A Study on the Scanning Electron Microscopy of the Buffalo Mammary Gland

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ABSTRACT : Scanning Electron Microscopy of twelve lactating buffalo mammary gland was done. The lactating mammary gland showed alveolus, arrangement of blood vessels and myoepithelial cells on the alveolus, the formation of lobules and interlobular connective tissue. From the exposed alveolar lumen fat globule formation can be seen which is still attached to the alveolar surface by microvilli. This technique should further be extended to study the alveolar structure in detail during different stages of mammary gland development in buffaloes. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 101-103)*

Key Words : Buffalo, Scanning Electron Microscopy, Mammary Gland, Lactation

INTRODUCTION

The technique of Scanning Electron Microscopy helps in better understanding of the microtopography of the objects examined. It provides easily comprehensible, quasi-three-dimensional representations of the objects examined, at a wide range of magnifications and is probably it most valuable characteristics for biological research, morphology and evolutionary interpretation. (Heywood, V. H., 1971). This technique has successfully been used to study the mammary structures of rat and mice in detail (Nemanic and Pitelka, 1971; Caruolo, 1980; Soloff et al., 1980). In the present pioneer study, efforts have been made to scan and unreveal the surface structures of the lactating buffalo mammary gland.

MATERIALS AND METHODS

Selection of animals

Twelve Murrah buffaloes having reproductive problems (repeat breeding and adhesion of uterus) were taken from the Institute's buffalo herd and divided into two groups of six each. The buffaloes were kept in loose housing system in buffalo herd and received daily rations as per the practice followed in the institute's farm. Free choice fresh tap water to all the buffaloes was available at all the times of the day.

Treatment of animals

To buffaloes of Group I, Estradiol-17β was administered at the rate of 0.1 mg/kg body weight/day and Progesterone at the rate of 0.25 mg/kg body weight/day for 7 days. To buffaloes of Group II same treatment was given for 14 days. From day 15th of the start of treatment, udder massage was given in the morning and evening, daily to simulate milk let-down. This practice was followed till the udders were turgid of milk, followed by milking (on day 28).

Preperation of samples

To study the ultrastructure of mammary gland about 1.5 to 2 gm of mammary tissue was surgically obtained from the left fore quarter of individual buffalo on day 28. The animals were operated at the animal health complex of the institute. SEM was first standardized (Dang and Ludri, 1999) by taking sheep mammary gland from the slaughter house and then the method was extended to the buffalo mammary tissue. Mammary gland samples were sliced into 1-2×3 mm strips and transferred to micro beakers containing 2.5% buffered glutaradehyde fixative (in 0.1 M Cacodylate buffer, pH 7.2) for 2.5 h at 4℃ and then in 1% buffered OsO_4 for 1 hour. The slices were washed with cacodylate buffer (5-10 min) and air dried in ascending order of ethanol : water series. The samples were first kept in 50% and 70% ethanol each for 10 min., than in 90% and 95% ethanol for 20 min each which was followed by dehydration in 100% absolute alcohol twice for a duration of 30 minutes each.

Processing of samples

After dehydration the processed samples were kept in dessicator till use. The processed samples were tampered to expose the natural surface and mounted on aluminium stubs with silver paint (silver scetate solution) and sputter coated with gold at approximately 200 A° thickness in Hitachi IB-3 ion coater. The ion current was maintained at 6 mA at fine vacuum of 0.05-0.07 torr for 4 min. The samples on aluminium stubs were placed in specimen holder and inserted into vacuum chamber. Hitachi S-405A Scanning Electron Microscope was operated at 15 KV using secondary electron mode. The observations were recorded with

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the help of attached camera asembly and the film was developed.

RESULTS AND DISCUSSION

The technique of SEM helped in effectively presenting the morphological structures of the buffalo mammary gland. SEM of the prelactating mammary tissue of buffalo showed only arrangement of connective fibres along with some empty spaces, which will accommodate the developing parenchyma of the gland during pregnancy and lactation. Results of SEM have been presented in figure 1~5. Figure 1 shows group of alveoli closely packed with each other and their lumen exposed. Alveolar epithelium is also clearly visible. These results are in agreement to the histological structures of induced lactating mammary glands of cows (Fleming et al., 1986). Black spots or craters can also be seen in the alveolus. According to Nemanic and Pitelka (1971) these shallow craters present on the cell surfaces are left by extraction of fat during tissue processing. Figure 2 shows a fat globule which is still attached to the alveolar surface by microvilli.

According to Cowie (1972) the lactating mammary gland resembles bunch of grapes or clusters which are

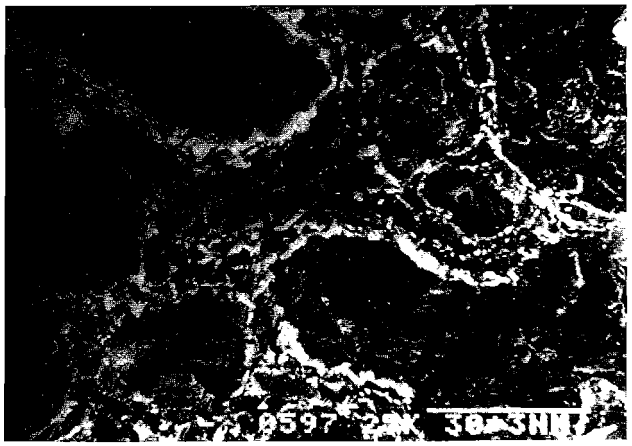

Figure 1. Scanning electron micrograph of lactating mammary tissue (×500). Alveoli are closely packed with their lumen exposed. Alveoli epithelium and craters left by exit of fat droplets are clearly seen

