Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 16 (3): 49-55
July-September, 2016
DOI: 10.3923/jms.2016.49.55

Mouse Mesonephros in Fetus Period is Necessary for Differentiation of Primordial Germ Cells in Ectopic Kidney Capsule

Kazuei Matsubara

Mouse Primordial Germ Cells (PGCs) proliferate and migrate into the Genital Ridges (GRs) and the urogenital complex (UGC) is then constructed from the GR and the mesonephros. However, the mesonephros disappear at an early stage of subsequent embryonic development and the interaction between mesonephros and PGCs are not well understood. In the present study, it has been investigated the need of mesonephros in PGC differentiation by the ectopic transplant. The GR or UGC of female mouse 12.5 days post coitum (dpc) were transplanted into the kidney capsules of female immunodeficient mice. At 4 and 8 weeks after transplantation, the kidney capsules were recovered and the cell types in the grafts were identified by histological analysis. In UGCs graft of 4 weeks after transplantation, the follicles containing with growing oocyte were observed in all recipient kidney. The structures had the characteristics of secondary follicles with zona pellucida and multi-layered granulosa cells. Antibody staining indicated that these follicles differentiated from PGCs and were not apoptotic. But, no follicles were observed at 4 weeks in transplantation of GR without mesonephros. Similarly, follicles were not present at 8 weeks after transplantation of GR or UGC. The mesonephros is necessary for forming the follicles into kidney capsules of recipient immunodeficient mouse. However, the transplantation of UGCs in ectopic kidney capsules should be performed within 4 weeks in order to study the processes of follicular formation/development.

Key words: Ectopic transplantation, gamete formation, mouse PGCs, mesonephros

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued four times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Kazuei Matsubara
Laboratory of Animal Sciences,
Iwate University, Iwate, Japan

Tel/Fax: +81-19-621-6160

ANSInet
Asian Network for Scientific Information

Laboratory of Animal Sciences, Iwate University, Iwate, Japan

INTRODUCTION

Mouse Primordial Germ Cells (PGCs), which are first identified in the extra-embryonic mesoderm by alkaline phosphatase staining, proliferate and migrate into the Genital Ridges (GRs) at 11.5~13.5 days post coitum (dpc) (Ginsburg *et al*., 1990). At 13.5 dpc, the mouse female fetus has about 25,000 PGCs in the GRs (Mintz and Russell, 1957; Tam and Snow, 1981) and these gradually differentiate to form oocytes from 13 dpc. At about the same time, the female GRs are formed into the ovary.

On the other hand, the kidney is derived from intermediate mesoderm and the primordial kidney, mesonephros and metanephros are formed. The GRs forms the urogenital complex (UGC) in association with the mesonephros. However, in a process of the kidney development, the primordial kidney and mesonephros disappears during the differentiation. The mesonephros produces the Retinoic Acid (RA) synthesizing enzyme (RALDH2) and RA moves from the mespnephros into the adjacent gonad through open mesonephric tubles (Bowles and Koopman, 2007). Germ cells do not initiate meiosis in cytoplasmic protein *Stra8*-null mice (Baltus *et al*., 2006), so RA must trigger meiosis by inducing *Stra8* (Oulad-Abdelghani *et al*., 1996).

The understanding of the formation and development of oocytes from PGCs has improved in recent years (Barnett *et al*., 2006), however, there are still some areas of uncertainty, because these processes occur over a very short time scale. One approach used to study of these processes is culture of mouse fetal gonads, GRs or UGCs. Recent advances in culture technology have made it possible to produce oocytes from cultured gonads (Klinger and de Felici, 2002; Obata *et al*., 2002; Shen *et al*., 2006, 2007). Mayanagi *et al*. (2003a) achieved proliferation of PGCs using culture of mouse UGCs in combination with intra-species serum, but the inducing differentiation of PGCs in GR proved difficult. However, in the recent study, it was reported that 12.5 dpc mouse PGCs differentiated to ova by the culture technique and completed *in vitro* generation of fertile oocytes (Morohaku *et al*., 2016). Another approach is to transplant embryo gonads into ectopic tissue of other animals in order to provide an *in vivo* environment. This approach has been used for various cell and tissue types (Byskov *et al*., 1977; Byskov, 1974; Donnez *et al*., 2006; Qing *et al*., 2008; Snow *et al*., 2002; Stevens, 1970).

