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Ultrasound Imaging of Mammary Glands in Dairy Heifers at Different Stages of Growth

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ABSTRACT. To obtain B-mode ultrasound images of mammary glands in dairy heifers at different stages of growth, 25 clinically normal Holstein heifers were used. The heifers were divided into 5 groups (n=5/group) by stage of their growth: 2-month-old (group 1), 5-month-old (group 2), postpuberty (group 3), mid (group 4), and late (group 5) pregnancy. Furthermore, the sections of mammary glands were observed grossly at postmortem examination in one heifer in each group. Ultrasound images varied with the development of mammary glands. In group 1, the mammary glands had distinctive ultrasonographic findings: an oval to fusiform homogeneous hypoechoic structure. In all groups except group 1, mammary tissue consists of two major areas: a homogeneous, medium echogenic area and a poorly-defined, heterogeneous, hypoechoic area mostly in the superficial part. The superficial hypoechoic area spread more extensively and more irregularly with the development of mammary glands. Most pregnant heifers had irregular and extremely hypoechoic or anechoic areas like lactiferous sinus in the glands. The gross findings of mammary glands suggested that the hypoechoic areas of various shapes represented the lactiferous sinus and ducts. Thus, these results indicate that B-mode ultrasound imaging can visualize the internal structures of udders and could be a useful tool for evaluation of mammary glands in heifers.
KEY WORDS: dairy heifer, mammary gland, ultrasound.

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The mammary glands of heifers develop through distinct stages, and puberty and pregnancy significantly affect their development. Therefore, mammary gland growth around puberty and during pregnancy could predict the milk yield potential of cows.

Mammary gland development until the first parturition is very complicated. The basic structure of mammary glands is formed at birth [22], and the mammary glands of a newborn ruminant are composed of a teat, a primary duct and several secondary ducts [17]. In the first few months after birth, mammary glands grow at the same rate as the rest of the body and only the nonepithelial tissues grow in this period. This growth period is termed isometric growth [4, 22]. At 2 to 3 months old, the mammary glands start to grow at a faster rate than the rest of the body, and this situation is termed allometric growth [22, 25]. In this phase the mammary fat pads also increase in size, and epithelial ducts penetrate the stromal tissue [3]. Most studies suggest that this accelerated mammary growth continues until around puberty [23, 25]. After that, mammary glands grow isometrically again and the isometric growth continues until conception [25]. After puberty onset, with each estrous cycle, a surge of estrogen stimulates mammary gland proliferation and lactiferous ducts show lengthening, thickening and branching [4]. The duct network formation during the first year of life could determine the extent of lobulo-alveolar development [27].

The main parts of mammary gland growth occur during pregnancy [4, 22], and it depends on the response to hormones secreted by the conceptus and the maternal body. With conception, lactiferous ducts lengthen further and alveoli are formed. Then, the adipose cells of the fat pad are slowly eroded away and the fatty tissue throughout the mammary glands is gradually replaced with ducts, lobuli, alveoli, vessels, and connective tissues [4, 32]. The growth of mammary ducts continues in early pregnancy, on the other hand, lobuli and alveoli develop extensively from mid pregnancy [24]. The fat cells start to decrease from the beginning of the third trimester and the amount of adipose tissue around the lobule further decreases in an eight- to nine-month pregnancy [21]. Thus, mammary gland development until parturition is very complicated, and is very important to milk production after parturition in dairy heifers.

Recent studies suggested that intramammary infections (IMI) were prevalent in dairy heifers [1, 11, 12, 20, 30]. Prepartum IMI could be one of the risk factors for clinical and subclinical mastitis in heifers [8], and some studies indicated that IMI in unbred heifers could be associated with reduction in alveolar epithelial and luminal areas and increase in connective tissue stroma [6, 29].

Ultrasound is a noninvasive technique, which provides

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real-time visualization of various tissues with gray-scale and a 2-dimensional image. Ultrasonographic characteristics of mammary glands affected with mastitis have been described in cows [2, 7, 10, 31], ewes [15], sows [5], and bitches [28]. Particularly in dairy cows, teats and mammary glands parenchyma have been examined via ultrasonography in both healthy [7, 13, 14, 18, 31] and diseased cases [7, 9, 10, 13]. However, to the best of our knowledge, ultrasound imaging of mammary glands in dairy heifers has not been investigated as yet.

