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microscopy confirmed dense labeling of the cell membrane of fibroblast-like stromal cells for LOX-1 (Fig. 4F).

In the DB, the fibroblast-like cells formed a meshwork around the GMG cells (Fig. 5A), but in a rather looser fashion than in the MG. The loose arrangement made it easier to determine that LOX-1-expressing cells were fibroblast-like stromal cells (Fig. 5B). Electron and immunoelectron microscopy showed positive LOX-1 staining on plasma membranes of these fibroblast-like cells (Fig. 5C, D).

After E12.5, clusters of glycogen trophoblast cells with clear cytoplasm were noted among spongiotrophoblasts in the JZ (data not shown). Cord-like clusters of glycogen trophoblasts in the JZ were strongly positive for LOX-1 protein (Fig. 5E) and mRNA (Fig. 5F). After E14.5, some glycogen trophoblasts crossed the giant cell layer and migrated into the DB, where they were still positive for LOX-1 but at a weaker intensity (Fig. 5E). Spongiotrophoblasts and giant trophoblasts showed no positive staining (Fig. 5E, F). Electron microscopically, these glycogen trophoblast cells had a characteristic pale vacuolated cytoplasm (Fig. 5G), and LOX-1 was localized along the cell membrane (Fig. 5H). In the LZ, moderate staining of LOX-1 protein (Fig. 5I) and moderate mRNA expression (Fig. 5J) were demonstrated in labyrinthine cytotrophoblasts.

From these data, we concluded that in the maternal compartment LOX-1 localized in fibroblast-like stromal cells, whereas in the fetal compartment LOX-1 was strongly expressed in glycogen trophoblast cells and weakly expressed in labyrinthine cytotrophoblasts.

Expression of LOX-1 mRNA in human placenta

To compare the expression levels of LOX-1 mRNA in first trimester and term human

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placentas, we performed real-time RT-PCR. Significantly stronger expression of LOX-1 mRNA was found in first trimester placenta compared with term placenta (Fig. 6).

Identification of LOX-1-expressing cells in human placenta

In the first trimester human placenta, strong positive immunoreactivity for LOX-1 was seen along the cell membrane of most villous cytotrophoblasts but not in syncytiotrophoblasts (Fig. 7A). No positive staining was observed in a negative-control section (fig. 7B). In situ hybridization of first trimester placenta demonstrated a positive reaction for LOX-1 mRNA in villous cytotrophoblasts (Fig. 7B,C). Localization pattern of LOX-1 did not change in the term placenta. Lox-1 is positive on flattened cytotrophoblasts located beneath the syncytiotrophoblasts (Fig. 7E). No staining was observed in a control section (Fig.7F).

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Discussion

Since the discovery of LOX-1 by Sawamura et al. (1997), many reports have suggested its possible involvement in the pathogenesis of atherosclerosis (Mehta et al. 2006). Recently, Mehta et al. (2007) reported that deletion of LOX-1 in atherogenic LDL receptor knockout mice reduced atherosclerosis as well as proinflammatory and prooxidant signals. LOX-1 expression is enhanced in some pro-atherogenic settings such as hypertension and hyperlipidemia in vivo and in atherosclerotic lesions (Mehta et al. 2006). LOX-1 is expressed at very low levels in healthy endothelium (Sawamura et al. 1997) and the physiological role of LOX-1 is not well understood. Northern blotting data for normal mice and humans (Sawamura et al. 1997) demonstrated that the placenta is the organ with the greatest expression of LOX-1 mRNA, which indicates that LOX-1 may play an important role in maintaining pregnancy.

During pregnancy, the placenta is a major source of prooxidant and endogenous antioxidant synthesis (Walsh 1998). Excessive generation of free radicals depletes antioxidant pools and causes oxidative damage to lipids, proteins, and DNA. The level of placental oxidative stress increases rapidly at the end of the first trimester, when placental vascular development is occurring, and such stress tapers off later in gestation (Jauniaux et al. 2000). Our present finding of higher expression of LOX-1 mRNA during early and midgestational stages compared with late gestation in both mouse and human indicates induction of LOX-1 by increased oxidative stress at this gestational stage and active involvement of LOX-1 in the functioning of early gestational stage placenta.

In maternal decidual compartments of murine placenta, namely, the MG and DB, the major cellular components are vascular endothelial cells, uterine NK cells (GMG cells), and fibroblast-like stromal cells. It is interesting to note that LOX-1 localized in fibroblast-like

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cells—the cells that are closely associated with uterine NK cells—but not in vascular endothelial cells. Certain studies indicate the possible role of uterine NK cells in control of trophoblast invasion and remodeling of uterine spiral arteries during the first half of pregnancy (Bulmer and Lash 2005). Intimate association between uterine NK cells and fibroblast-like stromal cells is believed to be important for survival of the uterine NK cells (Stewart 2000). Because ox-LDL binding of LOX-1 in fibroblasts induces expression of various adhesion molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (Chen et al. 2005), LOX-1 may be involved in strengthening the close association of these cells. The number of uterine NK cells decreases in late pregnancy, and apoptotic uterine NK cells were observed at day 16 in rat placenta, with their number increasing thereafter (Fukazawa et al. 1998). Because LOX-1 functions in the elimination of aged or apoptotic cells (Oka et al. 1998), LOX-1 expression by fibroblast-like stromal cells may also contribute to the elimination of apoptotic uterine NK cells.

