Systematic Review

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Salivary factors related to caries in pregnancy

A systematic review and meta-analysis

Mahtab Yousefi, DDS; Parvin Parvaie, MSD; Seyed Mohammad Riahi, PhD

ABSTRACT

Background. The authors of this meta-analysis aimed to assess saliva-related caries risk factors, including calcium and phosphate, hydrogen ion concentration, buffer capacity, *Streptococcus mutans* and *Lactobacillus* counts, flow rate, and decayed, missing and filled teeth index in each trimester during pregnancy.

Types of Studies Reviewed. The authors searched electronic databases up to July 1, 2019. Eligible observational studies were included. The authors assessed the quality of the included studies by using the Joanna Briggs Institute scale. To estimate the effects of pregnancy, standardized mean differences with 95% confidence intervals were pooled using the random-effects model. Subgroup analysis and meta-regression were used to explore heterogeneity. Publication bias was assessed using Begg and Egger tests.

Results. Twenty-nine studies were included in the meta-analysis, representing 1,230 pregnant women in the case groups and 715 in the control groups (nonpregnant women). The results showed that salivary calcium concentration decreased in the third trimester, salivary phosphate decreased in the second and third trimesters, saliva hydrogen ion concentration decreased in the first and third trimesters, stimulated saliva flow rate increased in the third trimester, and salivary *S mutans* count increased in the second and third trimesters. In addition, the results showed that saliva calcium, phosphate, *S mutans*, and buffer capacity amounts had changed from the first trimester to the third.

Conclusions and Practical Implications. In the third trimester, most salivary factors related to caries change and can increase the risk of developing caries in the future. Interventions and screening for caries prevention in pregnancy should start in the first or second trimesters.

Key Words. Pregnant women; saliva; caries; calcium; *Streptococcus mutans*; hydrogen ion concentration; phosphates; buffer capacity; flow rate; DMFT; meta-analysis.

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Pregnancy affects many organ systems and results in physical and physiological changes in various parts of body, including the oral cavity,[1] and researchers have reported a higher incidence of caries during pregnancy.[2] However, caries onset and activity are complex; saliva is a primary modifying factor[3] and alterations in saliva property during pregnancy might explain the increased incidence of caries.[2] These alterations might be related to estrogen effects, dietary changes, oral hygiene habits, and taste changes in pregnancy.[4,5] Inhibition of bacteria and their substrates, dilution, and elimination of bacteria and their substrates, buffering bacterial acids, and remineralization are effects of saliva on caries.[6] Assessment of caries activity by evaluating salivary biomarkers, hydrogen ion concentration (pH), buffer capacity (BC), calcium, phosphorous, and acidogenic oral bacteria has been reported in some studies.[7,8]

Researchers around the world have reported inconsistent findings on salivary changes during pregnancy; some investigators reported a decrease in salivary flow rate (FR) during pregnancy[9] and others found an increase.[10] *Streptococcus mutans* and *Lactobacillus*, the bacteria that are the main pathogens associated with caries,[11] were counted in saliva during pregnancy and conflicting results were derived from several studies.[12-14] Because salivary calcium and phosphate concentration are

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Table 1. Participants, exposure, comparison, outcomes, and study design questions.

PARTICIPANTS	EXPOSURE	COMPARISON	OUTCOMES	STUDY DESIGN
Pregnant Women (First, Second, and Third Trimesters)	Pregnancy	Nonpregnant women	Salivary factors related to caries (calcium, phosphate, hydrogen ion concentration, buffer capacity, *Streptococcus mutans* count, *Lactobacillus* count, and flow rate) Decayed, missing, and filled teeth index	Observational studies (longitudinal and cross-sectional)
Pregnant Women (Third Trimester)	During pregnancy	First trimester	Salivary factors related to caries (calcium, phosphate, hydrogen ion concentration, buffer capacity, *Streptococcus mutans* count, *Lactobacillus* count, and flow rate) Decayed, missing, and filled teeth index	Observational studies (longitudinal and cross-sectional)

the main determinants of critical pH and play an important role in the remineralization of carious lesions,[6] assayers detected these salivary electrolyte changes in pregnancy but did not obtain a uniform result.[14-16]

Considering that the therapeutic treatment of caries includes improving host resistance via biofilm control, increasing the pH of the biofilm, and enhancing remineralization,[3] identifying the salivary changes in different groups of patients, such as pregnant women, can be helpful in choosing the best treatment.

The goal of our meta-analysis was to compare amounts of salivary factors related to caries (that is, calcium, phosphate, pH, BC, *S mutans* and *Lactobacillus*, and FR) and decayed, missing, and filled teeth (DMFT) index in pregnant and nonpregnant women. In addition, a comparison between the first and third trimesters was done. Participants, exposure, comparison, outcomes, and study design questions are found in Table 1.

METHODS

This systematic review and meta-analysis is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocol guidelines.[17] This article was extracted from a thesis and the protocol was submitted to the Birjand University of Medical Sciences in Iran (registration code Ir.bums.REC.1398.68).

Data sources and searches

Two independent reviewers (R.S.M., Y.M.) conducted a systematic search in English electronic databases (that is, PubMed, Embase, Web of Science, and Scopus) and Persian electronic databases (that is, Magiran, Scientific Information Database, IranMedex, and Irandoc) up to July 1, 2019, to identify quantitative studies. We placed no restrictions on language. The searches were supplemented by screening the reference list of included studies. Search of gray literature was performed using Google Scholar and OpenGrey. Medical Subject Headings and Embase subject headings (Emtree) were used to develop search strategy and the strategy was revised for each database. Specific search strategies are detailed in eTables 1 through 4 (available online at the end of this article).

Eligibility criteria

Observational studies were considered eligible if they used spitting method for collecting whole saliva and cannula or Lashley cup for parotid saliva, and evaluated 1 or more of the following items in the saliva of healthy pregnant women: pH, FR, BC, calcium or phosphate concentration, *S mutans* or *Lactobacillus* count, and DMFT index. Having a control group (healthy nonpregnant women) was not an inclusion criteria and studies that measured the factors mentioned during pregnancy (first trimester-third trimester) were also included. Some studies were not excluded but their data were eliminated if they were reported in a qualitative scale.[4,12,18]

Study selection

After removal of duplicates, 2 reviewers (R.S.M., Y.M.) independently screened the titles and abstracts of search results to identify studies based on the predetermined selection criteria. Interrater

ABBREVIATION KEY

BC: Buffer capacity.
Ca^{2+}: Calcium.
DMFT: Decayed, missing and filled teeth.
FR: Flow rate.
HR: High risk.
LC: *Lactobacillus* count.
OCP: Oral contraception.
P: Phosphorus.
pH: Hydrogen ion concentration.
PO_4^{3-}: Phosphate.
SMC: *Streptococcus mutan* count.

