Mechanistic understanding of the epithelial rests of Malassez (ERMs) in tooth overeruption

2025 Research Aid Awards (RAA)

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FollowUp Form

Award Information

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Title of Project:*

Mechanistic understanding of the epithelial rests of Malassez (ERMs) in tooth overeruption

Award Type

Research Aid Award (RAA)

Period of AAOF Support

July 1, 2025 through June 30, 2026

Institution

University of Pittsburgh, School of Dental Medicine, Department of Orthodontics and Dentofacial Orthopedics

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)

Dr. Andrew Jheon

Amount of Funding

$6,000.00

Abstract

(add specific directions for each type here)

Mechanistic understanding of the epithelial rests of Malassez (ERMs) in tooth overeruption
Arshia Ashjaei, DDS
University of Pittsburgh, 2026
Background: Overeruption of unopposed teeth is a common clinical problem that complicates dental treatment and often requires orthodontic intervention. The epithelial rests of Malassez (ERMs), remnants of Hertwig's epithelial root sheath that persist in the periodontal ligament (PDL), may contribute to periodontal homeostasis. Notably, both ERM number and eruptive tooth movement decline with age, suggesting a mechanistic link. However, the role of ERMs in unopposed tooth overeruption remains uncharacterized. Using mouse models, we examined the association between ERMs and overeruption during aging (Aim 1) and the effect of disrupting Perp, a key cell-attachment gene in ERMs (Aim 2).
Methods: For Aim 1, KRT14Cre;Ai9(RCL-tdT) reporter mice fluorescently labeled epithelial cells, including ERMs. Right maxillary molars were extracted in a split-mouth design to create unopposed mandibular molars in 6-, 12-, and 24-week-old mice, and hemi-mandibles were harvested three weeks later. Overeruption was measured by micro-computed tomography (microCT) using anatomical landmarks, and ERMs were visualized by 3D tissue clearing (PEGASOS) and confocal microscopy. For Aim 2, KRT14CreERT+/-;Perpfl/fl mice were generated for tamoxifen-inducible inactivation of Perp in ERMs.
Results: A total of 33 KRT14Cre;Ai9fl/fl mice were analyzed. Unopposed molars overerupted across all four linear measurements relative to the contralateral control. Overeruption decreased significantly with age: 6-week-old mice showed the greatest differences (all four measurements significant; three at $p<0.001$ and measurement 2 at $p<0.01$), 12-week-old mice intermediate differences (three of four significant), and 24-week-old mice no significant differences. Significant overeruption was detected more consistently in males than females, likely reflecting larger male sample sizes.
Conclusions: Using a mouse molar extraction model, we quantified unopposed tooth overeruption with age in vivo. Overeruption was age-dependent, decreasing significantly from 6 to 24 weeks. A decline in ERM number and activity may underlie this reduced eruptive potential in older animals. Ongoing 3D quantification of ERMs and tamoxifen-inducible disruption of Perp (KRT14CreERT+/-;Perpfl/fl) will define the functional role of ERMs in overeruption. These findings advance mechanistic understanding of ERMs in tooth eruption and may inform clinical strategies to prevent unopposed tooth overeruption.

Respond to the following questions:

Detailed results and inferences:*

If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".
OR
Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

Tables and Figures.pdf

Overeruption of teeth that lose their opposing occlusal partner is a common clinical problem in restorative and orthodontic practice, yet the biological mechanisms that drive it, and that cause it to vary so widely between patients, remain poorly understood. Our project investigated whether the epithelial rests of Malassez (ERMs), small clusters of epithelial cells embedded in the periodontal ligament since tooth development, contribute to this eruptive process, and whether age-related decline in ERM number could help explain why younger patients tend to overerupt more readily than older ones.

Aim 1 asked whether unopposed tooth overeruption itself is age-dependent in a controlled, quantifiable animal model. We used KRT14-Cre;Ai9 reporter mice, in which all epithelial cells, including ERMs, are permanently and fluorescently labeled, allowing us to later visualize ERMs directly in three dimensions in the same animals used for the eruption measurements. In a split-mouth design, we extracted the three right maxillary molars in mice at 6, 12, or 24 weeks of age, leaving the right mandibular molars unopposed while the left mandibular molars served as an internal, same-animal control. Three weeks after extraction, hemi-mandibles were collected and scanned by micro-computed tomography (microCT), which let us measure tooth position with high precision using four linear measurements: distance from the mesial cusp tip to the buccal alveolar bone (1A), to the lingual alveolar bone (1B), to the root apex (Measurement 2, reflecting crown-root length), and to the inferior border of the mandible (Measurement 3, reflecting global vertical tooth position).

Sample size needs were established by power analysis using pilot data (estimated effect size 0.56, a medium effect by conventional benchmarks), indicating that roughly 11 animals per age group would be needed to detect a meaningful difference with 80% power at a significance level of 0.05. A total of 48 mice have been bred for this study, and data collection is ongoing toward that target; to date we have analyzed 33 mice: 10 at 6 weeks (7 male, 3 female), 13 at 12 weeks (5 male, 8 female), and 10 at 24 weeks (6 male, 4 female). Across the full cohort, paired t-tests comparing the unopposed (right) side to the control (left) side showed that overeruption was real and measurable: Measurements 1A, 1B, and 3 differed significantly between sides ($p<0.001$), and Measurement 2 also differed significantly ($p<0.05$).

