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Original article

# A model for estimation of testicular volume in different age male rats – suitability of various testicular volume calculation formulas for living animals

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## Abstract

A change in testicular volume can indicate decreased male fertility and is useful for comparing spermatogenesis before and after experiments in animals, or before and after treatment of especially valuable domestic breeding animals with pharmaceuticals affecting spermatogenesis, such as antitumor drugs. Various formulae can be used to calculate the volume of living males' testes. This study examined the suitability of four testicular volume calculation formulas for male Wistar rats of different ages (2-months, 1-year, and 2-years-old) and the changes in testicular volume during 90 days of observation. In 2-months-old rats, the solid ellipsoid triaxial body calculation formula and the prolate ellipsoid formula were most suitable for the calculation of testes' volume, and the volume of both testes increased very significantly during the 90 days of observation. In 1-year-old rats, the solid ellipsoid triaxial body calculation formula was optimal, and the volume of both testes increased insignificantly. For 2-years-old rats, the solid ellipsoid triaxial body calculation and prolate ellipsoid formulas or their average was recommended, and the volume of both testes decreased slightly during 90 days of observation.

**Keywords:** calliper, rat, testis, ultrasound, volume



## Introduction

Assessing testicular volume provides valuable information about spermatogenesis and male fertility. Testicular volume reflects spermatogenesis, as about 70% to 80% of testicular mass is generated by the seminiferous tubules. Consequently, testicular volume correlates significantly with various semen parameters (total sperm count, concentration, motility, morphology, percentage of live sperm) and hormone (follicle stimulating, luteinizing hormone and testosterone) levels (Takahara et al. 1987, Goede et al. 2011, Khan et al. 2015).

Changes in testicular size can result from experiments, drug treatments (especially for oncological diseases), and natural aging processes, making accurate measurement crucial. Therefore, various experiments on live animals, their testes and genital ducts, and the testing of various substances and drugs can negatively affect spermatogenesis and testicular size. The most accurate method for determination of testis size and volume is manual measurement with calliper and water displacement after autopsy or castration (Gouletsou et al. 2008, Mbaeri et al. 2013a). Also, some authors suggest that the weight of the testis is equal to its volume since testis density is nearly 1.0 (~1.03-1.04). This method can be used for male animals euthanized after experiment but is not suitable for volume determination before or during experiment on living animals (Borges et al. 2023, Sadeghinezhad et al. 2023).

Sometimes it is necessary to measure testis size and volume before, during and after an experiment or before and after treatment with various drugs to understand how these parameters change in the individual organism. However, preserving of fertility during such procedures is challenging, especially in living animals.

Various methods, including testis measurement by orchidometer, calliper and ultrasound, and calculation formulas, are employed for this purpose (Taskinen et al. 1996, Karaman et al. 2005, Carlsen et al. 2006, Cha et al. 2006, Sakamoto et al. 2008, Goede et al. 2011, Mbaeri et al. 2013b). Human and animal testis volume can be calculated using various formulas such as the prolate ellipsoid formula, the prolate spheroid formula, the Lambert's empiric formula and the solid ellipsoid triaxial body calculation formula (Sakamoto et al. 2008, Stegani et al. 2008, Hsieh et al. 2009, Mbaeri et al. 2013a, Khan et al. 2015, Love et al. 2015, Brêtas et al. 2016, Wei et al. 2024). We did not find data in the literature on which formulas are best for calculation of the testicular volume in individuals of different ages. Therefore, in this investigation, we aimed to determine the most suitable testicular volume calculation formula in different age male rats and to assess changes in testicular volume during 90 days of observation.

## Materials and Methods

### Ethical approval

This study was conducted in accordance with the relevant laws and institutional guidelines, and permission from the State Food and Veterinary Service (No. B1-135, 16.03.2017) was obtained for the use of animals in this investigation.

### Animals

Eighteen Wistar male rats were used in this study. The animals were purchased from the Animal Facility of the Veterinary Academy at the Lithuanian University of Health Sciences. The investigation was carried out at the Animal Research Center at the Lithuanian University of Health Sciences. The rats were divided into three age groups: 2-months-old, 1-year-old and 2-years-old, with six rats in each group.

### Study design

Before the observation period, each rat was individually marked with different colours of acrylic dyes and weighed using a KERN 440-21N balance. Ultrasound examination of the right and left testis size (length, width and thickness) was performed on each living rat.

