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Allometric Growth of Prenatal Organs as a Function of Age in the Japanese Quail Embryo, *Coturnix japonica*

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Abstract: The Japanese quail is widely being used as a model in developmental biology. Embryonic organs are sensitive indicators of pathological and treatment-induced alterations. There is a shortage of well characterized allometric information in the literature for assessing the prenatal growth in the Japanese quail. This study collected baseline information on the relative growth of various internal organs and external morphological structures during the prenatal life of the Japanese quail from day 10 to hatchling for use in comparative and pathological studies. The organs examined were the brain, eyes, liver, gizzard, proventriculus, heart, lungs and kidneys. This information would be potentially valuable to the scientific community for defining the processes involved in organogenesis, teratogenesis, comparison of growth among different age and genetic groups, physio-pathological responses to drugs, disease conditions and the evaluation of therapeutic drugs and environmental stresses.

Key words: Japanese quail, environmental stresses, embryonic organs

INTRODUCTION

An evaluation of embryonic organs in terms of size, shape and weight is an integral part of studies involving growth, toxicology, pathology, drug testing and the conduction of necropsies in both human and veterinary medicines (Luecke *et al*., 1995; Bindhu *et al*., 2007). The Japanese quail is fast replacing the chicken embryo as an animal model of choice due to its small size, cost effectiveness, rapid growth rate, sexual maturation and its adaptability to the laboratory environment. Both Japanese quail and chick embryos currently serve as experimental models in developmental studies (Padgett and Ivey 1960; Romanoff, 1960; Grahams and Meier, 1975; Dieterlen-Lievre, 1997; Tsudzuki *et al*., 1998), toxicology and drug testing (Scane and AnneMcnabb, 2003; Javed *et al*., 2008; Smith, 2008), screening of endocrine disruptors (Kamata *et al*., 2006), environmental toxicants (Cooke, 2007), hypoxic and hyperoxic environments (McCutcheon *et al*., 1982; Soldatov *et al*., 2007), intergeneric hybridization (Poynter *et al*., 2009), reproductive toxicology (Quinn *et al*., 2007), thermal stresses (Givisiez *et al*., 2001); altitude studies (Smith *et al*., 1969), maternal effect (Al-Murrani, 1978), comparative embryonic development (Lilja *et al*., 2001) and pre-incubation storage of eggs (Arora and Kosin, 1966). In the above listed studies, the assessment of treatment effects were based on various embryonic parameters including growth rate, embryonic mass, development of specific organs, structural malformations and viability during the prenatal life as well as various anatomical and physiological alterations observed during postnatal life. These studies also pointed out that growing tissues and organs exhibit marked differential sensitivity to the treatments. A well known example is abnormal and missing limbs in neonates following the use of the drug, Thalidomide, by women to relieve morning sickness during their first trimester of pregnancy. Similar target specific responses were reported in laboratory animals resulting from the use of retinoic acid (Kwasingroch and Kocher, 1980). Differential growth responses in brain, eyes, heart, liver, intestines and kidneys have been reported when embryos were exposed to hypoxic and hyperoxic environments (McCutcheon *et al*., 1982; Stock *et al*., 1983), differential photoperiods (Lauber, 1975), prenatal nutritional deficiencies (Pond *et al*., 1991) and high altitude (Smith *et al*., 1969). An accurate determination of shape, size and weight of individual organ would be a valuable tool to aid in the study and understanding of organogenesis, malformations, growth rate comparison, aging process, toxicology, drug safety studies, environmental stress, relative distribution of therapeutic drugs and toxicants in various organs. This may also assist investigators in their ability to successfully harvest tissues within a specific time frame or stage of embryonic growth for evaluation and study. This study was therefore designed to: 1. Characterize the allometric growth of internal organs relative to age and 2. Establish baseline reference values for use in comparative and pathological research in the Japanese quail.

MATERIALS AND METHODS

Eggs of uniform size and weight (~10 g) were collected, between 3-6 pm, from a randomly breeding colony of Japanese quail. The birds were 75-days old, hatch-mates weighing 130-135 g at the peak of production and fertility. They were housed in cages under a 14L:10D lighting system and provided with commercial quail feed

ad libitum. Eggs were incubated in forced-draft incubator with automatic rotation at 99-100°F and 65-70% relative humidity. Each egg was weighed to the nearest milligram (mg) prior to incubation. Starting at day (d) 10 of incubation, a group of eggs were removed from the incubator at two-day intervals and opened into a petri-dish containing saline solution. The embryos were devoid of the extra-embryonic tissues and residual yolk found in older embryos, washed with fresh saline, blotted with absorbent paper and weighed individually to the nearest mg. Embryos which appeared abnormal or retarded were discarded. The embryos were cut open, internal organs excised and weighed to the nearest mg. The external morphological structures such as third toe, leg, beak and metatarsus were measured to the nearest millimeter (mm) mimicking a study carried out by Grahams and Meier (1975). The hatchlings were euthanized approximately 10 h after hatching with CO_2 gas, cleared of residual yolk, weighed and processed similarly to pre-hatch embryos mentioned above. The gender of the embryos and hatchlings was not identified. Other organs and tissues such as digestive tract, adrenals, thymus, spleen and other tissues were not included in the measurements. The data on embryonic growth in relation to age, growth between intervals, growth rate, ratios and correlations among different internal organs and external embryonic structures to body mass were analyzed and presented as means± SEM, correlations (R^2) and regression analyses.

