Proliferative and Non-Proliferative Lesions of the Rat and Mouse Mammary, Zymbal's, Preputial, and Clitoral Glands

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INTRODUCTION

The INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP) to develop an internationally-accepted nomenclature for proliferative and non-proliferative lesions in laboratory animals. The purpose of this publication is to provide a standardized nomenclature for classifying proliferative and nonproliferative lesions observed in the mammary, Zymbal's, preputial, and clitoral glands of laboratory rats and mice. Standardized nomenclature of proliferative mammary gland changes (Mann *et al.*, 1996) in rats were previously published by the STP and WHO. The standardized nomenclature of mammary, Zymbal's, preputial, and clitoral gland lesions presented in this document are also available electronically at the goRENI website on the internet (http://www.goreni.org/).

While our charge and primary goal has been to provide a morphology-based terminology applicable to the rat and mouse, the extended scientific community is working towards the harmonization of nomenclature and diagnostic criteria across species. This is born of the genomic revolution and the one gene, one medicine concept that now permeates medicine and is actualized by the genetic engineered mouse (GEM). Clearly, "harmonization" will come through genomics. The molecules that are associated with breast and other cancers in humans are also likely to be associated with cancers in the same organ in mice. The molecular biologists are proving that the entire gene expression pattern of these tumors in mice match closely that of different classes of human breast cancers. The GEM models of human breast cancer are creating tumor phenotypes that have never been seen in spontaneous or carcinogen-induced mouse mammary tumors, some of which are also morphologically similar to human cancers. An entirely new nomenclature will be required to accommodate these newly created animals. The nomenclature will need to begin with the molecule and include the microscopic anatomy. Although the "mapping" of these terms across species is not complete, the toxicological pathology community will have increasing contact with these newly created animals. We will need to be familiar with their pathology and will need to understand their similarities to and difference from their human counterparts. For this reason, throughout this publication there has been an attempt to incorporate the emerging data from GEM models as it relates to nomenclature and diagnostic criteria.

The mammary gland of laboratory rodents is an important organ for the evaluation of effects of xenobiotics, especially those that perturb hormonal homeostasis or are potentially carcinogenic. Mammary gland cancer is a leading cause of human mortality and morbidity worldwide (Jemal *et al.*, 2009) and is a subject of major research efforts utilizing rodent models (Cardiff *et al.*, 2000). Zymbal's, preputial, and clitoral glands are standard tissues that are evaluated in animal models that enable human risk assessment of xenobiotics. A widely accepted and utilized international harmonization of nomenclature for mammary, Zymbal's, preputial, and clitoral gland lesions in laboratory animals will decrease confusion among regulatory and scientific research organizations in different countries and will provide a common language to increase and enrich international exchanges of information among toxicologists and pathologists.

Proliferative lesions in laboratory rodents may arise from infectious agents or changes in hormonal homeostasis as part of the aging process or experimentally as a result of genetic engineering, but the most toxicologically important proliferative mammary gland lesions are caused by exposure to potentially toxic test materials that effect hormonal homeostasis or cause cellular damage. Cellular damage from repeated exposure to toxicants induces a repair process in which the damaged tissue, if return to normal morphology is not complete, may continue to proliferate (hyperplasia) and/or undergo metaplasia to a different, more resistant cell type. Site of these changes is heavily dependent upon the nature of the toxicant and the type of tissue exposed.

Non-proliferative lesions in general are also associated with experimental perturbation or are a result of degenerative changes frequently associated with aging. Modern laboratory animal management practices within rodent facilities are such that spontaneous infectious processes should be infrequently encountered; thus the lesions related to infectious diseases are not described in detail in this document. For both proliferative and non-proliferative lesions, genetic engineering in an animal may also result in a model with novel and important lesions that require consistent terminology across laboratories and descriptions that aid in translation to human disease. These are described or referenced in this document.

Genetic Engineered Mouse Models:

Genetic engineering of mice has introduced literally hundreds of new mouse models of human breast cancer. The new models can be divided into four categories (Cardiff *et al.*, 2007): Models that 1. Recapitulate spontaneous and chemically induced models; 2. Develop unique Genotype-specific "signature" phenotypes; 3. Mimic human morphological phenotypes; and 4. Simulate human genotypes or molecular phenotypes.

<u>Mouse models that recapitulate spontaneous and chemically induced mouse models</u> (Figures 34-41)

1. *Virus-induced tumors*. Activation of mouse DNA by integration of mouse mammary tumor virus (MMTV) is the primary mutagenic event leading to "spontaneous" mouse mammary tumors. The original descriptions of mouse mammary tumors by Apolant and Haaland are based on "spontaneous" tumor induced by MMTV (Apolant, 1906, Haaland, 1911). The classification of Dunn is also based on MMTV-induced tumors (Dunn, 1959). Molecular analysis revealed that most of these tumors have activation of wnt, FGF and/or notch in various combinations. Interestingly, when mice were specifically engineered using molecular constructs containing wnt or FGF behind the MMTV-LTR promoter, they developed Type A, Type B or Type P tumors (Sass and Dunn, 1979). Equally important, activation of one oncogene, such as wnt, resulted in co-activation of the other complementary oncogene, such as FGF (van Leeuwen and Nusse, 1995).

