The main causative factor of increased mortality rate in experimental rat

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Abstract Rats are still often used in vital areas of biological study. Animal management, behavior, compliance and technical processes directly relevant to research projects are just a few of the many duties placed on the shoulders of those conducting such studies. This study was designed to fill a gap in the literature by providing a rapid reference source for researchers and animal care takers responsible for the care and/or usage of rats in research settings, since no prior study has detailed the death rate of experimental rats. A total of 103 adult wister albino rats, aged (12-15) weeks, were included in this study. The rats were subjected to orthodontic treatment using a metal spring coil measuring 6 mm in length, with force of 100 gm. to induce tooth movement. The experimental study involved a total of 56 rats, with 47 rats being excluded from the study due to mortality during the course of the study. The total death rate was determined. The greatest fatality rate was found to be 32%, and it was caused by rats related factors. The anesthetic death rate was 23%. The mortality rate in a controlled laboratory environment was 26%. The lowest cause of death rate was found to be the environmental factors at 19%. To maintain the reliability and repeatability of study results, it is crucial to implement a health monitoring programme for the experimental rat, whose death rate may be significantly impacted by factors such as biological diversity, stress, and an exhausted mechanism.

Keywords: animal management, anesthesia, experimental rat, mortality, orthodontic

1. Introduction

Animal models play an indispensable role in testing medical biomaterial histopathology and pharmacology for understanding their histoconductivity, mechanical properties, biocompatibility, interaction with host tissues, and degradation. Rodents, particularly laboratory rats, represent a reliable and affordable model for conducting basic research. This has been done with a wide variety of animal species, although male rodents, particularly rats, are the usual favorites (Aljuboury, 2022). Due to their hormonal properties, high reproductive rates, affordable prices, easy maintenance, commercial availability, established methods, and low animal fatality rates, rats are a popular choice for experimental animals (Yildirimturk, 2016). In addition, after the publication of the rat genome sequence in 2004, Maurer & Quimby reported that more than 90% of rat genes in 2015 had a single human ortholog (Maurer & Quimby, 2015). Reports catalog rat attrition during experimental research (Harran, 2009; Gavrilova & Gavrilov, 2014; Alnajar & Al Groosh, 2020). Potential factors that contributed to the high death rate observed in the experimental rats are outlined in Figure 1 as follows:

1.1. Laboratory management and breeding

According to the Federation of European Laboratory Animal Science Associations 2002 and Patrick et al. (2013). The following factors, summarized in Figure 1, indicate the primary causes of rat mortality.

1.2. Environmental provisions

The microenvironment of an animal is affected by the conditions of the cage, even though environmental measurements are performed at the room level (macroenvironment). The rate of air exchange, ammonia buildup, temperature, and humidity may all be regulated by the use of individually ventilated cages (IVCs) (Graham, 2021). Infections, ammonia and carbon dioxide levels, cleaning needs, worker hours, and allergies are all factors that may be mitigated with the use of IVC systems.

1.3. The environmental provisions are subdivided into the following factors

1.3.1. Ventilation
The metabolic heat needs of the animals should be taken into account. Maintaining an adequate quantity of ammonia within the immediate habitat of an animal is more critical than performing air changes. When handling animals, employees’ health must be considered because they might be exposed to allergenic substances such as dander and urine. Maintaining specified pathogen-free or gnotobiotic rats requires the use of high-efficiency particulate air (HEPA) or comparable filtering of incoming air to minimize the risk of airborne entry of infectious illnesses into the animal room (Robert & James, 2010; Butler et al., 2022).

![Figure 1: Mortality factor in experimental rats.](image)

1.3.2. Temperature

The temperature of the environment has been demonstrated to affect various hematological, reproductive, nutritional, and dietary variables. Based on these results, mouse housing should be kept between 20 and 26°C. (Fischer et al., 2019).

1.3.3. Humidity

It is important to avoid extremely high or low humidity and rapid changes in humidity. At low humidity levels, mice may suffer from respiratory problems due to excessive dustiness of bedding and feed and drying of mucous membranes. Furthermore, infections of the upper airways may occur more easily (Federation of European Laboratory Animal Science Associations 2002 (FELASA)).

