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Ovarian Teratomas in Mice Lacking the Protooncogene c-*mos*

Yasuhide Furuta,[1,2] Yasuyo Shigetani,[1,2] Naoki Takeda,[1,2] Kuniko Iwasaki,[2] Yoji Ikawa[2,3] and Shinichi Aizawa[1,2,4]

[1]*Department of Morphogenesis, Institute of Molecular Embryology and Genetics (IMEG), Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto 860,* [2]*Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki 305 and* [3]*Department of Biochemistry, Tokyo Medical and Dental University School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113*

Parthenogenesis has been suggested to be tightly coupled with development of ovarian teratomas. Indeed, ovarian tumors developed in c-*mos*-deficient female mice, which are characterized by the parthenogenetic activation of oocytes. The tumors appeared at a frequency of 30% between 4 and 8 months of age, and did not develop in younger or older mice. Most of the tumors were benign and consisted of multi-focal cysts most notably with mature ectodermal components, but also with mesodermal and endodermal components. One among 17 tumors observed consisted of extra-embryonic tissues alone, and two bore malignant components with metastasis to peritoneal organs. The results strongly suggest the involvement of c-*mos* mutations in human germ cell tumors.

Key words: Ovarian teratoma — c-*mos* — Parthenogenesis — Mutant mouse

v-*mos* is an oncogene found in Moloney murine sarcoma virus; it encodes a serine protein kinase that is located in cytoplasm.[1-3] The difference in sequence between v-*mos* and its cellular counterpart, c-*mos*, is subtle,[4] and the c-*mos* gene could transform fibroblasts such as NIH3T3 cells when its expression was directed by viral LTR.[4] The oncogenic activation of c-*mos* by transcriptional activation and without structural activation has been observed in mouse myeloma.[5] *In vivo*, the expression of c-*mos* is tightly restricted in germ lineages,[6] and the c-*mos* gene product (Mos) has been suggested to play a crucial role in oogenesis in *Xenopus* as a cytostatic factor[7-11]; the transforming activity is closely associated with the activity as a cytostatic factor in *Xenopus* oocytes.[12]

The c-*mos*-deficient mice were generated to examine the c-*mos* functions in mammals.[13,14] Somatic cell divisions, spermatogenesis and initiation of oogenesis were apparently normal in these mice, suggesting that c-*mos* is not essential in these processes. Mos was, however, essential in arresting mature oocytes at the MII phase, and c-*mos*-deficient oocytes were prone to be parthenogenetically activated. Parthenogenetic activation of oocytes has been suggested to be associated closely with development of ovarian teratomas,[15] and so in this study we examined the tumor development in detail.

MATERIALS AND METHODS

The genetic background of the c-*mos*-deficient mice used in the present experiment is as follows. The c-*mos* gene was targeted in TT2 ES cells derived from an F1 embryo between C57BL/6 and CBA mice.[13,16] The chimera from the mutated ES cells was crossed with C57BL/6 mice to yield heterozygous mice (F1), and the heterozygotes were intercrossed to yield homozygotes in the F2 generation. F3 homozygotes were obtained by crossing mostly F2 heterozygous females and some homozygous females with homozygous males.[13] The mice were housed in an environmentally controlled specific pathogen free facility with a 12 h light:12 h dark cycle. C57BL/6 mice were purchased from Charles River Japan.

Complete autopsies were performed whenever possible and organs were observed both grossly and histologically. For the latter, tissues were fixed with 4% formaldehyde in phosphate-buffered saline, embedded in paraffin, sectioned at 4-μm thickness and routinely stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

Fig. 1 outlines the incidence of ovarian tumors in c-*mos*-deficient female mice; tumors were never found in other tissues. The total incidence was about 30%, and the tumors developed between four and eight months of age; tumors did not develop in younger or older females. To confirm this, 20 females were autopsied and their ovaries were examined at the age of three months; none of them bore apparent tumors. At the age of 10 months, ovaries of 28 surviving female mice were also examined macroscopically and/or microscopically, and no apparent tumors were found. Fig. 2 shows the macroscopic appearance of the tumors. Their size varied, and most of