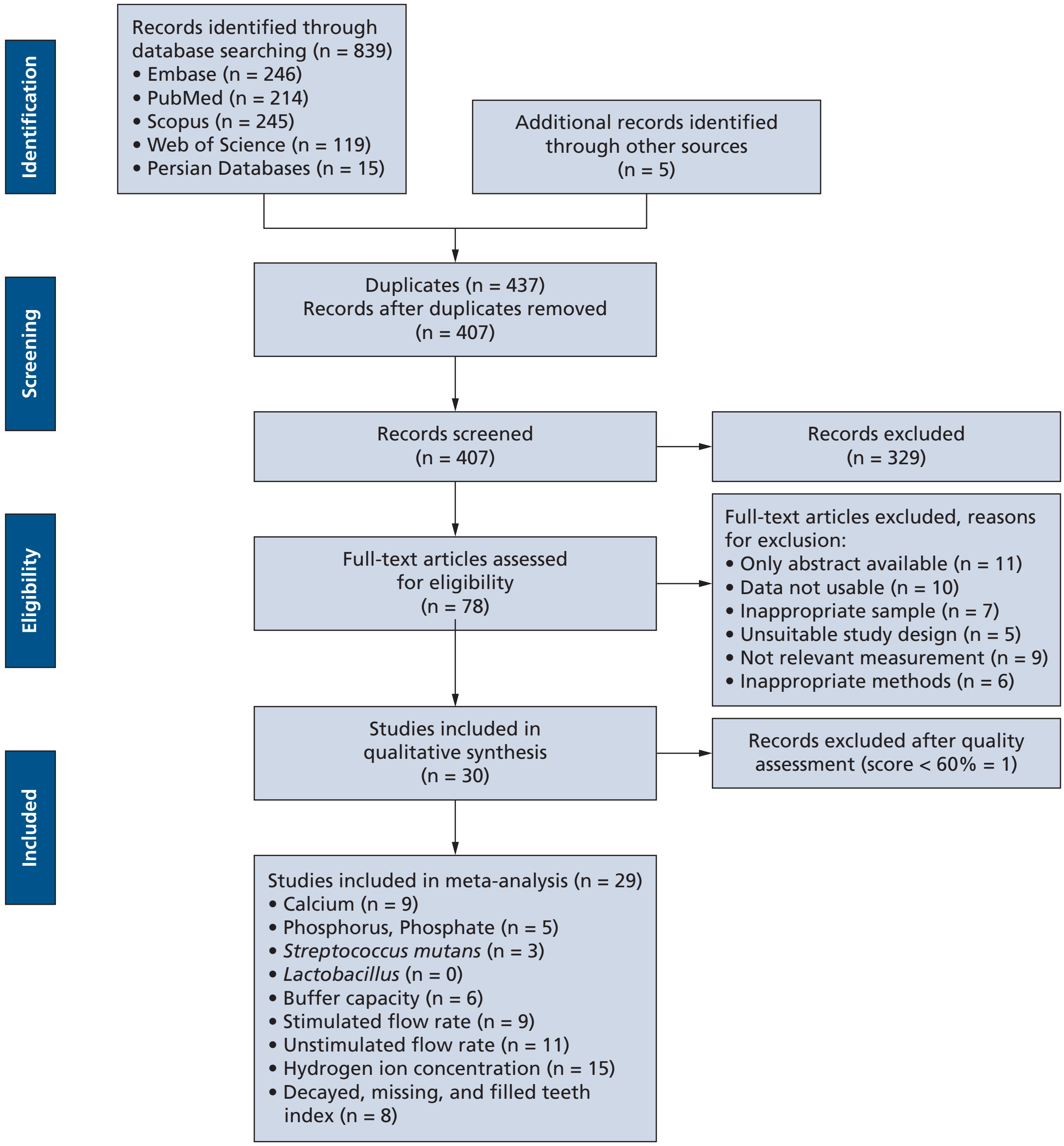

Figure 1. Flowchart of study selection process for the systematic review and meta-analysis.

agreement was 0.98 and any disagreements were resolved by means of discussion or consulting a third reviewer (P.P.). The full texts of all relevant and potentially relevant studies were assessed to determine the final list of included studies. The excluded full-text articles were archived with the reasons for exclusion. Flow of the records is depicted in a Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram (Figure 1).

Data extraction

A standard dedicated data extraction form was designed in Excel software (Microsoft). Two authors (R.S.M., Y.M.) extracted data independently. The following information was included: general information, including first author, study design (cross-sectional or longitudinal), publication year, country of study (Human Development Index and World Health Organization region), quality grade of study; population characteristics, including minimum, maximum, and mean age by groups (pregnant and control), trimester (first, second, and third), inclusion and exclusion criteria, matching variables, measured outcomes, mean and standard deviation of each outcome in groups and trimesters; and methodological information, including saliva collection method (stimulated or unstimulated and time of collection), sample size by groups and trimesters, and laboratory method for determination of each outcome.

Unification of measurement units

To enable comparison, phosphate and calcium measures were converted from milligrams per deciliter to millimoles per liter using their molar mass. FR amounts were converted to milliliters per minute. Phosphate and phosphorus moles were considered equivalent because saliva contains an equal number of moles of phosphorus and phosphate.[19]

Risk of bias assessment

After a calibration exercise, evaluation of the included study quality was conducted by 2 authors (R.S.M., Y.M.) independently using the Joanna Briggs Institute scale for observational cohort and analytical cross-sectional studies.[20] Interrater agreement was 0.95 and any disagreements were resolved by means of discussion or consulting a third reviewer (P.P.). According to the authors' agreement, studies with quality scores less than 60% were considered a high risk of bias and were excluded.

Statistical analysis

All statistical analyses were done using Stata software, Version 14.0 (Stata). The pooled effect size in all outcomes was calculated by standardized mean difference (SMD) and 95% confidence interval (CI) for each trimester of pregnancy. The random-effect model was used to estimate pooled SMD. Heterogeneity among studies was assessed by graphical and statistical tests (that is, Galbraith diagram, Cochran Q, and I^2 statistics). The range of I^2 statistics was from 0% through 100%, and values of 70% or higher were considered heterogeneous.[21,22] Meta-regression and subgroup analysis were conducted to explore the sources of heterogeneity among studies.[23] Publication bias was evaluated graphically and statistically by Begg and Egger plots and tests. In all analyses, the significance level was considered 0.05. The significance level in analyses with fewer than 5 studies was considered 0.1.

RESULTS

Search results

Figure 1 illustrates the flow of the study selection process. According to the search strategy (eTables 1 through 4; available online at the end of this article), 844 articles were found and, after removal of duplicates, 407 studies remained. Title and abstract screening identified 78 studies for full-text assessment. In the next step, 48 articles were excluded for the following reasons: only abstracts were available, data were not usable, samples were inappropriate, study designs were unsuitable, measurements were not relevant, and the methods of saliva collection or outcome measurements were inappropriate. Quality assessment of 30 studies was performed and 1 study was removed (score $< 60\%$, $n = 1$) to reduce the risk of bias.[24] Overall, 29 studies were included in the meta-analysis.