When we stratified by age, a clear and consistent trend emerged. At 6 weeks, all four measurements differed significantly between the unopposed and control sides (1A, 1B, and 3 at $p<0.001$; Measurement 2 at $p<0.01$), demonstrating a strong eruptive response in young animals. At 12 weeks, three of the four measurements (1A, 1B, and 3) remained significantly different, while Measurement 2 no longer reached significance, suggesting a measurable decline in the eruptive response. By 24 weeks, none of the four measurements differed significantly between sides, indicating that the unopposed molars in older mice essentially stopped overerupting relative to controls. This progressive loss of significance from 6 to 24 weeks is, to our knowledge, one of the first quantitative, in vivo demonstrations that the capacity for unopposed tooth overeruption declines with age, rather than the eruptive response simply varying randomly across the lifespan.

We also examined whether sex influenced the eruptive response. Significant differences between sides were detected more consistently in males than females across age groups; however, when we directly compared the magnitude of the right-left difference between sexes at 6 weeks, the values were comparable, and in two of the four measurements the female mean difference was actually larger than the male mean difference. We believe the more frequent statistical significance in males most likely reflects larger and more balanced male sample sizes at this age (7 males versus 3 females) rather than a true sex difference in the biological magnitude of overeruption. This distinction matters for interpreting any future sex-based clinical inferences, and it is one of the questions we continue to investigate with larger, sex-balanced cohorts.

Aim 2 was designed to test directly whether ERMs are mechanistically required for this eruptive process, rather than simply correlating with it. We generated KRT14-CreERT;Perp(fl/fl) mice, in which tamoxifen administration conditionally inactivates Perp, a gene required for desmosome assembly and ERM structural integrity, specifically within ERMs. Maxillary molar extraction is performed one day after the first tamoxifen dose, so the overeruption model proceeds in parallel with ERM disruption; the prediction is that mice with disrupted ERMs will show reduced overeruption compared to controls if ERMs are truly required for the response. Collection of these animals at 9, 15, and 27 weeks of age is ongoing. In parallel, using PEGASOS tissue clearing and confocal microscopy on KRT14-Cre;Ai9 reporter tissue, we have confirmed that ERMs are clearly detectable within the periodontal ligament space of the mandibular first molar in three dimensions. This sets up direct quantification of ERM number and distribution and correlation with the degree of overeruption measured in Aim 1, once data collection is complete.

Taken together, these findings support a model in which the eruptive potential of unopposed teeth declines progressively with age, and in which ERMs, already known to decrease in number with age in both rodents and humans, may be one of the biological drivers of that decline. ERMs in our tissue express KRT14, Egfr, and Perp, markers associated with epithelial stem-cell-like function and tissue maintenance; the presence of Egfr is notable given that EGF signaling has separately been shown to accelerate eruption of the mouse incisor, raising the possibility of a shared signaling mechanism in molar eruption.

We recognize several limitations. Although the split-mouth design controls for animal-to-animal variability, microCT-based linear measurements capture positional change rather than underlying cellular biology directly, and our observation window was limited to three weeks following extraction, so we cannot yet say whether longer-term overeruption follows the same age-dependent pattern. Sample sizes, particularly for females at certain ages, remain modest, and we are actively working to balance and expand these cohorts.

For the orthodontic community, we believe these results are clinically relevant in two ways. First, they offer a possible biological explanation for a phenomenon orthodontists see constantly and currently manage largely by clinical judgment, why some unopposed or partially erupted teeth drift dramatically out of the occlusal plane while others in older patients remain comparatively stable and they suggest that patient age itself may be a meaningful biological variable, not merely a proxy for time-since-tooth-loss, when anticipating how much overeruption to expect and when to intervene. Second, if Aim 2 confirms that ERMs are functionally required for overeruption, ERM biology could eventually represent a novel pharmacological target for slowing or preventing unwanted tooth movement in unopposed teeth, an approach distinct from the purely mechanical and restorative strategies orthodontists currently rely on.

We are grateful to the AAOF for supporting this work, and we look forward to completing the Perp inactivation studies and 3D ERM quantification, which will allow us to determine definitively whether ERMs are required for tooth overeruption and to better characterize the cellular and molecular pathways involved. We hope these findings, and the reproducible mouse model we have developed, will be useful to other investigators studying eruption biology and periodontal adaptation, and that they will ultimately help inform more individualized, age-aware approaches to managing unopposed teeth in clinical orthodontic practice.

Were the original, specific aims of the proposal realized?*

Yes, the original specific aims of the proposal were realized.

We proposed 2 aims:

AIM 1: To confirm an association between a reduction in ERMs and overeruption in older mice.

AIM 2: Temporal- and site-specific inactivation of Perp will disrupt ERMs and we will measure the amount of overeruption to determine a definitive role for ERMs in overeruption.

Were the results published?*

No

Have the results of this proposal been presented?*

Yes

To what extent have you used, or how do you intend to use, AAOF funding to further your career?*

The AAOF funding was used to cover the cost of laboratory reagents, specifically the required mouse lines, mouse husbandry, reagents and tools for mouse molar extraction procedures, mouse collection, micro-computed tomography, and histological reagents.

Accounting: Were there any leftover funds?*

If "yes", enter your best estimate and work with your grants manager to finalize financial reports and send refund payable to: AAOF
Attn: George
401 N. Lindbergh Blvd.
St. Louis, MO. 63141-7839

If "no", enter zero.

$0.00

Not Published

Are there plans to publish? If not, why not?*

The study will be "published" as my MSc thesis.
But once the study is completed (we are still collecting, processing, and analyzing data from our last set of mouse experiments), we will submit for peer review to the Journal of Dental Research.

