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# Gross anatomy, topography and histology of murine mammary fat pads with study of ductal architecture using whole mount technique#

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**Abstract**

*Mice are commonly used as a model organism in human breast cancer research. Understanding the normal histology of the mouse mammary gland is necessary to appreciate any abnormality in the tumour microenvironment. This study focuses on the morphological variations occurring in the mouse mammary gland in relation to their parity with special emphasis on orthotopic sites for tumour induction. The gross anatomy, topography and histology of the mammary gland has been described. The predominance of adipose tissue makes mice mammary fat pad unique. In nulliparous mice, ductal structures could be identified in all the five pairs of mammary fat pads. Adipose tissue was present in all the five mammary fat pads being the least in the cervical (first) mammary fat pad. Understanding the ductal architecture of murine mammary fat pads will be great importance to researchers for constructing mouse intra-ductal models (MIND) models. From the whole mount technique of the fourth mammary fat pad, it was observed that the mammary fat pad in mouse contained a single lactiferous duct, branched into four to five secondary ducts, which in turn branch into numerous tertiary ducts and ended in a bulbous structure called terminal end buds (TEBs). No division into separate lobules unlike in humans. The sub-iliac lymph node was located within the fourth mammary fat pad. In parous animals, the terminal end bud structures elongate and proliferate to form lobulo-alveolar units. Adipose tissue was scant as compared to the nulliparous gland. For the whole mount technique, an extended fixation time of 12 hours in*

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*Carnoy's fixative was recommended for the parous mammary gland.*

**Keywords:** *Mammary gland, terminal end buds, whole mount, ductal structures*

Mice are extensively employed in cancer research as experimental models. Due to their close evolutionary relationship, physiological resemblance to humans, convenience of laboratory maintenance, breeding and abundance of diverse inbred varieties, mice have been a long-standing choice for mimicking human biology and studying diseases. A thorough knowledge of anatomy and histology of the mammary glands are necessary to avoid erroneous interpretation in disease and in tumour microenvironment studies related to breast cancer. There are few studies on the anatomy and histology of the mouse mammary gland, however there is a paucity in the structural details reported.

Mammary fat pads are five in number corresponding with the nipples and are located in the cervical, thoracic, abdominal and inguinal regions. Mammary glands develop from the ectodermal thickening. Milk buds are the more distinct thickening that forms nipple. Each gland terminates into a single collecting duct that releases milk through the nipple. The pubertal age for mice is approximately five weeks (McNally and Stein, 2017). Male mice lack nipples because of degeneration of bud epithelium during embryonic development (Kratochwil, 1971).

This study focuses on the morphological and histological study of the five pairs of mouse mammary fat pads and detailed study on the duct and the differences observed in relation with parity.

## Materials and methods

Six mice (nulliparous) in the age group of six to eight weeks weighing 20-25 grams were selected for the study. Samples of parous mammary glands were collected from post-mortem samples acquired from small animal breeding station (SABS), CVAS, Mannuthy. Samples were formalin fixed, paraffin embedded and routine staining procedures were carried out with hematoxylin and eosin staining. Special staining with Masson's trichrome and van Gieson's method for collagen fibres, Gomori's method for elastic fibres and McManus method for glycogen was done. Whole mount technique with carmine alum was performed to visualize epithelial structures (Plante *et al.,* 2011). All animal experiments were done according to the committee for the purpose of control and supervision of experiments on animal guidelines and Institutional Animal Ethics Committee approval (CVAS/MTY/IAEC/23/29).

## Results and discussion

Mice are the most commonly used animal models in breast cancer studies. Orthotopic implantation of tumour cells are characterised by implantation of tumour cells into the same anatomical site, from where it was developed (Soares-Sousa *et al*., 2019). Transplantation of tumour cells into the mice mammary fat pad is the most widely used technique for generating induced breast cancer models, and hence, understanding the normal anatomy is crucial in interpretation of results.

### *Gross anatomy of the mammary fat pads*

Mammary fat pads are placed symmetrically on both sides and are pale white in colour. The cervical, thoracic, abdominal and inguinal mammary fat pads are continuous with the subcutaneous fascia and extend laterally.

