MIGRATION OF THE PRIMORDIAL GERM CELLS AND GONAD FORMATION IN THE EARLY CHICKEN EMBRYO

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Summary

In this study, characteristics of chick primordial germ cells (PGCs), which is the founder cell of the germline, and gonadal development of the chick embryo between 12 hrs and 6 day of incubation were investigated by transverse serial sections of chick embryos under the light microscopic observation.

In embryo stage 20 (3 day of incubation), there are a lot of PGCs at the mesenchym, which were moving to the thickened epithelium (gonadal ridge). The PGCs arrive at both right and left gonad primordia in equal number prior to stage 24 (4 day of incubation), but in the following stages, the distribution of the PGCs became asymmetrical. More PGCs colonized the left than the right gonad, but the reason for the unequal distribution of PGCs is uncertain. The PGCs have mostly settled in the gonadal ridge (GR) at 6 day embryo.

This study was conducted to investigate characteristics of the PGC migration and gonadal formation and observe the best condition for PGC isolation, culture and to attempt the possibility of the production for transgenic germline chimeras with manipulated PGCs.

(**Key Words** : Primordial Germ Cell, Gonadal Ridge, Serial Section, Chick Embryo)

Introduction

Since the first described the primordial germ cells (PGCs) in the gonad of the chick embryo, numerous studies have been conducted to determine the origin, deposition, and ultimate fate of these cells. PGCs are the founder cells of the germline and their descendants will form the functional gametes of the adult animal. PCGs are present in the central portion of the area pellucida in preoviposition (stage X) embryo (Eyal-Giladi and Kochav, 1976). The characteristics of PGCs are morphologically large, round, eccentric nucleus and histochemically stained with the periodic acid-schiff (PAS) that stain those a deep purplish red due to abundant glycogen content in the intracytoplasm (Meyer, 1960, 1964). In birds, it is now generally accepted that PGCs first appear in the epiblast (Eyal-Giladi et al., 1981), then migrate to the hypoblast within the first hour of incubation (Eyal-Giladi et al., 1981; Sutasurya et al., 1983; Urven et al., 1988). During gastrulation, the PGCs migrate anteriorly via the hypoblast and reside in the extraembryonic area referred as the germinal crescent (Swift, 1914; Ginsberg and Eyal-Giladi, 1986). At stages 15-18 (2-3 day of incubation) (Hamburger and Hamilton, 1951), they separate from the endoderm, temporarily, circulate via blood vascular system, leave the blood vessels, and invade in the thickened coelomic epithelium, which is the first morphological indication of gonad formation (Ando and Fujimoto, 1983; Ukeshima et al., 1985, 1991). However, it is unclear how the PGCs can invade the thickened epithelium of the gonadal ridge after extravasation. But it has been speculated that PGCs showed directional movement (amoeboid movement) toward gonadal ridge by stages 20-24 (Fujimoto et al., 1975, 1976; Ukeshima et al., 1984) and probably GR, the superficial epithelium of the gonadal ridge, releases some chemical factors to PGCs (Dubois et al., 1976; Kuwana et al., 1986).

PGCs begin participitation in gonadal soma formation between day 6 and 7, it is the time of sexual differentiation (Rodemer-Lenz, 1989). Sexual differentiation of the gonad occurs following the arrival of

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the germ cell. It is unclear whether the arriving PGCs contribute to the differentiation or products of the gonadal differentiation process attracts PGCs. Germ cell multiplication occurs at the next stage. The proliferation stage of the germ cell in the female embryos take place between 8 and 11 day of incubation whereas in male, between 13 and 15 day of incubation (Romanoff, 1960).

The purpose of this study was to investigate characteristics of the PGC migration and gonadal development, observe the best condition for PGC isolation, culture and ultimately apply for the production for transgenic germline chimeras with manipulated PGCs.

Materials and Methods

1. Preparation of embryos

Embryos of White Leghorn fowl were used to observe the PGCs. Fertilized eggs were incubated at 37.5℃ for 0.5 to 6 days and 60-70% relative humidity in a forced-draft incubator. At the end of incubation each embryos were removed from the eggs.

