International Journal of Enhanced Research in Science Technology & Engineering, ISSN: 2319-7463
Vol. 2 Issue 6, June-2013, pp: (51-54), Available online at: www.erpublications.com

Repeated axial length measurements in *Gallus gallus domesticus*

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Abstract: This study was undertaken to establish a non-invasive axial length measurement in *Gallus gallus domesticus* to enable repeated measurements of various ocular components. Three chicks were obtained from a local hatchery and raised in stainless steel brooders for a total of 9 days. The axial lengths of anaesthetized chicks were measured by high frequency ultrasonography in both eyes at day 3, day 6 and day 9. The measurement from day 3 to day 6 showed irregular patterns in ocular components growth compared to the following three days of measurement that showed increased ocular component thickness growth except for decreased pattern of sclera and retinal thickness. This repeated technique used to measure various ocular components in-vivo has the advantages of fast and non-invasive approach, cost effective due to less sample needed and suitable for longitudinally studies. This non-invasive axial length measurement technique was suggested for future research to investigate the peripheral refraction.

Keywords: axial length, chick, *Gallus gallus domesticus*.

Introduction

Chickens were the most robust animal model for experimental myopia studies [1]. Responses to visual cues tend to be more consistent compared to other animals [2], [3]. The chick eye is similar to humans in that it was cone-dominated and accommodation occurs by deformation of the lens. However, many other differences exist, such as skeletal intraocular muscles [4], [5], tetrachromatic retina [6] and the distribution of receptors and ganglion cells are dense in more than one area of the retina, at the area centralis, but also in the superior-temporal retina [7]. The shape of the chick eye is prolate, tending to be flatter in the axial dimension than in the equatorial direction [8–12]. The popular method to measure axial lengths was to enucleate the eye. However, the method was susceptible to freezing artefact, where various tissues shrank at least 3% [13], or, if the eye was fixed, fixation artefact. The relative data was valid with the assumption that all parts of the eye freezed or were fixed at similar rates, but whether freezing occurs equally across the globe remain inconclusive. This study was undertaken to establish a non-invasive axial length measurement in *Gallus gallus domesticus* as alternative for repeated measurement of various ocular components.

Materials and Methods

Three chicks were obtained from a local hatchery and raised in stainless steel brooders for a total of 9 days. Chicks were fed chick starter and water *ad libitum*. The temperature of the brooder was maintained at 32°C with a light regime of 14 hr light/10 hr dark, under fluorescent lighting. At day 3, the chicks were anaesthetized with 1% isoflurane in oxygen prior to ultrasound measurements. Lid retractors were used to keep the eyes open. Axial lengths were measured by high frequency ultrasonography in both eyes. The measurements were recorded on day 3, day 6 and day 9.

Results

The raw data of ocular growth for the chicks used in day 3, day 6 and day 9 was showed in Table 1. However, the raw data need to be normalised for represent the better actual ocular growth data with our sample before it could be used to analyse. The normalised data was detailed in Table 2. The formula for the normalization process as follow:

Normalised value, $N_n = X_n – (X_a – X_{av})$
$X_{n/a/av}$ = value of ocular component thickness or depth
n = measurement day
a = first measurement day
av = average of all first measurement day

Sample:
Scleral thickness at day 6, X_6 = 0.100477 mm
X_a = 0.085137 mm
X_{av} = 0.086607083 mm
Thus, normalised value of scleral thickness at day 6,
$N_6 = X_n – (X_a – X_{av})$
$= X_6 – (X_a – X_{av})$
= 0.100477 mm – (0.085137 mm – 0.086607083 mm)
= 0.101947083 mm

Table 1: Ocular components of chick raw data (mean ± SE) at day 3, day 6 and day 9

