FULL PAPER

Anatomy

Histochemical properties of bovine and ovine mammary glands during fetal development

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ABSTRACT. In order to obtain more information on the development of bovine and ovine fetal mammary glands, a series of mammary glands from fetuses of different ages were analyzed. A total of 16 bovine fetuses with curved crown rump lengths ranging from 12 cm (80 days) to 75 cm (240 days) and 15 ovine fetuses ranging from 55 days to 131 days were examined. We used hematoxylin and eosin stain and Oil-Red-O stain to analyze the developmental and morphogenetic processes of mammary glands. In addition, we used immunohistochemical staining to determine the pattern of expression of cytokeratin 18 (CK18) during luminal epithelial differentiation, α-smooth-muscle actin (α-SMA) for myoepithelial differentiation, Ki-67 for cell proliferation, and estrogen receptor α (ERα). Our analyzes showed: (a) The primary mammary duct begin to proliferate in a lengthwise within the teat at 90 days in bovine fetuses and 63 days in ovine fetus; (b) luminal epithelial cells and myoepithelial cells appeared from 90 days in bovine fetuses and 63 days in ovine fetus; (c) proliferation of epithelial cells appeared to coincide with the development of the primary and secondary ducts; and (d) ERα was not found in the fetal mammary gland, but adipocytes showed the presence of ERα. Overall, these results indicate that the sequence of events in the prenatal development of the mammary gland of sheep is similar to that of cattle.

KEY WORDS: α-SMA, bovine, CK18, fetal mammary gland, ovine

The mammary gland is a milk-producing organ that is characteristic of all female mammals. They are composed of lobes, which contain a network of glandular tissue consisting of branching ducts and terminal secretory lobules in a connective tissue stroma [30]. The basic structures of mammary glands of ruminants are formed during the fetal period and development of the mammary ducts is confined to a very limited area around the cistern of the gland [7, 23]. These developments in the mammary glands are accompanied by the proliferation and differentiation of mammary epithelial and stromal cells. Thus, the identification of cell differentiation stages during fetal development is critical for gaining a better understanding of mammary gland development and stem cell mechanisms. Previous studies have used a range of markers to study mammary epithelial cell status during development. Several cytokeratin proteins can be used as markers within the mammary epithelium. For example, CK5 and CK14 identify basal/myoepithelial cells, and CK8/18 expression is characteristic of luminal cells in the mouse mammary gland [25]. Furthermore, differentiated cells in the mammary gland are characterized by the expression of either CK8/18 or α-smooth muscle actin

Table 1. Details of bovine fetal samples

CRL[a] (cm)	Estimated age (days)	N
12.0–12.5	80	2
14.5–16.0	90	3
19.0	95	1
20.0–21.0	100	2
23.0–24.0	110	2
30.0	130	1
37.5	150	1
39.0	155	1
43.0	160	1
47.0	170	1
75.0	240	1

a) CRL: Crown rump length.

Table 2. Details of ovine fetal samples

Age (days)	N
55	1
63	1
68	1
76	2
90	1
97	1
103–105	2
108–110	2
118–120	3
131	1

(α-SMA) alone [4]. Immunohistochemical studies on mouse fetuses have revealed the expression of CK8 in the mammary gland at embryonic day 15.5 [25]. Furthermore, it has been reported that basally located cells in the mammary buds that originate from the epidermis do not express smooth muscle markers at the fetal stage in mice [9, 17]. In human fetuses, expression of α-SMA occurs in the basal cells of the mammary gland at 24 to 28 weeks of gestation [19]. Further information on protein expression during fetal stages will facilitate our understanding of how cell lineages differentiate during mammary gland development. Moreover, determining the differentiation stages of cells in mammary glands during the fetal period will provide insights into the development of the mammary gland. To date, such studies have not been conducted on bovine and ovine fetuses. The present study used histochemical and immunohistochemical analyzes to determine cell differentiation stages in bovine and ovine mammary glands at different times during the fetal period.

MATERIALS AND METHODS

In this study, we used 16 bovine (*Bos taurus*) and 15 ovine (*Ovis aries*) fetuses. The details of these samples are shown in Tables 1 and 2. All experiments in this study were performed in accordance with the Utsunomiya University Guide for Experimental Animals (A17-0012).

Fetal mammary gland

Bovine fetuses of different gestational ages were obtained from pregnant Holstein cows slaughtered at the Chikusei slaughterhouse (Chikusei, Ibaraki, Japan). Fetal age (full term is 280 days) was estimated from the measurement of fetal crown rump length (CRL) [22]. Pregnant ewes at gestational ages of 55 to 131 days (full term is 147 days) were anesthetized with a 2.5–3.0% sevoflurane-oxygen mixture. The uterus was exposed through a midline laparotomy incision. The ovine fetus (Suffolk × Corriedale crossbred) was then removed from the uterus by cutting the umbilical cord.