Byskov *et al*. (1977) and Byskov (1974) transplanted the mouse ovary of 12 dpc into subcutis of nude mouse (BALB/c Nu/Nu). The transplanted ovary was divided to three parts: 'Pure' gonadal tissue without extraovarian rete tubuli, adherent extraovarian rete tubuli and mesonephric remnants. As the result, the 'Pure' gonadal tissue without extraovarian rete tubuli contained only oogonia and oocytes and follicles were never seen in the graft for 14 days. But, in another graft tissue, oocyte and small and medium seized follicles were seen. The rete tubuli had not yet invaded to the gonads at 12 dpc, but it was lying entirely outside the ovary as extra ovarian rete. Also, the meiotic prophase had not started. Therefore, Byskov (1974) reported that the rete system (rete ovarii and rete tubuli) derived from mesonephros were important to subsequent ovarian follicle formation and meiosis initiation, this small organ was not the simple persistence for fetus and associated with the differentiation of the germ cell. Transplantation of mouse embryos into the kidney capsules of nude mice resulted in the formation of teratoma (Van Berlo *et al*., 1990). Similarly, the teratomas developed after the transplantation of bovine GRs at 34 and 37 dpc (Choi and Anderson, 1998) or after transplantation of embryo germ cells (Durcova-Hills *et al*., 2003). With regard to oocyte growth, normal mouse oocytes and follicles can develop after transplantation of infant ovarian cells (Eppig *et al*., 2000) in combination with follicle culture techniques (Liu *et al*., 2000). Essentially, the same approach has been used with oocytes of pigs (Kaneko *et al*., 2003) and cattle (Senbon *et al*., 2004).

On the other hand, Ozdzenski (1972) reported that ovaries were observed at 8-16 days after transplantation in the kidney capsules of an inbred strain mice which 11.5 dpc GRs of same strain were transferred. However, the oocytes observed in these ovaries were inviable. Qing *et al*. (2008) transplanted the mouse GR or UGC of 12.5 dpc in kidney capsule of recipient female mouse which surgically removed both ovaries and confirmed an ovarian follicle in kidney capsule over 3-4 weeks after transplantation of GR groups without mesonephros. As a result, they concluded that the mesonephros was not necessary for the ectopic transplantation of the gonad of 12.5 dpc. Furthermore, Ohta and Wakayama (2004) succeeded in producing round spermatids at 9-10 weeks after transplantation of 12.5 dpc mouse GRs under the dorsal skin of nude mice, but no mature sperm were formed. However, the contradiction exists in these reports, because in order to initiate meiosis, RA secreted from mesonephros is need. In spite of these accumulated findings, the behaviors of PGCs and a role of mesonephros in the grafts are still far from sufficiently understood and have be contradiction. Therefore, if the mesonephros disappears during development is necessary for the differentiation of PGCs, the formation of mature gametes might occur in recipient kidney capsules after transplantation of 12.5 dpc UGC. In the present study, in order to determine whether mesonephros is necessary for differentiation of PGCs and growth of follicle, the female mouse GR or UGC of 12.5 dpc was transplanted into the kidney capsules of immunodeficient mice.

MATERIALS AND METHODS

Animals: Eight weeks old SPF/VAF Crlj: CD1 mice (ICR mice) and SPF/VAF Crlj: CD1-Foxn1nu mice (ICR-nu mice) were obtained from Charles River Laboratories Japan Inc. (Kanagawa, Japan). Female mice were mated with males of

the same strain and 12:00 pm on the day of vaginal plug detection was defined as 0.5 dpc. All mice were housed under a 12 h (7:00-19:00) light-dark cycle at 22-24°C in a barrier cage. The ICR and ICR-nu mice were fed *ad libitum* with rodent diet CE-2 (CLEA) and rodent diet CL-2 (CLEA), respectively. All mice had *ad libitum* access to sterilized water. The experimental protocols were reviewed and approved by the Animal Ethics Committee of Iwate University.