[4] To whom all correspondence should be addressed.

them consisted of multiple cystic parts frequently containing fluid and solid portions in various proportions. In four of the total of 17 cases observed tumors developed bilaterally. Most of the ovarian tumors could be easily distinguished from normal tissues, but in two cases tumor cells were seeded widely into surrounding peritoneal organs so that the tumors fused with each other; the primary tumors in the ovaries were large and solid. No tumors were found in wild-type or heterozygous females by nine months of age, or in homozygous mutant males (data not shown).

When examined histologically, most of the tumors were found to be composed of tissues originating from three germ layers in various proportions; ectodermal components were most numerous. Smaller cysts were common and lined with squamous epithelia; they were often filled with keratin laminae in whorls (Fig. 3a). Skin appendages such as hair follicles, adipose tissues and sebaceous glands were also frequently noted in the vicinity of these epithelial linings (Fig. 3b). Mature neural tissues were abundant in non-cystic parts and contained various cell types of both neuronal and glial origin (Fig. 3c).

Cartilage (Fig. 3d) and immature cartilaginous condensations were widely recognized as mesodermal tissues, though they were not as abundant as ectodermal components. Bones were also found in many tumors and they occasionally contained normal hematopoietic bone marrow cells (Fig. 3e). Tumors also had larger cysts lined with simple squamous (Fig. 3f) or simple columnar epithelia (Fig. 3g). Many of them were ciliated (Fig. 3, g and h), and some secreted mucus. Intestinal tubule-like structures were also common (Fig. 3i). Smooth muscle-like tissues usually underlay these endodermal cysts (Fig. 3, f and h), but striated muscles were not observed.

In one case, a different type of cyst had developed, pressing the follicles and the corpora lutea (Fig. 3j). Large syncytial cells similar to trophoblast giant cells were found in the periphery of the cyst (Fig. 3k). There

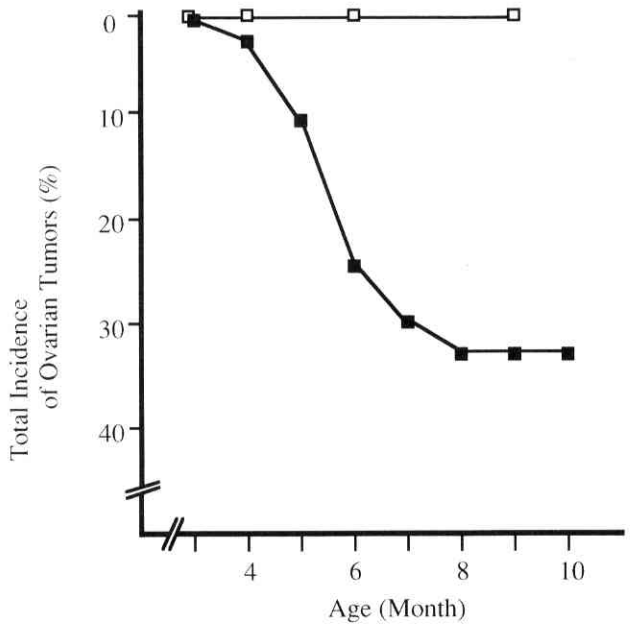


Fig. 1. Incidence of ovarian tumors in c-*mos*-deficient females. The incidence is given at the age when tumor development was assessed by palpation and confirmed by anatomization. Seventy-eight homozygous females from F2 and F3 generations were subjected to the present analysis. Of these, 20 were anatomized at three months, 14 developed tumors from four to six months, 13 were anatomized at six months, three developed tumors from seven to eight months and the remaining 28 were anatomized at ten months of age. Fifty-four wild-type and heterozygous females from F1 and F2 generations served as controls. Open boxes indicate control females, and solid boxes homozygous mutant females.

