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Usage of anti-usag-1 (Uterine sensitisation–associated gene-1) therapy for tooth regeneration: A systematic review

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Abstract
Background: Tooth regeneration as an idea was debatable and improbable. Anti USAG-1 is being widely discussed as a breakthrough for tooth regeneration. Uterine sensitisation associated with gene-1 is a protein thought to influence the teeth' regeneration indirectly. USAG-1 acts on BMP-7 (bone morphogenetic protein-7) protein, inhibiting its action and hindering teeth regeneration. Suppressing the said protein has been shown to promote supernumerary tooth formation in mice. Animal studies are undergoing tremendous breakthroughs, and soon human trials can be initiated. USAG-1 suppression can provide a new curative treatment plan for edentulous and anodontia patients.
Aim: To evaluate the effectiveness of anti-USAG therapy for tooth regeneration.
Method: A literature search was performed using PubMed, Cochrane central register of controlled trials (CENTRAL), science direct, Wiley, and PMC using MeSH Terms-Anti USAG-, tooth regeneration. Of 108 articles screened, eight full-text articles were assessed for eligibility, and five articles were taken for the qualitative analysis. This review was outlined according to the PRISMA guidelines.
Results: Articles collected showed positive effects of Anti USAG-1 inducing supernumerary teeth formation in genetically modified mice models.
Conclusion: In the available literature, anti-USAG-1 can stimulate third dentition or induce teeth regeneration in mammals.

Keywords: USAG-1, BMP-7, tooth regeneration, mouse model, third dentition

Introduction
Tooth agenesis has been a widely discussed topic in the field of dentistry. There is a congenital lack of a set number of teeth in agenesis, while an increase in the said number leads to a supernumerary dentition. Humans are diphyodont, meaning we have both a deciduous and a permanent sets of teeth. Genetic anomalies, usually about MSX1, PAX9, IRF6, GREM2, AXIN2, LRP6, SMOC2, LTBP3, PITX2, and WNT10B WNT10A, have been shown to induce hypo/oligodontia [8]. Missing teeth or hypodontia is estimated to be around 2-8% in the general population, and oligodontia is around 0.09-0.3%. Prosthesis with dental implants and dentures has been the treatment for missing teeth. Recent advancements in tooth regeneration through stem cells from the late cap stage and bell stage, adult bone marrow and dental pulp containing odontogenic stem cells, and material and molecular biology are being analysed for their safety in clinical application. In a study, the formation of tooth structures was observed after embryonic tooth primordia were transferred into the adult jaw, demonstrating that an embryonic primordium can develop in its adult environment [6].
The tooth is a vertebrate organ, and its development has been studied extensively at a molecular level. Tooth phenotype is not determined by the number of dental papillae but by interactive protein mechanisms [13]. Dentition is a time and position-specific epithelial-mesenchymal interaction [14, 15, 16, 17]. It has been deduced that a signalling centre in the enamel knot, like the other signalling centre in the embryo, seemingly regulates the tooth phenotype. Tooth morphogenesis is an epithelial-mesenchymal single transduction interactive network involving Wnts, BMPs, and fibroblast growth factors that design the shape of the individual tooth [14, 15, 16, 17].

According to genetic rescue experiments, BMP and Wnt signalling pathways contribute to cell proliferation regulation in the dental epithelium, with Wnt signalling also influencing the odontogenic destiny [11].

USAG-1 is a bone morphogenetic protein and interactive pathway antagonist. Sclerostin Domain Containing 1 (SOSTDC1), the human homolog gene for Usag-1, shares 85 per cent of its sequence with mouse Usag-1 [10]. BMPs are highly conserved signalling molecules that are members of the transforming growth factor (TGF)-beta superfamily and play a role in the patterning and morphogenesis of a variety of organs and the development of dentition [1]. In addition, they are essential in structuring and designing an embryo and regulating apoptosis during its development. Previous studies demonstrated that BMP7 null mice undergo agenesis of maxillary teeth [18].

Further analysis shows USAG-1 binding to the BMP-7 receptors inhibiting its action [18]. USAG-1 is a BMP antagonist that regulates BMP signalling locally by binding and neutralising BMP activities and contributes as a regulator of Wnt signalling [7]. Another study demonstrated that suppressing USAG-1 has been shown to induce supernumerary dentition by retaining its dental tooth germs [4]. Thereby supporting and validating the hypothesis of a third dentition that is inducible.