Transplantation into and recovery of recipient kidneys: Transplantation was performed on a sterile bench. Pregnant ICR mice were sacrificed at 12.5 dpc by cervical dislocation and the embryos were dissected into a petri dish containing Hank's Balanced Salt Solution (HBSS). The GRs or UGCs were then obtained from the embryos by microsurgery and were collected in a 96-well microplate (Falcon; Becton Dickinson Labware, Franklin Lakes, NJ, USA) containing HBSS. The GR and mesonephros were separated from the UGC. The plates were placed on ice until use. Female gonads were selected by morphology (Fig. 1) for transplantation into the kidney capsule of recipient ICR-nu mice. Eight weeks old female mice were anaesthetized using sodium pentobarbital (Nembutal 0.01 mL g^{-1} i.p.) and held in a prone position. A single GR or UGC from female ICR mice was transplanted using fine forceps under a kidney capsule of the recipient ICR-nu mice. In total, 16 GRs or 16 UGCs were transplanted into the kidney capsules of 8 recipient ICR-nu mice, respectively. The kidneys of the ICR-nu recipient mice were analyzed at either 4 or 8 weeks after transplantation according to the reports of Qing *et al*. (2008) and Ohta and Wakayama (2004). In the present study, both ovaries of recipient female mouse were not removed.

Histological analysis: After removal from the mice, the kidneys were fixed with 15% formalin solution and processed for preparation of 10 μm sections that were cut using a with microtome. The sections were attached to glass microscope slides, treated with Periodic Acid Schiff (PAS) kit (Sigma-Aldrich, USA) and stained with hematoxylin and eosin. The sections were also treated with rabbit anti-mouse PGC IgG polyclonal antibody labeled with FITC (Mayanagi *et al*., 2003a, b) produced in author's laboratory. This antibody specifically recognizes mouse 11.5-12.5 dpc PGCs. Skim milk was used as the blocking reagent. Fluorescence was observed by fluorescence microscopy (Olympus, AX-80, Japan). Slides on which secondary follicles were observed were treated with rabbit anti-single-stranded (anti-ss) DNA monoclonal antibody (Dako Cytomation, Carpinteria, CA, USA); this antibody detects apoptotic cells. Normal donkey serum was used as the blocking reagent. Donkey anti-rabbit IgG labeled with biotin (Jackson Immune Research, USA) was used as the secondary antibody and the tissues were stained with ABC solution (Vector Laboratories, Inc., USA) and DAB reagent (Dojindo, Japan).

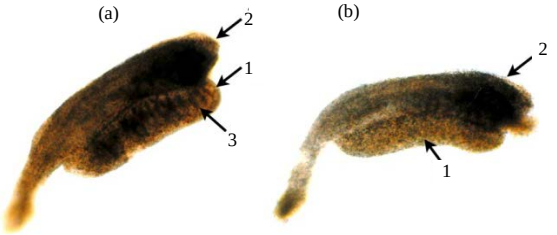

Fig. 1(a-b): Mouse male and female 12.5 dpc urogenital complexes identified by morphology, (a) Male UGC and (b) Female UGC. These gonads could be distinguished by the presence of primitive testicular cords (3) in the male. (1) A genital ridge and (2) A mesonephros

RESULTS

Eight GRs (6 at 4 weeks, 2 at 8 weeks) had completely disappeared from the transplantation sites (Table 1). However, GRs were identified in the other 8 kidney capsules (2 and 6 grafts recovered after 4 or 8 weeks, respectively). These grafts showed histological changes compared to their appearance before transplantation: Two grafts had formed a water vacuole at 8 weeks (Fig. 2a), the other 6 grafts (2 grafts at 4 weeks, 4 grafts at 8 weeks) had enlarged (Fig. 2b).

With respect to the transplantation of UGCs, 12 grafts were identified, the other 4 grafts had disappeared at 4 (1 graft) or 8 (3 grafts) weeks after transplantation. Five of the identified grafts did not show any change to their appearance after transplantation (Fig. 2c) (3 and 2 grafts recovered after 4 and 8 weeks, respectively), 4 grafts had formed a water vacuole (each 2 grafts at 4 and 8 weeks) and 3 grafts had formed fatty material (2 and 1 grafts at 4 and 8 weeks, respectively). Three grafts that showed no apparent change after 4 weeks had some follicles in the kidney medulla (Fig. 3a-c). These follicles were secondary follicles with multi-layered granulosa cells around a growing oocyte. Formation of the zona pellucida around the oocytes was confirmed by PAS staining (Fig. 3d, e). However, the react of the zona pellucida and PAS was very weak and indistinctness.

