Research Note: Developmental Ability of Twin Embryos Produced by Microinjection Treatment into Chick Blastoderm

M. NAITO,[1] M. WATANABE,[2] K. NIRASAWA, and T. OISHI

National Institute of Animal Industry, Tsukuba Norindanchi, P.O. Box 5, Ibaraki 305, Japan

(Received for publication September 20, 1990)

ABSTRACT Twins and triplets of chick embryos were produced by microinjection treatment of cell suspension into the blastoderm. One pair of the twin embryos had an ability to develop normally and survived up to Day 21 of incubation, but did not hatch. The other twins and triplets died within 7 days.
(*Key words*: developmental ability, twin embryos, microinjection, blastoderm, chick)

1991 Poultry Science 70:1261–1264

INTRODUCTION

Multiple embryos in Aves can be produced by cutting a single blastoderm. In ducks, up to four complete embryos can be produced, and similar results were obtained in chickens (Spratt and Haas, 1960, 1961a,b; Slack, 1983). Because these experiments were conducted using blastoderms with the vitelline membrane removed from the yolk and cultured *in vitro* up to the stage of 10 to 15 somites (Stages 10 to 12; Hamburger and Hamilton, 1951), it is unknown about the subsequent developmental ability of multiple embryos produced by cutting the blastoderm.

Twins and triplets of chick embryos were produced in an experiment by injecting dissociated quail blastoderm cells into the chick blastoderm (Naito *et al.*, 1991). These embryos had an intact yolk and vitelline membrane; therefore, embryonic development was able to progress further. In the present experiment, the developmental ability of the twins and triplets of embryos by culturing them in recipient eggshells was investigated.

MATERIALS AND METHODS

Fertilized eggs (Stage X; Eyal-Giladi and Kochav, 1976) laid by White Leghorns were cracked and the contents were placed in glass vessels. A glass micropipette was inserted into the central area of the chick blastoderm and quail blastoderm cells (1 to 5 μL in cell suspension) were injected into the subgerminal cavity (Naito *et al.*, 1991). The injected embryos were cultured in small recipient eggshells followed by large recipient eggshells, according to the method of Naito *et al.* (1990), which was a modification of the method of Perry (1988). The development of the embryos was observed through cling film placed over the aperture of the recipient eggshells.

RESULTS AND DISCUSSION

Incidences of twins and triplets of the chick embryos are shown in Table 1, which summarizes the results performed in the authors' laboratory (Naito and Perry, 1989; Naito *et al.*, 1990, 1991; Naito, unpublished observations). A pair of twin embryos survived up to Day 21, but the other twins and triplets died within 7 days. It is clearly demonstrated that these twins and triplets were produced by the microinjection treatment, because no twins and triplets were observed in the embryos without microinjection treatment except the one cultured from a fertilized ovum. The twins observed in the embryos cultured from fertilized ovum were probably produced by the physical effect of transfer between culture systems.

Figure 1 shows a series of photographs illustrating the development of the twins. On Day 3 of incubation, twin embryos were located at a distance with one staged 18 and the other staged 16 (Hamburger and Hamilton, 1951). In these, the twins were cut perpendicular to the future axis of the blastoderm, because the two embryos did not orient parallel (Slack, 1983). Although the two

[1]To whom correspondence should be addressed.
[2]Ehime University School of Medicine, Shigenobu, Ehime 791-02, Japan.