Subsequently, the male rats were housed under standard conditions in standard colony cages, fed and watered with tap water *ad libitum* for 90 days. At the end of the observation period, the animals were euthanized using CO<sub>2</sub> cages with minimal distress. The bodies of the rats were weighed, and ultrasound measurements of the right and left testes were done. Fresh testes were obtained during the autopsy 20 minutes *post mortem*. The left testes were marked with a cotton thread. The testes were rinsed in streaming tap water, dried with blotting paper, and the remnants of epididymis, adipose tissue and ligaments were removed. The length, width and thickness of each testis were measured using a sliding calliper. The volume of each testis was determined by water displacement using the Archimedes principle.

Blind ultrasound measurements of all testes before and after the observation period were performed by the same examiner.

### Data and statistical analysis

All obtained data were tabled in the Microsoft Excel 2003 program. The volume of each testis was calculated using four formulas: (1) the solid ellipsoid triaxial body calculation formula:  $\text{volume} = 4 \times \pi \times \text{half the thickness} \times \text{half the width} \times \text{half the length} / 3$ ; (2) the prolate ellipsoid formula:  $\text{volume} = \text{length} \times$

Table 1. Weight of 2-months, 1-year, and 2-years-old male rats before and after observation.

| Age               | Body weight, g (mean±SD) |                   |                           |
|-------------------|--------------------------|-------------------|---------------------------|
|                   | Before observation       | After observation | Difference (%)            |
| 2-months-old      | 258.25±18.97             | 367.75±17.35      | 109.5±6.03 a1<br>(42.61%) |
| 1-year-old        | 369.6±20.72              | 390.6±24.36       | 21.0±5.79 a2<br>(5.34%)   |
| 2-years-old       | 383.17±29.83             | 388.33±30.24      | 5.17±2.71 a3<br>(1.33%)   |
| a1:a2:a3; p<0.001 |                          |                   |                           |

Table 2. Size of the 2-months, 1-year, and 2-years-old male rats testes measured before and after observation.

| Age                 | Size, cm (mean±SD)                        |             |             |                                     |             |             |                                      |             |            | p values      |
|---------------------|-------------------------------------------|-------------|-------------|-------------------------------------|-------------|-------------|--------------------------------------|-------------|------------|---------------|
|                     | Sliding calliper after observation period |             |             | Ultrasound after observation period |             |             | Ultrasound before observation period |             |            |               |
|                     | Length                                    | Width       | Thickness   | Length                              | Width       | Thickness   | Length                               | Width       | Thickness  |               |
| Mean of both testes |                                           |             |             |                                     |             |             |                                      |             |            |               |
| 2-months-old        | 1.88±0.12a1                               | 1.05±0.09b1 | 0.93±0.09c1 | 1.91±0.11a2                         | 1.04±0.12b2 | 0.94±0.09c2 | 1.59±0.11a3                          | 0.84±0.12b3 | 0.73±0.1c3 | a1:a3, a2:a3, |
| 1-year-old          | 2.03±0.14                                 | 1.09±0.11   | 0.99±0.17   | 1.99±0.16                           | 1.06±0.1    | 0.95±0.12   | 1.95±0.18                            | 1.04±0.14   | 0.93±0.12  | b1:b3, b2:b3, |
| 2-years-old         | 2.1±0.15                                  | 1.04±0.09   | 0.97±0.1    | 2.08±0.18                           | 1.04±0.12   | 0.94±0.14   | 2.09±0.22                            | 1.06±0.12   | 0.97±0.14  | c1:c3, c2:c3; |
| Left testes         |                                           |             |             |                                     |             |             |                                      |             |            |               |
| 2-months-old        | 1.85±0.1a1                                | 1.08±0.1b1  | 0.98±0.05c1 | 1.9±0.14a2                          | 1.08±0.15b2 | 1.0±0.08c2  | 1.6±0.08a3                           | 0.88±0.1b3  | 0.78±0.1c3 | a1:a3, a2:a3, |
| 1-year-old          | 2.04±0.09                                 | 1.12±0.05   | 1.0±0.17    | 2.0±0.16                            | 1.1±0.1     | 0.98±0.12   | 1.98±0.22                            | 0.96±0.16   | 0.96±0.16  | b1:b3, b2:b3, |
| 2-years-old         | 2.18±0.12                                 | 1.07±0.1    | 0.97±0.1    | 2.17±0.15                           | 1.08±0.12   | 0.95±0.16   | 2.18±0.24                            | 1.1±0.13    | 0.98±0.1   | c1:c3, c2:c3; |
| Right testes        |                                           |             |             |                                     |             |             |                                      |             |            |               |
| 2-months-old        | 1.9±0.14a1                                | 1.03±0.1b1  | 0.88±0.1c1  | 1.93±0.1a2                          | 1.0±0.08b2  | 0.88±0.05c2 | 1.58±0.15a3                          | 0.8±0.14b3  | 0.68±0.1c3 | a1:a3, a2:a3, |
| 1-year-old          | 2.02±0.18                                 | 1.06±0.15   | 0.98±0.19   | 1.98±0.18                           | 1.02±0.08   | 0.92±0.13   | 1.92±0.15                            | 1.0±0.14    | 0.9±0.07   | b1:b3, b2:b3, |
| 2-years-old         | 2.02±0.13                                 | 1.02±0.08   | 0.97±0.1    | 1.98±0.16                           | 1.0±0.13    | 0.93±0.15   | 2.0±0.18                             | 1.02±0.12   | 0.95±0.19  | c1:c3, c2:c3; |

