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Histochemical Observations during Uterine Involution in Meishan Pigs

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Abstract. Postpartal changes of the endometrium in primiparous Meishan pigs during the process of uterine involution were examined by morphological and immunohistological methods. Reproductive organs were collected from lactating sows (Days 1, 13, 20 and 31 after parturition) and a weaned sow (weaned on Day 28 and sacrificed on Day 31 after parturition). Cryosectioned uterine tissues were stained with Mayer's hematoxylin and eosin for histological observations. To observe membranous extracellular matrix localization, samples were treated with antibodies against laminin and type IV collagen. The diameter of the uterine horn decreased dramatically by Day 13 and slightly decreased during Days 13 to 31 after parturition. Luminal and glandular epithelial cells were low and cuboidal during lactation but became highly columnar just after weaning. During lactation, laminin and type IV collagen were localized in a winding pattern in the basal lamina of the endometrial epithelium. After weaning, they were found in the basal lamina of the endometrial epithelium in a stratum. These results suggest that uterine involution is completed just after weaning.

Key words: Uterine involution, Postpartum, Pig, Laminin, Type IV collagen

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Uterine involution in mammalian species is necessary for establishment of the next pregnancy. Uterine involution includes a reduction of uterine size and morphological and histological changes. Among domestic animals, most morphological and histological studies on uterine involution have focused on cows [1–6], because they are the most important domestic animal for meat and milk production. We have previously described morphological and histological changes that occur in the bovine uterus postpartum [2–5]. To understand the process of postpartum uterine involution in cows, an ultrasonic scanning device has also been used [7]. These studies provide many useful data to promote the next conception

postpartum and to shorten the calving interval. On the other hand, changes in the porcine uterus postpartum have received little attention with the exception of the reports by Palmer *et al.* [8, 9], who showed that the uterus returned to its pregravid size within 3–4 weeks after parturition [8]. The weight and the length of the uterus decreased until 3 weeks after parturition [9]. The development of luminal and glandular epithelial cells was not seen until postweaning [9].

In the rat and mouse, the size reduction of the uterus during involution is related to the rearrangement of tissue components. In this process, the extracellular matrix (ECM) is rearranged [10, 11] with both breakdown and resynthesis of ECM components [10, 12]. ECMs are composed of glycoproteins, proteoglycans and

glycosaminoglycans. ECMs have effects on cell adhesion, proliferation, migration and differentiation, and provide structural support for organs. ECMs also determine the morphologies of various tissues.

In the present study, we examined the localization of membranous ECMs (laminin and type IV collagen) in the porcine uterus during involution by immunohistochemistry. In addition, we confirmed the macroscopical and histological changes in the endometrium during postpartum uterine involution in pigs.

Materials and Methods

Tissue collection

Reproductive organs were collected from lactating Meishan sows (Days 1, 13, 20 and 31 after parturition) and a weaned sow (weaned on Day 28 and sacrificed on Day 31 after parturition). Endometrial tissues dissected from uteri were frozen in an embedding O.C.T. compound (SAKURA Finetechnical, Tokyo, Japan) in liquid nitrogen and stored at -80°C until immunohistochemical staining.

Antibodies

Rabbit polyclonal antibody against mouse laminin was generously donated by Dr. Fumio Nakamura (Hokkaido University, Sapporo, Japan). Rabbit polyclonal antibody against bovine type IV collagen was purchased from LSL Co., Ltd. (Tokyo, Japan).

Immunohistochemistry

Frozen tissues were sectioned ($6\text{--}8\text{ }\mu\text{m}$ thick) with a cryostat (HM 500; Microm Lab., Walldorf, Germany) at -20°C . Cryosections were mounted on 3-aminopropyl triethoxysilane-coated slides, air dried quickly and completely and stored at -80°C . Samples were fixed in -80°C acetone for 5 min and air dried. For general histological observations, sections were stained with Mayer's hematoxylin and eosin. For immunohistochemical observations, nonspecific staining was blocked with 3% goat serum and 5% skim milk in PBS for 30 min at room temperature. The sections were then incubated with the primary antibodies overnight at 4°C . After the primary antibody treatment, the sections were stained with a Pathostain ABC-POD Kit (Wako

Chem., Osaka, Japan) and then nuclei were stained with Mayer's hematoxylin. Rabbit serum was substituted for the primary antibodies as negative controls. Immunostained sections were examined and photographed with a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan).

Results

The diameter and length of the uterine horn decreased markedly from Day 1 to Day 13 postpartum and slightly decreased during Days 13 to 31 postpartum (Fig. 1). This change was due to the recovery from hypertrophy of the uterine myometrium that occurred during pregnancy. The uterine cross sections were oval on Days 1, 13 and 20 postpartum and circular on Day 31. Uterine sections stained with Mayer's hematoxylin and eosin are shown in Fig. 2. Large blood vessels, which maintained the pregnancy, remained in the endometrium adjacent to the uterine myometrium of lactating sows. Glandular epithelial cells on Days 1 and 13 postpartum were low and cuboidal, and the cytoplasm stained poorly. The same appearance was observed throughout lactation (Days 1 to 31) (Fig. 2A-D). After weaning, the glandular epithelial cells were highly columnar (Fig. 2E). The luminal epithelial cells on Day 1 after parturition had two forms: a low columnar or cuboidal form with shrunken nuclei (Fig. 2A), and a high cuboidal form (Fig. 2B). On Day 13 postpartum, the luminal epithelial cells were very low and flattened in appearance (Fig. 2C). The nuclei had become so numerous that the epithelium had a stratified appearance. On Day 20 postpartum, the luminal epithelial cells were slightly higher than on Day 13. On Day 31 postpartum, the luminal epithelial cells had the same appearance as on Day 20 (Fig. 2D). After weaning, the luminal epithelial cells were highly columnar in appearance (Fig. 2E).