RESULTS AND DISCUSSION

Prenatal growth of Japanese quail embryos and their internal organs (viscera) were examined and weighed from d10 to hatchling. The organs included were the eyes, brain, liver, gizzard, proventriculus, heart, lungs and kidneys. The aforementioned organs were observed to be growing at a differential rate with some organs growing larger and faster than others. The larger organs included eyes, brain, liver and gizzard, whereas, the smaller organs included proventriculus, heart, lungs and kidneys. The weights of paired organs such as eyes, lungs and kidneys were pooled for the purpose of this study. The sizes of the larger organs were quite diverse from each other, whereas, small slow growing organs were closely associated to one another with respect to growth pattern (Table 1 and Fig. 1). The external structures such as third toe, leg, metatarsus and beak were interrelated with age, weight and internal organs of the embryo.

Embryonic growth: From d10 to d16 of incubation, the embryos acquired an average weight of 1.44±0.07 g, 2.52±0.09 g, 3.71±0.08 g and 5.38±0.14 g by the end of d10, 12, 14 and 16 (hatch), respectively ($p<0.05$). The growth was rapid during d10-12, somewhat slower during d12-14 then regained velocity during d14-16, adding 1.08, 1.19 and 1.67 g during these intervals (Table 1, 2 and Fig. 1). When expressed as percentage of hatchling, the embryos gained 25.1%, 43.9%, 64.5% and 98.7% of the weight by d10, 12, 14 and 16, respectively and the remaining 1.3% occurring post hatching affecting mostly liver, gizzard and proventriculus. The data reflects that the embryos did not reach maximum maturity within all aspects prior to hatching (Table 5). Age and weight of embryos were highly correlated (Fig. 8) and these parameters could be easily and accurately estimated on the basis of morphological appearance and the size of external structures (Table 5, Fig. 9, 10 and 11).

Growth of internal organs of embryo: The growth of internal organs as a function of age and embryonic mass are given in Table 4 and Fig. 5. Divergent growth

Table 1: Absolute mean wet weight of internal organs of Japanese quail embryo in relation to age and body mass (Mean±SEM; n = 15)

Age (days)	10	12	14	16	Hatchling
Wt. of embryo (g)	1.44±0.07	2.52±0.09	3.71±0.08	5.38±0.14	5.74±0.16
Organs (mg)					
Liver	22.80±1.13	63.50±2.63	98.20±2.03	144.10±2.15	163.00±2.12
Heart	16.20±0.17	29.10±0.82	34.70±1.38	47.90±1.95	48.90±1.74
Kidney	9.53±0.90	26.40±1.01	31.30±0.88	40.70±2.24	40.60±0.97
Brain	109.70±3.17	187.30±40.0	224.10±4.77	285.60±3.70	285.40±4.02
Gizzard	25.30±1.24	90.10±2.85	131.70±2.00	254.10±3.70	283.70±4.48
Proventriculus	8.60±0.52	23.60±0.91	37.90±1.51	54.60±1.83	62.10±1.58
Lungs	10.20±1.15	28.00±1.18	31.40±1.04	43.31±1.68	45.50±1.58
Eyes	205.00±3.80	279.20±3.54	294.40±2.63	298.10±3.70	295.10±2.66

Table 2: Increment in mean organ weights (mg) during 2-day Intervals*

Between (days)	WOE	Brain	Lungs	Gizzard	Liver	Proventriculus	Heart	Eyes	Kidneys
10-12	1.08	77.60	17.8	64.8	40.7	15.0	12.9	74.1	17.1
12-14	1.19	36.80	3.6	41.6	34.7	14.3	5.6	15.2	4.9
14-16	1.67	61.50	11.7	122.4	45.9	16.7	13.2	12.2	9.4
16-Hatchling	0.36	-0.02	2.2	29.6	19.3	7.5	1.0	2.0	-0.1

*n = 15 embryos. Weight of embryos in grams and weight of organs in milligrams. WOE = Wt. of Embryo (g)