Study characteristics

Ten longitudinal and 19 cross-sectional studies were included in the meta-analysis. Publication years of articles ranged from 1959 through 2018. Twelve studies were conducted in Asia, 8 studies in Europe, 7 studies in the United States, and 2 studies in Africa. In total, 1,230 pregnant women were included in the case groups and 715 in the control groups. Mean age of participants was 26.57 years and in 16 studies age was a matching variable. Researchers in only 2 studies collected parotid saliva; the others used whole saliva. In 14 studies, detection was conducted in unstimulated saliva, in 9 studies researchers collected stimulated saliva and in 5 studies researchers used both of them. Researchers mentioned inclusion or exclusion criteria or both in 23 studies and in almost all of these studies emphasized an absence of systemic diseases. The characteristics of the studies and participants are summarized in Table 2.

Table 2. Characteristics of studies and participants included in the meta-analysis.

STUDY	COUNTRY	STUDY DESIGN	PARTICIPANTS			
			Age Range (Y)	Pregnant		Not Pregnant, No.
				No.	Trimester	
Rosenthal and Colleagues,[25] 1959	United States	Cross-sectional	14-38	61	NA*	52
Marder and Colleagues,[26] 1972	America	Longitudinal	15-42	59	First, second, third	NA
Laine and Colleagues,[27] 1988	Finland	Longitudinal	19-34	16	First, second, third	NA
D'Alessandro and Colleagues,[28] 1989	Argentina	Cross-sectional	17-44	107	First, second, third	7
Guidozzi and Colleagues,[15] 1992	South Africa	Cross-sectional	18-31	25	Third	9
Salvolini and Colleagues,[29] 1998	Italy	Cross-sectional	20-30	45	First, second, third	15
Laine and Colleagues,[30] 2000	Finland	Longitudinal	26-41	8	Third	15
Kivelä and Colleagues,[31] 2003	Finland	Longitudinal	24-39	9	Third	17
Rockenbach and Colleagues,[16] 2006	Brazil	Cross-sectional	18-38	22	NA	22
Molnar-Varlam and Colleagues,[18] 2011	Romania	Longitudinal	20-35	35	First, second, third	NA
Ortiz-Herrera and Colleagues,[32] 2011	Mexico	Cross-sectional	16-45	25	NA	25
Seifi and Colleagues,[33] 2011	Iran	Longitudinal	19-27	30	First, second, third	NA
Bakhshi and Colleagues,[34] 2012	Iran	Cross-sectional	18-35	60	First, second, third	60
Lasisi and Colleagues,[35] 2014	Nigeria	Cross-sectional	20-42	50	NA	50
Martinez-Pabon and Colleagues,[12] 2014	Colombia	Longitudinal	16-42	35	Second, third	NA
Naveen and Colleagues,[10] 2014	India	Cross-sectional	19-34	30	Third	30
Purushothama and Colleagues,[36] 2014	India	Cross-sectional	20-35	30	First, second, third	30
Saluja and Colleagues,[37] 2014	India	Cross-sectional	15-55	30	Third	30
Bakhshi and Colleagues,[38] 2015	Iran	Cross-sectional	20-35	90	First, second, third	30
Jain and Colleagues,[39] 2015	India	Cross-sectional	18-35	120	First, second, third	40
Karnik and Colleagues,[9] 2015	India	Cross-sectional	18-37	30	First, second, third	30
Pancu and Colleagues,[40] 2015	Romania	Cross-sectional	20-40	15	NA	15
Rio and Colleagues,[41] 2015	Portugal	Longitudinal	18-40	30	First, third	30
Hegde and Colleagues,[42] 2016	India	Cross-sectional	19-34	30	Third	30
Rio and Colleagues,[43] 2016	Portugal	Longitudinal	20-40	30	First, third	30
Kamate and Colleagues,[14] 2017	India	Longitudinal	18-28	50	First, second, third	50
Singh and Colleagues,[13] 2017	India	Cross-sectional	NA	25	NA	25
Sonbul and Colleagues,[4] 2017	Saudi Arabia	Cross-sectional	16-54	80	First, third	41
Méndez Monge and Colleagues,[44] 2018	Mexico	Cross-sectional	18-37	53	NA	32

* NA: Not applicable. † pH: Hydrogen ion concentration. ‡ Ca^{2+}: Calcium. § FR: Flow rate. ¶ BC: Buffer capacity. # OCP: Oral contraception. ** PO_4^{3-}: Phosphate. †† P: Phosphorus. ‡‡ HR: High risk. §§ SMC: *Streptococcus mutans* count. ## DMFT: Decayed, missing, and filled teeth.

Table 2. (Continued)

