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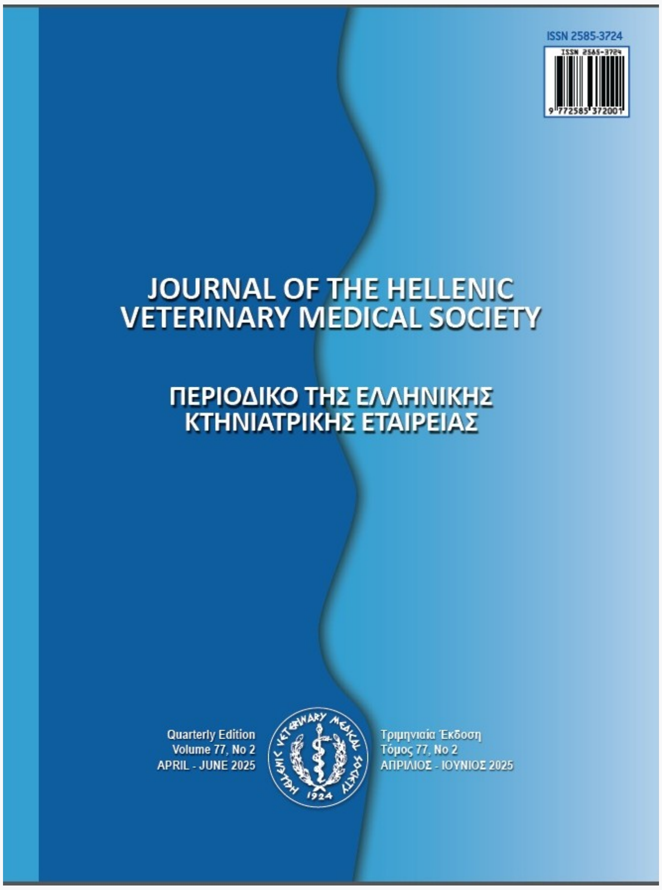

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Research article
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Relationship between growth and development of rat pups and their head and teeth development

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ABSTRACT: Laboratory rats are indispensable experimental animal models used instead of humans. In translational medicine research, the lifespan of the animal model must be similar to the human life period in which the research question arose for the validity and applicability of research results. In this study, we aimed to examine the laboratory rats used instead of humans during postnatal lactation, childhood, adolescence, preadolescence, and young adult life periods while also contributing to laboratory animal science. Life periods, which are years for humans, are expressed in days for laboratory rats. Therefore, if researchers do not perform experimental procedures in the animal life period appropriate for the human life period in which the research questions arise, the research results will not be valid, and applicable information cannot be obtained. Because each animal exhibits anatomical and physiological changes specific to its life period, in laboratory rats, the upper and lower incisors emerge in the first 8-10 days after birth, and the premature period is completed. In the 30-day period after birth, laboratory rats complete the lactation period and childhood period and reach a body weight of approximately 12 times their birth body weight. In addition, there was no significant difference between male and female rat pups regarding head length, head width, jaw width, and incisor length/width measurements up to 30 days after birth. In contrast, these measurements were higher in male rat pups than female pups after the 30th day ($p<0.05$). It was determined that the effects of pubertal physiological changes in male rats began to be seen after 30 days after birth. In connection with this, this situation should be taken into consideration when evaluating and interpreting the effects of experimental procedures in both pediatric animal models and adult rat models.

Keyword: growth rat pups; head; incisor teeth development.

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INTRODUCTION

Translational medicine is critical in scientific research and the transition to clinical practice (Worboys et al., 2021). Animal experiments have significantly contributed to the development of modern medicine and continue to do so. Experimental animal models allow researchers to conduct research that is impossible to perform in humans due to ethical and legal regulations (Mukherjee et al., 2022). Selecting an experimental animal model is a challenging and complex process. Researchers often struggle to select an appropriate animal model due to the unique limitations of each. The most basic starting point in selecting an appropriate animal model is to start from the validity and relevance of the knowledge and experience gained from the experiment to humans. Animal models have become the most crucial step in the biocompatibility testing of drugs, vaccines, and medical devices for humans (Pehlivanović B et al., 2019). In this context, the selected animal model must closely mimic the physiological or pathophysiological processes in humans relevant to the research question.

Rats play an essential role as animal models in this field of research. (Weber et al., 2019) Rats are the most preferred animals in translational medicine due to their genetic and physiological similarities with humans, their modeling of various human diseases, and their use in biocompatibility testing of drugs (Wang et al., 2022; Zou et al., 2022).

The laboratory rat was the first mammal species introduced into the laboratory about a century ago. It has been used as a human surrogate in experimental modeling of a wide variety of human diseases, including addiction, aging, autoimmunity, cardiovascular disease, hypertension, metabolic disorders, and cancer. Approximately 80–85% of animals used in experimental research are strains of mice and rats. The laboratory rat is used more than other animal models due to its advantages, such as having over 300 subspecies with defined genetic and microbiological characteristics, a short gestation period (21 days), many offspring in one litter, the ability to house more animals per unit area, low cost, ease of transportation, and adaptability to laboratory conditions (Yılmaz O. Gökmen N, 2015; Yılmaz. O & Yılmaz C, 2021).

Significant changes in physiological characteristics and anatomical structures exist in people's normal life periods after birth. There is a correlation between the time when babies' teeth come out after birth and the baby's age. Baby tooth development is an indicator of general health and is essential in terms of nutrition, speech development, and the formation of the jaw structure (de Oliveira et al., 2019; Fernández-Escudero et al., 2020). The order and time of teeth coming out in babies are considered a reference for babies' normal growth and development (Aruede & Pepper, 2024). Newborn babies do not have teeth in the first 6 months after birth. At this stage, there are milk (temporary) and some permanent tooth buds under the gum in the upper and lower alveolar parts of the mouth (Brecher & Lewis, 2018). The teething process in babies begins with the emergence of the incisors, and 20 milk teeth will erupt in the mouth by age 3. In children, permanent teeth appear for the first time at approximately age six and the development is usually completed by age 13 (Muthu et al., 2024). Standard growth curves of babies are used to monitor and evaluate their growth process. The main parameters in monitoring and assessing the average growth and development of a baby, organized according to specific age and gender groups, are height, weight, and head circumference measurements (Van den Broeck et al., 2009; WHO Multicentre Growth Reference Study Group, 2006).

Tooth eruption, head circumference measurement, and other parameters have not yet been used to monitor the average growth and development of laboratory rat pups. There are studies covering the post-pubertal period in rats, including differences in incisor growth rates (Madalena et al., 2022; Silva et al., 2016). Laboratory rats are also used as model animals in dentistry due to their anatomical and physiological similarity to humans (Drevensek et al., 2009; Hayano et al., 2018).

Average term births of rat pups are premature, and they live the first ten days after birth with their ears closed, eyes closed, hairless and naked, utterly dependent on their mothers. For this reason, mother rats provide intensive severe care services for the survival of the many premature pups they give birth to and ensure their well-being ("Highlights for the Research Involving the Lactation Period in Laboratory Rats," 2023). The period when rat pups weigh approximately 5-6 grams at birth and 7-10 days after birth is considered the intrauterine premature period of humans. The period between 10-21 days after birth of rat pups is considered the infantile period of human babies, and during this period, they reach a body weight of approximately 30-50 grams. The period between 21-35 days after birth of rat pups is considered as the childhood period, and they reach a body weight of approximately 50-150 grams. The period between 34-38 days in females and 39-47 days in males after the birth of rat pups is considered the puberty period, and their body weight reaches 150-200 grams. The period between 49-70 days after the birth of rat pups is defined as the preadolescent period. The period after 70 days after the birth of rat pups is stated as the young adult period (Dutta & Sengupta, 2016; Sengupta, 2013; Garmash O, 2012).

