



Comparative Analysis of *Nkx2-5/GATA4/TBX5* Expression in Chicken, Quail and Chicken-quail Hybrids during the Early Stage of Cardiac Development in Embryos

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ABSTRACT: The present study makes an investigation into expression of genes related to cardiac development in chicken, quail and chicken-quail hybrids during the early stage of embryogenesis. Real-time PCR was used to detect mRNA expressions of *Nkx2-5*, *GATA4* and *TBX5* in the heart of chicken, quail and chicken-quail hybrids embryos during the 3rd to 7th days of incubation. Results showed that *NKX2-5* mRNA displayed a similar expression trend in chicken, quail and chicken-quail hybrids. The initial and highest expression of *Nkx2-5* was focused on the 3rd day of incubation, then it declined till 5th day of incubation, thereafter, it fluctuated. Expression of *Nkx2-5* gene in quail was significantly higher than in chicken and chicken-quail hybrids, and no significant difference was observed between the two latter species. *GATA4* mRNA showed a similar expression trend between chicken and quail, which displayed a steady increase from 3rd to 6th d, then, the expression level decreased. However, *GATA4* mRNA expression in chicken-quail hybrids was significantly higher than that in chicken and quail from 3rd to 5th d ($p < 0.01$), but significantly lower than that in chicken and quail during the later stage of the experiment ($p < 0.05$), due to the dramatic drop from 5th d onwards ($p < 0.01$). *TBX5* mRNA expression in chicken and quail showed the same trend as *GATA4* expressed in the two species. Furthermore, *TBX5* expression in chicken-quail hybrids was significantly higher than that in chicken and quail during the whole course of experiment, although relatively lower *TBX5* expression was detected in the early stage. In conclusion, *Nkx2-5*, *GATA4* and *TBX5* genes showed dynamic changes during the process of cardiac development in chicken, quail and their hybrids embryos. In addition, the expression trend in chicken was similar to that in quail, and there was no significant difference for gene expression level, except *NKX2-5*. However, expression of these genes in chicken-quail hybrids was significantly different from their parents, the difference mechanism needs to be further explored. (**Key Words:** Cardiac Development, Embryogenesis, Gene Expression, Chicken, Quail, Chicken-quail Hybrids)

INTRODUCTION

The heart is the first organ to be formed and function during the embryogenesis in vertebrate animals (Olson, 2006). Its development includes: cardiac crescent formation, linear heart tube formation, looping heart and initiation of chamber morphogenesis, followed by chamber maturation and separation, and finally, conduction (Bruneau, 2002). Additionally, the molecular events play an important role underlying these processes (Srivastava and Olson 2000; Bruneau, 2002; Wagner and Siddiqui 2007). It has been demonstrated that a battery of transcription factors involved or suspected of involvement in these processes, such as

GATA family, including *GATA4* (Grepin et al., 1997; Gillio-Meina et al., 2003) and *GATA6* (Gove et al., 1997; Davis et al., 2001); *Nkx2-5* (Searcy et al., 1998; Schwartz et al., 1999), *TBX5* (Liberatore et al., 2000; Plageman and Yutze, 2004), regulate different aspects of cardiac morphogenesis. Among these genes, *Nkx2-5*, *GATA4* and *TBX5* have become hot topic recently, due to their important function in the cardiac development process. *Nkx2-5* is a key regulator in heart development and has been demonstrated to be associated with the initial stage of cardiac development in vertebrate animals (Warkman et al., 2008). *GATA4* has been identified as being expressed early in the cardiogenic area and persisted later in the developing heart (Bisping et al., 2006). It plays an essential role in promoting cardiac development and differentiation of the myocardium (Pu et al., 2004; Bisping et al., 2006). *TBX5* has been demonstrated to participate in the occurrence of the heart,

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formation of tubular heart ring, and the formation of the initial differentiation of the cardiac chambers and interval (Plageman and Yutze, 2004). Owing to the increased knowledge of the function of these genes research on cardiac development has recently become a topic of vigorous investigation in a variety of model animals, ranging from mammalian animals like mice (Arceci et al., 1993; Gong et al., 2006) to avian such as chicken (Brand et al., 1997; MacNeill et al., 1997; Krause et al., 2004).