Further justification for the cause was shown during the research. The topical application of BMP-7 on mice caused an increased number of teeth, demonstrating the formation of organic tooth germ by a single target molecule [4]. However, a clear link has yet to be seen between inhibition of USAG-1 and the Runx2 gene for tooth formation.

Detailed studies have been conducted regarding tooth redevelopment, and they have pragmatic results in animal studies. To identify molecular and cellular causes of tooth agenesis, gene function investigations in mammalian cells and animal models should be carried out [8]. USAG-1 inhibition and BMPs usage have much clinical potential in dentistry for tooth regeneration as it demonstrates the benefits of monoclonal antibodies on tooth regeneration and provides a new therapeutic framework. However, extensive research and experimentation are needed to shortly progress with positive clinical applications. Hence, this systematic review aims to analyse whether inhibition of USAG-1 can lead to tooth redevelopment.

Materials and Method

Study Design

A systematic review of clinical trials was done using Anti USAG-1 (uterine sensitisation associated gene-1) therapy for tooth redevelopment.

Search strategy

Published results on Anti USAG-1 usage for tooth regeneration include original articles and research papers. Databases such as Ovid medicine, PubMed, PMC, Science direct, Wiley online library, CINAHL, Cochrane library, OSF, and Scopus were considered for the Nov- Dec 2021 study review. In addition, a literature search to collect relevant data was performed using the MeSH terms "Anti USAG-1 for tooth regeneration".

According to the Prisma guidelines, the MeSH terms were altered in each search engine when the results were too many or too few.

Eligibility criteria

Inclusion criteria

- Studies were conducted during the 1990 - 2021-time frame.
- Full-text articles
- Studies include randomised control trials.
- Animal trials

Exclusion criteria

- Systematic review.
- Articles not having full text.
- Articles in different languages.
- Pilot studies.
- Studies without Anti USAG-1 for tooth regeneration were excluded.

Search engines

1. PubMed
2. Wiley
3. Science Direct
4. PMC
5. Google Scholar
6. Cochrane

Results

The search generated 108 publications, of which eight were independently evaluated as being eligible. After applying inclusion-exclusion criteria, five related articles were selected for further assessment.

Figure 1 depicts a diagram flow of the three tables considered for the reports found, vetted and evaluated for eligibility, excluded, and included for the review.

