Tear Production and Intraocular Pressure in Healthy Canine Neonates

Sander Mes
3259552
Project tutor: A. P. M. Van Schaik-Verboven

Table of contents

Summary

Diseases such as keratoconjunctivitis sicca sometimes need lifelong therapy with lacrimomimetics and lacrimostimulants and several forms of uveitis and glaucoma need an aggressive approach when treating. Therefore, when a puppy is presented in a clinical situation, it is necessary to use and interpreted diagnostic tools such as STT and tonometry in a correct way. Previous veterinary research does not provide this information. The aim of this study is to measure the development of basal and total tear production and intraocular pressure in canine neonates.

Eight healthy beagle puppies were used to measure aqueous tear production using a standardized Schirmer Tear Test with (STT1) and without (STT2) the application of topical anesthesia. Also, intraocular pressure (IOP) was measured using a TonoVet® rebound tonometer. These measurements were performed once a week starting from the moment the eyes opened at the age of 2 week until the age of 12 weeks.

STT1, STT2 and IOP values at the age of 2 weeks were all lower than adult values and increased significantly until the age of 9, 10 and 6 weeks, respectively. IOP values decreased significantly between 9-10 weeks of age, increased significantly between 10-11 weeks, and decreased significantly again between 11-12 weeks. There was a significant effect of time on all parameter.

Introduction

Anatomy and Physiology of the Tear Film

The tear film is an important biological structure with different components. It consists of an inner mucoid layer, an aqueous layer and a superficial lipid layer. The inner mucoid layer covers the cornea and conjunctiva. It lubricates the cornea, binds the aqueous layer to the corneal epithelium and prevents desiccation.[1] It is composed by mucins, immunoglobulins, urea, salts, glucose, leukocytes, cellular debris and enzymes.[2] Mucins are produced in the apocrine conjunctival goblet cells in response to mechanical, immune, histamine, antigenic, or neural stimulation.[3-5] With the corneal epithelium being hydrophobic, the hydrophilic mucoid layer facilitates the evenly distribution of the aqueous layer over the ocular surface. The mucoid layer itself connects to the cornea using the glycocalyx layer, which consists of glycoproteins and glycolipids and is associated with the corneal and conjunctival microvilli and microplicae[6,7]

The middle aqueous layer of the tear film is active in the lubrication and protection of the ocular surface. It flushes foreign material from the conjunctiva and the cornea and contains antibacterial factors. It consist of 98,2% water and 1,8% solids, made up predominantly of proteins.[8] The aqueous layer contains inorganic salts, glucose, urea, proteins, glycoproteins,[9] and soluble mucins.[10] Production takes place in the lacrimal gland, the superficial gland of the nictitating membrane, and the accessory lacrimal glands in the conjunctiva.[11]

The superficial lipid layer provides a smooth optical surface, reduces the evaporation of tears, and prevents tear overflow onto the eyelids.[11] It also decreases the surface tension of the tear film, which draws water into the aqueous layer and thus increases the film thickness.[12] It consists of both polar and nonpolar lipids. The polar fraction, composed of mainly phospholipids, covers the aqueous layer of the tear film and the nonpolar fraction lies more superficial.[13] It is produced by the holocrine tarsal and meibomian glands of the eyelids.[11]

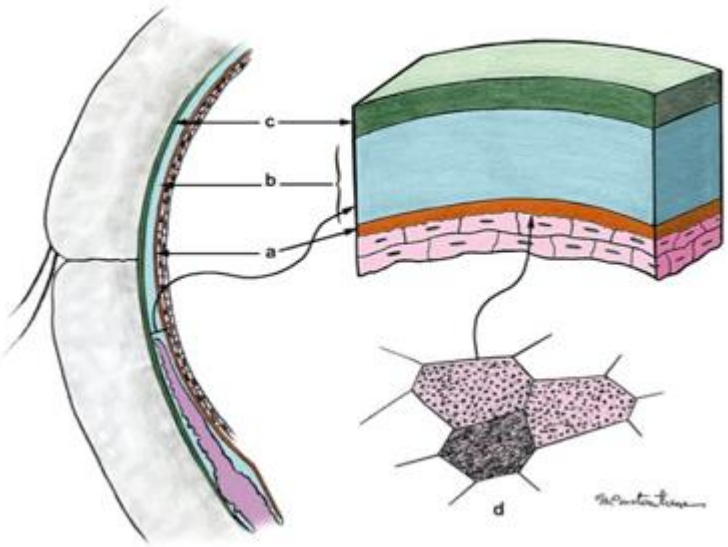

Figure 1. Components of the tear film with a. mucoid layer, b. aqueous layer, and c. lipid layer (from: Veterinary Ophthalmology, Gelatt, 2013).

