HATCHING OF CULTURED EMBRYOS OF THE PEKING DUCK

Z. D. Li and S. Z. Qi

Department of Animal Biochemistry, Beijing Agricultural University, Beijing 100094, China

Summary

Peking duck embryos incubated for 82 hrs after oviposition, stage 18 to 20 (Hamberger and Hamilton, 1951) were transferred into the recipient eggshells and further incubated at 38.0℃, RH(relative humidity) 60 to 70%, with rotation at an angle of 30° and the frequency of 4 times an hour. The survival rate was 100, 98, 82, 82, 70, 46, 21 and 16%, after the incubation period of 4, 8, 12, 16, 20, 24, 28 and 29 days, respectively. The present result clearly shows that the ex ova culture is possible for the Peking duck just as in the cases of the chicken or the quail. This culture technique could be useful for experimental manipulation of the embryos.

(**Key Words** : Culture, Embryo, Peking Duck)

Introduction

In 1976, Dunn and Boone attempted to culture chicken embryos in a wrap film, but they died 15.5 days later. Ono and Wakasugi (1984) succeeded in hatching Japanese quail embryos cultured in chicken shells. Rowlett and Simkiss (1987) did a similar experiment for chicken embryos using turkey shells or large chicken shells to obtain the hatching rate of 21.7% and 20%, respectively. Furthermore, Perry (1988) and Naito and Perry (1989) developed a more complex method and made possible ex ova culture of chicken embryos from the single cell stage to hatching. The Peking duck needs a longer incubation period than the chicken or the quail and, therefore, different conditions must be required for successful hatching in ex ova culture. In this experiment, we examined the possibility of the ex ova culture of Peking duck embryos.

Materials and Methods

Fertilized eggs laid by the Peking ducks were used for the present study. The eggs were initially incubated for 82 hrs at 38.0℃, RH 60 to 70%, with the rotation at an angle of 90° at hourly intervals. Double-yolked eggs of the Peking duck were used as the recipient shells, which were about 30 g heavier than the eggs laid by the donor duck. They were sterilized with 75% ethanol and the content was discarded from a hole with the diameter of 40 mm made at the blunt end of the shell (figure 1). The empty

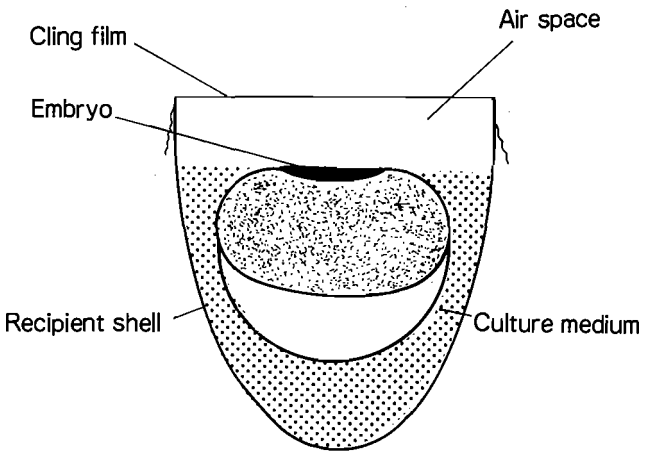

Figure 1. Culture system (Perry, 1988).

shells were washed with a mixture of penicillin, streptomycin and Fazizon (GIBCO, kindly supplied from Dr. N. Fujihara). The Peking duck embryos after 82 hrs of incubation, stage 18 to 20 (Hamberger and Hamilton, 1951), were used as donors and transferred into the recipient shells. The recipient shells were sealed with polyethylene film and further incubated at 37.8 to 38.0℃, RH 60 to 70%. They were rotated 4 times an hour at an angle of 30°. After 16 days, the incubation

[1]Address reprint requests to Dr. Z. D. Li, Dept. of Animal Biochemistry, Beijing Agricultural University, Beijing 100094, China.

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temperature was lowered to 37.4℃ and water of 20.0 to 25.0℃ was sprayed on the cultured embryos four times a day. And, from the 24th day of incubation on, the water spraying was done 12 times a day but without any rotation.