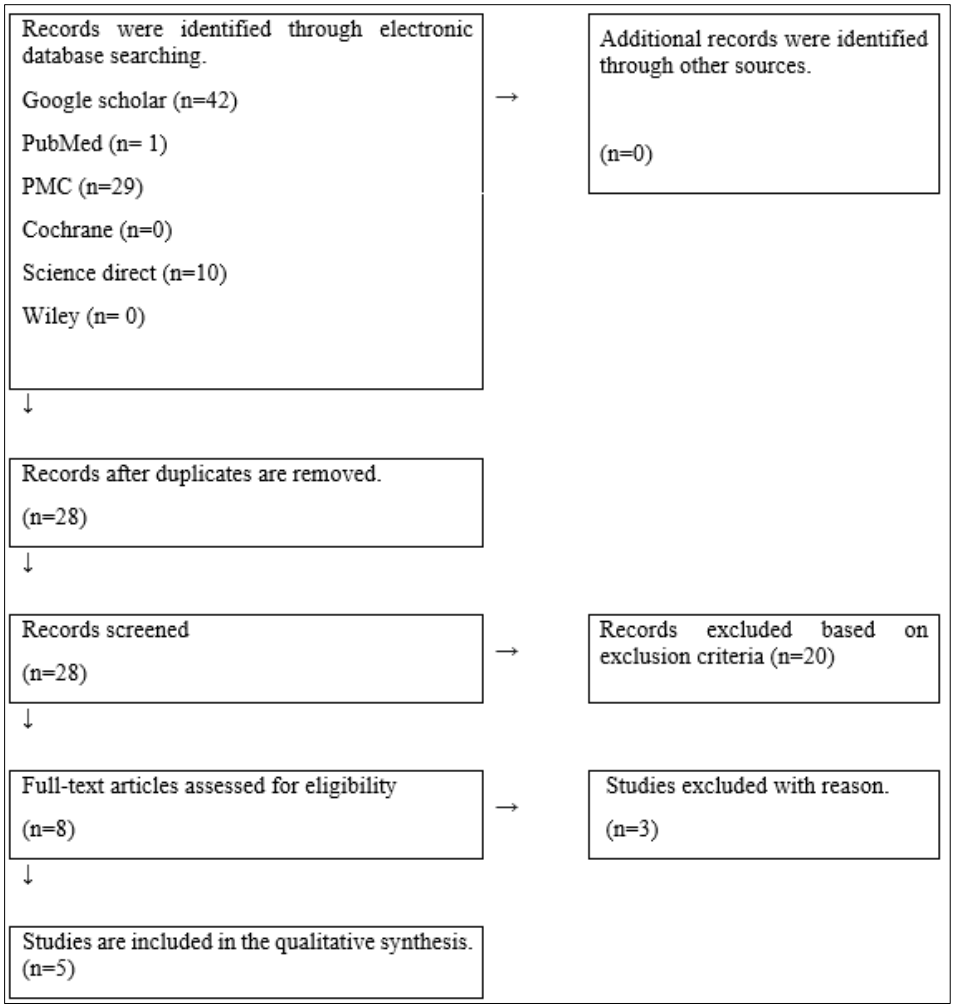

Fig 1: Flow diagram showing the number of studies identified, screened, assessed for eligibility, and included for systematic review

Table 1: Characteristics of intervention in study

Author name	Year	Sample size	Subject characteristics	Duration	Number (case/control)
Honoka Kiso *et al.* [2]	2014	88	USAG-1/LacZ mice and BMP-7/LacZ mice were used in this study. To reduce the influence of mouse background, only F2 progeny were used.	20 days	Test group- BMP-7 was colocalised in the maxillary rudimentary incisor tooth germ area. Control group- BMP-7 was colocalised in the normal maxillary incisor tooth germ region.
Kazuyuki Saito *et al.* [1]	2016	151 (63 F1 gen & 88 F2 gen)	Analysis of adult Bmp7-LacZ knock-in mice, 22 WT samples and 20 Bmp7+/− samples collected from mice two months after birth. Twenty-one samples of the stated genotypes were analysed after three months. In addition, F2 progeny were analysed after four months.	Four months (In 2,3,4-month intermissions)	Test group-BMP7- and USAG-1-LacZ knock-in mice treated with the bacterial LacZ (β-galactosidase) gene. Control group- WT-type mice were treated with the bacterial LacZ (β-galactosidase) gene.
Katsu Takahashi *et al.* [1]	2016	78	Analysis of Cebpb+/+ Runx2+/− mice was undertaken.	15days	Test group-Cebpb+/+ Runx2+/− mice were treated with OSECs molecular marker. Control group-WT-type mice were treated with OSECs molecular marker.
Sayaka Mishima *et al.* [4]	2021	23	*In vivo* experiments using Runx2−/−, mice were done. In addition, wild-type siRNA-treated mouse mandibles are examined.	10days	Test group-Runx2−/− were treated with USAG-1 siRNA (903 &304) Control group-Wild-type mice were treated with USAG-1 siRNAs.
A Murashima-Suginami *et al.* [5]	2021	151	USAG-1 and EDA1 mice are analysed. In addition, msx1 and USAG-1 mice are examined.	35days	Test group- EDA1- deficient mice were administered with USAG-1 neutralising antibodies. Control group- WT-type mice were administered with USAG-1 neutralising antibodies.

The features of the studies chosen for the systematic review are listed in Table 1: The following traits were investigated: The author's name, the year of the study, and the sample number, as well as information about the participants, such as gender and the interventions used in the study. All the included studies were clinically controlled animal studies conducted strictly in the lab.

