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Physio-pathological Responses in Japanese Quail Layers to Blood Collection via Cardiac Puncture

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Abstract: The Japanese quail is a very popular animal model for various biomedical disciplines such as growth and development, reproduction, physiology, endocrinology, nutrition, drug testing and toxicology. Hematological and clinical chemistry parameters are important diagnostic tools and experimental studies invariably draw on them for evaluating the outcomes of treatments. For blood collection, a wide variety of sites are utilized for sampling blood from animals including the heart. Cardiac puncture is being practiced in rodents, birds and rabbits particularly when larger quantities are needed for diagnostic and serological work. One should, however, be fully aware of possible complications that may result from this method. On this aspect, information on the post-collection responses are lacking in the Japanese quail. This report highlights some important observations made on the Japanese quail following a one-time collection of blood via cardiac puncture. The results were very considerably variable. Of the nineteen (19) experimental birds used in this project, one (1) bird succumbed to the procedure and died instantaneously and the others (n = 18) experienced some temporary loss in body weight, cessation in egg production and regression of the reproductive organs. The loss in body weight was the maximum by d6 post-collection. At this time (d6), five (5) birds which weighed between 115-118g during the pause and had lost a significant amount of body weight (loss of ~ 12% from the initial weight at day 0) were, after blood collection, euthanized with carbon dioxide and their hearts and reproductive organs were examined and fixed in 10% formalin. Most of the surviving birds (n = 8) regained weight between d3 to d12 post-collection and resumed egg production; however, some birds (n = 2) did not lay eggs even by d12 post collection. Both Hemoglobin (Hb) and Packed Cell Volume (PCV) were significantly elevated during the pause and then returned to their initial values of d0 upon resumption of egg production after the healing process had been compromised. It was implicit that cardiac puncture traumatized the heart and triggered various changes in hormonal and physio-chemical parameters. Post collection pathological alterations included hemopericardium, ventricular hemorrhage and the formation of fibrous tissues in the myocardium and other complications. These points should be kept in mind while planning an experiment or evaluating the outcome of an experiment involving cardiac puncture.

Key words: Japanese quail, cardiac puncture, blood collection, reproduction

INTRODUCTION

The Japanese quail is a very popular animal model for research and its continued refinement is essential for obtaining accurate and reliable results. The selection of a blood collection site is very crucial in this respect as diverse techniques could bring about different effects on the animal and influence the outcome of the results (Arora, 1979; Campbell, 1988; Samour, 2008; Ballard and Cheek, 2010). In the Japanese quail, the brachial (wing) vein and right jugular vein are the sites of choice and are readily accessible. However, the major complication in the use of these sites is the occurrence of low grade hematomas which heal within 3-4 days post collection without any significant tissue damage (Arora, 2010). On the other hand, cardiac puncture is a blind and invasive procedure and it is being used in rodents, rabbit and birds where larger samples are needed for diagnostic and serological work (Besch and Chou, 1971; Bellinger and Mendel, 1975; McClure, 1999; Ness, 1999). Data on reference values and responses to cardiac puncture in the Japanese quail are missing in the literature. The objective of this report is to highlight various physio-pathological changes observed in the Japanese quail layers following blood collection via cardiac puncture.

MATERIALS AND METHODS

Twenty-five 12-week old Japanese quails were used in this study and kept in two groups: Control (n = 6) and Cardiac (n = 19). To minimize the effects of gender, age, body weight and physiological status in the egg-laying cycle, only females from the same hatch, of similar body weights (between 130-135 g) having a hard-shelled egg in the shell gland were used. The birds were housed and cared for according to the recommended guidelines (National Research Council, 1996) and were housed in a temperature controlled room at 23±2°C under 16L:8D hrs of lighting system and were provided *ad libitum*

