# Intraocular pressure in the smallest primate aging model: the gray mouse lemur

Suggested running title: Intraocular pressure in aging mouse lemurs

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**Abstract**

**Objective:** The aim of this study was to assess the practicability of common tonometers used in veterinary medicine for rapid IOP screening, to calibrate IOP values gained by the tonometers and to define a reference IOP value for the healthy eye in a new primate model for aging research, the gray mouse lemur.

**Studied Animals & Procedures:** TonoVet® and the TonoPen™ measurements were calibrated manometrically in healthy enucleated eyes of mouse lemurs euthanized for veterinary reasons. For comparison of the practicability of both tonometers as a rapid IOP assessment tool for living mouse lemurs, the IOP of 24 eyes of 12 animals held in the hand was measured. To define a standard reference value for IOP in mouse lemurs, 258 healthy animals were measured using the TonoVet®.

**Results**: IOP measurements for the TonoVet® can be corrected by using the formula: $y = 0.981 + (1.962 * \text{TonoVet® value})$, and those for the TonoPen™ by using that of $y = 5.38 + (1.426 * \text{TonoPen™ value})$. The calibrated IOP for a healthy mouse lemur eye was 20.3 ± 2.8 mmHg. The TonoVet® showed advantages in practicability, e.g. small corneal contact area, short and painless corneal contact, shortened total time spent on investigation as well as the more accurate measured values. IOP measurements of healthy mouse lemur eyes were not affected by age, sex, eye side or colony.

**Conclusion:** Tonometry using TonoVet® is the more practicable assessment tool for IOP measurement of the tiny eyes of living mouse lemurs. Pathological deviations can be identified based on the described reference value.

**Keywords:** intraocular pressure, tonometer, reference value, mouse lemur, primate, aging

## Introduction

Mouse lemurs belong to the smallest living primates worldwide. (1) Due to this fact, their maintenance and breeding is more cost-efficient than in larger primate species which makes them a useful primate model for research. Additionally, mouse lemurs have a life expectancy which is much shorter than that of other non-human primate aging models, with about 8 years in the wild and up to 18.5 years in captivity. (2, 3) The genome of mouse lemurs has recently also been sequenced by the Broad Institute (GenBank accession number ABDC00000000). Besides the importance of mouse lemurs for biomedical as well as aging research, (4-9) they are also important for evolutionary research, based on their high and cryptic species diversity, their uneven distribution and their flexible adaptation to their natural habitat. (10)

Mouse lemurs are nocturnal and have relatively small absolute eye sizes with an average diameter of 9.4 mm. (11, 12) Aged gray mouse lemurs were reported to suffer from different eye diseases such as cataract, retinal atrophy and buphthalmia, an abnormal enlargement of the eyeball. (13) Whether this malformation was due to glaucoma, which is associated with ocular hypertension, still needs to be clarified. Due to the difficulty in handling non-anesthetized animals, however, IOP has not previously been documented. Applanation and rebound tonometry are commonly used in veterinary medicine to determine IOP in domestic animals such as dogs, (14, 15) cats, (16-18) and birds (19, 20) as well as in laboratory animals such as rats, (21, 22) rabbits (23) and macaques. (24, 25) Since factory settings for TonoPen™ and TonoVet® are only available for common species in the veterinary clinics such as dogs, cats and horses, for uncommon species it is necessary to calibrate measurements by manometry to achieve a true IOP (tIOP) before defining standard reference IOP values for a given species. (e.g. rabbits, (26) birds (20) and macaques. (25))

The TonoPen™ is an applanation tonometer often used for intraocular measurements in veterinary medicine. (14, 16, 21) IOP measurement gives an indirect assessment of the IOP

by using the Imbert-Fick law. (27) It measures the counter pressure that is necessary to flatten a thin membrane surrounding a sphere filled with liquid. Its use in dogs and cats is easy and fast but requires a local anesthesia of the cornea.

The TonoVet® is a rebound tonometer based on a patented measurement system which uses a small, disposable probe which is brought into contact with the cornea. (17, 18, 20, 24, 28) The probe rebounds with a determined speed, correlating to the IOP. Basically, the higher the IOP, the higher the speed of the return bounce. Its use is easy and fast and requires no local anesthesia.