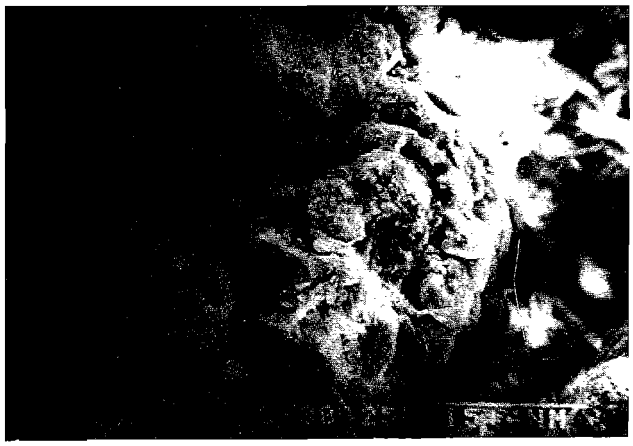

Figure 3. Scanning electron micrograph of lactating mammary tissue (×200). Arrangement of group of alveoli into one complete lobule can be seen

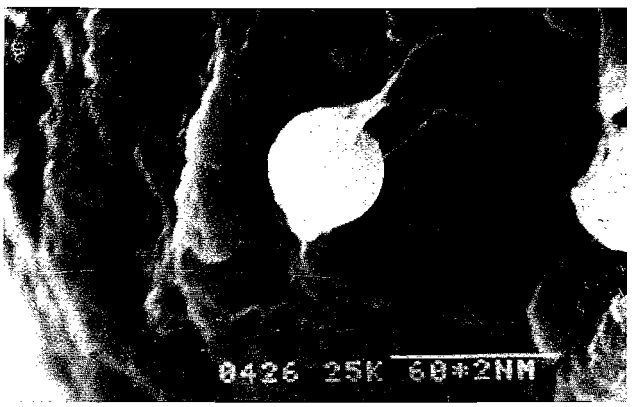

Figure 2. Scanning electron micrograph of lactating mammary tissue (×500). From the exposed alveolar lumen the fat globule formation can be seen. The fat globule is still attached to the alveolar surface by microvilli

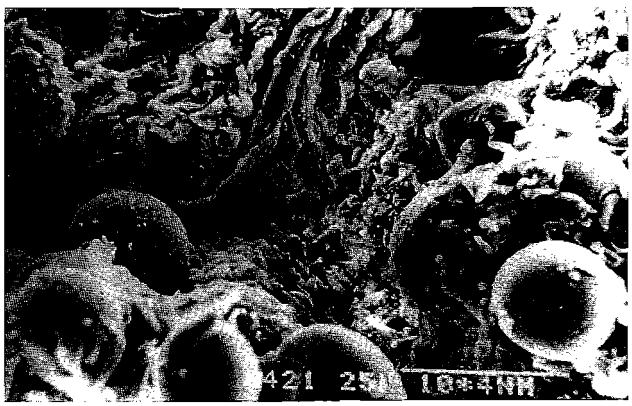

Figure 4. Scanning electron micrograph of lactating mammary tissue (×300). Two separate clusters or bunches of alveoli are visible. These bunches are arranged into two lobules which are separated by interlobular connective tissue

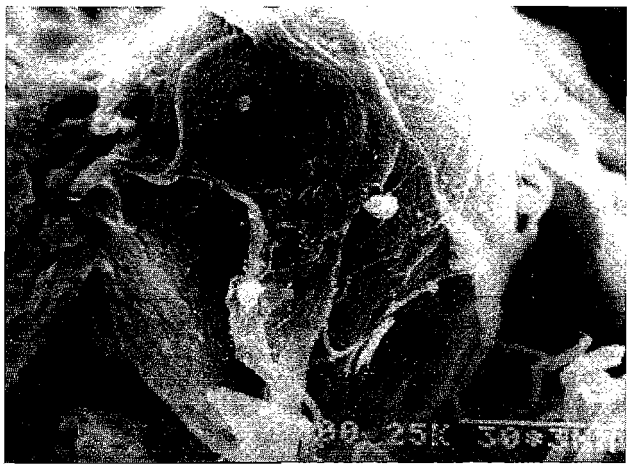

Figure 5. Scanning electron micrograph of lactating mammary tissue (×1000). A single alveolus is visible which is surrounded by the blood vessels embedded in the fascia. On the extreme right side of the alveolus, the body of the myoepithelial is exposed from where the nucleus is clearly visible

grouped together into lobes. Figure 3 shows one complete lobule having several alveoli of different sizes. In figure 4 bunches of spherically shaped alveoli showing the two lobules seperated by interlobular connective tissue can be seen. Nemanic and Pitelka (1971), however, reported that structure of mice mammary gland as bunches of grapes does not fit the three dimensional structure revealed by scanning microscopy. Figure 3 clearly shows a single alveolus which is surrounded by the blood vessels and by myoepithelial cells which are very intimately attached as reported by Richardson (1949). The nucleus of one of the myoepithelial cells is clearly visible. Caruolo (1980) and Nagato et al. (1980) were able to scan and count the myoepithelial cells present per alveolus in the lactating rat mammary lobule by this technique.

By the use of SEM large surface areas of the buffalo mammary gland could be examined in detail. This technique confirmed the three dimensional concepts of mammary gland gathered through light and transmission electron microscopy. Our study was limited to few samples, however, work of SEM on the alveolus can further be initiated by removing the connective tissue from the alveolus by enzyme and acid treatment (Caruolo, 1980), which can help in future elucidation of the myoepithelial cells role in milk ejection of buffaloes. Thus, there seems to be a good scope of using this technique on the buffalo mammary gland as it will not only provide new information about the surface structures but will also act as good indices for knowing the phsiological state of the gland.

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