

Research Note: Embryonic viability by weight difference between donor and surrogate eggs in a surrogate eggshell incubation system

Hyeon Yang, Bo Ram Lee, Sun Keun Jung, Hwi-Cheul Lee, Yong Jin Jo, Jingu No, Ji-Youn Kim, Haesun Lee, Seokho Kim, Keon Bong Oh, and Sung June Byun¹

Animal Biotechnology Division, National Institute of Animal Science, Rural Development Administration, Wanju-gun 55365, South Korea

ABSTRACT A surrogate eggshell incubation system is a well-defined method to apply to avian genetic modification. In this study, we tried to investigate whether the egg weight differences between donor and surrogate eggs have an effect on donor viability. The groups were divided by egg weight differences between the donor and surrogate eggs into 4 in each system. The viability at d 4 was evaluated at the end of System II, the embryos alive were transferred into the second surrogate eggshells, and

the viability at d 5, 6 was evaluated at early phase of System III. Then, the viability of System III was evaluated at different incubation period: d 6–12, d 13–18, d 19–21, and hatching rate was evaluated at d 22. Although the effect of egg weight differences between the donor and surrogate eggs was not observed, a specific group in System III showed higher survival and hatching rate than other group ($P > 0.05$).

Key words: chicken, surrogate eggshell incubation system, viability, weight

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INTRODUCTION

Transgenic chickens have been produced by different approaches than those used for mammals due to the unique reproductive mechanisms in chicken. The formation of eggshells in shell gland makes it difficult to directly apply to genetic modification to chicken blastoderm. Therefore, many attempts, such as surrogate eggshells (Mozdziak et al., 2003), windowing eggshells (Speksnijder and Ivarie, 2000), and shell-less incubation systems (Ono, 2000) have been exploited.

The surrogate eggshell incubation system has been verified to be more efficient, owing to its higher hatchability (Borwornpinyo et al., 2005). The incubation established for the first time in 1988 (Perry, 1988), consists of three different systems, System I (in vivo fertilized donor egg), System II (the first surrogate eggs larger than donor eggs), and System III (the second surrogate eggs larger than System II eggs) (Liu et al., 2012). The surrogate eggshell incubation system has the potential to support comparatively easily manipulation of chicken blastoderm. This results from a broad eggshell

opening made by grinding the pointed-end of the surrogate eggshell in System II.

Although the surrogate eggshell incubation system has been improved to enhance hatchability, only a few studies have reported the eggs weights of donor, first and the second surrogate eggs. In the current study, we tried to investigate whether the egg weight differences between the donor and surrogate eggs have an effect on donor viability. The weight differences between the donor and surrogate eggs were set up as four groups in each System II and III, according to the weight distribution of the eggs. Then, donor viability was evaluated in two systems.

MATERIALS AND METHODS

Experimental Animals and Animal Care

The care and experimental use of White Leghorn (WL) chickens was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Institute of Animal Science (NIAS-2020-468), Republic of Korea. All procedures, including chicken maintenance, feeding, reproduction, and treatment, followed the standard operating protocols of the Animal Biotechnology Division at the National Institute of Animal Science.

Egg Collection and Grouping

Fertile eggs for donor of System I and fertile or unfertile eggs for the first surrogate eggs of System II were

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¹Corresponding author: pcs1778@korea.kr

obtained from the WL population of the same age (37–46 wk) at the institutional poultry farm. Infertile eggs used for the second surrogate eggs of System III were purchased from a regional HyLine Brown (HLB) poultry farm. All eggs weights were labeled on the eggshell surface, and divided into 4 different groups in System II (the weight of the first surrogate eggs – the weight of the donor eggs) and System III (the weight of the second surrogate eggs – the weight of the donor eggs), respectively. The groups for the first surrogate eggs were 4.0 g or lighter (Group A), 4.1 to 4.4 g (Group B), 4.5 to 4.8 g (Group C), and 4.9 g or heavier (Group D) than donor eggs. The groups for the second surrogate eggs were 28.5 g or lighter (Group E), 28.6 to 30.5 g (Group F), 30.6 to 32.5 g (Group G), and 32.6 g or heavier (Group H) than donor eggs. The donor and first surrogate eggs were used within two days after oviposition. The purchased second surrogate eggs were stored at 4°C, 70 to 80% relative humidity.