### *Topography of the mammary fat pads*

The position of the mammary fat pads have been depicted in the schematic diagram in Fig. 1a. Cervical mammary fat pad is small in size compared to the other fat pads and is distributed on lateral aspects of the cervical region Fig. 1b. The thoracic mammary fat pad was located lateral to the thoracic vertebrae Fig. 1c. The abdominal mammary fat pad was distributed around the lumbosacral region Fig. 1d. The inguinal mammary fat pad was continuous with the abdominal mammary fat pad and extended into the inguinal region Fig. 1d. In comparison to nulliparous animals, mammary glands of parous animals were grey in colour, voluminous and thicker, had lesser fat and extended to a region of greater extent. In

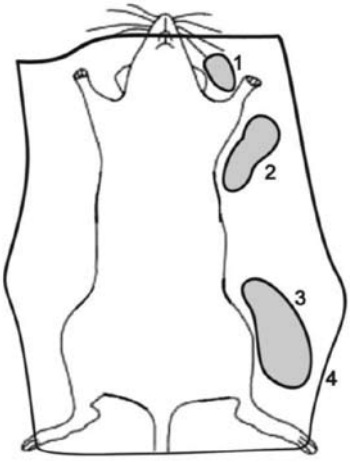


**Fig.1a.** Schematic diagram representing the topographical location of mammary fat pads in mice (1-cervical, 2-thoracic, 3-abdominal, 4- inguinal)

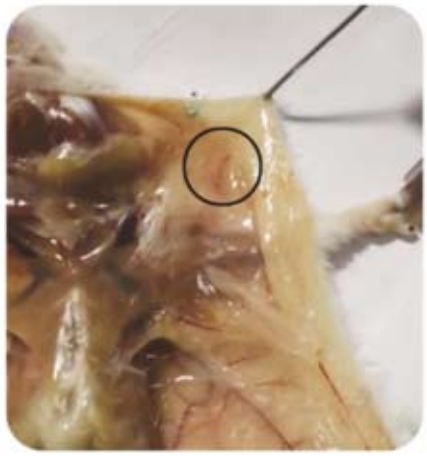

**Fig.1b.** Gross appearance of cervical mammary fat pads

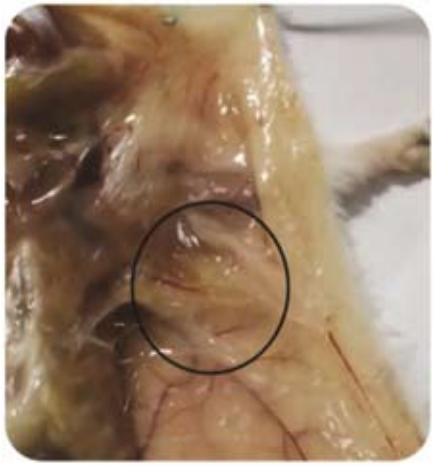

**Fig.1c.** Gross appearance of thoracic mammary fat pads

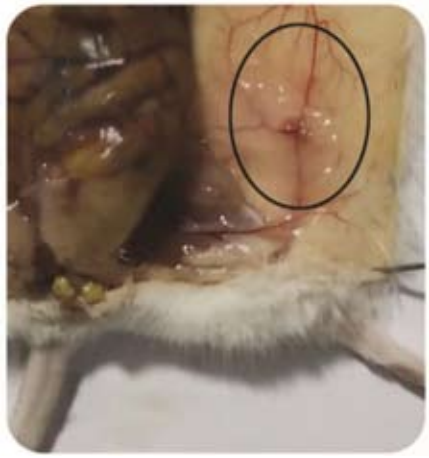

**Fig.1d.** Gross appearance of abdominal and inguinal mammary fat pads

**Plate 1.** Gross and topographic anatomy of murine mammary fat pads

comparison to nulliparous animals, mammary glands of parous animals were grey in colour, voluminous and thicker, had lesser fat and extended to a region of greater extent.

***Histology of the mammary fat pads***

Histologically, the pubertal mammary fat pad of mice was composed chiefly of adipose tissue and few ducts (Plate 2a). The lactiferous ducts were lined by two layers of cells, luminal epithelial cells and basal myoepithelial cells. The myoepithelial cells formed a basal layer of stellate cells with pale to clear cytoplasm. Radice *et al.* (1997) identified myoepithelial cells by immunohistochemical techniques using antibody targeting smooth muscle actin. Scanty connective tissue fibres and fibroblasts were seen surrounding the blood vessels and ducts and shown in (Plate 2b and 2c). Presence of elastic fibres in the blood vessels of mammary fat pad and glycogen in the ductal epithelium were studied by special staining techniques (Plate 2d and 2e). Keratinized squamous epithelium of the skin over the mammary fat pad was found in close association with the mammary gland. Sebaceous glands and hair follicles could be found overlying the gland. In the thoracic mammae, muscle cells were interspersed among them.