In this study we applied TonoPen™ and TonoVet® as rapid IOP assessment tools to the gray mouse lemur to

1. calibrate IOP measurements of the tonometers by manometry,
2. assess the practicability of the tonometers to measure the IOP of mouse lemurs´ eyes in-vivo to screen colonies,
3. apply the most practicable technique for screening IOP in two of the world's largest colonies to investigate the effect of eye position, sex, colony and age on IOP and establish a reference value for IOP.

## Methods

*Animals and maintenance*

Mouse lemurs (*Microcebus murinus*) tested in this study belonged to two licensed breeding colonies housed at the Institute of Zoology at the University of Veterinary Medicine Hannover, Germany (for details in housing conditions see (29) Hannover breeding license number 42500/1H) and the University of Montpellier 2 (Agreement $N^{br} \neq$ C-34-172-23). Of 349 investigated animals 258 animals, which showed no ocular pathologies, were used for analysis, 75 (38 females; 37 males) from Hannover and 183 (101 females; 82 males) from Montpellier, France, ranging from 0.5 to 10 years. All animals were born in captivity. Since mouse lemurs are nocturnal, the captive animals were maintained under artificial light conditions with a reversed light cycle. Additionally, animals in Montpellier were maintained under an accelerated photoperiodic regime. This means that the photoperiodically triggered reproductive "year" lasted 8 instead of 12 months. It has been shown that these conditions accelerate aging processes in gray mouse lemurs by the factor 1.5. (30-32)

*Ophthalmological investigations*

Handling for ophthalmological examinations was similar to the weekly caretaker handling of the animals resulting in reduced stress for the lemurs. All examinations were conducted at the end of the sleeping period/beginning of the activity period to minimize disturbances in the animals' activity. All procedures applied in this study were licensed by the respective authorities (Hannover license number, 33.9-42502-05-11A200, LAVES to Elke Zimmermann; Montpellier license number, 34-124 to Jean-Michel Verdier).

Both eyes of a lemur were investigated with a slit-lamp bio-microscope (SL-14; Kowa, Eickemeyer, Germany) and indirect ophthalmoscope (Omega 100; Heine, Ettenheim, Germany) to determine potential eye pathologies with a possible effect on IOP or corneal texture. To gain a view of the lens and retina, mydriatic eye-drops (Mydrum®, Chauvin ankerpharm GmbH, Berlin, Germany) were used to dilate the pupil.

*Manometry*

We determined the IOP value of an enucleated eye with the TonoVet® and TonoPen®, respectively, at manometrically defined IOP pressure steps (DD-890, ATP Messtechnik GmbH, Ettenheim, Germany). The used manometer was calibrated by the Bureau of Standards in Hannover (Mess- und Eichwesen Niedersachsen Betriebsstelle Eichamt Hannover, Goethestraße 44, 30169 Hannover). The pressure measured by the manometer in this set-up (including the pressure in the examined eye) was labeled as the true IOP (tIOP). The values in the more relevant sector for clinical use between 5 mmHg and 50 mmHg were taken in steps of 5 mmHg ± 0.1 mmHg. Between 50 mmHg and 100 mmHg measurements were taken in steps of 10 mmHg ± 0.1 mmHg.

Eight healthy eyes of four animals euthanized for veterinary reasons (incurable pathologies) were enucleated transconjunctivally immediately after euthanasia. These eyes were referred to as healthy since they were found to be inconspicuous and showed no signs of pathological disease according to an ophthalmological investigation performed not more than 6 months previously. After enucleation, the eyes were stored in 0.9% NaCl solution at 6°C for up to a maximum of 4 hours before measuring. A small bowl of modeling clay was adjusted to ensure the fixation of the enucleated eye. The cannula (24 G, length 25 mm, B. Braun Melsungen AG, D-34209, Germany) was inserted transsclerally into the vitreous and was not moved or reinserted while taking the measurement. The pressure was constant and measured