In avian species, cardiac development in chicken has received much more attention in recent years, from morphology to molecular research (Brand et al., 1997; MacNeill et al., 1997; Krause et al., 2004). Quail belongs to the same order (Galliformes) and the same family (Phasianidae) as chicken. It has also been used as a model animal for a wide range of research, varying from embryology (Huss et al., 2008; Poynter et al., 2009) to space-related science (Minvielle et al., 2007). To our knowledge, little is known on the molecular mechanism of cardiac development in quail embryos, although there are a few publications on the morphology of cardiac development and even a comparison was made with chicken (Virágh et al., 1993; Saber et al., 2008). Chicken-quail hybrids were produced as early as the 1980s by artificial insemination of chicken sperm to quail (Taksashima et al., 1982; Greenfield et al., 1986). As an interspecies hybrid, it has a significant heterosis for growth rate, body size, meat quality and other production performances (Lucotte <http://www.sciencedirect.com/science/article/pii/0305049177900347> - AFF1#AFF1 et al., 1977; Liao et al., 2012). Chicken-quail hybrids could be used as a good model for avian studies (Liao et al., 2012), although some problems and challenges still exist. Recently, more studies have focused on its embryo development, however, the molecular mechanism of cardiac development has not been explored.

The present study, therefore, investigates the expression of genes related to cardiac development in chicken, quail and chicken-quail hybrids during the early stage of embryogenesis. Real-time PCR was used to detect mRNA expression of *Nkx2-5*, *GATA4* and *TBX5* in the heart of

chicken, quail and chicken-quail hybrids embryos during 3rd to 7th days of incubation.

MATERIAL AND METHODS

Sampling

Females from 200 Korean quails (*Coturnix coturnix*) were artificially inseminated with semen from 5 *Anak* chickens (*Gallus gallus*), provided by the animal experiment station, Shihezi University, Xinjiang, China. Eggs from quails which had received chicken semen (200 eggs of chicken-quail hybrids) were collected. Fertilized eggs of 200 chicken and 200 quail were also obtained. All of the fertilized eggs, including fertilized eggs of chicken, quail and chicken-quail hybrid, were incubated in humidified atmosphere (55% to 60%) at 37.8±0.5°C (control incubation conditions). Thirty embryos were used from chicken, quail, and chicken-quail hybrids, respectively. Embryos were removed from eggs at 3rd, 4th, 5th, 6th and 7th d of incubation (n = 6 per d), during which, the heart were isolated, frozen immediately in liquid nitrogen, and then stored at -80°C for RNA extraction.

RNA extraction and reverse transcription-PCR (RT-PCR)

Total RNA from embryonic hearts was extracted using TRIzol reagent according to the manufacture's instruction (Invitrogen, USA). The quantity of total RNA was examined by ethidium bromide-stained denaturing agarose gel electrophoresis, and RNA concentration was determined by spectrophotometry at 260 nm. RNA samples were stored at -80°C prior to use.

To obtain cDNA, total RNA of each sample (500 ng) was reversely transcribed by the PrimeScript RT Master Mix (Perfect Real Time) (TaKaRa Biotechnology Co., Ltd. Dalian, China), according to the manufacturer's instructions.

Primers (Table 1) were designed for amplifying target genes, according to the mRNA sequence of chicken in the GenBank, by Primer premier 5.0 and synthesized by Beijing BGI Company, China. The PCR reaction volume

Table 1. Primer sequences, PCR product size, annealing temperature, and GenBank accession number for the target genes in chicken, quail and their hybrids

Gene	Primer sequence (5'-3')	Product size/bp	Annealing temp. (°C)	Acc. No.
<i>GATA4</i>	F:GTGTCACCTCGCTTCTCCTT	360	59	XM_420041 ^a
	R:GTGCCCTGTGCCATCTCT			
<i>TBX5</i>	F:CAAGAGAAAAGATGAGGAATG	105	56	NC_006102 ^a
	R:GGGTAACCTGGACCTGTAGAAA			
<i>Nkx2-5</i>	F:TAGCCCTGTGACGACGACTC	357	58	NM_205164 ^a
	R:GGTTTCCTCCTCTCCTCTGT			
<i>β-actin</i>	F:CTGTGCCCATCTATGAAGGCTA	139	55	NM_205518.1 ^a
	R:ATTCTCTCTCGGCTGTGGTG			