Table 2: Characteristics of outcome and effect measures

Author name	Year	Effect measure	Result
Honoka Kiso *et al.* [2]	2014	Assessment of:	
		1) Colocalisation of USAG-1 and BMP-7 near maxillary rudimentary incisor tooth germ.	USAG-1 and BMP-7 expressions were detected at E13, E14, and E15. At E13, their activity was present near the enamel tooth organ and labial epithelium. At E14 and E15, transcripts were detected near maxillary rudimentary incisor tooth germ and were colocalised.
		2) USAG-1 acts antagonistically towards BMP-7.	A Series of experiments with USAG-1 and BMP-7 genotypic mice were conducted. Positive results were seen concerning USAG-1 antagonistic nature towards BMP-7.
		3) Increased BMP-7 signalling in USAG-1 deficient mice	In USAG-1 deficient mice near the supernumerary tooth, increased BMP- signalling was seen by BMP-7 abrogation.
		4) BMP-7 induces supernumerary tooth formation.	BMP-7 induced normal teeth formation in USAG-1+/- and supernumerary teeth in USAG-1 deficient mice.
Kazuyuki Saito *et al.* [1]	2016	Assessment of:	
		1) BMP-7 and USAG-1 activity on teeth size of different murine models.	Micro CT analysis to measure the volume and cross-sectional area of the mandibular incisor was done on different genotypic mice. Positive findings concerning tooth size variations showed that BMP-7 induces more cell proliferation in dental mesenchyme, resulting in increased tooth size, and USAG-1 has a generalised decrease in teeth dimensions.
Katsu Takahashi *et al.* [3]	2016	Assessment of: Cebpb and OSECs.	Positive application of OSECs shows the potential to differentiate the odontogenic cells and formation of the whole tooth. Furthermore, Cepbp has been shown to maintain OSEC activity.
Sayaka Mishima *et al.* [4]	2021	Assessment of:	
		1) Efficiency of USAG-1 and Runx2 stealth siRNA application on tooth development.	It was confirmed that the application of USAG-1 stealth siRNA and Runx2 stealth RNA has increased tooth germs in USAG-1 knockdown and decreased tooth germs in Runx2 knockdown with comparable staging.
		2) Efficacy of cationised gel as DDS for the administration of siRNA.	Renal capsule transplantation of mandibular explants with cationised gelatin delivery of siRNA was done. The positive result of cationised gelatin as DDS was demonstrated.
		3) Local application of USAG-1 stealth siRNA to recover arrested tooth development.	According to the findings, the topical treatment of USAG-1 stealth siRNA #304 partially restored the inhibition of tooth development in Runx2/ mice.
A Murashima-Suginami *et al.* [5]	2021	Assessment of:	
		1) USAG-1 neutralising antibody for recovering missing teeth.	Experiments with mouse monoclonal antibodies and USAG-1 recombinant protein from E. coli were conducted. It showed that USAG-1 antibodies interfered with the binding of USAG-1 to BMP and Wnt pathways leading to the recovery of teeth.
		2) USAG-1 neutralising activity for generating whole teeth by affecting BMP-7 signalling.	Examination of USAG-1 neutralising antibodies activity on BMP and Wnt signals was done. Analysis showed that the antibodies have a better antagonistic effect of USAG-1 on BMP signals, achieving better phenotypic changes in mice.

Table 2 shows the outcome and result of the effectiveness of Anti-USAG-1 therapy in tooth regeneration. The outcome and results were positive in the above studies showing that anti-USAG-1 therapy can be used as a potent treatment plan for congenital tooth agenesis and other genetic disorders.

Table 3: Characteristics of bias in different studies taken for review

Author name	Randomised sequence generation	Allocation concealment	Blinding of outcome	Incomplete outcome	Selective bias	Other bias
Honoka Kiso *et al.* [2]	-	-	+	+	+	+
Kazuyuki Saito *et al.* [1]	-	-	+	+	+	+
Katsu Takahashi *et al.* [3]	-	-	+	?	-	?
Sayaka Mishima *et al.* [4]	-	-	+	+	+	+
A Murashima- Suginami *et al.* [5]	-	-	+	+	+	+

The bias assigned as + Low risk of Bias; - High risk of Bias;? Unclear risk of Bias.

Table 3 shows the bias analysis of the studies included, categorised as high risk of Bias, low risk of Bias, and unclear risk of Bias. The categorisation was carried out according to the Cochrane risk of bias tools for randomised controlled trials.

Discussion

This systematic review considered five studies to analyse if anti-USAG-1 therapy can induce tooth regeneration in mice. Cell-based tissue engineering has been the mainstream treatment for curing in regenerative medicine. *In vitro* organ culture and *in vivo* implantation under a tooth cavity, the bioengineered tooth germ produces a structurally correct tooth with blood vessels and nerve fibres [9]. Much research has been done on tissue-based engineering, but the treatment has not been viable due to its high cost and safety issues. In

tissue engineering, a new tooth germ formation is desired, but we see arrested rudimentary tooth primordia in this review. Activation of this rudimentary primordia has been shown to promote tooth regeneration and achieve third dentition.