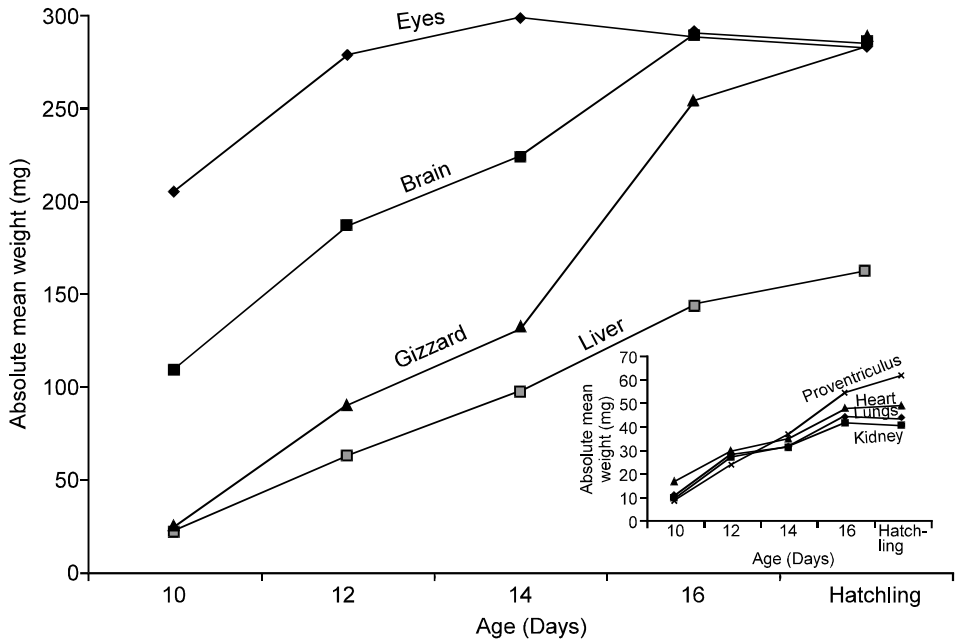


Fig. 1: Absolute mean weight of organs as a function of age (days)

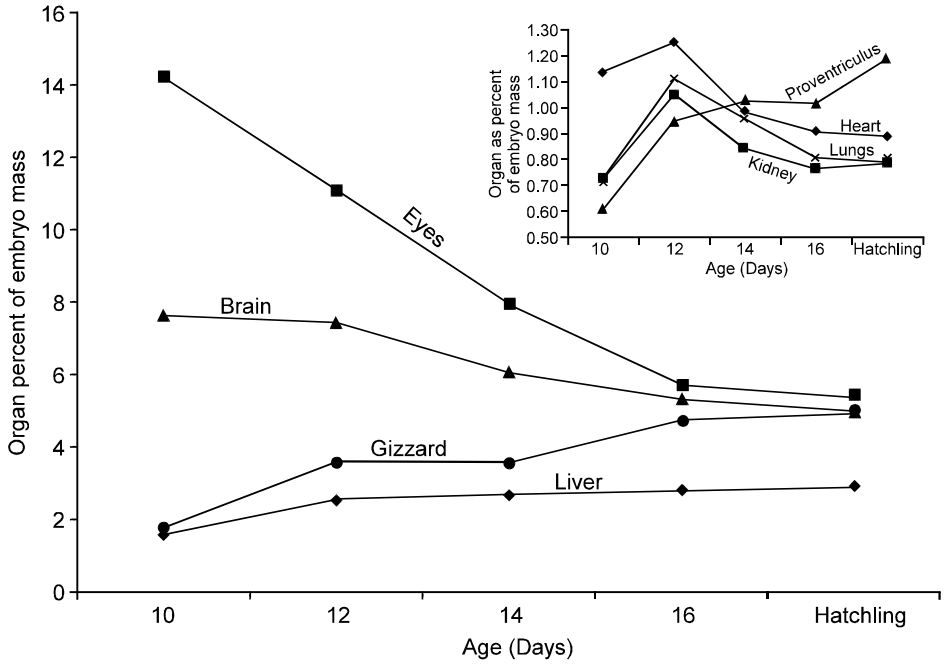


Fig. 2: Organs as a percent of embryo mass

rate was more evident among the large organs (eyes, brain, gizzard and liver) as compared to small organs. The growth was biphasic with rapid growth during d10-12 and d14-16 and somewhat slow growth during d12-14, reflecting some anatomical and physiological adjustments taking place within the embryo during this period (Table 2).

