Altern. Animal Test. EXperiment. 7 (2-3),59-66 (2001)

Color Doppler Echocardiograms Can Measure Hemodynamic Changes of Cardiovascular Systems in Chick Embryos

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Abstract

The aim of the present study was to evaluate the hemodynamic changes of chick embryos by using color Doppler echocardiography, which is one of the essential tools used to determine cardiovascular abnormalities in humans. Fertylisin (FTS), which induces cardiovascular abnormalities in rodents, was injected into the air sac of fertile eggs of White Leghorn chickens on the 2nd day of incubation and the chick embryos were observed grossly and physiologically on the 18th day of incubation. A high frequency of cardiovascular abnormalities including double aortic arch etc. was observed in the FTS-treated chick embryos . Color Doppler flow imaging echocardiograms and first Fourier transformation (FFT) patterns of the heart and aorta of FTS- or calmelose (control)-treated chick embryos were recorded on the 18th day of incubation by using echocardiography with 5 MHz transducer. The values of maximum velocity (MV), flow integration (FI) and acceleration time (AT) obtained from the FFT patterns in FTS-treated chick embryos were decreased compared with those of the control embryos, but the heart rate was increased. These findings suggest that color Doppler echocardiography could be applicable to evaluate the anatomical and physiological abnormalities of chick embryonic cardiovascular systems.

Keywords: *chick embryo, Doppler echocardiography, cardiovascular anomaly, fertylisin*

Introduction

We studied the toxicological and pharmacological effects of cardiovascular drugs by using physiological techniques in chick embryos, to help advance the development of animal alter-

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native methods (Miyazaki et al., 1994, Sugiyama et al., 1996, 1997, 2000, Yoshiyama et al., 1997). Chick embryonic hearts have been studied in the field of embryology and teratology, because they have similar atrioventricular form and develop through similar process to rodents and human (Butler and Juurlink, 1987). However, the hemodynamic functions in chick embryos have scarecely been studied.

Color Doppler echocardiography is one of the fundamental diagnostic tools in the field of clinical cardiology. It is now generally accepted as the most reliable and easy method to evaluate abnormalities of the heart and vessels in clinical medicine (Shimada and Ishikawa, 1978, Come, 1985). We have tried to record color Doppler images from the chick embryonic heart since 1990 (Saito et al., 1992) and recently obtained stable and good tracings. In the present study, we demonstrate the normal and abnormal tracings obtained from the chick embryos, with cardiovascular abnormalities induced by injection of bis (dichloroacethyl) diamine, fertilysin (FTS). FTS is an agent that inhibits polyamine synthesis and induces severe cardiovascular abnormalities in mammals (Corn et al., 1984, Okamoto et al., 1984). We have previously been experienced in many cases with cardiovascular abnormalities in chick embryos induced by FTS injection. When these FTS-induced anomalies in chick embryos were compared with those in rats treated with the same agent, in the abnormal sites of cardiovascular anomalies, *i.e.* double aortic arch, no difference was found between two species (Sugiyama et al., 1992, Okamoto et al., 1984).

In the present study, we demonstrated color Doppler images and first Fourier transformation (FFT) patterns of normal and abnormal cases with respect to anatomical findings. Furthermore, we estimated whether color Doppler echocardiography can be used to examine cardiac functions in chick embryos as an animal alternative method.

Materials and Methods

Eggs and Incubation

Fertile eggs of White Leghorn chicks were obtained from Ohmiya Poultry Science (Saitama, Japan). They were incubated at 37.5 ± 0.2℃ in a relative humidity of about 65.5%, turned automatically every hour in an egg incubator (P-1 type, Showa Incubator Laboratory, Saitama, Japan).

Drugs used

Fertilysin (FTS, Aldrich Chemical Co., USA) was commercially purchased and suspended to desired concentrations in 0.5% calmellose (CMC, Wako Pure Chemical Industries. Ltd., Osaka, Japan) and sterilized by an autoclave. Urethane and α-chloralose (Sigma Chemical Co., St. Louis, Mo, USA) were commercially purchased and dissolved in saline.