2. *Chemically induced tumors.* A limited number of chemically-induced tumors have been analyzed but the common carcinogens such as DMBA or MCA result in mutational activation of the H-ras gene (Cardiff *et al.*, 2000) and activation of the Wnt1 pathway(Currier *et al.*, 2005). These tumors are frequently keratoacanthomas or adenosquamous carcinomas with squamous metaplasia. The tumors with squamous metaplasia have activation of the wnt-APC-B-Catenin pathway (Tsukamoto *et al.*, 1988, Gaspar *et al.*, 2009, Michaelson and Leder, 2001). Other tumor types have not been analyzed.

Mouse Models that develop unique genotype-specific "signature" phenotypes

Most GEM develop tumor phenotypes that never have been previously seen in the mouse. The key observation is that most oncogene activation results in tumors with gene-specific signature phenotypes and oncogene activation from the same pathway results in tumors with similar phenotypes (Rosner *et al.*, 2002). The myc, ras and neu tumor phenotypes are unique and distinguishable (Cardiff *et al.*, 2000). Tg(Myc) mice develop tumors with large pleomorphic nuclei and coarse chromatin with abundant amphophilic cytoplasm. These resemble a mammary counterpart to Burkitt's lymphoma. Tg(Ras) tumors form papillary structures with small cells with oval nuclei and red cytoplasm (Sinn *et al.*, 1987), resembling human transitional cell carcinomas of the urinary bladder. The Tg(cNeu) tumors tend to be solid nodules with intermediate sized nuclei and light pink-orange cytoplasm (Muller *et al.*, 1988, Komitowski *et al.*, 1982). With central necrosis, they resemble human comedo-carcinomas. The previously mentioned wnt phenotype is distinctive but well characterized. Moreover, mutation of other molecules in the Wnt pathway, such as APC, B-catenin result in tumors with similar morphologies (Gaspar *et al.*, 2009). These phenotypes may be primarily based on cytological characteristics as a recent study of Tg(Myc) with different mutations share cytological characteristics but different histological patterns (Andrechek *et al.*, 2009). Mammary tumors in Tm(PTEN-/-) mice characteristically are adenomyoepitheliomas (Stambolic *et al.*, 2000). No molecular studies of sporadic, spontaneous adenomyoepitheliomas have been performed. The Epithelial-Mesenchymal-Transition (EMT) tumor phenotype is a special type that has been previously recognized as a carcinosarcomas (Barnes *et al.*, 2005). In GEM, EMT occurs in the context of loss of the expression of the initiating oncogene and p53 mutations (Debies *et al.*, 2008). Such tumors have a range of phenotypes ranging from a mixed adenocarcinoma and spindle cell tumor to a pure spindle cell tumor (Damonte *et al.*, 2007, Cardiff, 2010, Radaelli *et al.*, 2009). EMT is most easily verified using the criteria of dual staining with mesenchymal and epithelial markers (Damonte *et al.*, 2007). Some observers have speculated that these are metaplastic carcinomas. However, molecular evidence to support this hypothesis is lacking. There are numerous additional examples; further discussion is beyond the scope of this article.

Mouse tumors that mimic the histopathology of human tumors

With genetic engineering, numerous candidate oncogenes have been tested by targeting the mouse mammary gland. Tumors that most closely resemble their human counterparts include the Tg(c-ErbB2) and Tm(CDN1-/-xp53-/-). Many Tg(c-ErbB2) tumors resemble their human counterparts that over express this same gene (Bouchard *et al.*, 1989, Ursini-Siegel *et al.*, 2007). The Tm(CDN1-/-xp53-/-) tumors have the typical single file configuration of human lobular carcinoma (Derksen *et al.*, 2006).

Mouse models and molecular classification

Recently, molecular classifications have been adapted to the clinical classification of human no-specific type (NST) breast cancer (Weigelt and Reis-Filho, 2009). This has been followed by attempts to classify mouse mammary tumors with the same expression arrays. Since the vast majority of mouse tumors promoted by the MMTV-LTR do not express ER, PR or Her2, they all fall into the "triple negative" or basal category of human NST breast cancer (Weigelt and Reis-Filho, 2009). In "lumping" clinical outcomes with expression profiles they circumvent the traditional morphological classifications. These attempts to match

mouse and human molecular expression profiles have not included rigorous attempts to match histological patterns with the expression profiles. Attempts to match the human molecular categories also ignore the rich variation in GEM tumors leading to a potentially misleading grouping of genotypes. The challenge for the next iteration of INHAND will be to anticipate the movement towards molecular classifications of breast cancers and to harmonize them with the traditional structure-based classifications.

MORPHOLOGY

I. MAMMARY GLAND:

Embryologic development of the rat and mouse mammary gland has been investigated extensively and will only be briefly considered here (for reviews see (Ceriani, 1970, Knight and Peaker, 1982, Russo *et al.*, 1989). The male and female rat and mouse mammary gland develops from a single layer of cuboidal epithelium originating from the milk bud that forms the primitive nipple, progressing in a cephalo-caudal sequence (Myers, 1917, Cardiff and Wellings, 1999). Islands of epithelium thicken into hillocks of cuboidal cells resting on an indistinct basal membrane; simultaneously there is atrophy of the epithelial cells between the hillocks (Myers, 1917). In the male or female fetus, mammary glands remain as rudimentary buds of epithelium without development into distinct lobules or alveoli (Knight and Peaker, 1982).