Eyes: This paired organ was the largest and fastest growing organ in the embryo and weighed 66.5, 90.5, 95.4 and 99.4% of the hatchling eye weight by d10, 12, 14 and 16, respectively, thus attaining its full growth before hatching (Table 5 and Fig. 5). The eyes grew very rapidly during d10 and 12. The ratios between the eyes and body mass were 7.0, 9.0, 12.6, 17.5 and 18.6 at d10,

12, 14, 16 and hatchling, respectively, increasing with age and mass (Table 7). When expressed as percent of embryonic mass, the weight of the eyes decreased drastically from 14.20% at d10 to 5.70% at d16 and ultimately to 5.38% in the hatchling (Table 4 and Fig. 2).

Brain: The brain was the second largest and faster growing organ after the eyes, growing almost linearly during d10-16, reaching 38.4, 65.5, 78.5 and 100% of hatchling by d10, 12, 14 and 16, respectively (Table 5 and Fig. 5). The growth was very rapid during d10-12 and again during d14-16. When expressed as percent of body mass, the brain weighed 7.64, 7.43, 6.04, 5.31 and 4.97% on d10, 12, 14, 16 and hatchling, respectively, decreasing with age (Table 4 and Fig. 2). The ratio between brain and eyes were 1.86, 1.49, 1.31, 1.07 and 1.08, decreasing with age. The ratios between brain and body mass were 13.2, 13.5, 16.6, 18.8 and 20.1 at d10, 12, 14, 16 and hatchling, respectively, increasing with age and body mass (Table 7 and Fig. 6). The ratios between eyes and brain was isometric, both of them reaching maximum weights by d16.

Gizzard: The gizzard was the third largest and faster growing organ after eyes and brain. Its growth was almost linear from d10 to d16; however, the velocity of growth was somewhat slow during d12-14. It weighed 8.29, 31.8, 46.4 and 89.6% of the hatchling at d10, 12, 14 and 16, respectively and continued to grow another 10.4% post hatching (Table 5). When expressed as a percent of body mass, the gizzard weighed 1.76, 3.58, 3.55, 4.72 and 4.94% at d10, 12, 14 and 16, respectively and continued to grow with increasing age and embryonic mass (Table 4 and Fig. 2). The ratios between gizzard and body mass were 56.9, 28.0, 28.2, 21.2 and 20.2 at d10, 12, 14, 16 and hatchling, respectively, decreasing with age and mass (Table 7 and Fig. 6).

Liver: The liver was the fourth largest and faster growing organ. It weighed 1.58, 2.52, 2.64, 2.68 and 2.85% of the body mass at d10, 12, 14, 16 and hatchling, respectively (Table 4). Its growth was almost linear with age reaching 88.2% of hatchling at d16, continuing to grow further by 11.8% after hatch (Table 5). The ratio between liver and body mass was 62.6, 39.4, 37.8, 37.4 and 36.2 at d10, 12, 14, 16 and hatchling, respectively, decreasing with age and body mass (Table 7 and Fig. 6).

Proventriculus: The proventriculus like the gizzard grew in a linear fashion from d10-16 and attaining 87.6% of hatchling at d16 with an additional 12.4% growth occurring after hatching (Table 5). It weighed 0.60, 0.94, 1.02, 1.01 and 1.08% of body mass at d10, 12, 14, 16 and hatchling, respectively (Table 4). Its growth was the fastest from d14 onward to hatchling. The ratios between proventriculus and body mass were 167.4, 106.8, 97.9, 98.5 and 92.4 at d10, 12, 14, 16 and hatchling, respectively, decreasing with age and body mass (Table 7 and Fig. 6).

Table 3: Growth rate of internal organs as a function of age (R-values)

Age (days)	12	14	16	Hatchling
Eyes	36.2	5.4	1.3	0.0
Brain	70.7	19.6	27.4	0.0
Gizzard	256.1	46.2	92.9	11.7
Liver	179.1	54.6	46.7	13.1
Proventriculus	174.4	260.6	44.1	13.7
Heart	79.6	19.6	38.0	2.9
Lungs	174.5	121.4	37.9	5.0
Kidney	177.1	18.6	30.0	0.0

R-values were calculated by the formula = W2-W1/W1 x 100

Table 4: Organ weight as a percent of body mass

Age (days)	10	12	14	16	Hatchling
Embryonic mass (g)	1.44	2.52	3.71	5.38	5.74
Organs (mg)					
Liver	1.58	2.52	2.65	2.68	2.85
Heart	1.13	1.15	0.94	0.89	0.85
Kidneys	0.65	1.05	0.84	0.76	0.71
Brain	7.64	7.43	6.04	5.31	4.97
Gizzard	1.76	3.58	3.55	4.72	4.94
Proventriculus	0.60	0.94	1.02	1.01	1.08
Lungs	0.71	1.11	0.85	0.80	0.79
Eyes	14.20	11.08	7.94	5.70	5.38