Tear production quantification

Quantitative evaluation of tear production requires the Schirmer tear test (STT), which measures the aqueous portion of the tear film.[14,15] The STT1 evaluates basal (which is continuous) and reflex (after stimulation of the cornea) tear production during a wetting period of 60 seconds.[14] The STT2 includes the application of a topical anesthesia and drying of the conjunctival sac, to measure the basal tear production solely.[15] STT1 and STT2 values are significantly different in the normal dog.[14,16] Normal STT1 values in dogs range between 18.64 ± 4.47 mm/min to 23.90 ± 5.12 mm/min.[14,16-18] STT2 values

are more variable and range between 3.6 ± 2.8 mm/min to 13.95 ± 4.40.[14,16,17] In the clinical setting, STT1 readings are interpreted as follows:[19]

- >15 mm/min = normal production
- 11-14 mm/min = early or subclinical KCS
- 6-10 mm/min = moderate or mild KCS
- <5 mm/min = severe KCS

To perform the STT in small animals with small palpebral fissures, some studies use a modified version of the standardized STT. This method requires a commercial STT strip which is aseptically transected using a scalpel blade. This leads to two 35 mm. long/2.5 mm. wide strips. For the test itself, only the unnotched half is used using the same procedures as the standardized STT, i.e. placement in the ventral conjunctival sac for 1 minute.[20]

Quantitative evaluation of the aqueous portion of the tear film is also possible using the phenol red thread (PRT) tear test.[21,22] This test is performed using a standardized cotton thread which is impregnated with phenol red, a pH-sensitive indicator. After placement in the lower conjunctival fornix for 15 seconds, the length of the color change from contact with the aqueous tears is read from the end of the thread.[23]

Keratoconjunctivitis Sicca

Abnormalities in either the quantity or quality of any tear film component can lead to tear film dysfunction. Quantitative abnormalities are considered to have an origin in the aqueous portion of the tear film, and can be measured with the STT, while qualitative abnormalities have their origin in the other parts (i.e. mucin or lipid layer), which needs different diagnostics. Keratoconjunctivitis sicca (KCS) is an important ocular disease and is the result of a quantitative aqueous tear deficiency, with an incidence of approximately 1-4% in canine patients.[24,25] Insufficient or absent secretion from the lacrimal and nictitans glands, which may result from a single disease process or a combination of conditions, can lead to this condition.[25]

Quantitative tear deficiency causes a number of events which eventually lead to inflammation of the ocular surface. The lack of aqueous tear component leads to hypertonicity and dehydration of the conjunctival and corneal epithelia, hypoxia of the corneal epithelium and subepithelial corneal stroma,[26] irritation of the ocular surface by the eyelids and third eyelid, and a more optimal environment for microorganism colonization on the ocular surface.[27] These events result in inflammation, which in turn causes more cell damage leading to a deteriorating cycle.[28,29] Eventually, these events lead to keratoconjunctivitis, which can result in ocular pain, ocular discharge, vascularization, ulcer and descemetocele formation, and reduced vision. Chronic cases are characterized by pigmentation and keratinization of the cornea.[19]

Diagnosis of KCS is made on the presence of typical clinical signs, positive ocular staining, and reduced quantitative aqueous tear readings. Rose Bengal stain can be used to detect devitalized cells, subtle corneal or conjunctival epithelial defects, and adherent mucus tags.[30] The STT is still the standard for measuring the aqueous tear production.[14] Medical treatment of KCS has extensive possibilities and include lacrimostimulants, lacrimomimetics, antibiotics, mucinolytic-anticollagenase agents, and anti-inflammatory therapy.