Histology

Teats with the glandular portion were fixed in 10% neutral buffered formalin for 3 days and then embedded in paraffin after dehydration through an ethanol series. Sections (5 μm) were cut and stained with hematoxylin and eosin (HE). Other fixed tissues were washed 3 × 5 min in phosphate-buffered saline (PBS) and incubated overnight in 20% sucrose in PBS at 4°C; the tissues were then embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan) and kept at −80°C. Sections (7 μm) were cut and stained with Oil-Red-O (ORO).

Immunohistochemistry

Paraffin sections were deparaffinized, rehydrated through an ethanol series to distilled water, and treated for antigen retrieval with Histo VT One (pH 7; Nacalai Tesque, Kyoto, Japan) at 90°C for 20 min. The slides were then cooled to room temperature. After rinsing with PBS, they were dipped in 3% H_2O_2 in methanol for 30 min to quench endogenous peroxidase activity and rinsed in PBS. Background labeling was blocked by incubating the sections with 2.5% normal horse serum (NHS) for 30 min at room temperature. Information on the primary antibodies is provided in Table 3. The slides were incubated with each primary antibody at 4°C overnight (CK18, Ki-67, ERα) or at room temperature for 1 hr (α-SMA). Negative controls were obtained by omitting the primary antibodies. Before and after incubation with the secondary antibody, sections were rinsed in PBS. The slides were incubated in biotinylated secondary antibody and then with streptavidin-HRP (SA-5004; Vector Laboratories, Burlingame, CA, U.S.A.) for 30 min. The slides were rinsed in PBS before staining with the diaminobenzidine tetrahydrachloride visualization agent (SK-4105; Vector ImPACT DAB substrate, Vector Laboratories) for 8 min to produce coloration. The slides were counterstained with hematoxylin for 1 min and rinsed in running tap water for 10 min.

Table 3. List of primary antibodies

Primary antibody	Supplier	Cat No	Antigen	Iso type	Dilution	Cell type	Conjugate
CK18	Abcam, Cambridge, U.K.	ab668	Cytoskeleton preparation of epidermal carcinoma cell line A431	mouse IgG	1:1,000	luminal cell	no
α-SMA	Sigma-Aldrich, St Louis, MO, U.S.A.	A2547	Human α-SMA	mouse IgG	1:10,000	myoepithelial cell	no
Ki-67	Dianova, Hamburg, Germany	DIA-670	Human Ki-67	mouse IgG	1:200	proliferating cell	no
Ki-67	BD PharMingen, San Diego, CA, U.S.A.	558617	Human Ki-67	mouse IgG_1	1:200	proliferating cell	Alexa555
ERα	Santa Cruz Biotechnology, Dallas, TX, U.S.A.	sc-787	Bovine ERα	mouse IgG	1:500	−	no

Double immunofluorescence staining

Double immunofluorescence staining was performed on frozen tissue sections. Antigen retrieval was carried out using Histo VT One at 70°C for 20 min. The slides were then cooled to room temperature. Sections were incubated in blocking buffer (5% NHS/1% bovine serum albumin /0.2% Triton X-100 in PBS) for 30 min at room temperature. The sections were incubated with anti-CK18 antibody (diluted 1:1,000) overnight at 4°C. After rinsing 3 × 5 min in PBS, the sections were incubated in anti-IgG (H+L) mouse, horse-poly biotin (Vector Laboratories) diluted at 1:200 for 1 hr. The sections were then incubated in streptavidin, Alexa Fluor® 488 conjugate (Invitrogen, Carlsbad, CA, U.S.A.) diluted at 1:200 for 1 hr, followed by three PBS washes. The sections were then incubated with Alexa Fluor®555 mouse anti-Ki-67 antibody (B56; BD Pharmingen, San Diego, CA, U.S.A.) overnight at 4°C. The slides were mounted in Antifade medium (Vectashield H-1400, Vector Laboratories). The stained sections were examined using a confocal microscope (Fv10i; Olympus, Tokyo, Japan).

Evaluation of DAB staining

Cytoplasmic staining was considered positive for CK18 and α-SMA the intensity of the DAB reaction was categorized as follows: (i) +++, strong (dark brown); (ii) ++, moderate (normal brown); (iii) +, weak (light brown); and (iv) +/− or −, negative.

Cell counting of Ki-67 and ERα-positive cells

Only cells distinctly stained for Ki-67 and ERα were counted as previously described [8]. The total number of stromal, ductal cells, myoepithelial cells, Ki-67 and ERα positive cells were scored in 10 random microscopic fields (0.04 mm^2) of each sample under a 400 × magnification. The number of Ki-67 positive cells was shown as a percentage of ductal or myoepithelial cells counted per microscopic fields. The number of ERα positive cells was shown as a percentage of stromal cells counted per microscopic fields.

