Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

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Acta Scientiarum. Biological Sciences, vol. 33, núm. 2, 2011, pp. 215-217
Universidade Estadual de Maringá
.png, Brasil

DOI: 10.4025/actascibiolsci.v33i2.8187

Morphological analysis of testes from post-hatch chicks submitted to temperature variation during incubation

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ABSTRACT. This study aimed to evaluate the effects of temperature variation during incubation on testicular morphology of post-hatch chicks. We utilized 60 eggs incubated under different temperatures: Group 1 – 37.5°C; Group 2 – 39.5°C; and Group 3 – 34°C. Chicks were weighted and the testes were histologically analyzed. All eggs from Group 3 showed embryonic death. There were no significant differences in body weight and testicular morphology between Groups 1 and 2; however, there was a difference in the diameter of the seminiferous tubules ($p < 0.001$). The increase in temperature to 39.5°C during incubation causes a decrease in diameter of the seminiferous tubules.

Keywords: *Gallus gallus domesticus*, thermal stress, embryonic development, seminiferous tubules, morphogenesis.

RESUMO. Análise morfológica dos testículos de pintainhos recém-eclodidos submetidos à variação de temperatura durante a incubação. Este estudo objetivou avaliar os efeitos da variação de temperatura durante a incubação na morfologia testicular de pintainhos recém-eclodidos. Foram utilizados 60 ovos incubados em diferentes temperaturas: Grupo 1 – 37,5°C; Grupo 2 – 39,5°C; e Grupo 3 – 34°C. Os pintainhos foram pesados e seus testículos analisados histologicamente. Todos os ovos do Grupo 3 apresentaram morte embrionária. Não houve diferenças significativas no peso e na morfologia testicular entre os Grupos 1 e 2, porém, houve diferença no diâmetro dos túbulos seminíferos ($p < 0,001$). A elevação da temperatura para 39,5°C durante a incubação causa a redução do diâmetro dos túbulos seminíferos.

Palavras-chave: *Gallus gallus domesticus*, estresse térmico, desenvolvimento embrionário, túbulos seminíferos, morfogênese.

Introduction

Embryonic development in vertebrates is usually correlated with environmental, physical, chemical and biological factors, which can interfere in the embryogenic process. Previous studies reported alterations in embryonic development and hatch time after alterations in temperature and humidity during incubation (DAHLKE et al., 2008; LEANDRO et al., 2000; POLOVINTSEVA; SULEIMANOV, 2008).

Incubation temperature in chickens is 37.5°C, and temperatures over than 42°C or under than 30°C cause embryonic death (KRAUSOVA; PETERKA, 2007; PETERKA et al., 1996; ROMANOFF, 1960). However, small variations in incubation temperature can accelerate or slow down the morphogenesis process and/or cause teratogenicity (KRAUSOVA; PETERKA, 2007; LEANDRO et al., 2000; PETERKA et al., 1996).

The embryogenesis of the reproductive system is strongly correlated with the formation of the excretory system (SMITH; SINCLAIR, 2001). Under normal conditions of incubation, the reproductive organs start their development on the 5th day of development, together with sexual differentiation (HAMBURGER; HAMILTON, 1992; LI et al., 2007; SMITH; SINCLAIR, 2001). A detailed anatomical description of the reproductive system was made by Bull et al. (2007). Until the age of 20 weeks, the testes are oval, elongated, curved and tortuous structures displaced at the sides of the body median line over the cranial portion of the kidneys. From the 21st week on, they show a high increase in size and oval shape, overlaying to the medial portion of the kidneys.

The aim of this study was to evaluate the effects of temperature variation during incubation on the weight of post-hatch chicks and the diameter of seminiferous tubules.

Material and methods

This study was carried out under regulations set by the Animal Use Ethical Committee of the Biological Sciences Institute of the University of Brasília. We utilized 60 fertilized eggs of domestic fowl (*Gallus gallus domesticus*) from small farms around Distrito Federal, Brazil. The eggs were divided in three experimental groups under different temperature protocols during incubation: Group 1 – incubated at 37.5°C (Control group); Group 2 – incubated at 37.5°C during 20h and increased to 39.5°C; and Group 3 – incubated at 37.5°C during 20h and decreased to 34°C. The eggs were kept at these temperatures until the end of the incubation period, 21 days. All eggs were incubated in automatic incubators (J-80, JMM Chocadeiras, Franca, São Paulo State, Brazil), with periodical rotation of the eggs and temperature and humidity controls. The humidity was kept at 60%.