INCLUSION/ EXCLUSION CRITERIA	MATCHING VARIABLES	SALIVA COLLECTION METHOD; GLAND	COLLECTION TIME	MEASUREMENTS	QUALITY SCORE
Inclusion: healthy participants	Age	Unstimulated; whole	NA	pH,[†] Ca^{2+}[‡]	60.00
NA	NA	Stimulated; parotid	NA	FR,[§] Ca^{2+}	60.00
Inclusion: healthy participants, good oral health	NA	Stimulated; whole	7:30-10:00 AM	FR, pH, BC,[¶] sialic acid, protein, anions	60.00
NA	NA	Stimulated; parotid	NA	FR, pH	60.00
Exclusion: systemic disease, medication, using OCP[#]	NA	Unstimulated; whole	9:00-11:00 AM	Ca^{2+}, PO_4^{3-}[**]	60.00
Inclusion: No systemic disease, 20 ≤ body mass index ≤ 25.9, standard diet, normal glucose tolerance and blood pressure	Age	Unstimulated; whole	9:30-11:30 AM	Ca^{2+}, P[††]	100
NA	Age	Stimulated, unstimulated; whole	8:00-10:00 AM	FR, pH, BC	70.00
Inclusion: healthy noncigarette smokers, regular dental examinations, good oral health	Age, hormonal status	Stimulated; whole	8:30 AM-1:00 PM	FR, BC	80.00
Inclusion: healthy participants, no xerostomia Exclusion: HR[‡‡] pregnancy	Age, oral hygiene	Unstimulated; whole	7:30-10:30 AM	pH, Ca^{2+}, PO_4^{3-}	71.42
Inclusion: no orthodontic braces, regular dental examination Exclusion: preexisting infection, low-level education, job risk factors	NA	Stimulated; whole	NA	pH, SMC,[§§] *Lactobacillus* count, BC	70.00
Inclusion: no systemic disease Exclusion: HR pregnancy	NA	Unstimulated; whole	NA	DMFT index,[##] pH	60.00
Exclusion: systemic disease, medication, cigarette smoker, xerostomia	NA	Unstimulated; whole	15-17	Ca^{2+}, P	80.00
Inclusion: first pregnancy, normal blood pressure, normal weight gaining, no history of abortion Exclusion: systemic disease, medication, cigarette smoker	Age	Unstimulated; whole	9:30-11:30 AM	Ca^{2+}, P	100
Exclusion: using OCP, hypertension, diabetes mellitus, gingival bleeding, toothache, intraoral swelling	Age	Unstimulated; whole	8:00-9:00 AM	FR, pH, Ca^{2+}, PO_4^{3-}	100
NA	NA	Stimulated; whole	NA	FR, pH, BC, acidogenic bacteria count	60.00
Exclusion: systemic disease, salivary gland disorder, oral mucosal diseases		Stimulated, unstimulated; whole	9:00-11:30 AM	FR, pH, BC	71.42
Inclusion: 5 ≤ DMFT index ≤ 15 Exclusion: systemic disease, medication, xerostomia, DMFT index ≤ 5	Age, DMFT index	Stimulated; whole	9:00-11:00 AM	SMC	100
Inclusion: no systemic disease, no salivary gland disorders, good oral hygiene Exclusion: cigarette smoker, HR pregnancy		Stimulated; whole	11:00-14:00 AM	FR, pH,	71.42
Exclusion: systemic disease, medication, cigarette smoker, pocket depth ≥ 3 mm	Age	Unstimulated; whole		pH, SMC, BC	100
Exclusion: systemic disease, medication, cigarette smoker, tobacco chewing habit		Unstimulated; whole		pH	71.42
Inclusion: not using OCP Exclusion: systemic disease, xerostomia, cigarette smoker	Age	Unstimulated; whole	9:00-11:00 AM	DMFT index, FR, pH	100
Inclusion: no systemic disease	NA	Stimulated, unstimulated; whole	NA	FR, BC	60.00
Exclusion: systemic disease, drug addiction, cigarette smoker, menopause, HR pregnancy, ≥ 16 teeth	Age, education	Stimulated, unstimulated; whole	8:00 AM-12:00 PM	FR, pH, Ca^{2+}, PO_4^{3-}	90.00
Inclusion: Not using OCP Exclusion: systemic disease, salivary gland disorder	Age	Stimulated, unstimulated; whole	9:00-11:30 AM	DMFT index, FR, pH, Ca^{2+}, BC	71.42
Exclusion: systemic disease, drug addiction, menopause, HR pregnancy, ≥ 16 teeth	Age, oral hygiene, education, cigarette smoker	Unstimulated; whole	NA	FR, pH	70.00
Exclusion: systemic disease, medication, DMFT index ≥ 15	Age	Unstimulated; whole	9:00-11:00 AM	DMFT index, FR, pH, SMC, Ca^{2+}	100
NA	NA	Stimulated; whole	NA	SMC	60.00
Inclusion: not systemic disease, not medication, ≤ 20 teeth	Age, socioeconomic	Stimulated, unstimulated; whole	NA	DMFT index, FR, pH, SMC, *Lactobacillus* count	100
NA	Age	Stimulated; whole	NA	DMFT index, FR, pH	85.81

Meta-analysis results

The results are presented in the sections that follow and Tables 3 and 4.

All Trimesters Versus Control

The decreased salivary calcium concentration in pregnancy was marginally significant, although considerable heterogeneity was present (SMD, −0.52 [95% CI, −1.08 to −0.03]; $P = .065$, $I^2 =$ 91.40%). We found significant differences in reducing salivary pH during the pregnancy but the heterogeneity was high (SMD, −0.77 [95% CI, −1.07 to −0.48]; $P < .001$, $I^2 = 79.50\%$). The meta-analysis results showed that salivary *S mutans* count had a statistically significant increase during pregnancy compared with control (SMD, 0.79 [95% CI, 0.51 to 1.08]; $P < .001$, $I^2 =$ 0.00%). There were no statistically significant differences between the saliva phosphate, pH, BC, FR, and DMFT index during all trimesters compared with control.

Third Trimester Versus Control

Our results suggest that salivary calcium and phosphate concentration reduced statistically in the third trimester compared with control (for calcium: SMD, −1.11 [95% CI, −1.59 to −0.62]; $P < .001$, $I^2 = 75.50\%$; for phosphate: SMD, −1.22 [95% CI, −1.90 to −0.49]; $P =$ .001, $I^2 = 68.30\%$). Stimulated FR was statistically higher in the third trimester relative to control (SMD, 0.28 [95% CI, 0.06 to 0.50]; $P = .014$, $I^2 = 6.70\%$). There was a significant negative association between the third trimester and salivary pH (SMD, −0.86 [95% CI, −1.28 to −0.45]; $P < .001$, $I^2 = 79.90\%$). Salivary *S mutans* count increased significantly during the third trimester compared with control (SMD, 1.73; 95% CI, 1.13 to 2.33; $P < .001$, $I^2 =$ 41.70%). Differences in other outcomes in the third trimester compared with control participants were not significant.

Second Trimester Versus Control

There was an association between the second trimester and decreased salivary phosphate concentration (SMD, −1.41 [95% CI, −1.91 to −0.90]; $P < .001$, $I^2 = 12.70\%$). Salivary *S mutans* count statistically decreased in the second trimester relative to control, although there were 2 related articles (SMD, 0.83 [95% CI, 0.33 to 1.34]; $P = .010$, $I^2 = 35.70\%$). There were not enough studies available to perform meta-analysis for BC and FR at the second trimester. Comparisons of salivary calcium, pH, and DMFT index in the second trimester relative to control participants were not significant.

First Trimester Versus Control

Only salivary pH significantly decreased in the first trimester compared with control participants (SMD, −0.5 [95% CI, −0.99 to −0.02; $P = .042$, $I^2 = 75.5\%$). Other outcomes were not significantly different in this trimester compared with control participants.

Third Trimester Versus First Trimester

From the first trimester to the third trimester, increasing salivary *S mutans* count and decreasing salivary calcium, phosphate, and BC were significant (for *S mutans*: SMD, 1.29 [95% CI, 0.45 to 2.13]; $P = .003$, $I^2 = 64.90\%$; for calcium: SMD, −0.82 [95% CI, −1.53 to −0.1]; $P = .025$, I^2 =87.40%; for phosphate: SMD, −0.82 [95% CI, −01.64 to 0.01]; $P = .052$, $I^2 = 79.3\%$; and for BC: SMD, −0.67 [95% CI, −1.09 to −0.24]; $P = 0.002$, $I^2 = 0.00\%$).