RESULTS

HE staining and ORO staining

Bovine fetus: At around Day 80 of bovine fetal age (Day 80), breasts were mainly formed of stromal cells. The round form cluster of the cells was observed within the teat. The cluster consisted of the cells formed in a cube or polygon shape (Fig. 1A). Subsequently, at Day 90, these cells formed a cord-like structure and elongated into the stroma (Fig. 1B). These cord-like structures became the primary mammary ducts. Cells containing a vesicle were observed in the connective tissue at Day 95 (Fig. 1C and 1D). At Day 100, canalization resulted in the formation of a lumen, and secondary ducts branched from this primary duct. The ducts were embedded within an intralobular stroma and separated from the adipose tissue at Day 150 (Fig. 1E). At Day 95, 110 and 160, the cells containing a vesicle showed ORO staining within their cytoplasm (Fig. 2A).

Ovine fetus: At Day 55 of ovine fetal age, there was no ductal structure in the teat. At Day 63, a ductal structure (primary duct) has elongated toward the connective tissue (Fig. 1F). The cells in the connective tissue containing a vesicle showed ORO staining within their cytoplasm at Day 68 (Fig. 2B). At Day 76, the mammary gland contained round, oval and elongated ducts, sometimes with branching (Fig. 1G and 1H). The ducts were lined with two or three layers of epithelial cells. The ducts were embedded within loose connective tissue at Day 131.

Immunohistochemistry

We used antibodies against four proteins to investigate the histochemical properties of bovine and ovine mammary glands (Tables 4 and 5).

Epithelial cell markers (CK18 and α-SMA)

Bovine fetus: Lumen formation in the ducts of mammary glands was associated with the expression of CK18 and α-SMA in the ductal cells (Table 4). At Day 80, CK18 and α-SMA were not detected in the mammary gland (Figs. 3A and 4A). At Day 90, two or three layers of epithelial cells constituted the mammary ducts. CK18 was only detected in the inner layer of cells of the ducts
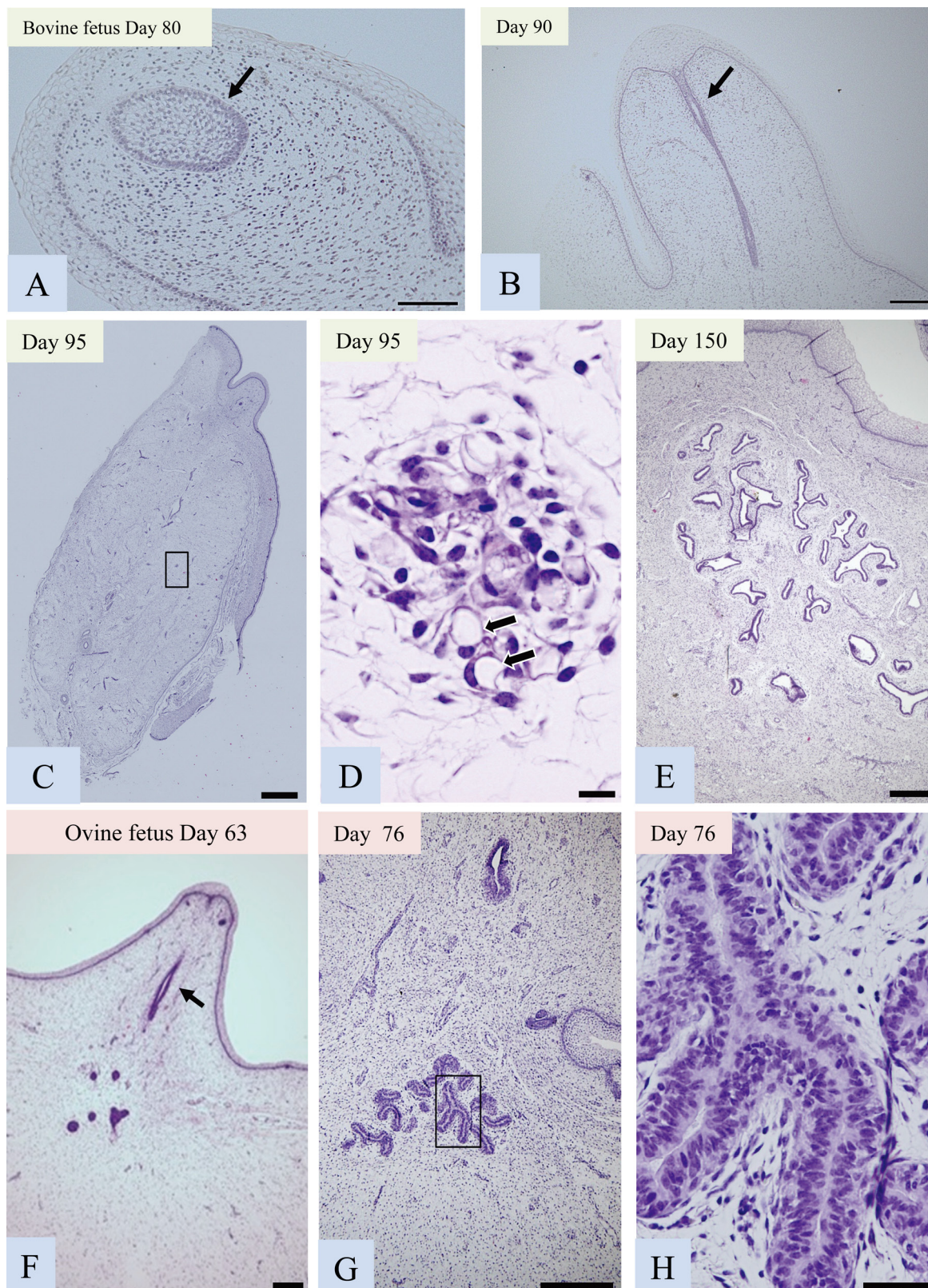
Fig. 1. Photomicrographs of bovine and ovine fetal mammary glands stained with HE. (A) The round form cluster in the teat (black arrow). (B) Primary mammary duct in the bovine mammary gland (black arrow). (C) Bovine fetal mammary gland stroma. (D) Higher magnification of the boxed area in C showing cells that have a vesicle in their cytoplasm (black arrows). (E) Secondary ducts in the bovine fetal mammary gland. (F) Primary mammary duct in the ovine mammary gland (black arrow). (G) Secondary ducts in the ovine fetal mammary gland. (H) Higher magnification of the boxed area in G. Scale bar: (A, H) 100 μm (B, E, F) 200 μm, (C) 1,000 μm, (D) 20 μm and (G) 250 μm.