The uvea and the intraocular pressure

The eye consists of three layer. An outer fibrous layer, with the sclera and cornea. A middle vascular layer, the uvea. And an inner neuroectodermal layer, with the retina and the optic nerve (Figure 2). The uvea has a posterior part, consisting of the choroid, and an anterior part, consisting of the iris and the ciliary body. The choroid is a thin vascular tissue between the sclera and the retina and connects at the ciliary body. It provides nourishment to the outer retinal layers. The iris contains a constricting and a dilating muscle, which control the amount of light entering the eye. The ciliary body lies posterior of the iris and consists of an anterior pars plicata and a posterior pars plana. The para plicata consists of a ring of 70-100 ciliary processes, depending on the species.[31] These processes increase the surface area of the aqueous humor production site and serve as a region for attachment of the lenticular zonules, which connect the lens to the ciliary body. The ciliary processes consist of a center of stroma and blood vessels, covered with a layer of pigmented and a layer of non-pigmented epithelium.[32]

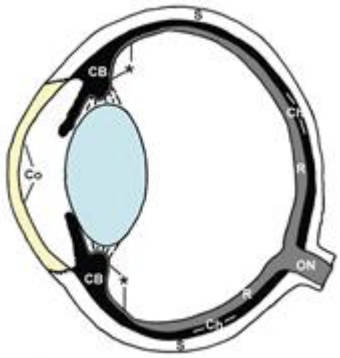

Figure 2. The eye with the fibrous layer (white), the vascular layer (black), and the neuroectodermal layer (grey) (from: Veterinary Ophthalmology, Gelatt, 2013).

The formation of aqueous humor is based on diffusion, ultrafiltration, and active secretion by the nonpigmented ciliary epithelium. Diffusion and ultrafiltration from the capillaries into the posterior chamber are driven by oncotic pressure, hydrostatic pressure, and concentration gradients. Active secretion with a Na^+,K^+-ATPase regulated pump in the non-pigmented epithelium is the most important factor in aqueous humor formation.[33,34] Na^+, which is transported from the blood to the aqueous humor, creates an osmotic gradient. This leads to water movement from the non-pigmented epithelium to the posterior chamber, creating an elevated intraocular pressure.[35] Also, carbonic anhydrase (CA) catalyzes the reaction $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$ in the epithelium. This creates more HCO_3^- available for entry into the aqueous humor, which elevates the intraocular pressure by more water movement[36] (Figure 3). In a normal eye, the rate of aqueous humor production is the same as the outflow through the pathways. This is influenced by humoral, sympathetic and parasympathetic innervation. The equilibrium between the production and outflow creates the intraocular pressure which is measured during an ophthalmologic examination.[11]

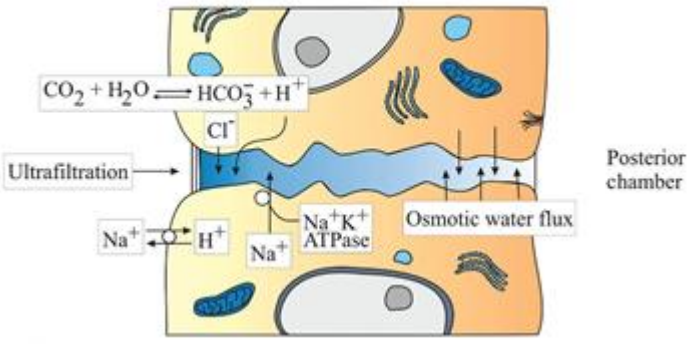

Figure 3. Diffussion, ultrafiltration, and active secretion in the nonpigmented ciliary epithelium (from: Veterinary Ophthalmology, Gelatt, 2013)

After production, the aqueous humor enters the posterior chamber and flows through the pupil into the anterior chamber, where it leaves the eye through the corneoscleral trabecular and uveosceral outflow pathways. The biggest proportion of the aqueous humor leaves through the corneascleral pathway, which consists of the trabecular meshwork between the base of the cornea and the iris. A small percentage (about 15%) of the aqueous humor leaves the anterior and posterior chamber using the uveoscleral pathway, which consists of diffusion through the iris and ciliary body.[37]

Tonometry

There are three different types of tonometers available; indentation (Schiötz), applanation, and rebound tonometers. An indentation tonometer measures the depth which is produced by a standard force on the surface of the cornea. The indentation of the cornea is indirectly proportional to the IOP, and can be used to calculate the IOP using the Imbert-Fick law.[38] This method is not recommended for veterinary use.[39]Applanation tonometry consists of a probe which flattens the cornea. The force which is required for this is also used to calculate the IOP using the Imbert-Fick law.[40] Rebound tonometry is a relatively new technique which uses a probe inside of a magnetic field. It measures the IOP by quantifying the deceleration of the probe after its impact on the corneal surface.[41] IOP values may vary in the dog, due to age and diurnal variation. General values in the dog are between 15-18 mmHg,[11] but it varies between 11-29 mmHg.[42] Lower values can be an indication of uveitis, higher values of glaucoma.