F: forward primer; R: reverse primer; Acc. No: GenBank accession number. ^a Chicken sequence.

for each gene was 25 μ l, which contained 1 μ l cDNA templates, 1 μ l each primer (10 μ mol/L), 0.5 μ l Taq DNA polymerase (2.5 U/ μ l) cDNA, 2.5 μ l 10 \times Taq Buffer, 1.3 μ l MgCl₂ (25 mmol/L), 2.0 μ l dNTP mixture (2.5 mM) and 11.8 μ l ddH₂O. After 5 min of denaturation at 94°C, the PCR reaction was performed at 94°C for 30 s, annealing for 30 s, 72°C for 30 s for 35 cycles, with an extension at 72°C for 10 min.

cDNA cloning for target genes

PCR products were separated by electrophoresis on 2% agarose gel and purified by a gel extraction kit (Tiangen Biotech, CO., LTD, Beijing, China). The purified PCR products were ligated into pGEM-T vector (Tiangen Biotech, CO., LTD, Beijing, China), and then the vector was transformed into DH5 α cells. After culture in LB medium containing IPTG, X-gal and Ampicillin for 12 to 16 h at 37°C, the positive clones were selected and send to BGI Company (Beijing, China) for sequencing.

Real-time PCR

Real-time PCR was performed under the following condition: an initial denaturing step at 94°C for 4 min, followed by 35 cycles of 94°C for 15 s, annealing at 55°C for 20 s, and extension at 72°C for 20 s, and a final extension step of 10 min at 72°C. Each reaction was carried out in a total volume of 20 μ l, consisting of 12.5 μ l SYBR[®] Premix Ex, 0.5 μ l each primer (10 μ mol/L), 2 μ l cDNA and 4.5 μ l ddH₂O. Amplification reactions in triplicate for each sample were performed. β -actin was used as an internal control.

Statistical analysis

The relative mRNA expression level of genes was calculated by “normalized relative quantification” method, and statistical analysis was carried out using SPSS version 17.0. One-way ANOVA test and repeated measure of ANOVA were used for statistical analysis of normalized gene copy number, with a p-value of 0.05 considered indicative of a statistically significant difference.

RESULTS

In the present study, expression of the three genes: *Nkx2-5*, *GATA4* and *TBX5*, were determined in the heart of chicken, quail and chicken-quail hybrids embryos during the 3rd, 4th, 5th, 6th and 7th days of incubation. Results showed that these genes underwent dynamic changes during the process of cardiac development in chicken, quail and their hybrids.

For *Nkx2-5* gene, although there was a similar expression trend between chicken and quail during the whole course of experiment: the initial and highest expression of *Nkx2-5* were focused on 3rd day of incubation, then it declined till 5th day of incubation, and fluctuated thereafter. Expression of *Nkx2-5* gene in chicken was significantly lower than that in quail at the same point of time ($p < 0.01$ on incubation d 3, 4 and 5; $p < 0.05$ on incubation d 6 and 7) (Table 2 and Figure 1). In addition, the current study revealed that *Nkx2-5* expression in chicken-quail hybrids is similar to chicken and no significant difference was observed between the two species ($p > 0.05$) (Table 2 and Figure 1). Thirdly and generally, expression of *Nkx2-5* gene in quail was significantly higher than that both in chicken and chicken-quail hybrids (Figure 1).