Laminin was detected at the basal lamina of the endometrial and glandular epithelium and blood vessels (Fig. 3). In the uteri of lactating sows, laminin was localized in the basal lamina of the endometrial epithelium on Day 1 after parturition (Fig. 3A). Between Days 13 and 31 postpartum, the staining area was thick and was wedged into stromal tissue like a stick (Fig. 3B, C). After weaning, laminin was found at the basal lamina of

Fig. 1. Reproductive organs from lactating sows. (A) Day 1, (B) Day 13, (C) Day 20 and (D) Day 31 postpartum. Bars = 5 cm.

the endometrial epithelium in a stratum (Fig. 3D). Type IV collagen was detected in the basal lamina of the endometrial and glandular epithelium, the apical surface of the endometrial epithelium and the blood vessels (Fig. 4). The expression of type IV collagen was similar to that of laminin during lactation (Fig. 4A-C) and after weaning except that type IV collagen was observed in fibers in the stroma postweaning (Fig. 4D). The localization of all these ECMs after weaning had the same pattern as that observed in non-pregnant sows during the estrous cycle (data not shown).

Discussion

This is the first report about the localization of laminin and type IV collagen in porcine uteri during uterine involution. Localization of both laminin and type IV collagen during lactation was different from that in the postweaning period. During postpartum involution in rat [13, 14] and

mouse [15, 16], collagen decreased rapidly, and collagenase including matrix metalloproteinases (MMPs) increased. These changes correlate with uterine tissue remodeling. MMPs may also play a role in changing the localization of laminin and type IV collagen and in tissue remodeling in the porcine uterus during involution.

The luminal and glandular epithelial cells developed after weaning. This result was in agreement with the results of Palmer *et al.* [9]. Cell-ECM interactions have effects on cell proliferation and differentiation [17–19]. In particular, components of basement membranes such as laminin and type IV collagen influence the differentiation and growth of epithelial cells [20, 21]. In the present study, the luminal epithelial cells did not develop during lactation. During lactation, laminin and type IV collagen were localized in a winding pattern in the basal lamina of the epithelial cells. After weaning, the luminal epithelial cells were well developed and these ECMs localized in the basement membranes in a

Fig. 2. Mayer's hematoxylin and eosin staining in the endometrium during lactation and after weaning. Uteri from lactating sows on Day 1 (A, B) and Day 13 (C) postpartum. Uteri from sows on Day 31 after farrowing (D, E). A lactating sow (D) and a weaned sow on Day 28 after farrowing (E). LE: luminal epithelium, GE: glandular epithelium, S: stroma. Bars=100 μ m.

stratum. Therefore, the arrangement of the basement membrane components affects the proliferation and growth of the luminal epithelial cells during porcine uterine involution.

On Day 31 postpartum, the shapes of the luminal epithelial cells and the localization of laminin and type IV collagen in the postweaning sow were

different from those in the lactating sow. However, the uterus of the lactating sow on Day 31 postpartum had the same appearance as on Day 20 postpartum. These results suggest that uterine involution is suppressed during lactation. The suppression of uterine involution may be due to the sucking stimulus provided by the piglets. On the

Fig. 3. Immunohistochemical localization of laminin in the endometrium during lactation and after weaning. Uteri from lactating sows on Day 1 (A) and Day 13 (B) after farrowing. Uteri from sows on Day 31 after farrowing (C, D). A lactating sow (C) and a weaned sow on Day 28 after farrowing (D). LE: luminal epithelium, S: stroma, B: blood vessel. Bars = 100 μ m.

other hand, on Day 31 postpartum, the uterine appearance of the postweaning sow did not differ from that of the sexually matured and non-pregnant sows. Consequently, uterine involution is completed within a few days (no more than 3 days) after weaning. The results of this study suggest that the uterine involution completed after weaning is correlated with the rearrangement of ECM components in the uterus.

A complete understanding of the process of uterine involution postpartum is essential for the proper management of domestic animals because

this process plays an important role in preparing for the next pregnancy and in maintaining a high conception rate. The results of this study should help to understand the process of porcine uterine involution.

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Fig. 4. Immunohistochemical localization of type IV collagen in the endometrium during lactation and after weaning. Uteri from lactating sows on Day 1 (A) and Day 13 (B) after farrowing. Uteri from sow on Day 31 days after farrowing (C, D). A lactating sow (C) and a weaned sow on Day 28 after farrowing (D). LE: luminal epithelium, S: stroma, B: blood vessel. Bars = 100 μ m.

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