Additional analyses

Subgroup analysis and meta-regression were performed to analyze sources of heterogeneity. Publication year, Human Development Index, mean age of participants, and quality grade of study were analyzed in meta-regression and saliva detection method (stimulated or unstimulated) and laboratory detection method for outcomes were applied for subgroup analysis. None of these factors were found to affect the heterogeneity through meta-regression, but we found a significant association for salivary pH in the subgroup analysis using the saliva collection method. In stimulated saliva, differences in pH were not significant in the third trimester compared with first and in the third trimester and all trimesters compared with control, but there was a significant association in unstimulated saliva, and the heterogeneity declined (for stimulated saliva in the third trimester:

Table 3. Meta-analysis results of salivary biomarkers of caries in pregnancy compared to control.

VARIABLE	NO.	TOTAL SAMPLE, NO.	CASE, NO.	CONTROL, NO.	SMD* (95% CI†)	P VALUE FOR SMD = 0	Cochran Q	I^2, %	P VALUE FOR EGGER TEST
All Trimesters Versus Control									
Ca^{2+}‡	9	691	373	318	−0.52 (−1.08 to 0.03)	.065	93.84	91.40	.69
P,§ PO_4^{3-}¶	5	318	182	136	−0.5 (−1.26 to 0.23)	.178	35.96	88.90	.73
pH#	15	1079	646	433	−0.77 (−1.07 to −0.48)	< .001	68.31	79.50	.83
BC‡‡	6	319	182	137	−0.49 (−1.20 to 0.21)	.170	37.82	86.80	.82
Streptococcus mutans count	3	210	105	105	0.79 (0.51 to 1.08)	< .001	0.27	0.00	.58
Stimulated FR††	9	579	362	217	0.10 (−0.14 to 0.33)	.419	12.35	35.20	.83
Unstimulated FR	11	745	392	353	0.08 (−0.25 to 0.42)	.620	49.90	80.00	.66
DMFT index**	8	696	418	278	0.05 (−0.27 to 0.38)	.742	29.33	76.10	.26
Third Trimester Versus Control									
Ca^{2+}	6	364	170	194	−1.11 (−1.59 to −0.62)	< .001	20.40	75.50	.88
P, PO_4^{3-}	3	144	60	84	−1.22 (−1.94 to −0.49)	.001	6.31	68.30	.33
pH	9	556	304	252	−0.86 (−1.28 to −0.45)	< .001	39.70	79.90	.67
BC	5	255	133	122	−0.62 (−1.37 to 0.12)	.103	30.40	86.90	.67
S Mutans count	2	140	60	80	1.73 (1.13 to 2.33)	< .001	1.72	41.70	NA§§
Stimulated FR	7	383	213	170	0.28 (0.06 to 0.50)	.014	6.43	6.70	.37
Unstimulated FR	6	422	210	211	0.09 (−0.42 to 0.60)	.730	33.37	85.00	.73
DMFT index	4	321	160	161	0.21 (−0.40 to 0.83)	.499	86.60	86.60	.69
Second Trimester Versus Control									
Ca^{2+}	3	210	85	125	−0.89 (−2.06 to 0.28)	.138	26.09	92.30	.34
P, PO_4^{3-}	2	110	35	75	−1.41 (−1.91 to −0. 90)	< .001	1.15	12.70	NA
pH	3	208	121	87	−0.71 (−1.57 to 0.16)	.100	13.70	85.50	.68
BC	1	60	30	30	−0.08 (−0.59 to 0.43)	.750	'NA	100.00	NA
S Mutans count	2	140	60	80	0.83 (0.33 to 1.34)	.010	1.56	35.70	NA
Stimulated FR	1	48	41	7	0.22 (−0.58 to 1.02)	.600	NA	100.00	NA
Unstimulated FR	1	100	50	50	0.64 (0.24 to 1.04)	.002	NA	100.00	NA
DMFT index	2	180	90	90	0.35 (−0.34 to 1.04)	.321	5.29	81.10	NA
First Trimester Versus Control									
Ca^{2+}	4	270	115	155	−0.17 (−0.51 to 0.17)	.330	5.17	42.00	.94
P, PO_4^{3-}	3	170	65	105	0.05 (−0.76 to 0.86)	.900	11.74	83.00	.97
pH	5	528	166	147	−0.5 (−0.99 to −0.02)	.042	16.30	75.50	.49
BC	1	60	30	30	−0.03 (−0.53 to 0.48)	.920	NA	100.00	NA
S mutans count	2	140	60	80	0.04 (−0.44 to 0.52)	.870	1.56	36.10	NA
Stimulated FR	3	174	96	78	−0.06 (−0.37 to 0.24)	.685	1.19	0.00	.66
Unstimulated FR	4	301	150	151	0.11 (−0.11 to 0.34)	.320	2.83	0.00	.60
DMFT index	3	261	130	131	0.05 (−0.37 to 0.48)	.807	6.09	67.10	.86

* SMD: Standardized mean difference. † CI: Confidence interval. ‡ Ca^{2+}: Calcium. § P: Phosphorus. ¶ PO_4^{3-}: Phosphate. # pH: Hydrogen ion concentration. ** DMFT: Decayed, missing, and filled teeth. †† FR: Flow rate. ‡‡ BC: Buffer capacity. §§ NA: Not applicable.

SMD, −0.21 [95% CI, −0.50 to 0.08]; $P = .156$, $I^2 = 0\%$; and for unstimulated saliva: SMD, −1.34 [95% CI, −1.58 to −1.10]; $P < .001$, $I^2 = 0\%$) (Figure 2). In addition, subgroup analysis by means of detection method of BC found that saliva-check buffer kit method would give a different result compared with the other methods. Saliva-check buffer kit results showed significant decreasing BC

Table 4. Meta-analysis results of salivary biomarkers of caries during pregnancy, the first trimester compared with the third trimester.

VARIABLE	NO.	TOTAL SAMPLE, NO.	CASE, NO.	CONTROL, NO.	SMD* (95% CI†)	*P* VALUE FOR SMD = 0	Cochran *Q*	I^2, %	*P* VALUE FOR EGGER TEST
Calcium	5	290	145	145	−0.82 (−1.53 to −0.10)	.025	31.69	87.40	.41
Phosphorus, Phosphate	3	130	65	65	−0.82 (−1.64 to 0.01)	.052	9.66	79.30	.26
Hydrogen Ion Concentration	8	528	271	257	−0.27 (−0.84 to 0.30)	.352	70.00	90.00	.47
Buffer Capacity	2	92	46	46	−0.67 (−1.09 to −0.24)	.002	0.81	0.00	NA‡
***Streptococcus mutans* Count**	2	120	60	60	1.29 (0.45 to 2.13)	.003	2.85	64.90	NA
Stimulated Flow Rate	4	252	126	126	0.13 (−0.22 to 0.48)	.471	5.44	44.90	.72
Unstimulated Flow Rate	4	300	150	150	0.06 (−0.24 to 0.36)	.690	5.06	40.70	.55
Decayed, Missing, and Filled Teeth Index	3	260	130	130	0.24 (−0.19 to 0.68)	.274	6.20	67.80	.52

* SMD: Standardized mean difference. † CI: Confidence interval. ‡ NA: Not applicable.