access to quail feed and fresh water. Feed and water were withheld for ~ 2 hrs prior to weighing and blood sampling. Birds were taken out from the cages very gently and sedated quickly with Isoflurane or methoxyflurane to minimize distress and physical movements. After sedation, the birds were laid on the table top in lateral decumbency on the right side with their legs and wings secured using adhesive tape and then allowed to lie undisturbed for approximately two minutes before blood sampling. On day 0, approximately 0.25 mL of blood was collected from the heart in the Cardiac group using a tuberculin syringe attached with a 1-inch 25-gauge needle while applying light negative pressure in the syringe; the blood was immediately transferred into EDTA-coated vials. Blood collected directly from the needle hub into EDTA-coated vials also worked very well. The heart was entered from the lateral thoracic area between the 4th and 5th rib close to the sternum while the heart beat was located through digital palpation or stethoscope. The blood sampling was carried out between 9:00 and 11.00 AM and blood was processed for Hemoglobin (Hb) content using the Drabkin's cyanmet-Hb method (Crosby *et al*., 1964) and for PCV using a standard microhematocrit centrifuge (14,000 RPM for 5 min) within twenty minutes of the collection. Birds were weighed on alternate days between 15:00 to 17:00 PM and egg production record was maintained. The birds having an egg in the shell gland on day 0 were laid the same day. One bird died instantaneously following cardiac puncture. From the remaining eighteen (18) birds, five (5) birds which had lost a significant amount of body weight (a loss of ~ 12% from the original weight at d0) were, after blood collection, euthanized with carbon dioxide and their hearts and reproductive organs were examined and fixed in 10% formalin. Among the remaining thirteen (13) cardiac birds, three (3) birds continued to lay eggs as usual with a little or no change in weight, eight (8) birds regained weight and resumed egg production following a pause of d3 to d12 and two (2) birds did not lay eggs even up to the end of the experiment on d12 post collection; they were euthanized and their reproductive organs were found to be partly developed. On d12, blood was collected from all the birds via brachial vein (cardiac puncture was not attempted) for comparing the control and recovered cardiac birds. Data collected on the body weight, Hb and PCV values in euthanized, control and recovered groups were compared statistically using the paired t-test method. Data are presented as mean± standard deviation using the 0.05% level of significance.

RESULTS

A total of 25 layers (Control group = 6 and Cardiac group = 19) were used in this experiment. The responses from the cardiac group very considerably variable; One (1) bird died instantaneously following cardiac puncture. Among the remaining eighteen (18) cardiac birds, three (3) continued to lay egg as usual with a little or no loss in weight; five (5) were euthanized on d6 post collection; eight (8) resumed egg production after a pause ranging from d3 to d12 and two (2) did not lay eggs even up to the end of the experiment on d12 post collection and their reproductive organs were found not to be fully developed. None of the cardiac birds exhibited any problem in the utilization of feed and water and in their physical movements. Five (5) birds from the cardiac group which were euthanized on d6 presented, in addition to ~12% loss in body weight, a significant elevation in Hb and PCV values (PCV = 51.3±2.8%; Hb = 14.3±2.9 g/dl ($p<0.05$) when compared to the previous samples collected from them at the start of the experiment (cardiac puncture) on d0 (PCV = 43.3±2.1% and Hb = 10.8±1.4 g/dl). By d12 post collection, all the cardiac birds had regained their body weights comparable to their original weights at d0 and to the control birds. Furthermore, both Hb and PCV values returned to values comparable to that of the control group (PCV = 41.8±2.7% and Hb = 10.9±0.9 g/dl; $p>0.05$). In addition to changes in Hb and PCV values, the euthanized birds were found to have a marked regression of oviducts and follicular involution in the ovaries (Fig. 1). The hearts from two birds exhibited hemopericardium (Fig. 2) which, upon histological examination, revealed extensive hemorrhage in the ventricular myocardium (Fig. 3) and the formation of fibrous tissues along the path of entry of the needle (Fig. 4).

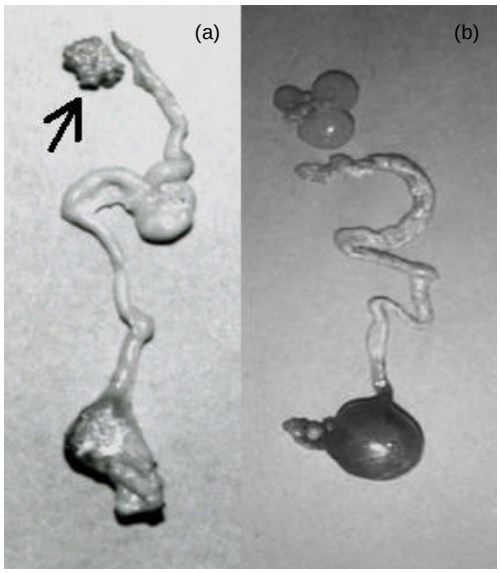

Fig. 1: Photograph of ovary and oviduct showing follicular atresia resulting from cardiac puncture (a) and an active ovary from control bird (b)

Fig. 2: Photograph of the heart exhibiting extensive hemopericardium resulting from cardiac puncture in a Japanese quail layer

