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Reference physiological and biochemical values of adult albino rats

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Abstract

The physiological and health status of control animals used in studies and experiments worldwide can vary. Because of this variation, it is critical to establish acceptable ranges of the hematology, hormonal, and biochemical, along with essential physiological parameters for control groups, better to understand the detrimental and non-adverse impacts of test substances. Because the reference values and intervals are crucial tools in scientific papers. and the lack of accurate values for animals explicitly found in Mosul, as well as the difficulties the researcher encounters when comparing the values of control animals with the international values and even the values placed, for instance, in a blood cell counting device, served as the impetus for the research project. Fifty mature male rats were raised in the animal house from one month of birth to maturity under norms that included adequate lighting, food, water, and ventilation. Before taking any samples, the animals were restrained using buckles made of stainless steel to keep them under control. The research team decided to obtain samples from as many measures as possible, incorporating values and intervals of vital signs and hematological, hormonal, and biochemical variables. Most values were as expected compared to other rodent species from different countries, and books talk about rodent parameters, which are very diverse in vital, hormonal, and biochemical parameters. However, the blood picture needed to be more distinct.

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Introduction

The order Rodentia is the biggest group of mammals, consisting of more than 440 genera and over 2000 distinct species. The order consists of five primary families: Muridae, Sciuridae, Echimyidae, Heteromyidae, and Dipodidae. The Muridae account for approximately 66% of all rodent taxa (1). Rodents exhibit remarkable intelligence according to their physiological characteristics (2). Many rodent species are characterized by their small size, short gestation periods, early sexual maturity, secretive behavior, nocturnal activity, adaptability, and highly developed senses of touch, taste, and smell (3). Researchers noticed that sex and age substantially impacted hematological and biochemical markers in India's Wistar (Han) rats (4). Rodents, such as mice and rats, are the preferred preclinical

animal models in pharmaceutical research due to their strong resemblance to humans and other mammals. They are also more suitable for comparison than larger and longer-lived species (5). Most human diseases can be replicated in these rats by altering corresponding genes or physicochemical stimuli. Additionally, rodents are crucial for conducting toxicity and safety studies while developing novel medications (6). Therefore, any variations in hematological, biochemical, or vital signs deemed significant are documented in reference studies. Based on prior research, the lymphocyte counts in rats typically vary according to age. Generally, as rats age, the number of lymphocytes falls while the percentage of neutrophils, eosinophils, and monocytes increases (7). However, a separate study indicates that age does not substantially influence the quantity of monocytes (8). Although the basophil numbers in rats fall

within the usual range for most species, they make up less than 1% of blood leukocytes (9). Both factors, whether they positively affect biology, could potentially result in discovering new biomarkers or therapeutic targets for different diseases (10). Although the normal range for RBC count in rats is not mentioned, further research is needed to establish reference values (11). Understanding the typical range of hematocrit is essential for differentiating between normal and pathological conditions. In awake hamsters, reducing HCT levels led to a rise in mean arterial blood pressure and a consistent increase in cardiac output (12). Rats within the normal range of mean corpuscular volume (MCV) exhibit distinct physiological and behavioural responses compared to other strains of rats (13). The behavioural patterns commonly observed in rats within the MCH group are affected by various factors, including anxiety levels, which are internally related to measures of inquisitive and seeking activities (14). The abstracts presented do not directly specify the normal range of Red Blood Cell Distribution Width (RDW) in rats. However, they do include information about the importance and association of RDW with various diseases and disorders. RDW is a metric that quantifies the variability in size and volume of red blood cells (RBCs) and is correlated with anisocytosis. Elevated RDW levels are connected to unfavourable cardiorenal outcomes (15). RDW-SD, a metric for erythrocyte mean corpuscular volume (MCV) variability, exhibits variation among rat species. A study conducted by Chan et al. revealed notable variations in reported baseline RDW-SD values among different rat strains and detection methods (16). These findings indicate that variations in RDW-SD outcomes can be attributed to factors such as species and detection methods. The study suggests utilizing corresponding negative control models as a fundamental RDW-SD reference range for each investigation assessing RDW-SD levels in rats (17). Platelet indices, such as platelet count, plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW), undergo significant changes in rats with the duration of diabetes. Additionally, platelet count is a crucial test used in drug development studies to evaluate the well-being of rats (18). Platelet-large cell ratio (P-LCR) is a regularly computed measure in hemogram examinations. The literature does not provide a particular reference to the typical range of P-LCR in rats (19). The abstracts do not expressly state the specific range of triglyceride levels considered normal in rats. Nevertheless, the abstracts indicate that rats' triglyceride levels were within the range of normal values in multiple trials (20). In addition, the rate of cholesterol breakdown in rats decreases as they age, resulting in prolonged cholesterol retention in the body (21). The abstracts do not specify the typical range of blood urea values in rats (22). Investigating the disparities in ALT U/L metabolism between rats and humans holds promise for comprehending sickness and wellbeing (23). Understanding the variations in respiratory rate

