05 while there is no significant difference between enamel thickness in treated group pups on the 9th postnatal day and enamel space thickness in treated group pups on the 14th postnatal day at p value ≥ 0.05. However, there is a significant difference between enamel thickness of control and treated groups at both time points **(Table 1)**.

- ***Dentin thickness:***

Data revealed that there is a significant difference between dentin thickness of control group pups on the 9th postnatal day and control group pups on the 14th postnatal day, treated group pups on the 9th postnatal day and treated group pups on the 14th postnatal day at p value ≤ 0.05. Additionally, there is a significant difference between both groups in both time points **(table 2)**.

Table (1) *Showing statistical results of enamel thickness in the developing tooth germ of the control and treated groups on the 9th and 14th postnatal days:*

Enamel thickness				
Tukey HSD[a]				
Groups	Number of samples	Subset for alpha = 0.05		
		1	2	3
Control 9 days	6	45.625167		
Control 14 days	6		57.099667	
Treated 9 days	6			69.821500
Treated 14 days	6			67.986667
Significance		1.000	1.000	.829
Means for groups in homogeneous subsets are displayed.				
[a]. Uses Harmonic Mean Sample Size = 6.000.				

Table (2) *Showing statistical results of dentin thickness in the developing tooth germ of the control and treated groups on the 9th and 14th postnatal days:*

Dentin thickness				
Tukey HSD[a]				
Groups	Number of samples	Subset for alpha = 0.05		
		1	2	3
Control 9 days	6	99.049167		
Control 14 days	6		110.194000	
Treated 9 days	6		108.788167	
Treated 14 days	6			132.813833
Significance		1.000	.966	1.000
Means for groups in homogeneous subsets are displayed.				
[a]. Uses Harmonic Mean Sample Size = 6.000.				

DISCUSSION

Vaccination by COVID-19 vaccines has become obligatory among the population due to COVID-19 pandemic. Pregnant females are a high-risk group to COVID infection. Therefore, the safety of vaccination must be precisely considered regarding the fetal development and the mother health [(11)].

In the present study, the effect of maternal COVID-19 vaccination using the Oxford vaccine on the development and eruption of lower first molar of the rat offspring was studied.

The current study was performed using Wistar albino rats as experimental animals because of simple handling and similarity of metabolic reactions to humans, specially the physiological processes that rule bone and dentin metabolism (odontogenesis). This offers an excellent experimental model for studies on calcified tissues [(15)].

The developmental stage of the lower first molar tooth germ in the pups was captured and assessed at different time points; at zero day (the day of birth), 14 days, and 30 days postnatal. At all timepoints, the developmental stages of the lower first molar tooth germ in control and treated pups were nearly comparable with no histological differences among them.

The normal development and timing of rat lower first molar from birth to 500 days was first reported by **Hoffman and Schour** [16,17]**.** then by **Nagai *et al*** [18] who assessed development in the prenatal and postnatal stages and reported that the bud stage develops on the 15th and 16th and the cap stage on the 17th and 20th gestation day (just prior to delivery). On the 21st gestation day (day zero), the first odontoblast of the lower first molar starts differentiation while dentin matrix begins to calcify on the first day after birth (day 1).

The results of the present study coordinate largely to these findings, on the day of birth (day zero), the teeth germ of lower first molar of both control and treated pups showed transition from cap to early bell stages of odontogenesis. On the 14th postnatal day, enamel space begins to appear as an artifactual cleft due to the strong mineralization of enamel matrix and disintegration of its scanty organic matrix during preparation. However, enamel matrix is still evident in some cervical areas below the ameloblastic layer. Sections on the 14th postnatal day showed a decreased bone area above the lower first molar preparing for the tooth to erupt.

The thickness of enamel and dentin were investigated in the concurrent study. The enamel thickness has increased normally and significantly from 9 days to 14 days in the control group. However, this was not the case in the treated group where the increase in enamel thickness from day 9 to day 14 was not statistically significant. The reason is not clear. Nevertheless, the difference between both groups at the two timepoints was statistically significant, indicating a favorable enamel formation in the treated group.

Enamel formation and mineralization is regulated with TGF-β1 and Runx2 in ameloblasts with direct relationship between the two genes [19]. Here, we demonstrated an increase in TGF-β1 signaling via upregulation of Bmp-2 expression in treated group.

Likewise, a statistically significant difference was found concerning measured dentin thickness on top of developing cusp tip between control and treated groups. Dentin thickness has increased notably from day 9 to day 14 in each group and also between control and treated groups at the two timepoints. Dentin formation is intimately related to the expression of BMP-2 and Dentin sialophosphoprotein (DSPP). BMP-2 is necessary for odontoblast differentiation [20].

Overall, the histological and histomorphometric findings of this experiment showed that the Oxford vaccine has no effect on the developmental stage of molar tooth germ in rat offspring. However, hard tissue thickness may be affected as the enamel thickness was increased in a non-statistically significant manner in the treated group from day 9 to day 14 while dentin thickness was increased significantly in the treated group at the same timepoints in the lower first molar tooth germ. Some limitations have been faced in the current study including the difficult handling of newborn pups and the need of micro-computed tomography (μCT) imaging to evaluate exactly the eruption process. Further studies are needed to emphasize the effect of vaccination of pregnant mothers on the ultrastructure of dental tissues in the offspring.

CONCLUSION

After conducting the present experiment, the following could be concluded:

1. Using the Oxford vaccine for vaccination of pregnant mothers albino rats did not influence the appearance or the development of lower first molar tooth germ in the albino rat offspring on zero day, 14th day and one month postnatal.
2. Hard tissue thickness in the offspring may be affected by the vaccination.

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