1.3.4. Light

The lighting in its habitat is crucial for the mouse, which is mostly nocturnal. External light variations may be mitigated with proper lighting management. Long-term exposure to bright light in rats has been associated with retinal atrophy, and animals are subjected to a regular light–dark cycle, generally 12 hours light:12 hours dark; however, there is some debate concerning the effect of creating artificial ‘dawn’ and ‘dusk’ periods on animal behavior (Burn, 2008).

The participants were advised to seek comfort via behavioral means such as increased salivation, burrowing, and shade, with cold adaptation being superior to heat adaptation (Hankenson et al., 2018).

2. The laboratory setting

Many factors contribute to the misconception that laboratory rats exhibit stereotypical behavior involving the following factors:

**A- Cages**: Research shows that living in a poor cage may hinder mental health and, in extreme situations, even cause death. Rats benefit greatly from their personal space, and they have evolved to live in such environments (Abou-Ismail & Mahboub, 2011). Cleaning schedules should strike a balance between preserving hygiene and preserving smell patterns produced by animals. Most cages were cleaned once a week. This is especially true for clinical chemistry studies. For example, elevate urea nitrogen in the blood (Balcombe, 2010).
B- Social organization: Any social structure of laboratory rodent species should be taken into consideration to ensure their health and happiness. Keep the burrow system in place until proven otherwise. Male rats of all ages studied thus far are gregarious creatures who like to spend their downtime near one another. Preference for social interaction during periods of activity increases with age (Ben-Ami Bartal et al., 2014). The transfer of previously used nesting material to clean cages during cage cleaning (but not through the use of a filthy bedding mate) has been shown to minimize aggression (Liss et al., 2015). Animals may have strong, unpleasant disputes when they are moved to new groups, and the evidence suggesting that individual housing in rats can be deleterious is not convincing (Massari et al., 2019).

C- Transport: Because of the persistent consequences of stress on behavior and physiology, long-distance transportation should be avoided in rats wherever feasible (Arts et al., 2012). After obtaining the plants from a commercial breeder, it is recommended that the rats be allowed to accoporate for at least one week—usually two to three weeks. Due to the stress of transportation, animals are unlikely to drink or eat, but if a trip continues for more than 24 hours, they must be given access to food and water (Castelhano & Baumans, 2009).

D- Handling: Unless required for the treatment, the rats were not touched. If handling is necessary, it must be performed with as little contact as possible for the sake of both the handler and the rodent. Careful handling of experimental rats is essential since their bites and scratches may spread a wide variety of zoonotic illnesses. Risk evaluations should be performed before any contact with the rat (Hubrecht & Kirkwood, 2010).

E- Water: Pathogenic microorganisms, metals, and organic compounds might all be found in the water we drink. Depending on where the water comes from, the level and kind of pollution might be different. It is not safe to put a bottle of tap water in a cage since the water is already tainted with germs. As a means of sanitation, some mice attempt to force their waste and bedding materials into drinking tubes. Water bottles and drinking tubes can be sources of Pseudomonas aeruginosa and other coliform bacteria. P. aeruginosa kills mice that have been immunosuppressed (by irradiation or other means) and has been associated with otitis media and encephalitis in recently shipped mice. Filled water bottles and drinking tubes can be autoclaved to kill any bacteria that may have gotten in from the water source or improperly washed containers, but this will not stop germs from growing in the water while it is being used. Acidification (pH 2-3) or chlorination (15-20 ppm active chloride) can be used to reduce bacterial contamination (Whipple et al., 2021). Water quality varies depending on its origin and the pipes through which it travels, which can increase or decrease the percentage of metals and organic compounds in the water (Hubrecht & Kirkwood, 2010).

An increase in serum bilirubin, a decrease in albumin and globulin, and an increase in alkaline phosphatase enzymes are all indicators that contaminated water interferes with liver function (Patrick & Jason, 2013).

F- Fungal infection: Fungi are relatively uncommon causes of disease in healthy and immunocompetent humans and other mammals, even though hosts are constantly exposed to infectious propagules. However, an increasing number of recalcitrant fungal diseases in animals have occurred over the last two decades; these diseases originate from opportunistic and pathogenic fungi that can be disseminated across a wide range of gauges, and bottle feeding via experimental labs or even zoonotic agents is naturally transmitted from vertebrate animals to humans and vice versa (Kohler et al., 2014).

Different categories of fungal infections can be encountered in rats and other mammals, originating from environmental sources without transmission to humans; in addition, endemic infections can be indirectly transmitted from the environment (Ibrahim & Abbas, 2012).

