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≪Research Note≫

Simple Culture System for Bobwhite Quail and Japanese Quail Embryos from the Blastoderm Stage to Hatching using a Single Surrogate Eggshell

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The *ex vivo* culture of avian embryos is a technique for the long-term culturing of embryos outside of their own shell and shell membrane. It allows easy access to the developing embryos and embryo manipulation. The two-step system is widely applied when the culture is performed after oviposition. Japanese quail as well as bobwhite quail are used as models for avian safety assessment as recommended by the Organisation for Economic Co-operation and Development (OECD) guidelines. However, biological studies on the bobwhite quail have been more limited than those on the Japanese quail. We have developed a more simplified *ex vivo* culture protocol for the two species of quail embryos from the blastoderm stage through to hatching using a single surrogate eggshell. Hatchabilities of 31% and 27% were obtained in bobwhite quail and Japanese quail embryos, respectively. The simple system described in the present study is an easy and acceptable procedure.

Key words: bobwhite quail, culture, embryo, Japanese quail, New World quail, Old World quail

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Introduction

The *ex vivo* culture of avian embryos is a technique used for the long-term culturing of embryos outside of their own shell and shell membrane. In contrast to shell windowing techniques, the *ex vivo* culture allows easy access to the developing embryos and embryo manipulation. Avian embryos are subjected to various environmental conditions in the course of normal development. For example, development takes place on the first day in the oviduct of the chicken embryo where egg formation is completed by the deposition of albumen, uterine fluid, chalaza, shell membrane, and shell; for the next 21 days, the enveloping layers act as a buffer between the embryo and egg's environment (Perry, 1988). Current technologies now permit the culture of chicken and Japanese quail embryos from the single-cell stage, which is normally in the oviduct, through to hatching (Perry, 1988; Naito *et al*., 1990; Ono *et al*., 1994).

Chicken development is divided into three periods for the purpose of the *ex vivo* culture: fertilization to blastoderm formation lasts for 1 day, embryogenesis for 3 days, and embryonic growth for 18 days (Perry and Mather, 1991). Cultures are divided into three steps, corresponding to these three periods, respectively. The two-step system is widely applied when the culture is performed after oviposition.

Quail are divided phylogenetically into two groups, Old World quail such as the Japanese quail (*Coturnix japonica*) and New World quail such as the bobwhite quail (*Colinus virginianus*) (Silbley and Monroe, 1990). Both Japanese quail and bobwhite quail are used as models for avian safety assessment as recommended by the Organisation for Economic Co-operation and Development (OECD) guidelines (OECD, 2010). However, biological studies on the bobwhite quail have been more limited than those on the Japanese quail.

One of the advantages of avian embryos for the experimental analysis of developmental events is the relative ease with which they can be cultured *ex vivo*, manipulated, and observed. We previously developed a two-step *ex vivo* culture protocol for bobwhite quail embryos from the blastoderm stage through hatching (Kato *et al*., 2013) as well as the Japanese quail (Ono *et al*., 1994). We have now developed a more simplified *ex vivo* culture protocol for the two species of quail embryos from the blastoderm stage through to hatching.

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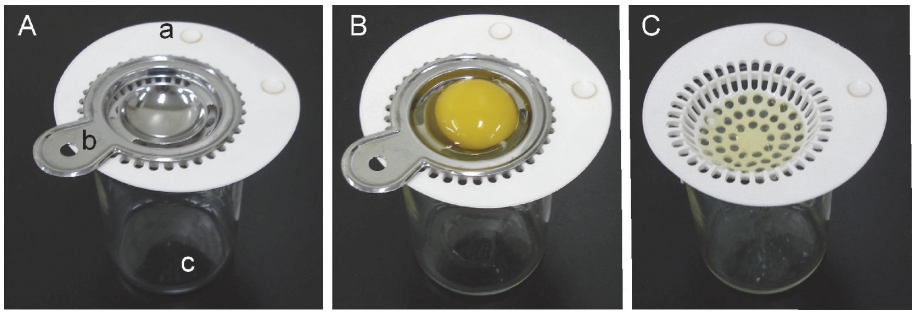