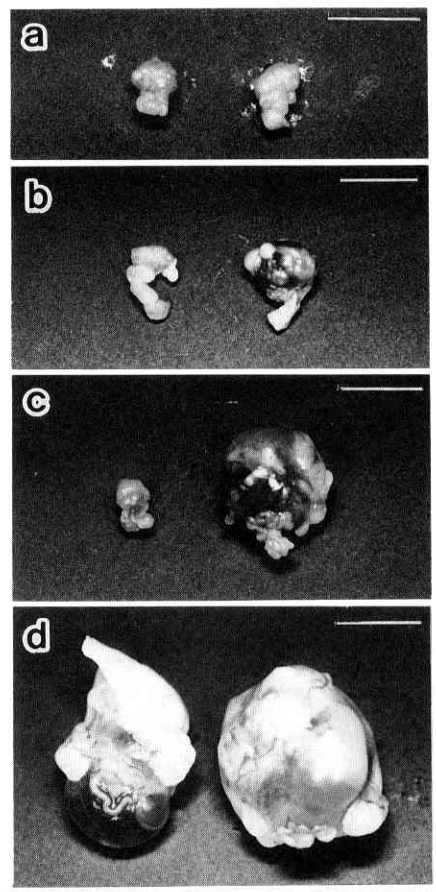
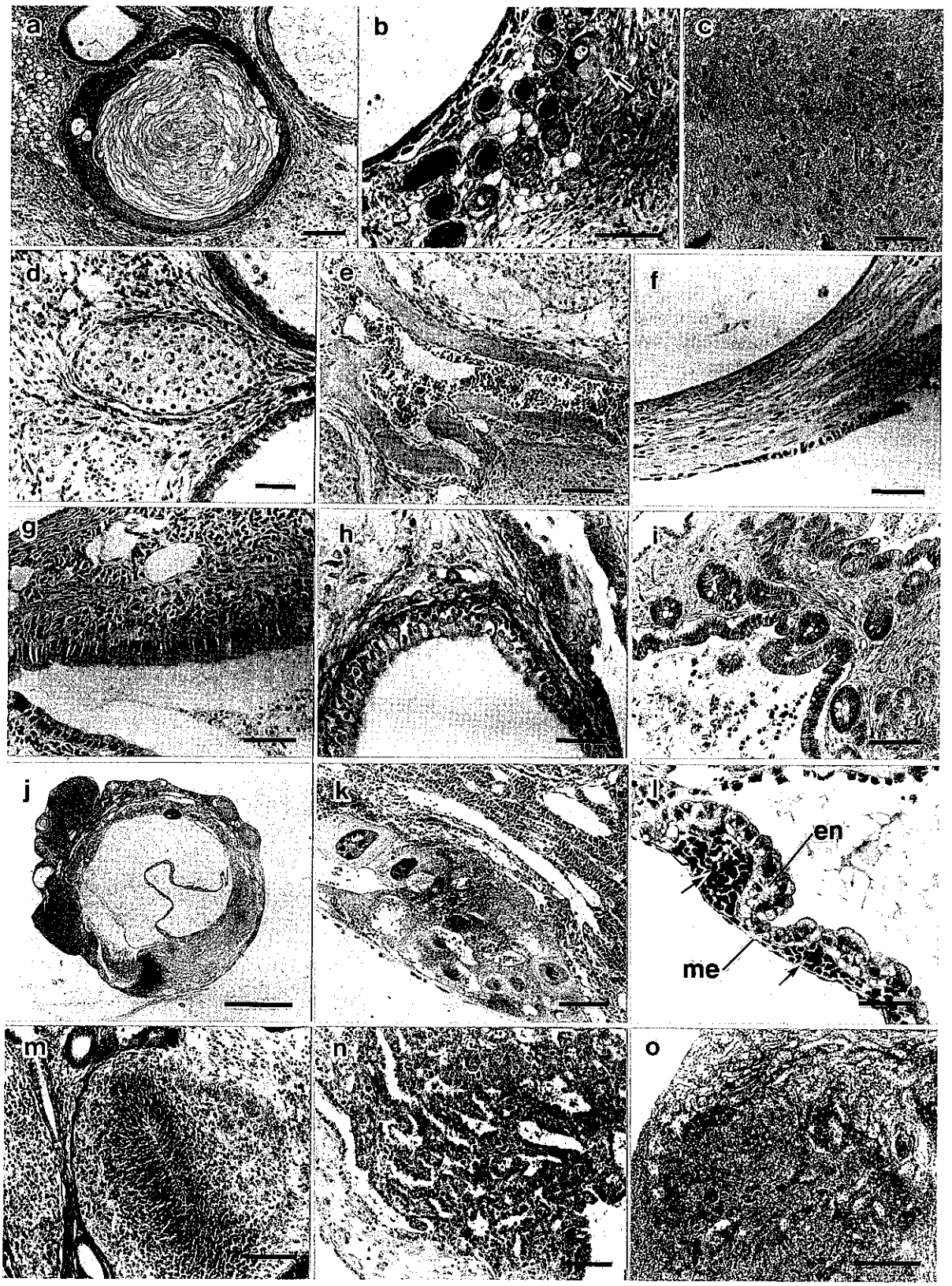