Mice are diphyodont animals with similar dental patterns to humans. Experimentation with USAG-1 has shown that the protein acts on both BMP and Wnt pathways. BMP and Wnt are necessary for proper growth and development of the body, and any drugs are avoided that affect their activity. USAG-1 suppresses these pathways; thus, experiments were done to inhibit its action. Micro CT analysis, staining, dissection and tests were used to assess the competency of the therapy on the mice models.

Honoka Kiso *et al.* (2014) [2] researched 88 mice models. BMP-7 was colocalised with maxillary rudimentary and normal maxillary incisor tooth germ. The results from the experiment showed that USAG-1 is a novel BMP-7 antagonist. In a USAG-1 deficient mouse, it was analysed that increased BMP-7 signalling led to supernumerary tooth formation and could be prohibited with BMP-7 abrogation. Explant culture and subsequent sub-renal kidney capsule culture showed that BMP-7 can induce supernumerary teeth but cannot induce extra teeth. In USAG-1 deficient mice, supernumerary teeth were found in 100% of the maxillary incisor region, while partial formation was seen in the mandible.

Kazuki Saito *et al.* (2016) [1] researched 151 mice models. Here it was portrayed that the genetic control for tooth organ size and shape plays an important role in teeth regeneration. BMP-7 and USAG-1 are shown to influence tooth size. Furthermore, there is a marked difference in the mechanism of action of BMP-7 and USAG-1 in the bell and cap stages of tooth morphogenesis. These results suggest that local regulation of gene expression can alter the size of the teeth in humans when dental stem cells are used for the regeneration of teeth *in vivo*.

Katsu Takashi *et al.* (2016) [3] researched 78 mice models to assess that molecular targeted therapy is beneficial for tooth regeneration. Recent advances regarding the rescue of rudimentary tooth germs and the contribution of OSECs are discussed in this article. The paper results showed that USAG-1 is an antagonist of BMP-7, and a candidate molecule can help in the formation of a whole tooth in the right conditions. Furthermore, OSECs are analysed to differentiate the odontoblast by loss of stemness and EMT and help in the regeneration of the whole tooth. Thus, using Cebpb protein, which regulates acute-phase reaction and inflammation, targeted molecular therapy with OSECs and USAG-1 can cause tooth regeneration.

Sayaka Mishima *et al.* (2021) [4] researched 23 mice models to assess the effectiveness of USAG-1 stealth sRNAs in tooth regeneration. Stealth siRNAs strains #903 and #304 were used in analysing tooth regeneration in arrested teeth. In addition, runX2-/- mice were utilised, manifesting with arrested tooth organs. USAG-1 stealth siRNA showed therapeutic potential to promote teeth formation via catatonic gelatin as DDS. Thus, suggesting that people suffering from congenital tooth agenesis and RunX2 mutation can be treated.

A Murashima-Suginami *et al.* (2021) [5] researched 151 mice models. Here USAG-1 neutralising antibodies were utilised. In EDA1 deficient mice, USAG-1 neutralising antibodies were shown to rescue arrested tooth formation. However, further analysis showed that USAG-1 neutralising antibodies did not affect certain genotypes, suggesting that they affect all tooth agenesis. Still, certain biomarkers in causative mutations, thus indicating that USAG-1 neutralising antibodies, must be focused on congenital agenesis with mutations in specific genes. In this systematic review, all eligible articles have shown that the application of Anti USAG-1 (uterine sensitisation associated gene-1) therapy on genotypically specific mice models positively affects tooth regeneration. However, much research is needed to establish Hedgehog, FGF, Wnt, and BMP families' roles in developing human supernumerary teeth [12]. Further insight and advances are necessary to progress and establish this treatment as a primary cure for teeth agenesis.

Conclusion

The study concluded that suppression of USAG-1 (uterine sensitisation associated gene-1) therapy could provide constructive remedies for congenital tooth agenesis and other genetic diseases. Furthermore, targeted therapy towards USAG-1 *in vivo* can lead to the rescue of arrested tooth germ and the formation of a third dentition.

