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Influence of Ovo Injecting IGF-1 on Weights of Embryo, Heart and Liver of Duck During Hatching Stages

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Abstract: IGF-1 is one of important factors which can make an effect on differentiation and development of tissues and organs. The present study was to observe the effects of IGF-1 to duck's embryo, liver and heart's development, by *in ovo* feeding IGF-1 to hatching duck eggs. Firstly, 0.5mL IGF-1 diluted in saline at different concentrations including 0, 80, 100 and 120ng/mL were injected into duck eggs and the results showed that 100ng/mL was the adjust concentration which had significant effects on duck embryo, liver and heart's development in later periods of hatching stages comparing with the control group. Then, the adjusted concentration of IGF-1 was used to investigate the developmental roles of IGF-1 on duck embryo and organ's development. The results showed that IGF-1 had a positive effect on duck embryonic development, especially had an obvious effect on liver. It was concluded that IGF-1 may play an important role in the process of duck liver development during the hatching stages.

Key words: IGF-1, *in ovo* injecting, duck, heart, liver, embryo

INTRODUCTION

Insulin growth factor-1 (IGF-1) is considered as one of crucial regulatory factors functioning in tissues' differentiation and development. It is secreted most by liver and mainly determined by growth hormone which can maintain the whole IGF-1 level in animal's blood.

Researchers have reported that IGF-1 played an important role in cell's proliferation and organs' formation through different physiological ways. IGF-1 is considered as a potent neurotrophic factor in motoring the neurons' development. It has an effect on the recovery of skeletal muscles after injury and the recovery of neural cells, by playing a role of adjustment factor through affecting the initial process of increasing the number of satellite cell differentiation and proliferation (Rabinovsky *et al*., 2003). The IGF family also plays a significant role in embryonic/fetal growth and development (Tollefsen *et al*., 1989; McMurtry *et al*., 1997). Besides of that, it also plays an important role in cardiovascular homeostasis and it has been proven that plasma levels of IGF-1 is correlated inversely with systolic function in heart failure (Täng *et al*., 2012).

In recent years, researches about IGF-1 in poultry become more and more usual. Researches made investigation on how diets affect the amount of IGF-1 in Japanese quail, the results showed that Glycerol can cause lower expression of IGF-1 and growth hormone (Gasparino *et al*., 2012). What's more on chick eyes' development is that, IGF-1 was also an inhibiting factor of growth hormone in retinal growth. IGF-1 also has an effect on the muscle development of poultry. It was reported that *in ovo* feeding of IGF-1 can increase both the diameter and the number of muscle fibers in Japanese Quails (Turgay and GüLMEZ, 2011). Moreover, there also had reports about the relationships between *in ovo* injecting IGF-1 and the development of breast muscle as well as the effect on expression of Follistatin (Ding Qing-feng *et al*., 2011) and the studies showed *in ovo* feeding IGF-1 into duck eggs can up regulate expression of Follistatin which is a positive regulator of muscle development. In chick, researchers found that the pectoral muscle of broilers exhibited increased myoblast proliferation on ED15 followed by increased differentiation from ED16 leading to increased muscle fiber hyperplasia and mass by ED18 compared to layers and this is also proved to accompany with expression of IGF-1 (Al-Musawi *et al*., 2011). From these studies about IGF-1, we can figure out one of the most important effects of IGF-1 is that it can cause cell differentiation during embryonic stages. Genetic studies showed single nucleotide polymorphism was proved to have close correlation with low production of breast muscle chick line (Sato *et al*., 2012). What's more, it has been reported that the muscle tissue weight, muscle fiber parameters and myoblast proliferation rate in leg and breast muscle, as well as the expression levels of the transcription factors MyoG and MRF4 was higher in ducks injected with IGF-1 at 12 embryonic day (Liu *et al*., 2011). Due to all influences of IGF-1 on poultries, we presumed that it was meaningful to investigate the

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relationship between *in ovo* feeding IGF-1 and the weight of heart, liver and the whole embryo in duck. Therefore, the present research was to *in ovo* inject IGF-1 into duck eggs, using a proven technique which has been used as a model in many experiments in poultry embryo (Clare Ashton *et al*., 2005), to investigate the roles on IGF-1 on weighs of embryo, heart and liver during duck embryonic ages. These works, perhaps, are meaningful to make a primary understanding of IGF-1 on avian's organ and embryo development.

MATERIALS AND METHODS

Egg resources: 400 laboratory eggs were selected from Waterfowl farm of Sichuan Agricultural University for *in ovo* feeding IGF-1 (Tianfu duck, weighing 90±5g), grouped all eggs into four groups (marking A, B, C and D, containing 100 eggs randomly in each group). Then set all eggs into incubator for hatching under the same conditions. All experiments and procedures were allowed by animal welfare.