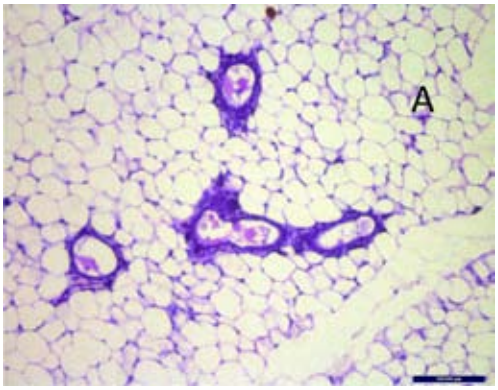


**Fig. 2a.** Transverse section of the nulliparous mice mammary fat pad containing predominant adipocytes (A) with ductal structures. H&E x 100

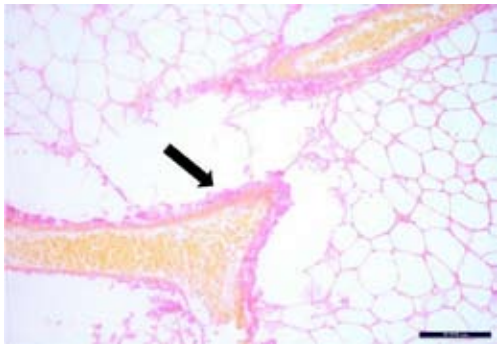

**Fig. 2c.** van Gieson's method x 200 (collagen- red)

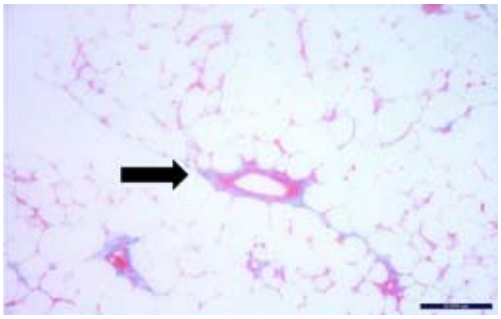

**Fig. 2b.** Masson trichrome staining x 200 (collagen-blue)

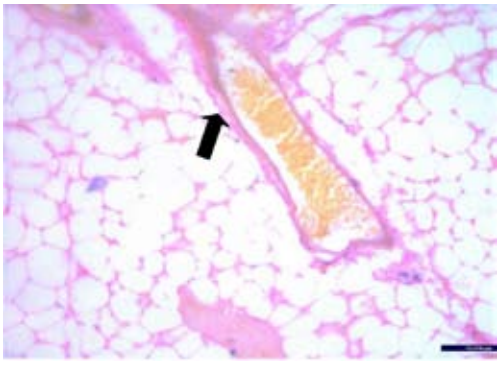

**Fig. 2d.** Gomori's method for elastic fibres x 200

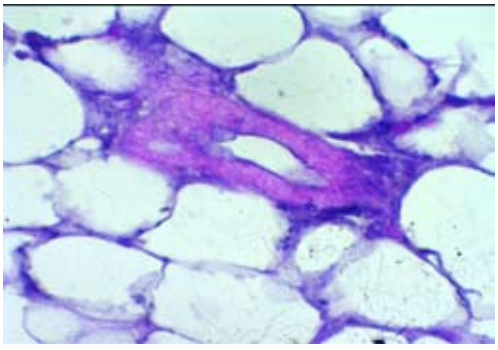

**Fig. 2e.** McManus method for glycogen (PAS x 1000)

**Plate 2.** Histological study on mouse mammary fat pad

In nulliparous mice, ductal structures could be identified in all the five pairs of mammary fat pads. Adipose tissue was present in all the five mammary fat pads being the least in the cervical (first) mammary fat pad. In parous mice, ductal structures with secretions could be identified in all the five pairs of mammary fat pads. Adipose tissue was present in all the five mammary fat pads.

***Ductal Architecture by whole mount technique***

From the whole mount technique of the fourth mammary fat pad, it was observed that the mammary fat pad in mouse contained a single lactiferous duct which was also named as primary duct (Plate 3a). This duct branched into four to five secondary ducts, which in turn were found to branch into numerous tertiary ducts. Tertiary ducts further branched within the adipose tissue of the mammary fat pad and ended in a bulbous, club-like structure called terminal end buds (TEBs) (Plate 3b). Silberstein (2001) explained that when the extremities of the fat pad are reached, the end buds shrink in size and become mitotically inactive and there will be sufficient room still exists to allow for the expansion of this network, enabling the formation of tertiary lateral branches that emerge during each diestrum

and pregnancy. Treuting *et al.* (2012) reported similar observations that mice mammary gland contains single lactiferous duct which branches into secondary ducts. with terminal end bud structures. Humpreys *et al.* (1996) described that terminal end buds contain body cells and cap cells of which body cells give rise to mammary epithelial cells and the cap cells are myoepithelial precursors. There were no division into separate lobules unlike in humans. Peixoto *et al.* (2015) noted the presence of sub-iliac lymph node in the abdominal mammary fat pad and can also be easily observed in the whole mount technique of the gland.