(Fig. 3B). The cytoplasm of cells in the ducts stained weakly for α-SMA (Fig. 4B). At Day 100, nearly all ductal cells displayed strong CK18 immunoreactivity; expression of CK18 was strongest in the cytoplasm of cells on the luminal side (Fig. 3C). α-SMA was detected in the basal cells of the ducts, and α-SMA positive cells formed a single layer around the ducts (Fig. 4C). Staining for α-SMA persisted to later developmental stages with no modification in its distribution.

Ovine fetus: CK18 was detected in the inner cells of the ducts on Day 63 (Fig. 3D). The CK18 positive cells were scattered in the duct prior to ductal cavitation. Expression of α-SMA was first evident at Day 63 and increased by Day 76 (Table 5). Basal cells with α-SMA positive staining formed a single layer surrounding the ducts at Day 68 (Fig. 4E). At this stage, some cells displayed stronger CK18 immunoreactivity in the ducts. Expression of CK18 was observed homogeneously in the cytoplasm (Fig. 3E). At Day 76, all ductal cells expressed CK18 (Fig. 3F), and strong expression of α-SMA was detected in the cytoplasm of basal cells (Fig. 4F).

Proliferating cell marker (Ki-67)

Bovine fetus: At Day 90, Ki-67 expression was observed in the cell nucleus of ductal and basal cells (Fig. 5A). From Day 100 to 160, the number of Ki-67 positive cells was decreased and nearly all Ki-67 positive cells were located at the inner part of secondary ducts (Fig. 5B). The percentage of Ki-67 positive cells to ductal cells and myoepithelial cells tended to decrease during the late period of fetal development (Day 160–240) (Table 4).

Ovine fetus: In the fetal mammary gland, there were many Ki-67 positive cells in the duct at Day 63 (Fig. 5C). The percentage of Ki-67 positive cells in the ducts was 2.8% (Table 5). The number of Ki-67 positive cells in the ducts at Day 103 was very small (Fig. 5D). The percentage of Ki-67 positive cells tended to decrease during late fetal development (Day 103–131) (Table 5).

Estrogen receptor α

ERα expression was not found in epithelial cells in mammary glands at fetal development stages. Nor expression was detected in connective tissue at Day 90 in bovine fetuses or Day 63 in ovine fetus. However, staining was observed in the nuclei of cells with vesicles on Day 100 (bovine fetuses, Fig. 5E) and Day 68 (ovine fetus, Fig. 5F). The percentage of ERα positive cells to stromal cells in bovine (Table 4) and ovine (Table 5) mammary glands tended to decrease during late development.

Double immunofluorescence staining

We examined the expression of Ki-67 in CK18 positive cells during bovine mammary gland development. At Day 100 and Day 155, all ductal cells were CK18 positive (Fig. 6A and 6D) and some cells in the inner layer of the ducts co-stained for Ki-67 (Fig. 6C and 6F).
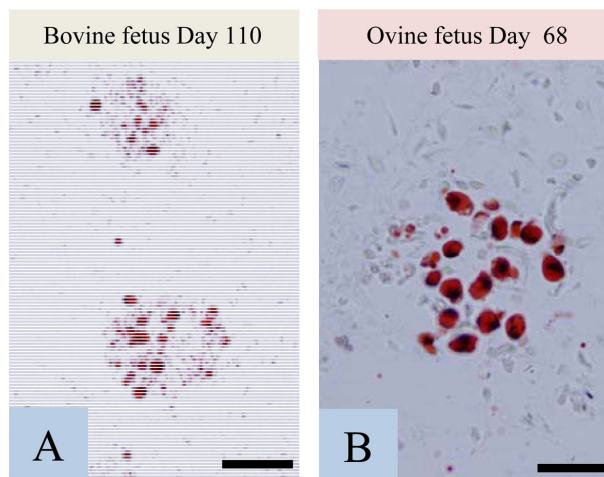
Fig. 2. Photomicrographs of Oil-Red-O stained of bovine (A) and ovine (B) fetal mammary gland stroma. The vesicles within cytoplasm were stained with ORO. Scale bar: (A) 100 μm, (B) 50 μm.