Injection of FTS into fertile eggs

FTS was injected at concentrations of 0 (CMC), 1, 5, 10, 25, 50, 100 or 500 μg/egg into the air sac of fertile eggs on the 2nd day of incubation. All eggs were candled daily for viability. On the 18th day of incubation, surviving embryos were sacrificed and observed grossly using stereomicroscopy. The value of 50% lethal dose (LD_{50}) of FTS on the 18th day of incubation was calculated using the method of Litchfield-Wilcoxon (Litchfield and Wilcoxon, 1949).

Color Doppler echocardiogram recording systems for chick embryos

The surviving 18th day-embryos treated with 0, 25 or 50 μg/egg of FTS were used for this experiment. First, a mixture (0.1 mL/egg) of urethane (45 mg/mL) and α-chloralose (450 mg/mL) was injected into the air sac of egg. Twenty minutes after injection of the anesthetic, the shell around the air sac of each egg was removed using scissors and then filled with warm jelly (Aquasonic 100, Patker Lab., USA)

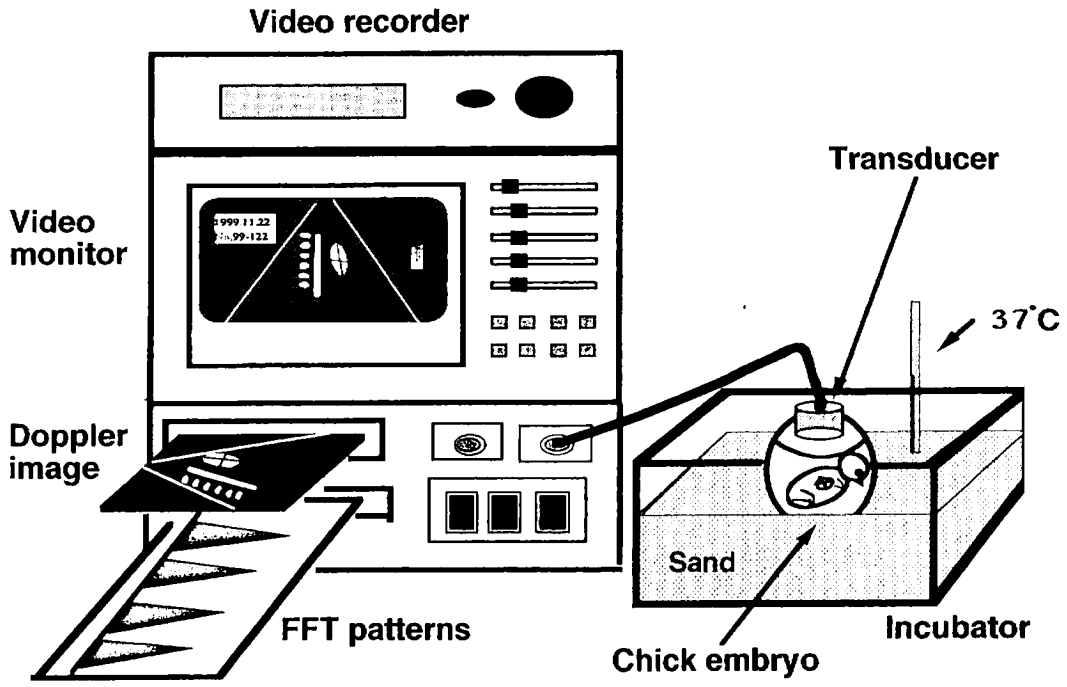

Fig. 1 Schema of the color Doppler echocardiogram recording system for chick embryo in egg shells

on the outer membrane to obtain good coupling with a transducer. Then color Doppler images and FFT patterns of the heart and aorta of the 18th day-chick embryos were recorded using a YHP 77520A system (Yokokawa Co., Tokyo, Japan) with a 5 MHz transducer (Fig. 1).

We analyzed the FFT patterns obtained from the descending aorta as a method to examine the cardiovascular dynamics of the chick embryos. On the color Doppler images, red signals represent upward blood flow and blue signals represent downward flow inside the heart and aorta. Dark colors show increased flow volume and light colors show decreased flow volume.

Since the descending aorta run in parallel with the vertebrae we used the vertebrae as a starting point to determine the location of the heart and aorta. Values for the maximum velocity (MV), acceleration time (AT), flow integration (FI) and R-R intervals were calculated using a computer from the FFT patterns. Electrocardiograms (ECG) and heart rates (HR) were also recorded simultaneously (not shown in Fig. 1). All procedures were performed in the condition that the eggs were maintained at 37℃.