Fig. 2. Macroscopic appearance of ovarian tumors in c-*mos*-deficient mice. a, Control ovaries from a normal female at 6 months of age. b–d, Unilaterally or bilaterally swollen ovaries from c-*mos*-deficient females. The size of tumors was variable, but most of them consisted of multiple cysts. Bar = 5 mm.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was a bimembranous protrusion in the cyst, and nucleated primitive blood cells were present between the membranes (Fig. 3l). Thus, the tumor architecture resembled the extraembryonic structure in normal embryos. The embryonic components were not visible in this tumor.

Most of these cystic tumors were thus apparently benign, composed of a variety of mature tissue types. In

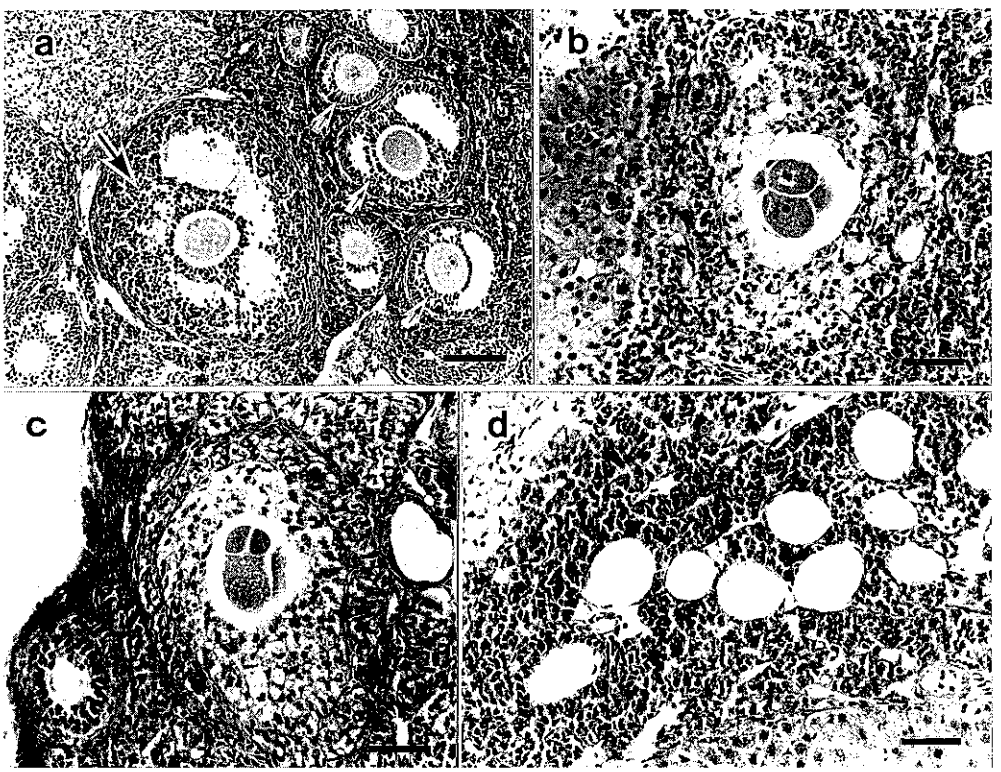

Fig. 4. Histological appearance of non-tumorous ovaries. a, An apparently normal Graafian follicle is seen at the center (large arrow), but small follicles containing only a few granulosa cells were frequent, though oocytes were of reasonably mature size (small arrows). b, A relatively normal 4-cell embryo in a follicle. The number of surrounding follicular cells was reduced and they were less granulous. c, An abnormally dividing oocyte surrounded by a degenerating follicle. Many cleaving oocytes were unequally divided and pyknotic. Some had undergone a few cycles of cell division to more than 8 cells, but no blastocyst- or egg cylinder-like structures were seen. d, Numerous vacuoles common in non-tumorous c-*mos*-deficient ovaries. They sometimes contained fragments of degenerated oocytes, suggesting an atretic follicular nature. Bar = 100 μm in a; 50 μm in b–d.

Fig. 3. Histological appearance of ovarian tumors in c-*mos*-deficient mice. a–c, ectodermal components; d–f, mesodermal components; g–i, endodermal components; j–l, extraembryonic components; m–o, malignant phenotypes. a, A stratified squamous epithelial lining with cornealization. These cysts were usually small-sized and often had keratin whorls. b, Skin appendages including