Therefore, the objective of this study was to demonstrate the ultrasonographic features of normal mammary glands in dairy heifers at different stages of growth as fundamental information to evaluate the developmental and the pathological changes in mammary glands.

MATERIALS AND METHODS

The present investigation was carried out at the Field Center of Animal Science and Agriculture, Obihiro University, Japan. The protocol and experimental design were approved by Obihiro University of Agriculture and Veterinary Medicine, Laboratory Animal Care and Use Committee.

In this study, 25 healthy heifers aged from 2.2 to 25.2 months were evaluated. Based on age and pregnancy period, heifers were allocated into 5 groups (n=5 per group): 2-month-old (group 1), 5-month-old (group 2), postpuberty (group 3, 12.6- to 14.7-month-old), mid (group 4, 4.8- to 5.9-month pregnant), and late pregnancy (group 5, 21 to 24 days prepartum). All heifers in group 3 had experienced some estrous cycles before the ultrasound image was obtained and were examined in luteal phase which was confirmed by rectal examination.

Ultrasound examination was performed on the 2 mainly left quarters of each heifer with a B-mode ultrasound scanner (HS-2000: Honda Electronics Ltd., Toyohashi, Japan) and a convex array transducer (HCS-452M: Honda Electronics Ltd.) operated at 5.0 MHz. Animals were examined while they were in standing position without sedation. The hair over the udders was clipped, and then sufficient amount of acoustic gel (Biosonic: AMITIE Co., Ltd., Korea) was applied to the cow's skin after alcohol impregnation.

The mammary gland was visualized from the ventral side in all animals. Additionally, the front and rear quarters were visualized from the lateral side and the caudal side, respectively in groups 4 and 5. The ultrasound probe was manipulated caudal to teats in all quarters when the mammary glands were visualized from the ventral side. But, in groups 3, 4, and 5, the probe was placed at the cranial position to teats of the front quarters because the front quarters mammary parenchyma were indistinguishable from that of the rear quarters in these groups. Each mammary gland was evaluated from two directions: transverse and sagittal section from the ventral side, transverse and dorsal section from the lateral side, and sagittal and dorsal section from the caudal side. Structure, echogenicity and homogeneity of each tissue were assessed. A detailed evaluation of mammary gland size was not performed because pressure by the probe could change the shape and the size of the mammary glands to some degree. For later analysis, we recorded the still images for each location with a printer (UP-880 Video Graphic Printer: Sony; Tokyo, Japan) and a digital video camera (DM-FV10: Canon, Tokyo, Japan).

Furthermore, one heifer in each group, which had shown typical ultrasound findings at each stage of growth, was euthanized to compare the ultrasound images and sections of the mammary glands. The cutting sites in mammary glands were marked either by painting on the skin with saturated solution of picric acid or by injection of methylene blue solution under ultrasonic guidance. After euthanizing, the udders were removed and transverse and sagittal sections of the tissues were observed. Furthermore, the dorsal section of right front quarter was observed in the late pregnant heifer.

RESULTS

Appearance of udders was significantly different in each group (Fig. 1-1a to 5a), and ultrasound images of the mammary glands varied at the different stages of growth. There was no significant difference in findings between front and rear quarters, so, the common findings of all quarters in each group would be described here.

In group 1, the mammary glands were shown as oval to fusiform in both transverse and sagittal sections. They were homogeneous hypoechoic structures and surrounded by a hyperechoic band-like area (Fig. 1-1b). The definition and the homogeneity of the band-like area differed in individuals. The oval to fusiform hypoechoic area was about 5 mm thick (front quarters; 4.0 to 4.9 mm, rear quarters; 4.2 to 8.5 mm).

After 5 months of age, the oval to fusiform structure was not visualized. Mammary tissues had homogeneous and medium echogenicity with the inclusion of a poorly-defined and irregular hypoechoic area mostly in the superficial part. In group 2, all mammary glands were about 1 to 2 cm thick, and the extension and the echogenicity of the superficial hypoechoic area differed among individuals. In the sagittal section, extremely hypoechoic cleft-like or linear structures were observed in the superficial hypoechoic area (Fig. 1-2b).