Ocular components	Measurement in mm					
	Day 3		Day 6		Day 9	
	Right	Left	Right	Left	Right	Left
Scleral thickness	0.08 ± 0.007	0.09 ± 0.005	0.08 ± 0.014	0.07 ± 0.027	-0.04 ± 0.055	-0.05 ± 0.031
Choroidal thickness	0.14 ± 0.029	0.11 ± 0.010	0.14 ± 0.012	0.13 ± 0.006	0.21 ± 0.041	0.20 ± 0.022
Retinal thickness	0.26 ± 0.012	0.21 ± 0.019	0.27 ± 0.013	0.28 ± 0.004	0.26 ± 0.004	0.25 ± 0.011
Vitreous chamber depth	5.21 ± 0.101	5.17 ± 0.104	5.09 ± 0.152	5.09 ± 0.072	5.49 ± 0.128	5.45 ± 0.146
Lenticular thickness	1.73 ± 0.009	1.72 ± 0.019	1.87 ± 0.028	1.84 ± 0.015	2.11 ± 0.103	2.09 ± 0.109
Anterior chamber depth	1.14 ± 0.047	1.19 ± 0.009	1.17 ± 0.051	1.21 ± 0.031	1.23 ± 0.050	1.27 ± 0.030

Table 2: Ocular components of chick normalised data (mean ± SE) at day 3, day 6 and day 9

Ocular components	Measurement in mm					
	Day 3		Day 6		Day 9	
	Right	Left	Right	Left	Right	Left
Scleral thickness	0.09	0.09	0.09 ± 0.016	0.06 ± 0.032	-0.03 ± 0.058	-0.06 ± 0.028
Choroidal thickness	0.12	0.12	0.12 ± 0.019	0.15 ± 0.014	0.12 ± 0.065	0.22 ± 0.012
Retinal thickness	0.23	0.23	0.24 ± 0.011	0.30 ± 0.023	0.24 ± 0.015	0.28 ± 0.010
Vitreous chamber depth	5.19	5.19	5.07 ± 0.074	5.11 ± 0.052	5.48 ± 0.063	5.47 ± 0.044
Lenticular thickness	1.73	1.73	1.86 ± 0.033	1.85 ± 0.020	2.11 ± 0.104	2.09 ± 0.108
Anterior chamber depth	1.16	1.16	1.19 ± 0.071	1.19 ± 0.022	1.26 ± 0.033	1.25 ± 0.023

The changes of six ocular components in day 3, day 6 and day 9 were summarised in Figure 1. Scleral thickness initially decreased in the left eye but increased in the right eye for the first 3 days before decreased and levelled off (1A). Choroidal thickness initially increased in the left eye but decreased in the right eye for the first 3 days before increased and levelled up (1B). Retinal thickness initially increased before decreased and levelled off (1C). Vitreous chamber depth (VCD) thickness initially decreased before increased and levelled up (1D). Lenticular thickness increased for both left and right eyes (1E). Anterior chamber depth (ACD) increased for both left and right eyes (1F).

Discussion

The repeated technique used to measure various ocular components in-vivo has the advantages of fast and non-invasive approach, cost effective due to less sample needed and the same animal can be used for longitudinally studies. The freeze technique was required to sacrifice the animal and the need of histology preparation section before the data can be collected and analysed. A high correlation was found previously between high-frequency ultrasonography and laser interferometry techniques although both techniques cannot replace each other due to different types of measurement principle [14]. This high-frequency ultrasonography allowed fine (8–20 μm) resolution of anterior chamber depth, vitreous chamber depth, choroidal thickness and axial length. The accuracy of these measurements depend on the consistency of the wave forms representing the various interfaces at the back of the eye, and the ability to consistently choose the equivalent peaks within the wave forms [15]. The measurement from day 3 to day 6 showed irregular patterns in ocular components growth compared to the following three days of measurement that showed increased ocular component thickness growth except for decreased pattern of sclera and retinal thickness. The values of ocular components thickness or depth found from this research were supported by data using histological sections [16] and also by data using laser interferometry [14]. Thus, this non-invasive axial length measurement technique was recommended for future research to investigate the peripheral refraction.