Table 3 and Figure 2 illustrate the mRNA expression of *GATA4* gene in the heart from the embryos of chicken, quail and their hybrids during the early stage of embryogenesis. From this table, it can be clearly observed that *GATA4* mRNA showed a similar expression trend between chicken and quail, which displayed a steady increase from 3rd to 6th d, and from d 6 onwards the expression level decreased. There was no significant difference between the two species during the whole course of experiment ($p > 0.05$), although expression in quail was a little higher than that in chicken. However, *GATA4* mRNA in chicken-quail hybrids showed a significantly different expression trend from their parents. The highest expression level of *GATA4* mRNA, in chicken-quail hybrids, occurred on d 3 of incubation, then, it declined till the end of the experiment. *GATA4* mRNA in chicken-quail hybrids was significantly higher than that in

Table 2. *Nkx2-5* mRNA expression during the cardiac development of chicken, quail and chicken-quail hybrids embryos

Gene	Species	Days of embryogenesis				
		3	4	5	6	7
<i>Nkx2-5</i>	Chicken	0.152 \pm 0.013 ^a	0.053 \pm 0.006 ^a	0.028 \pm 0.0067 ^a	0.082 \pm 0.0088 ^a	0.052 \pm 0.006 ^a
	Quail	20.557 \pm 3.261 ^A	12.133 \pm 1.110 ^A	5.337 \pm 0.773 ^A	11.707 \pm 1.096 ^b	5.847 \pm 1.367 ^b
	Chicken-quail hybrids	2.913 \pm 0.303 ^b	1.188 \pm 0.420 ^a	0.607 \pm 0.084 ^a	1.600 \pm 0.342 ^a	0.935 \pm 0.048 ^{ab}

Each mRNA of the gene in the heart from chicken, quail and chicken-quail hybrids embryos detected by Real-time PCR is displayed as a relative to β -actin. The letter in the up right corner of the value represents significant differences between any other two data. If the letter between the two values is the same, it means that there is no significant difference ($p > 0.05$). Whereas, if the letter is different between the two data, it means there was significant difference. A different lowercase letter means $p < 0.05$, while a capital letter means $p < 0.01$. Each value is the mean \pm SE of six embryos. The same below.

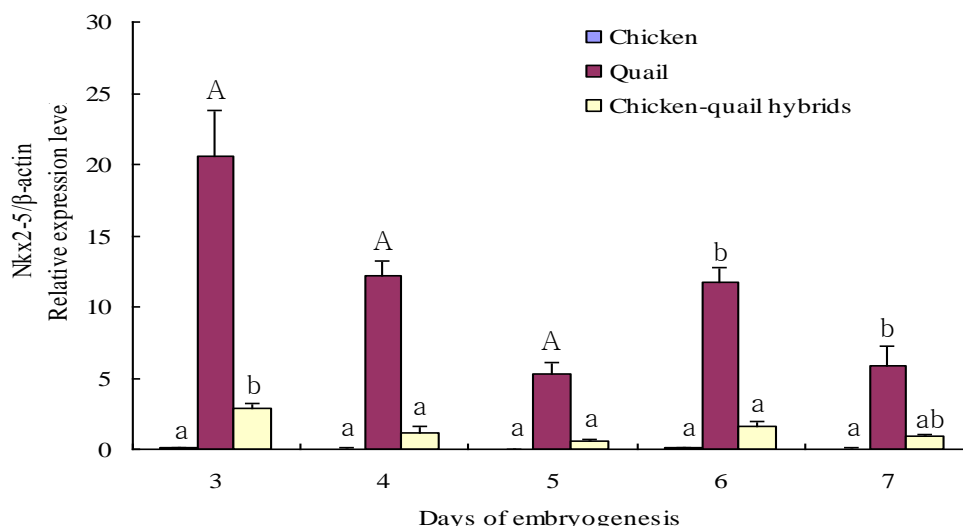


Figure 1. *Nkx2-5* mRNA expression during the cardiac development of chicken, quail and chicken-quail hybrids embryos.

chicken and quail from d 3 to d 5 ($p < 0.01$), however, due to the significant drop from d 5 onwards ($p < 0.01$), it was significantly lower than that in chicken and quail during the later stage of the experiment ($p < 0.05$). During the period of experiment, *GATA4* expression in chicken-quail hybrids fluctuated largely, and differed significantly from chicken and quail.