**Table 4.** The results of DAB staining of the bovine fetal mammary gland

CRL (cm)	12.0–12.5	14.5–16.0	20.0–21.0	23.0–24.0	30.0	37.5–39.0	43.0	47.0	75.0
Age (days)	80	90	100	110	130	150–155	160	170	240
CK18	−	+	++	++	+++	+++	+++	+++	+++
α-SMA	−	+	++	+++	+++	+++	+++	+++	+++
Ki-67[a] (%)	0.6	12.1	5.0	2.1	0.5	0.5	0.3	0.1	0.1
ERα[b] (%)	−	−	2.5	2.9	5.1	1.3	1.1	1.9	2.0

+++; strong staining, ++; moderate staining, +; weak staining, −; negative staining. a) percentage of Ki-67 positive cells to ductal cells and myoepithelial cells, b) percentage of ERα positive cells to stromal cells.

Table 5. The results of DAB staining of fetal ovine mammary gland

Age (days)	55	63	68	76	90	97	103–105	108–110	118–120	131
CK18	−	+	+	+++	+++	+++	+++	+++	+++	+++
α-SMA	−	+	+	+++	+++	+++	+++	+++	+++	+++
Ki-67[a] (%)	−	2.8	3.8	4.4	3.2	0.9	0.4	0.7	0.3	0.4
ERα[b] (%)	−	−	2.8	2.2	1.6	1.7	1.5	1.7	1.5	1.2

+++; strong staining, ++; moderate staining, +; weak staining, −; negative staining. a) percentage of Ki-67 positive cells to ductal cells and myoepithelial cells, b) percentage of ERα positive cells to stromal cells.
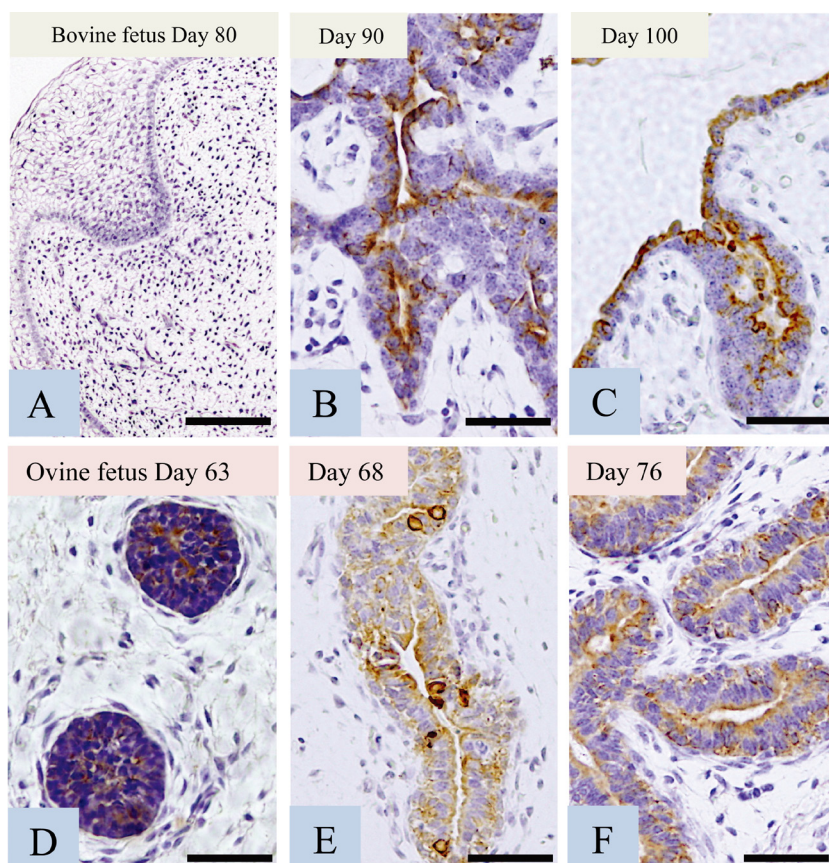
Fig. 3. Paraffin sections of bovine and ovine fetus mammary gland showing CK18 staining. (A) The teat of Day 80 of bovine fetus. (B) Bovine fetal primary mammary duct, (C) bovine fetal primary and secondary ducts. (D) Mammary ducts and (E, F) secondary ducts of ovine fetus. Scale bar: (A) 100 μm, (B–F) 50 μm.