In group 3, the irregular, poorly-defined hypoechoic area in the superficial part spread deeply into medium echogenic area in a radial pattern or multidirectional pattern to a varying degree (Fig. 1-3b). The extension of the hypoechoic area was more apparent in sagittal section than that of the transverse section, and it tended to spread caudally. Furthermore, irregular and extremely hypoechoic areas were found in the superficial hypoechoic area.

In groups 4 and 5, namely in the pregnant heifers, the superficial hypoechoic areas spread more extensively and more irregularly in shape than that in nonpregnant heifers. Eight of the ten pregnant heifers had irregular and extremely

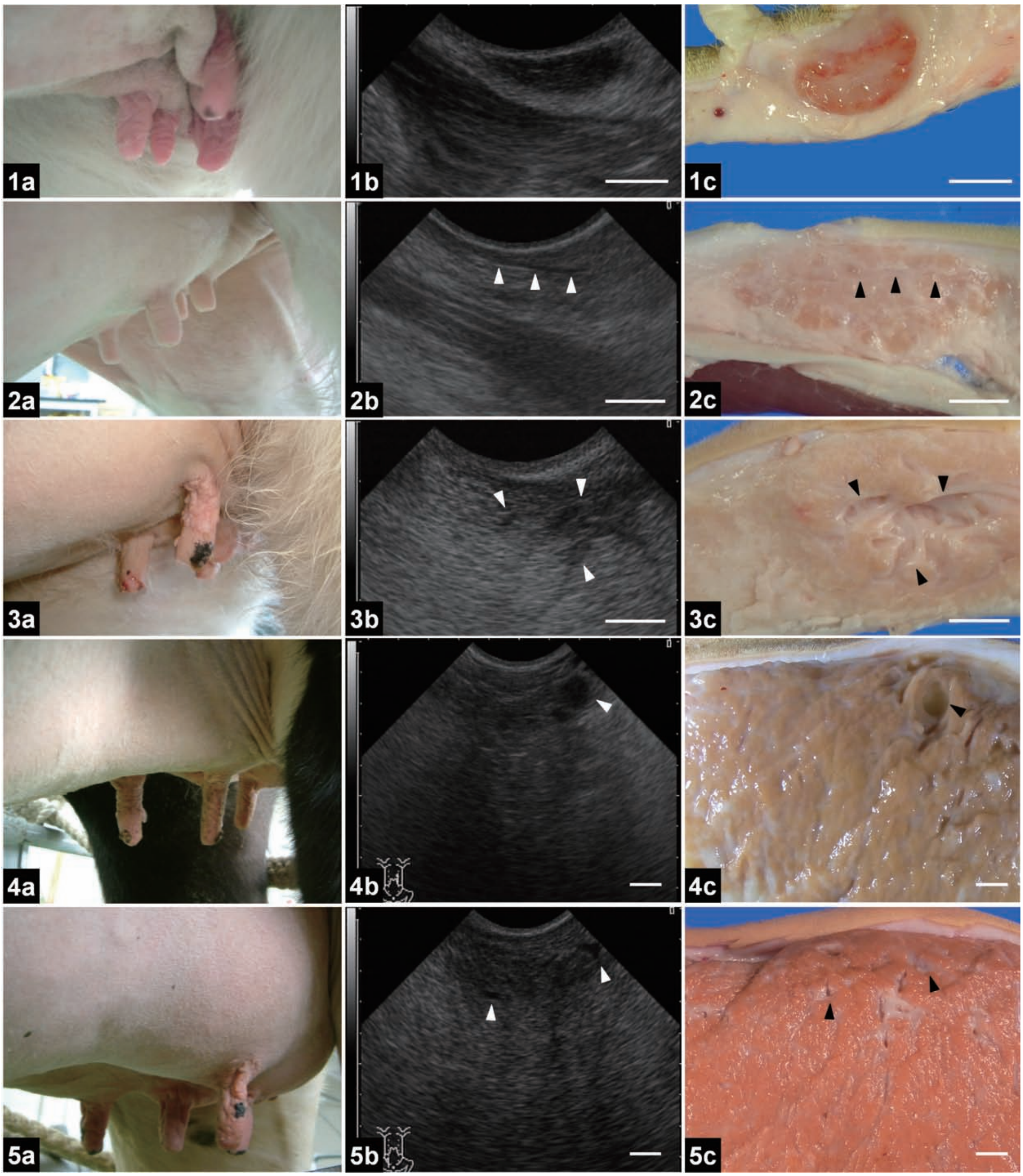

Fig. 1. Appearance of udders (1a-5a), ultrasound images (1b-5b) and sections (1c-5c) of mammary gland in heifers at different stages of growth. Each number corresponds with the group number. Ultrasound images and sections of mammary glands are shown in sagittal sections. White arrowheads indicate the irregular or liner hypoechoic structure in 2b to 5b. Black arrowheads indicate each structure (lactiferous sinus or ducts in 2c to 5c) corresponding to the structures indicated by white arrowheads in ultrasound images (2b to 5b). White bar=1 cm.