Table 1: Outcomes of transplantation of mouse genital ridges and urogenital complexes under the kidney capsules of ICR-nu mice

	No. of GR		No. of UGC	
Morphology	4 weeks	8 weeks	4 weeks	8 weeks
Unchanged	0	0	3	2
Enlarged	2	4	0	0
Vacuolated	0	2	2	2
Fatty material	0	0	2	1
Disappeared	6	2	1	3
Total No. of grafts	8	8	8	8

GR: Genital ridges and UCG: Urogenital complexes

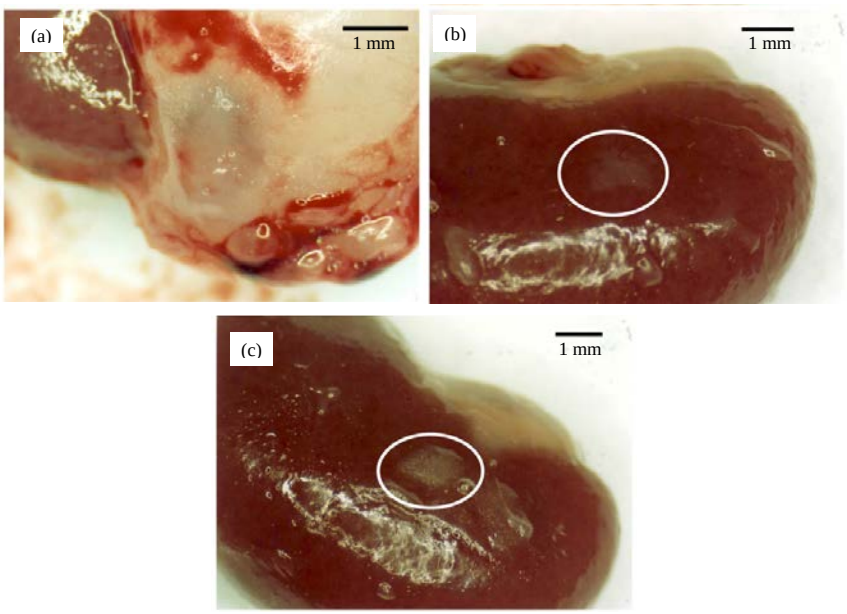

Fig. 2(a-c): Morphology of transplanted GR/UGC in ICR-nu mouse kidneys at 4 and 8 weeks after transplantation, (a) Water vacuole at the transplantation site, (b) Enlarged graft at the transplanted site (ellipses) and (c) The graft shows little or no change from the appearance at transplantation (ellipses)

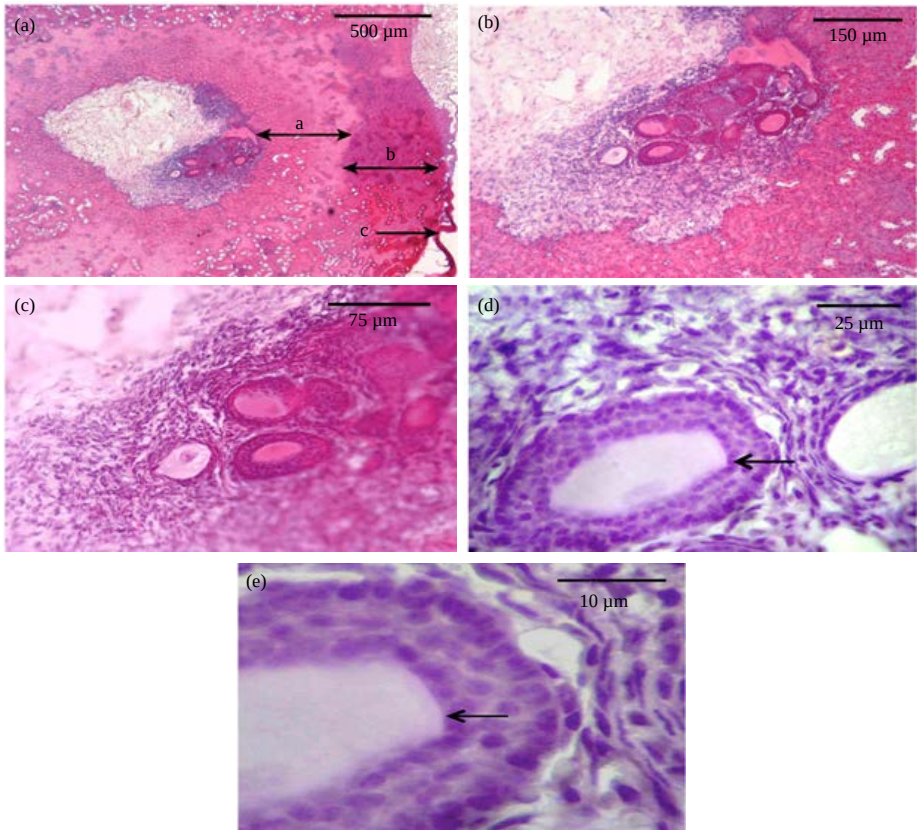

Fig. 3(a-e): Histological sections of the kidney of ICR-nu mouse with secondary follicles at 4 weeks after transplantation of UGC. (a) Overview of follicles in the kidney cortex (HE stain), (b, c) The same follicles at higher magnification, showing that they are enclosed by a granulosa cell layer(s) and (d, e) Oocyte, in a secondary follicle with zona pellucida (arrow), PAS: Stain positive