Uveitis

Uveitis is an inflammation inside the uvea. Terminology to describe which portion of the uvea is inflamed include iritis (iris), iridocyclitis or anterior uveitis (iris and ciliary body), choroiditis or posterior uveitis (choroid), and panuveitis (inflammation of the entire uvea).[43] Causes of uveitis are infectious or non-infectious, which include trauma, lens-induced (e.g. as a result of cataract), neoplastic, and idiopathic.

Uveitis occurs after damage to uveal tissue or vasculature which disrupts the blood-aqueous barrier (in the non-pigmented ciliary epithelium) or the blood-retina barrier. Prostaglandins and leukotrienes induce vasodilation and increase the vascular permeability, which results in the breakdown of the blood-aqueous barrier.[44] Prostaglandins also induce hyperemia and constrict the iris sphincter muscle, which causes miosis and pain. Breakdown of the blood-aqueous barrier results in the entry of proteins, cells, and inflammatory components into the aqueous humor. In the anterior chamber, the prostaglandins increase uveoscleral outflow. Together with a decreased production in the ciliary epithelium as a results of a disrupted blood-aqueous barrier, this leads to a lowered IOP.[45]

Diagnosis of uveitis is based on the observation of clinical signs. These can be specific in the case of anterior uveitis, such as aqueous flare (due to elevated levels of proteins and cellular components in the anterior chamber), hypopyon and decreased IOP, but can also be aspecific like miosis, corneal edema and conjunctival hyperemia. Symptoms of posterior uveitis can also be aspecific and include vitreous opacity, optic neuritis and retinal detachment. If a cellular infiltrate is present in the anterior chamber, cytological evaluation of the aqueous humor can contribute to a diagnosis. Some agents which cause uveitis and neoplastic cells can be identified.[46]

Therapy primarily focusses on reducing the inflammation, stabilizing the blood-aqueous barrier, decreasing pain, and preserving vision. Agents used to reach these goals include topical mydriatics, topical corticosteroids, and systemic NSAID's.[44] If there is an underlying problem, therapy should focus on this problem as well.

Glaucoma

Glaucoma is a group of ocular diseases that exhibit increased level of IOP that are harmful to the maintenance of vision and health of the eye. They can be classified on the basis of the possible cause (primary, secondary, or congenital), the gonioscopic appearance of the filtration angle (open, narrow, or closed iridocorneal angle and open, narrow, or collapsed ciliary cleft), and the duration or stage of the disease (Figure 4). In the primary glaucomas, there is an increase in IOP without concurrent ocular disease. It is hereditary in many canine breeds and often develops bilaterally. Primary glaucoma has several possible causes, including abnormal cells in the outflow system, pupillary blockage of the iridocorneal angle, and pectinate ligament dysplasia. In secondary glaucomas, there is an ocular disease which causes a physical obstruction of the aqueous outflow pathways, leading to higher IOP values. Congenital glaucomas are characterized by an increase in IOP soon after birth, caused by anomalies in structures of the iridocorneal angle and ciliary cleft (i.e. the filtration angle). Diagnosis of glaucoma is mainly based on tonometry as elevated values of the IOP are very specific. It is also possible to use gonioscopy to examine the filtration angle, but this is primarily useful for classification of the type of glaucoma.[47]

A. Primary glaucomas:
Open/normal angle/cleft: Acute/chronic
Narrow/closed angle/cleft: Acute/chronic

B. Secondary glaucomas:
Uveitis
Lens luxations
Intumescent cataract
Phakolytic/phacoclastic uveitis
Hyphema
Intraocular neoplasia
Aphakic
Malignant/ciliary block
Melanocytic/Pigment cell proliferation
Pigment cell exfoliation/anterior uveal cysts
Giant retinal tears (Schwartz-Matsuno syndrome)
Anterior chamber silicone oil
Postoperative ocular hypertension

C. Congenital glaucoma
Pectinate ligament dysplasia
Goniodysgenesis

Figure 4. Causes of primary, secondary and congenital glaucoma (from: Veterinary Ophthalmology, Gelatt, 2013).