Fig. 1. **How to collect thin albumen from chicken eggs. (A)** Materials and setup. a, albumen separator (FE-078, LEC Inc., Tokyo); b, egg separator (C-9613, Pearl Metal Co. Ltd., Sanjo, Niigata); c, 300-mL beaker. **(B)** Crack open an egg on the egg separator. **(C)** Remove an egg separator and yolk. Thin albumen falls and thick albumen remains in the albumen separator. An approximate amount of 12.5 mL is yielded per egg.

Materials and Methods

Animals

This study was performed in accordance with the policies on animal care developed by the Animal Care and Management Committee of Shinshu University. Bobwhite quail (flight-type) and Japanese quail (egg-laying and meat-producing types) were maintained in our laboratory at Shinshu University, and fertilized eggs were collected daily. The developmental stage of embryos was determined on the basis of the normal tables of Hamburger and Hamilton (1951). Thin albumen of chicken eggs was prepared as shown in Fig. 1.

Ex vivo Culture of Bobwhite Quail Embryos

(1) Simple culture: The eggshell was wiped with 70% ethanol before use. A detailed explanation of the culture procedures is given in Fig. 2. Briefly, the albumen capsule-removed yolk was transferred to a large-sized surrogate shell of a Japanese quail egg (Figs. 2A−G). The open space in the shell was then filled with thin albumen from a chicken egg, and the open surface of the surrogate shell was sealed tightly with a piece of cling film (Fig. 2H). The culture set (Fig. 2H) was incubated at 38.5℃ and 60 to 70% relative humidity, with rocking along the long axis of the shell at a 90-degree angle and at 7.5-min intervals, until the embryo had developed to between stages 12 and 14 (~66 h, Fig. 2I). A portion of albumen (~3.5 m*l*) was then removed in order to adjust to the normal volume of the egg (Figs. 2J−K). Open surface of the shell was sealed tightly (Fig. 2L) and then the embryo was cultured similarly with the exception that the film surface was directed upward and rocking was performed along the short axis of the shell at a 30-degree angle and at 30-min intervals. The film was perforated one or two days before the expected hatching time to facilitate embryonic respiration (Fig. 2M) and rocking was stopped. Immediately before hatching, the film was removed and the open surface was covered with a plastic dish. Chicks were considered to have hatched when they were completely free from the shell (Fig. 2N).

(2) Two-step culture: The first-step procedure was basically according to Kato *et al*. (2013). The size of the surrogate eggshell was 11.5 to 13.0 g. However, the size of the surrogate eggshell was 15.0 g in the second-step.

Ex vivo Culture of Japanese Quail Embryos

The culture protocol was basically similar to that of the bobwhite quail embryos, with the exception that the timing (~54 hr, stages 12−14) and amount (~3.0 m*l*) of the removal of albumen. Incubation period of Japanese quail embryos is shorter than that of bobwhite embryos (16 vs. 23 days) and the normal volume of albumen is less in bobwhite quail eggs than Japanese quail eggs.

Statistical Analysis

Data were analyzed with two-tailed Fisher's exact test or extended Fisher's exact test as appropriate (Freeman and Halton, 1951; Mehta and Patel, 1983). A P value of <0.05 was considered significant.

Results and Discussion

Table 1 shows the viability and hatchability of bobwhite quail and Japanese quail embryos cultured from the blastoderm stage. Good viability, 88−95%, was obtained in both species of quail embryos.

In bobwhite quail embryos, 31% and 37% hatchabilities were obtained in the simple and two-step culture, respectively, and these were not significantly different ($P=0.57$). When 25 g, 38 g and 45 g (whole egg weight) chicken surrogate shells were used as the second-step culture, 25%, 39% and 26% hatchabilities were available, respectively (Kato *et al*., 2013).