In the JZ and LZ, LOX-1 expression was restricted to glycogen trophoblast cells. Glycogen trophoblast cells are of undetermined origin that arise in the JZ (Fukazawa et al. 1998). Although these cells were once thought to be differentiated from spongiotrophoblasts (Adamson et al. 2002; Georgiades et al. 2002), more recent evidence suggests that they are distinct from spongiotrophoblasts (Rampton et al. 2005; Bouillot et al. 2006). Because we found no LOX-1 expression in spongiotrophoblasts at the mRNA and protein levels, we believe that LOX-1 is one of the molecules that discriminates between glycogen trophoblast cells and spongiotrophoblasts.

This is the first study to describe the distinct localization of LOX-1 in murine placenta. It is interesting to note that LOX-1 expression is not observed in vascular endothelial cells of

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murine placenta. Since vascular endothelial cells are major cell type for LOX-1 expression in lung, aorta and other organs (Sawamura et al., 1997), LOX-1 expressed by fibroblast-like stromal cells and glycogen trophoblast cells may possess unique functions in murine placenta.

As the glycogen trophoblast cells invade decidua they are considered to analogous to the human invasive extravillous trophoblasts (Malassine et al. 2003, Coan et al. 2006). Pavan et al. (2004) reported that ox-LDL, but not native LDL, inhibited human extravillous trophoblast invasion in a concentration-dependent manner, as evidenced in the trophoblast invasion assay. They also discovered that LOX-1 was the main scavenger receptor responsible for uptake of ox-LDL by cytotrophoblasts (Pavan et al. 2004). Taken these data together, LOX-1 expression by glycogen trophoblast cells may regulate their invasion of the DB. The weaker expression of LOX-1 by invading glycogen trophoblast cells in the DB, which we observed, supports this hypothesis.

In the first trimester placenta of human, we confirmed that localization of LOX-1 in villous cytotrophoblasts but not syncytiotrophoblasts nor vascular endothelial cells by means of immunohistochemistry and in situ hybridization. We also found LOX-1 localization in villous cytotrophoblasts in term placentas. Our result in term placenta is inconsistent with the previous observation by Lee et al. (2005), who observed LOX-1 expression in syncytiotrophoblasts in normal term and preeclamptic placentas with stronger staining in the latter. LOX-1 staining in Figure 3 of their paper (Lee et al. 2005), however, seems to localize in cytotrophoblast layer. In a recent paper, Mori et al. (2007) reported that cytotrophoblast layer in term placenta becomes thinner by spreading their cell surface over the basal lamina and 80% of its continuity is still preserved. Our data of LOX-1 localization in term placenta support their observation.

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For murine placenta, we clearly demonstrated that LOX-1-expressing cells were fibroblast-like stromal cells in the MG and DB and glycogen trophoblast cells in the JZ and LZ, as well as labyrinthine cytotrophoblasts in the LZ. LOX-1 seems not to be essential to maintain placental function, because LOX-1-deficient mice were fertile (Mehta et al. 2007) and had no detectable abnormal pregnancy. However, LOX-1-expressing placental cells may be involved in management of oxidative stress during pregnancy. Further investigation is required, however, to clarify the exact role of LOX-1 in pregnancy.

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This article may be cited as DOI: 10.1369/jhc.2008.950543

Literature Cited

Adamson SL, Lu Y, Whiteley KJ, Holmyard D, Hemberger M, Pfarrer C, Cross JC (2002) Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. Dev Biol 250:358-373

Aoyama T, Chen M, Fujiwara H, Masaki T and Sawamura T (2000) LOX-1 mediates lysophosphatidylcholine-induced oxidized LDL uptake in smooth muscle cells. FEBS Lett 467:217-220

Aoyama T, Fujiwara H, Masaki T and Sawamura T (1999) Induction of lectin-like oxidized LDL receptor by oxidized LDL and lysophosphatidylcholine in cultured endothelial cells. J Mol Cell Cardiol 31:2101-2114

Bonet B, Chait A, Gown AM and Knopp RH (1995) Metabolism of modified LDL by cultured human placental cells. Atherosclerosis 112:125-136

Bouillot S, Rampon C, Tillet E, Huber P (2006) Tracing the glycogen cells with protocadherin 12 during mouse placenta development. Placenta 27:882-888

Branch DW, Mitchell MD, Miller E, Palinski W, Witztum JL (1994) Pre-eclampsia and serum antibodies to oxidized low-density lipoprotein. Lancet 343:645-646

Bulmer JN, Lash GE (2005) Human uterine natural killer cells: a reappraisal. Mol Immunol 42:511-521

Burton GJ, Jauniaux E (2004) Placental oxidative stress: from miscarriage to preeclampsia. J Soc Gynecol Invest 11:342-352