Table 5: Organ weight as percent of hatchling

	10	12	14	16
Embryo	25.10	43.9	64.6	98.7
Organ weight (mg)				
Eyes	66.50	90.5	95.4	99.4
Brain	38.40	65.6	78.5	100.0
Gizzard	8.92	31.8	46.4	89.6
Liver	13.95	38.9	60.1	88.2
Proventriculus	13.80	38.0	61.0	87.9
Heart	33.10	59.5	71.0	98.0
Lungs	22.40	61.5	69.5	95.2
Kidneys	22.90	65.0	77.1	100.0

Heart: The heart increased in weight linearly with age reaching 98.0% of the hatchling at d16 and increasing further by 2.0% after hatch (Table 5). Its growth was rapid during d10-12. When expressed as percent of body mass, the weight of heart increased during d10-12 and then decreased with increasing body mass to hatchling. The ratios between heart and body mass were 88.9, 86.6, 106.9, 112.3 and 117.4 at d10, 12, 14, 16 and hatchling, respectively, increasing with age and body mass (Table 7 and Fig. 6). It should be noted that this is the first embryonic structure commencing physiological function for supplying oxygen and nutrients to other parts of the body.

Lungs: The rapid growth of this paired organ was observed during d10-12 and d14-16 attaining a maximum weight of 95.2 % of hatchling at d16 and an additional growth of 4.8% occurring post hatching (Table 5). When expressed as percent of embryonic mass, the lungs increased rapidly during on d12 and then decreased gradually thereafter (Table 4 and Fig. 2). The

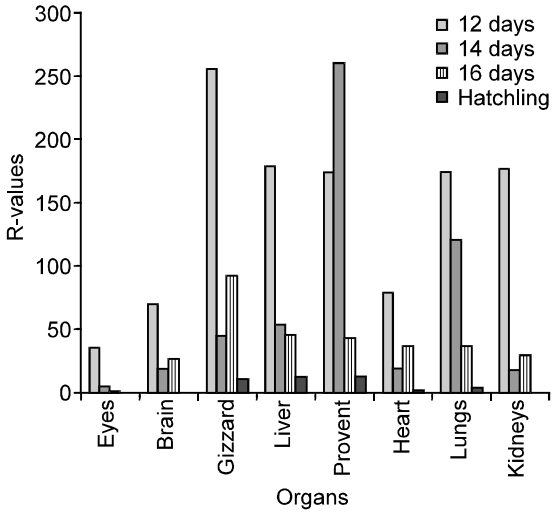


Fig. 3: Growth rate of organs (R-Values). Provent = Proventriculus

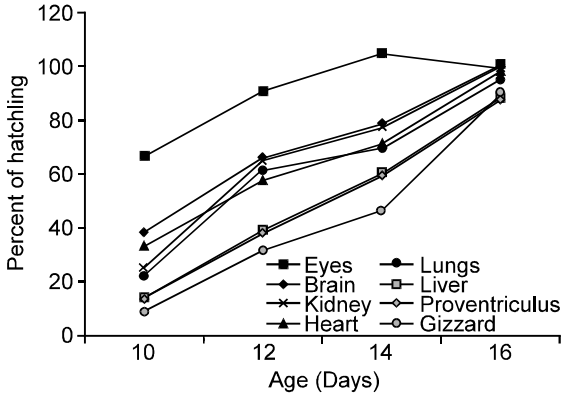


Fig. 4: Organ weight as percent of hatchling

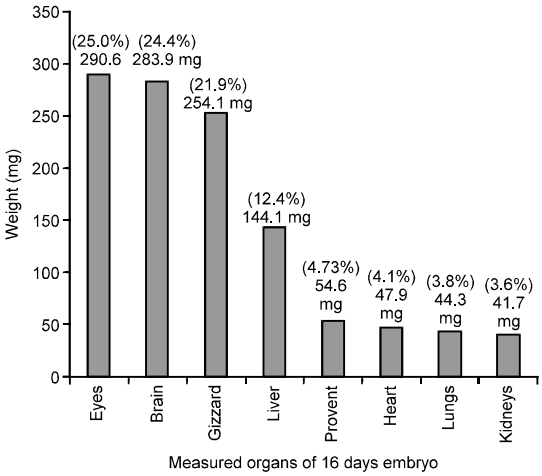


Fig. 5: Proportional Contribution of organs to embryonic mass at d16

ratios between lungs and body mass were: 131.2, 90.0, 118.2, 124.2 and 126.2 at d10, 12, 14, 16 and hatching, respectively; higher at d10, decreasing at d12 and then increased gradually on to hatchling (Table 7 and Fig. 6).