is valuable for studying disease and health. Knowing the normal values is necessary to differentiate between physical activity, exercise, disease, and healthy conditions. This knowledge is crucial for preventing metabolic diseases and promoting longevity (23,24).

Finally, the study aims to integrate all physiological, hematological, hormonal, and biochemical indicators affected by various causes in normal conditions. Consequently, we must remain current with these principles, especially in each country.

Materials and methods

Rats' management

This study utilized a sample of 50 male adult Wistar rats weighted at an average of 223.310-243.169 g raised in the animal facilities at Mosul University's Veterinary College, Iraq. Throughout the experiment, conducted in October, November, and December of 2023, the rats were raised in a controlled environment with a consistent daily cycle of twelve hours of light and darkness at a temperature of 25°C±3 after two acclimations ten cages made of plastic with a lid made of steel. Before collecting any parameters, the rats were upraised from one month to mature (3-3.5 months) and provided unrestricted access to fresh water and a standard regular diet. The rats were handled and managed following the regulations of the Ethics Committee at the College of Veterinary Medicine, University of Mosul. The study was approved under the reference number UM.VET.2023.080. Each enclosure has a total of 50 rats.

Limitation procedures

Handling and restraining exotic animals can be difficult because of their strong jaws and the requirement for pharmacological immobilization and sedation. In a recent study, sedative medications were omitted because they tend to affect regular readings. Consequently, physical constraint was employed as an alternative. We employed stainless steel buckles to manage and confine the creatures.

Vital signs

The individuals at the resting stage were gathered using a specialized instrument for veterinary purposes called the Veterinary Monitor Operation Manual (25), which is included in table 1.

Hematological parameters

The initial portion of the blood samples was collected from the animals' eyes using an ocular puncture technique into a capillary tube containing heparin and subsequently transferred into a 0.5 mL tube containing EDTA. Following persistent agitation, the blood tube is inserted into the Veterinary auto-hematology analyzer (26-28). Table 2 display the parameters for full blood counts (CBC).

Parameters	Abbreviation	Unit
Noninvasive blood pressure	NIBP	mmHg
Systolic pressure	SBP	mmHg
Diastolic pressure	DBP	mmHg
Pulse pressure	PP	mmHg
Oxygen saturation	SpO2	%
Body temperature (rectal)	Temp	°C
Respiratory rate	RR	BrPM
Heart rate	HR	bpm

Table 1: The table displays vital signs using a Veterinary Monitor

 Table 2: The table displays complete blood counting (CBC)

 using Automatic Hematology Analyzer

Full English name	Abbreviation	units
White blood cell count	WBC	10 ⁹ /L
Neutrophils count	Neu	10 ⁹ /L
Lymphocyte count	Lym	10 ⁹ /L
Monocyte count	Mon	10 ⁹ /L
Eosinophils count	Eos	$10^{9}/L$
Basophils count	Bas	$10^{9}/L$
Neutrophils cell percentage	Neu%	%
Lymphocyte percentage	Lym%	%
Monocyte cell percentage	Mon%	%
Eosinophils cell percentage	Eos%	%
Basophils cell percentage	Bas%	%
Red blood cell count	RBC	$10^{12}/L$
Red cell distribution width CD	RDW-CD	%
Red cell distribution width SD	RDW-SD	fL
Hemoglobin count	HGB	g/L
Hematocrit	HCT	%
Mean corpuscular volume	MCV	fL
Mean corpuscular hemoglobin	MCH	pg
Mean corpuscular hemoglobin	MCHC	g/L
Platelet count	PLT	10 ⁹ /L
Mean platelet volume	MPV	fL
Platelet distribution width	PDW	fL
platelet crit	PCT	%
Platelet-large cell ratio	P-LCR	%
Platelet-large cell count	P-LCC	10 ⁹ /uL

Hormonal parameters

To determine hormone levels, the second blood sample was collected in a gel tube, subjected to centrifugation to obtain pure serum, and subsequently stored at a temperature of -26°C until analysis using a Microplate Reader (29,30), the following kit as shown in table 3 (Shanghai Ideal Medical Technology CO; LTD, Chani).