DISCUSSION

Development of mammary gland is a multistep complex process involving mesenchymal–epithelial interactions and hormone regulations [11, 12]. These processes have been described in some animal models [6, 10, 28]. For ruminants, several studies to date have provided information of mammary gland morphogenesis in cattle [23, 27], buffalos [7] and sheep [23]. However, analysis of the expression of terminal differentiation markers during fetal mammary development is currently lacking. Therefore, at first, we confirmed early stages of mammary gland morphogenesis in the fetal ruminant, and next, we analyzed the expression of CK18 and SMA.

In the present study, we observed round form cluster of the cells in bovine teat at Day 80. The invagination of the mammary bud has been shown to begin to grow into the center of the teat at around Day 80 [23]. Therefore, the round form cluster found in this study may be early form of the mammary bud. We observed a morphologically similar pattern of development of mammary gland structures in bovine and ovine fetuses. Epithelial cell proliferation and cord-formation were observed in the teat at the middle of fetal development. Canalization of the primary duct was observed at Day 63 (ovine fetus) and Day 100 (bovine fetuses). Secondary ducts were found at Day 76 (ovine fetuses) and Day 100 (bovine fetuses), respectively. This observation of morphological changes supports the observations of Rowson [23]. In buffalo, secondary ducts are observed at around 100 days of gestation [7]. These results suggest that changes in mammary gland structure occur at similar developmental stages in these animals.

Previous studies have proposed that the mammary fat pad is essential for the development of mammary epithelium by providing signals that mediate ductal morphogenesis [20]. In the stroma, the cells containing a vesicle were stain with ORO, indicating the presence of triglycerides and cholesteryl oleate [21]. Therefore, the cells containing a vesicle were considered adipocytes. From Day 68 (ovine fetus) and Day 95 (bovine fetus), adipocytes began to accumulate lipid inclusions in their cytoplasm and fat pad started development. On embryonic day 14 in the mouse, fat pad precursor tissues appear in subcutaneous stroma [13]. Then, at the late embryonic day 16 stage, these cells start to differentiate into adipocytes and complete the conversion to adipocytes 48 to 72 hr after birth [24]. In contrast to the mouse fetus at late fetal age, where the mammary gland penetrated the fat pad [29], the mammary gland of bovine fetus and ovine fetus were separated from the fat pad by multiple layers of fibroblastic tissue.

Cytokeratins are intermediate filament proteins found in most epithelial cells. Among the cytokeratins, CK5 and CK14 marks basal/myoepithelial cells, whereas CK8/CK18 expression marks luminal cells in the mouse mammary gland [17]. It has been
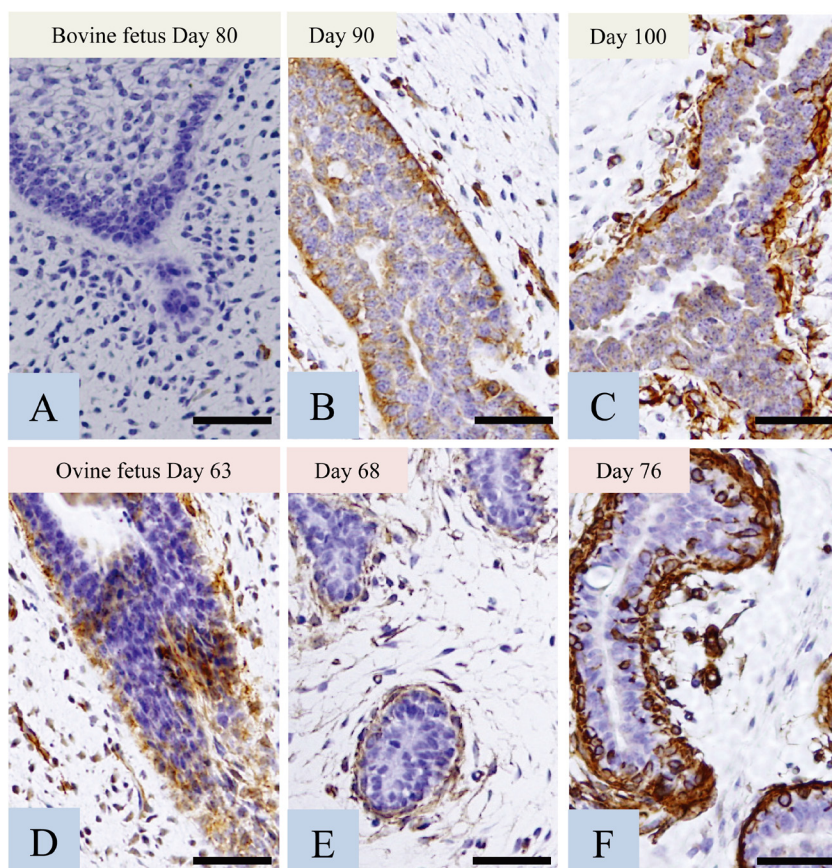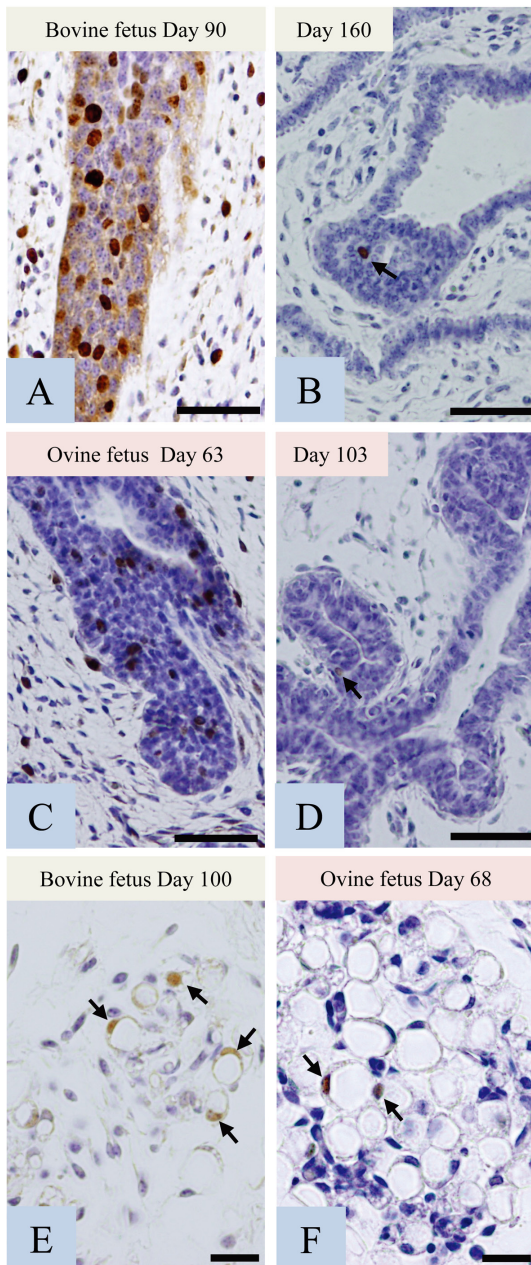
Fig. 4. Paraffin sections of bovine and ovine fetus mammary gland showing α-SMA staining. (A) The teat of Day 80 of bovine fetus. (B) Bovine fetal primary mammary duct, (C) primary and secondary ducts. (D) Ovine fetal mammary ducts and (E, F) secondary ducts. Scale bar: (A–F) 50 μm.