Antifungal resistance in rats is also a causative factor; resistance to antifungals can occur in different animal species in addition to rats that receive these drugs, although the true epidemiology of resistance in animals is unknown, and options for treating infections caused by resistant infections are limited (Gonçalves et al., 2016). The contamination of foods and animal feeds with mycotoxins is a worldwide problem. Contaminated feeds containing these products cause negative metabolic responses and enzyme activity, resulting in reduced body weight gain, tissue necrosis and the formation of mycotoxins by many important phytopathogenic and food spoilage fungi; these products are undoubtedly among the most significant risk factors for mammalian health (Aupanun et al., 2017; Seyedmojtaba et al., 2018).

3. Anesthesia

General anesthesia can be induced either by inhalation or by injection of anesthetics via the intraperitoneal, intramuscular or intravenous route. There are excellent standard works available with detailed information on anesthesia and pain management in rodents (Navarro et al., 2021).

After administering an anesthetic, the animal must be monitored to ensure that it is at the required depth of anesthesia and that vital body functions have not become dangerously depressed. Respiratory and cardiac function must both be monitored closely (National Research Council, 2009).

Injectable anesthetics may be used to induce anesthesia, which is then maintained with a gaseous anesthetic. In some instances, particularly for small animals such as highly active rodents, the opposite may apply, with short-term anesthesia induced by inhalation and maintained by injection. Many anesthetics interfere with the thermoregulation of animals. Therefore, body temperature should be carefully monitored during anesthesia. An electric blanket controlled by a thermostat and integrated with a rectal temperature probe could be used (Baumans, 2005; Berry, 2015). It should be mentioned that...
when the ketamine component is used to anesthetize rodents, ketamine with drawl suppresses the hypothalamus, which is thought to be the body's thermostat, during anesthesia revival in rats (Sotoudeh & Namavar, 2022); this leads to a decrease in body temperature, which appears clinically as a repetitive trimmer episode that may lead to death if the rodent leaves during anesthesia revival in a cold or low-temperature environment (Roughan & Flecknell, 2003).

Behavioral scoring systems and food and water intake can be used in postoperative assessments. Twitching, flinching, stretching, reduced climbing and locomotion can be observed and assessed for recovery (Patricia et al., 2019).

4. Rat-related factors

A-The husbandry and welfare: When male and female rats are housed together, they are less stressed by common husbandry and experimental procedures performed in the animal room than when they are housed separately. Before parturition, one to four males are usually grouped with a large number of females, and pregnant females are separated (Bailoo et al., 2020).

B-Stressful or exhausted procedures: Stressful procedures can affect certain analytes, most of which are directly related to the stress induced by these procedures. Short-term stress causes the release of epinephrine, whereas long-term stress causes the release of corticosteroids, which induces a decreased lymphocyte and eosinophil count. This can lead to an increase in the immune system serum concentration of corticosteroids in response to fasting, pen and large group housing, and even in homing gauge-type rats housed in standard solid bottom polycarbonate cages, the corticosterone serum concentration is greater than that in rats housed in standard wire bottom cages. Fecal corticosterone has been used as a noninvasive technique for monitoring rat stress hormones, and more immune-reactive fecal corticoid metabolites are detected in male rats in clinical laboratory data (Rowland & Toth, 2019).

Since albino rats are a genetically pure strain, they have not undergone stress or labor-intensive procedures in their unique breeding environment. These stimuli can potentially exceed the capacity for self-healing, which can suppress the immune system. Increased cortisone secretion could result from this process, impairing the immune system and making infections more common. In extreme circumstances, it may even be lethal (Pound & Ritskes-Hoitinga, 2018).

C-Biological variety: Rats can be divided into two genetic groups: inbred and outbred. Inbred strains have more background data and exhibit greater stability, accuracy, and homogeneity than outbred strains, which may all contribute to their greater survival rate (Festing 2010). The disadvantage of using outbred strains is that each breeding colony may be different due to genetic drift. Hence, a Wistar or Sprague-Dawley rat from one breeder may be genotypically and phenotypically different from those obtained from a different source. Considerable differences in neuroanatomy, behavior and pharmacology have been reported, for example, in Sprague-Dawley rats obtained from different commercial breeders and for examples of frequently used strains: Wistar albino, Sprague-Dawley albino and long-even hooded.