Presented

Please list titles, author or co-authors of these presentation/s, year and locations:*

Title: Mechanistic understanding of the epithelial rests of Malassez (ERMs) in tooth overeruption
Presented at 2026 American Association of Orthodontists Annual Session in Orlando
Author: Dr. Arshia Ashjaei
Co-authors: Dr. Andrew Jheon and Jin Yie

Was AAOF support acknowledged?

If so, please describe:

Yes, AAOF support was acknowledged in the Acknowledgement slide and the Title slide

Internal Review

Reviewer comments

The abstract is not uploaded. Please upload the abstract.

Reviewer Status*

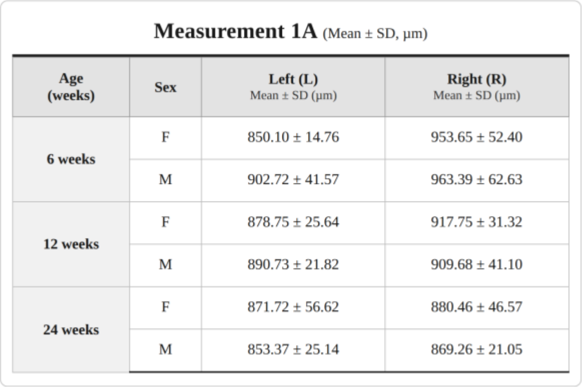
Approved

File Attachment Summary

Applicant File Uploads

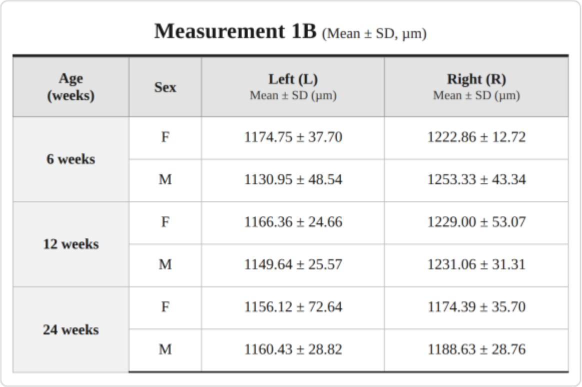
- Tables and Figures.pdf

Table 1 - Linear Measurements of Mandibular Molars by Age, Sex, and Side (Mean ± SD)



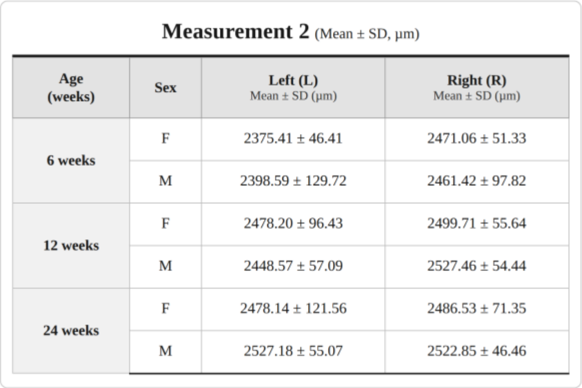
Measurement 1A (Mean ± SD, µm)

Age (weeks)	Sex	Left (L) Mean ± SD (µm)	Right (R) Mean ± SD (µm)
6 weeks	F	850.10 ± 14.76	953.65 ± 52.40
	M	902.72 ± 41.57	963.39 ± 62.63
12 weeks	F	878.75 ± 25.64	917.75 ± 31.32
	M	890.73 ± 21.82	909.68 ± 41.10
24 weeks	F	871.72 ± 56.62	880.46 ± 46.57
	M	853.37 ± 25.14	869.26 ± 21.05



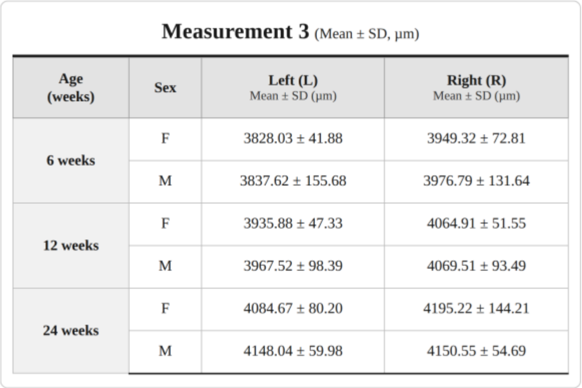
Measurement 1B (Mean ± SD, µm)

Age (weeks)	Sex	Left (L) Mean ± SD (µm)	Right (R) Mean ± SD (µm)
6 weeks	F	1174.75 ± 37.70	1222.86 ± 12.72
	M	1130.95 ± 48.54	1253.33 ± 43.34
12 weeks	F	1166.36 ± 24.66	1229.00 ± 53.07
	M	1149.64 ± 25.57	1231.06 ± 31.31
24 weeks	F	1156.12 ± 72.64	1174.39 ± 35.70
	M	1160.43 ± 28.82	1188.63 ± 28.76



Measurement 2 (Mean ± SD, µm)

Age (weeks)	Sex	Left (L) Mean ± SD (µm)	Right (R) Mean ± SD (µm)
6 weeks	F	2375.41 ± 46.41	2471.06 ± 51.33
	M	2398.59 ± 129.72	2461.42 ± 97.82
12 weeks	F	2478.20 ± 96.43	2499.71 ± 55.64
	M	2448.57 ± 57.09	2527.46 ± 54.44
24 weeks	F	2478.14 ± 121.56	2486.53 ± 71.35
	M	2527.18 ± 55.07	2522.85 ± 46.46