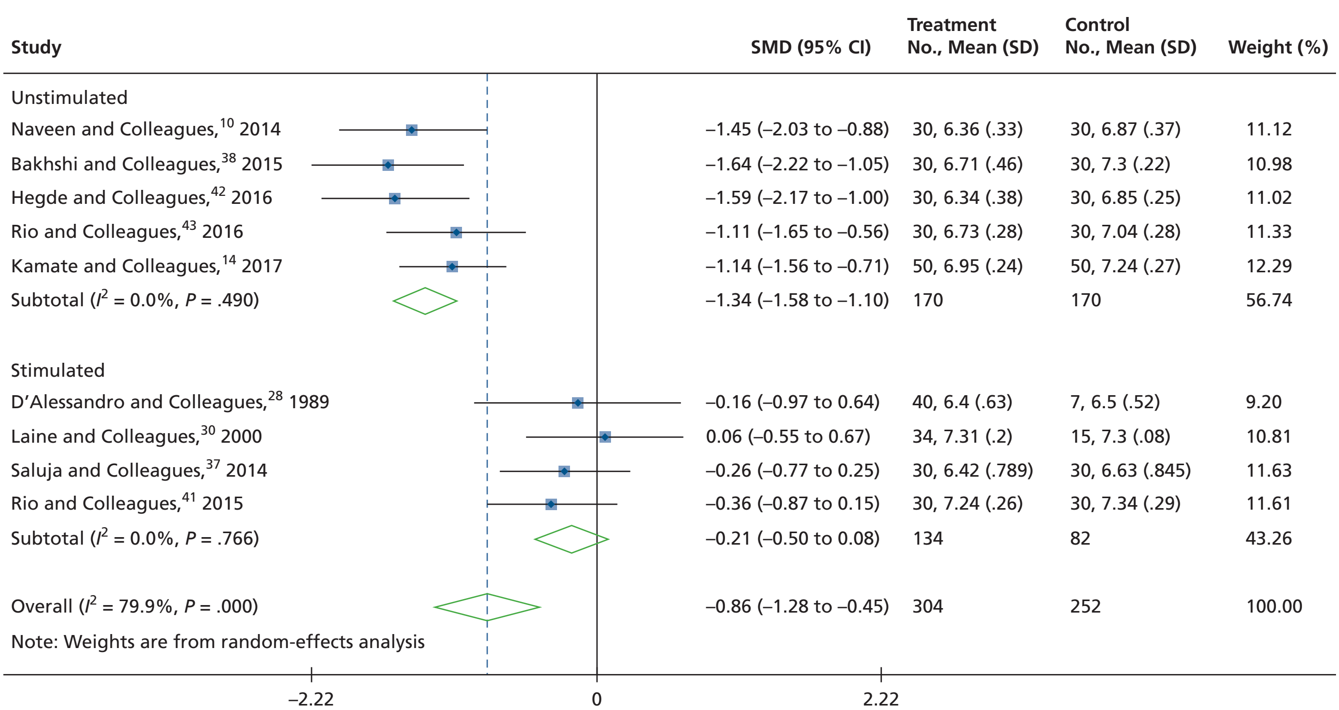

Figure 2. Subgroup analysis for salivary pH via saliva collection method in the third trimester compared with control group. CI: Confidence interval. SD: Standard deviation. SMD: Standardized mean difference.

of saliva in the third trimester and all trimesters, but other methods did not show significant changes (for saliva-check buffer kit in the third trimester: SMD, −1.52 [95% CI, −1.93 to −1.11]; $P < .001$, $I^2 = 0\%$; for other methods: SMD, −0.06 [95% CI, −0.51 to 0.45]; $P = .81$, $I^2 = 36.80\%$) (Figure 3).

Publication bias

There was no evidence of publication bias from the funnel plot and Egger test was not significant for all analyses (Tables 3 and 4).

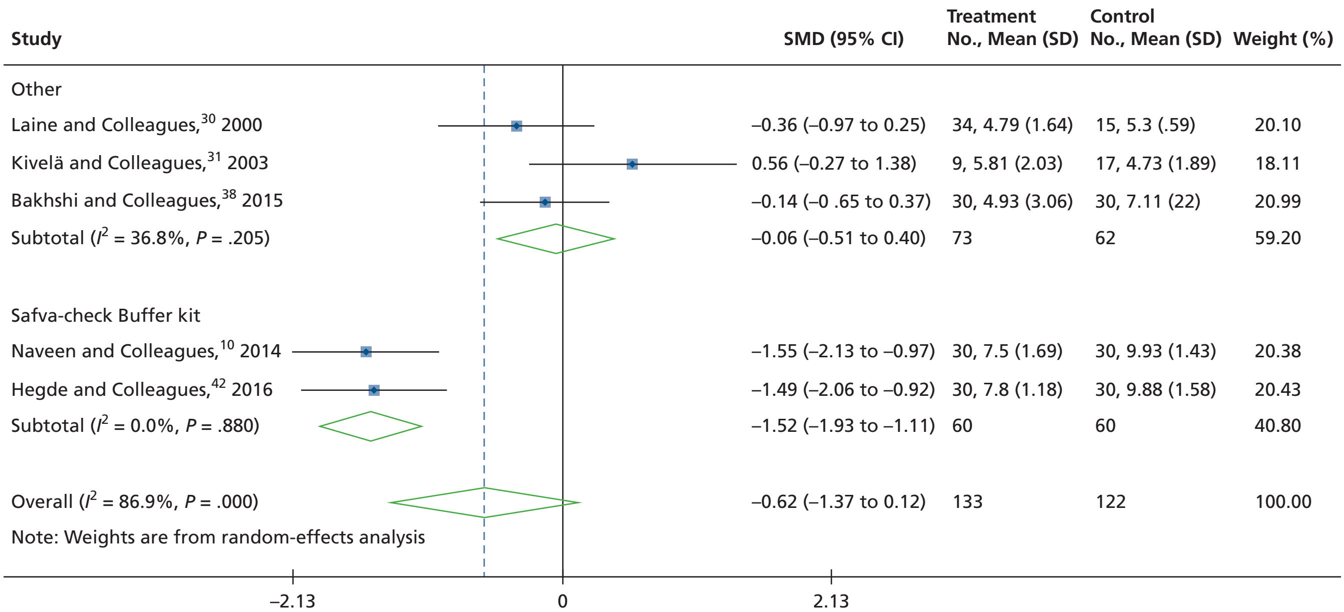

Figure 3. Subgroup analysis for buffer capacity via detection method in the third trimester compared with control group. CI: Confidence interval. SD: Standard deviation. SMD: Standardized mean difference.