width× thickness× 0.52; (3) the prolate spheroid formula: volume=length× width<sup>2</sup>× 0.52 and (4) the Lambert's empiric formula: volume=length× width× thickness× 0.71.

The Statistica program (Statistica Version 5, StatSoft inc.) Basic statistics was used for the calculation of the mean and standard deviation of the data. The one-way ANOVA Fisher LSD Post-hoc test was used for statistical comparison of testis parameters in age groups (p values). Data were presented as mean ± standard deviation (SD), and p<0.05 was taken as statistically significant.

## Results

### Animal and testes data

During this observation period, the body weight increased in all male rat age groups. The weight increase in young (2-months-old) male rats was significantly greater (42.61%) than in 1-year-old rats and 2-years-old rats (5.34% and 1.33% respectively; p<0.01), as the young animals were still growing during this observation period (Table 1).

Both testes of each male rat were measured using a sliding calliper after the observation period and ultra-

sound examinations were made before and after the observation period (Table 2). Comparison of testes' size measurements by ultrasound and sliding calliper showed that the mean length, width and thickness of the left and right testes, did not differ significantly in all age groups of male rats (p>0.05). These results indicated that the testicular ultrasound examination results can be equated with the actual (measured by sliding calliper) size of the testes in all age groups of rats.

Ultrasound examination of the testes before and after the observation period showed that the size of 2-months-old rats' left and right testes significantly increased during the 90 days of observation (p<0.05), which is related to the growth of the young rats. During this observation period, the length, width and thickness of testes changed slightly in the 1-year-old and 2-years-old rats' groups (p>0.05, Table 2).

### Study of suitability of testicular volume calculation formulas

To determine the best formula for calculation of testicular volume, the results of four calculation formulas using the testicular size measured with a sliding calliper were compared with water displacement volume

Table 3. Comparison of water displacement volume and volume calculated using three formulas for the 2-months, 1-year, and 2-years-old male rat testes measured by sliding calliper after observation.