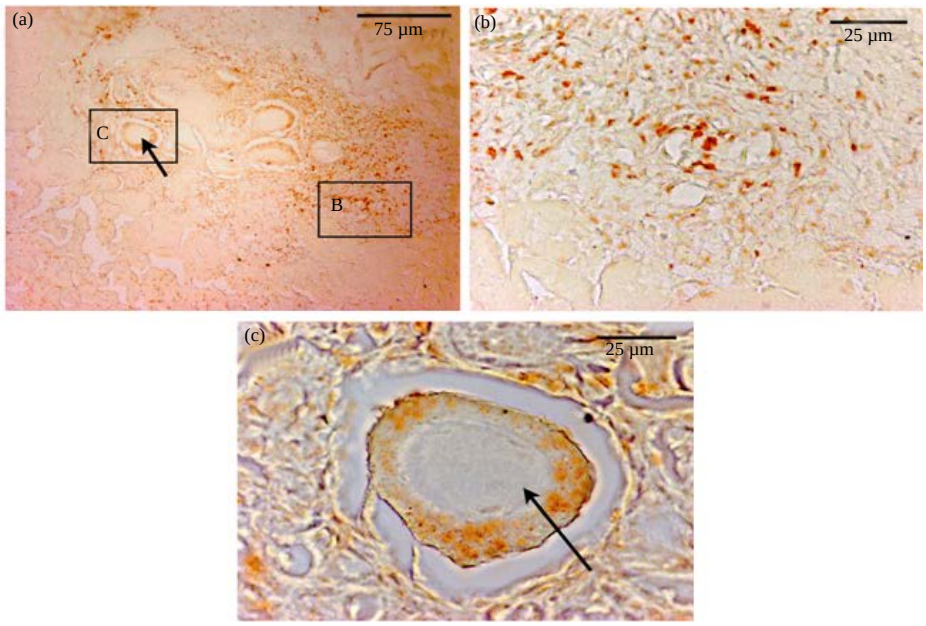

Fig. 4(a-c): Kidney of an ICR-nu mouse, 4 weeks after transplantation of UGC with secondary follicles stained with the anti-ss DNA antibody. (a) Overview of the tissue including secondary follicles (arrow), (b) Tissues of except secondary follicles and (c) Granulosa cells stained with the anti-ss DNA antibody. The oocyte and zona pellucida (arrow) were not stained

Some of the follicle had a thin layer of granulosa cells. The oocyte in follicles did not react with rabbit anti-mouse PGC IgG nor with rabbit anti-ss DNA monoclonal antibody. However, the granulosa cells around of the zona pellucida reacted with the rabbit anti-ss DNA monoclonal antibody (Fig. 4). In addition to clearly identifiable follicles, other follicle-like structures were present in the graft.

DISCUSSION

In the present study, PGCs in 12.5 dpc UGCs transplanted into recipient kidney capsules formed the secondary follicles with oocyte surrounded by zona pellucida. And the follicles were enclosed from granulosa cells at 4 weeks. But, no follicle was recognized at GR transplantation group without mesonephros. Therefore, under the conditions of this study, it is concluded that the mesonephros is necessary for the differentiation of PGCs in recipient kidneys at early period of development. However, the role of mesonephros for follicle development is still in confusion.