values showed no fluctuation. Minimal leakages were observed sporadically between the three-way stopcock and the silicon tubes, but sealed by themselves with higher pressure. A three-way stopcock was connected to the cannula, the manometer and a NaCL solution reservoir via three silicon tubes. Pressure was adjusted by changing the height of the NaCl solution reservoir starting at low pressure and constantly increasing it until 100mmHg. The whole system was open all the time to avoid fluctuations (see Fig. 1). Once the manometer displayed a constant pressure, the pressure was read using the TonoVet® and TonoPen™. Each complete measurement by the TonoVet® represented the mean of four single values, for the TonoPen™ the mean consisted of four to five single values. The measurement was carried out until three complete measurements per eye were successfully obtained.

*In-vivo application of TonoPen™ and TonoVet®*

The tonometers TonoPen™ and TonoVet® were used to measure IOP in the eyes of 12 young mouse lemurs (6 males, 6 females; aged between 2 and 3 years; Colony Hannover). Both eyes of these mouse lemurs were unremarkable and showed no signs of pathological diseases according to an ophthalmological investigation performed on the previous day. Measurements of the TonoVet® and TonoPen™ were taken 24 hours after the ophthalmological investigation to minimize influences of the mydriatic eye-drops on the IOP. Both eyes of each animal were evaluated until a successful measurement with the respective tonometer was achieved. A successful measurement is indicated by an audible tone. Measurements with the TonoVet® were always taken before measurements with the TonoPen™ in order to avoid any unexpected effect of local anesthetic.

175

176 *Rebound tonometry (TonoVet®)*

177 Six single values of the IOP were taken per eye by the TonoVet® tonometer (TonoVet®;
178 ICare, Finland Oy, Finland). The TonoVet® then automatically deleted the lowest and
179 highest value and calculated the mean from the remaining four values. Only successfully
180 completed measurements displaying a mean on the TonoVet® were recorded. Due to the
181 large sample size and to minimize disturbance to the animals, one successfully completed
182 measurement per eye was taken. Only measurements with a deviation $\leq 1.0$, indicated by a
183 missing bar on the display, were used. For all 12 animals no anesthesia or forced fixation of
184 the eyelids was necessary (see Fig. 2).

185

186 *Applanation tonometry (TonoPen™)*

187 The TonoPen™ tonometer (TonoPen™; Reichert® Technologies, Eickemeyer, Germany)
188 was used to measure IOP of the same 12 animals as for the TonoVet®. Before measurements
189 were taken, the eyes were locally anesthetized with eye-drops (Proparakain-POS® 0.5%). To
190 prevent a reflexive blink of the eyelids of an animal, the examiner had to fix the eyelids with
191 his fingers in an open position (see Fig. 3). Between four and five single values per eye were
192 necessary for the TonoPen™ to calculate a mean. Only successfully completed measurements
193 displaying a mean on the TonoPen™ were recorded. One successfully completed
194 measurement per eye was taken.

195

196 *Determination of the IOP in two colonies*

197 To determine a reference value for the healthy mouse lemur eye, a large sample size is
198 required.

241 animals in Montpellier and 108 animals in Hannover were investigated. To determine the IOP the TonoVet® was used. Both eyes of all animals were investigated until a measurement without SD-error was achieved (indicated by a missing bar on the display). The animals were investigated at the end of the sleeping/beginning of the activity period. In the case of those animals in Montpellier the investigation period ranged from 09:00-15:00 (beginning of activity period at 12:00 for all animals), for Hannover from 09:00-17:00 (beginning of activity period at 10:00, 12:00 or 14:00, respectively, according to the room). After IOP measurement, an ophthalmological investigation was performed for each animal as described in the section on ophthalmological investigation to select animals with healthy eyes for this study. Fifty-eight animals in Montpellier and 33 animals in Hannover showing eye malformations were thereby excluded from further analysis.

**Data analysis**

*Manometric calibration of the IOP measured by TonoVet® and TonoPen™ in enucleated eyes*

For the manometric calibration of the TonoVet® and TonoPen™ IOP measurements, we performed a regression analysis based on the eight means (= eight eyes) per step mmHg per instrument.