Results

Mortality of chick embryos treated with FTS

As shown in Fig. 2, the total mortality of chick embryos treated with FTS on the 2nd day of incubation and observed on the 18th day of incubation increased in a dose-dependent manner. When the relation between the death time of embryos and the dosage of FTS was observed, the mortality of embryos was highest within 3 days after injection and decreased gradually with incubation. LD_{50} value for a single injection of FTS was 40.5 ±11.3 μg/egg on the 18th day of incubation.

Cardiovascular abnormalities in chick embryos induced by injection of FTS

Cardiovascular abnormalities including double aortic arch, single and complex retarda-

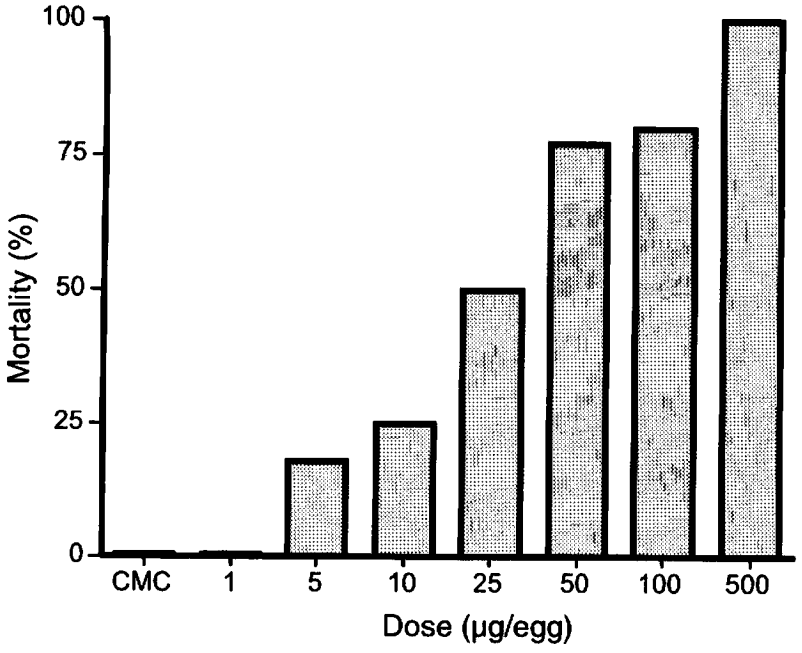

Fig. 2 Mortality in fertilysin-treated chick embryos on the 18th day of incubation
Fertilysin was injected into the air sac of eggs on the 2nd day of incubation and the chick embryos were observed on the 18th day of incubation.

Table.1 Frequency of cardiovascular anomalies in chick embryos induced by fertylisin

	Dose (μg /egg)							
	CMC	1	5	10	25	50	100	500
No. of eggs used	10	12	12	12	12	30	30	30
No. of surviving embryos	10	12	10	9	6	7	6	0
Total no. of abnormal embryos	0	0	6	8	6	7	6	0*
Percent of abnormalities	0	0	60	89	100	100	100	0*
Classification of cardiovascular anomalies	No. of abnormalities							
Abnormal positions of cardiovascular systems	0	0	4	5	5	6	5	0*
Overriding aorta	0	0	2	2	1	1	1	0*
Double aortic arch	0	0	0	2	2	3	4	0*

Fertylisin was injected into the air sac of eggs on the 2nd day of incubation and the chick embryos were observed on the 18th day of incubation.
*; abnormalities of the cardiovascular system in chick embryos could not be found because of too early death.

tion of the pulmonary artery and branchial arteries or common carotid artery were observed in 60% of embryos by injection of 5 μg of FTS and in 100% by 25 μg or more (Table 1).

Doppler image and anatomical findings obtained from an abnormal chick embryo treated with FTS. Hemodynamic findings obtained from the FFT patterns show in Fig. 5. The values of MV, AT and FI in aortae of FTS-treated embryos were lower compared with those in controls. However, the HR was more rapid than that of the controls.