shown that single-lineage cells, such as those expressing only CK14 or CK8 or CK19, are already specified during embryonic mammary gland development in the mouse [25]. In the mature gland, the luminal epithelial cells express CK18 and CK19 [3, 17, 25]. Individual luminal cells express higher levels of CK8/18 than other nearby luminal cells after ductal cavitation [17]. In this study, CK18 expression was localized to the bovine and ovine fetal ducts. This result is in agreement with that for the mouse mammary gland. Therefore, our results suggest that the cells that compose the ducts had differentiated into luminal epithelial cells from around Day 90 (bovine) or Day 63 (ovine). There were differences in the expression pattern of CK18 between bovine and ovine fetal mammary glands. In the ovine fetus at Day 68, nearly all luminal cells were weakly positive for CK18, and strong CK18 positive cells were scattered in the mammary ducts. In the bovine fetus, a high intensity of CK18 immunoreactivity was present at Day 100, compared with that at Day 90. Mammary luminal epithelial cells acquire CK18 during differentiation [3, 5]. Therefore, the cells that showed stronger CK18 immunoreactivity were supposed to have differentiated into luminal epithelial cells.
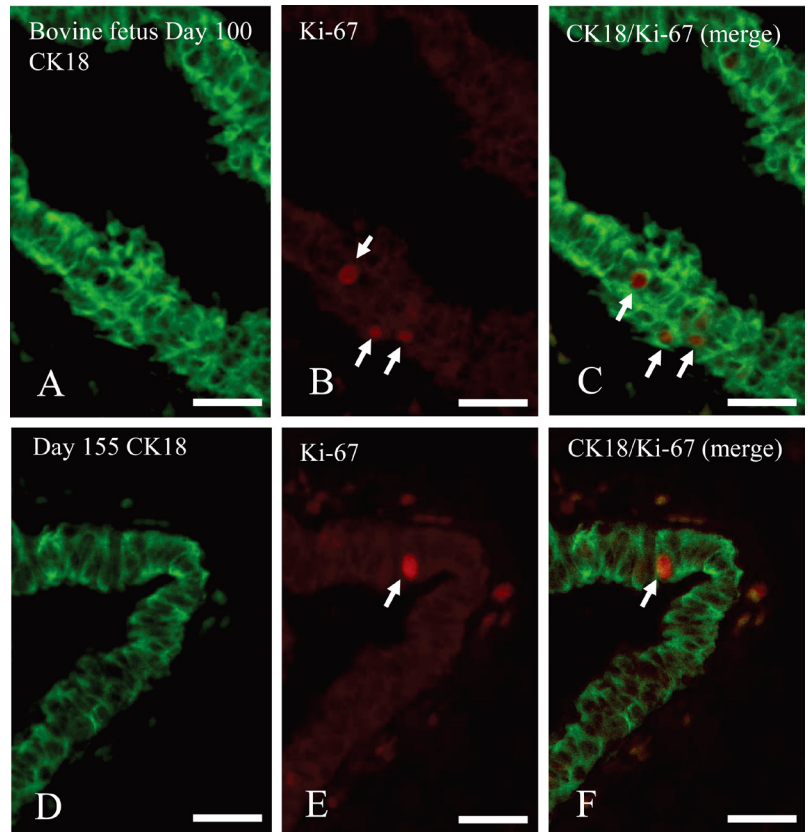
Immunohistochemical staining for α-SMA is commonly used to identify mammary myoepithelial cells [1]. In this study, α-SMA was observed in the basal cells of the duct from Day 90 (bovine fetuses) or Day 63 (ovine fetus). The observed morphological changes and distribution in the myoepithelial cells in the bovine fetus before birth were almost identical to those in the ovine fetus. The human fetal mammary gland shows myoepithelial differentiation at approximately 24 to 28 weeks of gestation [19]. Therefore, it is likely that differentiation of myoepithelial cells in bovine and ovine fetal mammary glands is faster than that in human fetuses.