Measurement 3 (Mean ± SD, µm)

Age (weeks)	Sex	Left (L) Mean ± SD (µm)	Right (R) Mean ± SD (µm)
6 weeks	F	3828.03 ± 41.88	3949.32 ± 72.81
	M	3837.62 ± 155.68	3976.79 ± 131.64
12 weeks	F	3935.88 ± 47.33	4064.91 ± 51.55
	M	3967.52 ± 98.39	4069.51 ± 93.49
24 weeks	F	4084.67 ± 80.20	4195.22 ± 144.21
	M	4148.04 ± 59.98	4150.55 ± 54.69

Figure 1 - Comparison of Mandibular Molar Measurements Between Control (left) and Overeruption (right) Sides

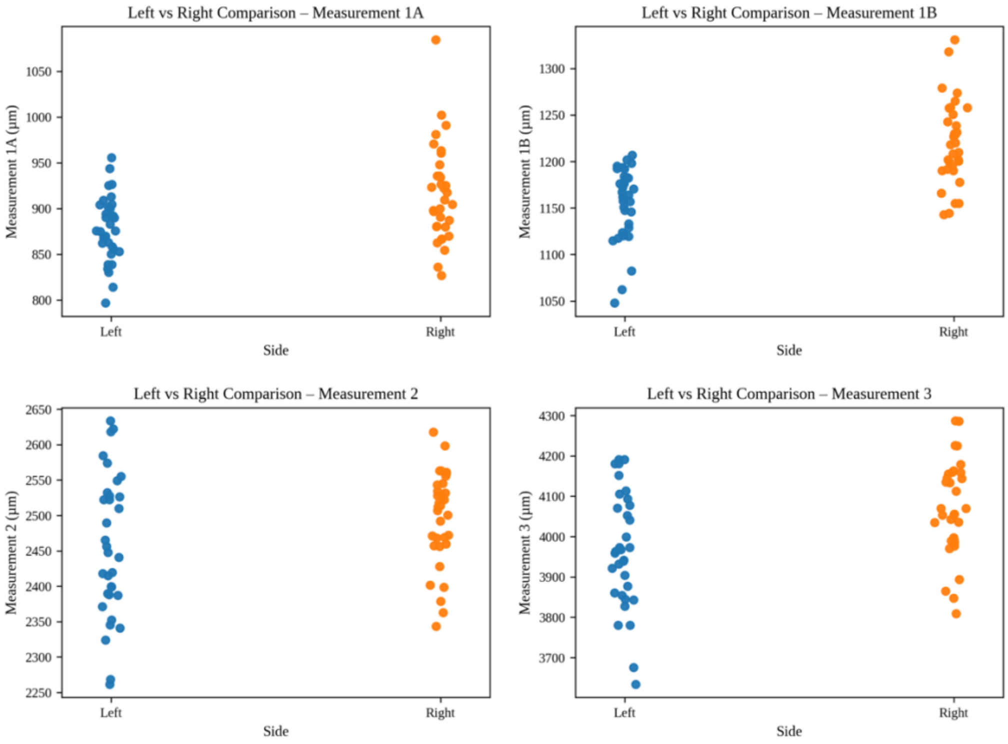


Figure 2 - Age-Dependent Differences in Mandibular Molar Linear Measurements (1A, 1B, 2, and 3)

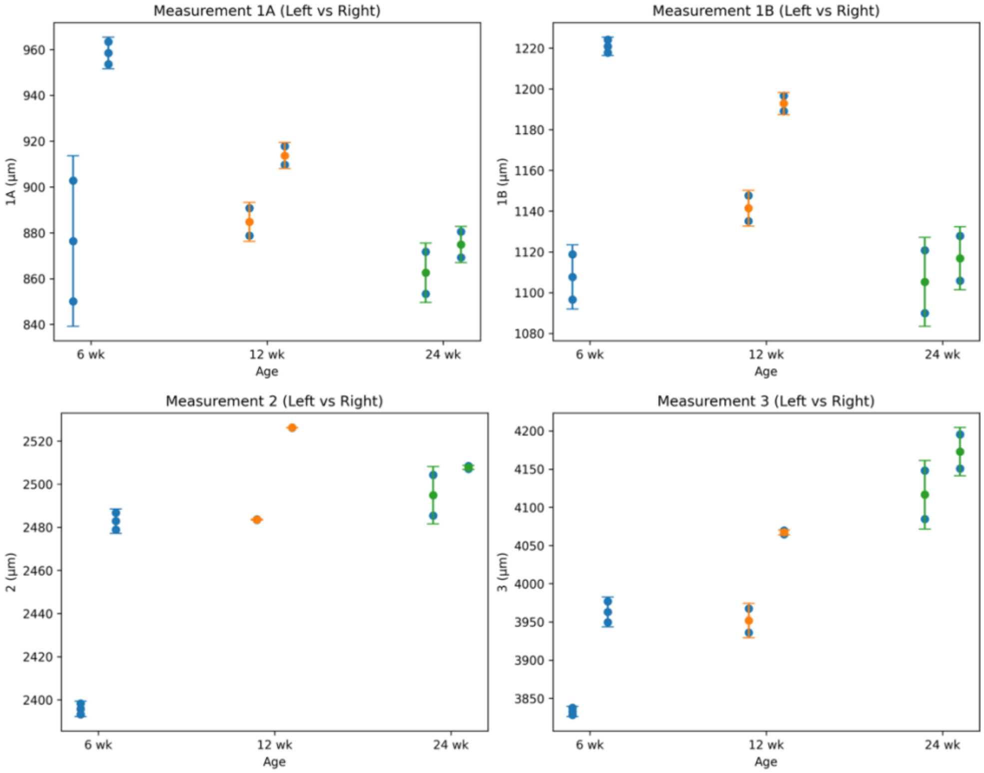
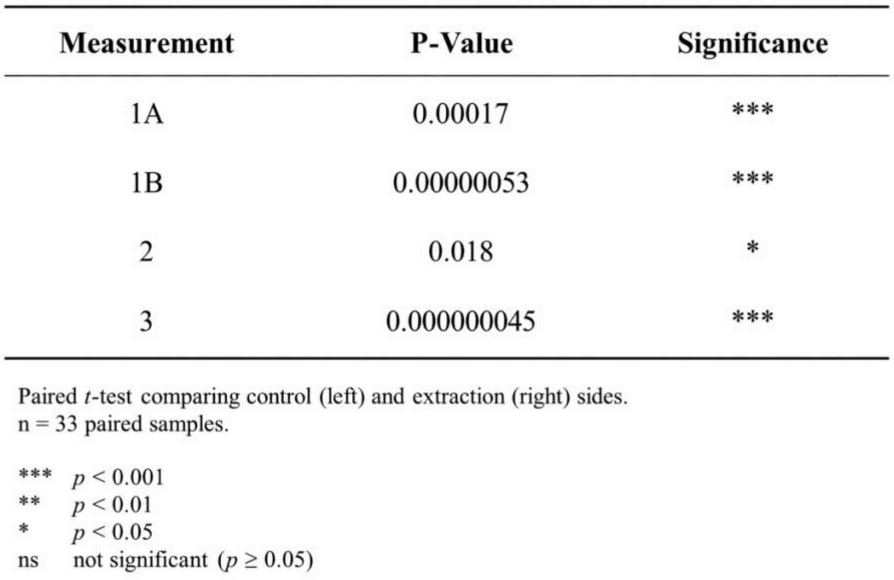