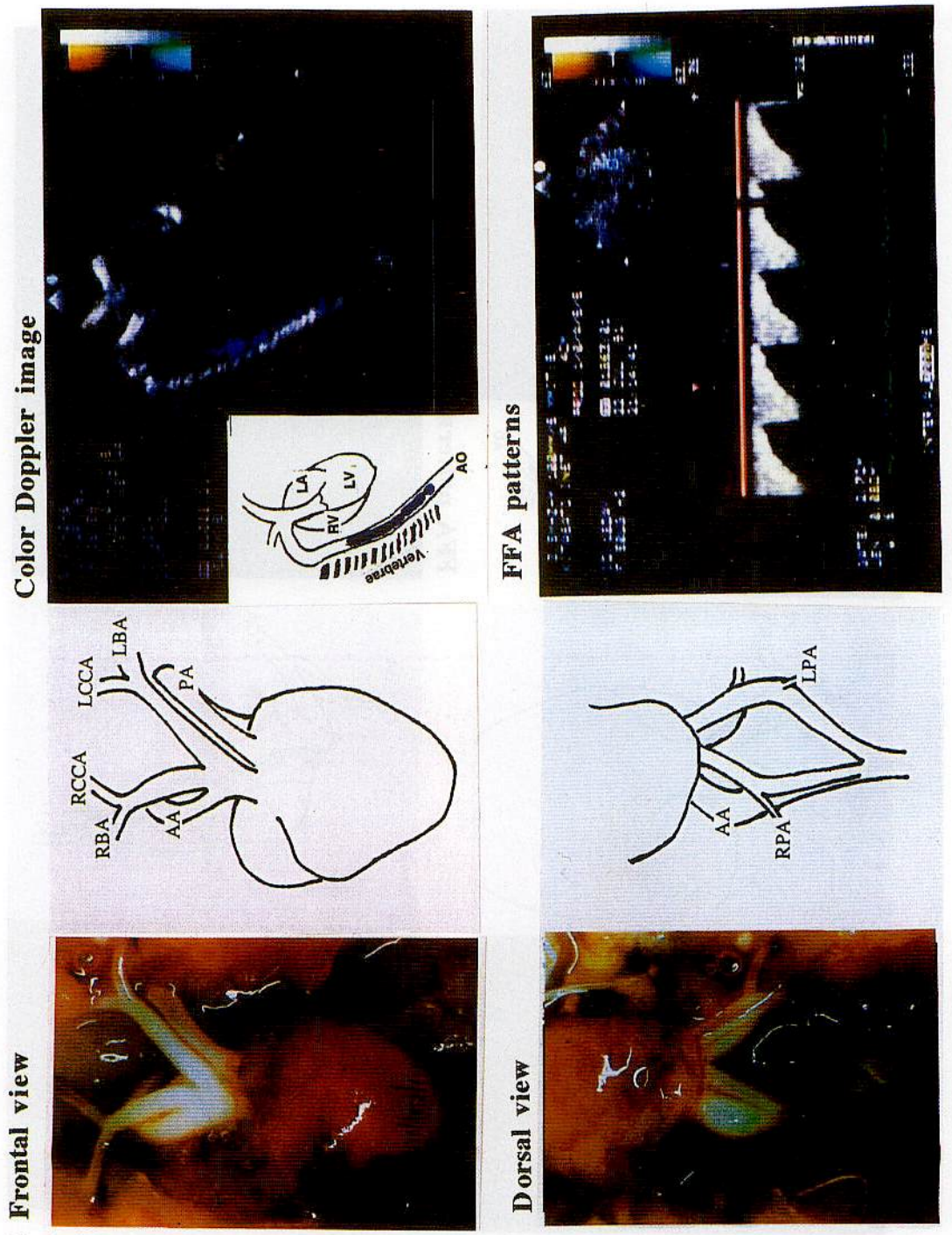

Fig. 3 Color Doppler images and FFT patterns from normal cardiovascular systems in 18 day-chick embryos and anatomical findings

AO: Aorta, RV; Right ventricular, LA: Left LV; Left ventricular.

Frontal view: AA; Aortic artery, PA; Pulmonary artery, RBA; Right branchial artery, LBA; Left branchial artery, RCCA; Right common carotid artery, LCCA; Left common carotid artery.

Dorsal view: AA; Aortic artery, RPA; Right pulmonary artery, LPA; Left pulmonary artery.

