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Physiological effects of hypoxic conditions during the plateau period on the chicken embryo

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ABSTRACT

The chick embryo employs several adaptive responses to hypoxic challenges, affecting both metabolism and oxygen (O_2) transport. The present study assessed the effects of hypoxic conditions (17% O_2) during the plateau phase on embryonic metabolic rate, cardiovascular parameters, and development up to hatching. The study was divided into 2 experiments: (1) Control; 17% O_2 for 6 h/d on E16–E18 (6H), and 17% O_2 for 12 h/d on E16–E18 (12H), and (2) Control; 12H, and 17% O_2 continuously for 72 h on E16–E18, (72H). Hypoxic embryos exhibited a significant increase in heart rate and an upward trend starting on E17 in hematocrit and hemoglobin levels. We observed a decrease in metabolism in 12H and 72H embryos during the plateau period; their oxygen consumption as well as yolk consumption were lower compared to Control and they hatched with a significantly lower body temperature, indicating lower heat production. There was no evidence of adaptation or long-term effects of exposure to 17% O_2 for 6 h/d. Exposure to 72 h of hypoxic conditions led to significant physiological changes and had a detrimental influence on embryonic development and growth. In contrast, exposure to 12 h/d produced moderate hypoxic changes, which helped the embryo to cope with the stress without significant influences on its growth and development. The decrease in metabolism may represent a metabolic adaptation through a decrease in resting metabolic rate and lower heat production. Such alterations may affect post-hatch performance and energy allocation between maintenance and growth, especially under stress when there is increased oxygen demand.

1. Introduction

The metabolic rate of the developing chicken embryo is influenced by various incubation conditions, such as temperature, humidity, and oxygen concentration (Romanoff, 1967; Deeming and Ferguson, 1991; Druyan et al., 2012). Rahn et al. (1974) suggested that as incubation progresses, metabolic processes are increasingly limited by the restricted diffusion capacity of the chorioallantoic membrane (CAM), and that is the explanation for the plateau phase of gas exchange and heat production during days 16 to 18 of incubation (E16–E18). As embryogenesis progresses towards hatching, the capacity for oxygen diffusion across the CAM must increase to match the gradual increase of O_2 demand (Tazawa et al., 1976; Tazawa and Mochizuki, 1977). In light of the restrictions imposed by the relatively fixed conductance of the shell and the limited diffusion capacity of the CAM, it is expected that hypoxemia will develop. The embryo can employ several potential adaptive responses to hypoxic challenges that may be protective or potentially pathological. These can affect both metabolism and oxygen transport, in ways including alteration of cardiac output and redistribution of oxygenated blood from the periphery to vital organs (Mulder et al., 1998). An increased cardiac output means that there is an increase in the amount of oxygen delivered by the blood. This can be achieved by increasing the heart rate and/or stroke volume. However, Burggren and Keller (1998) found that an increase of heart rate and/or stroke volume following prolonged exposure of chick embryos to hypoxia can lead to an increased heart mass and ventricular hypertrophy.

Increased blood oxygen-carrying capacity can be achieved by various means: polycythemia (Dusseau and Hutchins, 1988), modification of hemoglobin (Liu et al., 2009), an increase of vascularization (Dusseau and Hutchins, 1989) or combinations of these. Furthermore, hypoxia acts as a suppressor of non-shivering thermogenesis in mammals, cooling the embryo without increasing the metabolic rate (MR) (Singer and Muhlfeld, 2007). These adaptive responses result in a more efficient consumption of residual oxygen. Under conditions of hypoxia, the decrease in oxygen consumption is a very common response in all classes of animals. In mammals, it is particularly apparent in the neonatal period, and several lines of evidence suggest that it is a regulated process, rather than simply a passive response to the decrease in O_2 availability (Mortola, 2001). In avian embryos oxygen

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consumption is reduced during hypoxia (Mortola, 2009) with minimal increases in lactate levels (Bjonnes et al., 1987; Hoiby et al., 1987). However, while some previous studies reported that there was no post-hypoxic elevation in O_2 consumption above the pre-hypoxic level (Ar et al., 1991; Mortola and Besterman, 2007), we have found that embryos that were exposed to hypoxia in an early phase of incubation had elevated hematocrit and hemoglobin concentrations, that affected their oxygen consumption rate and residual yolk intake (Druyan et al., 2012). Mortola et al. (2012) have suggested that the avian embryo reduction in O_2 consumption under hypoxic conditions is a real adaptive response, causing a hypometabolic state, with no or minor participation of anaerobic pathways involved in energy balance.

Therefore, the aim of the present study was to assess the effects of exposure to hypoxic conditions (17% O_2) during the plateau phase on the embryonic MR, cardiovascular parameters, and embryonic development during exposure and until hatch.

2. Materials and methods

The study was approved by the Agricultural Research Organization Committee for Ethics in Using Experimental Animals, and has been carried out in compliance with the current laws governing biological research in Israel (Approval number: IL-158/08).