DISCUSSION

Main findings

To our knowledge, this is the first systematic review and meta-analysis that has investigated changes in salivary factors related to caries and DMFT index during pregnancy and in each trimester compared with nonpregnant women.

Calcium and phosphate are protective factors against caries as remineralization agents. They are a part of hydroxyapatite unit cell in enamel and these salivary components are involved in maintaining supersaturation of teeth. Phosphate also plays a buffering role in saliva.[6] Our study results found that decreases in salivary calcium occur in the third trimester and decreases of salivary phosphate start in the second trimester, with a large effect size. We found that both of them are reduced during pregnancy from the first trimester to the third trimester. Our results suggest that salivary phosphate concentration is reduced in pregnant women compared with nonpregnant women, but changes in calcium were marginally significant in this comparison. The heterogeneity between studies for this result was substantial and did not reduce with subgroup and meta-regression analysis.

The other factor we investigated was saliva pH. According to previous researchers, salivary pH can enhance the demineralization process by 2 effects. First, low saliva pH has been proposed to cause a shift in acid-tolerant and acid-producing bacteria[45] like *S mutans*, which is the main etiologic agent for caries. Second, low saliva pH can cause decreased saliva phosphate concentration as a remineralization agent.[6] We found decreasing saliva pH in pregnant women compared with control participants. With subgroup analysis, we found that decreasing pH occurs in unstimulated saliva during the first and third trimesters relative to control participants. As pregnant women are susceptible to gastroesophageal reflux disease[46] and because of a relationship between saliva pH and the volume and value of esophageal pH,[47] a hypothesis of decreasing saliva pH in pregnancy can be supported. In addition, some investigators reported lower saliva pH as a result of progesterone action on the level of plasma bicarbonate in pregnancy.[10] Differences in dietary and oral hygiene habits and taste changes can also be reasons for lower saliva pH in pregnancy.[4,5]

The results of saliva BC were different with respect to its measuring method. The saliva check buffer method indicated significant decreasing of saliva BC during pregnancy,[10,42] but other methods did not show significant differences.[30,31,34,40] Our results indicated decreasing BC from the first trimester to the third trimester. There are 2 systems of BC in saliva: bicarbonate buffer system and phosphate buffer system.[6] Lasisi and Ugwuadu[35] reported that salivary bicarbonate was reduced during pregnancy after an increase in progesterone and it caused lower BC. Decreased BC results in increasing acidity of the oral environment and its associated effects.

The main microorganisms implicated in the initiation and progression of caries are *S mutans* and *Lactobacillus*, and salivary counts of these pathogens are associated with an increased caries frequency.[48] We found increased *S mutans* counts in saliva from the second trimester compared with control participants with a low heterogeneity and large effect size. Changes continued in the third trimester with a higher effect size. Unfortunately, for analysis of *Lactobacillus* count, we did not find any suitable study based on our inclusion criteria, which indicates the need for future studies.

FR is another important factor because it can change the composition of saliva and decreased salivary FR is a risk factor for caries.[49] We concluded that the stimulated saliva FR increased during the third trimester. This result is inconsistent with some previous studies.[28,31,37,41] The differences in results can be explained by low sample sizes, parotid sampling, and normal intraindividual changes of saliva content. Presence of estrogen receptor-beta has been identified in oral epithelium and salivary glands,[50] and an increase in salivary FR was reported during the use of hormonal replacement therapy[51]; therefore, increasing stimulated saliva FR in pregnancy might be due to the estrogen effect. As saliva FR is also a modulator of salivary pH,[47] increasing stimulated salivary FR in pregnancy can clarify our result, indicating that salivary stimulated pH was not statistically altered during pregnancy. Our study results showed that unstimulated saliva did not change significantly during pregnancy. Unstimulated FR is more important in oral health because the usual state of the glands is at rest.[49]

No significant changes in DMFT index during pregnancy were expected because caries can progress for a long period and most of the changes in saliva occur in the third trimester. Time plays an important role in the formation of caries, as caries is considered a chronic and ongoing disease.[52] However, in a longitudinal study, Kamate and colleagues[14] reported that increased *S mutans* and decreased pH and calcium in saliva continued in the postpartum period and these changes were reflected in an increased DMFT index.

Strengths and limitations

Our study has several strengths. First, all trimesters of pregnancy were included in meta-analysis. Second, we considered several caries-related factors in saliva. Third, the effect of stimulated and unstimulated saliva on all of the salivary factors was investigated in subgroup analysis. Fourth, publication bias was not detected. In addition, our study has some limitations that must be acknowledged. First, in some analyses, the number of included studies was low, especially in the second trimester. Second, there was high heterogeneity among the studies in some analyses, which was mostly reduced via application of subgroup analysis and meta-regression. Third, there were not sufficient related studies for analyzing salivary *Lactobacillus* count. Finally, age matching was not mentioned in some included studies; however, the age range of pregnancy was narrow.

CONCLUSIONS

According to the results of our study, maximum changes in salivary pH and *S mutans* count occur during the third trimester, which can increase the risk of developing caries postpartum; however, future studies are suggested to investigate salivary changes postpartum. Decreases of salivary calcium and phosphate were observed with high effect size but substantial heterogeneity.

Based on our results, interventions and screening for caries prevention in pregnancy should start in the first or second trimesters. A strategy for the prevention of caries might include increasing saliva pH by means of BC with use of neutralizing mouthrinse, reducing refined carbohydrates exposures (quantity and numbers), decreasing saliva *S mutans* count by using xylitol, and maintaining good oral hygiene habits. ■

SUPPLEMENTAL DATA

Supplemental data related to this article can be found at: https://doi.org/10.1016/j.aime.2020.04.021.

Dr. Yousefi is a general dental practioner, School of Dentistry, Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran.

Dr. Parvaie is an assistant professor, Department of Oral and Maxillofacial Medicine, School of Dentistry, Birjand University of Medical Sciences, Birjand, Iran.

Dr. Riahi is an assistant professor, Cardiovascular Diseases Research Center, Department of Epidemiology and Biostatistics, School of Health, Birjand University of Medical Sciences, Birjand, Iran. Address correspondence to Dr. Riahi, Cardiovascular Diseases Research Center, Department of Epidemiology and Biostatistics, School of Health, Birjand University of Medical Sciences, Ghafari St, Birjand, Southern Khorasan, 9717853577 Iran, e-mail Riahim61@gmail.com.

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eTable 1. Search strategy in Embase database.