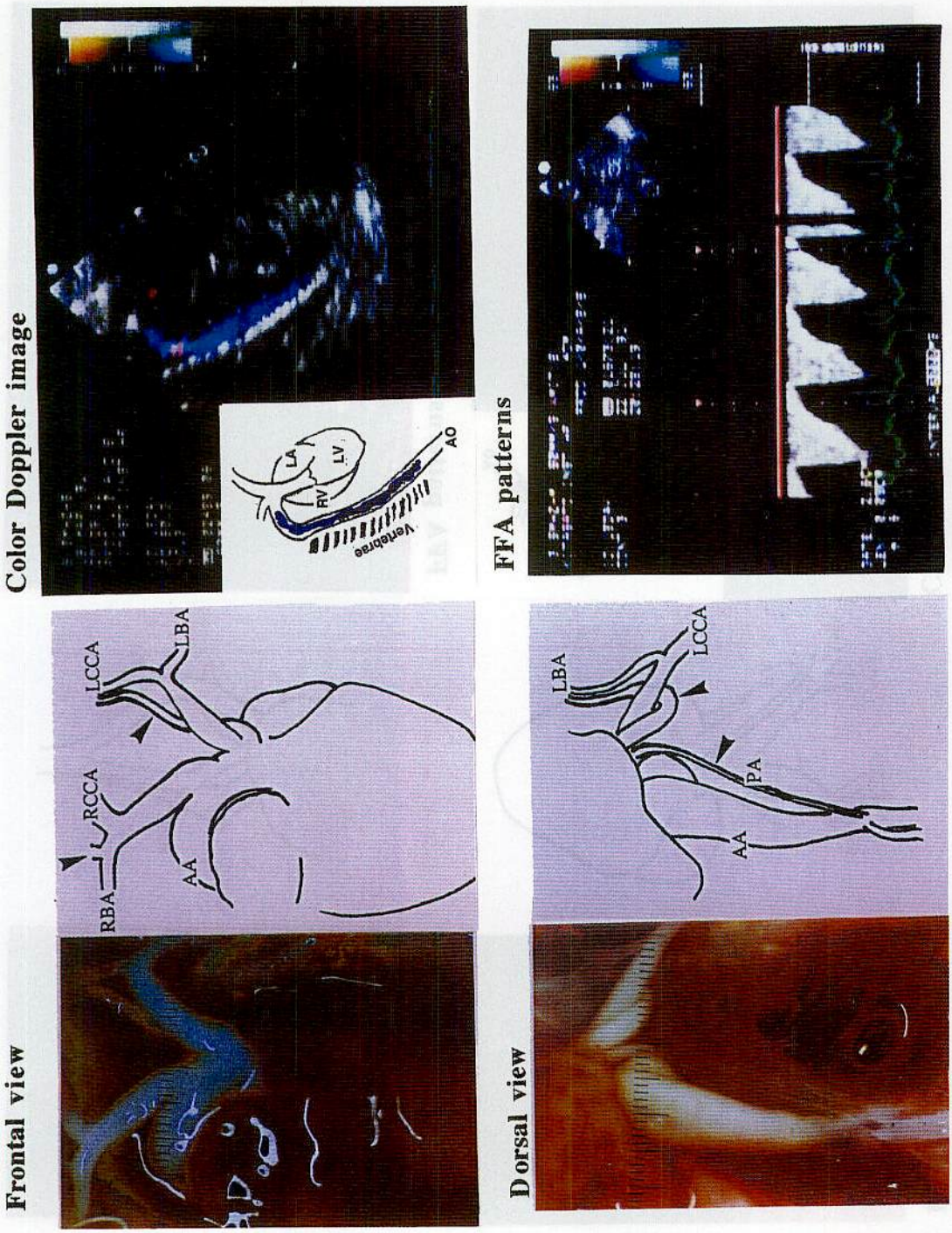

Fig. 4 Color Doppler images and FFT patterns from cardiovascular anomalies in the 18 day-chick embryos induced by fertilysin.

Fertilysin (50μg/egg) was injected into the air sac of eggs the 2nd day of incubation and the chick embryos were observed on the 18th day of incubation. Arrows in figures indicate cardiovascular anomalies.