Biochemical parameters

The biochemistry analyzer measures biochemical parameters using serum sample. Its components are listed in table 4.

Table 3: The table displays hormonal assay using a Microplate Reader

Hormones	CAT No.	Unit
Rat LEP	YBS-21877	ng/mL
Rat TSH	YBS-22345	Mu/L
Rat INS	YBS-20822	µIU/mL
Rat T4	YBS-28766	ng/mL
Rat Cortisol	YBS-22098	ng/mL

 Table 4: The table displays biochemical parameters using

 Automatic Biochemistry Analyzer

Parameters	Abbreviation	Unit
Glucose	Glu	mg/dL
Total Cholesterol	TC	mg/dL
Triglyceride	TG	mg/dL
High-density lipoprotein	HDL	mg/dL
Blood Urea Nitrogen	BUN	mg/dL
Serum Creatinine	sCR	mg/dL
Alanine transaminase	ALT	U/L
Aspartate transferase	AST	U/L
Total protein	TP	g/dL

Statistically analyze

Analyzed data was subjected to descriptive and inferential statistical analysis using JMP Pro16.1 software (2021 SAS Institute Inc., North Carolina, USA). The descriptive statistics encompassed the measures of central tendency, such as the mean median, and the measures of dispersion, such as the range and quantiles. The impact of the treatments on the animals was assessed by analyzing variance (ANOVA) in the data. The treatment groups, chosen from a population with a normal distribution, were compared using the student's t-test and the Duncan multiple range test. The results were considered statistically significant at a significance level of P<0.05 (31).

Results

After assessing various health indicators, all rats appeared to be in good health (Table 5). These indicators encompass oxygen saturation, rectal temperature, heart rate, respiratory rate, and blood pressure (systolic, diastolic, and even plus) as a range (11.137-13.752, 8.012-9.547, 51.644-66.255, 81.882-88.197, 33.532-34.727, 60.489-66.990, 71.130 \pm 75.549) respectively. All of these parameters were taken in the morning and obtained by a patient monitor specialized in veterinary medicine. The average rat weight was 233.24 g, with a 223.310-243.169 g range.

The complete blood picture of adult male rats involves leukocytic count (14.217-16.675) and differential leukocyte count (DLC) (1.959-2.661, 9.305-11.360, 2.683-3.128, 0.078-0.096, 0.015-0.020) and their percentage (13.879-18.116, 63.814-69.525, 18.431-20.292, 0.538-0.681, 0.1080.113), whereas the range of counting erythrocytes (11.434-13.010) and their distribution width (13.739-14.164 and 33.064-34.067) respectively. The hemoglobin concentration in grams per 100 milliliters ranges from 181.492 to 207.147, while the hematocrit percentage ranges from 67.283 to 78.320. On a median, the anemic index comprises MCV (fl), MCH (pg), and MCHC (g/100 l) (58.258-60.037, 15.616-16.079, 265.53-272.662). The mean platelet count (597.942-688.557) and percentage mean platelet crit are 0.445-0.519, respectively, along with the mean platelet volume (7.474-7.281) and platelet distribution width (10.367-10.792). Yet, the mean platelet-large cell ratio (4.824-5.751) and platelet-large cell count (28.621-34.138) are all parameters illustrated in table 6.

Table 7 displays the normal range of hormones for adult male rats, which includes leptin, TSH, insulin, T4, and cortisol (2.935-3.142, 3.097-3.445, 25.993-27.549, 81.207-87.307, and 45.979-52.888). Table 8 reveals the average value of biochemical analysis for control rats, encompassing glucose (117.39-104.56), total cholesterol (79.21-73.98), triglycerides (71.45-61.50), high-density lipoprotein cholesterol (37.796-33.923), blood urea nitrogen (43.303-39.816), serum creatinine (0.546-0.513), Alanine transaminase (64.562-60.117), aspartate transferase (235.20-220.173), and total protein (6.046-5.729).