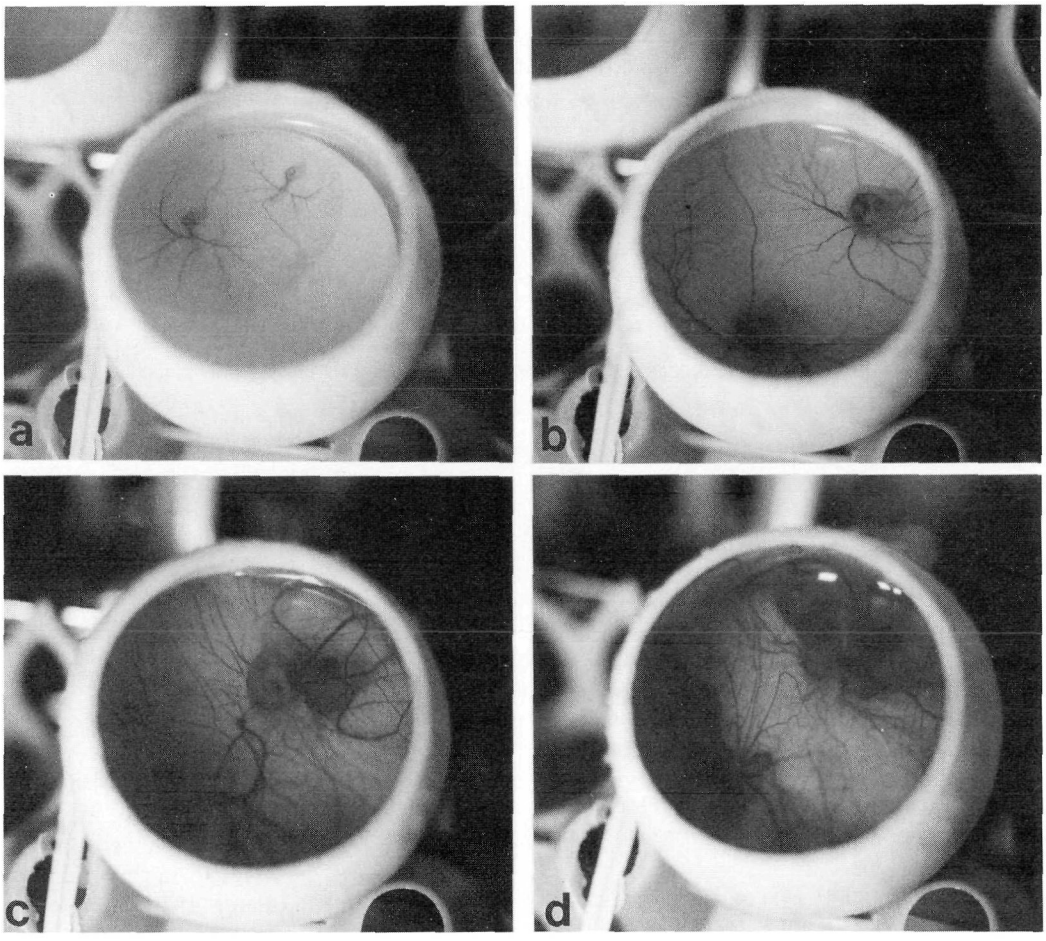

FIGURE 1. Twin embryo development: a = Day 3; b = Day 5; c = Day 7; d = Day 9; e = Day 11; f = Day 13; g = Day 17; and h = Day 21.

TABLE 1. *Incidence of twins and triplets of chick embryos*

Cultured embryo	Treatment	Eggs treated	Twin (days)[1]	Triplet (days)[1]	References[2]
		(n)	(days)		
Fertilized ovum	Culture	69	1 (3)	0	1
Uterine egg (16 to 64 cells)	Culture	113	0	0	2, 4
Blastoderm	Culture	184	0	0	1, 2, 3
Blastoderm	Microinjection	560	4 (4, 4, 7, 21)	1 (4)	3

[1]Survival days from blastoderm stage.

[2]Key to references: 1. Naito *et al.* (1990); 2. Naito and Perry (1989); 3. Naito *et al.* (1991); 4. Naito, unpublished.