The current understanding of tooth, head development, and growth in laboratory rat pups, commonly used in experimental studies, is limited. This study aims to explore the correlation between rat pup development and human infant development by tracking growth, incisor eruption, and head development. Additionally, it will assess the impact of experimental procedures on incisor and head size development in the pediatric rat model.

MATERIAL AND METHODS

This study was approved by the Dokuz Eylül University Medical Faculty Multidisciplinary Experimental Animal Laboratory Ethics Committee (Protocol 49/2023 01.11.2023). Wistar Albino Rat offspring produced in the laboratory were used. The sample size of the study was determined using the G*Power 3.1.9 software, based on similar reference studies (Franco et al., 2012; Zaman et al., 1997) with an effect size of 2.0, power = 0.95, and an alpha error probability of 0.05. Taking into account a potential dropout rate of 25%, the study was completed with 10 male and 10 female rats. For this purpose, ten male and ten female offspring born to three healthy random mother rats who had given their second birth in the breeding colony were included in the study, and the offspring's development was followed. Each mother rat and her offspring were housed together in the same cage. Mothers and offspring were housed under standard laboratory conditions with a temperature of 22±2°C, humidity (55±5%), and a 12/12 h light/dark photoperiod cycle. Mothers and weaned offspring were fed with standard rat pellet feed ad libitum. After the lactation period, the offspring rats included in the study were separated from different mothers and placed in separate cages with five offspring in each cage. During the measurements and follow-up of the puppies included in the study, care was taken not to touch the puppies with bare hands and to use gloves. To easily follow the puppies' development after birth, their tails were marked with a non-removable pin.

After the lactation period, the feed and water consumption of the rat pups in each cage was measured. The weights (gr) of the newborn rat pups were measured daily on a scale (CAS Corporation serial number 0811ED150826). The head length, width between the ears, the distance between the eyes, and upper jaw width of the rat pups were monitored from birth to puberty (67 days after birth) by daily measurements at the points indicated in Figure 1 with a GFB Elekronin Caliper brand compass. The length and width of the baby rats' lower and upper incisors were measured and recorded in millimeters (mm) with an electronic caliper from the 21st day after birth (Figure 2). Each baby rat was monitored daily from birth regarding eye-opening, ear flap opening, hair growth, and incisor tooth emergence time, and their photographs were taken and archived. The process in which the incisors of the baby rats penetrated the mucosa and emerged onto the oral mucosa was also recorded daily. The rats included in the study were returned to the breeding colony after 67 days. This study monitored the normal developmental stages of laboratory rat pups. No adverse

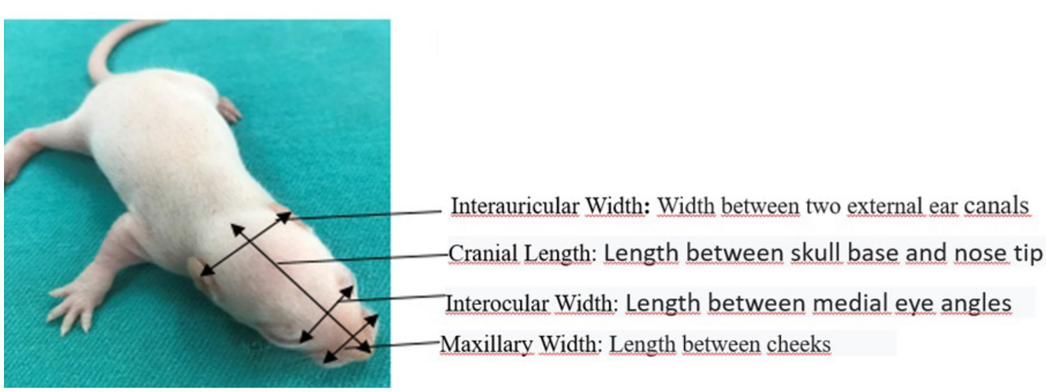

Figure 1. Measurement Locations of Head Measurements.

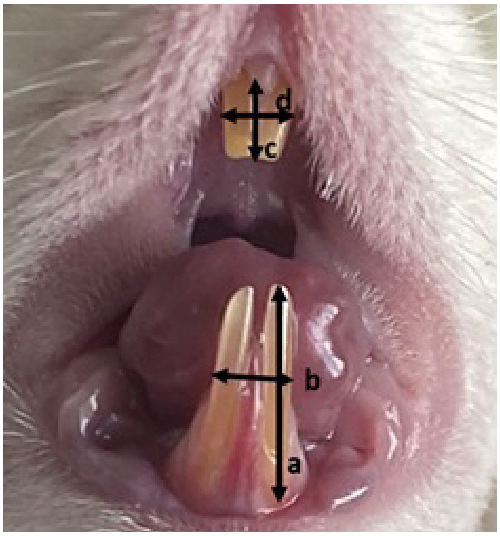

Figure 2. a. Lower incisor length, b. Lower incisor width, c. Upper incisor length, d. Upper incisor width.

events were encountered during the follow-up of 20 rat pups from birth to the 67th day.

Evaluation Methods

In this study, the period from birth to puberty of rat pups covers different life periods. To statistically reveal the differences in the development of rat pups in these periods, the 3rd, 10th, 21st, 30th, 54th, and 67th days were taken as reference(Dutta & Sengupta, 2016; Sengupta, 2013). Postnatal life periods of rats are given in Figure 3, and the measurements of these life periods are compared.

Statistical Analysis

IBM SPSS Statistics for Window (version 26.0. Armonk, NY:IBM Corp) statistical package program was used in the analysis phase of the data. Descriptive statistics of the evaluation results were given as number and percentage for categorical variables, mean and standard deviation for numerical variables. The conformity of the groups to normal distribution was determined by the Shapiro-Wilk test. In comparing the numerical values of two independent groups, when the normal distribution condition was provided, the t test was used in independent groups, and when it was not provided, the Mann-Whitney U test was used. The Spearman correlation test was used to evaluate the relationships between numerical data. The statistical alpha significance level was accepted as $p<0.05$.

RESULTS

Follow-up and Observations on Development of Rat Pups

Although the lower incisors of baby rats were observed under the mucosa one day after birth, it was observed that they penetrated the mucosa on the 8th-9th day after birth. Although the upper incisors were observed under the mucosa on the second or third day after birth, they emerged by penetrating the mucosa on the 9th-10th day. In baby rats, the lower incisors generally emerge by penetrating the mucosa one day before the upper incisors. Even in the babies of the same mother, the eruption time of the lower and upper incisors is not completed on the same day, and all incisors emerge in one or two days.

Figure 3. Life Stages and Incisor Development in the Laboratory Rat.

It has been observed that rat pups generally start to grow feathers on the 8th-9th day after birth, their eyes start to open on the 11th-12th day, and they react to sound on the 13th-14th day. The head, face, and teeth development and changes of the postnatal rat pups are photographed and the daily changes are given in Figure 4.

