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≪Research Note≫

Culture System for Bobwhite Quail Embryos from the Blastoderm Stage to Hatching

Atsushi Kato[1,2], Daichi Miyahara[1], Hiroshi Kagami[1], Yusuke Atsumi[3], Shusei Mizushima[4], Kiyoshi Shimada[5] and Tamao Ono[1]

[1] Faculty of Agriculture, Shinshu University, Minamiminowa, Japan
[2] Aichi Agricultural Research Center, Nagakute, Japan
[3] Avian Bioscience Research Center, Nagoya University, Nagoya, Japan
[4] Faculty of Agriculture, Shizuoka University, Shizuoka, Japan
[5] WCU, Major in Biomodulation, College of Agriculture and Life Science, Seoul National University, Korea

Quail are divided phylogenetically into two groups, Old World quail and New World quail. Old World quail, such as the Japanese quail (*Coturnix japonica*), belong to the Phasianidae and distributed in the Palaearctic region (Europe, North Africa, and Asia), whereas New World quail, such as the bobwhite quail (*Colinus virginianus*), belong to the Odontophoridae and are restricted to North and South America. Both the bobwhite quail and the Japanese 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 limited compared with those on the Japanese quail. We have therefore now developed an *ex vivo* culture protocol for bobwhite quail embryos from the blastoderm stage through hatching. Of the various culture conditions examined in the present study, a good hatching rate (39%) was obtained when the embryos were cultured *ex vivo* in a two-step procedure. Unincubated embryos (with egg yolk) were first cultured inside the shell of a Japanese quail egg (11.5 to 13.0 g whole egg weight) together with chicken thin albumen for 63 to 65 h and were then transferred to the shell of a small-sized chicken egg (38 g whole egg weight) until hatching. This *ex vivo* culture system should provide to be widely applicable to the maintenance and generation of manipulated birds for basic and applied studies on the bobwhite quail.

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

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Introduction

Quail are divided phylogenetically into two groups: Old World quail and New World quail. Old World quail, such as the Japanese quail (*Coturnix japonica*), the common quail (*Coturnix coturnix*), and the blue-breasted quail (*Coturnix chinensis*), belong to the Phasianidae and are distributed in the Palaearctic region (Europe, North Africa, and Asia). In contrast, New World quail, including the bobwhite quail (*Colinus virginianus*), the California quail (*Callipepla californica*), and the scaled quail (*Callipepla squamata*), belong to the Odontophoridae and are restricted to North and South America (Sibley and Monroe, 1990). Among the various quail species, only the Japanese quail is widely used for egg and meat production and as a model animal for poultry and biological research. The advantages of the Japanese quail include its ease of handling, short generation time, and prolific nature. It requires only 16 days to hatch and 6 to 8 weeks to achieve an adult body weight of 110 to 140 g or 250 to 300 g for egg-laying and meat-producing strains, respectively. On the other hand, the bobwhite quail has long been a favorite game bird throughout the eastern United States and is so named because of the loud call of the male. Many commercially available strains have been selected for marketing traits. The bobwhite quail requires 23 days to hatch and 24 weeks to mature to an adult body weight of at least 200 g (flight-type) or 400 g (meat-type). Like the Japanese quail, the bobwhite quail is used as a model for avian safety assessment as recommended by the Organisation for Economic Co-operation and Development (OECD) guidelines (OECD, 2010).

One of the advantages of avian embryos for experimental analysis of developmental events is the relative ease with which they can be cultured *ex vivo*, manipulated, and observed. The early embryo is the most challenging stage of

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Correspondence: Dr. T. Ono, Faculty of Agriculture, Shinshu University, 8304 Minamiminowa, Kamiina, Nagano 399−4598, Japan.
(E-mail: tamaoon@shinshu-u.ac.jp)

avian development with regard to culture and manipulation. It has proved possible, however, to culture embryos of certain avian species *ex vivo* from the single-cell stage, which normally exists in the oviduct, through hatching (Perry, 1988; Naito and Perry, 1989; Naito *et al*., 1990; Ono *et al*., 1994). Given that biological studies on the bobwhite quail are more limited than those on the Japanese quail, we have now developed an *ex vivo* culture protocol for bobwhite quail embryos from the blastoderm stage through hatching. We have compared various culture conditions such as the timing of transfer from the first to the second step culture, the type of albumen as a culture medium and the size of surrogate shell in the second step culture.

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 strains) 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). Small-sized chicken eggs including first eggs from strains such as Red Junglefowl, Fayoumi, and Silky were obtained for use as surrogate shells from the Avian Bioscience Research Center at Nagoya University. Thin albumen of chicken, Japanese quail, and bobwhite quail eggs was prepared in our laboratory at Shinshu University.