In the Japanese quail embryos, 27% and 40% hatchabilities were obtained in the simple and two-step culture, respectively, and these were not significantly different ($P=0.17$). When the 45g chicken surrogate shell was used as the second-step culture, 43% hatchability was obtained (Kato *et*

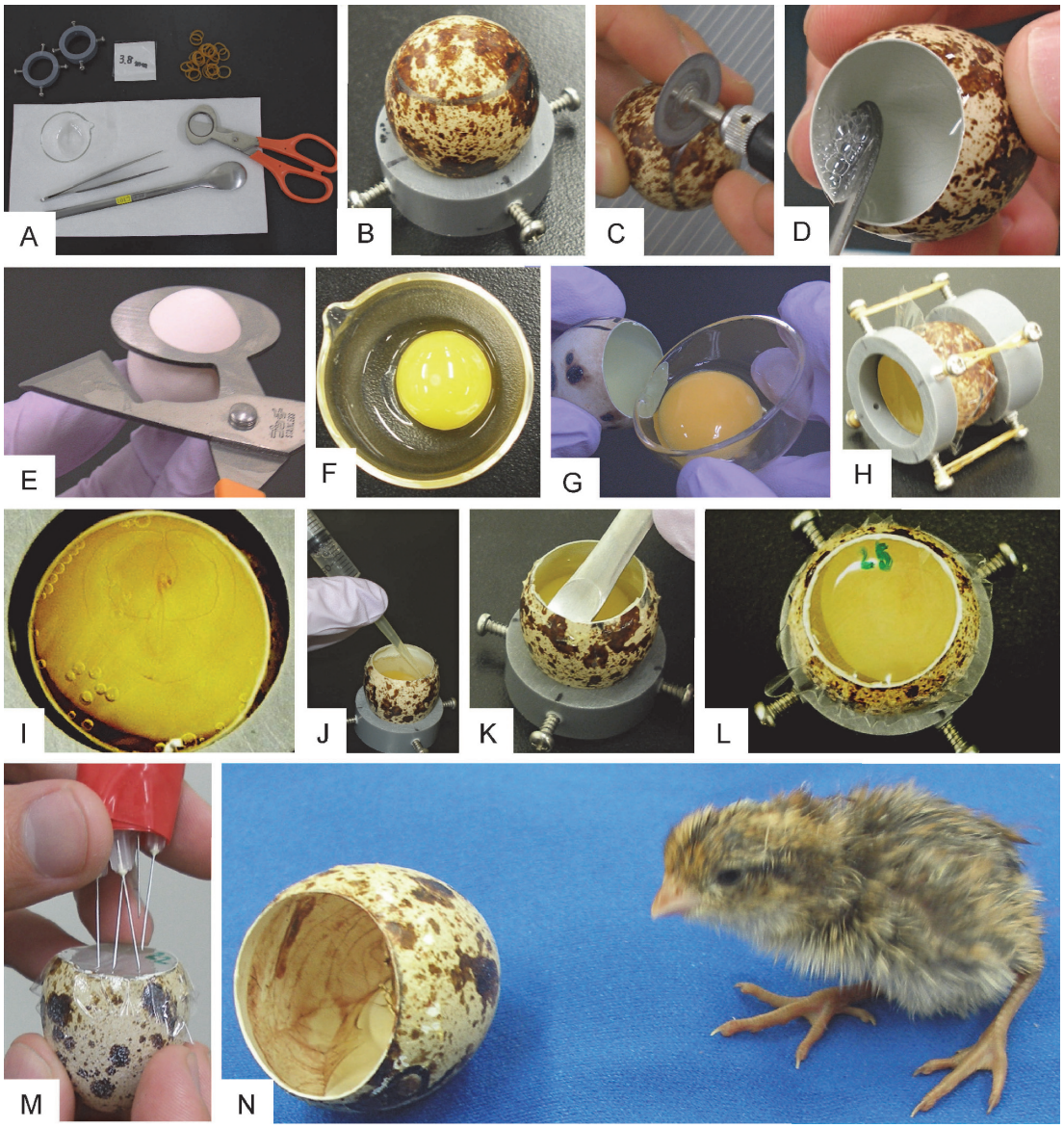

Fig. 2. **Simple culture system for the bobwhite quail embryos. (A)** Materials for the culture. **(B)** A large-sized Japanese quail egg (15 g whole egg weight) for the surrogate shell with the cutting line at the dull end marked at a level of 23 mm in diameter. **(C)** Cut the shell along with the line using a diamond disk (MC 1221, Minitor, Tokyo, Japan) attached to an electric drill (No. 28400, Proxxon, Tokyo, Japan). **(D)** Dump out the content and remove the foam. **(E)** Cut the sharp end of the bobwhite quail egg to be cultured using the quail egg cutter (Pucchi, Kitasho, Seki, Japan). **(F)** Transfer the content to a glass evaporating dish and remove the thick albumen capsule surrounding the egg yolk. **(G)** Pour chicken thin albumen (~5 m*l*) to the surrogate shell and transfer the yolk to the shell. **(H)** The culture setup. **(I)** A bobwhite quail embryo after 66 hr of culture. **(J)** Remove a portion of albumen (~3.5 m*l*) in order to adjust to the normal volume of the egg. **(K)** Remove the foam with a spatula. **(L)** Apply the thin albumen as an adhesive around the cut end of the shell. Cover with a new cling film and adhere the cling film to the the cut end of the shell. **(M)** Prick ~10 holes in the cling film with needles when the embryo has holed the chorioallantoic membrane with its beak. **(N)** A newly hatched bobwhite quail chick from the surrogate Japanese quail shell.

Table 1. **Viability and hatchability of cultured bobwhite quail and Japanese quail embryos from the blastoderm stage**

Type of embryo	Size of the surrogate shell (g)[1]		No. of embryos		
	before stages 12–14	after stages 12–14	cultured	surviving at stages 12–14 (%)	hatched (%)
Bobwhite quail	15	15[2]	51	47 (92%)	16 (31%)
	11.5–13	15	73	69 (95%)	27 (37%)
Japanese quail	15	15	52	46 (88%)	14 (27%)
	11.5–13	15	65	59 (91%)	26 (40%)