The average weights, head measurements and maxillary widths of rat offspring on the 3rd, 10th, 21st, 30th, 54th and 67th days are shown in Table 1. The average values and statistical differences of the length and width of the lower and upper incisors on the 21st, 30th, 54th and 67th days are given in Table 2.

Weights of Rat Pups

When the body weights of rat pups are generally compared between females and males up to 30 days after birth (PN), there is no significant difference between the two genders (Figure 5). However, after 30 days, male rats had higher body weights than female rats, which was statistically significant (p<0.001). In

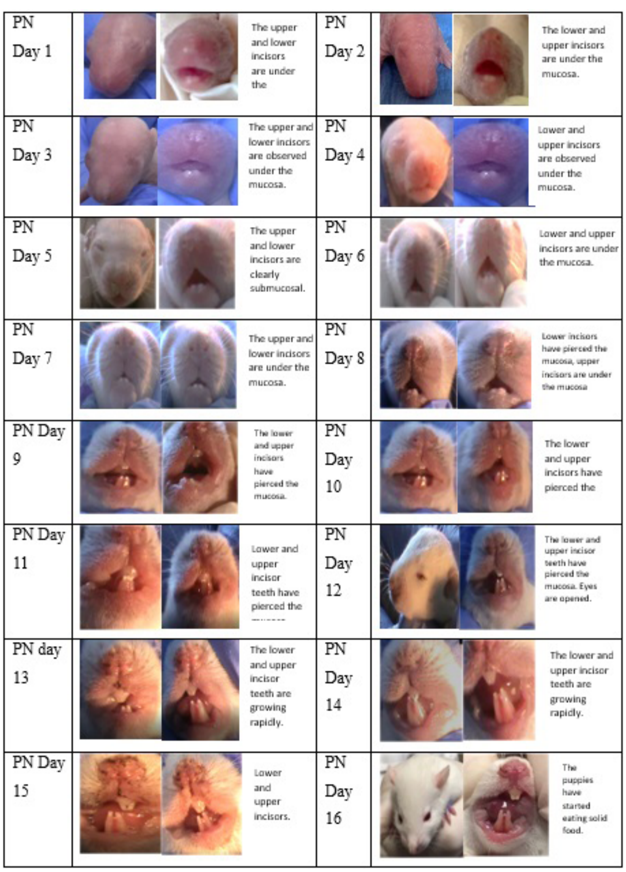

Figure 4. Incisor Teeth Development of Rat Pups.

Table 3, the mean weights of female and male rats between days 2 and 67 are presented.

Head Length of Rat Pups

When comparing the head length of male and female rat pups up to 30 days PN, there is no significant difference between the two sexes. However, after 30 days, the head length of male rats was higher than that of female rats, and this difference was statistically significant (p<0.05). Figure 6 shows the head length changes of male and female rat pups. In Table 4, the average head lengths of female and male rats from days 2 to 67 are presented.

Distance Between the Two Ears of Rat Pups

When the distance between the two ears of rat pups is compared up to 30 days PN, there is no significant difference between the two genders (Figure 7). However, after 30 days, although the inter-ear distance of male rats was higher than that of females, this difference was not statistically significant (p>0.05).

Distance Between the Eyes of Rat Pups

When the distance between the eyes of rat pups is compared up to 30 days PN, there is no significant difference between the two genders. However, after 30 days, although the distance between the eyes of male rats was higher than that of females, this difference was not statistically significant (p>0.05) (Figure 8).

Maxillary Width of Rat Pups

When comparing the maxillary width of rat pups in males and females up to 30 days PN, there is no significant difference between the two sexes. However, after 30 days, the maxillary width of male rats was higher than that of females, and this difference was statistically significant (p<0.05) (Figure 9).

Width of Mandible Incisor Teeth in Rat Pups

When comparing the mandibular teeth width of rat pups in females and males up to 30 days PN, there is no significant difference between the two sexes. However, after 30 days, the mandibular teeth width of male rats was higher than that of females, which was statistically significant (p<0.05) (Figure 10).

Length of Mandibular Incisor Teeth in Rat Pups

When comparing the size of mandible incisors of rat pups in females and males up to 30 days PN, there is no significant difference between the two sexes. However, after 30 days, the mandibular teeth length of male rats was found to be higher, and this differ-

Table 1. Comparison of weight, cranial length, interaural width, interorbital width, and maxillary width averages between genders.

	Day 3	Day 10	Day 21	Day 30	Day 54	Day 67
Weight (gr)						
Female	6 (0.15) [5-6]	14.34±0.83	30.71±1.15	62.50±2.87	178.43±8.32	220.43±9.54
Male	6 (0.11) [6-6.5]	15.50±0.71	32.17±1.21	68.67±2.64	228.17±12.77	318.67±26.08
p*	0.073**	**0.022**	**0.049**	**0.002**	**<0.001**	**<0.001**
Cranial Length (mm)						
Female	15.7±0.73	24.2 (0.17) [24-25]	31.90±0.56	37.86±0.73	42.91±0.91	44.73±1.40
Male	15.72±0.45	24.1 (0.16) [24-25]	32.75±0.90	39.98±1.06	43.62±1.02	46.00±1.41
p*	0.884	0.731**	0.062	**0.001**	**0.045**	**0.032**
Interauricular Width (mm)						
Female	10.53±0.34	14.5 (0.11) [13.8-14.7]	16.17±0.81	17.56±0.70	21.67±1.29	22.20±2.05
Male	10.79±0.32	14.5 (0.06) [14.3-14.7]	16.65±0.45	17.83±0.63	22.52±1.37	23.57±1.48
p*	0.178	0.445**	0.226	0.475	0.276	0.204
Interocular Width (mm)						
Female	6.97±0.47	8.23±0.46	8.90±0.43	10.49±0.53	11.80±0.50	12.46±0.45
Male	6.51±0.59	8.13±0.55	9.38±0.72	10.33±0.42	12.73±0.95	12.87±0.57
p*	0.147	0.738	0.163	0.583	0.066	0.175
Maxillary Width (mm)						
Female	6.27±0.56	8.26±0.70	9.34±0.26	10.63±0.67	11.41±0.90	12.64±0.46
Male	6.44±0.20	8.63±0.41	9.63±0.43	10.40±0.46	12.47±0.48	13.95±0.98
p*	0.485	0.276	0.164	0.499	**0.027**	**0.009**

*** T Test in Independent Groups, **Mann-Whitney U Test**

Note: Mean±Standart deviation for T Test in Independent Groups

Median (Standart error of mean) [Minimum – Maximum] for Mann-Whitney U Test

ence was statistically significant (p<0.05) (Figure 11).

Maxillary Incisor Width of Rat Pups

When comparing the maxillary incisor width of rat pups up to 30 days PN, there is no significant difference between the two sexes. However, after 30 days, the width of male rats' maxillary teeth was higher than that of females, but this difference was not statistically significant (p>0.05) (Figure 12).

Maxillary Incisor Length of Rat Pups

When comparing the maxillary incisor length of rat pups in females and males up to 30 days PN, there is no significant difference between the two sexes. However, after 30 days, the maxillary tooth length of male rats was higher than that of females, and this difference was not statistically significant (p>0.05) (Figure 13).

In Table 5, the correlation of head length, weight, and tooth development in male rats is presented, while Table 6 shows the same correlations for female rats. The data for both male and female rats exhibit a strong positive correlation.