Results

Viability of the cultured embryos of Peking duck during the experiment is shown in the table 1. Seventy-three fertilized eggs in total were used in 5 series of experiments. All embryos survived up to 7 days, 82% up to 16 days and 46% up to 24 days of incubation. The table shows that the survival rate in the end of culture period, especially after 20 days, is very low. But the present result clearly shows that the ex ova culture is possible in the Peking duck just as in the cases of the chicken and the quail. In detail, the optimal time of

TABLE 1. CHANGE IN THE PERCENTAGE OF THE SURVIVAL RATE OF PEKING DUCK EMBRYO WITH ADVANCING AGE

Days of incubation	experimental series (Number of embryos)					Average Survival rate (n=73)
	I (12)	II (8)	III (19)	IV (17)	V (17)	
4	100	100	100	100	100	100
8	100	88	100	100	100	98
12	33	75	100	100	100	82
16	33	75	100	100	100	82
20	25	50	74	100	100	70
24	25	38	42	59	65	46
28	8	25	11	41	18	21
29(hatched)	8	25	6	29	12	16

transplantation was reported to be 60 and 72 hrs of incubation for the quail and chicken, respectively. The time for the Peking duck was 82 hrs of incubation, which are later than the case of chicken and quail. Figure 2. indicated that an embryo of Peking duck is nearly hatching. Its weight was a little smaller than normal and the culture period was prolonged for 1~1.5 days. There were malformation in some legs of Peking ducks. After hatching, Peking ducks grew normally. When reaching sex maturity, they produced normal offsprings.

Figure 2. Embryo of Peking duck nearly hatched.

Discussion

1. Embryo transferring time

In the ex ova culture system, the best transferring time of quail embryo is about 60 hrs (Ono and Wakasugi, 1984) and that of chicken is about 72 hrs (Rowlett and Simkiss, 1987). In this experiment, it was found that the best transferring time for Peking duck embryo is about 82 hrs. That was proved in a primary experiment. If the time is less than 82 hrs, the development area of blood vessels on yolk was small which may facilitate embryo transfer but the embryo tended to die so readily with the very low rate of viability. If it is longer than 82 hrs, the blood island of embryo has developed so large that it is very easy to damage the blood vessels which causes bleeding. About half of embryos died in the transfer operation in these cases.

2. Embryo transferring method

If the eggs were cracked in the middle, thin albumen would flow out first that damaged thick albumen mechanically and the embryoes would not locate in the

center. Most of those embryos with abnormal position would die within a week. In our experiment we put the Peking duck eggs with sharp end above for 5 min and then peeled off egg shell and outer shell membrane carefully. At last, inter shell membrane was quickly opened and all contents were poured into recipient shell.

3. Rocking egg

In normal culture of Peking duck, the rotation carried out once every two hours at the angle of 110~130°. But in this experiment, the rotation angle was only 30° to avoid the touching of embryo to warp. As a remedial measure, the rocking frequency was increased to once every 15 min (eight fold), but it was still not sufficient and deformed embryos were produced. Goto et al. (1988) claimed that the hatching rate of healthy chicken was only 21.3%, if eggs were not being rocked during culture. From this we can see that rocking eggs are very important for the embryo development. But how to increase rotation angle needs further research.

4. Cooling egg

The culture period of Peking duck is 28 days. When culture was advanced to about 16 days, lipid metabolism is accelerated to increase the inner temperature of egg. Because the ratio of surface area to weight of Peking duck egg is smaller than that of chicken or quail, it makes the exchange of heat difficult. If eggs could not be cooled properly, high ratio of death will be occurred. In ex ova culture system, the development of embryo is a little delayed than normal, which means that cooling frequency should be reduced. In contrary, in ex ova culture, above the recipient shell is wrap film whose gaseous permeability is far lower than eggs and uneasy to loss heat, so the cooling frequeney shall be increased. This is a very difficult problem. In order to solve the problem, we reduced cooling time and increased cooling frequency (Several times a day, were needed, instead of three times a day for normal culture) and obtained a good result. But it still needs further research.

In ex ova culture of Peking duck embryos, the difficulty of egg cooling, the insufficiency of calcium supply in recipient shells and other problems cause the hatching rate low. If the hatching rate can be increased by continuing research to solve those problems and improving culture conditions, the ex ova cuture system will be a good model for genetic engineering.

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