hair follicles, sebaceous glands (arrow) and adipose tissues juxtaposed to a slightly cornealized cyst. c, Mature neural tissue consisting of cells of both neural and glial origin. These types of tissues were most abundant in solid parts. d, A cartilagenous nodule adjacent to cysts with cornealized and ciliated epithelia. e, Highly ossified bone with hematopoietic bone marrow cells. f, Smooth muscle cell layers underlying a simple squamous cyst. g, A simple columnar epithelium. Epithelial linings of this type and simple squamous type shown in f usually formed large cysts. h, A tubule with ciliated simple columnar epithelium surrounded by several layers of smooth muscle cells. i, Highly differentiated intestinal mucosa containing numerous goblet cells. j, A cystic tumor developed within a slightly swollen ovary, pressing the corpora lutea and follicles. k, Trophoblast-like large syncytial cells at the periphery of the cyst. l, Magnified view of membranous structure protruding into the cyst. It was a bilayer, apparently corresponding to endoderm (en) and mesoderm (me) components of the embryonic yolk sac, and contained nucleated primitive blood cells (arrows). m, Neural tube-like structure with immature neural epithelial-like cells. n, Malignant immature cells. They formed partly tubular or papillary architecture. o, Invasion of malignant cells into kidney. Bar = 1 mm in j; 200 μm in o; 100 μm in a–c, e, i, j, m, n; 50 μm in d, f, g, h, k, l.

two cases, however, tumors metastasized into peritoneal organs. The primary tumors in ovaries were solid on the whole; there existed a number of cysts, but they were small. They contained immature components in addition to various types of mature tissues. Immature neuroepithelial cells with neural tube-like structure (Fig. 3m) and immature epithelial-like cells, some forming papillary or tubular architecture (Fig. 3n), were seen. The latter had numerous active mitotic figures and seemed to be the cells that were actively invading the surrounding organs (Fig. 3o).