System II

The surrogate eggshell incubation procedures were based on previous studies (Borwornpinyo et al., 2005) with simple modification. Briefly, the donor and first surrogate eggs were sterilized in a water bath for 3 min at 37.5°C supplemented with a sodium hypochlorite solution (1/4,000 dilutions, Sigma, Saint Louis, MO). The pointed-end of the first surrogate egg was ground, entire contents of the egg were discarded, and washed with distilled water. The donor egg was carefully broken, the yolk with blastoderm and thick albumen were transferred into the first surrogate eggshell. Then, only thin albumen from other eggs was added to fill the eggshell. The reconstituted eggs were sealed with Saran Wrap and albumen, the eggs were well-fixed using a pair of plastic rings and rubber bands. The eggs positioned pointed-end up were incubated at 37.8°C, 60 to 70% relative humidity, and rocked through an angle of 90° at 30 min intervals for 3 d. During incubation, donor viability was visually monitored daily. During this period, the formation and circulation of embryonic blood and blood vessel were mainly considered to be criteria of survival.

System III

The second surrogate eggs were also sterilized like the donor and first surrogate eggs. The blunt-end of the second surrogate egg was ground, entire contents of the eggs were discarded, and washed with distilled water. Only alive donor developing to the Hamburger and Hamilton Stage 17-to-18 (HH stage 17-to-18; Hamburger and Hamilton, 1951) were transferred into the second surrogate eggshell. The reconstituted eggs were sealed with Saran Wrap and albumen, the eggs positioned blunt-end up were incubated at 37.8°C, 60 to 70% relative humidity, and rocked through an angle of 30° at 30 min intervals for 15 d. Then, the eggs were incubated at 37.3°C, 70 to 80% relative humidity,

without rocking by hatching. During incubation, embryonic viability was visually monitored daily. The development of blood circulation system, cardiac impulse and embryonic movement were observed in viable embryos. After beginning pulmonary respiration, embryonic movement and air burlbles on the inside of Saran Wrap were criteria of survival.

Statistical Analysis

The viability of chicken embryo at d 4 by the end of culture in system II was analyzed by chi-square test. The total number of embryos at d 6 in each group was considered as the initiation number to evaluate the viability in System III. The viability of chicken embryo at d 6–12, d 13–18, d 19–21, and hatching rate at d 22 were analyzed by chi-square test. All statistical analysis was performed on SPSS software (IBM SPSS statistics version 28.0.1.0). The significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

A transfer of a yolk with blastoderm into surrogate eggshells twice is a feature of surrogate eggshell incubation system. This system consisted of the three types of eggs: in vivo fertilized eggs for donor and the first and second surrogate eggs for use as surrogate eggshells in System II and III. The optimal egg weight differences between the donor and surrogate eggs in the systems have not yet been seriously considered.

In this study, the donor and first surrogate eggs were collected from a WL population of same age; on average, 59.0 ± 3.6 g of fertilized eggs ($n = 94$) and 63.4 ± 3.4 g of fertilized or unfertilized eggs ($n = 94$) were used as the donor and first surrogate eggs, respectively. The entire contents of the first surrogate eggs were discarded. Additionally, 89.8 ± 5.2 g of the second surrogate eggs ($n = 81$) collected from a HLB poultry flock were used, and the entire contents of the second surrogate eggs were also discarded.

The donor viability on each incubation period and hatching rate were shown in Table 1 and Table 2. Total 94 donors were transferred into the first surrogate eggshells and they were incubated 72 h, and 81 embryos were confirmed to be alive at d 4 in System II. The viability of the donor by egg weight differences in System II ranged from 84.4 to 90.4%, and there was no significant difference ($P > 0.05$). After transfer of live embryo to the second surrogate eggshells at d 4, the viability of the embryos was decreased among 3 groups (Group B, C, and D) at early phase of System III. However; on average, 86.7% at d 5 and 81.5% at d 6 of the embryonic viability were confirmed (Table 1). Then, the embryos at d 6 were counted as the initiation number in System III.