2. Paraffin embedding and microdissection

Embryos were employed in the bouin fixative solution for 2 days, and then dehydrated in graded series of ethyl alcohol (50, 70, 80, 90, 95, and 100%) for 60 min and transfer to xylene : ethyl alcohol mixture (1:3, 1:1, 3:1), xylene for each 60 min at room temperature. Embryos were embedded in paraffin (melting point 60℃) : xylene mixture (1:3, 1:1, 3:1), paraffin for each 60 min. And then samples were molded and trimmed in PIKA (Seiko, Ltd.) microtome where 2 μm sections were obtained. They were loosened in 50-70% ethyl alcohol and hot water (40-50℃), and mounted on the slide glass, dryed on the hot plate (30℃) for 3 hrs. Sections were hydrated, stained with Harris haematoxylin for 2 min, washed with tap water for 5 min and eosin Y staining for 20 min. Stained section were dehydrated and mounted with Paramount mounting solution. Samples were investigated and photographed using light microscope (Zeiss, Ltd.).

3. Comparison of both embryonic gonad PGCs and statistical analysis

The gonadal PGCs on the disected plane of right and left were counted from 4-6 day embryos and student's t-test was used to compare the number of PGC between left and right gonad.

Results and Discussion

In this study, gonadal development and PGCs migration to the gonads at stage 3 to 29 (between 12 hrs and 6 days of incubation) were observed at the light microscopic level by means of transverse serial sections.

1. Stage 3 to 6 (Incubation time : 12 to 24 hrs)

At this stage, PGCs arise from the epiblast and migrate into the hypoblast. During gastrulation, the PGCs migrate anteriorly via the hypoblast and reside in the extraembryonic area, which is a so-called germinal crescents located in the anterior region of the blastodisc at early developmental stage (Eyal-Giladi et al., 1981; Urven et al., 1988). We observed the PGCs which are arisen from epiblast have been migrating into the anterior region of the embryos (figure 1).

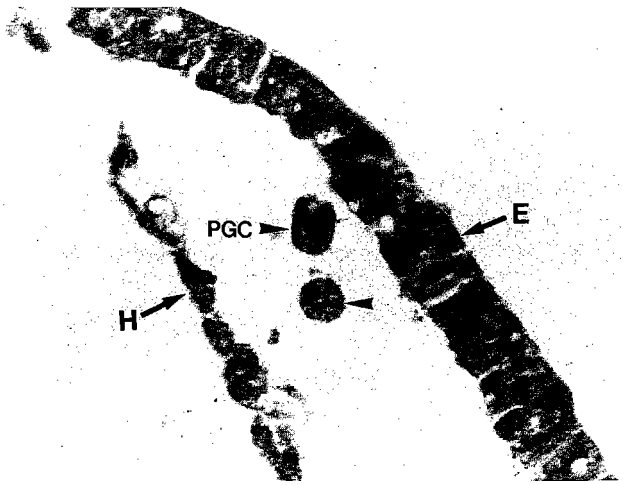

Figure 1. Stage 3. The PGCs arise from the epiblast and migrate into the hypoblast.
E : epiblast, H : hypoblast.

2. Stage 7 to 17 (Incubation time : 25 to 64 hrs)

The number of somites appears to be the simplest criterion for stage 7 to 14, but beyond stage 14 the number of somites becomes increasingly difficult to determine with accuracy. After 30 hrs of incubation, PGCs began to migrate via the circulatory system to the future gonadal region at stage 9 to 10 (30 to 38 hr of incubation) (Eyal-Giladi et al., 1981; Urven et al., 1988). With thin sections, chick PGCs were easily identifiable, because they were large in size (12 to 14 μm in diameter), possess a conspicuously large and round nucleus (7 to 10 μm in diameter) with a frequently fragmented nucleus and also had abundant cytoplasmic glycogen.

In embryo stage 17, many PGCs with large and round nucleus were shown in the vitelline artery (VA) as well as other blood cells (figure 2). After leaving to the gonadal

ridge at stage 16 to 17, the PGCs finally penetrate into the developing gonad by day 2.5 of incubation (Swift, 1914).

Figure 2. Stage 17. The PGCs are in the vitelline artery (►) and the other blood cells are shown.

3. Stage 18 to 20 (Incubation time : 65 to 72 hrs)

Figure 3 showed a transverse section of the embryonic gonadal ridge area at stage 20 (70-72 hrs of incubation). The site of the thickened coelomic epithelium get the site near prospective gonadal area between coelomic angle and mesonephros. At this developmental stage, no PGCs were observed anywhere in coelomic epithelium and its neighbors, but some were occasionally found in the blood vessels.