In the rat and mouse the development and function of the mammary gland is directed by numerous hormones including estrogens, androgens, progesterone, prolactin, growth hormone, insulin, catecholamines, and ACTH (Russo and Russo, 1996). Androgens initiate differentiation of the male phenotype by promoting atrophy of the rudimentary buds in the male (Goldman *et al.*, 1976, Sourla *et al.*, 1998b, Sourla *et al.*, 1998a). This atrophy of the buds is triggered by testosterone-induced condensation of the stroma (Topper and Freeman, 1980). Mammary gland morphology in the male rat is altered (feminization) to the morphology of the female rat if androgens are eliminated and androgens administered to female rat fetuses cause mammary glands to appear male-like (masculinization) (Goldman *et al.*, 1976). Prolactin is an important pituitary hormone controlling rodent mammmary gland growth. While the precise roles of estrogens (E) and progesterone (P4) in the rat fetus are less clear, studies in mice and ex vivo mammary gland explants suggest that progesterone is not critical to embryonic development of mammary glands in mice and its absence in utero does not affect the potential for maturation in adulthood (Freeman and Topper, 1978). Estrogens promote mammary gland development in the mouse fetus; however E inhibits development in the rat fetus (Ceriani, 1970).

The neonatal male and female rat and mouse have 6 and 5 pairs of glands, respectively, with a single, central lactiferous duct, several branching secondary ducts and numerous tertiary ducts (Ceriani, 1970, Cardiff and Wellings, 1999). In the rat, the glands are distributed in pairs along the milk line, with 1 pair cervical, 2 thoracic, 1 abdominal and 2 inguinal (Astwood *et al.*, 1937). Distribution in the mouse is in the neck, chest wall (2 pairs), abdominal wall, and inguinal region (Cardiff and Wellings, 1999). Mammary tissue is embedded in a mass of adipocytes, pre-adipocytes and fibroblasts, referred to as the fat pad, with a thin layer of stroma separating the epithelial cells from the fat pad (Hovey *et al.*, 1999, Imagawa *et al.*, 2002, Silberstein, 2001). In the pre-pubescent female rat, mammary growth is mainly influenced by growth hormone (GH) and prolactin (PRL) with minimal influences of estradiol (E2) and P4 (Knight and Peaker, 1982).

Mammary growth in the male and female mouse and rat during puberty is dependent on normal gonadal

function, as indicated by the absence of development in ovariectomized or gonadectomized rats (Cowie and Folley, 1961). While a detailed description of pubescent mammary gland development in males is not published, in females, growth of the mammary gland during puberty is characterized by differentiation of the epithelium into terminal end bud units (TEBs); rapid expansion by elongation and branching of the ducts; and hypertrophy of the fat pad (Cowie and Folley, 1961, Russo *et al.*, 1989, Knight and Peaker, 1982, Cardiff and Wellings, 1999). New end buds form from lateral branching of mature ducts and have the potential to form lobules. The TEBs are the major hormone sensitive area of the mammary glands for sexually mature rats and mice. Prolactin, estrogen, and progesterone are the predominant hormones controlling rodent TEB development to lobular alveolar structures in the virgin rodent (Richards *et al.*, 1983) In rats, which have a sexually dimorphic mammary gland (Figures 4, 5), androgens are also important steroid hormones that influence adult mammary gland morphology (Rudmann *et al.*, 2005, Lucas *et al.*, 2007).

In female mice and rats, ducts, ductules, and alveoli are lined by 1 or 2 layers of epithelial cells and surrounded by myoepithelial cells (Figures 1-3). Within these epithelial structures are 3 types of epithelial cells: clear cells, dark cells, and intermediate cells. The morphologic differences between these cell populations are the result of variability in the number of ribosomes, mitochondria, lipid droplets, and secretory vacuoles (Greaves, 1990). The rat mammary gland is sexually dimorphic (Lucas *et al.*, 2007). In contrast to the female rat, ducts in male rats are infrequent and when observed are lined by a stratifed epithelium consisting of vacuolated tall cuboidal to short columnar epithelial cells (Figures 4, 5). Alveoli are predominant in the male and also lined by a stratified epithelium. Imbalance of mammotrophic hormones like prolactin and androgens may result in the male and female rat mammary gland converting to the morphologic appearance expected of the opposite sex (Rudmann *et al.*, 2005, Lucas *et al.*, 2007).

A. Degenerative Changes

Degeneration **Ductular/alveolar epithelium**

Pathogenesis/Cell of Origin: Ductular, alveolar epithelial cell; myoepithelial cell.

Diagnostic Features:

- Epithelial cells swollen
- Epithelial vacuolization/bleb formation
- Increased intercellular spaces
- Loss of organization of the cell layers
- Dilatation (ectasia) of alveoli or ducts with accumulation of secretory material

Differential Diagnoses:

- Postmortem autolysis: uniform dissolution of entire tissue with no change in organization or depth of cell layers
- Atrophy: thinning of affected ducts or alveoli

Comment: Degenerative changes in the mammary gland are rare but can be observed with toxic agent exposure and as a consequence of aging. Surrounding tissues such as the fat pad, skin, and local lymph nodes can also undergo a variety of degenerative changes.