Medical treatment is aimed at maintaining the IOP below 20 mmHg, because values between 20 and 25 mmHg still cause damage in the eye. The therapy is aimed at three different aspect of the IOP. First, the reduction of aqueous production with the use of carbonic anhydrase inhibitors and beta-adrenergic blocking agents. Second, the improvement of outflow by stimulation of parasympathic nerve endings or prostaglandin analogues. And third, the reduction of intraocular volume with the use of hyperosmotic agent IV or PO. Surgical treatment is also possible, but none of them have proved to be optimal.[48]

Aim of this study

In dogs, little information is available about the tear production and intraocular pressure during the neonatal period and when these parameters reach adult values. Human literature presented some controversy regarding tear production in newborns. In 1944, Mutch reported that infants do not produce tears until a few weeks after birth.[49] In 1955, Sjögren reported that 13% of newborns do not produce any tears, and 65% had subnormal values.[50] More recent studies show that newborns do produce tears, but have lower STT1 and STT2 values.[51] Also, weight and post-conceptional age positively correlated with tear production.[51-53] In veterinary literature, Broadwater performed single STT1 and STT2 measurements in puppies of various breeds <6 months of age. He reported a significant effect of age, weight, and gender on STT1 results and a significant effect of weight and gender on STT2 results. Also, STT1 measurements reached adult values at the age of 9-10 weeks.[54] Da Silva compared STT1 values of four-week-old puppies with adults, using a modified Schirmer Tear Test. This study reported that canine neonates have reduced total tear production at the age of four weeks, compared to adult values.[20] Regarding the IOP, human data suggests that newborns are born with an IOP which declines in the first weeks of life, after which it gradually increases to adult values in several years.[55-58] This could be due to different measuring methods, different positions of the subjects, or the use of an anesthetic.

Mughannam *et al.* measured the IOP in Labrador Retriever dogs using a Tonopen XL at the age of 6 weeks and one year later. With a mean of 13.4 and 14.9 at the age of 6 weeks and one year, respectively, there was a marginal difference but not statistically significant.[59]

Diseases such as keratoconjunctivitis sicca sometimes need lifelong therapy with lacrimomimetics and lacrimostimulants and several forms of uveitis and glaucoma need an aggressive approach when treating. Therefore, when a puppy is presented in a clinical situation, it is necessary to use and interpreted diagnostic tools as STT and tonometry in a correct way. Previously mentioned studies provide an introduction in the knowledge about tear production and intraocular pressure in canine neonates, but do not provide sufficient information to use these diagnostic tools in the correct way. Therefore, the aim of this study is to measure the development of basal and total tear production and intraocular pressure in canine neonates.

Material and Methods

Animals

Eight healthy Beagle puppies from the same litter were included in this study, four male and four female. The dogs were bred as a replacement of dogs used for educational purposes at the Faculty of Veterinary Medicine at Utrecht University. Each individual dog was frequently observed to witness the moment of the eyes opening.

Each dog underwent a complete ophthalmic examination, which included fluorescein staining, a slit-lamp examination (SL-15®, Kowa Optimed Inc., Torrance, CA, USA), and indirect ophthalmoscopy (Video Omega 2C®, Heine Optotechnik, Herrsching, Germany). Dogs with signs of ocular disease that could affect tear production or intraocular pressure, were excluded from the study.

Figure 5. Performing the STT1 on a Beagle puppy at the age of 2 weeks using the Schirmer Tear Test.

Experimental protocol

Schirmer Tear Test 1 (STT1), Schirmer Tear Test 2 (STT2) and intraocular pressure (IOP) were measured once a week. Starting at the age of 2 weeks, 1-3 days after the eyes opened, until the age of 12 weeks. Before each research moment, a slit-lamp examination was performed to check for signs of ocular disease. After this, for the STT 1, a standardized tear strip (Schirmer Tear Test, Intervet Inc., Roseland, NJ, USA) was placed in the middle to lateral third of the ventral conjunctival sac of the right eye for 60 seconds (Figure 5). Immediately after placing the strip in the right eye, the same procedure was performed for the left eye . After completion of the STT1, the IOP was measured in both eyes, starting on the left, using a rebound tonometer (TonoVet®, Icare, Helsinki, Finland) (Figure 6). Subsequently, a drop of lidocaine 4% (ASTfarma B.V. Oudewater, The Netherlands) was placed in both eyes. Five minutes after application of the lidocaine, the residual volume in the ventral conjunctival sac was absorbed using two ophthalmic Sugi® sterile mounted swabs per eye. Immediately after this, the STT2 was performed using the same procedure as the STT1. All STT strips came from the same lot number to avoid any variation in the strips. All research moments were performed in the morning and the dogs were examined in a fixed order. At the age of 8 weeks, the male dogs were replaced to new owners outside the Faculty of Veterinary Medicine. For the remaining weeks of the study, the owners returned to the clinic with their dogs for each research moment.