4.1. Frequent check-ups for the general health of Rats

The most important indicators of disease and/or lack of well-being in rats according to the National Research Council Guide for the Care and Use of Laboratory Animals published in 2008 are summarized below.

- **Appearance:** Piloerection and a rough greasy or matted pelage, sometimes accompanied by loss of hair, loose skin, signs of muscle wasting on the back, dehydration and reduced body weight may all be observed. The eyelids are half or fully closed, and the eyes appear sunken. Red secretions from the lacrimal glands accumulate around the eyes (chromodacryorrhea).
- **Feces:** Soft feces, diarrhea, a dirty tail and an unpleasant smell are indicative of an intestinal infection.
- **Behavior:** Initially, animals may be more alert and aggressive but will become progressively more passive; They stop eating and drinking and reduce exploratory behavior. Sometimes, rats gnaw affected parts of the body.
- **Posture:** The animal frequently lies down, initially curled up with the head touching the abdomen, and later stretched with the tail extended. A hard belly indicates abdominal pain. A tilted head is indicative of an infected middle ear.
- **Locomotion:** A diseased rat moves slowly with a stiff-legged gait and arched back.
- **Vocalization:** Squeaking when handled.
- **Physiology:** Sneezing may be the first sign of a respiratory infection. When the condition of the animal worsens, breathing is audible and labored, and the respiratory frequency increases. Hypothermia indicates a serious condition, and a pale appearance is indicative of anemia or loss of blood.

5. Materials and Methods

The study was approved by the Scientific Research and Ethics Committee at the Department of Orthodontics-College of Dentistry/University of Baghdad (ID number: 623, date 12/4/2022).

Experiments were carried out to accomplish the objectives of this study, which aimed to fill a gap in the literature by providing a simple reference for researchers and animal care providers involved in the care and use of rats in research settings. This project is critical because no previous research has specifically investigated the intricate aspects of the death
rate among experimental rats. A total of 103 adult albino rats aged 12-15 weeks were included in the orthodontic experimental study. These rats were divided into five groups of different durations and from different treatment regimens, and only 56 rats were included in the orthodontic study. Another 47 rats were excluded due to differences in the causes mentioned above, as summarized in Figure 2.

Figure 2 Total number of experimental rats used in the study and the number of drop-out rats.

5.1. **Preparation of experimental rats**

The rats were acclimated for two weeks before the experiments were started. Each rat was kept in an individual cage. The animals were subjected to 12/12 hours of dark/light cycles. The housing temperature was 25°C±2°C, and the relative humidity was 30%-50% (Koolhaas, 2010). The animal was fed a standard laboratory pellet in addition to water ad libitum. The weight and general condition of each rat were checked periodically twice per week.

5.2. **Anesthetizing the animals**

The rats were anesthetized with an intramuscular injection of ketamine (87 mg/kg) (ketamine 10%) and xylazine (10 mg/kg) (XYL-M2 injectable solution 25 ml). The two drugs were mixed at a ratio of 2:1 (ketamine:xylazine) and administered intramuscularly to the thigh muscle (Flecknell, 2009). This process was repeated twice—during the placement and removal of the orthodontic appliance.

5.3. **Insertion of orthodontic appliance**

Each rat was examined to ensure that it had all its teeth. An orthodontic appliance that had been modified was fixed with the incisors serving as anchors, while a closed coil spring exerted a force of 100 grams to shift the first molar in the mesial direction. The cervical surfaces of both maxillary incisors were grooved using an angled handpiece and an inverted cone bur (Yadav et al., 2016). A stainless steel ligature wire 0.009 in diameter was wrapped around the cervical region of the first maxillary molar and then placed interdentally between the teeth. A 6-mm long closed coil spring was attached to the wire, and its free end was carefully curved toward the buccal surface of the tooth to prevent mechanical disturbance to the surrounding oral tissue and slippage of the coil. The tube was then ligated tightly to ensure maximum stabilization of the
Another short prefabricated stainless-steel ligature wire measuring 0.009 inches in diameter was coiled around the grooves produced on both incisors as a mechanical retention to account for the conical shape of the incisors of the rats. A pressure gauge indicated that the closed coil spring of the fixed orthodontic device exerted a total force of 100 grams for mesial traction of the maxillary first molar. The opposite end of the closed coil spring was secured using a ligature wire. After 60 seconds of exposure to an etching solution of 37% ortho-phosphoric acid, the enamel was quickly washed with water. A lightly cured filling composite material was inserted after the teeth had been etched and dried with cotton rolls and an air bulb. The surfaces of the etched and dried teeth as well as the grooves made by the ligature wire were coated with a thin coating of a lightly cured bonding compound using a disposable brush. Before being exposed to light for 20 seconds, the labial and palatal wires were completely submerged in the filling material in the grooves. A disposable spatula was used to apply the filling. No protective filling material was placed over the closed coil spring at the tip of the maxillary incisor. Each week, the appliance was inspected for signs of loosening or damage. With the incisors as anchors, the application of an orthodontic force in the medial direction on the maxillary first molar led to mesial traction of the first molar and the formation of a space between the first and second molars.