The PGCs were found in blood vessel (dorsal aorta) up to 72 hr incubation, but Petitte et al. (1991) reported that PGCs are in the blood vessel up to 65 hrs. PGCs begin to arrive at the region of the future gonad at the stage 19 (68 to 72 hr of incubation) and prior to 2.5 days of incubation when the epithelial thickening, it appears at the gonadal site. The PGCs after extravasation are found to penetrate into the coelomic epithelium of the splanchnopleure (Ando and Fujimoto, 1983).

4. Stage 21 to 24 (Incubation time : 3 to 4 days)

At the developmental stage 21 (3 1/2 day incubation), many PGCs were migrating in the mesenchym and several PGCs were under the epithelium of the gonadal ridge (figure 4).

Chick gonads in the stage 24 (4 day incubation) were forming with the advance of development and most PGCs with irregular shape were located in the gonad, but some were found in the mesenchym nearby (figure 5).

Also, the PGCs showed directional movement toward

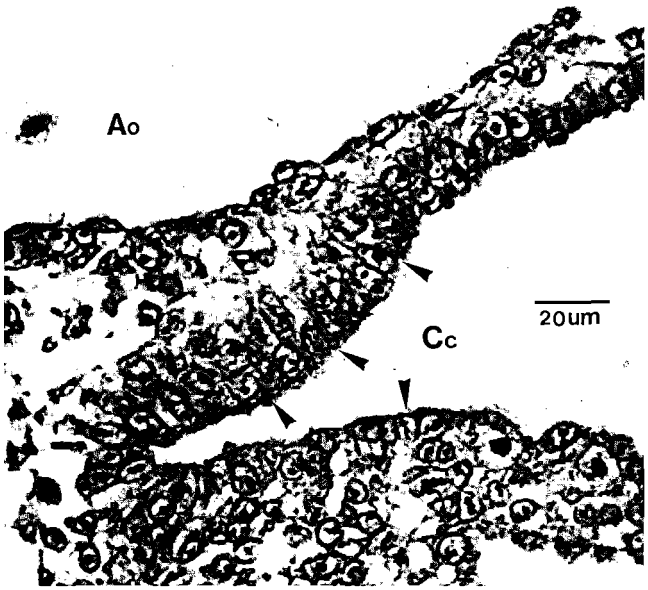

Figure 3. Transverse section of the stage 20 embryionic gonad. Gonadal soma formation is begun at the coelomic epithelium of the region between the coelomic angle and mesonephros (►). Ao : Dorsal aorta, Cc : Coelomic cavity.

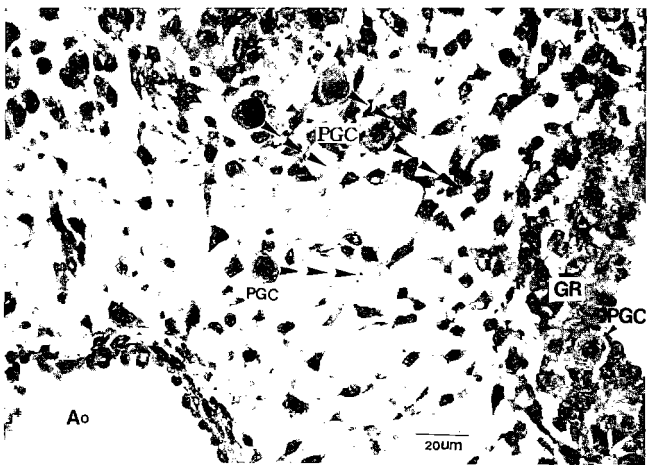

Figure 4. Early arrived PGCs are shown under the epithelium of the gonadal ridge and some PGCs (►) are migrating toward the gonadal ridge (GR). Ao : Dorsal aorta, GR : Gonadal ridge.

gonadal ridge and it showed higher attractive activity to the gonadal ridge than the other tissues. It was considered to be due to an active amoeboid movement of the PGCs

(Kuwana and Fujimoto, 1983). Kuwana et al. (1986) suggested that the superficial epithelium of the gonadal ridge release some chemical attractant to PGCs. One mechanism of the PGC migration possibility is, therefore, that the developing gonad produces a chemotactic substance that attracts PGCs and retain them in the capillaries bordering the gonad (Rogulska, 1969). Another possibility is that the endothelial cells of the gonadal capillaries have a cell surface compound that cause the PGCs to adhere there specifically. The PGCs arrived at both the right and the left gonad primordia in equal number prior to 4 day of incubation, but following this period the distribution of the PGCs became asymmetrical. More PGCs colonized in the left (70%) than right (30%) gonad, and PGC numbers between the left and the right gonad were different significantly ($p < 0.0001$) after 4 day of incubation (table 1). An active migration to the left gonad occured in both sexes which resulted in a greater concentration of PGCs on the left side. The underlying cause of the unequal distribution of PGCs is uncertain (Maraud et al., 1986).