Figure 6. Measuring the IOP on a Beagle puppy at the age of 2 weeks using a TonoVet® rebound tonometer.

Statistical analysis

All of the data (STT1, STT2, IOP, weight, gender, and age) was entered into a spreadsheet and descriptive statistics were performed to calculate means and standard deviations. The One-Sample Kolmogorov-Smirnov Test was used to test for normal distribution of the data. Paired samples t-tests were used to test for left-right and STT1-STT2 differences at every research moment. A one way ANOVA with repeated measures was performed to compare the effect of time on STT1, STT2, IOP values. Results were considered significant if $P = <0.05$. All statistical analyses were made using IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL, USA). Graphs were generated using Sigmaplot version 12.0 (Systat Software Inc., Germany).

Results

The eyes opened at 11 (n=2), 12 (n=5), and 13 (n=1) days of age. All dogs showed mild to moderate diffuse corneal edema, a non-pigmented iris, and pupillary membranes at the age of two weeks. The corneal edema had disappeared at the age of three (n=1), four (n=2), and five (n=5) weeks. The iris was fully pigmented at the age of six (n=4) and seven (n=4) weeks. The pupillary membranes were fully atrophied at the age of four (n=4) and five (n=2) weeks and two dogs showed persistent pupillary membranes.

STT2 was lower than STT1 (P = <0.05) at every time point. No difference was found between values from OD and OS (P = <0.05). Consequently, we chose to perform further statistics on readings from the right eyes, as the eyes are not independent. Based on the measurements of 8 right eyes at the age of 2 to 12 weeks, the results were as follows:

STT1

The means with S.D. of every week are presented in Table 1 and Figure 7. The mean STT1 started at 5.13 ± 2.45 at the age of two weeks and increased significantly every week until 20.99 ± 1.66 at the age of 9 weeks (P = <0.05), after which it reached a plateau phase with no significant changes. There was a significant effect of time on STT1 (P = <0.001) with a partial eta squared of 0.829, which demonstrates a large effect. Gender did not affect STT1 at any age.

STT2

The means with S.D. of every week are presented in Table 1 and Figure 8. The mean STT2 started at 2.28 ± 1.64 at the age of two weeks and increased significantly every week until 14.94 ± 4.26 at the age of 10 weeks *(*P = <0.05), after which it reached a plateau phase with no significant changes. There was a significant effect of time on STT2 (P = <0.001) with a partial eta squared of 0.792, which demonstrates a large effect. Gender affected STT2 at the age of 2 (P = 0.005) and 7 (P = 0.008) weeks, with higher values in male dogs.

IOP

The means with S.D. of every week are presented in Table 1 and Figure 9. The mean IOP started at 5.63 ± 2.67 at the age of two weeks and increased significantly every week until 12.63 ± 1.77 at the age of 6 weeks (P = <0.05), after which it decreases significantly between 9-10 weeks of age, increases significantly between 10-11 weeks of age and decreases significantly again between 11-12 weeks. There was a significant effect of time on IOP (P = <0.001) with a partial eta squared of 0.683, which demonstrates a large effect. Gender affected IOP at the age of 10 (P = 0.01) and 12 (P = 0.018) weeks, with higher values in male dogs.

	n	STT1 (mm/min)	STT2 (mm/min)	IOP(mmHg)
2 weeks	8	5.13 ± 2.45	2.28 ± 1.64	5.63 ± 2.67
3 weeks	8	7.25 ± 1.98	4.25 ± 2.51	9.50 ± 1.92
4 weeks	8	11.75 ± 2.19	7.38 ± 2.97	11.13±1.25
5 weeks	8	14.25 ± 2.20	8.06 ± 2.81	10.50 ± 1.77
6 weeks	8	14.69 ± 2.34	10.31 ± 3.28	12.63 ± 1.79
7 weeks	8	17.75 ± 2.05	11.31 ± 4.17	12.88 ± 1.64
8 weeks	8	18.56 ± 4.20	10.99 ± 3.46	12.00 ± 2.73
9 weeks	8	20.88 ± 1.66	12.13 ± 3.29	13.25 ± 1.75
10 weeks	8	18.88 ± 2.80	14.94 ± 4.26	11.50 ± 1.93
11 weeks	8	19.63 ± 4.83	16.44 ± 2.97	14.75 ± 1.39
12 weeks	8	21.44 ± 2.85	15.75 ± 3.02	12.63±1.85