Table 5: The table displays vital signs in male adult rats

Parameters	Mean±SE	Mean±DV	Ranges
Systolic Pressure	12.445±0.624	12.445±2.793	11.137-13.752
Diastolic Pressure	8.78 ± 0.366	8.78±1.683	8.012-9.547
Pulse pressure (PP)	58.95±3.490	58.95±15.608	51.644-66.255
Oxygen saturation (SpO ₂)	85.04±1.571	85.04±11.109	81.882-88.197
Body temperature (rectal Temp)	34.13±0.297	34.13±2.101	33.532-34.727
Respiratory rate (RR)	63.74±1.617	63.74±11.438	60.489-66.990
Heart rate (HR)	73.34±1.099	73.34±7.773	71.130±75.549

Table 6 displays complete blood counting (CBC) in male adult rats

Parameters	Mean±Se	Mean±DV	Range
White blood cell counts (WBC)	15.446 ± 0.611	15.446±4.325	14.217-16.675
Neutrophils count (Neu)	$2.310{\pm}0.174$	2.310±1.233	1.959-2.661
Lymphocyte count (Lym)	10.333 ± 0.511	10.333±3.614	9.305-11.360
Monocyte count (Mon)	2.906 ± 0.110	2.906 ± 0.781	2.683-3.128
Eosinophils count (Eos)	$0.087{\pm}0.004$	0.087 ± 0.032	0.078-0.096
Basophils count (Bas)	$0.017 {\pm} 0.001$	0.017 ± 0.008	0.015-0.020
Neutrophils cell percentage (Neu%)	15.998 ± 1.054	15.998±7.453	13.879-18.116
Lymphocyte percentage (Lym%)	66.67±1.420	66.67±10.046	63.814-69.525
Monocyte cell percentage (Mon%)	19.362 ± 0.462	19.362±3.273	18.431-20.292
Eosinophils cell percentage (Eos%)	0.61 ± 0.035	0.61±0.250	0.538-0.681
Basophils cell percentage (Bas%)	$0.12{\pm}0.005$	0.12 ± 0.040	0.108-0.131
Red blood cell count (RBC)	12.222±0.392	12.222±2.772	11.434-13.010
Red cell distribution width CD (RDW-CD)	13.952 ± 0.105	13.952±0.749	13.739-14.164
Red cell distribution width SD (RDW-SD)	33.566±0.249	33.566±1.765	33.064-34.067
Hemoglobin count (HGB)	194.32±6.383	194.32±45.136	181.492-207.147
Hematocrit (HCT)	72.802 ± 2.745	72.802±19.416	67.283-78.320
Mean corpuscular volume (MCV)	59.148±0.442	59.148±3.130	58.258-60.037
Mean corpuscular hemoglobin (MCH)	15.848 ± 0.115	15.858 ± 0.815	15.616-16.079
Mean corpuscular hemoglobin concentration (MCHC)	269.1±1.772	269.1±12.534	265.53-272.662
Platelet count (PLT)	643.4±22.620	643.4±159.950	597.942-688.857
Mean platelet volume (MPV)	$7.578 {\pm} 0.051$	7.578±0.363	7.474-7.681
Platelet distribution width (PDW)	10.58 ± 0.105	10.58 ± 0.746	10.367-10.792
plateletcrit (PCT)	$0.482{\pm}0.018$	0.482 ± 0.129	0.445-0.519
Platelet-large cell ratio (P-LCR)	5.288 ± 0.230	5.288±1.632	4.824-5.751
Platelet-large cell count (P-LCC)	31.38±1.372	31.38±9.707	28.621-34.138

Table 7: Tl	he table c	displays	hormonal	assay ir	ı male adı	ult rats

Hormones	Mean±SE	Mean±DV	Ranges
Leptin (LEP)	3.039±0.051	3.039 ± 0.327	2.935-3.142
Thyroid Stimulating Hormone (TSH)	3.271±0.086	3.271±0.551	3.097-3.445
Insulin (INS)	26.771±0.385	26.771±2.465	25.993-27.549
Thyroxine (T4)	84.257±1.509	84.257±9.662	81.207-87.307
Cortisol	49.433±1.709	49.433±10.944	45.979-52.888

Table 8: The table displays biochemical parameters in male adult rats

Parameters	Mean±SE	Mean±DV	Ranges
Glucose (Glu)	110.98±3.192	110.98±22.574	117.39-104.56
Total Cholesterol (TC)	76.6±1.302	76.6±9.209	79.21-73.98
Triglyceride (TG)	66.48±2.476	66.48±17.514	71.45-61.50
High-density lipoprotein cholesterol (HDL-C)	35.86 ± 0.963	35.86±6.812	37.796-33.923
Blood Urea Nitrogen (BUN)	41.56±0.867	41.56±6.136	43.303-39.816
Serum Creatinine (sCR)	$0.53{\pm}0.008$	0.53 ± 0.057	0.546-0.513
Alanine transaminase (concentration) (ALT)	62.34±1.106	62.34 ± 7.820	64.562-60.117
Aspartate transferase (concentration) (AST)	227.688±3.739	227.688±26.441	235.20-220.173
Total protein (TP)	$5.888 {\pm} 0.078$	5.888 ± 0.556	6.046-5.729

Discussion

This discussion shows that the normal physiological, hematological, hormonal, and biochemical levels of people with albinism are significant compared with control groups from other research from different countries. These differences between values may come from environmental, nutritional, genetic, geographical, managemental, and other factors that the researchers consider.