DISCUSSION

"The life cycle of humans, which is typically expressed in months or years, is described in days for laboratory rats. In laboratory rats, postnatal days

Table 2. Comparison of the average width and length of the lower and upper incisors between genders

	Day 21	Day 30	Day 54	Day 67
Mandibular Incisor Width (mm)				
Female	1.40 (0.02) [1.40-1.50]	1.90 (0.05) [1.80-2.10]	2.20 (0.04) [2.10-2.40]	2.49±0.27
Male	1.50 (0.05) [1.40-1.70]	2.10 (0.03) [2.00-2.20]	2.45 (0.05) [2.20-2.50]	2.70±0.27
p*	0.295**	0.138**	**0.014****	**0.033**
Mandibular Incisors Length (mm)				
Female	3.30 (0.31) [1.20-3.50]	6.44±0.21	7.80 (0.19) [7.60-9.00]	8.77±0.57
Male	3.65 (0.09) [3.30-3.80]	6.38±0.19	9.20 (0.13) [8.70-9.60]	9.80±0.59
p*	**0.022****	0.606	**0.002****	**0.009**
Maxillary Incisors Width (mm)				
Female	1.31±0.16	1.73±0.11	2.40 (0.06) [2.10-2.40]	2.51±0.11
Male	1.48±0.13	1.72±0.12	2.30 (0.07) [2.00-2.50]	2.62±0.37
p*	0.063	0.854	0.945**	0.534
Maxillary Incisors Length (mm)				
Female	1.40 (0.06) [1.30-1.70]	2.59±0.20	3.71±0.24	3.97±0.36
Male	1.50 (0.07) [1.30-1.70]	2.90±0.21	4.23±0.37	4.52±0.19
p*	0.628**	**0.019**	**0.011**	**0.007**

*** T Test in Independent Groups, **Mann-Whitney U Test**

Note: Mean±Standart deviation for T Test in Independent Groups

Median (Standart error of mean) [Minimum – Maximum] for Mann-Whitney U Test

0–21 can be considered the lactation period, days 21–32 the childhood period, days 32–70 the preadolescent period, and days 70–100 the young adult period (Sengupta, 2013). As can be seen, rats take approximately 100 days to reach young adulthood, which takes humans 20 years. When selecting a model organism, translational medicine researchers should choose one with a life cycle that aligns with the corresponding stage of human development related to the research question. This approach ensures that the knowledge and findings gained from the study are valid and applicable to humans.

Rats are a polyparous species that produce many offspring in one litter. Although laboratory rat pups have a live weight of 4-6 grams when they are born, they double their birth weight within ten days (Sampson & Jansen, 1984; Mocchegiani et al., 1997). Because researchers say that the change in live weight gain rate is the change in which the fastest effect will be observed in the experiment in pediatric models, live weight change is definitely monitored. In this study, while there was no significant difference in the live weight gain of male and female rat pups in the first 30 days, a significant difference was found between genders due to the faster live weight gain of male rats after 30 days. Researchers should consider this when evaluating study results.

The lower incisors of laboratory rat pups emerge in 8-9 days after birth, and the upper incisors emerge in 9-10 days. Even in the offspring of the same mother, the time for the lower and upper incisors to emerge is completed on a different day, and all incisors emerge in a day or two. In babies, the first lower incisors appear in approximately 6-10 months after birth, and the upper incisors in 8-12 months (Coradini et al., 1997). In rat puppies, incisors emerge

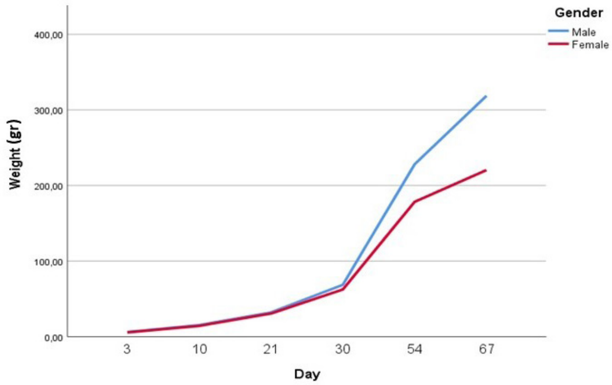

Figure 5. Body Weight Change of Female and Male Rat Pups

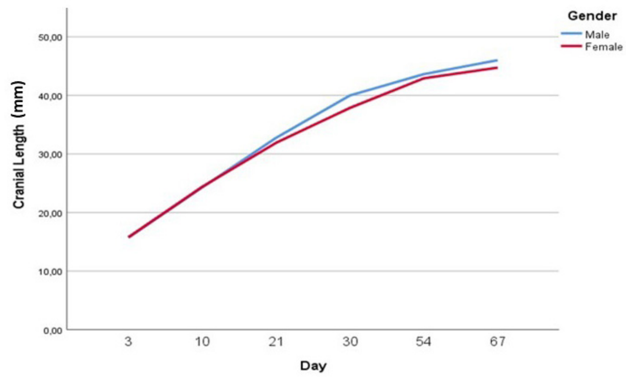

Figure 6. Head Length Change of Female and Male Rat Pups

Table 3. Weight (gr) averages in female and male rats

Weight					
Days	(Mean ± SD)		Days	(Mean ± SD)	
	Male	Female		Male	Female
2	5.75±0.27	5.4±0.35	35	95.49±2.87	84.85±3.43
3	6.17±0.26	5.71±0.39	36	98.62±3.04	88.15±3.59
4	7.33±0.61	6.79±0.57	37	101.13±3.2	90.8±3.73
5	8.25±0.76	7.93±0.53	38	111.17±3.97	101.36±4.31
6	10±0.63	9.07±0.67	39	118.48±4.5	106.17±4.5
7	10.83±0.68	9.86±0.69	40	125.79±5.03	110.99±4.71
8	12.33±0.41	11.86±0.56	41	133.1±5.57	115.81±4.93
9	14.08±0.49	13.36±0.75	42	140.42±6.11	120.63±5.16
10	15.5±0.71	14.34±0.83	43	147.73±6.66	125.44±5.39
11	16.42±0.97	14.41±0.58	44	155.04±7.21	130.26±5.64
12	19.42±0.8	18.64±0.94	45	162.35±7.76	135.08±5.89
13	20.58±0.97	19.5±0.82	46	169.67±8.31	139.89±6.14
14	21.92±0.58	21.14±1.31	47	176.98±8.86	144.71±6.4
15	23.42±0.74	22.79±1.35	48	184.29±9.42	149.53±6.67
16	25.08±0.58	24.07±1.21	49	191.6±9.98	154.34±6.94
17	26.83±0.61	25.21±0.99	50	198.92±10.53	159.16±7.21
18	27.83±0.61	26.5±1.15	51	206.23±11.09	163.98±7.48
19	29.67±0.75	28.29±1.11	52	213.54±11.65	168.79±7.76
20	30.25±0.76	28.57±1.21	53	220.85±12.21	173.61±8.04
21	32.17±1.21	30.71±1.15	54	228.17±12.77	178.43±8.32
22	36.49±1.14	34.5±1.17	55	235.13±13.7	181.66±8.36
23	39.72±1.36	37.41±1.3	56	242.09±14.67	184.89±8.41
24	42.9±1.72	40.22±1.39	57	249.05±15.65	188.12±8.47
25	46.11±2.1	43.13±1.55	58	256.01±16.65	191.35±8.53
26	49.33±2.27	45.86±1.65	59	262.97±17.67	194.58±8.61
27	52.42±2.29	48.93±2.17	60	269.94±18.69	197.81±8.7
28	57.83±2.46	53.43±2.37	61	276.9±19.73	201.04±8.79
29	61.33±2.99	56.36±2.64	62	283.86±20.77	204.27±8.9
30	68.67±2.64	62.5±2.87	63	290.82±21.82	207.51±9.01
31	74.67±2.54	66.86±2.78	64	297.78±22.88	210.74±9.13
32	80.67±2.52	71.21±2.84	65	304.74±23.94	213.97±9.26
33	86.67±2.58	75.57±3.05	66	311.71±25.01	217.2±9.39
34	91.57±2.7	80.73±3.25	67	318.67±26.08	220.43±9.54

Table 4. Head length (mm) averages of female and male rat pups.