Chen K, Chen J, Liu Y, Xie J, Li D, Sawamura T, Hermonat PL, Mehta JL (2005) Adhesion molecule expression in fibroblasts: alteration in fibroblast biology after transfection with LOX-1 plasmids. Hypertension 46:622-627

Copyright 2008 by The Histochemical Society

Accepted for publication on April 23, 2008
This article may be cited as DOI: 10.1369/jhc.2008.950543

Chen M, Nagase M, Fujita T, Narumiya S, Masaki T, Sawamura T (2001) Diabetes enhances lectin-like oxidized LDL receptor-1 (LOX-1) expression in the vascular endothelium: possible role of LOX-1 ligand and AGE. 287:962-968

Coan PM, Conroy N, Burton GJ, Ferguson-Smith AC (2006) Origin and characteristics of glycogen cells in the developing murine placenta. Dev Dyn 235:3280-3294

Divjak M, Glare EM, Walters EH (2002) Improvement of non-radioactive in situ hybridization in human airway tissues: use of PCR-generated templates for synthesis of probes and an antibody sandwich technique for detection of hybridization. 50:541-548.

Draude G, Hrboticky N, Lorenz RL (1999) The expression of the lectin-like oxidized low-density lipoprotein receptor (LOX-1) on human vascular smooth muscle cells and monocytes and its down-regulation by lovastatin. Biochem Pharmacol 57:383-386

Fournier T, Handschuh K, Tsatsaris V, Evain-Brion D (2007) Involvement of PPARg in human trophoblast invasion. Placenta 21:S76-S81

Kobayashi H, Sakashita N, Okuma T, Terasaki Y, Tsujita K, Suzuki H, Kodama T, Nomori N, Kawasuji M, Takeya M (2007) Class A scavenger receptor (CD204) attenuates hyperoxia-induced lung injury by reducing oxidative stress. J Pathol 212:38-46

Fukazawa Y, Yamamura Y, Sato T, Iguchi T, Ohta Y (1998) Mode of cell death in the rat metrial gland during peripartum regression. Anat Rec 252:369-377

Georgiades P, Ferguson-Smith AC, Burton GJ (2002) Comparative developmental anatomy of the murine and human definitive placentae. Placenta 23:3-19

Hubel CA (1999) Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med 222:222-235

Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ (2000) Onset of

Copyright 2008 by The Histochemical Society

Accepted for publication on April 23, 2008
This article may be cited as DOI: 10.1369/jhc.2008.950543

maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. Am J Pathol 157:2111-2122

Komohara Y, Terasaki Y, Kaikita K, Suzuki H, Kodama T, Takeya M (2005) Clearance of apoptotic cells is not impaired in mouse embryos deficient in class A scavenger receptor types I and II (CD204). Dev Dyn 232:67-74

Kume N, Murase T, Moriwaki H, Aoyama T, Sawamura T, Masaki T, Kita T (1998) Inducible expression of lectin-like oxidized LDL receptor-1 in vascular endothelial cells. Circ Res 83:322-327

Li D, Mehta JL (2000) Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells. Arterioscler Thromb Vasc Biol 20:1116-1122

Lee H, Park H, Kim YJ, Kim HJ, Ahn Y-M, Park B, Park JH, Lee BE (2005) Expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) in human preeclamptic placenta: possible implications in the process of trophoblast apoptosis. Placenta 26:226-233

Li DY, Zhang YC, Philips MI, Sawamura T and Mehta JL (1999) Upregulation of endothelial receptor for oxidized low-density lipoprotein (LOX-1) in cultured human coronary artery endothelial cells by angiotensin II type 1 receptor activation. 84:1043-1049

Malassine A, Frendo J-L, Evain-Brion D (2003) A comparison of placental development and endocrine functions between the human and mouse model. Hum Reprod Update 9:531-539.

Mehta JL, Chen J, Hermonat PL, Romeo F, Novelli G (2006) Lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1): a critical player in the development of atherosclerosis and related disorders. Cardiovasc Res 69:36-45

Mehta JL, Sanada N, Hu CP, Chen J, Dandapat A, Sugawara F, Satoh H, Inoue K, Kawase Y,

Copyright 2008 by The Histochemical Society

Accepted for publication on April 23, 2008
This article may be cited as DOI: 10.1369/jhc.2008.950543

Jishage K, Suzuki H, Takeya M, Schnackenberg L, Beger R, Hermonat PL, Thomas M, Sawamura T (2007) Deletion of LOX-1 reduces atherogenesis in LDLR knockout mice fed high cholesterol diet. Circ Res 100:1634-1642

Mori M, Ishikawa G, Luo S-S, Mishima T, Goto T, Robinson JM, Mtsubara S, Takeshita T, Kataoka H, Takizawa T (2007) The cytotrophoblast layer of human ghorionic villi becomes thinner but maintains its structural integrity during gestation. Biol Reprod 76:164-172

Moriwaki H, Kume N, Kataoka H, Murase T, Nishi E, Sawamura T, Masaki T and Kita T (1998) Expression of lectin-like oxidized low density lipoprotein receptor-1 in human and murine macrophages: upregulated expression by TNF-α. FEBS Lett 440:29-32