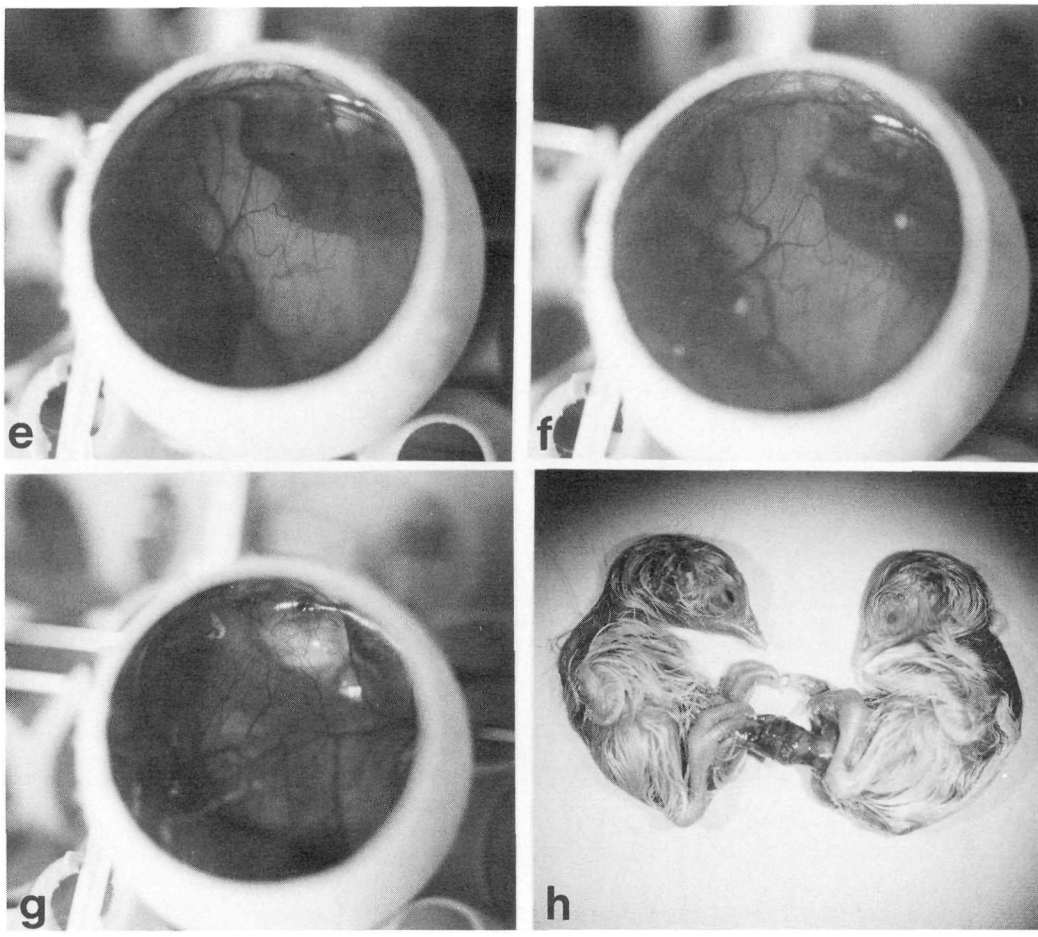

FIGURE 1. (continued) Twin embryo development: a = Day 3; b = Day 5; c = Day 7; d = Day 9; e = Day 11; f = Day 13; g = Day 17; and h = Day 21.

embryos were in the same area vasculosa, the blood vascular system developed independently in each. The twin embryos were grown normally and the size of the embryos appear to be similar. On Days 5 to 7, the limbs began to grow, and on Days 9 to 11 the eyes became large and the digits and toes conspicuous. The egg-tooth was clearly visible on Day 13. On Day 17, the embryos sank in the egg, becoming invisible. The yolk almost retracted away from the two embryos on Day 21, but they were unable to hatch. The other twin and triplet embryos were located contiguously on Day 3 of incubation, and they were not able to survive beyond Day 7. Therefore it seems probable that the distance between the embryos in the area vasculosa is related to their subsequent development.

In the present experiment, twins and triplets of the embryos were produced by the division of the future axis of the blastoderm by the microinjection treatment. It was shown that the twin embryos have an ability to develop normally up to Day 21 of incubation.

ACKNOWLEDGMENTS

The authors are grateful to the staff of the Poultry Management Section of National Institute of Animal Industry for their assistance.

REFERENCES

Eyal-Giladi, H., and S. Kochav, 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General morphology.

Dev. Biol. 49:321–337.

Hamburger, V., and H. L. Hamilton, 1951. A series of normal stages in the development of the chick embryo. J. Morphol. 88:49–92.

Naito, M., K. Nirasawa, and T. Oishi, 1990. Development in culture of the chick embryo from fertilized ovum to hatching. J. Exp. Zool. 254:322–326.

Naito, M., and M. M. Perry, 1989. Development in culture of the chick embryo from cleavage to hatch. Br. Poult. Sci. 30:251–256.

Naito, M., M. Watanabe, M. Kinutani, K. Nirasawa, and T. Oishi, 1991. Production of quail-chick chimaeras by blastoderm cell transfer. Br. Poult. Sci. (in press).

Perry, M. M., 1988. A complete culture system for the chick embryo. Nature 331:70–72.

Slack, J.M.W., 1983. From Egg to Embryo. Cambridge University Press, Cambridge, England.

Spratt, N. T., Jr., and H. Haas, 1960. Integrative mechanisms in development of the early chick blastoderm. I. Regulative potentiality of separated parts. J. Exp. Zool. 145:97–137.

Spratt, N. T., Jr., and H. Haas, 1961a. Integrative mechanisms in development of the early chick blastoderm. II. Role of morphogenetic movements and regenerative growth in systematic and topographically disarranged blastoderms. J. Exp. Zool. 147:57–93.

Spratt, N. T., Jr., and H. Haas, 1961b. Integrative mechanisms in development of the early chick blastoderm. III. Role of cell population size and growth potentiality in synthetic systems larger than normal. J. Exp. Zool. 147:271–293.