Cranial Length					
Days	(Mean ± SD)		Days	(Mean ± SD)	
	Male	Female		Male	Female
2			35	41.11±0.74	39.68±0.8
3	15.72±0.45	15.77±0.73	36	41.23±0.74	39.85±0.74
4	17.18±1.26	17.14±0.93	37	41.32±0.75	40±0.71
5	18.23±0.62	17.96±0.56	38	41.7±0.81	40.57±0.74
6	19.23±0.79	19±0.59	39	41.82±0.8	40.72±0.72
7	21.27±0.5	20.84±0.72	40	41.94±0.78	40.86±0.7
8	22.27±1.03	22.14±1.17	41	42.06±0.78	41.01±0.69
9	23.67±0.68	23.56±0.57	42	42.18±0.77	41.16±0.68
10	24.28±0.4	24.39±0.46	43	42.3±0.77	41.3±0.68
11	25.17±0.41	25.33±0.65	44	42.42±0.78	41.45±0.68
12	26.68±0.78	26.14±0.75	45	42.54±0.78	41.6±0.69
13	27±0.87	27.09±0.72	46	42.66±0.8	41.74±0.7
14	27.52±0.87	27.39±0.63	47	42.78±0.81	41.89±0.71
15	28.33±1.17	28.04±0.73	48	42.9±0.84	42.04±0.73
16	29.33±1.46	29.19±1.1	49	43.02±0.86	42.18±0.76
17	30.7±0.48	30.6±1.27	50	43.14±0.89	42.33±0.78
18	31.27±0.67	30.59±0.5	51	43.26±0.92	42.47±0.81
19	31.67±0.61	31.07±0.31	52	43.38±0.95	42.62±0.84
20	32.05±0.64	31.66±0.38	53	43.5±0.98	42.77±0.88
21	32.75±0.90	31.90±0.56	54	43.62±1.02	42.91±0.91
22	33.57±0.74	32.68±0.58	55	43.8±0.92	43.05±0.89
23	34.19±0.66	33.26±0.68	56	43.98±0.83	43.19±0.88
24	34.8±0.64	33.85±0.82	57	44.17±0.76	43.33±0.88
25	35.42±0.68	34.43±0.99	58	44.35±0.72	43.47±0.91
26	36.03±0.77	35.01±1.17	59	44.53±0.71	43.61±0.92
27	36.78±0.59	35.89±1.04	60	44.72±0.73	43.75±0.96
28	37.25±0.52	36.77±0.54	61	44.9±0.78	43.89±1.02
29	37.95±0.61	37.17±0.78	62	45.08±0.85	44.03±1.05
30	39.98±1.06	37.86±0.73	63	45.27±0.94	44.17±1.11
31	40.25±0.88	38.3±0.76	64	45.45±1.05	44.31±1.17
32	40.55±0.71	38.71±0.88	65	45.63±1.16	44.45±1.24
33	40.78±0.77	39.17±1.05	66	45.82±1.29	44.59±1.32
34	40.97±0.75	39.45±0.9	67	46.01±1.41	44.73±1.4

in 8-10 days (Gomes et al., 2013). An appropriate evaluation guide to reveal the delay in teething time may be important for pup development in pediatric rat pup models. Considering that the period when the lower and upper incisors emerge in rat pups is still in the premature lactation period, teeth appear at a much earlier stage than in humans.

A positive correlation exists between the daily growth and development of laboratory rat pups from birth and the lengthening of the head, jaw, and incisors. The head length, head width, interocular width, maxillary width, and length/width of lower and upper incisors of male and female rat offspring increased. In the study examining the effects of anterior crossbite applied to rats on tooth and jaw development over 120 days, starting from day 30, it was shown that in the control group, the development of the upper and lower jaws progressed in parallel with cranial development, and weight gain occurred in a

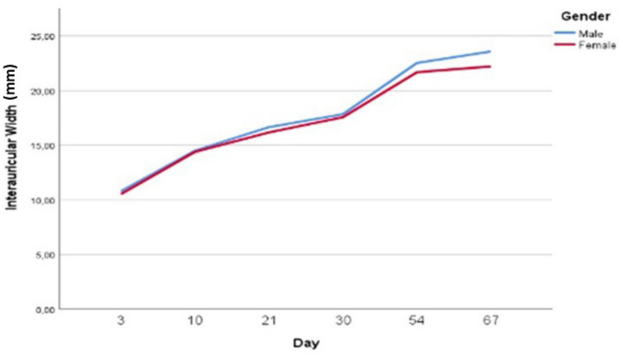

Figure 7. Change in Interauricular Distance of Female and Male Rat Pups.

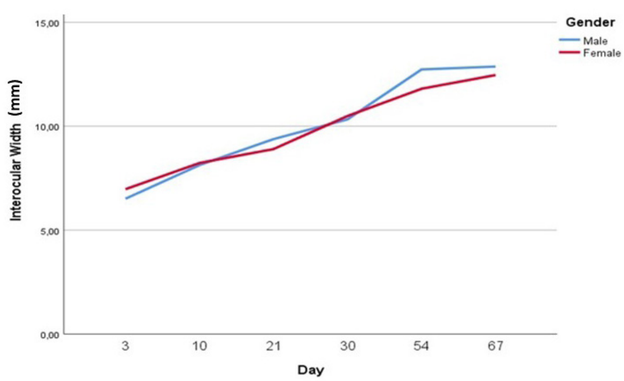

Figure 8. Change in Distance Between Two Eyes of Female and Male Rat Pups.

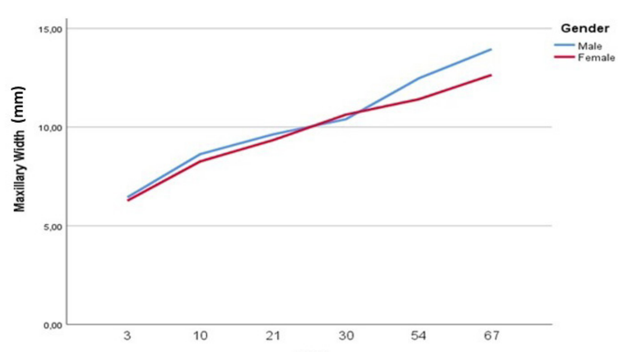

Figure 9. Maxillary Width of Female and Male Rat Pups.