*Comparison of IOP between TonoPen™ and TonoVet®*

For the in-vivo comparison of applanation (TonoPen™) and rebound (TonoVet®) tonometry, we calculated the mean, range, standard deviation and median IOP for each instrument based on the measurements of the 24 eyes of the 12 animals. For the TonoVet® tonometer and the TonoPen™ tonometer, we compared measured IOP values between left and right eyes using

the paired t-test and measured IOP values between sexes using the unpaired t-test since the values followed a normal distribution. The IOP values obtained by the TonoPen™ and TonoVet® were compared using the Wilcoxon-signed-rank test.

*Comparison of IOP across colonies*

To assess the effect of sex and eye position, IOP values (using TonoVet®; $N_{totalanimals}$ = 258, $n_{totaleyes}$ = 516; Hannover, N = 75; Montpellier, N = 183) obtained for each eye and animal per colony were compared between the left and right eye and between sexes using the Wilcoxon-signed-rank test and Mann-Whitney-U test, respectively.

If findings within colonies did not reveal any significant effect of eye position or sex, we used the median value of an animal per colony for further statistical analysis. To explore the effect of colony, we compared IOP values between colonies using the Mann-Whitney-U test. The effect of age on IOP was analyzed using a Spearman-Rank correlation. For the colony in Montpellier, the cycle age was multiplied by the factor 1.5 to calculate the chronological age in years. To define the reference IOP value for a healthy mouse lemur eye, we calculated the mean, range, standard deviation and median of the IOP of the healthy eye of both colonies.

## Results

*In vitro calibration of the rebound (TonoVet®) and applanation (TonoPen™) tonometry*

We found a linear correlation between both the rebound tonometry and direct manometry or applanation tonometry and direct manometry (see Fig. 4; n = 8 eyes; N = 4 mouse lemurs). The regression analysis showed consistent linear underestimation of IOP by the TonoVet® and TonoPen™. From 15 mmHg up to 100 mmHg, the regression analysis showed that the measured IOP (mIOP) for TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) can be corrected by using the function tIOP = 0.981 + (1.962 * mIOP) and for TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) by using the function tIOP = 5.38 + (1.426 * mIOP). In both tonometers it was not possible to obtain values below 15 mmHg. From 15 mmHg up to 100 mmHg the TonoVet® constantly underestimated the tIOP by 50 percent. While the TonoPen™ almost measured the same values at 20 mmHg as the TonoVet®, the measured values started to increase slightly compared to the TonoVet®, reaching around 66% of the tIOP at 100 mmHg.

*In-vivo IOP measurements with TonoPen™ and TonoVet®*

A comparison of IOP values between the left and right eye for each instrument showed no significant difference between the eye side (TonoVet®, paired t-test, N = 12, t = -0.538, p = 0.601; TonoPen™, paired t-test, N = 12, t = -0.794, p = 0.444, respectively).

No difference between sexes was found for IOP or for the TonoVet® (unpaired t-test, $N_{total}$ = 24, $N_{males}$=$N_{females}$ = 6, t = -0.130, p = 0.292) or for the TonoPen™ (unpaired t-test, $N_{total}$ = 24, $N_{males}$=$N_{females}$ = 6, t = -0.340, p = 0.198). Thus, we did not differentiate between sexes in all further analyses.

The mean IOP for the TonoVet® tonometer was 9.2 ± 1.5 mmHg, the median 9.0 mmHg and the range 6-12 mmHg, the tIOP amounting to 19.0 ± 2.2 mmHg.