Table 2 - Paired t-test Comparison of Linear Measurements Between Control (Left) and Extraction (Right) Mandibular Molars

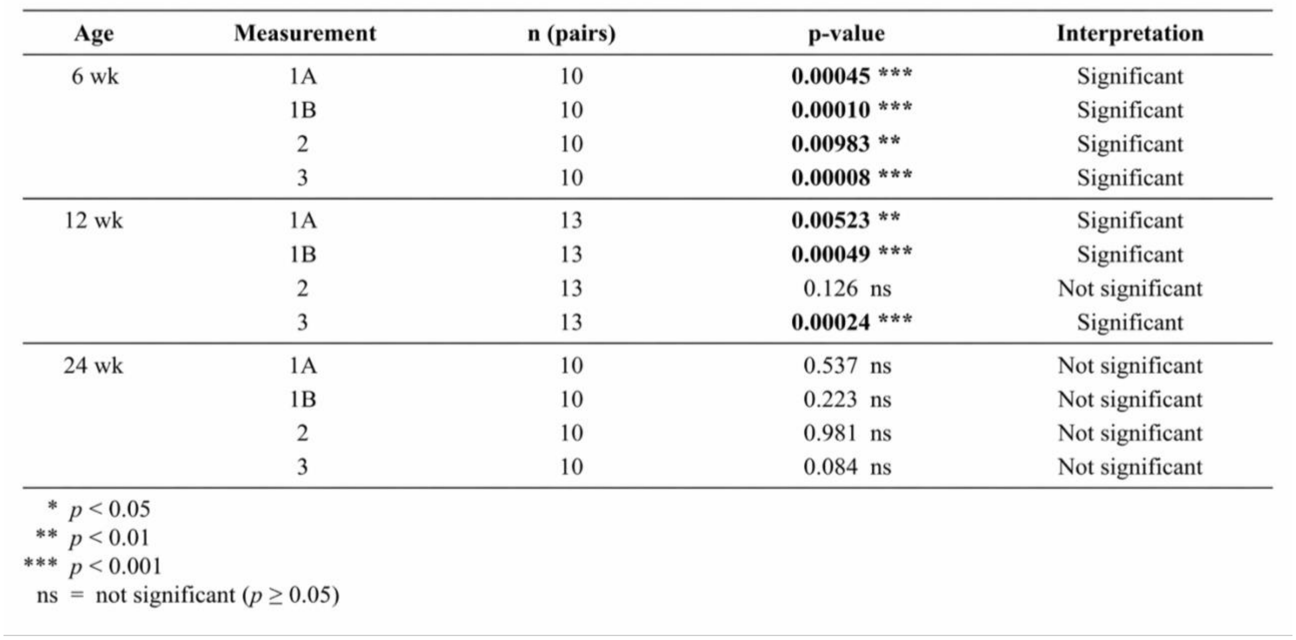


Measurement	P-Value	Significance
1A	0.00017	***
1B	0.00000053	***
2	0.018	*
3	0.000000045	***

Paired *t*-test comparing control (left) and extraction (right) sides.
n = 33 paired samples.