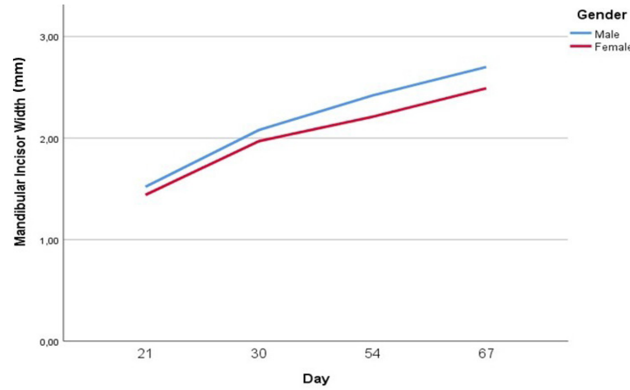

Figure 10. Width Variation of Mandibular Incisor Teeth in Female and Male Rat Pups.

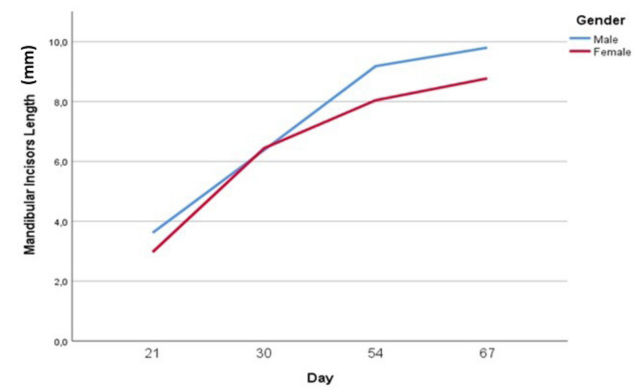

Figure 11. Change in Mandible Incisor Teeth Length of Female and Male Rat Pups.

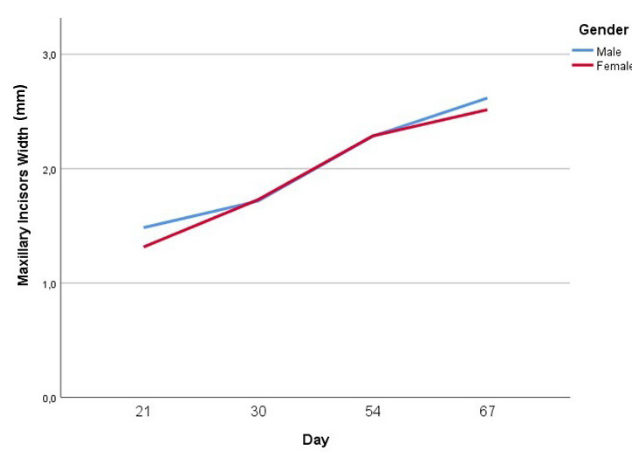

Figure 12. Maxillary Incisor Width Variation in Female and Male Rat Pups.

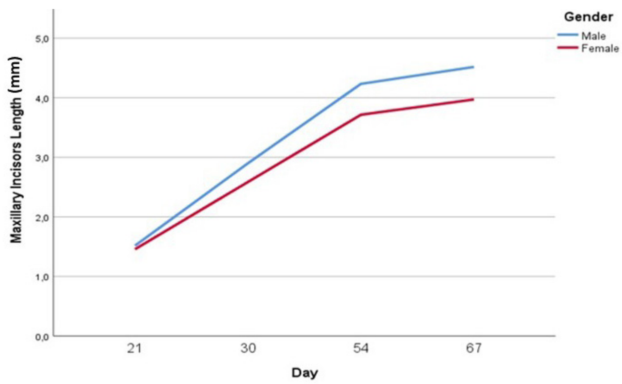

Figure 13. Change in Maxillary Incisor Teeth Length of Female and Male Rat Pups.

positive direction (Ohmura, 1990). A study examining the effects of masticatory muscle activity on dental and jaw development in male rats during the 30-day growth phase using posterior bite appliances revealed that weight gain, along with the development of the jaw and molar teeth, continued to progress positively at 14, 28, and 42 days, with variations in muscle tone significantly influencing both jaw and tooth development (Bresin & Kiliaridis, 2002). In a study, a positive correlation was found between the body weight of rats and the development of facial bones, including the width of the maxilla (Diamond et al., 1965). In another study, it was shown that in male rats, an increase in body weight was accompanied by a parallel increase in mandibular length (Lee et al., 2021).In our current study, when male and female rat offspring were compared, it was determined that there was no difference until 30 days after birth and that there was a faster increase in males after 30 days. In experimental studies planned during the pre-adolescent period, selecting male rats may be considered a better model for demonstrating growth changes in craniofacial and dental aspects. The main reason is that hormonal and physiological changes are different between genders during the same prepubertal life period (Ayyavoo et al., 2014; Garnett et al., 2004). Our results will allow researchers to evaluate and interpret the effects of procedures applied to rat pups in experimental pediatric models by comparing them with these normal values.

Body weight, head circumference, and height measurements are shown as a higher percentile in male babies than in female babies (WHO Multicentre Growth Reference Study Group, 2006). It has been shown that the eruption of the first milk tooth in babies and the number of teeth erupted over time are linked to the rate of weight gain (Mennella et al., 2020). When this literature information on human babies was correlated with our study, body weight gain was found to be higher in male rats than in females. However, in male rats, the increase in the length and width of the lower and upper incisors is also associated with increased body weight. It has been stated that the rate of teething and milk tooth growth in macrosomic children of different genders is more common in male babies. This may be the effect of sex hormones, which provide a better-developed muscle system in boys than in girls (Garmash, 2019). Growing evidence highlights the significant influence of sex hormones on bone architecture during the onset of puberty (Cavallo et al., 2021). Specifically, estradiol and testosterone, which are produced in increasing quantities during this period, have been shown to play a crucial role in regulating both bone growth and maturation (Fujita et al., 2004; Shu et al., 2024). A study has shown that increasing the levels of sex steroids in both female and male rats has positive effects on bone formation at the midpalatal suture in response to maxillary expansion (Birlik et al., 2016). Testosterone has been shown to have a direct effect on craniofacial development (Reis et al., 2021), particularly in rats during the pubertal period, by promoting the differentiation of prechondroblast cells, contributing to mineralization and vascularization, and increasing the synthesis of type I and type III collagen (Reis et al., 2023). In our study, the higher growth and development observed in male rats compared to female rats may be attributed to differences in the secretion of sex hormones, as other conditions such as nutrition and sleep were similar to those of female rats and considered standard. The examination of hormonal levels in relation to craniofacial growth differences between male and female rats during the pre-adolescent period could be planned for future studies.

In babies, primary teeth first form a bulge under the mucosa, then pierce the mucosa and erupt towards the occlusal, and at approximately the 6th month after birth, the lower jaw incisors erupt first (Muthu et al., 2024). In our study, the lower incisors were visible in the mucosa from the 8th day, and on the 10th day, the lower and upper incisors emerged by piercing the mucosa almost simultaneously. Tooth eruption in rats occurs at a rate where 1 day in their tooth development process corresponds to 15 days in humans.

Considering the study's limitations, the sample size could have been increased. In order to develop this percentile based on evidence, biochemical data,

Table 5. Correlation of weight, head, maxilla, and tooth development in male rats.