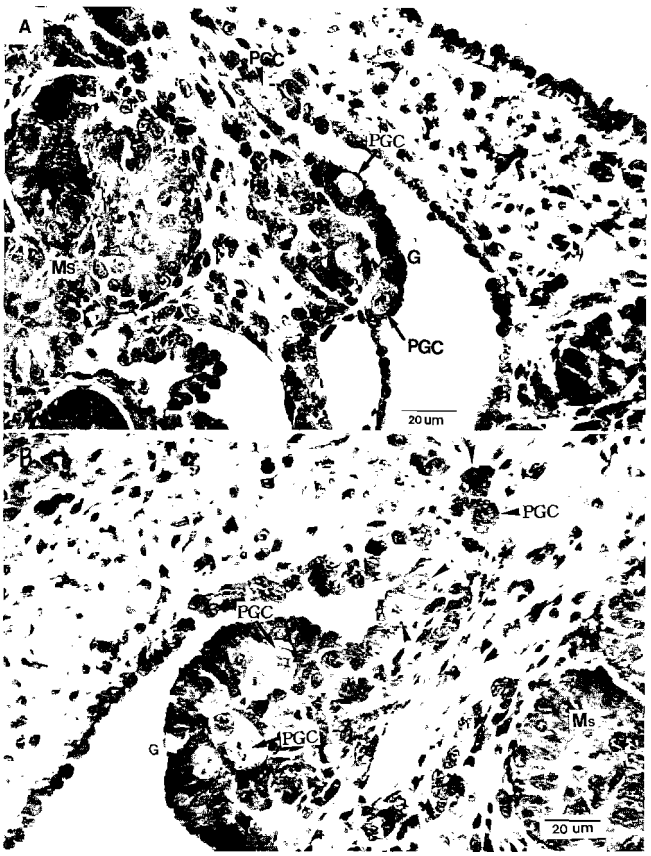

Figure 5. Stage 24. Some PGCs which arrived late are found in the mesenchyme (►). A : left gonad, B : right gonad, G : Gonad, Ms : Mesonephros.

TABLE 1. COMPARISON OF PGCs NUMBER AFTER SECTION BETWEEN LEFT AND RIGHT EMBRYONIC GONAD AT 4 TO 6 DAYS

Age (day)	No. of Embryo	Left gonad [a]Mean ± SD	Right gonad Mean ± SD
4	6	4.83 ± 1.17	3.67 ± 1.21
4.5	5	7.80 ± 1.10	3.2 ± 1.10***
5	5	11.75 ± 0.96	3.5 ± 0.58***
6	5	17.20 ± 1.30	4.0 ± 0.71***

[a] Values represent means ± SD for number of PGCs on the disected plane of left and right embryonic gonads.
*** $p < 0.0001$.

5. Stage 25 to 29 (Incubation time : 4 to 6 days)

At stage 29 (6 day incubation), most all PGCs were found in the sexually indifferent gonads in which the germ cells could be distinguished by their large size (about 11.2 μm in diameter), round nucleus (figure 6). The PGCs settle down in the gonadal primordium until the stage 27 to 29 (5 to 6 days) when they rapidly proliferate to form germ cells (Nakamura et al., 1988).

These results showed that the PGCs have mostly settled down in the gonadal ridge at 6 day embryo. This may reflect the fact that the attractive force of GR decrease after the completion of PGC migration into GR (Kuwana et al., 1986). The PGCs in males remain scattered randomly within a developing seminiferous tubule until day 13 of incubation (stage 39). After this period, they begin to differentiate into spermatogonia. In contrast to male embryos, the sexual differentiation of female embryos occurs in the left ovary at 8 day of incubation (stage 34). The PGCs begin active meiotic division, forming oogonia at this stage (Van Krey, 1990).