Kidneys: This paired organ continued to grow rapidly with age reaching almost 100% by hatching time at d16 (Table 6); the growth was rapid on d10 and then decreased gradually thereafter. When expressed as percent of body mass, the kidneys, similar to the lungs and heart, grew rapidly at d12 and decreased gradually thereafter (Table 4 and Fig. 2). The ratios between kidneys and body mass were 151.1, 95.5, 118.5, 131.2 and 141.0 at d10, 12, 14, 16 and hatchling, respectively with a sharp drop on d12, with ratios increasing markedly with increasing body mass (Table 7 and Fig. 6).

Growth rate of organs (R-values): On d10, at the beginning of this study, all of the organs had passed the process of organogenesis and were growing rapidly at their individual rates; some faster than the others (Tables 1, 3 and Fig. 3). The R-values for growth rate were calculated (formula = W2-W1/W1 x 100) from d10 to hatchling. At d12, among all the examined organs, the gizzard had the fastest growth rate, followed by the liver, kidneys, lungs, proventriculus, heart, brain and eyes, respectively. At d14, the growth rates among these very organs were quite different; the fastest growing organ was the proventriculus, followed by the lungs, kidneys, liver, gizzard, heart, brain and eyes in that order. At d16, the order of growth rate was the gizzard, liver, proventriculus, heart, lungs, kidneys, brain and eyes, respectively. Between d16 and hatching, the growth rate was mostly complete for the brain, kidneys and eyes and to some extent, for the lungs and heart as well. However, there was sizable post hatching growth for the liver, gizzard and proventriculus.

Proportional contribution of different organs to body mass: A major contribution to the hatching embryo came from the larger organs such as the eyes, brain, gizzard and liver and at a lesser degree from the smaller organs such as the kidneys, lungs, heart and proventriculus. Proportional contributions to the hatching embryo (d16) were: Eyes 26.05%, Brain 24.27%, Gizzard 21.59%, Liver 12.31%, Proventriculus 4.64%, Heart 4.07%, Lungs 3.68% and Kidneys 3.46% (Table 6 and Fig. 4). When expressed as percent of hatchling (=100%), the proportional contribution from various organs were: Brain 100.0, Kidneys 100.0, Eyes 99.4, Heart 98.0, Lungs 95.2, Gizzard 89.6, Liver 88.2 and Proventriculus 87.6%. The lungs, gizzard, liver and proventriculus continued to grow to a varying degree after hatching (Table 5, Fig. 5).

Table 6: Proportional contribution (%) of the organs to embryonic mass at d16 (hatch)

Organ	Day 10	Day 12	Day 14	Day 16
Eyes	32.64	35.30	33.30	26.05
Brain	17.44	23.68	25.35	24.27
Gizzard	4.03	11.39	14.90	21.59
Liver	3.63	8.03	11.11	12.31
Heart	2.56	3.68	3.93	4.07
Lungs	1.62	3.54	3.57	3.68
Proventriculus	1.37	2.98	4.29	4.64
Kidneys	0.67	3.34	3.54	3.46

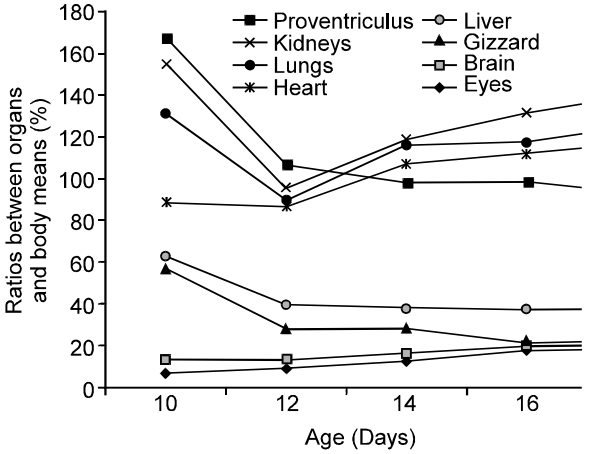


Fig. 6: Ratios between the organs and embryonic mass

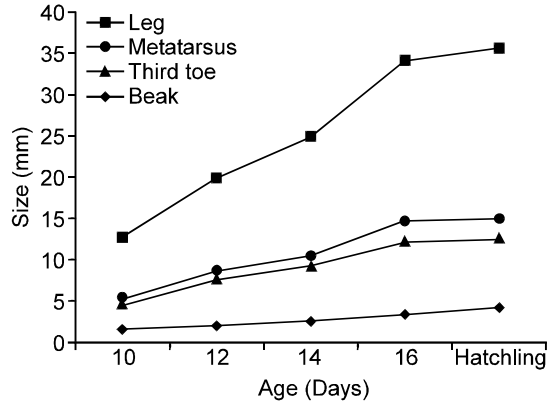


Fig. 7: Growth of external embryonic structures

Correlation between organs: Weight and age of the embryos were significantly correlated, 0.986 (Fig. 8). Hence, the weight of the embryo was predictable accurately from its age and either of them could serve as an index for embryonic growth rate. Correlations among three large and fast growing organs (eyes, brain, gizzard and liver) were highly significant (brain and gizzard, 0.994, brain and liver, 0.993, gizzard and liver, 0.976 and eyes and brain 0.978) reflecting the relative organization among these structures and the relative growth of these organs which could be judged from each others growth. Correlations between age and larger internal organs were: age and gizzard, 0.936; age and brain, 0.913 and age and liver, 0.951. Similarly, the embryonic mass (weight) was highly correlated with liver, gizzard and brain. It is therefore evident that a relative growth of large internal organs could be reliably predicted from the age and weight of the embryo.