Moriwaki H, Kume N, Sawamura T, Aoyama T, Hoshikawa H, Ochi H, Nishi E, Masaki T and Kita T (1998) Ligand specificity of LOX-1, a novel endothelial receptor for oxidized low density lipoprotein. Arterioscler Thromb Vasc Biol 18:1541-1547

Nakagawa T, Akagi M, Hoshikawa H, Chen M, Yasuda T, Mukai S, Ohsawa K, Masaki T, Nakamura T, Sawamura T (2002) Lectin-like oxidized low-density lipoprotein receptor 1 mediates leukocyte infiltration and articular cartilage destruction in rat zymosan-induced arthritis. Arthritis Rheum 46:2486-2494

Oka K, Sawamura T, Kikuta K, Itokawa S, Kume N, Kita T, Masaki T (1998) Lectin-like oxidized low-density lipoprotein receptor 1 mediates phagocytosis of aged/apoptotic cells in endothelial cells. Proc Natl Acad Sci USA 95:9535-9540

Pavan L, Hermouet A, Tsatsaris V, Therond P, Sawamura T, Evain-Brion D, Fournier T (2004) Lipids from oxidized low-density lipoprotein modulate human trophoblast invasion: involvement of nuclear liver X receptors. Endocrinology 145:4583-4591

Rampon C, Prandini MH, Bouillot S, Pointu H, Tillet E, Frank R, Vernet M, Huber P (2005)

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Accepted for publication on April 23, 2008
This article may be cited as DOI: 10.1369/jhc.2008.950543

Protocadherin 12 (VE-cadherin 2) is expressed in endothelial, trophoblast, and mesangial cells. Exp Cell Res 302:48-60

Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y, Tanaka T, Miwa S, Katsura Y, Kita T and Masaki T (1997) An endothelial receptor for oxidized low-density lipoprotein. Nature 386:73-77

Stewart IJ (2000) Mouse granulated metrial gland cells require contact with stromal cells to maintain viability. J Anat 197: 495-502

Tsujita K, Kaikita K, Hayasaki T, Honda T, Kobayashi H, Sakashita N, Suzuki H, Kodama T, Ogawa H, Takeya M (2007) Targeted deletion of class A macrophage scavenger receptor increases the risk of cardiac rupture after experimental myocardial infarction. Circulation 115:1904-1911

Tulppala M, Ailus K (1995) Antibodies to oxidized low-density lipoprotein and to cardiolipin in nonpregnant and pregnant women with habitual abortion. Fertil Steril 64:947-950

Walsh SW (1998) Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. Semin Reprod Endocrinol 16:93-104

Yoshida H, Kondratenko N, Green S, Steinberg D, Quehenberger O (1998) Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. Biochem J 334:9-13

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Figure Legends

Figure 1. Expression of LOX-1 mRNA in different murine organs (A) and in murine placentas at different gestational days (B). Among the organs, the placenta at E18.5 had the strongest LOX-1 mRNA expression; lung and aorta showed moderate expression (A). Via real-time PCR, the placenta at E15.5 had the strongest expression of LOX-1 mRNA, which decreased gradually thereafter (B). PCR product sizes were 522 bp for mouse LOX-1 and 392 bp for GAPDH.

Figure 2. Expression of LOX-1 mRNA in different compartments of murine placenta. For RT-PCR, the placenta (E14.5) was divided into four parts: myometrium, metrial gland (MG) + decidua basalis (DB), junctional zone (JZ) + labyrinth zone (LZ), and yolk sac + amnion. The MG + DB (lane 2) and the JZ + LZ (lane 3) showed strong mRNA expression.

Figure 3. Localization of LOX-1 protein and mRNA in murine placenta at E14.5. Four distinct compartments were easily distinguished by means of hematoxylin and eosin staining (A). Immunohistochemistry for LOX-1 (B) and in situ hybridization for LOX-1 mRNA (D) produced very similar localization patterns. The negative control for LOX-1 immunostaining using normal rat IgG showed no staining(C). In situ hybridization with the sense probe (E) showed no positive reaction except for nonspecific staining at the edge of the section. MG = metrial gland, DB = decidua basalis, JZ = junctional zone, LZ = labyrinth zone. Scale bars represent 300 μm.

Figure 4. LOX-1-expressing cells in the MG of murine placenta at E14.5. In the MG,

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fibroblast-like cells were closely associated with GMG cells, also known as uterine natural killer (NK) cells (arrowheads in A; hematoxylin and eosin staining). LOX-1 immunostaining was observed on fibroblast-like cells surrounding uterine NK cells (B). LOX-1 mRNA was also detected in fibroblast-like cells via in situ hybridization (C). Immunostaining of desmin, a marker for fibroblast-like cells, showed the same localization pattern as did that of LOX-1 (D). Electron microscopy demonstrated the close association of a uterine NK cell, which has a characteristic round shape, pale cytoplasm, and fibroblast-like cells surrounding it, with long cell processes of the fibroblast-like cells (arrows) (E). An immunoelectron micrograph revealed distinct localization of reaction products of LOX-1 on cell processes of fibroblast-like cells (arrows) (F). Inset in F is the higher magnification image of the boxed area. Scale bars represent 50 μm in A to D and 10 μm in E and F.