Table 1. Values (mean ± S.D.) for the STT1, STT2 and IOP at 2 to 12 weeks of age

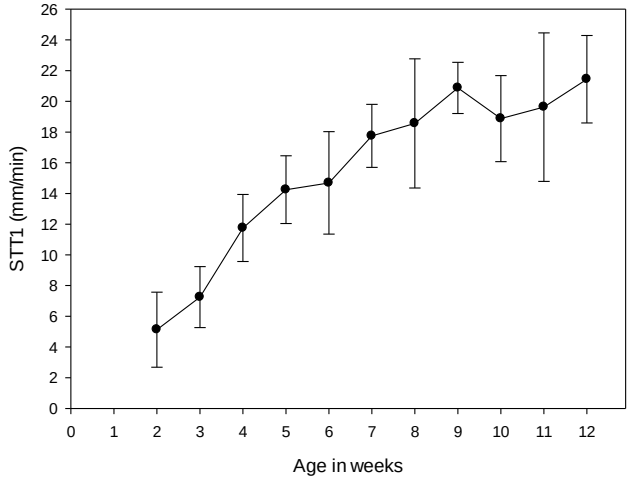

Figure 7. Mean STT1 values from the age of 2 to 12 weeks

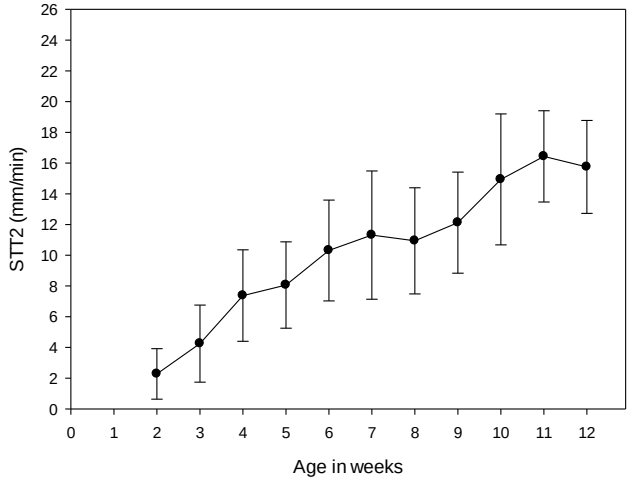

Figure 8. Mean STT2 values from the age of 2 to 12 weeks

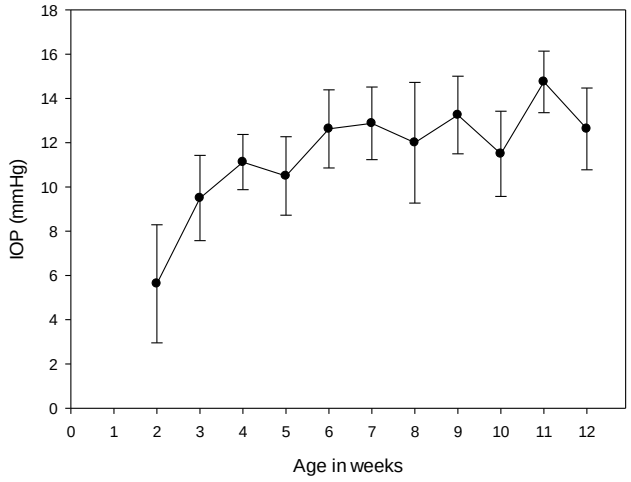

Figure 9. Mean IOP values from the age of 2 to 12 weeks

Discussion

Results of the current study show that canine neonates have reduced basal and total tear production and intraocular pressure after the palpebral fissures open, compared to adult values. Values for the STT1, STT2, and IOP increase significantly until the age of 9, 10, and 6 weeks respectively, with another significant increase for the IOP between 10-11 weeks of age. There was a significant effect of age on all parameters. These results are similar to two previous studies with canine neonates where reduced values for the STT1 and STT2 around the age of 3-4 weeks were found.[20,54] One of these noted a significant effect of age (STT1 and STT2) and reached adult values at the age of 9-10 weeks,[54] which corresponds with our findings. The other study found a mean value of 13.6 ± 3.07 mm/min at the age of 4 weeks (STT1).[20] But total tear production was measured using a modified STT, which showed no significant difference when compared with normal STT within their control group, but led to significant different testing results *in vitro* when compared to the normal testing strip.[60] Still, these findings are close to our measurements at the age of 4 weeks. The lower IOP values are comparable with results in young children and neonates.[57,58] One study in dogs noted that there was no significant difference in IOP values between puppies and adult dogs.[59] But their measurements were at the age of 6 weeks, when our dogs reached adult values.

The reason for these lower values is not fully understood. Several ocular structures in the dog continue to develop postnatally.[61] Therefore, the nerves that innervate the lacrimal gland, ciliary body and other ocular structures may not be fully developed at birth. Decreased corneal sensitivity is known to decrease reflex tearing,[62] so underdeveloped corneal innervation could lower reflex tearing, leading to a lower total tear production. Several intraocular structures, including the ciliary body, gradually increase in size with time, as visualized with ultrasonography.[63] This might be an indication that these structures continue to develop their production size. Furthermore, electron microscopy of tissue from the iridocorneal angle revealed maturation of the pectinate ligament from the third to eighth week of life. The uveal meshwork didn't appear mature until 8 weeks of age.[64] This underdevelopment could lead to a greater outflow of aqueous humor, resulting in a lower IOP.