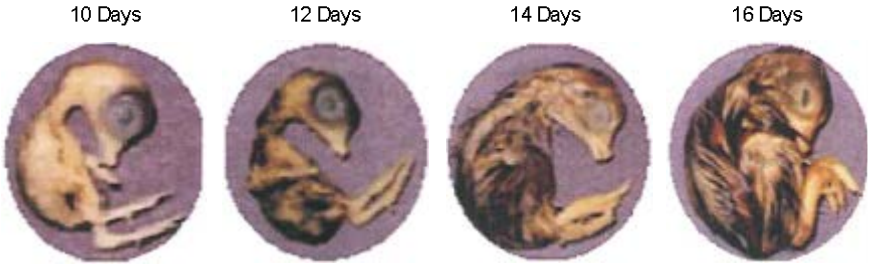
Relationships between internal organs and external structures: Four external embryonic structures: beak, leg, third toe and metatarsus were measured to assess embryonic weight and age, comparative growth rate and degree of embryonic maturation (Table 8 and Fig. 7). The growth of the beak was linear with age into the hatchling phase, whereas, the growth of the three other structures was linear only up to d16. All of the external structures were highly correlated in all combinations and ranged between 0.978 and 0.993. Therefore, any one of these structures could be utilized for assessing embryonic growth, age, retardation and malformation. Third toe and metatarsus were used successfully in this laboratory for assessing embryonic age (Fig. 9) and weight (Fig. 10) and were highly correlated, 0.994 (Fig. 11) and lend themselves to easy and quick measurements.

Furthermore, the external structures were highly correlated with internal organs as well; brain and leg, 0.996; liver and metatarsus, 0.995; third toe and brain, 0.948; metatarsus and gizzard, 0.980 and gizzard and leg, 0.960. Based on this information, the growth of the large internal organs could be predicted fairly from the size of any of the external structures. In conclusion, the age, body mass, larger internal organs (liver, gizzard and brain) and external structures (beak, third toe, leg and metatarsus) are highly correlated during the ontogenic process. Any aberration in the growth of the internal organs could be a basis for teratogenesis and embryonic mortality.

Table 7: Ratios between organs and body mass

Age (Days)	Eyes	Brain	Gizzard	Liver	Heart	Lungs	Kidneys	Proventriculus
10	7.0	13.2	56.9	62.6	88.9	131.2	151.1	167.4
12	9.0	13.5	28.0	39.4	86.6	90.0	95.5	106.8
14	12.6	16.6	28.2	37.8	106.9	118.2	118.5	97.9
16	17.5	18.8	21.2	37.3	112.3	124.2	132.2	98.5
Hatchling	18.6	20.1	20.2	36.2	117.4	126.2	141.0	92.4

Table 8: Growth of external embryonic structures



	10 Days		12 Days		14 Days		16 Days		Hatchling	
Organ (mm)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Beak	1.59	0.09	2.04	0.07	2.58	0.06	3.40	0.11	3.88	0.12
Third toe	4.51	0.16	7.62	0.17	9.26	0.29	12.15	0.15	12.50	0.12
Metatarsus	5.18	0.30	8.66	0.26	10.45	0.27	14.70	0.18	14.80	0.09
$Leg_{(M+T)}$	12.72	0.41	19.92	0.46	24.92	0.06	34.10	0.39	35.23	0.18

M+T = (Metatarsus + Tibiotarsus)