At the age of six months, 13 apparently healthy mice were killed so that their ovaries could be examined. In these non-tumorous ovaries, though normally maturing follicles and corpora lutea were present, they were less abundant, and atretic and abnormal follicles were more prevalent than in normal ovaries. Granular layers were reduced in a large number of the follicles, even if oocytes were apparently mature in size (Fig. 4a). A characteristic of c-*mos*-deficient ovaries was that nearly half of the follicles had activated oocytes, and many of them had undergone cleavage (Fig. 4b). Development was not observed beyond the early cleavage stage to the pre-implantation stage, however, and many of these cleaving oocytes were abnormally elliptic in shape and pyknotic. Surrounding granular cells were generally degenerated (Fig. 4c), and a number of vacuolar structures were noted (Fig. 4d), some of them containing traces of fragments of degenerated oocytes and granular cells. In the oviducts, the number of ovulated oocytes was limited and no cleaving eggs were found among them. Thus it is likely that most of the parthenogenetically activated oocytes deteriorated after a few divisions within the ovary.

The ovary gives rise to a wider range and variety of tumors than any other organ.[17] Ovarian teratomas, which have been thought to originate from germ cells, account for 25% to 30% of the ovarian tumors in human.[18,19] More than 90% of germ cell tumors are benign, mature cystic teratoma or dermoid cysts. Among various tissue types, ectodermal derivatives are the most dominant components.[20] Malignant change has been observed at a low incidence; neural and epithelial components are also plentiful. Nearly 90% of ovarian teratomas are diagnosed at reproductive ages,[21] while the incidence is quite low at non-reproductive ages.[22] These are characteristic features of the ovarian tumors observed here in c-*mos*-deficient mice, and it should be worthwhile to examine the status of the c-*mos* locus in human ovarian teratoma.

A high incidence of ovarian tumors has been found in the LT/Sv strain of mice.[23–25] The tumor development was shown to be a consequence of the high incidence of parthenogenetic activation of oocytes in this strain. The parthenogenesis proceeded in pre-antral stage follicles usually lined with a single layer of follicle cells (granulosa-cell-deficient [GCD] follicles),[24,26,27] and it was suggested that two atypical conditions were necessary for a high incidence of ovarian teratocarcinogenesis: competence for spontaneous parthenogenetic activation and a high frequency of GCD follicle formation.[28] The GCD follicle formation was also found in the c-*mos*-deficient mice, and in many respects the pathogenesis is similar between LT/Sv mice and c-*mos*-deficient mice. Several differences were noted, however. The most distinct is that in LT/Sv female mice the teratoma incidence was about 50% by 90 days of age, and parthenogenetic development to pre- and post-implantation stages was frequently observed in non-tumorous ovaries. In c-*mos*-deficient female mice, the onset was later, and the overall incidence was about 30%. In non-tumorous ovaries, development of activated oocytes beyond the early cleavage stage was rare, if any. The role of c-*mos*-deficiency in parthenogenetic activation of oocytes is in destabilization of MII arrest, and it is recessive and unlikely to reside in somatic components.[13] LT/Sv teratomas derive from oocytes that complete the first meiotic division,[15] but cleavage is also found among immature oocytes. Oocytes from the F1 progeny of matings of LT/Sv with other strains cleaved parthenogenetically and developed to blastocysts with the same frequency as LT/Sv oocytes, suggesting dominant genetic factors.[29] Analyses with chimera and reconstituted ovaries indicated that genetic changes for LT/Sv teratocarcinogenesis also affected somatic components in ovaries.[30] The B^{lt} mutation in C58/J strain was outcrossed to strain BALB/c and subsequently inbred to form the LT strain.[24] Thus, the effects of genetic backgrounds of mice alone, including endocrinological and immunological status, might account for the differences between LT/Sv mice and c-*mos*-deficient mice. It is also possible that mutations in several gene loci are involved in the LT/Sv phenotype or that parthenogenetic activation in LT/Sv mice occurs through a different mechanism. The status of the c-*mos* locus in LT/Sv mice should next be examined.

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