For the TonoPen™ tonometer the mean was 23.8 ± 5.9 mmHg, the median 24.5 mmHg and the range 14-38 mmHg with a tIOP of 39.4 ± 5.1 mmHg. (see Table. 1)

IOP values measured by the TonoVet® and the TonoPen™ differed significantly (Wilcoxon-test, N = 24, T = 0.00, n = 24, p = < 0.001). The comparison of measured values between instruments showed a higher estimation of the average IOP for the TonoPen™. The average value for the TonoPen™ was more than twice as high as the average value estimated by the TonoVet®. Measurements with the TonoPen™ for the same animal for the left and right eye showed high variability, while the values obtained with the TonoVet® were much more consistent, e.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). (see Fig. 5)

Unfortunately, several problems occurred when using the TonoPen™.in the examiner-animal context. The tip of the TonoPen™ was large in comparison to the small eyes of the mouse lemurs, this causing reflexive blinks of the animal due to contact with the animals' eyelashes. Furthermore, it was not possible to fixate the animals' eye-lids without causing indirect pressure on the eyeball. In addition, the examiner had to protect himself against bites since the hand which was fixating the animals' eye-lids was hazardously close to the animals' mouth and sharp teeth. The time required to complete one successful measurement per animal

varied between 5 and 10 minutes (without waiting time for the applied anesthetizing eye-drops).

When using the TonoVet® for IOP measurements, the animals showed no visible reaction when the probe touched the animals' cornea. Furthermore, almost no reflexive blink was visible. Since no forced fixation of the animals' eye-lids was necessary, bites never occurred. The investigation lasted on average only 30 seconds.

*Comparison of IOP between two mouse lemur colonies and the definition of a reference IOP value for the gray mouse lemur*

Both colonies were investigated using the TonoVet®. A comparison between measured IOP values in the left and right eye for each colony showed no significant differences (Hannover, Wilcoxon-test, N = 75, T = 22.05, n = 47, p = 0.361; Montpellier, Wilcoxon-test, N = 183, T = 55.27, n = 113, p = 0.336).

No difference between sexes was found for IOP (Hannover, Mann-Whitney-test, $N_{total}$ = 150, $N_{males}$ = 37 eye-pairs, $N_{females}$ = 38 eye-pairs, U = -1.314, p = 0.189; Montpellier, Mann-Whitney-test, $N_{total}$ = 366, $N_{males}$ = 82 eye-pairs, $N_{females}$ = 101 eye-pairs, U = -1.552, p = 0.121). Thus, the sexes were not further differentiated in further analyses.

The comparison of IOP values between both colonies showed no significant difference (Mann-Whitney-test, $N_{total}$ = 516, $N_{Hannover}$ = 75 eye-pairs, $N_{Montpellier}$ = 183 eye-pairs, U = -0.230, p = 0.818). Based on that, we did not further differentiate between colonies for subsequent analyses.

The effect of age on IOP was assessed by a Spearman-Rank correlation. No significant correlation between chronological age and IOP was revealed ($p = 0.418$, $r_s = -0.036$, $N = 516$, see Fig. 6). Thus, age did not affect IOP in the healthy eye for the investigated age-span. Based on that a reference value for IOP measured with the TonoVet® was calculated with a mean of 9.9 ± 1.66 mmHg, a median of 10 mmHg and a range of 5-15 mmHg. Using the regression function the calculated mean for healthy mouse lemur eyes amounted to tIOP = 20.3 ± 2.8 mmHg.

## Discussion

This is the first study measuring the IOP of mouse lemurs. Our findings suggest that the TonoVet® is the most suitable tool (compared to the TonoPen™) for rapid IOP screening of the tiny eyes of this smallest bodied primate. Results showed that IOP in the clinically healthy eye is not affected by age, sex, or eye side (left or right). Thus, a reference value for IOP could be defined based on a large sample-size of more than 250 individuals for this novel primate model for aging research.

*Calibration of IOP using manometry*

Every tonometer has to be calibrated for each species specifically due to the fact of different corneal attributes, especially corneal thickness, to attain the tIOP values. (33, 34) IOP measurements using eyes persisting in the eye socket in ex-vivo as well as enucleated eyes revealed no significant differences between these procedures. (35) Manometric in-vivo measurement of IOP is, however, known to potentially cause damage to intraocular structures and pain. Thus, for ethical reasons we decided to base our manometric investigations on enucleated eyes of animals which had died for natural reasons or had been euthanized due to incurable diseases which had no impact on IOP.

Our manometric calibration for the TonoVet® and TonoPen™ showed a consequent linear underestimation of the tIOP for both instruments which can be corrected by the established regression functions. For a fast clinical interpretation of the measurements displayed on the TonoVet® the values can be multiplied by 2. For the TonoPen™ a similar but not as easy to use formula is: tIOP = (1.5*measured value) + 5.