Necrosis **Ductular/alveolar epithelium**

Pathogenesis/Cell of Origin: Alveolar or ductular epithelium

Diagnostic Features:

- Cellular swelling or shrinkage
- Cytoplasmic eosinophilia
- Pyknosis or karyorrhexis of nuclei
- Exfoliation of cells.
- May result in thinning of epithelium
- May be associated with inflammation
- Luminal accumulations of fibrin and/or cell debris

Differential Diagnoses:

- Postmortem autolysis: uniform dissolution of entire tissue with no change in organization or depth of cell layers
- Apoptosis: Cells shrunken with distinct cell membranes, membrane budding, condensed nuclei; rarely associated with inflammation
- Degeneration: cell vacuolation but no inflammation or cell debris
- Atrophy: thinning of cell layers but no inflammation or cell debris
- Inflammation: cellular infiltrates and swelling but no exfoliation or cell debris.

Comments: Mammary gland epithelial necrosis is an infrequent finding in rodents. Fat necrosis occasionally occurs in the fat pad and is usually accompanied by granulomatous inflammation and fibrosis in the rat (Boorman *et al.*, 1990)

Regeneration **Ductular/alveolar epithelium**

Pathogenesis/Cell of Origin: Alveolar or ductular epithelium.

Diagnostic Features:

- Normal appearing epithelial cells with basophilic cytoplasm
- Increased nuclear:cytoplasmic ratio
- Epithelial architecture may appear irregular
- Mitoses
- Adjacent to or within areas of degenerating, necrotic, hyperplastic, or metaplastic epithelium

Differential Diagnoses:

- Hyperplasia: epithelium is thickened due to increased numbers of cells, resulting in undulating, rugose epithelial surface and irregular arrangement of cell layers (see proliferative lesion section of this document).

- Neoplasia: expansile nodule usually protruding into cavity, with cellular atypia and compression of adjacent structures. (See proliferative lesion section of this document).

Comments: Regeneration is a term indicating the growth of cells and tissues to replace lost or damaged structures, as opposed to hyperplasia, a term denoting an increase in the number of cells beyond normal in a tissue (Kumar *et al.*, 2009). Irregularity of epithelial cell arrangement may be observed in the ductular or alveolar epithelium in the process of regeneration. Degeneration, necrosis, and regeneration are often present together in mammary gland repeatedly injured by toxicants. See comments above in sections on degeneration and necrosis.

B. Inflammatory Changes

Inflammation

Synonym: Mastitis

While inflammation of the mammary gland is an important cause of morbidity in large animal veterinary species, because of animal husbandry procedures, inflammation of the rodent mammary gland is uncommon. More commonly, histologic changes are limited usually to infiltrates of foci of leukocytes.

Inflammation, Acute **Lobule (Figure 6)**

Pathogenesis/Cell of Origin: Alveolar or ductular epithelium and associated tissues.

Diagnostic Features:

- Vascular congestion
- Edema
- Accumulation of serous, mucous or fibrinous exudate
- Neutrophils
- Sloughed epithelial cells in tubular or alveolar lumina

Differential Diagnoses:

- Necrosis: pyknosis, karyorrhexis of nuclei, cytoplasmic swelling or shrinkage, cellular debris, accompanied by inflammatory infiltrate
- Chronic active inflammation: cellular infiltrate is a mixture of granulocytic, lymphocytic, histiocytic cells, fibrosis.

Comments: Migration of neutrophils into ductal and alveolar lumina produces a suppurative exudate. Eosinophils in exudate or mucosal infiltrate may indicate an immunologic or parasitic element to the inflammatory response.

Inflammation, Chronic **Lobule**

Pathogenesis/Cell of Origin: Alveolar or ductular epithelium and associated tissues.

Diagnostic Features:

- Cellular infiltrate is predominantly lymphocytes, plasma cells, and macrophages

- Regenerative, hyperplastic, and/or metaplastic changes in the affected epithelium and fibroplasia may be present

Differential Diagnoses:

- Other types of inflammation (see below)
- Early connective tissue or hematopoietic neoplasia

Comments: Chronic inflammation may have different characteristics depending on the longevity of the lesion and the initiating cause. It is uncommon in the rodent mammary gland but can occur as a result of leakage of secretory material outside the ducts.

Inflammation, Chronic Active **Lobule**

Pathogenesis/Cell of Origin: Alveolar or ductular epithelium and associated tissues.

Diagnostic Features:

- Cellular infiltrate is a mixture of granulocytic cells with lymphocytic and/or histiocytic cell types
- Congestion, edema, mucous exudate or other evidence of acute inflammation may be present
- Regenerative, hyperplastic, and/or metaplastic changes in the affected epithelium and fibroplasia may be present

Differential Diagnoses:

- Other types of inflammation (see below)
- Early connective tissue or hematopoietic neoplasia

Comments: The term chronic active inflammation implies recurrence or persistent presence of granulocytic inflammatory cells concurrent with ongoing chronic inflammation. Chronic active and granulomatous inflammation (see below) have many etiologic and morphologic similarities.

Inflammation, Granulomatous **Lobule**

Pathogenesis/Cell of Origin: Alveolar or ductular epithelium and associated tissues.