hypoechoic or anechoic areas in the tissue (Fig. 1-4b and 5b); there were large individual differences in the number and the size of these areas. The shapes of these areas were round, linear, irregular, or cleft-like with occasional posterior echo enhancement. Two of five heifers in group 5 had poorly-defined hypoechoic lines appearing like a branched duct in the medium echogenic area in the sagittal sections of the front quarters.

In the images from the lateral side of the front quarters and from the caudal side of the rear quarters, the mammary gland was nearly homogeneous with few striking structures in group 4. On the other hand, in group 5, extremely hypoechoic or anechoic, luminal structures were visualized from the lateral side in the front quarters. The echogenicity of the mammary gland in pregnant heifers tended to be slightly higher than that in unbred heifers.

On the adjacent tissue of the mammary glands, we could distinguish between the mammary gland and components of the abdominal wall in the prepubertal heifers. In some of the postpubertal and the pregnant heifers, although the size of the mammary glands was not so large, the boundary line was not visualized clearly.

In the comparison of ultrasound images with anatomical findings, the correspondence between the ultrasound images and the anatomical section could be observed. Grossly, in the 2-month-old heifer, the mammary glands were oval in transverse section and oval to semicircle in sagittal section. They were solid structure surrounded by connective tissue, and the mammary glands consisted of yellowish pink to orange-red colored tissue considered parenchyma and a small amount of creamy white tissue considered stroma. These results indicated that a homogeneous hypoechoic structure and a hyperechoic band-like area in ultrasound images represented mammary gland tissue and surrounding tissue of mammary gland, respectively (Fig. 1-1b and 1c).

In the 5-month-old heifer, mammary glands were surrounded by adipoid tissue and the mammary tissue appeared to be lobulated by reticulated stromal tissue. Tissue density in the base of the teat was low and small ductal structures extended caudally from the base of the teat along the stromal tissue in sagittal section. The hypoechoic linear structure observed in ultrasound images corresponded with this ductal structure (Fig. 1-2b and 2c).

In the postpubertal heifer, evident sinus was observed in all quarters and developed mainly caudally from teats. The stromal tissue penetrated among the parenchyma finely and lactiferous ducts extended radially from the lactiferous sinus. Compared with the sections of the mammary glands, the irregular and extremely hypoechoic areas and the hypoechoic areas, which extended radially, corresponded with lactiferous sinus and lactiferous ducts, respectively (Fig. 1-3b and 3c).

In pregnant heifers, lactiferous sinus developed around the base of the teat and lactiferous ducts extended more deeply than that in the postpubertal heifer. The tissue density of mammary gland in late pregnancy appeared to be higher than that in mid pregnancy. As in the postpubertal heifer, irregular and extremely hypoechoic or anechoic areas in the ultrasound images corresponded with lactiferous sinus (Fig. 1-4b, 4c, 5b and 5c). And these irregular areas were identified clearly in ultrasound images of the quarters containing voluminous secretion. Additionally, the extremely hypoechoic or anechoic luminal structures, which were visualized from the lateral side in front quarters in late pregnant heifers, corresponded to the course of the vessels extending in approximately dorsoventral direction. However, it was not clear how the artery and the vein, which run side by side, were visualized in gray scale images.

In the 2 pregnant heifers, honey-like, high viscosity secretion accumulated in 5 out of the 8 quarters and was variable in amount among quarters. Because of too little secretion in the other three quarters, the difference in the ultrasound findings such as echogenicity of the secretion was not determined between the five quarters and the other three quarters. The three nonpregnant heifers had no mammary secretion in the lactiferous sinus.