| Age                                       | Volume, cm <sup>3</sup> (mean±SD) |                                                   |                           |                          |                           | p values       |
|-------------------------------------------|-----------------------------------|---------------------------------------------------|---------------------------|--------------------------|---------------------------|----------------|
|                                           | Water displacement                | Solid ellipsoid triaxial body calculation formula | Prolate ellipsoid formula | Prolate spheroid formula | Lambert's empiric formula |                |
| Mean of both testes                       |                                   |                                                   |                           |                          |                           |                |
| 2-months-old                              | 0.99±0.11 a1                      | 0.95±0.11                                         | 0.94±0.11                 | 1.07±0.14                | 1.29±0.15 a2              | a1:a2; p<0.001 |
| Difference with water displacement volume | -                                 | -0.04±0.03 (-3.78%)                               | -0.04±0.03 (-4.4%)        | 0.09±0.15 (9.52%)        | 0.3±0.04 (30.55%)         |                |
| 1-year-old                                | 1.12±0.2 b1                       | 1.16±0.32                                         | 1.16±0.32                 | 1.28±0.3                 | 1.58±0.44 b2              | b1:b2; p<0.05  |
| Difference with water displacement volume | -                                 | 0.04±0.17 (2.87%)                                 | 0.04±0.16 (2.14%)         | 0.16±0.15 (13.26%)       | 0.46±0.27 (39.47%)        |                |
| 2-years-old                               | 1.13±0.2 c1                       | 1.12±0.25                                         | 1.12±0.25                 | 1.2±0.28                 | 1.52±0.34 c2              | c1:c2; p<0.05  |
| Difference with water displacement volume | -                                 | -0.01±0.07 (-1.66%)                               | -0.02±0.07 (-2.29%)       | 0.07±0.11 (5.53%)        | 0.39± 0.15 (33.42%)       |                |
| Left testes                               |                                   |                                                   |                           |                          |                           |                |
| 2-months-old                              | 1.05±0.06 d1                      | 1.01±0.06                                         | 1.0±0.06                  | 1.11±0.15                | 1.37±0.08 d2              | d1:d2; p<0.001 |
| Difference with water displacement volume | -                                 | -0.04±0.05 (-3.67%)                               | -0.04±0.02 (-4.28%)       | 0.06±0.16 (6.15%)        | 0.32±0.06 (30.7%)         |                |
| 1-year-old                                | 1.12±0.18 e1                      | 1.21±0.3                                          | 1.2±0.29                  | 1.33±0.14                | 1.64±0.4 e2               | e1:e2; p<0.05  |
| Difference with water displacement volume | -                                 | 0.09±0.15 (6.97%)                                 | 0.08±0.14 (6.29%)         | 0.21±0.05 (19.96%)       | 0.52±0.24 (45.12%)        |                |
| 2-years-old                               | 1.2±0.2 f1                        | 1.2±0.27                                          | 1.19±0.27                 | 1.31±0.31                | 1.62±0.36 f2              | f1:f2; p<0.01  |
| Difference with water displacement volume | -                                 | -0.01±0.08 (-1.29%)                               | -0.01±0.08 (-1.92%)       | 0.11±0.12 (8.35%)        | 0.42±0.17 (33.92%)        |                |
| Right testes                              |                                   |                                                   |                           |                          |                           |                |
| 2-months-old                              | 0.93±0.13 g1                      | 0.89±0.12                                         | 0.88±0.12                 | 1.04±0.15                | 1.21±0.16 g2              | g1:g2; p<0.05  |
| Difference with water displacement volume | -                                 | -0.04±0.02 (-3.89%)                               | -0.04±0.01 (-4.5%)        | 0.11±0.15 (12.88%)       | 0.28±0.04 (30.39%)        |                |
| 1-year-old                                | 1.12±0.25 h1                      | 1.12±0.38                                         | 1.13±0.37                 | 1.22±0.41                | 1.52±0.51 h2              | h1:h2; p<0.05  |
| Difference with water displacement volume | -                                 | 0.002±0.19 (-1.37%)                               | -0.01±0.19 (-2.0%)        | 0.1±0.21 (6.57%)         | 0.4±0.31 (33.81%)         |                |
| 2-years-old                               | 1.07±0.2 i1                       | 1.05±0.24                                         | 1.04±0.24                 | 1.1±0.22                 | 1.43±0.32 i2              | i1:i2; p<0.05  |
| Difference with water displacement volume | -                                 | -0.016±0.26 (-0.38%)                              | -0.02±0.07 (-2.65%)       | 0.03±0.09 (2.7%)         | 0.36±0.14 (32.92%)        |                |

(Table 3). These results indicated that the Lambert's empiric formula was completely unsuitable for calculation of the left and right testes' volume in all age rat groups ( $p<0.05$ ). Therefore, this formula was not used for further testicular volume calculations.

Using the solid ellipsoid triaxial body calculation, prolate ellipsoid and prolate spheroid formulas, the testicular volume was calculated based on ultrasound examination data of all age groups of male rat testes after the observation period and compared with the sliding calliper volume (Table 4). These results showed that all three formulas with a slight bias (from -8.63% to 5.46%) are suitable and can be used for testicular volume calculations.