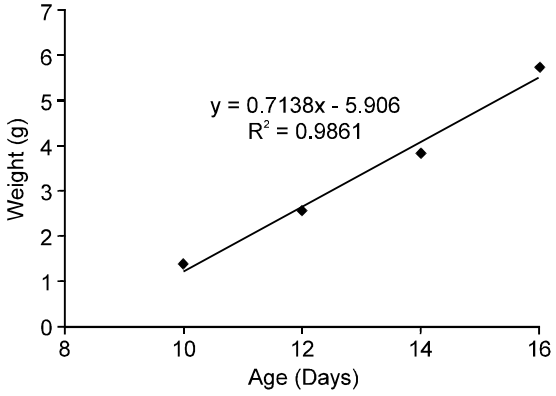


Fig. 8: Correlation between embryonic age and mass

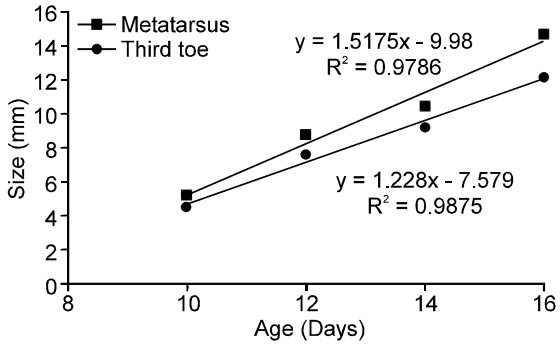


Fig. 9: Correlation between embryonic age and size of metatarsus and third toe

The Japanese quail remains a very valuable animal model in developmental biology as well as other biological disciplines like physiology, endocrinology, nutrition, reproduction and toxicology. Continuous characterization of Japanese quail is highly desirable for obtaining accurate and reliable results. An effort was made in this study to develop some background data and reference values for use by investigators in their individual studies, bringing about uniformity in the data originating from different laboratories. Landmark studies of Hamburger and Hamilton (1951) on staging of chicken embryo and later by Padgett and Ivey (1960) in the staging of Japanese quail embryo based on various external morphological structures made it easier to understand and visualize the normal and pathological growth of embryos. The assessment of treatment effect in experimental studies is, routinely, based on various embryological parameters such as growth of embryonic mass, development of specific organs, structural anomalies and mortality during the prenatal life and through various anatomical and physiological alterations during postnatal life. It is also well recognized that the size, shape and weight of the internal organs are sensitive indicators of growth, malformations and diseased conditions. Treatment-induced alterations in size, weight and growth of heart, brain and kidneys have been reported in embryos exposed to higher altitude (Smith *et al*., 1969), extended pre-incubation holding of eggs (Arora and Kosin, 1966), hormonal disruptors (Kamata *et al*., 2006), drugs (Scane and AnneMcnabb, 2003), differential photoperiods (Lauber, 1975), hyperoxic and hypoxic environments (McCutcheon *et al*., 1982), thermal stress (Givisiez *et al*., 2001) and environmental toxicants (Cooke, 2007). Assessment of internal organs is a routine regulatory practice in pharmaceutical, veterinary and medical medicines during performance of necropsies.

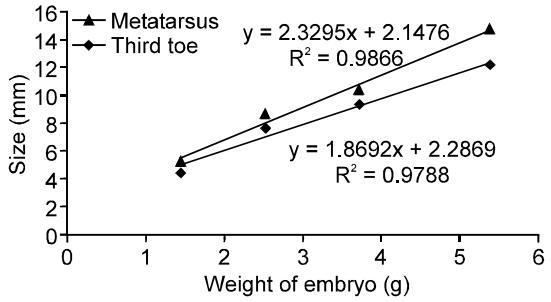


Fig. 10: Correlation between embryonic weight and size of metatarsus and third toe

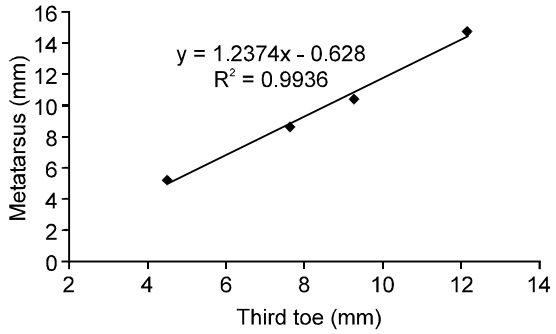


Fig. 11: Correlation between metatarsus and third toe

There is a deficiency of well characterized baseline information on the prenatal growth of various organs in the Japanese quail. For an earlier understanding of this subject on chicken embryos, the reader is referred to Romanoff (1960). The data from this study is presented on the differential growth of organs, contribution of organs to the embryonic mass, periods of accelerated growth, relationships between internal organs, relationship among external embryonic structures, relationships between internal and external structures, changes in ratios between organs and body mass and relative maturity of various organs prior to hatching. External embryological structures such as the beak, third toe and leg are very useful tools for staging embryonic growth and have been previously used by Hendrickx and Hanzlick (1965), Smith *et al*. (1969) and Grahams and Meier (1975). In summary, the growth of internal and external structures could be used alone or in conjunction with embryonic staging for comparing growth rate, determining the age and weight of embryos, identifying retardations and characterizing malformations. The baseline information presented here would prove to be valuable to the investigators in conducting a similar type of study for obtaining accurate and reliable data and avoiding confusion and inconsistencies.

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