Byskov *et al*. (1977) and Byskov (1974) indicated that the rete system derived from mesonephros were important to subsequent follicle formation and meiosis initiation. Also, Choi and Anderson (1998) reported that the teratomas developed in kidney capsule of nude mouse after transplantation of mouse 11.5 dpc GR and bovine 34 or 37 dpc GR. Other researcher also reported that GR transplanted into the mouse kidney could form teratomas (Van Berlo *et al*., 1990). On the other hand, according to the report of Ozdzenski (1972), the transplanted female 11.5 dpc GR formed ovaries in the kidney capsule at 8-16 days after transplantation, but the oocytes in these ovaries were very small and inviable. Qing *et al*. (2008) concluded that the mesonephros was not necessary for the ectopic transplantation of 12.5 dpc gonad. They indicated the possibility that other tissues in the host mice, such as kidney, secrete RA to promote the development of transplanted gonads. However, whether or not RA from the kidney of an adult mouse is secreted is not known. The difference between Qing *et al*. (2008) and the present study is whether or not removed the recipient ovaries. If there is no ovary in recipient mouse, PGC in GR without mesonephros will be able to initiate meiosis? Hashimoto *et al*. (1992) and Hayashi *et al*. (2012) surgically removed the 90-80% of a female mice ovaries and transplanted the reconstruction ovary which was removed the 12.5~14.5 dpc fetal mouse mesonephros in ovary capsule or ovarian bursa of the treated recipient mouse. As the results, they were formed follicles from GR transplanted into ectopic ovarian and succeeded to obtaining a litter from their follicles. On the other hand, in the kidney capsule of ovariectomized female mice, transplanted together with PGCs and gonadal somatic cells without mesonephros (Matoba and Ogura, 2011). In these studies, PGCs-derived follicle has been observed in the ectopic transplanted organ. The cause of these contradiction is unclear, but possibly the hormone secreted from recipient ovaries may decide subsequent fate of ectopic graft. The RA is generated

from mesonephros and the meiosis initiation start by this material (Bowles *et al*., 2006; Koubova *et al*., 2006). The RA generated from mesonephros of UGC graft may develop secondary follicles from PGCs. Eppig *et al*. (2000) transplanted the mouse infant ovarian cells into the kidney capsules of SCID mice and succeeded to producing normal mouse oocytes and follicles. Therefore, the mesonephros may be unnecessary at later stages of follicle development. Only when oocytes differentiated from PGCs (i.e., the initiation of meiosis), the mesonephros or rete system may be necessary.

In the present study, the absence of reaction by anti-mouse PGC IgG indicates that the secondary follicles differentiated from PGCs in UGC and the transplanted UGCs were able to form secondary follicles in the recipient kidney medulla at 4 weeks. Choi and Anderson (1998) observed teratocarcinoma cells in the kidney cortex. The unthinkable fact that the follicles developed in the kidney medulla indicates that some PGCs and mesonephros later developed to oocytes or rete system, respectively. However, it is not evidence that the mesonephros differentiate to rete system. The PGCs possess the ability to migrate and differentiate in the kidney medulla where nutrition is abundant. The kidney medulla may provide more suitable conditions for germ cell growth than the kidney cortex/capsule. The histochemical examination by PGCs specific antibodies (i.e., SSEA-1 (Solter and Knowles, 1978), Oct3/4 (Scholer, 1991) and MVH (Castrillon *et al*., 2000) etc.) will in future need. In the present study, follicles were not observed in kidneys at 8 weeks after transplantation of UGCs and the granulosa cells observed at 4 weeks reacted with the anti-ss DNA monoclonal antibody. Therefore, these follicles may disappeare between 4-8 weeks by apoptosis. Eight weeks may be too long to sustain mouse follicular organization in the ectopic kidney. The transplantation should be performed within 4 weeks in order to study the processes of follicular formation/development.

On the other hand, germ cells do not initiate meiosis in cytoplasmic protein *Stra8*-null mice (Baltus *et al*., 2006). Koubova *et al*. (2014) cultured 12.5 dpc mouse UGCs with the culture media supplemented with either pan-RAR inhibitor BMS-204493 or all trans. After 48 h, mesonephros was dissected off and ovaries were then processed for quantitative RT-PCR. As a result, *Rec8*, like *Stra8* could be a target of RA signaling and since *Dazl* expression was required for ovarian germ cells to respond to RA signaling, as with *Stra8* expression, expression of *Rec8* required both *Dazl* and RA. Also, in the resent study, Morohaku *et al*. (2016) succeeded to reconstitute of the entire process of mouse oogenesis *in vitro* from PGCs, using an estrogen-receptor antagonist that promotes normal follicle formation. Seven pups were borne from the cultured gonad. Therefore, the mesonephros is not necessary to support the meiosis of PGC *in vitro* culture, it seems to be able to growing the follicles by adding RA or estrogen etc. in the culture medium. However, in order to initiate meiosis of PGC *in vivo*, the organs (i.e., mesonephros) that secrete triggers such as RA prompting the expression of *Stra8* and *Rec8* is necessary.