Starting with the vital signs, Kyoto City, Japan recorded a slight difference in systolic and diastolic pressure but the pulse pressure values did not fall within the current reference range which was 51-66, and their result of 27.40 (32), while the albino rats in Zagazig, Egypt showed no such difference in both higher and lower pressure (33), on the contrary in research established in Abha, Sudia Arabia noted a clear difference in systolic pressure 80.3 and diastolic was 49.3 (34), besides in 3 remote countries documented a diverse pulse pressure from this study like Nigeria was incredibly greater than this research otherwise in Berlin, Germany, and Jamaica was a little lesser than the current study (35-37) Vital signs are regarded as the first line for determining healthy status; thus, any variation within a group or even geographical area is recorded as a pathological cause; however, when the variables are in different countries, ambient temperature, ration type, ventilation, humidity, and even animal manipulation can cause deviations from the norms of the trail results (38,39).

Research talks about a differential comparison of the oxygen saturation level in rats between 2 land levels starting in La Paz, Bolivia (3600 m above the sea level), documented 71% SPO2 at 90 mmHg and 95% SPO2 at 160 mmHg. In contrast, the sea level in Quebec City, Canada, was 89%

SPO2 at 160 mmHg and 72% SPO2 at 90 mmHg. All these values differed from this experiment value, which showed approximately 81-88% SPO2. These distinctions could clarify the disparity in the country distribution spotted in wild rats and mice (40).

A study done in the UAE in 2005 and in Bosnia and Herzegovina revealed the body temperature was different from the Mosul record by about 2.3°C (41), while the heart rate in Banaras, India, was enormously altered from ours; nevertheless, the respiratory was close to the same rate (42). According to a famed international website about rat guide of physiological and other parameters, their temperature was 37.7 C, above the manuscript record of about 33-34 C, blood pressure was the same, and the HR and RR were exactly dissimilar from the Mosul rat's heart and respiratory rates (43). Indeed, the surrounding temperature and other circumstances, such as circadian variations, have all been attributed to set point temperature shifts of rats or any other animal considered a homeotherm; these recent papers aim to address this issue (44).

In research found in Egypt, the hematological significances of the control group of RBC count, Hb, HCT, MCV, MCH, MCHC, and WBC count exhibited differences with the experiment values, especially in HCT and MCHC (45); however, in Ibadan, Nigeria the values of MCV and MCH is higher than this study but RBC count, Hb, HCT, and MCHC were exposed the lower from the Mosul albinos (46). In Nsukka, Nigeria, the total WBC count with each type did not present much difference (47), but in Uttar Pradesh, India, the DLC indicated little deviation from the study rates (48). The platelet indices in Calabar, Nigeria, were almost equal to this experiment's (49). The book covers rodent normal ranges, the RBC count, and PCV. The trial reports did not

fall in the standard range, and all forms of WBCs were in the same range, excluding monocytes, which were lower than our evidence, although thrombocytes were in virtually the same range (50). Since the blood criteria are regarded as a good indicator of animal health, variations in the feed component's type, amount, and state significantly affect the total blood picture (51).

During this study, the rats were not exposed to any stress agent, and the cortisol rates did not match with the control group of another study in Shiraz, Iran (52), while in Mumbai, India, they did not fall within the acceptable range of the current study (53). Likewise, in rodents, both hormones cortisol and corticosterone might change in ratio depending on the demands and physiological accordance to regulating metabolites (54).

The insulin evaluation in 2 studies in Cairo, Egypt, was lower than the Mosul study (55), and the 2nd one was higher than this trial (56). A study in Kurdistan, Iraq, measured the leptin level and was lower than the study evaluation (57); however, in Ankara, Turkey, it was the same (58), while the Medical Faculty of the LMU Munich evaluated some metabolic hormones using various methods and found a leptin value in the serum by ELISA close to and within the same range as current study (59). In these investigations in Harlan Labs, Indianapolis, Faculty of Georgia, male Sprague-Dawley rats (SD rats) were employed in the experiment to obtain results regarding serum insulin and leptin levels in line with the trial data (60). In general, leptin monitors calorie intake and spending; thus, its average level is determined by requests for energy, and variations in the common value do not differ at the physiological level. Also, the noted impacts of leptin on the production of thyroid hormone are contested, with research focusing primarily on energy imbalance (61). In 2 trials at Menoufiya University, Egypt, and Jorden, the hormone T4 of the control rats was lower than ours, but TSH was somehow like the manuscript levels (62,63).