Figure 5. LOX-1-expressing cells in the DB, JZ, and LZ of murine placenta at E14.5. In the DB, fibroblast-like stromal cells (arrowheads) formed a meshwork and surrounded uterine NK cells in a looser fashion than in the MG (A). Immunohistochemical LOX-1 localization coincided with the mesh-like structure of the fibroblast-like cells (B). Electron (C) and immunoelectron (D) microscopy showed distinct localization of LOX-1 on well-developed cell processes of the fibroblast-like cells. In the JZ, clusters of glycogen trophoblast cells had a strongly positive reaction for LOX-1 protein along their cell membranes (E) and for LOX-1 mRNA (F), whereas cells in the DB had a weaker reaction (E). Electron (G) and immunoelectron (H) microscopy showed strong LOX-1 immunoreactivity along the well-developed cell processes of glycogen trophoblast cells. In the LZ, weak staining of LOX-1 protein (I) and weak mRNA expression (J) were found in labyrinthine cytotrophoblasts. Scale bars represent 50 μm in A, B, E, F, I, and

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J; 2.5 μm in C and D; and 5.0 μm in G and H.

Figure 6. Expression of LOX-1 mRNA in human placenta. Real-time PCR revealed significantly greater LOX-1 mRNA expression in first trimester placenta than in term placenta $^*P < 0.05$.

Figure 7. LOX-1 immunostaining is observed along the cell membrane of cuboidal villous cytotrophoblasts of first trimester placenta (9w) (A). Inset is the higher magnification image of the boxed area. No positive staining is observed in a negative control section using normal mouse IgG (B). LOX-1 mRNA is detected in the cytoplasm of cytotrophoblasts in the first trimester placenta (9w) (C). The control section with the sense probe showed no positive reaction (D). In the term placenta, flattened cytotrophoblast layer is positive for LOX-1 immunostaining (E). Inset, higher magnification image of the boxed area, shows LOX-1-positive cytotrophoblasts subjacent to LOX-1-negative syncytiotrophoblasts. No positive reaction is observed in a control section using normal mouse IgG. Scale bars represent 100 μm in A, B, C and D, and 50 μm in E and F.