Ki-67 is a nuclear nonhistone protein that is expressed during late G1-, S-, G2- and M-phase of mitosis [2]. In bovine and ovine fetal mammary glands, Ki-67 positive epithelial cells were observed. In Day 90 (bovine fetuses) and Day 68 (ovine fetus), higher number of Ki-67 positive cells was observed in the innermost layer of the ducts. This cell proliferation appears to coincide with the development of primary and secondary ducts. Therefore, we suggest that cell proliferation occurs during the period of most active tissue structural development. As fetal age proceeded in ovine and bovine fetuses, the number of Ki-67 positive cells decreased. In the human fetus, proliferation of epithelial cells is consistently low during the late fetal period [19]. These observations suggest that fetal mammary gland develops mainly during the middle part of fetal development and thereafter shows little change until birth. We also observed in bovine fetuses that some Ki-67 positive cells co-express CK18. It has been reported that single-lineage cells are already specified during embryonic mammogenesis, indicating an embryonic origin for these putative multipotent/bipotent or luminally restricted progenitor cells [25, 26]. This finding indicates that Ki-67+/CK18+ cells found in this study may correspond to the progenitor cells that contribute to the tissue development.

Fig. 5. Paraffin sections of bovine and ovine fetus mammary gland showing Ki-67 and ERα staining. (A, B) Ki-67 staining (brown) showing proliferating cells in bovine fetal mammary ducts and (C, D) ovine fetal mammary ducts, black arrows indicate Ki-67 positive cells. (E) Mammary gland stroma in bovine fetus and (F) ovine fetus. The black arrows indicate ERα positive cells. Scale bar: (A–D) 50 μm, (E, F) 20 μm.

Fig. 6. Photomicrographs of cryostat sections of bovine fetal mammary gland double stained with CK18 (visualized with Alexa488, green) and Ki-67 (visualized with Alexa 555, red). (A–F) Sections of secondary ducts. White arrows: Ki-67 positive cells. Scale bar: 30 μm.

Estradiol is a regulator of mammary gland development and is thought to exert its effects through ERα expressed in the mammary gland stroma and epithelium [15]. It has been reported that stromal and epithelial ERα, in cooperation with epithelial progesterone receptor, play a pivotal role in epithelial growth regulation and morphogenesis in the mammary gland of sexually mature mice [15, 18]. Recent studies have demonstrated that amphiregulin is a critical paracrine regulator of estrogen action during ductal morphogenesis [14]. In bovine and ovine fetuses, the epithelial compartment of the fetal mammary gland lacked ERα immunoreactivity. On the other hand, ERα immunoreactivity was shown in the nuclei of cells composing the fat pad. ERα has been detected in adipocytes and fibroblasts within the adult mammary fat pads of mice [18], heifers [16] and humans [10]. The mammary gland may respond to the estrogen via stromal estrogen receptors because estrogen receptors were not detectable in mammary gland epithelium until birth in sheep and cow. However, we have no evidence that estrogen promotes mammary gland development during fetal period.

The mammary glands of bovine and ovine fetuses differ in size, and are correlated with body size. However, the architecture of the gland is similar between two species. Expression of CK18 and α-SMA was observed for the first time at Day 90 in the bovine fetuses and Day 63 in the ovine fetus. At this stage, secondary ducts began to branch from the primary duct. Ki-67 positive cells were observed in the middle stage of gestation. In fetal mammary glands of both species, secondary ducts were still present outside the fat pad and no lobular structures were found. These results indicate that developmental and morphogenic processes in the mammary glands of bovine and ovine fetuses are similar to each other.

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