In this study, we detected the expression of *TBX5* in chicken, quail and their hybrids. As shown in Table 4 and Figure 3, low expression of *TBX5* mRNA was observed

in the early stages (d 3 and 4) in both chicken and quail, but the level showed an upward trend till 6th d, from then onwards it decreased. During the whole course of experiment period the *TBX5* mRNA expression level was similar for both chicken and quail. On the other hand, in chicken-quail hybrids expression of *TBX5* was also low in the early stages (d 3 and 4) but increased sharply over the experiment period, with the significantly higher expression level occurred on d 5 ($p < 0.01$), 6 ($p < 0.01$) and 7 ($p < 0.05$), respectively, compared with chicken and

Table 3. mRNA expression of *GATA4* gene during the cardiac development of chicken, quail and chicken-quail hybrids embryos

Gene	Species	Days of embryogenesis				
		3	4	5	6	7
<i>GATA4</i>	Chicken	1.130±0.170 ^a	1.900±0.320 ^a	3.967±0.531 ^a	10.733±1.081 ^{ab}	5.667±0.890 ^b
	Quail	1.967±1.942 ^a	3.300±0.866 ^a	6.733±1.608 ^a	18.600±3.335 ^b	9.400±1.195 ^{Ab}
	Chicken-quail hybrids	41.600±4.100 ^A	25.330±3.745 ^A	21.600±1.943 ^A	1.900±0.235 ^a	0.533±0.108 ^a

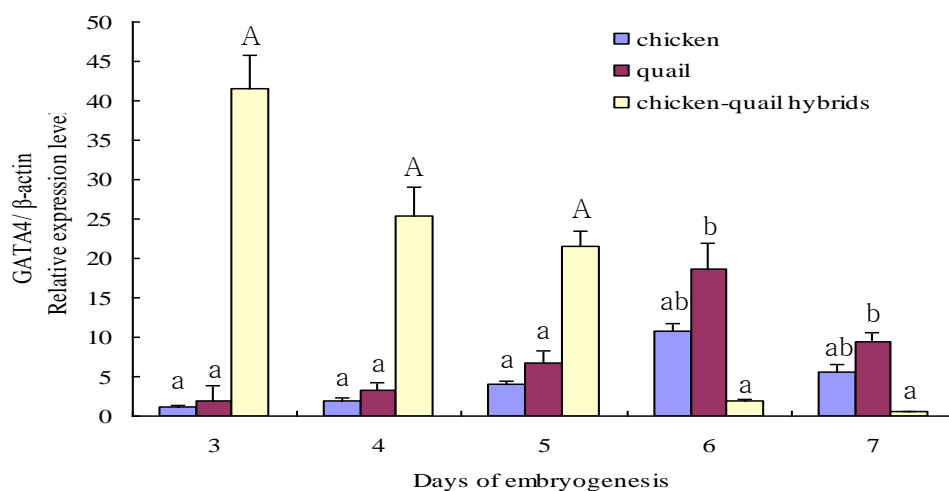
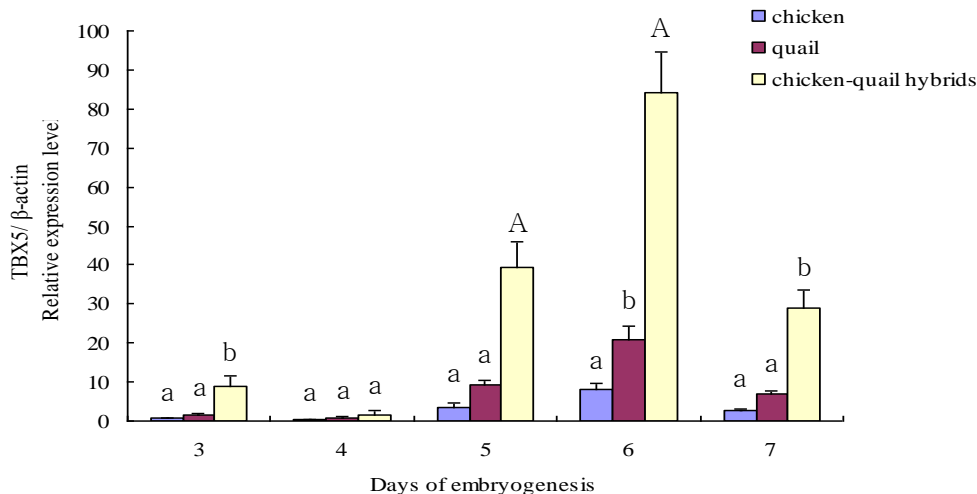


Figure 2. mRNA expression of *GATA4* gene during the cardiac development of chicken, quail and chicken-quail hybrids embryos.