The post-hatch chicks were weighed and euthanized by decapitation to collect the gonads. Only data from male chicks were used. The unhatched eggs after 21 days were opened to analyze the embryo.

Testes were fixed with Carnoy solution (60% absolute alcohol, 30% chloroform and 10% glacial acetic acid). The samples were dehydrated, embedded in paraffin wax, cut serially (5 μm thickness) and stained with hematoxylin-eosin. We made two laminas from each animal, and three non-adjacent sections of each lamina were analyzed. The sections were photographed in a light microscope (200x) and the diameter of seminiferous tubules on transversal section was measured using the software Image Pro Express 6.0 (Media Cybernetics Inc., Bethesda, MD, USA). Variable means of each group were compared by Student's *t* test ($p < 0.05$) using the software SPSS for Windows 13.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

After the incubation period, all eggs from Groups 1 and 2 hatched. All embryos from Group 3 were in the initial stage of development (most of embryo structures could be identified, including the beak) and dead. According to descriptions by Hamburger and Hamilton (1992), the embryos were 5-6 days old. Therefore, these embryos were not weighed and gonads were not collected.

From 20 eggs incubated in each group, we obtained 15 and 11 males for Groups 1 and 2, respectively. Approximately 60 seminiferous tubules were measured from each animal, totaling 700 tubules for each group.

There was no statistical difference between the weight of chicks from Group 1 and 2 (Table 1). During morphological analysis, no differences were observed between the analyzed groups, either. Both groups showed Sertoli cells and some spermatogonia inside the seminiferous tubules. Outside the tubules, a large amount of Leydig cells and connective tissue was observed. However, a statistical difference ($p < 0.001$) was observed between the diameter of seminiferous tubules of the analyzed groups (Table 1).

Table 1. Chick weight and diameter of seminiferous tubules after 21 days of incubation under two different temperature protocols (37.5 and 39.5°C).

Group	Chick weight (mean ± SD; g)	Diameter of seminiferous tubules (mean ± SD; μm)
Group 1 (37.5°C)	36.86 ± 7.13 (n=15)	35.66 ± 4.73 (n=700)
Group 2 (39.5°C)	34.79 ± 8.51 (n=11)	30.44 ± 6.96 (n=700)*

*$p < 0.001$; Student's *t* test.

The acceleration or slowing down of the embryonic development caused by temperature variation during the incubation period have been reported (DAHLKE et al., 2008; POLOVINTSEVA; SULEIMANOV, 2008). Those authors did not report embryonic death by thermal stress; however, those studies either analyzed the beginning of embryonic development (until 96h of incubation) or carried out a small temperature variation (increase or decrease of 1°C). On the other hand, Peterka et al. (1996) observed a high mortality rate after nine days of incubation at 34°C, corroborating the results found. Maybe 34°C is a critical temperature only after the 5th day of development, probably because this moment is the beginning of the embryo growth period and the embryo needs a high speed metabolism, but it is compromised by the low temperature.

Despite reports of development acceleration of embryo under temperature increase during the incubation period (DAHLKE et al., 2008; LEANDRO et al., 2000), we observed a negative effect on testicular development. According to Li et al. (2007), the migration of the primordial germ cells starts between the 2nd and 3rd day of incubation, and from the 5th day begins testicular morphogenesis and differentiation of the primordial germ cells to spermatogonia. Therefore, despite the fact that spermatogenesis in roosters occurs under high temperatures (40-41°C; BEAUPRÉ et al., 1997), perhaps the primordial germ cells are more sensitive than other spermatic cells and the increase of temperature during the incubation period reduced the number of primordial germ cells, causing a reduction in the diameter of seminiferous tubules.

Conclusion

Thus, we concluded that temperature variations during the incubation period can cause embryonic death and affect testicular morphogenesis. Therefore, temperature control is a critical factor for straight embryonic development and to guarantee the fertility of hatched animals.

Acknowledgements

We would like to thank CNPq for the scholarships, and Finatec for the financial support.

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Received on September 9, 2009.
Accepted on March 3, 2010.