Most studies find no effect of gender on STT1, STT2 and IOP.[15,16,59,65] We found a significant effect of gender on the STT2 at the age of 2 and 7 weeks, and on the IOP at the age of 10 and 12 weeks. This could be caused by the weight of the puppies, as the males were heavier than the females, on average. Also, at the age of 8 weeks, the male puppies were replaced to a new owner in a different environment. This led to different external factors, like temperature and handling, which possibly influenced the measurements. We attempted to exclude other external factors that could influence tear production and intraocular pressure. The dogs were housed together and fed and cleaned at a constant interval, but infrequent visits and petting by caretakers could not be prevented. Even though the puppies were very lively as they became older, measurements were taken when they were as calmly as possible. This was to prevent any effect of stress which could influence the innervation of lacrimal glands.[66] Also, pressure on the jugular vein was avoided, measurements were taken in the same order and at the same time in the morning (within 3 hours) to prevent different results due to the circadian rhythms of tear production and IOP.[67] Tear production shows the lowest values in the morning, as opposed to IOP, which is at its highest during the morning.[67] These physiological variations can lead to different readings when tear production and IOP are measured on a different time of the day.

Aqueous tear production was measured using standardized STT strips instead of a mSTT or the PRT tear test. The mSTT was created to fit in the smaller palpebral fissures of young and/or small animals[20] but is not commercially produced and has to be created from a normal STT. This leads to potential errors in readings due to test strips which could lack uniformity. The PRT tear test is also created to fit in smaller palpebral fissures, but is not widespread commercially available. We experienced to trouble with the standardized STT and the smaller palpebral fissures of the dogs and therefore chose to use this method.

This study measured the IOP using a TonoVet®. This choice was made because of the advantages this method has. The TonoVet® is developed for use in small-eyed laboratory animals such as rats and mice,[41] is evaluated in many other species, including dogs, and doesn't require the application of a topical anesthetic.[68] *In vivo* comparison to the golden standard in tonometry (the Mackay-Marg applanation tonometer) showed no significant difference in healthy canine eyes.[69] However, small errors from incorrect use of the TonoVet® or incorrect fixation of the dogs could have led to readings which are too high or too low.

In conclusion, STT1, STT2 and IOP values are significantly lower than adult values after birth. There was a significant effect of time on all 3 parameters. STT1 values significantly increased until 9 weeks of age, STT2 until 10 weeks of age, and IOP until 6 and between 10-11 weeks of age. These lower values show that STT outcomes and tonometry readings in young puppies must be interpreted in the right way and reassessed at an older age, before therapy is started. Still, this study measured these parameters in only 8 subjects from the same breed and same litter. In order to obtain better results which could be extrapolated to the rest of the population, more extensive research in different breeds should be performed.

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