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References

1. Saito K, Takahashi K, Asahara M, Kiso H, Togo Y, Tsukamoto H, *et al.* Effects of USAG-1 and Bmp7 deficiencies on murine tooth morphogenesis. BMC developmental biology. 2016 Dec;16(1):1-0. https://doi.org/10.1186/s12861-016-0117-x
2. Kiso H, Takahashi K, Saito K, Togo Y, Tsukamoto H, Huang B, *et al.* Interactions between BMP-7 and USAG-1 (uterine sensitisation-associated gene-1) regulate supernumerary organ formations. PloS one. 2014;9(5):e96938. https://doi.org/10.1371/journal.pone.0096938
3. Takahashi K, Kiso H, Murashima-Suginami A, Tokita Y, Sugai M, Tabata Y, *et al.* Development of tooth regenerative medicine strategies by controlling the number of teeth using targeted molecular therapy. Inflammation and regeneration. 2020 Dec;40(1):1-9. https://doi.org/10.1186/s41232-020-00130-x
4. Mishima S, Takahashi K, Kiso H, *et al.* Local application of *Usag-1* siRNA can promote tooth regeneration in *Runx2*-deficient mice. Sci Rep. 2021 Jul 1;11(1):1-9. https://doi.org/10.1038/s41598-021-93256-y
5. Murashima-Suginami A, Kiso H, Tokita Y, Mihara E, Nambu Y, Uozumi R, *et al.* Anti-USAG-1 therapy for tooth regeneration through enhanced BMP signalling. Science advances. 2021;7(7):eabf1798. https://doi.org/10.1126/sciadv.abf1798
6. Ohazama A, Modino SA, Miletich I, Sharpe PT. Stem-cell-based tissue engineering of murine teeth. Journal of dental research. 2004;83(7):518-522. https://doi.org/10.1177/154405910408300702
7. Murashima-Suginami A, Takahashi K, Kawabata T, Sakata T, Tsukamoto H, Sugai M, *et al.* Rudiment incisors survive and erupt as supernumerary teeth as a result of USAG-1 abrogation. Biochemical and biophysical research communications.

2007;359(3):549-555. https://doi.org/10.1016/j.bbrc.2007.05.148
8. Yu M, Wong SW, Han D, Cai T. Genetic analysis: Wnt and other pathways in nonsyndromic tooth agenesis. Oral diseases. 2019;25(3):646-651. https://doi.org/10.1111/odi.12931
9. Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, *et al.* The development of a bioengineered organ germ method. Nature methods. 2007;4(3):227-230. https://doi.org/10.1038/nmeth1012
10. Arikan V, Cumaogullari O, Ozgul BM, Oz FT. Investigation of SOSTDC1 gene in nonsyndromic patients with supernumerary teeth. Medicina oral, patologia oral y cirugia buccal. 2018;23(5):e531-e539. https://doi.org/10.4317/medoral.22520
11. Yuan G, Yang G, Zheng Y, Zhu X, Chen Z, Zhang Z, *et al.* The non-canonical BMP and Wnt/β-catenin signalling pathways orchestrate early tooth development. Development (Cambridge, England). 2015;142(1):128-139. https://doi.org/10.1242/dev.117887
12. Ge LH, Wang X. Beijing da xue xue bao. Yi xue ban = Journal of Peking University. Health Sciences. 2013;45(4):661-665.
13. Cai J, Cho SW, Kim JY, Lee MJ, Cha YG, Jung HS. Patterning the size and number of teeth and its cusps. Dev Biol. 2007;304(2):499-507.
14. Chai Y, Slavkin HC. Prospects for tooth regeneration in the 21st century: a perspective. Microsc Res Tech. 2003;60(5):469-79.
15. Koch WE. *In vitro* differentiation of tooth rudiments of embryonic mice. I. Trans filter interaction of embryonic incisor tissues. J Exp Zool. 1967;165(2):155-70.
16. Kollar EJ, Baird GR. The influence of the dental papilla on the development of tooth shape in embryonic mouse tooth germs. J Embryol Exp Morphol. 1969;21(1):131-48.
17. Thesleff I, Sharpe P. Signalling networks regulating dental development. Mech Dev. 1997;67(2):111-23.
18. Zouvelou V, Luder HU, Mitsiadis TA, Graf D. Deletion of BMP7 affects the development of bones, teeth, and other ectodermal appendages of the orofacial complex. Journal of experimental zoology Part B, Molecular and developmental evolution. 2009 Jun 15;312(4):361-74.