### Change of testicular volume during the observation period

Additionally, the ultrasound testicular volume was calculated using solid ellipsoid triaxial body calculation, prolate ellipsoid and prolate spheroid formulas before the observation period and compared with the volume after the observation period (Table 5). In 2-months-old male rats, the mean volume of both testes and volume of the left and right testes increased similarly when the solid ellipsoid triaxial body calculation formula and the prolate ellipsoid formula were used (97.82%, 91.61%, 104.03% respectively,  $p<0.005$ ), and the right testes' volume increased significantly more than the left during the 90 days of observation.

In 1-year-old rats' testes, comparison of the calcu-

Table 4. Comparison of volume calculated using three formulas in the 2-months, 1-year, and 2-years-old male rat testes measured by sliding calliper and ultrasound after 90 days of observation.

| Age                                                       | Volume, cm <sup>3</sup> (mean±SD)                 |                           |                          |                                                   |                           |                          | p values |
|-----------------------------------------------------------|---------------------------------------------------|---------------------------|--------------------------|---------------------------------------------------|---------------------------|--------------------------|----------|
|                                                           | Sliding calliper                                  |                           |                          | Ultrasound                                        |                           |                          |          |
|                                                           | Solid ellipsoid triaxial body calculation formula | Prolate ellipsoid formula | Prolate spheroid formula | Solid ellipsoid triaxial body calculation formula | Prolate ellipsoid formula | Prolate spheroid formula |          |
| Mean of both testes                                       |                                                   |                           |                          |                                                   |                           |                          |          |
| 2-months-old                                              | 0.95±0.11                                         | 0.94±0.11                 | 1.07±0.14                | 0.97±0.15                                         | 0.97±0.15                 | 1.07±0.21                | p>0.5    |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | 0.02±0.1 (2.42%)                                  | 0.03±0.1 (2.42%)          | -0.001±0.16 (-0.1%)      |          |
| 1-year-old                                                | 1.16±0.32                                         | 1.16±0.32                 | 1.28±0.3                 | 1.05±0.2                                          | 1.05±0.2                  | 1.17±0.23                | p>0.1    |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | -0.11±0.28 (-5.86%)                               | 0.11±0.27 (-5.86%)        | 0.1±0.35 (-3.62%)        |          |
| 2-years-old                                               | 1.12±0.25                                         | 1.12±0.25                 | 1.2±0.28                 | 1.09±0.39                                         | 1.08±0.3                  | 1.2±0.34                 | p>0.5    |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | -0.04±0.174 (-3.53%)                              | -0.04±0.17 (-3.53%)       | -0.01±0.22 (-0.41%)      |          |
| Left testes                                               |                                                   |                           |                          |                                                   |                           |                          |          |
| 2-months-old                                              | 1.01±0.06                                         | 1.0±0.06                  | 1.11±0.15                | 1.06±0.12                                         | 1.06±0.13                 | 1.14±0.24                |          |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | 0.05±0.15 (5.46%)                                 | 0.06±0.16 (5.16%)         | 0.03±0.16 (2.4%)         |          |
| 1-year-old                                                | 1.21±0.3                                          | 1.2±0.29                  | 1.33±0.14                | 1.13±0.16                                         | 1.12±0.16                 | 1.27±0.24                | p>0.5    |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | -0.08±0.32 (-3.09%)                               | -0.08±0.32 (-3.09%)       | -0.07±0.35 (-3.32%)      |          |
| 2-years-old                                               | 1.2±0.27                                          | 1.19±0.27                 | 1.31±0.31                | 1.18±0.3                                          | 1.18±0.3                  | 1.35±0.33                | p>0.5    |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | -0.01±0.13 (-1.23%)                               | -0.01±0.13 (-1.23%)       | 0.03±0.19 (2.73%)        |          |
| Right testes                                              |                                                   |                           |                          |                                                   |                           |                          |          |
| 2-months-old                                              | 0.89±0.12                                         | 0.88±0.12                 | 1.04±0.15                | 0.88±0.12                                         | 0.88±0.12                 | 1.01±0.18                |          |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | -0.006±0.022 (-0.62%)                             | -0.01±0.02 (-0.62%)       | -0.03±0.19 (-2.21%)      |          |
| 1-year-old                                                | 1.12±0.38                                         | 1.13±0.37                 | 1.22±0.41                | 0.98±0.22                                         | 0.98±0.22                 | 1.08±0.19                | p>0.1    |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | -0.14±0.26 (-8.63%)                               | -0.14±0.26 (-8.62%)       | -0.14±0.39 (-3.91%)      |          |
| 2-years-old                                               | 1.05±0.24                                         | 1.04±0.24                 | 1.1±0.22                 | 0.99±0.3                                          | 0.98±0.3                  | 1.05±0.31                | p>0.5    |
| Difference between volume (ultrasound – sliding calliper) | -                                                 | -                         | -                        | -0.06±0.21 (-5.82%)                               | -0.06±0.21 (-5.82%)       | -0.05±0.26 (-3.56%)      |          |