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Figure 1

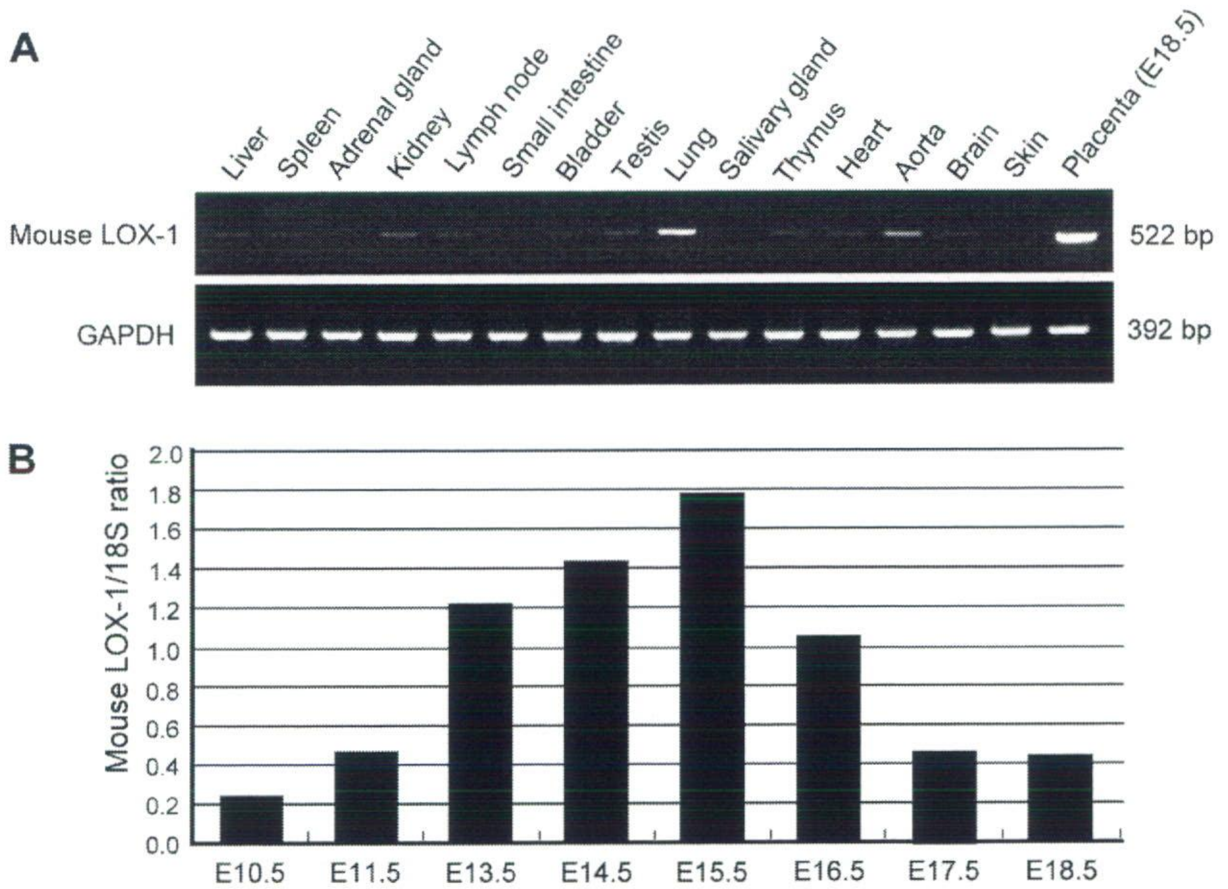

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Figure 2

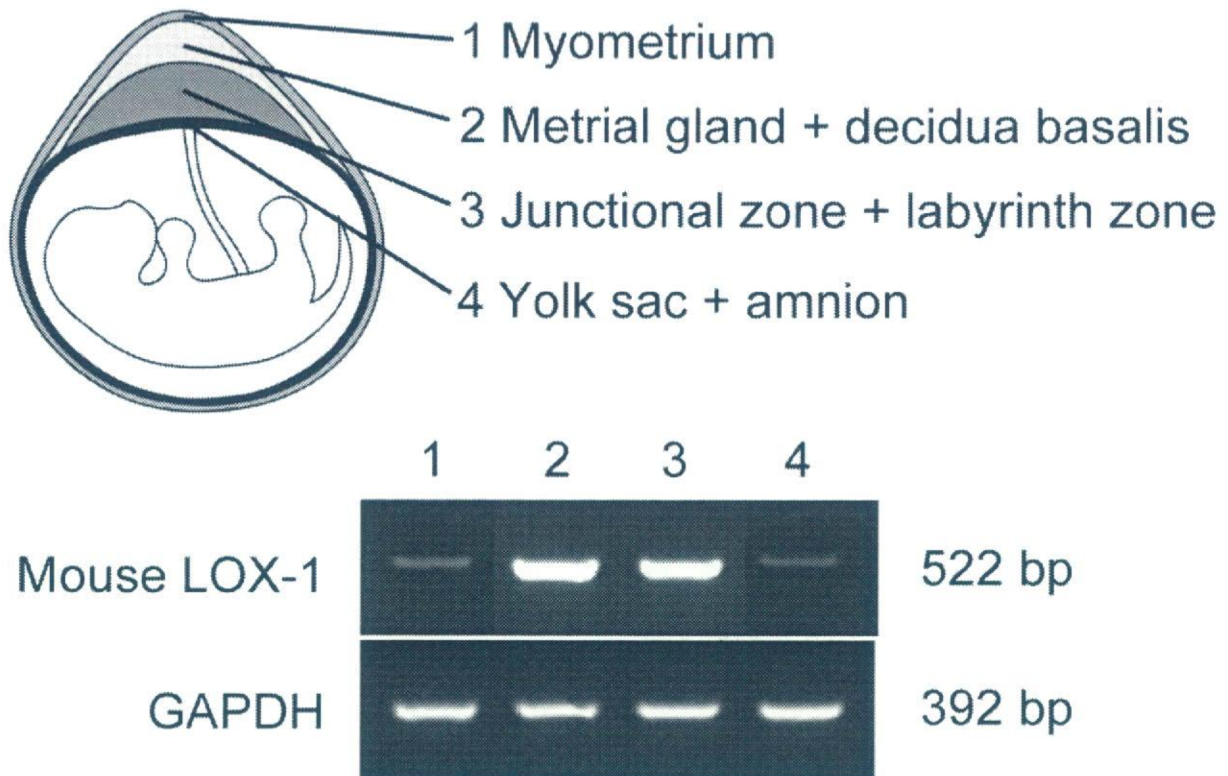