Table 4. mRNA expression of *TBX5* gene during the cardiac development of chicken, quail and chicken-quail hybrids embryos

Gene	Species	Days of embryogenesis				
		3	4	5	6	7
<i>TBX5</i>	Chicken	0.700±0.1718 ^a	0.367±0.092 ^a	3.667±0.907 ^a	7.933±1.726 ^a	2.800±0.480 ^a
	Quail	1.600±0.500 ^a	0.800±0.244 ^a	9.200±1.215 ^a	21.033±3.1143 ^b	6.900±0.740 ^a
	Chicken-quail hybrids	8.770±2.678 ^b	4.700±0.8198 ^a	39.567±6.276 ^A	84.167±10.527 ^A	28.900±4.616 ^b

**Figure 3.** mRNA expression of *TBX5* gene during the cardiac development of chicken, quail and chicken-quail hybrids embryos.

quail. In general terms, although *TBX5* mRNA in chicken-quail hybrids showed similar expression trend as their parents, its expression level was significantly higher.

DISCUSSION

In the present study, we found that there was a similar expression trend of *Nkx2-5* gene between chicken and quail during the whole course of experiment, but expression of *Nkx2-5* gene in chicken was significantly lower than that in quail at the same point of time. This difference may be due to the different incubation period of the embryos in chicken and quail: the former lasts 21 d while the latter only needs 16 to 17 d. On the other hand, it may be explained by the fact that the cardiac development in chicken lagged behind that in quail (Saber et al., 2008). Thirdly, there are different cardiac developmental stages in chicken and quail that require different expression of genes to regulate development, although *Nkx2-5* is a key regulator in heart development, it has been demonstrated to be associated with the initial stage of cardiac development in vertebrate animals (Warkman et al., 2008). In addition, the current study revealed that *Nkx2-5* expression in chicken-quail hybrids is similar to chicken, but significantly different from quail, the probable explanation may be due to the fact that chicken-quail hybrids arise from different species.

GATA4 is a member of the GATA family, which has

been identified as being expressed early in the cardiogenic area and persisting in the developing heart (Bisping et al., 2006). It plays an essential role in promoting cardiac development and differentiation of the myocardium (Pu et al., 2004; Bisping et al., 2006). In addition to that, research on *GATA4* expression in mouse cardiac development revealed *GATA4* might be closely related to the septal and ventricular development (Yang et al., 2010). In the present study, we detected the mRNA expression of *GATA4* gene in the heart from the embryos of chicken, quail and their hybrids during the early stage of embryogenesis. Results showed that *GATA4* mRNA displayed a similar expression trend between chicken and quail, which displayed a steady increase from 3rd to 6th d, and from d 6 onwards, the expression level decreased. Previous research showed that incubation period of 3 to 6 d was the rapid development phase of ventricles and myocardium in chicken, during which, *GATA4* expression showed an upward trend. Our result was in agreement with the opinion of Yang et al. (2010), who found that *GATA4* might be closely related to the septal and ventricular development in the mouse embryo. In this study, there was no significant difference between the two species during the whole course of experiment, although expression in quail is a little higher than that in chicken. This finding indicated that *GATA4* might be highly conserved between chicken and quail. However, *GATA4* mRNA in chicken-quail hybrids showed a significantly

different expression trend from their parents, chicken and quail, which may be due to the fact that chicken-quail hybrids are interspecies hybrids, which could cause genetic disorder (Cao et al., 2010).