lated volume data was complicated. It is unlikely that the testes' volume could increase 7.06-14.27% during 90 days of observation in adult healthy rats (these data were obtained using the prolate ellipsoid and prolate spheroid formulas). More realistic data were obtained using the solid ellipsoid triaxial body calculation formula, showing a fractional increase in the right testes' volume compared to the left (5.34% and 4.93% respectively).

In 2-years-old rats' testes, the obtained data were also complex when the results of the calculated volume before and after the observation period were compared. The results obtained using all three formulas showed that the volume of the testes decreased insignificantly

during the 90 days of observation ( $p>0.5$ ). However, the results varied widely. The data from the solid ellipsoid triaxial body calculation formula and the prolate ellipsoid formula showed that the volume of the left testes decreased more than the right. However, these results contrasted with results obtained using the prolate spheroid formula (the left testes volume decreased less than the right), therefore leading us to consider that this formula was least suitable for determination of testes' volume in this group of rats. We propose using the solid ellipsoid triaxial body calculation and prolate ellipsoid formulas, or taking the average of the data obtained from both formulas for calculation of the testes' volume.

Table 5. Volume calculated using three formulas in the 2-months, 1-year, and 2-years-old male rat testes measured by ultrasound before observation.

| Age                                                                                         | Volume, cm <sup>3</sup> (mean±SD)                 |                           |                          | p values |
|---------------------------------------------------------------------------------------------|---------------------------------------------------|---------------------------|--------------------------|----------|
|                                                                                             | Solid ellipsoid triaxial body calculation formula | Prolate ellipsoid formula | Prolate spheroid formula |          |
| Mean of both testes                                                                         |                                                   |                           |                          |          |
| 2-months-old                                                                                | 0.51±0.13                                         | 0.51±0.13                 | 0.58±0.14                | p<0.001  |
| Difference between ultrasound volume (after observation period - before observation period) | 0.46±0.11 (97.82%)                                | 0.46±0.11 (97.82%)        | 0.49±0.12 (89.1%)        |          |
| 1-year-old                                                                                  | 0.99±0.22                                         | 0.99±0.21                 | 1.12±0.31                | p>0.1    |
| Difference between ultrasound volume (after observation period - before observation period) | 0.06±0.18 (5.14%)                                 | 0.06±0.18 (8.07%)         | 0.05±0.29 (10.86%)       |          |
| 2-years-old                                                                                 | 1.14±0.31                                         | 1.13±0.31                 | 1.25±0.36                | p>0.5    |
| Difference between ultrasound volume (after observation period - before observation period) | -0.05±0.19 (-6.2%)                                | -0.05±0.19 (-3.75%)       | -0.05±0.27 (-1.92%)      |          |
| Left testes                                                                                 |                                                   |                           |                          |          |
| 2-months-old                                                                                | 0.57±0.11                                         | 0.57±0.11                 | 0.64±0.11                | p<0.001  |
| Difference between ultrasound volume (after observation period - before observation period) | 0.49±0.16 (91.61%)                                | 0.49±0.16 (91.61%)        | 0.5±0.16 (79.32%)        |          |
| 1-year-old                                                                                  | 1.07±0.19                                         | 1.06±0.19                 | 1.22±0.31                | p>0.5    |
| Difference between ultrasound volume (after observation period - before observation period) | 0.06±0.16 (4.93%)                                 | 0.06±0.16 (7.06%)         | 0.05±0.25 (7.44%)        |          |
| 2-years-old                                                                                 | 1.25±0.27                                         | 1.24±0.27                 | 1.39±0.34                | p>0.5    |
| Difference between ultrasound volume (after observation period - before observation period) | -0.06±0.21 (-7.62%)                               | -0.06±0.21 (-4.58%)       | -0.04±0.36 (-0.45%)      |          |
| Right testes                                                                                |                                                   |                           |                          |          |
| 2-months-old                                                                                | 0.45±0.14                                         | 0.45±0.14 c5              | 0.53±0.17 c6             | p<0.005  |
| Difference between ultrasound volume (after observation period - before observation period) | 0.43±0.05 (104.03%)                               | 0.43±0.05 (104.03%)       | 0.48±0.07 (98.88%)       |          |
| 1-year-old                                                                                  | 0.92±0.24                                         | 0.91±0.23                 | 1.02±0.31                | p>0.1    |
| Difference between ultrasound volume (after observation period - before observation period) | 0.06±0.21 (5.34%)                                 | 0.06±0.21 (9.08%)         | 0.05±0.36 (14.27%)       |          |
| 2-years-old                                                                                 | 1.03±0.34                                         | 1.02±0.34                 | 1.1±0.35                 | p>0.5    |
| Difference between ultrasound volume (after observation period - before observation period) | -0.04±0.19 (-4.78%)                               | -0.04±0.2 (-2.92%)        | -0.05±0.16 (-3.39%)      |          |