2.1. Experimental design

The study was comprised of two experiments (Exp.I and Exp.II), each with two replicated incubations. For each incubation, 900 fertile Cobb strain broiler chicken (*Gallus gallus domesticus*) eggs with an average weight of 64.0 ± 2.5 g were obtained from a breeder flock of hens during their optimal period of egg production (35–42 weeks old). All eggs were numbered and weighed individually prior to incubation. They were incubated in a Danki Medium-Size Incubator for 2500 eggs (Danki ApS, Ikast, Denmark) under standard incubation conditions of 37.8 °C and 56% RH, and were turned once per hour. The study facility is located at 31 m above sea level. At E16 after candling, 290 fertile eggs were randomly assigned to one of 3 treatments:

In Exp.I: 1. Control; 2. O_2 concentration of 17% for 6 h/d from E16 through E18 (designated as 6H); 3. O_2 concentration of 17% for 12 h/d from E16 through E18 (designated as 12H).

In Exp.II: 1. Control; 2. O_2 concentration of 17% for 12 h/d from E16 through E18 (designated as 12H); 3. O_2 concentration of 17% continuously from E16 through E18, a total of 72 h (designated as 72H).

2.1.1. 17% O_2 exposure

Exposure to 17% O_2 was accomplished by transferring the eggs to a low O_2 concentration kept in a Medium-Size Incubator (Danki ApS, Ikast, Denmark) equipped with a Model 2BGA-SP-MA O_2 and CO_2 Control System (Emproco Ltd., Ashkelon, Israel) for 6 h, 12 h, or continuously for 72 h as described before by Druyan et al. (2012).

On E19, all eggs were transferred into hatching trays. The experiment was terminated immediately after hatching. Body weight (BW) and body temperature (T_b) were measured and blood samples were drawn from the jugular vein of 10 randomly-selected chicks approximately 2 h after hatch (Druyan et al., 2012) for further analysis.

2.2. Embryo measurements

2.2.1. Eggshell temperature (T_{egg})

From E16, eggshell temperature (T_{egg}) of 15 eggs ($n = 15$) per incubation treatment was measured every 6 h with a ThermoScan type 6022 infrared thermometer (Braun, Kronberg, Germany) (Druyan, 2010; Piestun et al., 2008).

2.2.2. Heart rate (HR)

From E16 onward, HR of 15 embryos from each treatment was measured every 6 h with a Buddy Digital Egg Monitor (Avitronics, Torquay, UK). Use of infrared transmitters and sensors enabled the amplification of the cardiovascular signal of an embryo within the egg by as much as 20,000 times, allowing detection of the actual heartbeat of the embryo as early as 12 d after the incubation started (Druyan, 2010).

2.2.3. Oxygen consumption

In order to measure O_2 consumption of the embryos during incubation, every 12 h from E16 onward, 5 eggs from each treatment were placed individually in a small cylindrical metabolic chamber, measuring 7 × 7 cm in diameter and height, which was placed in a water container maintained at 37.8 °C. O_2 consumption was measured as previously described (Buffenstein and Yahav, 1991) in an open flow system and calculated by using the standard temperature, pressure, dry (STPD) method according to the following equation:

$$\text{O2 consumption}\left(\frac{\text{ml}}{\text{gmin}}\right) = \left[\frac{(20.94\ (\text{inflow}) - ?(\text{outflow})) \times 50}{100} \times \frac{60\ \text{min}}{\text{embryo weight g}}\right] \times \frac{273\ \text{K}}{(273 + \text{egg temperature})\ \text{K}} \times \frac{757}{760}.$$

Briefly, dried air was pumped at 50 ml/min into the metabolic chamber, which was fitted with a flow meter scaled from 0 to 60.56 ml/min (Aalborg Instruments and Controls, Orangeburg, NY, USA). Dried air from the metabolic chamber was measured for partial O_2 pressure with a model S-3A/I oxygen analyzer (Ametek, Pittsburgh, PA, USA), and O_2 consumption was measured continuously for 15 min, in light of previous studies (Druyan, 2010; Piestun et al., 2008). The embryos were euthanized by cervical dislocation at the end of the measurement and O_2 consumption was calculated per 1 g of embryo tissue.

2.2.4. Blood parameters

Between E16 and E19, approximately 0.5 ml of blood was drawn daily from the allantoic vein of 10 embryos per treatment into a heparinized syringe. After hatch, blood was sampled from the jugular vein. Whole blood samples were used to determine hematocrit and hemoglobin concentration.

Blood for hematocrit measurements was drawn into heparinized microcapillary tubes and centrifuged in a microliter centrifuge (Hettich, Tuttlingen, Germany) for 8 min at 4000 × *g*.

Hemoglobin concentration in whole blood samples was determined spectrometrically with a Hemoglobin Reagent Set, Catalog No. H7504 (Pointe Scientific, Canton, MI, USA), according to the manufacturer's instructions.

2.2.5. Egg, yolk, embryo, liver, breast muscle, and heart weights

Every day from the initiation of the low O_2 challenge (E16) until hatch (E21), 10 eggs per treatment were euthanized, dissected for detailed weight recording by Type E154 analytical scale (Gibertini, Novate, Italy, ±0.1 mg).

Since embryo or chick weight is commonly expressed as % of egg weight and organ weights are expressed as % of embryo or chick weight, the yolk-free absolute weight of each embryo (or chick) was used to calculate its relative weight (% of initial egg weight) using the following formula:

$$\text{Relative embryo weight}\ (\%) = [(\text{embryo weight})/(\text{initial egg weight})] \times 100.$$