[1] whole egg weight.
[2] Surrogate shell was not changed.
Normal hatchabilities of bobwhite quail and Japanese quail eggs were 50% (104/210) and 81% (92/114), respectively.
No significant differences were observed between the simple and two-step cultures in each species.

al., 2013).

Successful *ex vivo* hatching may depend on the technical experience and hatching rate of embryos' normal incubation. In the present study embryonic loss during the *ex vivo* culture might be higher in the bobwhite quail than the Japanese quail embryos because the hatching rate of normal incubation was 50% and 81% in the former than the latter.

Larger-sized shell is required for the surrogate shell of the second-step culture to have an enough space above the embryo. Otherwise the embryo gets contact with cling film when rocking at a 30-degree angle.

Stable supply of the small-sized chicken eggs for the surrogate shells is not easy. In the previous study we used first eggs from strains such as Red Junglefowl, Fayoumi, and Silky maintained at the Avian Bioscience Research Center at Nagoya University (Kato *et al*., 2013). Now we have a meat-producing type of Japanese quail to produce large-sized eggs (15.0 g whole egg weight) in our laboratory and enough eggs are available.

Thick albumen capsule was removed from the egg yolk in the present study because it was easier to locate the embryo upward (personal observation). However, it was dispensable for the embryo survival when the chicken egg was used as the surrogate shell to the blastoderm stage embryo. Nirasawa *et al*. (1992) transferred the blastoderm stage Japanese quail embryos with albumen capsule and Ono *et al*. (1994) did without it to the surrogate chicken eggshells and yielded 17.6% (9/51) and 20.0% (17/85) hatching rates, respectively, showing no statistical difference ($P=0.82$).

The simple *ex vivo* culture system developed here was easy and acceptable procedure. It should prove to be widely applicable to the maintenance and generation of manipulated birds for basic and applied studies on the bobwhite quail as well as the Japanese quail.

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