CONCLUSION

The mesonephros is necessary for differentiation of PGCs *in vivo* in ectopic kidney. Furthermore, its transplantation should be performed within 4 weeks in order to study the processes of follicular formation/development. Transplantation of mouse 12.5 dpc UGCs into the kidney of recipient mouse is an effective procedure to study the behavior of PGCs *in vivo* during development.

SIGNIFICANT STATEMENTS

These findings suggest that only PGCs affected of mesonephros is able to initiate the meiosis and differentiate into germ cells *in vivo*. Our method will assist in understanding the role of mesonephros on the meiosis of female primordial germ cells.

ACKNOWLEDGMENTS

Author wish to thank Professor Y. Yamamoto (Veterinary Medicine, Iwate University) for assessment of apoptosis and Charles River Laboratories Japan Inc. for the mice.

REFERENCES

Baltus, A.E., D.B. Menke, Y.C. Hu, M.L. Goodheart, A.E. Carpenter, D.G. de Rooij and D.C. Page, 2006. In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. Nat. Genet., 38: 1430-1434.

Barnett, K.R., C. Schilling, C.R. Greenfeld, D. Tomic and J.A. Flaws, 2006. Ovarian follicle development and transgenic mouse models. Hum. Reprod. Update, 12: 537-555.

Bowles, J., D. Knight, C. Smith, D. Wilhelm and J. Richman *et al*., 2006. Retinoid signaling determines germ cell fate in mice. Science, 312: 596-600.

Bowles, J. and P. Koopman, 2007. Retinoic acid, meiosis and germ cell fate in mammals. Development, 134: 3401-3411.

Byskov, A.G.S., 1974. Does the rete ovarii act as a trigger for the onset of meiosis? Nature, 252: 396-397.

Byskov, A.G.S., N.E. Skakkebaek, G. Stafanger and H. Peters, 1977. Influence of ovarian surface epithelium and rete ovarii on follicle formation. J. Anatomy, 123: 77-86.

Castrillon, D.H., B.J. Quade, T.Y. Wang, C. Quigley and C.P. Crum, 2000. The human *VASA* gene is specifically expressed in the germ cell lineage. Proc. Natl. Acad. Sci. USA., 97: 9585-9590.

Choi, S.J. and G.B. Anderson, 1998. Development of tumors from bovine primordial germ cells transplanted to athymic mice. Anim. Reprod. Sci., 52: 17-25.

Donnez, J., B. Martinez-Madrid, P. Jadoul, A. van Langendonckt, D. Demylle and M.M. Dolmans, 2006. Ovarian tissue cryopreservation and transplantation: A review. Hum. Reprod. Update, 12: 519-535.

Durcova-Hills, G., F. Wianny, J. Merriman, M. Zernicka-Goetz and A. McLaren, 2003. Developmental fate of Embryonic Germ Cells (EGCs), *in vivo* and *in vitro*. Differentiation, 71: 135-141.

Eppig, J.J., K. Wigglesworth and Y. Hirao, 2000. Metaphase I arrest and spontaneous parthenogenetic activation of strain LTXBO oocytes: Chimeric reaggregated ovaries establish primary lesion in oocytes. Dev. Biol., 224: 60-68.

Ginsburg, M., M.H. Snow and A. McLaren, 1990. Primordial germ cells in the mouse embryo during gastrulation. Development, 110: 521-528.

Hashimoto, K., M. Noguchi and N. Nakatsuji, 1992. Mouse offspring derived from fetal ovaries or reaggregates which were cultured and transplanted into adult females. Dev. Growth Differ., 34: 233-238.

Hayashi, K., S. Ogushi, K. Kurimoto, S. Shimamoto, H. Ohta and M. Saitou, 2012. Offspring from oocytes derived from *in vitro* primordial germ cell-like cells in mice. Science, 338: 971-975.

Kaneko, H., K. Kikuchi, J. Noguchi, M. Hosoe and T. Akita, 2003. Maturation and fertilization of porcine oocytes from primordial follicles by a combination of xenografting and *in vitro* culture. Biol. Reprod., 69: 1488-1493.

Klinger, F.G. and M. de Felici, 2002. *In vitro* development of growing oocytes from fetal mouse oocytes: Stage-specific regulation by stem cell factor and granulosa cells. Dev. Biol., 244: 85-95.