A comparable underestimation of the IOP measured by the TonoVet® and TonoPen™ was reported for dog eyes. (15) This underestimation was explained by corneal specification,

371 especially corneal thickness, calibration-standards and use by different examiners. For our
372 study, the same examiner performed the measurements so that the effect of the examiner on
373 the measured IOP values was minimized, whereas both corneal specification and different
374 calibration standards between TonoVet® and TonoPen™ are likely to explain the
375 underestimation of IOP values. In humans a significant effect on IOP measurements caused
376 by different thickness in different corneal areas using the rebound tonometry has already been
377 shown: higher values were determined when corneal thickness was higher. (33, 34) Our
378 measurements were taken at the center of the cornea to minimize this effect. We expect that
379 central corneal thickness in mouse lemurs is relatively thin, which would explain the linear
380 underestimation. Further investigations on corneal thickness of mouse lemurs, e.g. with an
381 ultrasound pachymeter are necessary to investigate this in more detail.
382
383 *Practicability of TonoPen™ or TonoVet® to screen the IOP of mouse lemur eyes*
384 The practical value of a tonometer is as important as the calibration. Thus, which of the
385 commonly used tonometers in veterinary science, TonoPen™ or TonoVet®, is the most
386 suitable tool to screen tiny eyes of a large number of mouse lemurs in colonies on a regular
387 basis? The eyes of the gray mouse lemur only have a diameter of 9.4 mm (11). Therefore, a
388 previous ophthalmological study on the gray mouse lemur had difficulties in using
389 applanation tonometry in non-anesthetized animals. (13) We applied both the TonoPen™ and
390 the TonoVet® for a subgroup of non-anesthetized animals to assess the practicability of these
391 instruments. We showed that both tonometers can be applied, but that there are huge
392 differences in practicability and ethical justifiability. The investigation with the TonoPen™
393 required a much longer time (up to 10 minutes) to assess the IOP of an animal compared to
394 TonoVet® (up to 30 seconds). The most time-consuming issue emerged with the high
395 number of failed IOP measurements (indicated by an alarm-signal of the TonoPen™). The

failure in measurement was due to the fact that the veterinarian had to pay attention to exerted pressure, contact-area and animal position while measuring the IOP. In contrast, unsuccessful measurements (indicated by an alarm-signal) and high SD measurements (which were considered unsuccessful and indicated by a bar on the display) were quite rare for the TonoVet®. Furthermore, the small eyeballs of the mouse lemurs necessitate manual fixation of the eye-lids, inflicting pressure on the globes, whereas no fixation of the animal's eye-lids was necessary for the TonoVet®. The animal itself must be fixated much more firmly when using the TonoPen™ eventually causing systemic hypertension and consecutively higher IOP.

Consequently, our experience using the TonoPen™ supports that of Beltran et al. (13) that using this instrument to measure IOP in the tiny eyes of mouse lemurs is problematic. The required extensive manipulation prevents the determination of physiologically expected IOP values. Usually physiological IOP ranges from 15-23 mmHg, e.g. in humans, (36) dogs, (14) cats, (18) horses, (28) rabbits, (23) rats (21) and macaques. (37) (see Table. 2) Therefore, non-physiologically high IOP as measured with the TonoPen™ in mouse lemurs (tIOP = 39.4 ± 5.1) may be the result of stress, high systemic blood pressure and unintended pressure on the globe, thus questioning the ethical justifiability of this method.

Other positive effects of the TonoVet® were that IOP of non-anesthetized animals can be measured rapidly and without any visible harm for the measured animal. Furthermore, a veterinarian can standardize the measurement quickly and quickly become routinized. The TonoVet® showed satisfactory results concerning reproducibility with a relatively small variation comparable to those of other studies using larger mammals such as rhesus macaques (25) or rabbits. (26) All in all, based on these findings we recommend the TonoVet® as a suitable IOP assessment tool for rapid screening of the eyes in non-anesthetized mouse lemurs.