In nulliparous animals, the tertiary ducts were encapsulated by bulbous structures, the terminal end buds (TEBs), however, in parous animals, the terminal end bud structures elongate and proliferate to form lobulo-alveolar units. Ingman and Robertson (2011) reported that during puberty, primary ducts elongate, TEBs bifurcate and generate additional primary ducts.Nandi (1958) and Richert *et al*. (2000) reported that lobulo-alveolar unit matures only at the time of pregnancy. In parous animals, the mammary stroma is filled with alveolar units and adipose tissue was scant as compared to the nulliparous gland (Plate 3c). Silberstein (2001) observed that fully developed terminal acinar units can be seen during pregnancy and lactation. An extended fixation time of 12 hours in Carnoy's fixative was recommended for the whole mount of parous mammary gland.

McNally and Stein *et al*. (2017) explained that the gland losses its lobuloalveolar structures, leading to a decrease in size and return to a morphological stage resembling that of the nulliparous state when the young ones are weaned. Treuting *et al.* (2012) described the involuted mammary gland resembled of the virgin type by losing the alveoli. It rapidly losses of epithelium and remodelling of the gland to the pre-pregnancy simple ductal structure devoid of lobuloalveolar units.

Bolander (1990) reported that among the mammary glands of mouse, the fifth inguinal gland displays the highest level of differentiation, while the first thoracic gland exhibits the lowest degree of differentiation, depicting a gradient of specification.

All the five pairs of mammary glands in the nulliparous animals bore ductal structures making them suitable for modelling breast cancer in various orthotopic sites. Adipose tissues were observed in the nulliparous animal and it was least in the cervical mammary fat pad. Hence all the five pairs of mammary

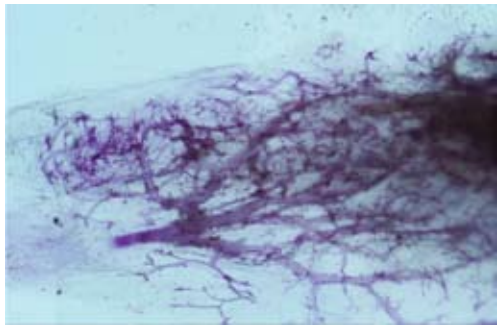

**Fig. 3a.** Mammary fat pad containing single lactiferous duct branching into secondary ducts which further divides to form tertiary ducts.

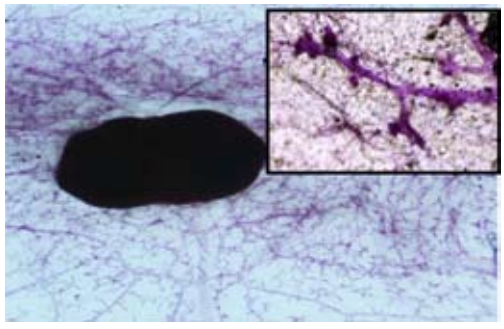

**Fig. 3b.** Nulliparous mammary fat pad of mice containing terminal end buds

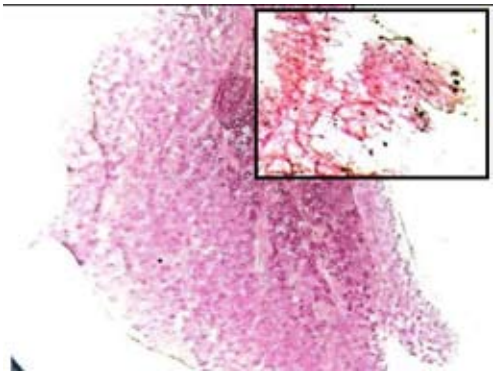

**Fig. 3c.** Parous mammary fat pad of mice containing lobulo-alveolar units

**Plate 3.** Whole mount technique of the mammary fat pad of nulliparous and parous mice

glands can be used for orthotopic models provided sufficient growth factors are provided. Fourth mammary fat pad is the most common orthotopic site for mice models of breast cancer.

**Conclusion**

Understanding the ductal architecture of murine mammary fat pads will be great importance to researchers for constructing mouse intraductal models (MIND) models. Thorough knowledge about the various morphogenetic changes occurring in the mammary parenchyma such as proliferation during pregnancy and lactation and involution during post partum period should be considered while planning the experiment. With these capabilities it can be stated that remodelling of stromal and parenchymal structures in the mammary gland during various physiological phases are closely related with the epithelial stromal interactions during progression of breast cancer. Hence vital clues may be obtained by closely studying the microenvironment. In the present work, a detailed ductal architecture and histological study has been conducted in all the five mammae with a comparison in relation to parity. Various alternative orthotopic sites for tumour induction have been suggested.

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**Conflict of interest**

The authors declare that they have no conflict of interest

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