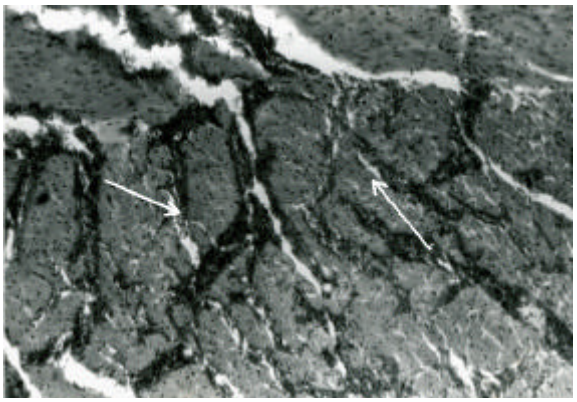

Fig. 3: Photomicrograph of a section of myocardium showing hemorrhage resulting from cardiac puncture

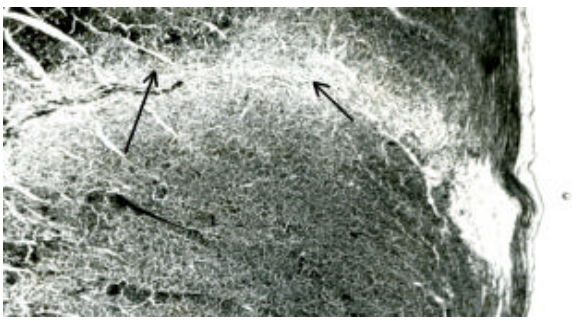

Fig. 4: Photomicrograph of a section of myocardium showing collagenous tissue formation along the path of needle entry

DISCUSSION

One-time blood collection via cardiac puncture gave considerably variable results; one of nineteen birds (19) used in this study died instantaneously. Three birds out of remaining eighteen (18) did not show any harmful effect of cardiac puncture and continued to lay eggs as usual showing a little or no loss in body weight, eight (8) recovered body weights after a pause ranging from d3 to d12 and two (2) of them did not resume production even up to the termination of the project on d12 post collection; their reproductive organs were found to be partially developed. This reflects that loss in weight, cessation in egg production and regression of reproductive organs following cardiac puncture depends upon the level and nature of trauma induced by the procedure. The affected birds lost weight mainly due to the regression of reproductive organs and their recovery was almost complete in most birds after an interruption of d3 to d12. The results are in accord with the earlier reports of Buckland *et al*. (1974) that broiler chicks and white leghorn pullets sampled via cardiac puncture had lower subsequent body weights than the corresponding non-bled control group and McClure and Cedeno (1955) for collecting blood from morning doves and pigeons. Hormonal status of the cardiac birds was not determined. Evidence from several studies support the idea that the Japanese quail experienced loss in body weight mainly due to the regression of reproductive organs (ovarian follicles involuted much faster than oviducts) following the interruption of the gonadotropins and induced a state alike that resulted from hypophysectomy (Opel and Nalbandov, 1961), blockage in the release of gonadotropin (Opel and Fraps, 1961) and lack of estrogen secretion from the ovary, the primary factors essential for the maintenance and functioning of reproductive organs in the layers. It seems that trauma from the cardiac puncture caused hormonal interruption either via the hypothalamus-pituitary-adrenal axis (Harvey *et al*., 1980) or from the secretion of a hypothalamic neuropeptide (termed as gonadotropin-inhibitory hormone; GnIH) which is known to inhibit the synthesis and release of gonadotropin in Japanese quail under stress (Tsutsui *et al*., 2000: Ubuka *et al*., 2008). Following the healing process, the gonadotropin was restored gradually, the reproductive organs were reactivated and resumed laying eggs. The birds which were euthanized on d6 following a loss of ~12% in weight from their original weight at d0 revealed, in addition to the loss in weight, cessation in egg productions and regression of reproductive organs, that trauma inflicted serious pathological changes in the heart. The hearts from two birds exhibited hemopericardium which, upon histological examination, revealed laceration and hemorrhage in the ventricular myocardium and formation of fibrous tissues.

The elevation of Hb and PCV values that occurred during the pause (and the ~12% loss of body weight) was probably due to the lack of estrogen secretion from the ovary, decreased blood volume and lack of adequate water consumption which was not studied. Conversely, the restoration of blood values upon recovery was due to the resumption of estrogen secretion from the ovary which is known to cause hemodilution from water retention and depression of erythrocyte volume (Hunsaker, 1968; Arneja and Arora, 1972; Nirmalan and Robinson, 1971). Higher hematological values have been reported in the non-layers as compared with layers (Atwal *et al*., 1964). In conclusion, this study indicates that blood sampling via cardiac puncture is traumatic and stressful to the bird. As a consequence, the birds experienced temporary weight loss, cessation in egg production, involution of reproductive organs, alteration in blood parameters and serious pathological changes in the cardiac tissues. Cardiac puncture as a method of blood collection is not recommended particularly when multiple parameters are being investigated simultaneously.

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