*** $p < 0.001$
** $p < 0.01$
* $p < 0.05$
ns not significant ($p \geq 0.05$)

Table 3 - Paired t-test Results Comparing Control (Left) and Extraction (Right) Mandibular Molars



Age	Measurement	n (pairs)	p-value	Interpretation
6 wk	1A	10	**0.00045 *****	Significant
	1B	10	**0.00010 *****	Significant
	2	10	**0.00983 ****	Significant
	3	10	**0.00008 *****	Significant
12 wk	1A	13	**0.00523 ****	Significant
	1B	13	**0.00049 *****	Significant
	2	13	0.126 ns	Not significant
	3	13	**0.00024 *****	Significant
24 wk	1A	10	0.537 ns	Not significant
	1B	10	0.223 ns	Not significant
	2	10	0.981 ns	Not significant
	3	10	0.084 ns	Not significant

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$
ns = not significant ($p \geq 0.05$)

Table 4 - Age-Dependent Changes in Overeruption: Measurement 1A

Age	Left Mean (µm)	Right Mean (µm)	Difference (R−L)
6 wk	886.93	960.47	73.54
12 wk	883.36	914.64	31.29
24 wk	860.71	873.74	13.03

Table 5 - Age-Dependent Changes in Overeruption: Measurement 1B

Age	Left Mean (µm)	Right Mean (µm)	Difference (R−L)
6 wk	1144.09	1244.19	100.10
12 wk	1159.93	1229.79	69.86
24 wk	1158.71	1182.94	24.23

Table 6 - Age-Dependent Changes in Overeruption: Measurement 2

Age	Left Mean (µm)	Right Mean (µm)	Difference (R−L)
6 wk	2391.64	2464.32	72.68
12 wk	2466.80	2510.38	43.58
24 wk	2507.57	2508.33	0.76

Table 7 - Age-Dependent Changes in Overeruption: Measurement 3

Age	Left Mean (µm)	Right Mean (µm)	Difference (R−L)
6 wk	3834.74	3968.55	133.81
12 wk	3948.05	4066.68	118.63
24 wk	4122.70	4168.42	45.72

Figure 3 - Age-Dependent Decrease in the Rate and Magnitude of overeruption in Male Mice (1A, 1B, 2 &3)

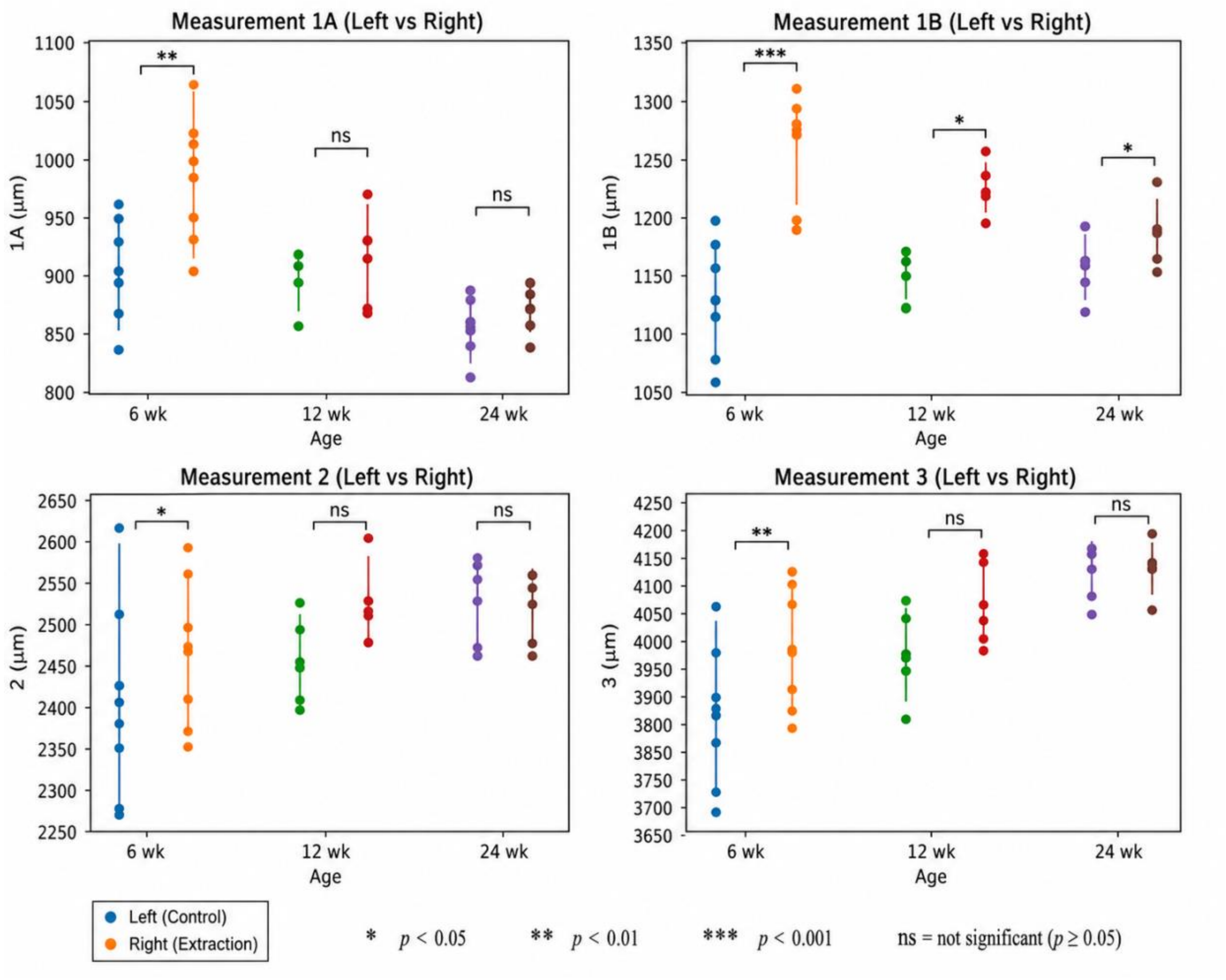
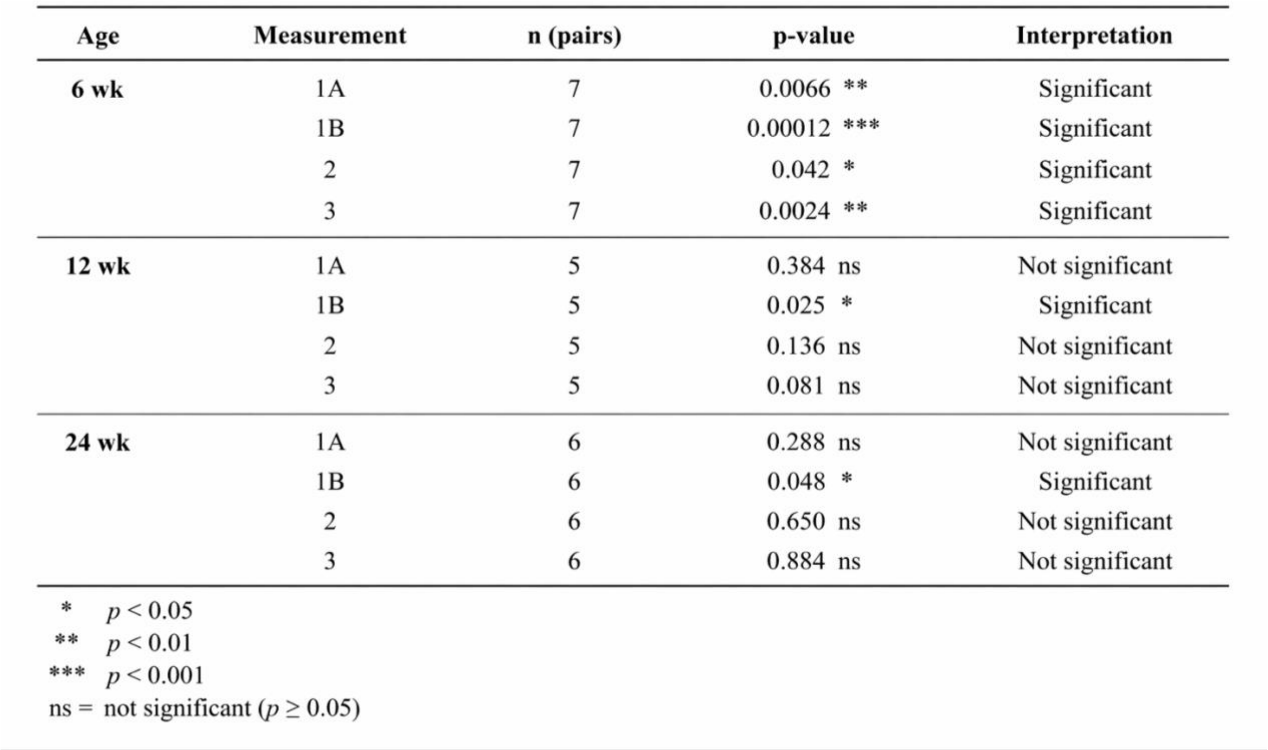