*Definition of a reference IOP for the healthy gray mouse lemur eye*

A reliable mean for IOP in mouse lemurs requires a large sample-size. To enlarge our sample-size we analyzed whether eye side (left or right), sex or age had any influence on IOP in mouse lemurs. Our study showed no significant differences between eye side and sex and no statistically significant correlation between age and IOP. The slight decrease in IOP between the age of 0.5 and 10 years was even smaller than the value of the determined standard deviation. Animals of ten years and older were excluded due to diagnosed ocular pathologies.

Our screening analysis of the two colonies, Hannover and Montpellier, included 258 animals with 516 healthy eyes in total and showed a tIOP of 20.3 ± 2.8 mmHg. This value matches quite well to the IOP range of 15-23 mmHg reported from humans (36) and mammals of veterinary and biomedical importance (see Table. 2) such as dogs, (14) cats, (18) horses, (28) rabbits, (23) rats (21) and macaques. (37) Since these mammals differ largely in size, activity and phylogeny, IOP seems to be independent from these factors.

Circadian rhythm is also described as affecting IOP, e.g. in cats, rabbits and the Tibetan monkey (23),(38),(39). To minimize this effect, we always determined IOP at the beginning of the animal's activity period.

Our study showed no correlation between age and IOP in the healthy mouse lemur eye comparable to e.g. Tibetan monkeys. (39) Age effects on IOP in animals and humans are ambiguous. Whereas investigations performed in rhesus monkeys and dogs showed a decrease in IOP with age (37),(14) studies in humans revealed both an increase and a decrease depending on the tested populations. (40),(36). High blood pressure, obesity and

other vascular deficiencies which are largely age dependent were discussed as explanations. (41-44).

It has to be taken into account that for our study we considered only ophthalmologically unremarkable animals. Pathologies influencing the state of health of the eye and leading to glaucoma or higher intraocular pressure have to be considered in follow-up studies.

## Conclusion

To conclude, we demonstrated the practicability, usefulness and reliability of the TonoVet® as a powerful tool for screening IOP in mouse lemurs, a novel primate model for human aging research. Average IOP of healthy mouse lemur eyes is not affected by eye side, sex and colony and does not correlate with age. Furthermore, the value of IOP of mouse lemurs coincides with those of other mammals. For future studies using this smallest-bodied primate aging model, our findings are an important foundation for clearly distinguishing peripheral from central pathologies.

## Software for statistical analysis

The statistical analysis was performed using SPSS 22.0 for Windows. Significance level was set at $P = 0.05$.

## List of Abbreviations

IOP: Intraocular pressure; tIOP: true intraocular pressure; mIOP: measured intraocular pressure

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

MD, MJ, EZ, IN, JS conceived, coordinated and designed the study. Manometrical data were acquired by MD. Data from the screening of both colonies were acquired by MD and MJ. Statistical analysis was conducted by MD. All authors contributed to drafting, reading and approving the final manuscript.

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**Figure 1. Set-up of the manometrical investigations.**

**Figure 2. Investigations of a 2-year-old mouse lemur with the TonoVet®.**

**Figure 3. Investigations of a 2-year-old mouse lemur with the TonoPen™**

**Figure 4. Values measured with the TonoVet® and TonoPen™ under manometrical control.** Manometrically determined IOP for the eight eyes of four animals are represented on the x-axis. TonoVet® and TonoPen™ values are represented on the y-axis. Each dot represents the mean of three measurements per step/per eye. The dashed line represents the regression line for the TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) and TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) measurements.

**Figure 5. Comparison of the in-vivo measured IOP for the TonoVet® and TonoPen™ in 12 animals.** The results show high variation in measurements for the TonoPen™ and much more consistent values for the TonoVet®. E.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). For exact values (see Table. 1).

**Figure 6**. **Relation between tIOP values and age in healthy mouse lemur eyes.** This scatterplot shows the tIOP values for all measured healthy animals from Hannover and Montpellier ($N = 258$) on the y-axis in relation to age on the x-axis. The decrease in IOP is statistically not significant ($p = 0.077$, $r_s = -0.110$).