As a results of transverse serial sections of the chick embryo stage 3 to 29, we obtained the information about the gonadal soma formation, and the characteristics of the PGCs from hypoblast to the embryonic gonad in sexually indifferent gonad. Several studies have been conducted to produce transgenic chicken by manipulated PGCs (Han et al., 1994; Tsai et al., 1992; Ono et al., 1994; Naito et al., 1994). PGCs were isolated from germinal crescent (Han et al., 1994; Tsai et al., 1992) or blood vessel (Ono et al., 1994) and exogenous DNA was introduced into the PGCs. Allioli et al. (1994) reported that primordial germ cells (PGCs) isolated from gonads of 5-day-old-chick embryo, were able to divide *in vitro*. Thus, it is possible to introduce and express exogenous DNA in chick PGCs maintained *in vitro* and produce germ line transgenic chicken with manipulated.

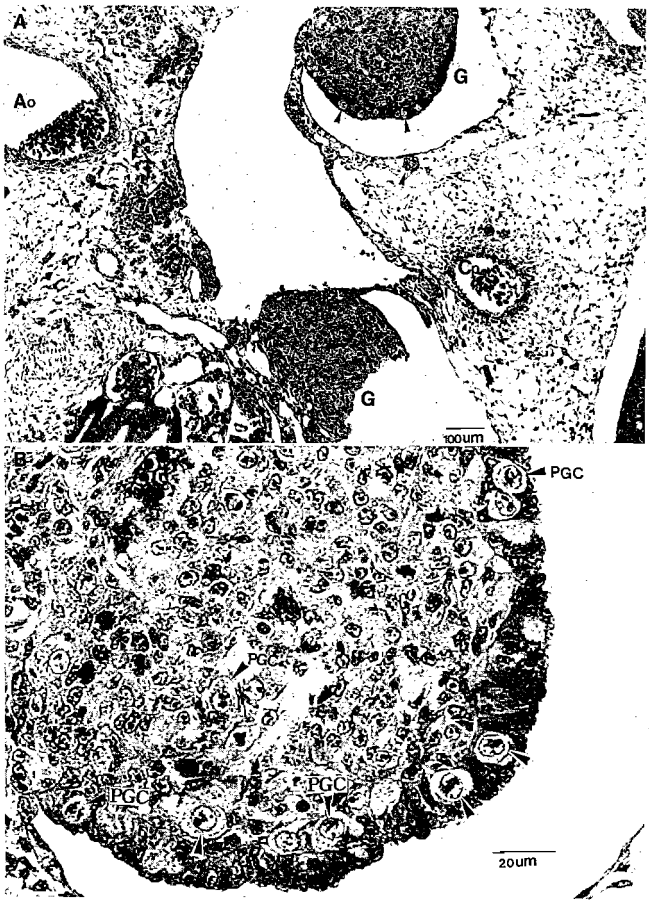

Figure 6. A 6-day chick embryonic gonads. The germ cells (►) can be distinguished by their large nucleus. The PGCs in the left gonad are much more than that of right gonad. G : Gonad, Ao : Dorsal aorta, Cp : Capillary.

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Literature Cited

Allioli, N., J. -L. Thomas, Y. Chebloune, V. -M. Nigon, G. Verdier and C. Legras. 1994. Use of retroviral vectors to introduce and express the β-galactosidase marker gene in cultured chicken primordial germ cells. Devel. Biol. 165:30-37.

Ando, T. and T. Fujimoto. 1983. Ultra structural evidence that chick primordial germ cells leave the blood-vascular system prior to migrating to the gonadal anlagen. Dev. Growth Differ. 25:345-352.

Dubois, R., D. Cumiage and J. Smith. 1976. Interpretation of some recent results in experimental embryology and the problem of the germ line. In: organ culture in Biological Research. Ball and Monnickendom, eds. Cambridge Univ. Press, London, pp. 61-93.

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. Dev. Biol., 49:326-337.

Eyal-Giladi, M., M. Ginsburg and A. Farbarov. 1981. Avian primordial germ cells are of epiblastic origin. J. Embryol. Exp. Morphol., 65:139-147.

Fujimoto, T., A. Ukeshima and R. Kiyofuji. 1975. Light- and electron microscopic studies on the origin and migration of the primordial germ cells in the chick. Acta. Anat. Nippon, 50:22-40.