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Figure 3

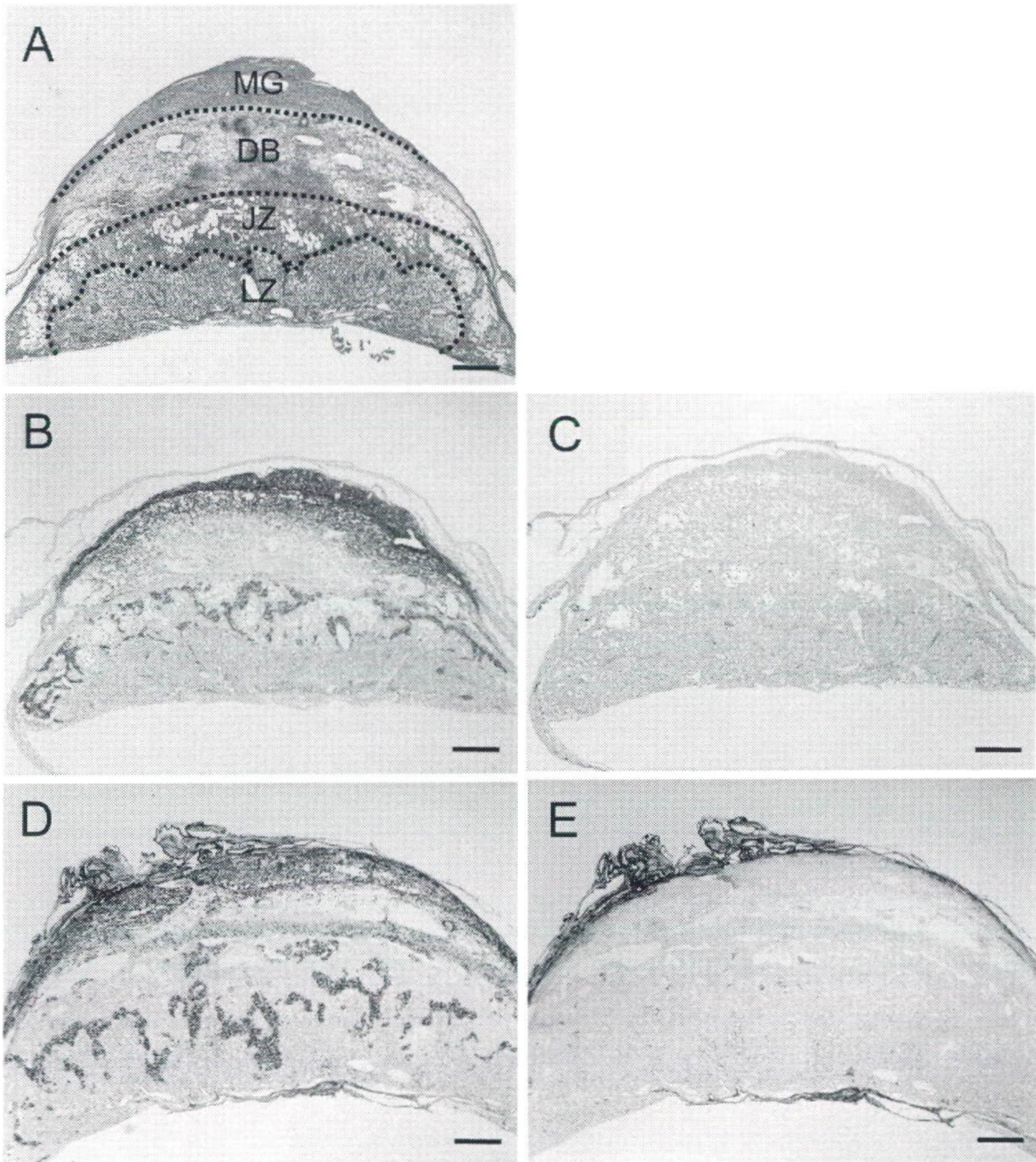

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Figure 4