## Discussion

Ultrasound examination of the testes is routinely used and provides valuable information in the assessing of testicular morphology and function. Using ultrasound, the echotexture and echogenicity of testes' tissues can be determined, and asymmetry, hypogonadism and other abnormalities of the testes can be estimated, and their size measured (Lotti and Maggi 2015, Pozza et al. 2020).

The main factors influencing the accuracy of these measurements include the inclusion of all or part of the epididymis, variability in the thickness and elasticity of the scrotal skin, compression of the scrotal contents, and the experience of the examiner (Junaidi and Martin 2013, Brétas et al. 2016). Therefore, it is important that all measurements would be performed by the same specialist.

In humans, Hsieh et al. (2009) and Mbaeri et al. (2013a) compared testes' volume calculated using three formulas with water displacement volume in patients aged 52-92 years. They concluded that the Lambert's empiric formula is optimal in clinical practice. Sakamoto and co-authors (2008) agreed with them after ultrasound measurements of the testes in patients aged 35.8±5.5 years.

According to our results, the Lambert's empiric formula was completely unsuitable for calculation of testes' volume in living male rats of all age groups.

We found very few reports concerning calculation of rats' testes volume. Only Khan and co-authors (2015) used the solid ellipsoid triaxial body calculation formula to investigate the volume of euthanized adult rats' testes, and Stegani et al. (2008) used the prolate ellipsoid formula for euthanized 45-day-old male rats.

We determined that different formulas were suitable for calculation of the testes volume in different age live male rats. The solid ellipsoid triaxial body calculation formula and the prolate ellipsoid formula were most suitable for calculation of testes volume in young (2-months-old) rats. In 1-year-old male rats, the solid ellipsoid triaxial body calculation formula was the best. The solid ellipsoid triaxial body calculation formula and the prolate ellipsoid formula, or an average of data obtained from both formulas, can be used for calculation of the testes volume in older (2-years-old) male rats.

Using these best formulas, we determined that during the 90 days of observation the volume of 2-months-old male rats' testes increased very significantly in accordance with their body growth. In 1-year-old rats, the volume of testes increased insignificantly. In older (2-years-old) male rats' testes, the volume of both testes decreased slightly.

Following our results we recommend using these appropriate formulas for calculation of the volume of the testes measured by ultrasound for live male rats of different ages.

In summary, this model demonstrated that different formulas are suitable for calculation of the testicular volume in male rats of different ages. This approach could also be applied to investigate changes of testicular volume in other live laboratory, company, and farm animals. However, further in-depth research is required.

## Conclusions

In 2-months-old rats, the solid ellipsoid triaxial body calculation formula and the prolate ellipsoid formula were most suitable for calculation of testes volume. During 90 days of observation, the volume of both testes increased very significantly as calculated using these two formulas.

For 1-year-old rats, the solid ellipsoid triaxial body calculation formula was determined to be the most suitable for calculation of testes volume. The volume of both testes increased insignificantly during 90 days of observation.

In 2-years-old rats, the solid ellipsoid triaxial body calculation formula and prolate ellipsoid formula showed promise for calculating testes volume. Nevertheless, it would be most appropriate to combine the data from both formulas and calculate their average. During 90 days of observation, the volume of both testes decreased slightly.

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