TBX5 expression and its relation to cardiac development have been well studied. It has been demonstrated that *TBX5* could be detected in early embryonic heart development, furthermore, it participates in the occurrence of the heart, formation of the tubular heart ring, and the formation of the initial differentiation of the cardiac chambers and interval (Plageman and Yutze, 2004). In this study, we detected the expression of *TBX5* in chicken, quail and their hybrids. We found that low expression of *TBX5* mRNA was observed in the early stages (d 3 and 4) in both chicken and quail, but the level showed an upward trend till 6th d, from which onwards, it decreased. During the whole course of experiment period, *TBX5* mRNA expression level was similar for both chicken and quail. The expression of *TBX5* both in chicken and quail suggested that it might be related to the rapid development of atrium, ventricles and atrial septum. On the other hand, in chicken-quail hybrids, expression of *TBX5* was also low in the early stages (d 3 and 4) but increased sharply over the experimental period, with the significantly higher expression level occurring on d 5, 6 and 7, respectively, compared with chicken and quail. In general terms, although *TBX5* mRNA in chicken-quail hybrids showed similar expression trend as their parents, its expression level was significantly higher than that in chicken and quail.

In conclusion, expression of these genes in chicken and quail showed similar expression trend, while chicken-quail hybrids showed a significantly different expression trend from their parents, the abnormal expression of genes in chicken-quail hybrids need to be further explored.

REFERENCES

- Arceci, R. J., A. A. King, M. C. Simon, S. H. Orkin and D. B. Wilson. 1993. Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. *Mol. Cell. Biol.* 13:2235-2246.
- Bisping, E., S. Ikeda, S. W. Kong, O. Tarnavsk, N. Bodyak, J. R. McMullen, S. Rajagopal, J. K. Son, Q. Ma, Z. Springer, P. M. Kang, S. Izumo and W. T. Pu. 2006. Gata-4 is required for maintenance of postnatal cardiac function and protection from pressure overload induced heart failure. *Proc. Natl. Acad. Sci. USA.* 103:14471-14476.
- Brand, T., B. Andrée, A. Schneider, A. Buchberger and H. H. Arnold. 1997. Chicken NKx2-8, a novel homeobox gene expressed during early heart and foregut development. *Mech. Develop.* 64:53-59.
- Bruneau, B. G. 2002. Transcriptional regulation of vertebrate cardiac morphogenesis. *Circ. Res.* 90:509-519.
- Cao, T. T. 2010. The study of related genes of causing abnormal sexual differentiation in the chicken and quail intergeneric hybrid. Master Thesis, Shihezi University, Xinjiang, China.
- Davis, D. L., A. V. Edwards, A. L. Juraszek, A. Phelps, A. Wessels and J. B. E. Burch. 2001. A GATA-6 gene heart-region-specific enhancer provides a novel means to mark and probe a discrete component of the mouse cardiac conduction system. *Mech. Develop.* 108:105-119.
- Gillio-Meina, C., Y. Y. Hui and H. A. LaVoie. 2003. GATA-4 and GATA-6 transcription factors: expression, immunohistochemical localization, and possible function in the porcine ovary. *Biol. Reprod.* 68:412-422.
- Gong, L., G. Qiu, X. Xu and K. Sun. 2006. Advances of heart-specific transcription factors: NKX2-5, *TBX5* and *GATA4* in congenital heart disease. *Int. J. Genetics.* 29:133-136.
- Gove, C., M. Walmsley, S. Nijjar, D. Bertwistle, M. Guille, G. Partington, A. Bomford and R. Patient. 1997. Over-expression of GATA-6 in *Xenopus* embryos blocks differentiation of heart precursors. *EMBO J.* 16:355-368.
- Greenfield, C. L., K. M. Lartin, F. S. Sanders and R. R. Diertel. 1986. Heterochromatin staining pattern of quail-chicken hybrid lymphocytes. *J. Hered.* 77:216-217.
- Grepin, C., G. Nemer and M. Nemer. 1997. Enhanced cardiogenesis in embryonic stem cells overexpressing the GATA-4 transcription factor. *Development* 24:2387-2395.
- Huss, D., G. Poynter and R. Lansford. 2008. Japanese quail (*Coturnix japonica*) as a laboratory animal model. *Lab. Anim.* 37:513-519.
- Krause, A., W. Zacharias, T. Camarata, B. Linkhart, E. Law, A. Lischk, E. Miljan and H. G. Simon. 2004. Tbx5 and Tbx4 transcription factors interact with a new chicken PDZ-LIM protein in limb and heart development. *Dev. Biol.* 273:106-120.
- Liao, H., X. Guo, L. Zhou, Y. Li, X. Li, D. Liang, D. Li and N. Xu. 2012. Expression of androgen and estrogen receptors in the testicular tissue of chickens, quails and chicken-quail hybrids. *Afr. J. Biotechnol.* 11:7344-7353.
- Liberatore, C. M., R. D. Searcy-Schrick and K. E. Yutzey. 2000. Ventricular expression of *TBX5* inhibits normal heart chamber. *Development. Dev. Biol.* 223:169-180.
- Lucotte, G., A. Perramon and M. Kaminski. 1977. Molecular basis for heterosis in the chicken-quail hybrid. *Comp. Biochem. Physiol. B.* 56:119-122.
- MacNeill, C., B. Ayres, A. C. Laverriere and J. B. Burch. 1997. Transcripts for functionally distinct isoforms of chicken GATA-5 are differentially expressed from alternative first exons. *J. Biol. Chem.* 272:8396-8401.
- Minvielle, F. 2007. The future of Japanese quail for research and production. *World. Poult. Sci. J.* 60:500-507.
- Olson, E. N. 2006. Gene regulatory networks in the evolution and development of the heart. *Science* 313:1922-1927.
- Plageman, T. F. and K. E. Yutzey. 2004. Differential expression and function of Tbx5 and Tbx20 in cardiac Development. *J. Biol. Chem.* 279:19026-19034.
- Poynter, G., D. Huss and R. Lansford. 2009. Japanese quail: an efficient animal model for the production of transgenic avians. *Cold Spring Harb. Protoc.* 1:112.
- Pu, W. T., T. Ishiwata, A. L. Juraszek, Q. Ma and S. Izumo. 2004. GATA4 is a dosage-sensitive regulator of cardiac morphogenesis. *Dev. Biol.* 275:235-244.