	Weight (gr)	Cranial Length (mm)	Interauricular Width (mm)	Interocular Width (mm)	Maxillary Width (mm)	Mandibular Incisor Width (mm)	Mandibular Incisors Length (mm)	Maxillary Incisors Width (mm)	Maxillary Incisors Length (mm)
Weight (gr)	-	-	-	-	-	-	-	-	-
Cranial Length (mm)	*0.983	-	-	-	-	-	-	-	-
Interauricular Width (mm)	*0.966	*0.935	-	-	-	-	-	-	-
Interocular Width (mm)	*0.959	*0.947	*0.942	-	-	-	-		-
Maxillary Width (mm)	*0.959	*0.953	*0.922	*0.942	-	-	-	-	-
Mandibular Incisor Width (mm)	*0.961	*0.937	*0.908	*0.921	*0.923	-	-	-	-
Mandibular Incisors Length (mm)	*0.979	*0.932	*0.954	*0.924	*0.927	*0.931	-	-	-
Maxillary Incisors Width (mm)	*0.940	*0.919	*0.912	*0.909	*0.869	*0.904	*0.904	-	-
Maxillary Incisors Length (mm)	*0.965	*0.932	*0.895	*0.888	*0.912	*0.968	*0.968	*0.873	-

*p<0.001, Spearman Correlation Coefficient

Table 6. Correlation of weight, head, maxilla, and tooth development in female rats.

	Weight (gr)	Cranial Length (mm)	Interauricular Width (mm)	Interocular Width (mm)	Maxillary Width (mm)	Mandibular Incisor Width (mm)	Mandibular Incisors Length (mm)	Maxillary Incisors Width (mm)	Maxillary Incisors Length (mm)
Weight (gr)	-	-	-	-	-	-	-	-	-
Cranial Length (mm)	*0.981	-	-	-	-	-	-	-	-
Interauricular Width (mm)	*0.947	*0.930	-	-	-	-	-	-	-
Interocular Width (mm)	*0.939	*0.911	*0.942	-	-	-	-		-
Maxillary Width (mm)	*0.959	*0.917	*0.922	*0.886	-	-	-	-	-
Mandibular Incisor Width (mm)	*0.888	*0.851	*0.908	*0.787	*0.739	-	-	-	-
Mandibular Incisors Length (mm)	*0.952	*0.922	*0.954	*0.840	*0.809	*0.910	-	-	-
Maxillary Incisors Width (mm)	*0.954	*0.956	*0.912	*0.858	*0.812	*0.885	*0.895	-	-
Maxillary Incisors Length (mm)	*0.941	*0.901	*0.895	*0.814	*0.775	*0.916	*0.923	*0.903	-

*p<0.001, Spearman Correlation Coefficient growth and sex hormone levels between genders can be investigated. Especially in the medical field, there are many genetic and environmental factors that affect the eruption time of teeth. In future studies, this developed percentile and newborn rats can be compared in terms of growth, development and tooth development under the influence of different disease models or environmental factors.

CONCLUSIONS

Laboratory rats are essential in biomedical research, but their anatomical, physiological, and biological differences across life stages must be considered when evaluating results. This study is the first to examine craniofacial and jaw incisor development in rat pups from birth to young adulthood, highlighting gender differences. It shows significant post-pubertal differences between male and female rats in weight, head length, jaw dimensions, and incisor size. The study concludes that parameters like incisor eruption time, head length, width, and jaw dimensions are useful for assessing gender differences in pediatric rat models.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

REFERANCES

Aruede G, Pepper T. Anatomy, *Permanent Dentition*. (2023). Treasure Island (FL): StatPearls Publishing.

Ayyavoo, A., Derraik, J. G. B., Hofman, P. L., Biggs, J., & Cutfield, W. S. (2014). Metabolic, cardiovascular and anthropometric differences between prepubertal girls and boys. *Clinical Endocrinology*, *81*(2), 238–243.

Birlik, M., Babacan, H., Cevit, R., & Gürler, B. (2016). Effect of sex steroids on bone formation in an orthopedically expanded suture in rats : An immunohistochemical and computed tomography study. *Journal of Orofacial Orthopedics = Fortschritte Der Kieferorthopadie : Organ/Official Journal Deutsche Gesellschaft Fur Kieferorthopadie*, *77*(2), 94–103.

Brecher, E. A., & Lewis, C. W. (2018). Infant Oral Health. *Pediatric Clinics of North America*, *65*(5), 909–921.

Bresin, A., & Kiliaridis, S. (2002). Dento-skeletal adaptation after bite-raising in growing rats with different masticatory muscle capacities. *European Journal of Orthodontics*, *24*(3), 223–237.

Cavallo, F., Mohn, A., Chiarelli, F., & Giannini, C. (2021). Evaluation of Bone Age in Children: A Mini-Review. *Frontiers in Pediatrics*, *9*, 580314.

Coradini, D., Biffi, A., Costa, A., Pellizzaro, C., Pirronello, E., & Di Fronzo, G. (1997). Effect of sodium butyrate on human breast cancer cell lines. *Cell Proliferation*, *30*(3–4), 149–159.

de Oliveira, A. J., Duarte, D. A., & Diniz, M. B. (2019). Oral Anomalies In Newborns: An Observational Cross-Sectional Study. *Journal of Dentistry for Children (Chicago, Ill.)*, *86*(2), 75–80.

Diamond, M., Rosenzweig, M. R., & Krech, D. (1965). Relationships between body weight and skull development in rats raised in enriched and impoverished conditions. *The Journal of Experimental Zoology*, *160*(1), 29–35.

Drevensek, M., Volk, J., Sprogar, S., & Drevensek, G. (2009). Orthodontic force decreases the eruption rate of rat incisors. *The European Journal of Orthodontics*, *31*(1), 46–50.

Dutta, S., & Sengupta, P. (2016). Men and mice: Relating their ages. *Life Sciences*, *152*, 244–248.

Fernández-Escudero, A. C., Legaz, I., Prieto-Bonete, G., López-Nicolás, M., Maurandi-López, A., & Pérez-Cárceles, M. D. (2020). Aging and trace elements in human coronal tooth dentine. *Scientific Reports*, *10*(1), 9964.

Franco, J. G., Fernandes, T. P., Rocha, C. P. D., Calviño, C., Pazos-Moura, C. C., Lisboa, P. C., Moura, E. G., & Trevenzoli, I. H. (2012). Maternal high-fat diet induces obesity and adrenal and thyroid dysfunction in male rat offspring at weaning. *The Journal of Physiology*, *590*(21), 5503–5518.

Fujita, T., Ohtani, J., Shigekawa, M., Kawata, T., Kaku, M., Kohno, S., Tsutsui, K., Tenjo, K., Motokawa, M., Tohma, Y., & Tanne, K. (2004). Effects of Sex Hormone Disturbances on Craniofacial Growth in Newborn Mice. *Journal of Dental Research*, *83*(3), 250–254.

Garmash O.(2012). A scientific review of age determination for a laboratory rat: how old is it in comparison with human age? *Biomedicine International*.2(2), 81-89.

Garmash, O. (2019). Dependence of Deciduous Tooth Eruption Terms and Tooth Growth Rate on the Weight-Height Index at Birth in Macrosomic Children over the First Year of Life. *Acta Medica (Hradec Kralove, Czech Republic)*, *62*(2), 62–68.