Diagnostic Features:

- Cellular infiltrate is predominantly plump macrophages (epithelioid cells) which may form interlacing bundles, accompanied by lymphocytes, plasma cells, and fibrosis, depending on duration and etiologic agent.
- Infiltrating macrophages may form multinucleated giant cells
- Etiologic agent may be visible, e.g., fungi, mycobacteria, or foreign bodies
- Granulocytes may be present in the affected area, in which case the process could be described as pyogranulomatous inflammation.
- Hyperplasia or metaplasia of affected epithelium may be present

Differential Diagnoses:

- Other types of inflammation (see below)
- Early connective tissue or hematopoietic neoplasia

Comments: Granulomatous inflammation suggests an etiologic agent which is resistant to dissolution or is immunogenic, e.g., fungi, mycobacteria, or foreign body (Kumar *et al.*, 2009). It is important to differentiate foreign body inflammation from other inflammatory lesions, because of the differences in their causes. Granulomatous inflammation is commonly seen in rats associated with rupture of a galactocele or dilated/ectatic duct.

Infiltration, Lymphocytic or Eosinophilic **Lobule**

Pathogenesis/Cell of Origin: Alveolar or ductular epithelium and associated tissues.

Diagnostic Features:

- Infiltration of a relatively pure population of lymphocytes, plasma cells or eosinophils into the lamina propria of the lobule and associated tissues

Differential Diagnoses:

- Acute inflammation: vascular congestion, edema, serous or mucous exudate, few neutrophils present
- Other types of chronic inflammation (see above)
- Hematopoietic neoplasia: homogenous lymphocyte population infiltrating entire tissue and other sites.

Comment: Leukocytic infiltrates consisting only of lymphocytes or plasma cells may indicate an immunologic effect of a xenobiotic but can also be observed as a spontaneous change.

Fibrosis, periductal **Lobule (Figure 7)**

Pathogenesis/Cell of Origin: In mice, animals treated with EGF/periductular fibroblasts

Diagnostic Features:

- Increased thickness in the connective wall of mostly medium size ducts

Differential Diagnoses:

- Fibrosis in chronic inflammation

Comment: This lesion is commonly seen in aged rats. In BALB/c mice treated with EGF the most evident histological feature is proliferation of small and medium size ducts. This proliferation is associated with an evident increase in the interlobal connective tissue as well as periductal fibrosis (Molinolo *et al.*, 1998).

C. Vascular Changes

Congestion **Lobule**

Synonym: Hyperemia

Pathogenesis/Cell of Origin: Vasculature of mammary gland and surrounding tissue

Diagnostic Features:

- Widely dilated, blood-filled vessels

Differential Diagnoses:

- Postmortem autolysis: uniform dissolution of entire tissue with lysis of red blood cells
- Angiectasia: Dilated vessels that distort normal architecture of the affected tissues

Comments: Congestion of the mammary gland and associated skin may be observed in rats dying or killed while moribund, related to terminal pooling of blood in the dependent portions of the rat.

Edema Interstitial **Fat Pad (Figure 8)**

Pathogenesis/Cell of Origin: Vasculature of mammary gland and surrounding tissue

Diagnostic Features:

- Proteinaceous fluid within fat pad, around vessels and free in lumen of ducts and alveoli

Differential Diagnoses:

- Postmortem autolysis: uniform dissolution of entire tissue with lysis of red blood cells
- Fibrinous exudate: smudged pink exudate with laminar appearance of fibrils visible at high magnification.

Hemorrhage **Lobule**

Pathogenesis/Cell of Origin: Lumen of ducts and alveoli and associated tissues

Diagnostic Features:

- Presence of free blood in duct and alveolar lumen or in adjacent fat pad

Differential Diagnosis:

- Angiectasia: blood present within dilated vascular lumens

Angiectasis **Lobule**

Pathogenesis/Cell of Origin: Vasculature of fat pad

Diagnostic Features:

- Increased profiles of blood vessels, distorting the normal architecture of the affected tissue

Differential Diagnoses:

- Hemangioma: blood-filled space lined with uniform endothelial cells, distorting the architecture of the affected tissue.
- Congestion: widely dilated, blood-filled vasculature not distorting the architecture of the affected tissue.
- Hemorrhage: free blood present outside vascular walls

Thrombosis **Lobule**

Pathogenesis/Cell of Origin: Vessels of mammary gland and fat pad

Diagnostic Features:

- Amorphous pink/grey, clearly laminated mass containing leucocytes and erythrocytes
- Attachment to lumen of vessel is rarely visible on routine sections

Differential Diagnosis:

- Postmortem clot: few or no leukocytes; lamination absent or very fine filaments

Comments: Thrombi are also often associated with mononuclear cell leukemia in rats, or a generalized debilitating condition.

D. Other Lesions

Corpora Amylacea **Ductular/alveolar epithelium (Figure 9)**

Pathogenesis/Cell of Origin: Alveolar or ductular epithelium and adjacent lamina propria, lumen of alveolus or duct.

Diagnostic Features:

- Small basophilic or amphophilic concretions
- Often laminar with mineralized areas
- May be associated with dilatation of ducts and alveoli

Differential Diagnoses:

- Necrosis: other evidence of tissue damage
- Degeneration: other evidence of tissue damage
- Mineralization: evidence of mineralization at other sites or tissues

Comments: Corpora amylacea are seen infrequently in untreated rats and mice. Corpora amylacea which stain eosinophilic with hematoxylin and eosin may represent amyloid.

Amyloid **Lobule**

Pathogenesis/Cell of Origin: Extracellular deposits of polypeptide fragments of a chemically diverse group of glycoproteins (Solomon *et al.*, 1999) in various tissues.