Table 8 - Age-Dependent Decrease in the Rate and Magnitude of overeruption in Male Mice



Age	Measurement	n (pairs)	p-value	Interpretation
6 wk	1A	7	0.0066 **	Significant
	1B	7	0.00012 ***	Significant
	2	7	0.042 *	Significant
	3	7	0.0024 **	Significant
12 wk	1A	5	0.384 ns	Not significant
	1B	5	0.025 *	Significant
	2	5	0.136 ns	Not significant
	3	5	0.081 ns	Not significant
24 wk	1A	6	0.288 ns	Not significant
	1B	6	0.048 *	Significant
	2	6	0.650 ns	Not significant
	3	6	0.884 ns	Not significant

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$
ns = not significant ($p \geq 0.05$)

Figure 4 - Age-Dependent Decrease in the Rate & Magnitude of overeruption in Female Mice (1A, 1B, 2 & 3)

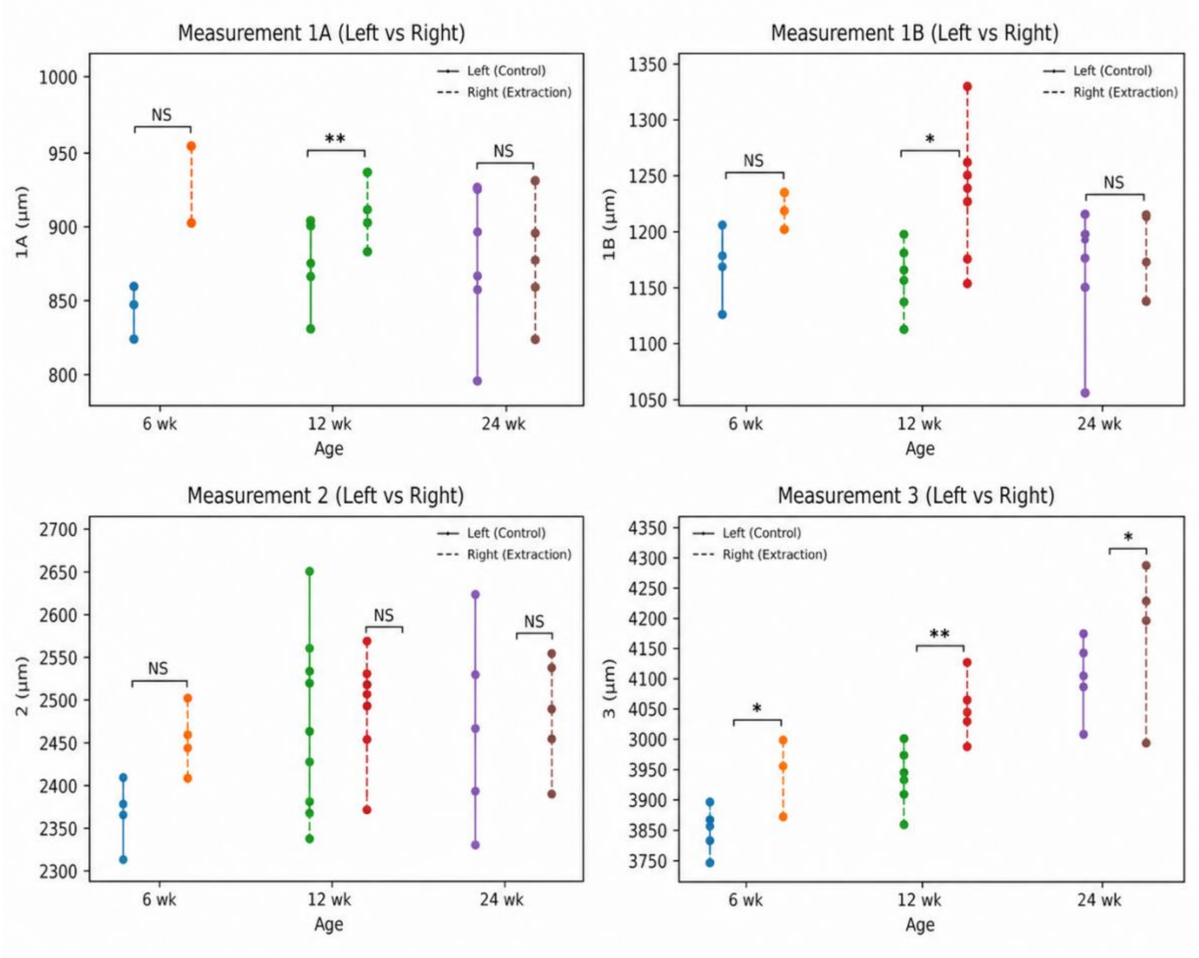
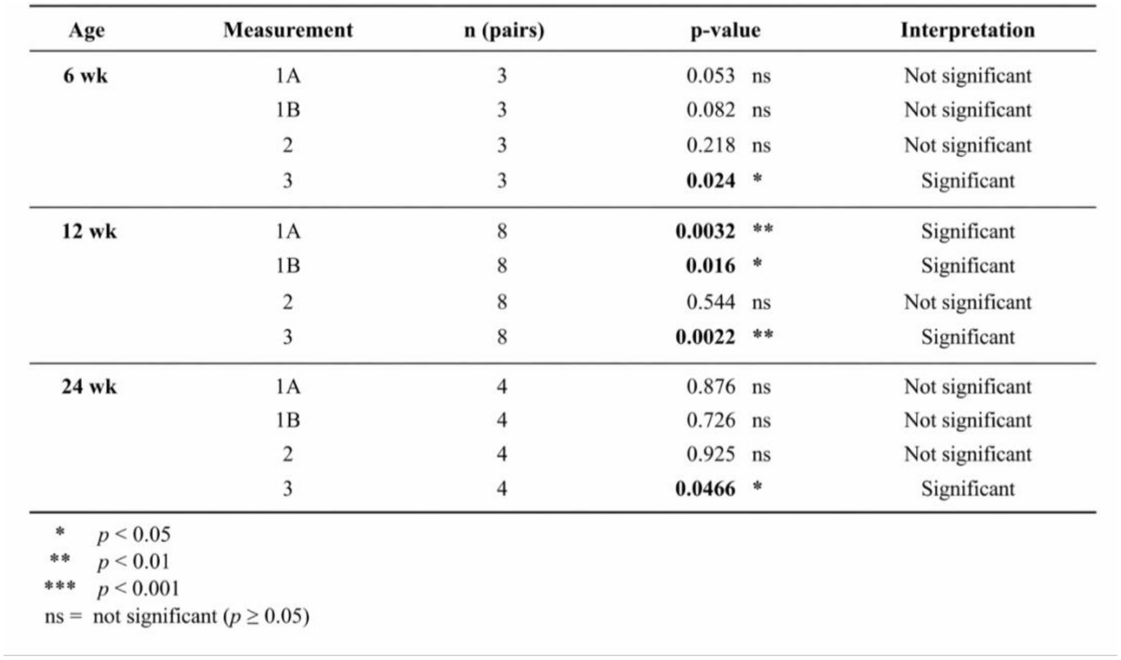


Table 9 - Age-Dependent Decrease in the Rate and Magnitude of overeruption in Female Mice



Age	Measurement	n (pairs)	p-value		Interpretation
6 wk	1A	3	0.053	ns	Not significant
	1B	3	0.082	ns	Not significant
	2	3	0.218	ns	Not significant
	3	3	**0.024**	*	Significant
12 wk	1A	8	**0.0032**	**	Significant
	1B	8	**0.016**	*	Significant
	2	8	0.544	ns	Not significant
	3	8	**0.0022**	**	Significant
24 wk	1A	4	0.876	ns	Not significant
	1B	4	0.726	ns	Not significant
	2	4	0.925	ns	Not significant
	3	4	**0.0466**	*	Significant

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$
ns = not significant ($p \geq 0.05$)