A force (100 grams) from a metal spring coil (6 mm) was used; the spring coil extends from the 1st molar anteriorly to the incisor on the left side for mesial retraction, as in one orthodontic research experiment (Abed & Albustani, 2013; Khamees & Al Groosh, 2023).

5.4. Statistical analysis

Normality and homogeneity of variance were checked. One-way ANOVA was performed to determine the significance of differences, and the data were analyzed with Bonferroni post hoc correction (SPSS V.20). A significance level of $p < 0.05$ was used for all the statistical tests.

6. Results

6.1. Statistical analysis of the factors predisposing patients to mortality

Table 1 shows the four main predisposing factors for mortality, the related subdivision causes and the number of dead experimental rats for each related cause that occurred during the experiment (Table 1).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Related causes</th>
<th>count</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental factor</td>
<td>Temperature</td>
<td>4</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>Humidity</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>1</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>Ventilation</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cages</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Handling</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Laboratory setting</td>
<td>Social organization</td>
<td>3</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>Transportation</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fungal</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Anesthesia</td>
<td>Induction of Anesthesia</td>
<td>5</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>Revival from Anesthesia</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Husbandry</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Rat related factor</td>
<td>Biological variety</td>
<td>4</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td>Stress and exhausted</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Work</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.2. The overall mortality rate of the experimental rats.

The overall mortality rate was calculated and is shown in Table 2. For the ratio-related factors, the mortality rate was 32%, which was the highest rate. For anesthesia, the mortality rate was 23%. In laboratory settings, the mortality rate was 26%. For environmental factors, the mortality rate was 19%, which is considered the lowest rate. A summary of the mortality rate data is provided in Figure 3.
6.3. Comparison of the subject effects

Using a binary regression test, we measured the effect of different factors (independent variables) on the mortality rate (dependent variable) (Table 3). In this table, we observed a significant effect of the mortality factor on the mortality rate ($p$ value=0.020); these 45 rats were divided into five groups with different durations of the experiment and different regimens, and there was no significant difference ($p$ value=0.483) between these subgroups concerning mortality (Table 3).

![Overall Mortality rate](image)

**Figure 3** The overall mortality rate of the rats.

### Table 3 Comparison of between-subjects effects.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>3.115 st</td>
<td>2</td>
<td>1.557</td>
<td>3.561</td>
<td>.051</td>
</tr>
<tr>
<td>Intercept</td>
<td>3.096</td>
<td>1</td>
<td>3.096</td>
<td>7.080</td>
<td>.016</td>
</tr>
<tr>
<td>Factors</td>
<td>2.890</td>
<td>1</td>
<td>2.890</td>
<td>6.608</td>
<td>.020</td>
</tr>
<tr>
<td>Groups</td>
<td>.225</td>
<td>1</td>
<td>.225</td>
<td>.514</td>
<td>.483</td>
</tr>
<tr>
<td>Error</td>
<td>7.435</td>
<td>17</td>
<td>.437</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>121.000</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>10.550</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .295 (Adjusted R Squared = .212)

6.4. Binary regression test for predictors of mortality rate:

Regression tests were used to test and evaluate the effect of the factors and groups on the mortality rate. In general, the results showed that the effect of the independent variable (factors and groups) on the dependent variable (mortality rate) was approximately .543. (Table 4, Figure 4).