Diagnostic Features:

- Lightly eosinophilic, amorphous extracellular material in submucosa or interstitium
- Green color using polarized light with Congo Red stain

Differential Diagnoses:

- Necrosis: Congo Red negative; other evidence of damage
- Degeneration: Congo Red negative
- Connective tissue hyaline: Congo Red negative
- Exudate or foreign bodies: Congo Red negative; other evidence of exudate or foreign body

Comments: Amyloidosis occurs in various tissues of aged mice of several strains (Korenaga *et al.*, 2004) but has only been rarely described in the literature in the mammary gland of rodents (Beems et al. 1978).

Dilation **Duct/alveolus (Figure 10-12)**

Synonyms: Cystic degeneration, cystic change, milk cysts, ectasia, galactocoele

Pathogenesis/Cell of Origin: Ducts and alveoli

Diagnostic Features:

- Dilated lumen with or without epithelial hypertrophy or hyperplasia
- Often luminal proteinaceous material, lipid, macrophages, or cell debris
- Often vacuolation of ductal/alveolar epithelial cells

Comments: Dilation may occur as a spontaneous aging change in association with mammary gland hyperplasia and metaplasia. In younger animals treated with xenobiotics, the change suggests perturbation of the hypothalamic-pituitary-gonadal axis. Severity grades and report descriptions may be used to indicate the extent of ductal and alveolar involvement and the size of the affected lumen diameters.

Pigment **Lobule (Figure 13)**

Synonyms: Pigmentation, accumulation

Pathogenesis/Cell of Origin: Intracellular deposits of endogenous substances which often may be differentiated using special histochemical, immunohistochemical, or electron microscopic procedures

Differential Diagnoses: Mineralization

Diagnostic Features:

- Various cell populations in the mammary gland contain intracytoplasmic endogenously-derived pigments

Comments: Pigments usually represent lipofuscin, hemosiderin or other hemoglobin breakdown products.

Mineralization **Fat Pad**

Pathogenesis/Cell of Origin: Fat pad

Diagnostic Features:

- Linear mineralization of vessels, visible on H&E sections, may be confirmed with stains for minerals
- Frequently accompanied by macrophages and inflammation

Comments: Mineralization of vessels may be present in severe cases of chronic nephropathy of aged rats.

E. Growth Disturbances

Non-neoplastic

Atrophy **Lobule (Figure 14)**

Synonyms: Feminization (male rat)

Pathogenesis/Cell of Origin: Ductular and alveolar epithelial cell

Diagnostic Features

- Tubular and alveolar profiles present but in decreased numbers or have thinned epithelium
- Tubular or alveolar lumina may appear dilated
- Relative amount of fat pad increased

Differential Diagnoses

- Hypoplasia: Tubular and alveolar profiles present but in decreased numbers due to developmental anomaly

Comments: Inconsistent sectioning of the mammary gland may result in the erroneous diagnosis of atrophy. Xenobiotics may perturb the hypothalamic-pituitary-gonadal axis resulting in a decrease in circulating, mammotrophic hormones resulting in glandular atrophy. In male rats, xenobiotics that increase prolactin will result in atrophy of tubular and alveolar profiles resulting in a tubulo-alveolar appearance of the gland similar to what is observed in the normal virgin female gland **(Figure 15)** (Ose *et*

al., 2009).

Hypertrophy, lobuloalveolar **Lobule (Figure 16)**

Synonyms: Masculinization, virilization (female rat)

Pathogenesis/Cell of Origin: Altered estrogen:testosterone balance results in ductular and alveolar epithelial cell alterations in female rat

Diagnostic Features

- Increased cell size
- A pseudostratified appearance to epithelium
- Increased abundance of cytoplasm
- Cytoplasmic basophilia and vacuolation
- May have increased ductular or alveolar secretions
- May accompany ductular and/or alveolar hyperplasia

Differential Diagnoses
Degeneration: Bleb formation, epithelial layer disorganization, and increased intercellular spaces

Comments: In female rats, xenobiotics that cause an increase in androgens may induce an alteration in the mammary gland characterized by hypertrophy and hyperplasia of alveolar and ductal epithelium resulting in a lobulo-alveolar appearance more like the normal male mammary gland, so called masculinization (Rudmann *et al.*, 2005, Lucas *et al.*, 2007).

Hyperplasia, lobular **Lobule (Figure 17, 18)**

Pathogenesis/Cell of Origin: Mammary gland epithelium of alveoli and ducts

Diagnostic Features

- Regular lobular architecture is maintained.
- Normal relationship between ductal and alveolar epithelial cells, myoepithelial cells and stroma.
- Alveolar epithelial cells form one layer and are mostly cuboidal.
- Cells lining the ducts vary from columnar to flattened.
- Cystic dilation may be present
- Alveoli and ducts may be distended with eosinophilic material as evidence of secretory activity.
- Little or no cellular pleomorphism.
- Focal squamous cell metaplasia may occur.
- Diffuse: The total mammary gland is affected. The mammary fat pads are filled with glandular acini.
- Focal: May occur as a single or multifocal lesion. One or more lobules are affected. Lack of compression or encapsulation. Lobule enlarged due to an increase in number and size of normal appearing alveoli and ducts. Hyperplastic lobules merge with the surrounding normal lobules. Usually lack of a prominent and homogenous fibrous connective tissue, but focal reactive fibrous connective tissue may occur.
- Focal with atypia **(Figure 19)**: One or more alveoli or ducts within a lobule are affected. Either focal, irregular proliferation of ductal or alveolar epithelium within a lobular hyperplasia or acinar, irregular proliferation of one or more alveoli or ducts within normal mammary gland tissue. Proliferation forms papillae, arches, nests extending into lumen. Small alveoli may be filled with

cells and appear solid. Small areas of cellular atypia and/or pleomorphism, nuclei may be enlarged, hyperchromatic or vesicular, cytoplasm may be secretory and stain basophilic or eosinophilic.