- Saber, A. S., K. M. Shoghy, A. M. Erasha and M. M. Nada. 2008. Cardiac looping and formation of the heart regions in Japanese quail embryo (*Coturnix coturnix*). *J. Vet. Anat.* 1:3-13.
- Searcy, R. D., E. B. Vincent, C. M. Liberatore and K. E. Yutzey. 1998. A GATA-dependent nkx-2.5 regulatory element activates early cardiac gene expression in transgenic mice. *Development* 125:4461-4470.
- Schwartz, R. J. and E. N. Olson. 1999. Building the heart piece by piece: Modularity of cis-elements regulating Nkx2-5 transcription. *Development* 126:4187-4192.
- Srivastava, D. and E. N. Olson. 2000. A genetic blueprint for cardiac development. *Nature* 407:221-226.
- Taksashima, Y. and Y. Mizuma. 1982. The sex ratio of chicken-quail hybrids. *Japanese. Poult. Sci.* 19:53-55.
- Virágh, S. Z., A. C. Gittenberger-de Groot, R. E. Poelmann and F. Kálmán. 1993. Early development of quail heart epicardium and associated vascular and glandular structures. *Anat. Embryol (Berl)*. 188:381-393.
- Wagner, M. and M. Siddiqui. 2007. Signal transduction in early heart development (II): ventricular chamber specification, trabeculation, and heart valve formation. *Exp. Biol. Med.* 232:866-880.
- Warkman, A. S., T. A. Yatskievych, K. M. Hardy, P. A. Krieg and P. B. Antin. 2008. Myocardin expression during avian embryonic heart development requires the endoderm but is independent of BMP signaling. *Dev. Dyn.* 237:216-221.
- Yang, X., J. Tian, C. Chen, H. Sun, L. Zhong, X. Wu and J. Zhu. 2010. Temporal regulation of p300 on heart-specific transcription factors during mouse cardiogenesis. *J. Chongqing Med. Univ.* 35:961-965.