Ex Vivo Culture of Embryos

Culture of bobwhite quail embryos consisted of two steps and was based on the protocols developed for Japanese quail (Ono *et al*., 1994; Ono, 2000) and blue-breasted quail (Ono *et al*., 2005). Eggshells were wiped with 70% ethanol before manipulation. Six series of culture experiments for bobwhite quail embryos (systems A through F) and one control series for Japanese quail embryos (system G) were performed until the embryos hatched or stopped developing.

System A: The eggshell (10.0 to 10.5 g whole egg weight) of an unincubated bobwhite quail egg was cut open, and the embryo (blastoderm), together with the egg yolk and albumen, was transferred to a glass evaporating dish (Fig. 1A). The thick albumen capsule surrounding the egg yolk was removed, and the naked yolk and blastoderm were transferred to a surrogate shell that had been prepared from a large-sized Japanese quail egg (11.5 to 13.0 g whole egg weight, meat-producing strain) by cutting of the sharp end at a level of 23 mm and removing the contents. 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 and a pair of plastic rings (with an inner diameter of 24 mm and four external screw bolt projections). The resulting construct (Fig. 1B) was incubated for 51 to 53 h 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 8 and 10.

For the second step, a small-sized surrogate chicken shell (~45 g whole egg weight) was prepared by cutting off a one-third part from the dull end of the egg and removing the contents. The embryo, together with the yolk and albumen, from the first culture step, was transferred to the new surrogate shell (Fig. 1C), and a portion of albumen was then removed for adjustment to the normal volume of the bobwhite quail egg (~1.5 m*l*). The shell was sealed with cling film, and the embryo was cultured under conditions similar to those described for the first step, with the exception that the

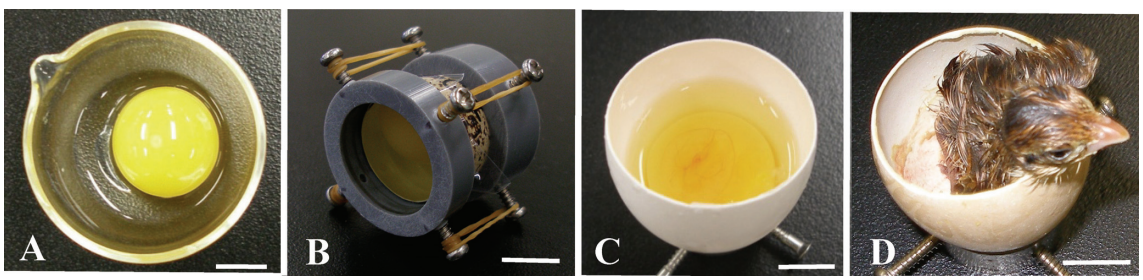

Fig. 1. **Culture system for bobwhite quail embryos.** (**A**) Contents of an unincubated bobwhite quail egg are transferred to a glass evaporating dish, and the thick albumen capsule surrounding the egg yolk is removed. (**B**) The naked egg yolk is transferred to a surrogate shell prepared from a Japanese quail (meat-type) egg. The open space in the shell is filled with thin albumen, and the shell is sealed tight with a piece of cling film and a pair of plastic rings. (**C**) The embryo, together with the yolk and albumen, from the first culture step is then transferred to a new surrogate shell prepared from a small-sized chicken egg. A portion of albumen is removed for adjustment to the normal volume of a bobwhite quail egg. (**D**) A newly hatched chick derived from a cultured bobwhite quail embryo. Bars, 1 cm.

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

System	First culture step			Second culture step	No. of embryos			
	Culture period (h)	Stage[1]	Type of thin albumen[2]	Type of surrogate shell[3]	Cultured	Surviving at transfer[4]	Hatched	Hatchability after second step
A	51–53	8–10	Ch	Ch (45 g)	52	51 (98%)	7 (14%)	14%
B	63–65	12–14	Ch	Ch (45 g)	58	52 (90%)	15 (26%)	29%
C	63–65	12–14	Jq	Ch (45 g)	62	59 (95%)	3 (5%)	5%
D	63–65	12–14	Bw	Ch (45 g)	45	39 (87%)	0 (0%)	0%
E	63–65	12–14	Ch	Ch (38 g)	59	55 (93%)	23 (39%)	42%
F	63–65	12–14	Ch	Ch (25 g)	51	44 (86%)	13 (25%)	30%
G[5]	51–53	12–15	Ch	Ch (45 g)	68	60 (88%)	29 (43%)	48%

[1] Developmental stage at the end of the first culture step.
[2] Ch, chicken; Jq, Japanese quail; Bw, bobwhite quail.
[3] Whole egg weight is shown in parentheses.
[4] Only viable embryos were transferred to the second culture step.
[5] Culture of Japanese quail embryos.

cling 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. One or two days before the expected hatching time, the film was perforated to facilitate embryonic respiration 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. 1D). Changes in the culture protocol for systems B through G are specified below, with other aspects of these systems being the same as for system A (Table 1).