Injecting process: All eggs were punctured at 13th embryonic age and injected 0.5mL different concentration of IGF-1 (bought from BioVison) diluted in saline which was 0 ng/mL, 80 ng/mL, 100 ng/m and 120 ng/mL, respectively. The detailed steps of puncturing were as following: At first check out the position of air chamber, amnion cavity, yolk and the embryo according to the exposure examination. Meanwhile, address the prepared saline with appropriate warming or cooling in order to keep them at 25°C. Then, before injecting IGF-1, invert the eggs for 10 min making the dull part of the egg down in order to make the embryo move to the sharp part of the egg. Then disinfect all instruments before puncturing all eggs with a hole of diameter of 1mm at the central part of the air chamber (Pay attention to not to hurt the shell membranes). Then inject different concentration of IGF-1 diluted in saline into the air chamber of eggs (Prevent broking the shell membrane). After injecting the cellophane was used to seal the holes (Pay attention that do not pollute the eggs). Repeat the puncturing and injecting steps to all 4 groups until all steps have done, then return all eggs into the incubator with normal incubation.

Data collect and analysis: Samples were collected at the 18, 21, 24 and 27 embryonic age. 10 hatching eggs from each group were selected by chance at each time point, weighing the embryo, peeled heart and peeled liver's weight of each egg. Make the coefficient of variation under 6%, then screen the statistics. Each group's results will be took significance comparison multiply under the situation $P<0.05$. All the results will be shown by bar chart and line graph. All analysis was done through SPSS 18.0.

RESULTS

The influence of different concentration of IGF-1 to duck liver, heart and embryo development at embryonic day 27: Focusing on the embryo weight (Fig. 1a), only group 80ng/mL had a significant decreasing trend comparing to the control group. Besides, the group 100ng/mL and 120ng/mL had no obvious increase on embryo weight comparing to the control group.

As well as comparing embryo weight, we also compared liver and heart weight of different concentration of IGF-1 (Fig. 1b), group 80ng/mL and 100ng/mL have no significant change on both liver and heart weight. By contrast, group 100ng/mL had a significant increase on both liver and heart weight and group 120ng/mL had a significant decrease on heart weight. Except all above that we also conducted the liver and heart ratio (Fig. 1c) which there was no group showed a remarkable increase or decrease. However, *in ovo* feeding IGF-1 had a positive effect on the liver's development with no significant difference comparing to the capricious change happened on heart's development. In addition, group 100ng/mL had the biggest increase on the liver's development.

Comparison of different organs and embryo's development between 0ng/mL and 100ng/mL: Firstly comes to the embryo's development (Fig. 2a) that at embryonic age 24, group 100ng/mL had a noteworthy ($P<0.05$) increase only comparing to the control group. Then comparing the heart's development (Fig. 2b) between 0ng/mL and 100ng/mL, there was barely difference through the chart and statistics ($P<0.05$). As same as the heart when comparing the liver's development between 0ng/mL and 100ng/mL (Fig. 2c), it can be seen that only at embryonic age 27, group 100ng/mL had a remarkable increase. The development of heart ratio (Fig. 2d) showed a capricious change during the whole embryonic age and there was also no significant change. To liver ratio's development (Fig. 2e), it was demonstrated from the chart and statistics that at embryonic age 27, group 100ng/mL had a remarkable change on liver ratio.

DISCUSSION

100ng/mL of IGF-1 is an appropriate concentration on duck's embryo, heart and liver development: IGF-1 is a kind of growth hormones which has a positive effect on embryo development. It has been reported that IGF-1 has an adjusted effect on some tissues and organs such as liver, heart and has a related effect with growth hormone and insulin (Iughetti *et al*., 2012). In this project, four groups of duck eggs were setted with injecting different concentration of IGF-1 ranging from 0ng/mL to 120ng/mL. IGF-1 has a positive effect on cell differentiation and embryo development, we tried to seek