Differential Diagnoses
ADENOMA: Tumor well demarcated from the adjacent mammary tissue. The regular architecture of the gland is distorted
FIBROADENOMA: Well defined. Uniform structures within the epithelial and connective tissue components. Ducts and acini are surrounded by layers of proliferative fibrous tissue
ADENOCARCINOMA: Structural or cellular pleomorphism. Detachment of epithelial cells from the basement membrane. Infiltrative growth; occurrence of distant metastasis

Comments: Diffuse hyperplasia is usually physiologic and is prominent in late gestation and during lactation. Lobular alveolar hyperplasia may result from xenobiotics that increase prolactin in female rats.

Neoplastic

Adenoma **Mammary gland (Figure 20, 21)**

Pathogenesis/Cell of Origin: Mammary gland epithelium.

Diagnostic Features

- Often macroscopically seen as nodule.
- Tumor is well demarcated from the adjacent mammary tissue.
- Compression of surrounding tissue may occur.
- May be encapsulated.
- The regular architecture of the gland is distorted
- Increase in size and variability in diameter of glandular lobules.
- Tightly packed epithelial structures.
- Alveoli may show varying size.
- Epithelium may be single or double layer.
- Epithelial cells are still attached to the basement membrane.
- Cells are well differentiated.
- Cells lining the alveolar structures are cuboidal
- Secretory activity may occur.
- Scanty connective tissue is present.
- Small focal areas of atypia and/or pleomorphism may be present.
- Focal squamous cell metaplasia may occur
- Subtypes:
    - Cystic: Simple or multiloculated cystic glandular structures.
    - Papillary: Papillary structures with fibrous core covered by cuboidal to columnar epithelium.
    - Alveolar/tubular: Round or oblong glandular structures, empty or with proteinaceous secretion.

Differential Diagnoses
HYPERPLASIA: Regular lobular architecture is maintained.
FIBROADENOMA: Well defined. Uniform structures within the epithelial and connective tissue components. Ducts and acini are surrounded by layers of proliferative fibrous tissue
ADENOCARCINOMA: Structural or cellular pleomorphism. Detachment of epithelial cells from the

basement membrane. Infiltrative growth; occurrence of distant metastasis

Fibroadenoma **Mammary gland (Figure 22)**

Pathogenesis/Cell of Origin: Mammary gland epithelium and connective tissue.

Diagnostic Features

- Well defined and may be encapsulated.
- Composed of proliferated glandular epithelium surrounded by layers of proliferated fibrous tissue.
- Lobular growth pattern is present and may involve significant proportions or a total mammary gland complex.
- Uniform structures within the epithelial and connective fibrous tissue components.
- Diversity of structural lobular patterns resulting from various proportions of epithelial and fibrous connective tissue can occur.
- Secretory epithelium usually composed of a single layer, but double layering may occur, focally.
- Small focal areas of atypia and/or pleomorphism may be present.
- Mitotic figures are rare.
- Subtypes:
    - Adenomatous: Consists mainly of the epithelial component.
    - Fibromatous: The connective tissue predominates.

Differential Diagnoses
HYPERPLASIA: Regular lobular architecture is maintained.
ADENOMA: Lacks any increased connective fibrous tissue. Tumor well demarcated from the adjacent mammary tissue. The regular architecture of the gland is distorted
FIBROMA **(Figure 23)**: Lacks any epithelial elements.
ADENOCARCINOMA ARISING IN FIBROADENOMA: Adenocarcinomatous change within fibroadenoma.

Comments: Adenocarcinomatous change within fibroadenomas should be diagnosed as "adenocarcinoma arising in fibroadenoma".

Tumor, mixed, benign **Mammary gland**

Pathogenesis/Cell of Origin: At least two different cell types including mammary epithelium and mesenchymal cells.

Diagnostic Features

- Contains at least two different benign components of mammary tissue.
- An epithelial component and connective tissue components other than fibrous tissue (e.g. fat, cartilage or bone) must be present.
- Adenolipoma type: Consists of uniform epithelial ductules or alveoli and mature adipose tissue.

Differential Diagnoses
FIBROADENOMA: Consists only of acinar and/or ductal epithelial cells and proliferating connective fibrous tissue.
TUMOR, MIXED, MALIGNANT: At least one of the components has malignant features.

Comments: "Tumor, mixed, benign" summarizes all possible mixtures of benign epithelial and mesenchymal tumor components. According to well established diagnostic criteria, "fibroadenomas" are

separately classified, although they are benign mixed tumors.

Carcinoma, adenosquamous (Mouse only) **Mammary gland**

Synonym: Adenoacanthoma, malignant; keratoacanthoma

Pathogenesis/Cell of Origin: Mammary gland epithelium.