System B: Timing of the transfer from the first step to the second step was changed to between 63 and 65 h of culture, when the embryos had developed to stage 12 to 14.

System C: The same as system B with the exception that thin albumen from a Japanese quail egg was used as a culture medium.

System D: The same as system B with the exception that thin albumen from a bobwhite quail egg was used as a culture medium.

System E: The first step was the same as that for system B, but a surrogate shell of a chicken egg with a whole egg weight of 38 g was used in the second step.

System F: The first step was the same as that in system B, but a surrogate shell of a chicken egg with a whole egg weight of 25 g was used in the second step.

System G: Embryos of an egg-laying strain of Japanese quail (~10.5 g whole egg weight) were cultured for 51 to 53 h to stage 12 to 15 in the first step.

Statistical Analysis

Data were analyzed with 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 statistically significant.

Results and Discussion

Table 1 shows the viability and hatchability of bobwhite quail and Japanese quail embryos cultured from the blastoderm stage according to systems A through G. We first cultured bobwhite quail embryos according to a protocol for Japanese quail embryos (systems A and H, respectively). Viability at the end of the first culture step was 98% (51/52) and 88% (60/68) for the bobwhite quail and Japanese quail embryos, respectively ($P=0.08$), but subsequent hatchability was significantly lower for the bobwhite quail embryos (14% versus 43%, $P\approx 0.00$). For system G, hatchability was essentially the same as that obtained in the original protocol (48%) (Ono *et al*., 1994), even though the opening of the surrogate Japanese quail egg (23 mm in diameter) was larger than that in the original protocol (19 mm in diameter) because larger eggs were available in the present study. Japanese quail embryos had progressed to developmental stages 12 to 15 after 51 to 53 h of culture, whereas bobwhite quail embryos had achieved stages 8 to 10 after the same culture period. The latter embryos required 63 to 65 h to reach stages 12 to 14. The latter embryos required longer incubation period (23 days) and thus showed slower development (Hendrickx and Hanzlik, 1965). The developmental stage of embryos is an important consideration with regard to the timing of transfer from the first to the second steps of the culture protocol.

We evaluated the effect of the timing of transfer from the first to the second steps of culture by comparing the results obtained with system A (stages 8 to 10) and system B (stages 12 to 14). The viability obtained with system B at the end of the first culture step was 90% (52/58), which did not differ significantly from that obtained with system A ($P=0.12$). The hatching rate for system B was 26% (15/58), which also did not differ significantly ($P=0.15$) from that for system A (14%, 7/52). Moreover, hatchability after the second culture step did not differ significantly between systems A and B (14% versus 29%, respectively; $P=0.09$). Given that system B yielded a better overall hatching rate, even though the difference with system A was not significant, we adopted a

culture period of 63 to 65 h (developmental stages 12 to 14) for subsequent comparisons.

The effect of the type of thin albumen (chicken, Japanese quail, or bobwhite quail) used as a culture medium was evaluated by comparing the results obtained with systems B, C, and D. The viability obtained at the end of the first step of culture was 87 to 95% for these three systems ($P=0.28$). Whereas the hatchability for system B was 26%, it was significantly lower for systems C and D (5% and 0%, respectively, $P\approx0.00$). The unsatisfactory hatching rates for systems C and D are thus probably attributable to contamination by fine fragments of eggshells or other factors, given that it was difficult to collect albumen from the quail eggs (Ono *et al*., 2005).

Finally, the effect of the size of the second surrogate shell was evaluated by comparing the results obtained with systems B, E, and F. The viability obtained at the end of the first culture step was 86 to 93% ($P=0.22$). The hatchability for systems E and F was 39% and 25%, respectively. System E thus showed the highest hatchability, although this parameter did not differ significantly among these three systems ($P=0.22$). An essential feature of the second step of the culture protocol concerns calcium absorption from the shell and its transport through the chorioallantoic membrane (Tuan and Zrike, 1978). About 80% of calcium in a neonatal chick is derived from the eggshell (Ono and Wakasugi, 1984). In cultured embryos, the area of the chorioallantoic membrane extending along the inside of the eggshell is less than that for normal embryos.

Of the various culture conditions examined in the present study, the combination of those in system E proved to be the best for the culture of bobwhite quail embryos from the blastoderm stage to hatching, and the hatching rate (39%) was equivalent to that of Japanese quail embryos (39% versus 43%, $P=0.72$). The bobwhite quail belongs to the phylogenetic group of New World quail and is a good candidate for biological research. Indeed, both the bobwhite quail and the Japanese quail are used as indicator species in avian toxicity tests (Romijn *et al*., 1995). The *ex vivo* culture system developed here should prove to be widely applicable to the maintenance and generation of manipulated birds for basic and applied studies on the bobwhite quail.

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