The embryonic viability by egg weight differences in System III was observed in 4 different incubation periods: d 6 to 12 (d 6–12), d 13 to 18 (d 13–18), d 19 to 21 (d 19–21), and d 22 (d 22, hatching). On average, 97.5% at d 6-12, 94.4% at d 13-18, 86.6% at d 19-21 of the viability

Table 1. Viability of chicken embryos by egg weight differences between donor and surrogate eggs in System II and success rate of transfer to System III.¹

Group	N	System II (%)		System III (%)	
		d 1	d 4	d 5	d 6
A	22	100.0	84.4	84.4	84.4
B	28	100.0	90.4	88.0	88.0
C	19	100.0	88.9	84.7	80.6
D	25	100.0	89.6	89.6	72.9

¹Viability of chicken embryos at d 4 in System II, at d 5 after transfer and d 6 in System III is indicated. Group A: 4.0 g or lighter, Group B: 4.1 to 4.4 g heavier, Group C: 4.5 to 4.8 g heavier, Group D: 4.9 g or heavier than donor eggs.

Table 2. Viability and hatchability of chicken embryos by egg weight differences between donor and surrogate eggs in System III.¹

Group	n	System III (%)				Hatchability (%)
		d 6	d 6–12	d 13–18	d 19–21	
E	13	100.0	96.3	88.9	85.2	74.1
F	22	100.0	97.8	97.8	80.9	66.4
G	21	100.0	100.0	95.0	95.0	95.0
H	19	100.0	96.0	96.0	85.3	72.0

¹Viability at d 6–12, d 13–18, d 19–21 and hatchability were evaluated on the basis of the total number of survived embryos at d 6. Group E: 28.5 g or lighter, Group F: 28.6 to 30.5 g heavier, Group G: 30.6 to 32.5 g heavier, Group H: 32.6 g or heavier than donor eggs.

and 76.9% at d 22 of hatching rate were observed and there were no significant differences by egg weight differences in each incubation period ($P > 0.05$). However, Group G showed higher survival and hatchability than others (Table 2). Borwornpinyo et al. (2005) reported that 43% of hatching rates was observed with Saran Wrap. The embryonic death was distinct between early phase of System III and shortly before hatching, indicating that this tendency is consistent with this study. The lack of statistically significant differences in System III may be caused by the number of observation. In the process of beginning of System II and III, some numbers of embryos should be excluded in total observation due to yolk burst and broken eggshells.

In the first study for developing the surrogate eggshell incubation system, the use of only second surrogate eggs 27.0 to 30.0 g heavier than donor eggs was mentioned (Perry, 1988). Borwornpinyo et al. (2005) reported that 97.5% at d 0–3 and 61.3% at d 19-hatching of the viability in the study in which 3.0 to 4.0 g and 35.0 to 40.0 g heavier first and second surrogate than donor eggs were used. Then, Liu et al. (2012) investigated the effect of interspecific egg white on the development of chicken embryos in the surrogate system. In the chicken egg white group, 98.3% in System II and 60.4% after d 21 of viability were observed, normal-sized eggs for the first

and 1.5-times heavier than donor eggs (50.0–55.0 g) for the second surrogate eggs were used. Egg weight differences in System III were approximately estimated by 25.0 to 27.5 g in the study.

Here, we showed that 88.3% of the donor viability in System II and 76.9% of hatching rate after System III, regardless of the egg weight differences. Although there were no significant differences in viability and hatching rate by egg weight differences in System II and III, the hatching rate may be progressed in this study. In common, egg weight is positively correlated with egg size (Chen et al., 2016). In our preliminary test, when the donor was incubated in narrow-spaced surrogate eggshells in System III, enriched blood vessels in the embryonic development were disturbed by sealing material in rocking process. So, the optimal egg weight differences needed to be investigated, and 4 different groups were set up in each system of this study. In conclusion, the optimal range of the second surrogate eggs in System III may be considered at least 30.6 to 32.5 g heavier than donor eggs.

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DISCLOSURES

The authors have no conflicts of interest to disclose.

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