EMBASE	SEARCH STRINGS	HITS
#1	Search 'pregnancy'/exp OR 'pregnancy'	928,232
#2	Search pregnant	237,900
#3	Search 'saliva'/exp OR 'saliva'	70,994
#4	Search 'salivary excretion'/exp OR 'salivary excretion'	249
#5	Search 'salivary flow'/exp OR 'salivary flow'	11,516
#6	Search 'salivary'	86,666
#7	Search 'streptococcus mutans'/exp OR 'streptococcus mutans'	12,549
#8	Search 'buffering capacity'/exp OR 'buffering capacity'	3,241
#9	Search 'bicarbonate'/exp OR 'bicarbonate'	67,300
#10	Search 'hydrogen ion concentration'/exp OR 'hydrogen ion concentration'	383,232
#11	Search 'ph'/exp OR 'ph'	731,088
#12	Search 'lactobacillales'/exp OR 'lactobacillales'	213,121
#13	Search 'flow rate'/exp OR 'flow rate'	96,961
#14	Search 'calcium'/exp OR 'calcium'	792,427
#15	Search 'phosphate'/exp OR 'phosphate'	496,500
#16	Search 'dental caries'/exp OR 'dental caries'	55,887
#17	Search 'dental caries activity'	78
#18	Search 'dmft index'/exp OR 'dmft index'	985
#19	Search #1 OR #2	981,904
#20	Search #3 OR #4 OR #5 OT #6	132,756
#21	Search #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #17 OR #18	2,196,299
#22	Search #19 AND #20 AND #21	246

eTable 2. Search strategy in PubMed database.

PUBMED	SEARCH STRINGS	HITS
#1	Search pregnancy[MeSH* Terms]	861,696
#2	Search pregnan*	963,727
#3	Search gestation	966,085
#4	Search pregnancy trimesters	42,481
#5	Search saliva[MeSH Terms]	39,648
#6	Search saliva*	112,970
#7	Search salivary excretion[MeSH Terms]	23
#8	Search salivary excretion	1,070
#9	Search salivary flow	5,033
#10	Search saliva release	1,968
#11	Search salivary secretion	10,670
#12	Search streptococcus mutans	11,369
#13	Search lactobacillus[MeSH Terms]	27,007
#14	Search lactobacillus	36,481
#15	Search buffering capacity	3,399
#16	Search Bicarbonates[MeSH Terms]	24,307
#17	Search Bicarbonates	24,599
#18	Search phosphate[MeSH Terms]	101,044
#19	Search phosphate	336,439
#20	Search hydrogen ion concentration[MeSH Terms]	298,244
#21	Search "salivary PH"	483
#22	Search "flow rate"	52,892
#23	Search calcium[MeSH Terms]	263,343
#24	Search calcium	572,722
#25	Search dental caries[MeSH Terms]	43,941
#26	Search dental caries	56,248
#27	Search dental caries activity test[MeSH Terms]	92
#28	Search dental caries activity test	687
#29	Search Dental Caries Susceptibility[MeSH Terms]	2,201
#30	Search Dental Caries Susceptibility	2,800
#31	Search tooth decay	56,886
#32	Search tooth caries	56,746
#33	Search "DMFT index"	837
#34	Search (#1 OR #2 OR #3 OR #4)	1,002,946
#35	Search (#5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11)	112,970
#36	Search (#12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 0R #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33)	1,256,350
#37	Search (#34 AND #35 AND #36)	214

* MeSH: Medical Subject Heading.

eTable 3. Search strategy in Scopus database.

SCOPUS	SEARCH STRINGS	HITS
#1	Search TITLE-ABS*-KEY† (pregnant)	224,045
#2	Search TITLE-ABS-KEY (pregnancy)	1,030,739
#3	Search TITLE-ABS-KEY (gestation)	143,955
#4	Search TITLE-ABS-KEY (gravidity)	3,773
#5	Search TITLE-ABS-KEY (pregnancy AND trimesters)	87,183
#6	Search TITLE-ABS-KEY (saliva)	76,281
#7	Search TITLE-ABS-KEY (salivary)	89,146
#8	Search TITLE-ABS-KEY (salivary AND excretion)	1,215
#9	Search TITLE-ABS-KEY (salivary AND flow)	6,196
#10	Search TITLE-ABS-KEY (saliva AND release)	4,212
#11	Search TITLE-ABS-KEY (salivary AND secretion)	10,052
#12	Search TITLE-ABS-KEY (streptococcus AND mutans)	14,422
#13	Search TITLE-ABS-KEY (lactobacillus)	59,276
#14	Search TITLE-ABS-KEY (buffering AND capacity)	8,760
#15	Search TITLE-ABS-KEY (bicarbonates)	82,779
#16	Search TITLE-ABS-KEY (phosphate)	662,141
#17	Search TITLE-ABS-KEY (hydrogen AND ion AND concentration)	286,024
#18	Search TITLE-ABS-KEY ("salivary PH")	618
#19	Search TITLE-ABS-KEY ("flow rate")	282,191
#20	Search TITLE-ABS-KEY (calcium)	997,880
#21	Search TITLE-ABS-KEY ("dental caries")	58,196
#22	Search TITLE-ABS-KEY ("dental decay")	916
#23	Search TITLE-ABS-KEY ("tooth caries")	675
#24	Search TITLE-ABS-KEY ("tooth decay")	1,714
#25	Search TITLE-ABS-KEY ("dental caries activity")	846
#26	Search TITLE-ABS-KEY ("Dental Caries Susceptibility")	2,179
#27	Search TITLE-ABS-KEY ("DMFT index")	1,116
#28	Search #1 OR #2 OR #3 OR #4 OR #5	1,106,934
#29	Search #6 OR #7 OR #8 OR #9 OR #10 OR #11	134,322
#30	Search #12 OR #13 OR #14 OR #15 OR #16 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27	2,234,404
#31	Search # 28 AND #28 AND #30	245

* ABS: Abstract. † KEY: Key words.

eTable 4. Search strategy in Web of Science database.

WEB OF SCIENCE	SEARCH STRINGS	HITS
#1	Search TS=pregnan*	440,336
#2	Search TS=gravidity	1,765
#3	Search TS=gestation	97,066
#4	Search TS="pregnancy trimesters"	114
#5	Search TS=saliva*	94,698
#6	Search TS=streptococcus mutans	11918
#7	Search TS=lactobacillus	48,887
#8	Search TS=buffering capacity	20311
#9	Search TS=bicarbonate	35,035
#10	Search TS=hydrogen ion concentration	17,517
#11	Search TS="salivary PH"	367
#12	Search TS=calcium	548,520
#13	Search TS=phosphate	359,887
#14	Search TS=flow rate	433,209
#15	Search TS= dental caries	21,853
#16	Search TS=dental decay	3,828
#17	Search TS=tooth caries	13,776
#18	Search TS=tooth decay	4,082
#19	Search TS="dental caries activity"	30
#20	Search TS="dental caries susceptibility"	58
#21	Search TS="DMFT index"	624
#22	Search #1 OR #2 OR #3 OR #4	481,593
#23	Search #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21	1,411,146
#24	Search #21 AND #5 AND #24	119