Koubova, J., D.B. Menke, Q. Zhou, B. Capel, M.D. Griswold and D.C. Page, 2006. Retinoic acid regulates sex-specific timing of meiotic initiation in mice. Proc. Natl. Acad. Sci. USA., 103: 2474-2479.

Koubova, J., Y.C. Hu, T. Bhattacharyya, Y.Q.S. Soh and M.E. Gill *et al*., 2014. Retinoic acid activates two pathways required for meiosis in mice. PLoS Genet., Vol. 10. 10.1371/journal.pgen.1004541.

Liu, J., J. van der Elst, R. van den Broecke, F. Dumortier and M. Dhont, 2000. Maturation of mouse primordial follicles by combination of grafting and *in vitro* culture. Biol. Reprod., 62: 1218-1223.

Matoba, S. and A. Ogura, 2011. Generation of functional oocytes and spermatids from fetal primordial germ cells after ectopic transplantation in adult mice. Biol. Reprod., 84: 631-638.

Mayanagi, T., K. Ito and J. Takahashi, 2003a. Association of culture of mouse urogenital complexes in media containing rodent sera with the appearance of primordial germ cell-like cells. Reproduction, 125: 519-526.

Mayanagi, T., R. Kurosawa, K. Ohnuma, A. Ueyama, K. Ito and J. Takahashi, 2003b. Purification of mouse primordial germ cells by Nycodenz. Reproduction, 125: 667-675.

Mintz, B. and E.S. Russell, 1957. Gene-induced embryological modifications of primordial germ cells in the mouse. J. Exp. Zool. Part A: Ecol. Genet. Physiol., 134: 207-237.

Morohaku, K., R. Tanimoto, K. Sasaki, R. Kawahara-Miki and T. Kono *et al*., 2016. Complete *in vitro* generation of fertile oocytes from mouse primordial germ cells. Proc. Nat. Acad. Sci., 113: 9021-9026.

Obata, Y., T. Kono and I. Hatada, 2002. Oogenesis: Maturation of mouse fetal germ cells *in vitro*. Nature, 418: 497-497.

Ohta, H. and T. Wakayama, 2004. Full-term development of offspring using round spermatids produced ectopically from fetal male germ cells. J. Reprod. Dev., 50: 429-437.

Oulad-Abdelghani, M., P. Bouillet, D. Decimo, A. Gansmuller and S. Heyberger *et al*., 1996. Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. J. Cell Biol., 135: 469-477.

Ozdzenski, W., 1972. Differentiation of the genital ridges of mouse embryos in the kidney of adult mice. Archives D'anatomie Microscopique Morphologie Experimentale, 61: 267-278.

Qing, T., H. Liu, W. Wei, X. Ye and W. Shen *et al*., 2008. Mature oocytes derived from purified mouse fetal germ cells. Hum. Reprod., 23: 54-61.

Scholer, H.R., 1991. Octamania: The POU factors in murine development. Trends Genet., 7: 323-329.

Senbon, S., A. Ota, M. Tachibana and T. Miyano, 2004. Xenografting of bovine secondary follicles into ovariectomized female severe combined immunodeficient mice. J. Reprod. Dev., 50: 439-444.

Shen, W., D. Zhang, T. Qing, J. Cheng and Z. Bai *et al*., 2006. Live offspring produced by mouse oocytes derived from premeiotic fetal germ cells. Biol. Reprod., 75: 615-623.

Shen, W., L. Li, Z. Bai, Q. Pan, M. Ding and H. Deng, 2007. *In vitro* development of mouse fetal germ cells into mature oocytes. Reproduction, 134: 223-231.

Snow, M., S.L. Cox, G. Jenkin, A. Trounson and J. Shaw, 2002. Generation of live young from xenografted mouse ovaries. Science, 297: 2227-2227.

Solter, D. and B.B. Knowles, 1978. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). Proc. Natl. Acad. Sci. USA., 75: 5565-5569.

Stevens, L.C., 1970. The development of transplantable teratocarcinomas from intratesticular grafts of pre- and postimplantation mouse embryos. Dev. Biol., 21: 364-382.

Tam, P.P.L. and M.H.L. Snow, 1981. Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. Development, 64: 133-147.

Van Berlo, R.J., B. de Jong, J.W. Oosterhuis, T. Dijkhuizen, J. Buist and A. Dam, 1990. Cytogenetic analysis of murine embryo-derived tumors. Cancer Res., 50: 3416-3421.