Garnett, S. P., Högler, W., Blades, B., Baur, L. A., Peat, J., Lee, J., & Cowell, C. T. (2004). Relation between hormones and body composition, including bone, in prepubertal children. *The American Journal of Clinical Nutrition*, *80*(4), 966–972

Gomes, J. R., Omar, N. F., Do Carmo, E. R., Neves, J. S., Soares, M. A. M., Narvaes, E. A., & Novaes, P. D. (2013). Relationship between cell proliferation and eruption rate in the rat incisor. *Anatomical Record (Hoboken, N.J. : 2007)*, *296*(7), 1096–1101.

ShimadaHayano, S., Fukui, Y., Kawanabe, N., Kono, K., Nakamura, M., Ishihara, Y., & Kamioka, H. (2018). Role of the Inferior Alveolar Nerve in Rodent Lower Incisor Stem Cells. *Journal of Dental Research*, *97*(8), 954–961.

Highlights for the Research Involving the Lactation Period in Laboratory Rats. (2023). *Journal of Laboratory Animal Science and Practices*, *3*(2), 45–50.

Lee, I.-S., Kim, D.-W., Oh, J.-H., Lee, S. K., Choi, J.-Y., Kim, S.-G., & Kim, T.-W. (2021). Effects of 4-Hexylresorcinol on Craniofacial Growth in Rats. *International Journal of Molecular Sciences*, *22*(16).

Madalena, I. R., Marañón-Vásquez, G. A., Omori, M. A., de Sousa, E. T., da Silveira, H. A., León, J. E., Baratto-Filho, F., Alves, S. Y. F., Stuani, M. B. S., Nelson-Filho, P., Kirschneck, C., & Küchler, E. C. (2022). Evaluation of tooth eruption rate of incisor teeth in rats with estrogen deficiency. *Clinical Oral Investigations*, *27*(1), 345–352.

Mennella, J. A., Reiter, A., Brewer, B., Pohlig, R. T., Stallings, V. A., & Trabulsi, J. C. (2020). Early Weight Gain Forecasts Accelerated Eruption of Deciduous Teeth and Later Overweight Status during the First Year. *The Journal of Pediatrics*, *225*, 174-181.e2.

Mocchegiani, E., Verbanac, D., Santarelli, L., Tibaldi, A., Muzzioli, M., Radosevic-Stasic, B., & Milin, C. (1997). Zinc and metallothioneins on cellular immune effectiveness during liver regeneration in young and old mice. *Life Sciences*, *61*(12), 1125–1145.

Mukherjee, P., Roy, S., Ghosh, D., & Nandi, S. K. (2022). Role of animal models in biomedical research: a review. *Laboratory Animal Research*, *38*(1), 18.

Muthu, M., Vandana, S., Akila, G., Anusha, M., Kandaswamy, D., & Aswath Narayanan, M. (2024). Global variations in eruption chronology of primary teeth: A systematic review and meta-analysis. *Archives of Oral Biology*, *158*, 105857.

Ohmura, K. (1990). [Experimental study of growth and development of the dentofacial complex in the rat. Influence of anterior displacement of mandible]. *Ou Daigaku Shigakushi*, *17*(3), 332–353.

Pehlivanović Kelle B, Dina F, Emina A, Ziga Smajic N, Fahir B. (2019). Animal models in modern biomedical research. *European Journal of Pharmaceutical and Medical Research*, 2019;6:35-38.

Reis, C. L. B., de Fátima Pereira Madureira, M., Cunha, C. L. R., Junior, W. C. R., Araújo, T. H., Esteves, A., Stuani, M. B. S., Kirschneck, C., Proff, P., Matsumoto, M. A. N., Küchler, E. C., & Silva Barroso de Oliveira, D. (2023). Testosterone suppression impacts craniofacial growth structures during puberty : An animal study. *Journal of Orofacial Orthopedics = Fortschritte Der Kieferorthopadie : Organ/Official Journal Deutsche Gesellschaft Fur Kieferorthopadie*, *84*(5), 287–297.

Reis, C. L. B., Guerra, K. C. C., Ramirez, I., Madalena, I. R., Almeida, A. C. P. de, & Oliveira, D. S. B. de. (2021). Does Suppression Levels of Testosterone Have an Impact in The Craniofacial Growth? A Systematic Review in Animal Studies/ A supressão de testosterona impacta o crescimento craniofacial? Uma revisão sistemática de estudos com animais. *Brazilian Journal of Development*, *7*(7), 75630–75648.

Sampson, D. A., & Jansen, G. R. (1984). Measurement of milk yield in the lactating rat from pup weight and weight gain. *Journal of Pediatric Gastroenterology and Nutrition*, *3*(4), 613–617.

Sengupta, P. (2013). The Laboratory Rat: Relating Its Age With Human's. *International Journal of Preventive Medicine*, *4*(6), 624–630.

VasSilva, M. A. D., Vasconcelos, D. F. P., Marques, M. R., & Barros, S. P. (2016). Parathyroid hormone intermittent administration promotes delay on rat incisor eruption. *Archives of Oral Biology*, *69*, 102–108.

Shu, W., Niu, W., Zhang, Y., & Li, H. (2024). Association between sex hormones and bone age in boys aged 9-18 years from China. *Journal of Cellular and Molecular Medicine*, *28*(7), e18181.

Van den Broeck, J., Willie, D., & Younger, N. (2009). The World Health Organization child growth standards: expected implications for clinical and epidemiological research. *European Journal of Pediatrics*, *168*(2), 247–251.

Wang, A. N., Carlos, J., Fraser, G. M., & McGuire, J. J. (2022). Zucker Diabetic-Sprague Dawley (ZDSD) rat: Type 2 diabetes translational research model. *Experimental Physiology*, *107*(4), 265–282.

Weber, B., Lackner, I., Haffner-Luntzer, M., Palmer, A., Pressmar, J., Scharffetter-Kochanek, K., Knöll, B., Schrezenemeier, H., Relja, B., & Kalbitz, M. (2019). Modeling trauma in rats: similarities to humans and potential pitfalls to consider. *Journal of Translational Medicine*, *17*(1), 305.

WHO Multicentre Growth Reference Study Group. (2006). WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatrica (Oslo, Norway : 1992). Supplement*, *450*, 76–85.

Worboys, M., Timmermann, C., & Toon, E. (2021). Before translational medicine: laboratory-clinic relations. *History and Philosophy of the Life Sciences*, *43*(2), 48.

Yılmaz O. Gökmen N. (2015). Experimental Animal Models in Orthopedics and Traumatology, Editör: Öztuna V. , Pelin and Tipo Printing, 1st Edition, Part 1-2, pp.1-25.

Yılmaz O, Yılmaz. C. (2021). Model animals and organisms in cancer research. Aktaş S, Alakavuklar M, editor. Essential Oncology for Clinicians. 1st Edition, *Türkiye Klinikleri, Medical Oncology, Special Topics*. pp.95-101.

Zaman, M. S., Lancaster, F. E., & Hupp, E. W. (1997). Physical and motor development in male and female rat offspring prenatally exposed to gamma radiation. *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes*, *32*(2), 313–325.

Zou, Y., Lin, H., Cai, J., Xie, Q., Chen, W., Lu, Y., & Xu, L. (2022). Effects of functional mandibular lateral shift on craniofacial growth and development in growing rats. *Journal of Oral Rehabilitation*, *49*(9), 915–923.