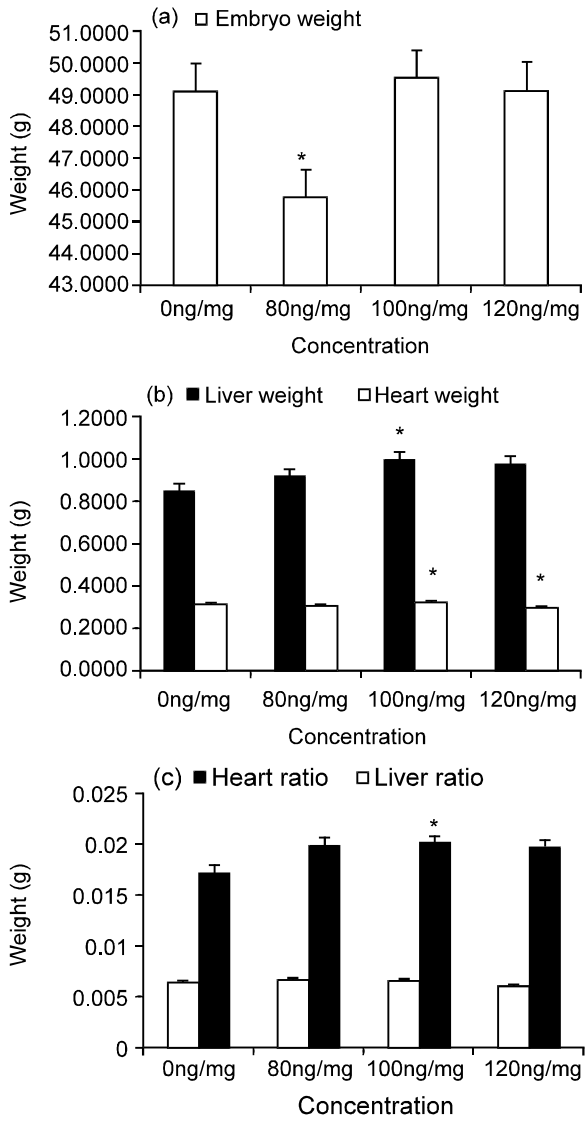

Fig. 1: Comparison of influence of different concentration of IGF-1 on duck embryo, heart and liver.
A Comparison of liver and heart weight.
B Illustration of embryo weight.
C Comparison of liver and heart ratio.
N = 10 for each group.
Label * means significant difference comparing to the control group at the P<0.05 level.

out the relationships between the effects of IGF-1 and the different concentrations of IGF-1. The results showed that the heart, liver and body weight of duck were different in groups due to the different concentration of IGF-1 injected, supporting that the effects of IGF-1 in ducks was also depended on a concentration manner. These phenomenon observed were consisted with a previous study in human tenocytes which has reported that different concentration of IGF-1 has different effect on human tenocytes (Qiu *et al*., 2012). In our experiment, statistics showed group 100ng/mL has the most obvious effect on embryo development. Except the embryo weight, heart and liver both showed significance increase. Although the embryo weight under 100ng/mL IGF-1 didn't show significant difference, it can still be found that embryo weight increased most under that condition. This result is similar with the results from the experiment mentioned above. Others proved that *in ovo* feeding IGF-1 has a positive effect on duck's muscle development, their results are similar with ours and gave explains of why embryo weight increased (Liu *et al*., 2012). High concentration may have an inhibited effect on any tissue's development and low concentration may not reach the lower limit of stimulating development. However, the reason for why tissues' weight would increase is complex, it can be only concluded that 100ng/mg of IGF-1 was the best concentration to stimulate duck embryo growth preliminarily.

IGF-1 may play vital roles in duck's liver development: IGF-1 are secreted in various kinds of cells like myocardial cell, hepatic cell and et al but circulating IGF-1 are almost produced in liver (Kasprzak and Adamek, 2012) which indicates that different tissues' IGF-1 level may be different and exogenous IGF-1 may have biggest influence on liver development. Related survey inferred what extent IGF-1 level do liver and local myocardium secreted using mouse as model (GAO Jun *et al*., 2007) which also indicated that the IGF-1 levels in hepatic cell and cardiac cell are different. As we all know that most tissues are produced during embryonic differentiation, in the present study, in order to discuss the relationship between IGF-1 and main organs in duck including liver and heart, we used *in ovo* feeding IGF-1 to duck eggs before hatching stage and results showed that IGF-1 had a bigger effect on liver rather than heart at late period of hatching stage. These results are similar with the findings of Liu's (2012) who proved that IGF-1 can affect duck muscle mass during late hatching stages after *in ovo* injected IGF-1 at 12 embryonic age. Although IGF-1 was injected into duck eggs at middle embryonic age, this study showed that IGF-1 cause effects at late embryonic age. We think that IGF-1's effect is in stages that IGF-1 play an important role in late development of ducks. We also find that IGF-1 has a more obvious effect on liver rather than cardiac and we don't find any similar research in this aspect. So this may point out a way for liver development on duck in the future.

