Journal of Integrative Agriculture 2017, 16(10): 2257–2263

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RESEARCH ARTICLE

Research on the appropriate way to transfer exogenous substances into chicken embryos

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Abstract

In biological research, chicken embryos are a classic experimental model for the exploration of the embryonic development and cell differentiation. Transferring exogenous substances into chicken embryos for producing medical antibodies has been widely used in the production practice. However, there are few studies about the effect of the different injection site and dosage on chicken embryos. The aim of this study was to explore the effects of different injection sites and dosages on chicken embryo hatching rate and development, so as to provide a basis for further studies using the chicken embryo model. Freshly laid eggs (Rugao yellow chicken) were injected with different doses of saline at the tip, equatorial plane and the blunt end of the egg shell, respectively. Egg hatching rate was recorded and compared among injection sites and different doses. A trypan blue stain was also injected at the aforementioned sites and the growth of chicken embryos was observed. The SPSS (statistical package for the social science) software was used to analyze the relationship between the chicken eggs hatching rate and the different injection sites or the different dosages. The experimental results showed that there were significant differences on egg hatching rates among the different injection sites and doses (P<0.05). The hatchability of the blunt end injection group was significantly higher than that of the other two sites. The egg hatching rate decreased with increased saline doses. The egg hatching rate of the 100 μL saline injection group was higher than the 200 and 300 μL dosage groups. Ultimately, we suggest that the optimal chicken embryo injection process is during early development, at the blunt end site with a dose less than 100 μL to minimize damage to the egg.

Keywords: chicken embryos injection, dosage, hatchability, chick embryo development

Received 9 November, 2016 Accepted 20 April, 2017

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doi: 10.1016/S2095-3119(17)61668-X

1. Introduction

Developmental biology has rapidly evolved since the 1990s and during this process many landmark research achievements have been reached through the use of some sort of biological models (Guo 2008). The unique biological characteristics of poultry make it an important model organism in the study of biological problems (Zhao *et al*. 2006). As a

non-mammal intermediate evolutionary organism between mammals and lower vertebrates, chickens are considered to be an important model animal for genetics research. The development of chicken embryos originates from eggs rather than the uterus and is convenient for operation and observation *in vitro* (Man *et al*. 2010). Chicken embryos also have many other advantages over the use of mammalian embryos, as they are inexpensive and easily handled (Wu 2009). For more than a century, chicken embryos have been an important model system in developmental biology studies. Nowadays, chicken embryos have become a classic experimental model for understanding embryonic development and the growth and differentiation of embryonic cells.

Since the advent of biotechnology, chicken embryos have become one of the best experimental systems (Stern 2005). Additionally, chicken embryos have been highly regarded for establishing medical models, specifically a model for tumor development. At present, the shell windowing method is mainly used when chicken embryos are utilized as the experimental model. Shell windowing is opening the eggshell and reclosing it without perturbing the embryo's growth for examining development. However, this method greatly influences the hatchability of the eggs. Windowing allows water evaporation and entry of air from the external environment, resulting in declined embryo hatchability (Li *et al*. 2014). It has also been reported that shell windowing causes irreversible mechanical damage on early-stage chick embryos (Liu *et al*. 2001). Meanwhile, the differences in the supply of calcium to the embryo that is caused by removing part of the shell adds to the increased mortality of the eggs (Ono *et al*. 1994). Here, we believe that egg injections may be an appropriate alternative to avoid the aforementioned negative effects of shell windowing. Injection openings would be small and development would be monitored through this hole to reduce mechanical damage on the early chick embryos. This method is widely used in the production of chick embryos in the medical field. In many western countries, automated egg inoculation injection systems are replacing the traditional manual inoculation. Currently, there is very little literature reporting the effects of different doses and injection sites on chicken embryo hatching rate and development.

The aim of this study was to explore the effects of different injection location and saline dosage on chicken embryo hatching rate and development, so as to provide the basis for delivering exogenous substances to chicken embryos with minimal damage.

2. Materials and methods

2.1. Reagents and materials

The following reagents and materials were used in this study: sodium chloride injection (250 mL, 200 g, Jiangsu Kangbao Pharmaceutic Co. Ltd., China); trypan blue (Sigma-Aldrich, USA); bromogeramine solution (Jiangxi Decheng Pharmaceutic Co. Ltd., China); 1 and 5 mL disposable sterilized syringe; hatching eggs (Jiangsu Institute of Poultry Science, China).

All procedures involving the care and use of animals conformed to U.S. National Institute of Health guidelines (NIH Pub. No. 85-23, revised 1996) and were approved by the Laboratory Animal Management and Experimental Animal Ethics Committee of Yangzhou University, China.

2.2 Methods

Prior to injection, shells were wiped with 75% alcohol and 0.01% benzalkonium bromide solution for sterilization. After sterilization, eggs were placed in egg cartons and marked at the injection site. Using a syringe needle, a hole was drilled for injection (Note: the operation should be soft and slow, in order to reduce the mechanical damage on the early chick embryos). A corresponding dose of the reagent was drawn with a syringe and 5 mm of the needle was inserted into the hole and the reagent was slowly injected. The needle was then removed gently and the injection site was wiped with 75% alcohol. A piece of paraffin was then placed on an alcohol lamp and heated until the paraffin oil appeared. The paraffin oil was dropped onto the injection site and allowed to set (Fig. 1).

After the injection treatment, the eggs were conventionally hatched: blunt end upwards during the first 18 days with an incubation temperature at 38.5°C and the relative humidity at 60%. The tile range was ±90 degree and the reversal rate was every 2 h. In the later hatching stage, the incubating temperature was reduced appropriately, and stop to tile the eggs in the last 3 days.

2.3. Experimental procedures

Egg injection site and dose preliminary study To determine the best injection site and saline dosage for hatching rates for further experiments, a wide range of doses and variable sites were tested. Varied saline doses (0, 250, and 500 μL) were injected into three different sites of the egg including the tip, equatorial plane and blunt ends, respectively. These sites were then sealed with paraffin. Each dose was used on 30 fertilized eggs.

Egg hatching measurements based on injection sites and saline doses Eggs were divided into three treatment groups based on injection site. Each injection site was given four dose treatments (0, 100, 200, and 300 μL) of saline. All three groups contained 120 breeder eggs total, with each dose having 30 breeder eggs. An untreated control group was also tested with 30 eggs. After the incubation