Fujimoto, T., A. Ukeshima and R. Kiyofuji. 1976. The origin, migration and morphology of the primordial germ cells in the chick embryo. Anat. Rec., 185:139-154.

Ginsburg, M. and H. Eyal-Giladi. 1986. Temporal and spatial aspect of the gradual migration of primordial germ cells from the epiblast into the germinal crescent in the avian embryo. J. Embryol. Exp. Morphol., 95:53-71.

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

Han, Jae Y., R. N. Shoffner and K. S. Guise. 1994. Gene transfer by manipulation of primordial germ cells (PGCs) in the chicken. Asian-Australasian J. of Animal Science. 7(3):427-434.

Kuwana, T. and T. Fujimoto. 1983. Active locomotion of human primordial germ cells *in vitro*. Anat. Rec., 209:337-343.

Kuwana, T., H. Maeda-Suga and T. Fujimoto. 1986. Attraction of chick primordial germ cells by gonadal anlage *in vitro*. Anat. Rec., 215:403-406.

Maraud, R., M. Rashedi and R. Stoll. 1986. Experimentally induced true hermaphroditism in genetically female fowl. Roux's Arch. Dev. Biol. 195:10-14.

Meyer, D. B. 1960. Application of the periodic acid-Schiff technique to whole chick embryos. Stain. Technol., 35:83-89.

Meyer, D. B. 1964. The migration of primordial germ cells in the chick embryo. Dev. Biol., 10:154-159.

Nakamura, M., T. Kuwana, Y. Myyayama and T. Fujimoto. 1988. Extragonadal distribution of primordial germ cells in the early chick embryo. Anat. Rec. 222:90-94.

Naito, M., A. Tajima, T. Yasuda and T. Kuwana. 1994.

Preservation of chick primordial germ cells in liquid nitrogen and subsequent production of viable offspring. J. of Reprod. and Fert. 102:321-325.

Ono, T. and R. Yokoi. 1994. Transfer of male and female primordial germ cells of quail into chick embryonic gonad of the same and opposite sexes. Proceeding of the 5th world congress on genetics applied to livestock production. 20:104-107.

Petitte, J. N., M. E. Clark and R. J. Etches. 1991. Assessment of functional gametes in chickens after transfer of primordial germ cells. J. Reprod. Fert., 92:225-229.

Rodemer-Lenz, E. 1989. On cell contribution to gonadal soma formation in quail-chick chimeras during the indifferent stage of gonadal development. Anat. Embryol., 179:237-242.

Rogulska. T. 1969. Migration of chick primordial germ cells from the intracoelomically transplanted germinal crescent into the ridge. Experientia 25:631-632.

Romanoff, A. L. 1960. The avian embryo. Macmillan Company, New York.

Sutasurya, L. A., S. Yasugi and T. Mizuno. 1983. Appearance of primordial germ cells in young chick blastoderm cultured *in vitro*. Dev. Growth Differ., 25:517-521.

Swift, C. H. 1914. Origin and early history of the primordial germ cells in the chick. Am. J. Anat., 15:483-516.

Tsai, H. and B. C. Wentworth. 1992. Long-term culture of chick primordial germ cells isolated from the blastodisc and germinal crescent. Poul. Abst. p. 72.

Ukeshima, T. and T. Fujimoto. 1984. Ultrastructure of primordial germ cells in the early chick embryo. In: ultrastructure of reproduction. J. Van Blerkom and P. M. Motta, eds. Martinus Nijhoff publishers, Boston, pp. 12-18.

Ukeshima, A., K. Yoshinaga, M. Kudo and T. Fujimoto. 1985. Primordial germ cells in the period when the cells are passing into the tissue from the blood circulation in the chick embryo. Abst. of the 12th International Anatomical Congress. London. p. 726.

Ukeshima, A., K. Yoshinaga and T. Fujimoto. 1991. Scanning and transmission electron microscopic observation of chick primordial germ cells with special reference to the extravasation in their migration course. J. Electron Microscop., 40:124-128.

Urven, L. E., C. A. Erickson, U. K. Abbot and J. R. McCarrey. 1988. Analysis of germ line development in the chick embryo using an antimouse EC cell antibody. Development, 103:299-304.

Van Krey, H. P. 1990. Reproductive biology in relation to breeding and genetics. In: *Poultry Breeding and Genetics.* edited by R. D. Crawford. Elsevier Science Publishers, Amsterdam, pp. 61-90.