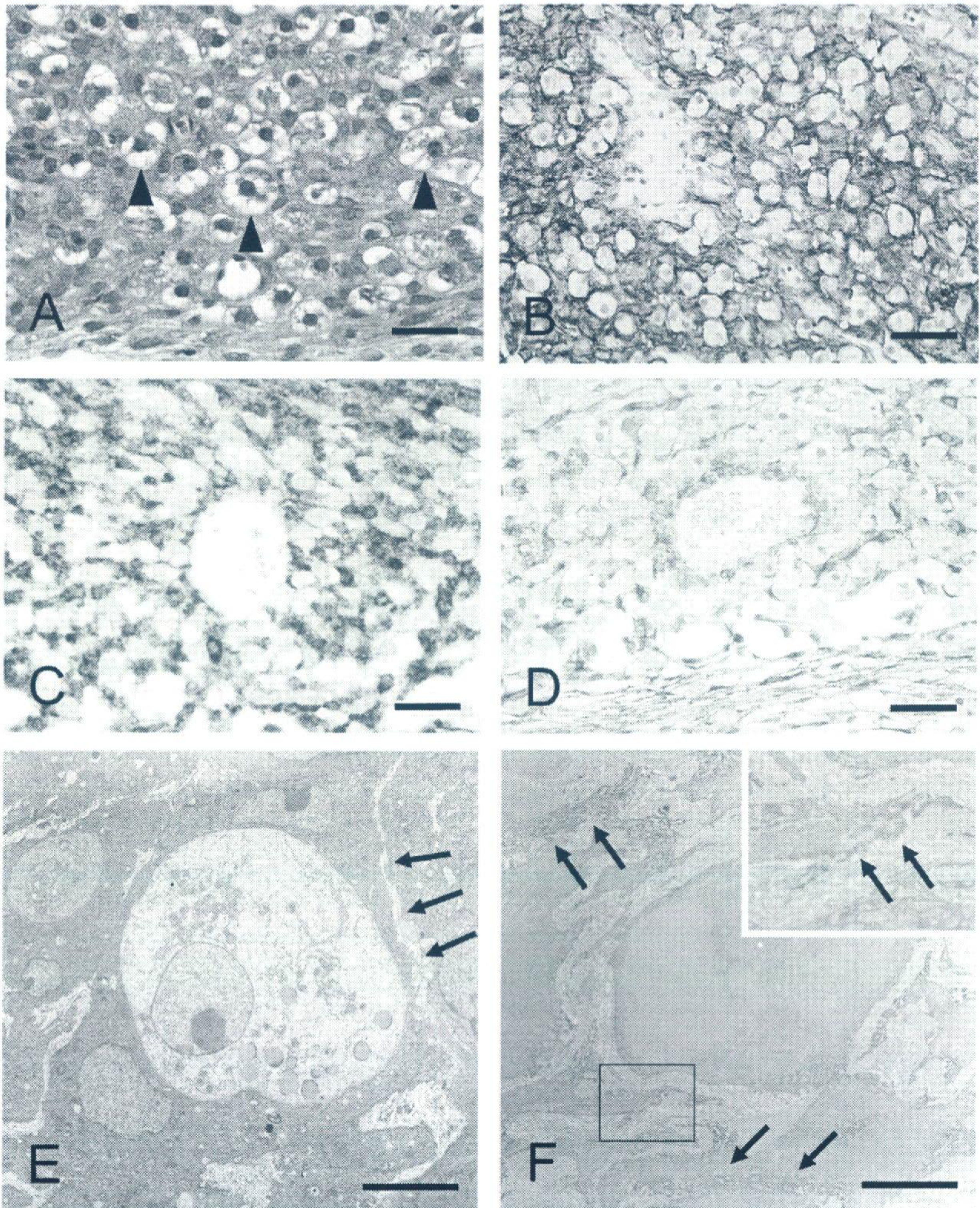

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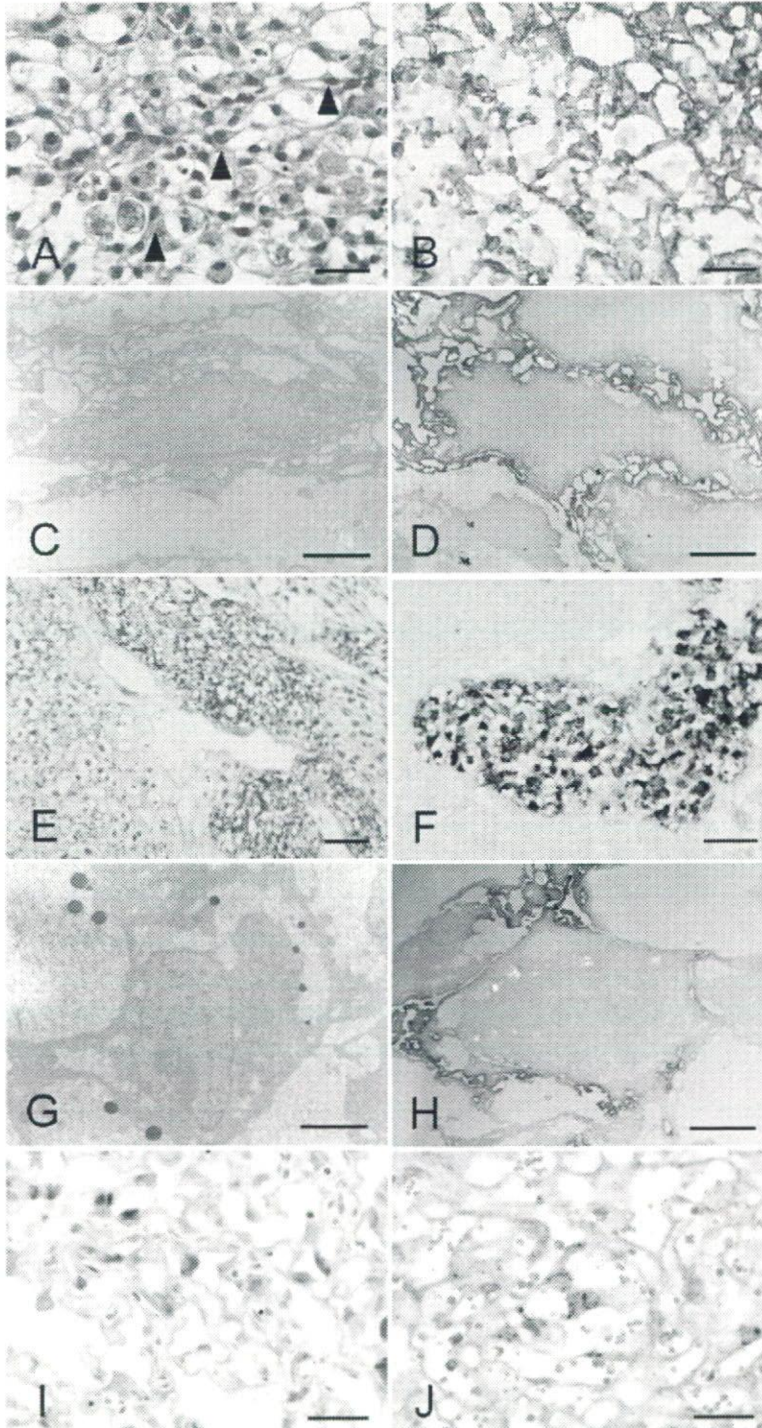
Figure 5



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