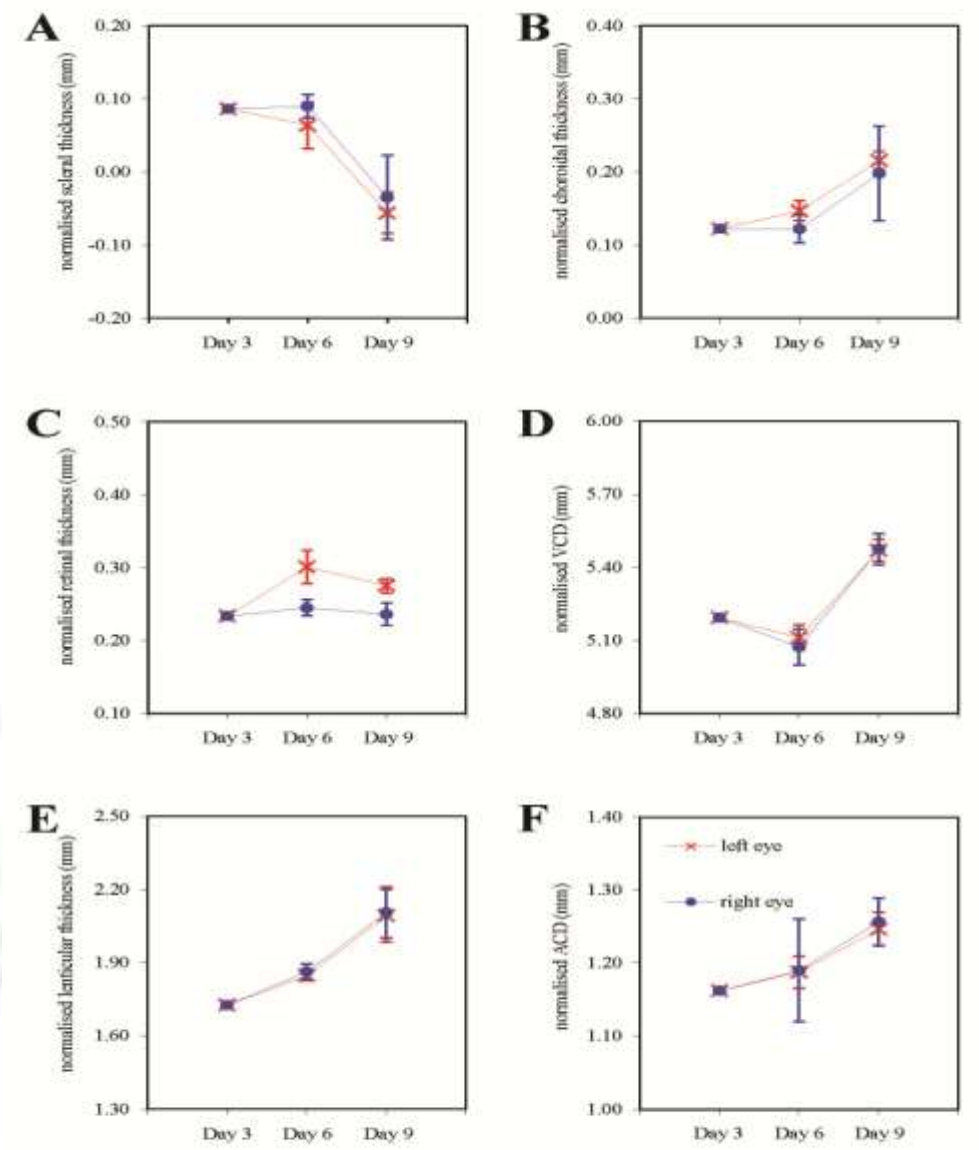

Figure 1: Changes of ocular component at day 3, day 6 and day 9; (A) Sclera, (B) Choroid, (C) Retina, (D) Vitreous chamber depth, (E) Lenticular and (F) Anterior chamber depth. Data were expressed as the mean ± SE. (×) – left eye, (●) – right eye.

Acknowledgments

The research was supported by Fundamental Research Grant Scheme (FRGS), 600-RMI/ST/FRGS 5/3/Fst (46/2011). The data was collected under the supervision of Assistant Professor Dr. Vivian Choh at live-animal housing facility in the School of Optometry and Vision Sciences at the University of Waterloo, Canada.

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