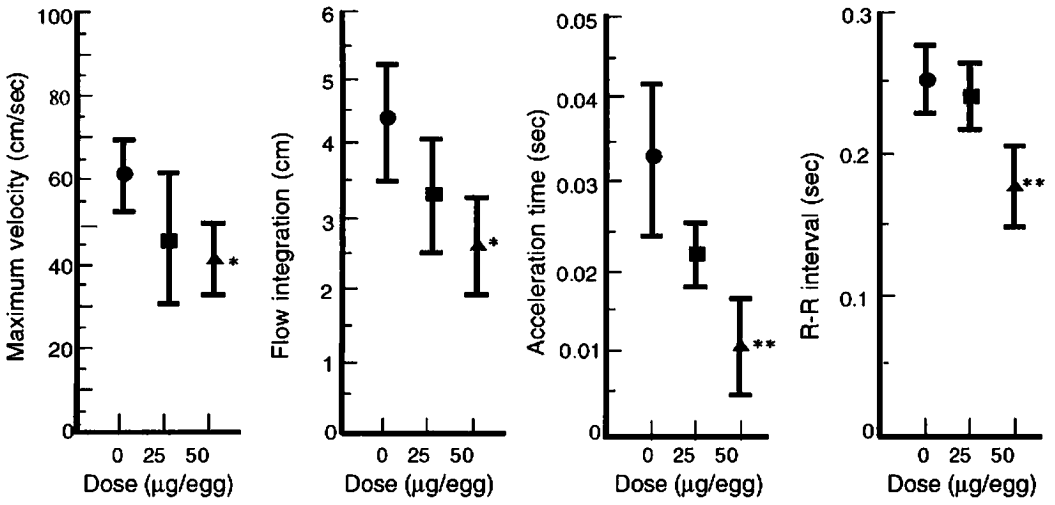

Fig. 5 Hemodynamic findings obtained from FFT patterns in chick embryos treated with fertilysin

0 μg/egg (CMC, ●), and 25 μg/egg (■) and 50 μg/egg (▲) of fertilysin were injected into the air sac of eggs on the 2nd day of incubation and the chick embryos were observed using color Doppler echocardiography on the 18th day of incubation. Data are the mean ± SE. *, ** Significantly different from the control (CMC) at $p<0.05$, $p<0.01$, respectively.

Discussion

In the present study, we attempted to evaluate the usefulness of the color Doppler echocardiography as a diagnostic tool for the chick embryonic heart. It was found that color Doppler echocardiography is very useful tool to investigate hemodynamic changes in chick embryos. Chick embryos getting an injection of FTS showed anomalies of their cardiovascular systems.

When FTS was injected into the air sac of fertile eggs on the 2nd day of incubation, the mortality and frequency of cardiovascular anomalies of the 18th day-chick embryos increased with increasing dose of FTS. The cardiovascular abnormalities observed were similar to those found in rats (Okamoto et al., 1984). This finding suggests that the heart and atrioventricular systems of chick embryos may develop in a similar way to those of mammals. However, further investigations are needed to clarify the mechanism of the action of FTS in chick embryos.

It was suggested that color Doppler echocardiograms may be the most reliable and easy method to evaluate abnormalities of the heart and vessels in clinical medicine (Shimada 1978, Come 1985).

Based on the findings obtained in normal embryos, it was possible to evaluate the anatomical abnormalities of the aorta, but very difficult to evaluate that of the heart mainly due to technical difficulties with the color Doppler system commercially available in Japan.

The values of MV, AT and FI in the FTS-treated embryos were significantly lower than those of the controls. Hemodynamic changes may be clarified by these factors with injection of FTS, which induced a weak contractility of the myocardium of chick embryos, and reached the maximum volume from the left ventricular outflow and decreased the blood flow value in the aorta. This indicated that the physiological function of the cardiovascular system of chick embryos may be disturbed by FTS. Accordingly, from the FFT patterns observed in the descending aorta, it was suggested that they offered a relatively reliable information to evaluate the cardiovascular dynamics of chick embryos treated with drugs.

In future studies, if the color Doppler system can be improved to record more precise information inside the heart and small vessels of chick embryos, it will be the most reliable method to evaluate the effects or toxicity of cardiovascular drugs in these models.

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