DISCUSSION

The objective of this study was to demonstrate the basic ultrasound images of normal mammary glands in dairy heifers at different stages of growth as fundamental information to evaluate the developmental and the pathological changes in mammary glands. The present study showed that the features of normal mammary glands at different stages of growth could be visualized by ultrasound.

Mammary development has been evaluated for a variety of purposes, such as the improvement of rearing method, in cows [23, 27]. A number of techniques including histological method and DNA content have been used for the evaluation of mammary development, and most of them are very invasive [19, 33]. In contrast, ultrasound imaging is a noninvasive technique and will allow researchers to examine the same animals repeatedly and readily. In previous studies, the physiological sonographic pattern of mammary glands represents medium homogeneous echogenicity and isolated anechoic lactiferous ducts and vessels in lactating cows [10]. In the area in which much milk accumulates, the glandular parenchyma shows mixed trabecular pattern of anechoic areas compartmentalized by hyperechoic partitions [2]. Therefore, mammary parenchyma can be better visualized from the lateral and the caudal side of udders than from the ventral side. On the other hand, heifers would have a little or no secretion in mammary glands, so the lactiferous sinus and the duct of heifers were expected to be less clear than that of lactating cows in ultrasound images. Though, the present results showed that mammary duct system was visualized as linear to irregularly-shaped hypoechoic structures in ultrasound images after 5 months of age with or without secretion. The change in shape and extent of the hypoechoic structures seems to reflect the developmental process of the mammary duct system: lengthening, thickening, and branching [3, 4]. Therefore, the shape and the extent of the hypoechoic structures could be useful as the index of the mammary gland development in ultrasound imaging.

In 2-month-old heifers, the mammary glands were visualized as a characteristic oval to fusiform hypoechoic area. The definition and the homogeneity of the hyperechoic band-like area differed in individuals, and the hyperechoic band was not observed after 5 months of age. These results indicate that the hyperechoic bands disappear with the development of the mammary glands. Therefore, the mammary gland development in 2-month-old heifers could be estimated from the definition of the hyperechoic band. The allometric growth of the mammary glands could occur at 2-month-old [22, 25], and the obscure hyperechoic band may be attributed to the onset of allometric growth. Thus, structure, echogenicity and homogeneity of the mammary parenchyma were varied among stages of growth, and this indicates that B-mode ultrasound imaging could provide useful information in assessing the development of mammary gland in heifers.

Ultrasound imaging of the mammary glands has been performed in lactating cows affected with mastitis, and it is known that some lesions, such as abscess, fibrosis, and gas formation, can be detected by ultrasonography [2, 10]. Additionally, although direct correlation between cell count and echogenicity is not clear, increased mixed-heterogeneous echogenicity of milk was observed in the cases with increased cell content in milk [2, 10, 31]. In a late pregnant heifer, the increased echogenicity in the dilated lactiferous sinus was encountered in a quarter; no milk was obtained from the quarter after parturition (data not shown). These ultrasound findings were different from the present findings obtained in late pregnant heifers.

In 8 of 10 pregnant heifers, extremely hypoechoic or anechoic areas of various shapes were observed; the comparison of ultrasound images with macroscopic findings suggested that these areas represent the lactiferous sinus and ducts. Of the 5 autopsied heifers, 2 pregnant heifers had the secretion in the lactiferous sinus; the visual appearance and the amount of the secretion varied with each quarter. A previous study showed that small alveoli began containing secretory material in the 5th month of pregnancy [26]. It is known that the visual appearance of secretions collected from primigravid heifers in late pregnancy may indicate the presence of infection, and many quarters that were not infected precalving had high viscosity secretions [16]. In the present study, it remains undetermined whether the nature of the secretion makes a difference in the ultrasound findings such as echogenicity. Though, the high viscosity secretion is expected to be difficult to move in the ducts; therefore, the distribution of the extremely hypoechoic or anechoic structures in mammary glands could vary according to the nature of the secretion.

This study demonstrated the ultrasonographic features of normal mammary glands in dairy heifers at different stages of growth, and suggests that ultrasound imaging could be a useful tool for evaluating the developmental and the pathological changes of mammary glands in heifers. In the future, further studies need to be done on a large scale of heifers under the field condition to establish the usefulness of ultrasonography as an evaluation tool for mammary gland disorders. Additionally, a comparison between ultrasound and histological findings in mammary glands is needed for the establishment of the accuracy, reliability and validity of the ultrasound method.

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