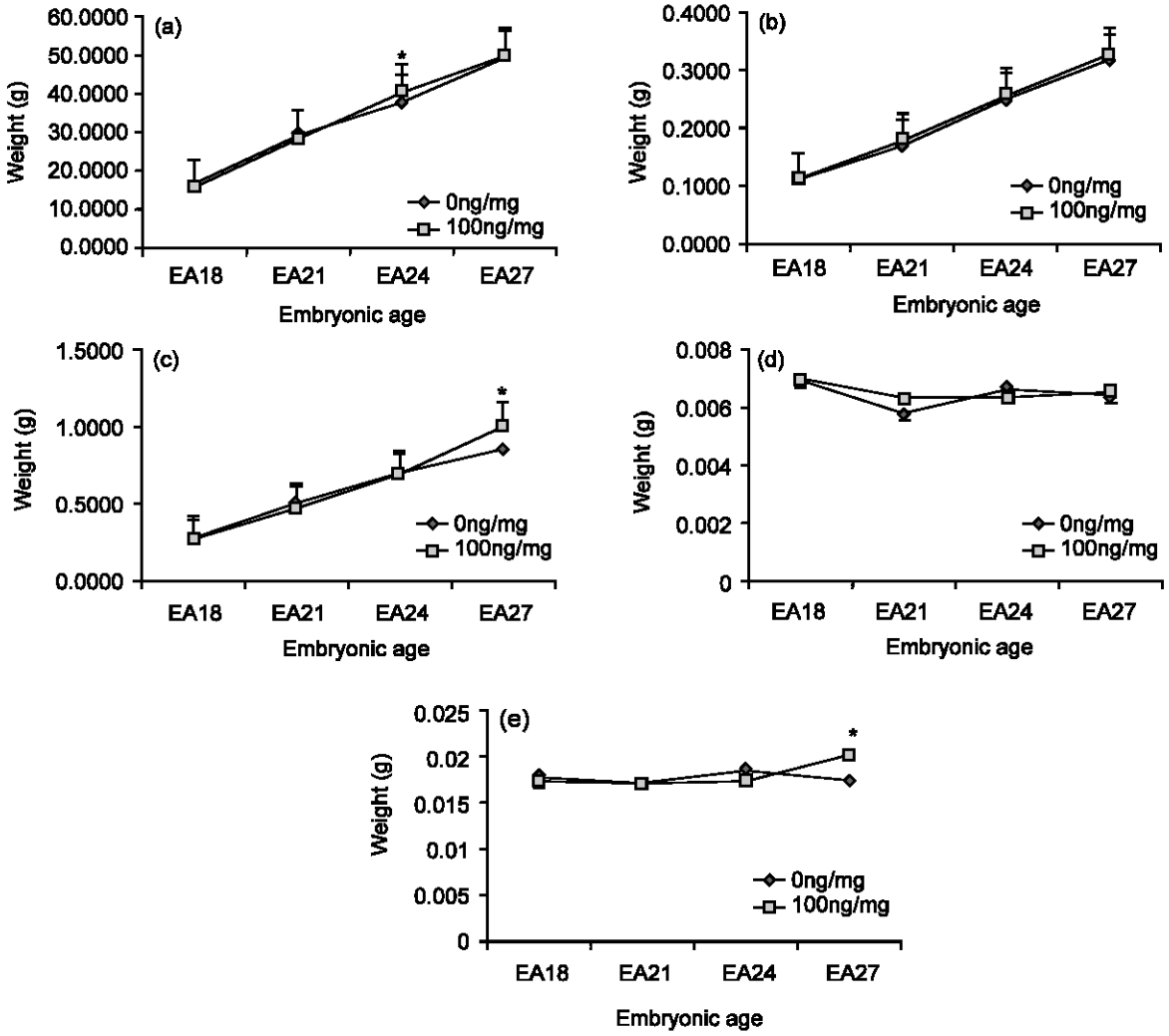

Fig. 2: Comparison of the influence of group 100ng/mL and group 0ng/mL IGF-1 on duck development during whole hatching stage,
A Comparison of the development of liver.
B Comparison of the development of heart.
C Comparison of the development of embryo.
D Comparison of the development of heart ratio.
E Comparison of the development of liver ratio. N = 10 for each group.
Signal * means significant difference comparing to the control group at the P<0.05 level.

Conclusions: 100ng/mL was the appropriate concentration which has significant effects on duck embryo, liver and heart's development in later periods of hatching stages comparing with the control group. IGF-1 may play an important role in the process of duck liver development during the hatching stages for liver was influenced most since *in ovo* injecting IGF-1.

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