In Giza, Egypt, a group of researchers calculated biochemical standards of ALT, AST, total protein, urea, creatinine, and glucose were roughly similar except AST was lower (64), while in Colombo, Sri Lanka the total cholesterol, total triglycerol were lower. However, the ALT was higher (65). Twenty-one days later, Wistar rats in Buea, Cameroon, noted a lipid picture of total cholesterol, triglycerol, and HDLC much higher than the experiment picture (66). In line with vet books about rats, the biochemical ranges of total protein, glucose, creatinine, TG, and cholesterol were roughly the same range; nonetheless, the urea nitrogen data was lower than ours even though the liver enzymes range was higher than the present experiment (67,68). Variations in various biochemical tests may solely be ascribed to individual variances, potentially linked to hereditary factors stemming from inbreeding and outbreeding (65). Others affirm that the solitary housing of rats induces alterations in specific biomarker markers (68).

Conclusion

The intervals are computed based on data gathered from a collection of healthy animals who have undergone statistical methods and are in comparable circumstances. Reference intervals are the primary instrument used in healthcare decision-making. They serve as the foundation for laboratory investigations and are crucial to the interpretation of findings, enhancing the accuracy of the results. After a months-long study, standards and levels were collected for 4 basic parameters covered by most research in Iraqi veterinary colleges for the sake of one goal: accuracy and scientific honesty in examining these standards. To prove this, these results were compared with those of other countries worldwide and from biochemistry and physiology books, indicating a noteworthy variance in vital, hormonal, and biochemical values and a slight variation in blood pictures.

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Conflict of interest

The authors declare that they have no conflict of interest

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القيم الفسلجية والكيموحيوية المرجعية في الجرذان البيضاء البالغة

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فرع الفسلجة والكيمياء الحياتية والأدوية، كلية الطب البيطري، جامعة الموصل، الموصل، تقسم التقنيات البصرية، تقسم تقنيات التخدير، جامعة النور، برطله، العراق

الخلاصة

يمكن أن تختلف الحالة الفسيولوجية والصحية للحيوانات السيطرة المستخدمة في الدر اسات والتجارب في العالم، وبسبب هذا الاختلاف، من الضروري تحديد نطاقات مقبولة لأمراض الدم والهرمونات و الكيمياء الحيوية، إلى جانب المعلمات الفسيو لوجية الأساسية لمجمو عات. السيطرة من اجل لحصول على أدراك أفضل للتأثيرات الضارة وغير الضارة لمواد الاختبار . لأن القيم و الفتر ات المرجعية تعتبر من الأدو ات الحاسمة في الأوراق العلمية وعدم وجود قيم دقيقة للحيوانات الموجودة تحديدا في الموصل، فضلا عن الصعوبات التي يواجهها الباحث عند مقارنة قيم حيوانات السيطرة مع القيم الدولية وحتى القيم الموضوعة، على سبيل المثال، في جهاز عد خلايا الدم، كان هذا بمثابة قوة دافعة لمشروع البحث. تمت تربية خمسين فأرا ذكرا ناضجا في بيت الحيوانات منذ شهر واحد من الولادة وحتى النضج وفقا لقواعد تشمل الإضاءة الكافية والغذاء والماء والتهوية. قبل أخذ أى عينات، تم تقييد الحيوانات باستخدام مشابك مصنوعة من الفولاذ المقاوم للصدأ لإبقائها تحت السيطرة. قرر فريق البحث الحصول على عينات من أكبر عدد ممكن من المقاييس، بما في ذلك قيم وفترات العلامات الحيوية والمتغيرات الدموية والهرمونية والكيميائية الحيوية. كانت معظم القيم كما هو متوقع مقاربة بأنواع القوارض الأخرى من مختلف البلدان، وتتحدث الكتب عن مؤشرات القوارض التي تختلف بشكل كبير في المؤشرات الحيوية والهرمونية والكيميائية الحيوية ولكن صورة الكاملة لعد خلايا الدم لم تكن مختلفة تماما