![Histogram](image)

**Figure 4** Histogram of regression tests

### Table 4 Model summary.

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.543 st</td>
<td>.295</td>
<td>.212</td>
<td>.66133</td>
</tr>
</tbody>
</table>

a. Dependent Variable: Mortality rates. b. Predictors: (Constant), Factors, Groups
7. Discussion

The development of any novel drug or procedure requires the interplay between clinical observations and studies in animal models. In contrast to other animals, rats seem to be the animal of choice for orthodontic tooth movement experimental studies for many reasons, such as being inexpensive; additionally, large samples can be used; transgenic strains can be developed recently in almost all small rodents; and most of the antibodies needed for molecular biological, immune-histochemical and cellular techniques are available for rodents and easier histological preparation than for other animals (Khamees et al., 2023).

Moreover, the most limiting factor in these experimental models is the high mortality and morbidity rate at the beginning or middle of the experimental study, similar to the findings of other experimental studies on rats (Harraa, 2009; Hussein & Saloom, 2020); thus, these models have a great effect on the duration of the experiment and the output of the results.

In this study, the highest percentage (approximately 32%) of the total mortality rate was related to rat-related factors, which included husbandry and welfare (Cait et al., 2022). When male and female rats are singly housed and housed apart from each other, they are under more stress from experimental procedures performed in the animal room because fighting occurs when the female is housed apart from the male in the harem and when the male is just housed together; this finding is consistent with what has been previously reported (Patrick & Jason, 2013). The second factor is stress-inducing or exhausting procedures. Rats are exposed to intraoral orthodontic appliances as part of the study of tooth movement, and such procedures can affect certain analytes, most of which are directly related to the stress induced by these procedures (Lombardo et al., 2016).

The least effective factors in this study were environmental factors, which included temperature, humidity, light, and ventilation. However, these factors had the least effect on mortality in the experimental rats (19%). Hence, it is important to control and check these conditions in the experimental rooms, and when the experiments are performed in the laboratory, this is extremely important because many behavioral patterns, including feeding patterns, are governed by these environmental factors since any disturbance in these factors leads to the disruption of many other behavioral and physiological circadian rhythms in rats; for example, in restriction experiments where rats are forced to eat and drink during the light phase, there are strong interactions between food intake and sleep, which is in line with previously published studies (Harraa, 2009; Hussein & Saloom, 2020; Alnajar & Al Groosh, 2020).

Infections of laboratory animals during stressful procedures and poor manipulation can severely influence the outcome of experiments, as also proven in previous research (Baumans, 2005; Seyedmojtaba et al., 2018; Pound & Ritskes, 2018).

Therefore, the following practical findings should be confirmed:

- Ability to recognize departures from good health and knowledge of actions to take where this is detected
- Knowledge and skills in the handling and care of animals, including breeding stock and animals undergoing experiments
- Awareness of normal behavior; ability to recognize the causes and signs of pain, discomfort or distress; and awareness of how to minimize these issues.
- Skill in the administration of medicines (as prescribed by a veterinarian), basic husbandry procedures such as identification methods, breeding and checking for physical signs of good health;
- Handling dexterity, the ability to choose an appropriate technique and perform euthanasia when needed, and knowledge of proper corpse disposal ability to interact well with other care personnel, including supervisors, as well as researchers who may be doing studies on animals in care. Awareness of the significance of excellent hygiene and biosecurity, as well as the processes required to maintain these effects, as well as the appreciation of health and safety issues that may arise in the animal facility and awareness of how these should be addressed.

Understanding the significance of keeping accurate records and aptitude for keeping records.

8. Conclusions

Animal management, behavior, compliance, and technical procedures are critical factors for any scientific research. This study intended to fill an important gap in the literature by offering quick access to information for researchers and animal caretakers involved in rat care and usage within studies, with a particular emphasis on the death rate of experimental rats. These findings highlight the importance of establishing a health observation system for experimental rats to ensure the consistency and reliability of the study results. The main factors contributing to the death rate were identified as biological diversity, stress, and exhaustion mechanisms, highlighting the significance of proactive steps in preserving the health and reliability of the experimental animals.

Acknowledgment
The researchers would like to extend their genuine pleasure and a deep sense of thanks and appreciation to all who worked, participated, and shared their expertise in making this study possible. The authors thank the adviser, panel examiners, research coordinator, and statistician for their great contribution, for imparting optimistic views, constructive comments, excellent suggestions, and recommendations for improving this study. To their family and friends for providing moral support

Ethical considerations
The project was approved by the Scientific Research and Ethical Committee of the College of Dentistry, University of Baghdad (Ref. 623. in 2022).

Conflict of interest
The authors declare no conflicts of interest.

Funding
This research did not receive any financial support.

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