Diagnostic Features

- May be well circumscribed.
- Differentiation is glandular and squamous.
- Glandular and squamous patterns are present in varying proportions.
- Squamous areas are well-differentiated.
- Squamous cell areas cover more than 25% of the lesion.
- Areas of gradual transition may be present from round or polygonal epithelial cells to flattened squamous cells.
- Squamous cells exhibit intracytoplasmic keratin and pearl formation.
- Metastasis exhibits squamous or glandular tissue.

Differential Diagnoses
ADENOCARCINOMA: Squamous areas may be present, but cover not more than 25% of the lesion.
CARCINOMA, SQUAMOUS CELL (Skin): Only squamous differentiation is present. No evidence of glandular pattern.

Adenocarcinoma arising in Fibroadenoma **Mammary gland**

Pathogenesis/Cell of Origin: Mammary gland epithelium and connective tissue.

Diagnostic Features

- Focally adenocarcinomatous change within a well defined primary fibroadenoma.
- The histological pattern of the adenocarcinomatous component is variable.
- Multilayering, pronounced pleomorphism, atypia of epithelial cells, and hyperchromasia in the adenocarcinomatous areas.
- Individual cells or nests of cells may infiltrate stroma.

Differential Diagnoses
FIBROADENOMA: Only uniform and well differentiated epithelial cells Composed of proliferated glandular epithelium surrounded by layers of fibrous tissue.
ADENOCARCINOMA: Develops as primary carcinoma, whereas "adenocarcinoma arising in fibroadenoma" is a focally malignant change within a primary fibroadenoma.

Adenocarcinoma **Mammary gland (Figure 24)**

Pathogenesis/Cell of Origin: Mammary gland epithelium.

Diagnostic Features

- Structural pleomorphism.

- Variability in growth pattern is high, tubular, papillary, cystic, solid, comedo, undifferentiated (see subtypes below).
- Different growths pattern may be present within a single lesion.
- Epithelium arranged in tubular or gland-like structures.
- Loss of tubulo-alveolar pattern possible.
- Acinar spaces show great variety in size and shape, but may sometimes be regular and orderly. In wnt tumors acinae are typically surrounded by myoepithelium.
- Often acinar spaces are completely filled by neoplastic cells detached from basement membrane.
- Intracystic papillary projections, solid cords, sheets, nests or tubes with no signs of glandular differentiation may be present.
- Acinar areas are frequently associated with cysts filled with blood, or fluid as evidence of secretory activity.
- Secretory activity may occur.
- Stroma within the tumor is usually scanty.
- Areas of necrosis, ulceration, and hemorrhage may occur.
- Neoplastic cells can be columnar or cuboidal and form one cell or several cell layers. Pronounced cellular and nuclear pleomorphism, nuclear hyperchromasia and mitotic Figure Xs are common; nucleoli are often prominent; atypia may occur.
- Areas of squamous differentiation of neoplastic epithelial cells may occur.
- Squamous areas cover not more than 25% of the lesion (mouse)
- Invasion of adjacent tissues, muscle or skin may be present.
- Metastasis may occur. Most mouse mammary tumors have an expansile growth margin. Metastasis may occur by “non-invasive intravascular metastasis” (Sugano et al)
- Subtypes:
    - Alveolar/tubular: Composed predominantly of alveolar or tubular structures.
        - Microacinar (Dunn’s Type A)
        - Solid Tubular (Dunn’s Type B)
        - Cystic (Dunn’s Type C)
    - Comedo: Multilayered epithelium surrounding central necrotic debris.
    - Cribriform: Solid sheets of neoplastic epithelial cells interrupted by round or irregularly shaped secondary lumina of variable size.
    - Cystic: Simple or multiloculated cystic glandular structures.
    - Medullary/solid: Rich in parenchymal tissue; sparse stroma.
    - Papillary: Papillary structures predominate.
    - Scirrhous: Rich in stroma, often sclerotic, sparse epithelial components.
    - Spindle cell: Spindle-shaped cells form solid tumor structures.
    - Do you want to add undifferentiated here, since it was noted above?

Differential Diagnoses

ADENOMA: Tumor well demarcated from the adjacent mammary tissue. The regular architecture of the gland is distorted. Epithelium single or double layered. Epithelial cells are still attached to the basement membrane.

ADENOCARCINOMA ARISING IN FIBROADENOMA: Regarded as a separate entity. Adenocarcinomatous change within fibroadenoma.

TUMOR, MIXED, MALIGNANT: Epithelial and mesenchymal neoplastic components. At least one of these is malignant.

CARCINOMA, ADENOSQUAMOUS: Squamous cell areas cover more than 25% of the lesion.

HYPERPLASIA WITH ATYPIA: Either focal, irregular proliferation of ductal or alveolar epithelium within a lobular hyperplasia or acinar, irregular proliferation of one or more alveoli or ducts within normal mammary gland tissue.

Comments: Tumors with focal adenocarcinomatous change within adenomas are diagnosed as

adenocarcinomas.

Tumor, mixed, malignant **Mammary gland**

Pathogenesis/Cell of Origin: At least two different cell types including mammary gland epithelium and mesenchymal cells.

Diagnostic Features

- This tumor is composed generally of epithelial cells and mesenchymal tissue elements.
- At least one of these components has malignant features.
- Mixtures of all types of carcinomatous and sarcomatous components may be present.
- Any element may predominate.
- Infiltrative growth, penetration of vessels, and metastasis may occur.

Differential Diagnoses
TUMOR, MIXED, BENIGN: Consists of different components, i.e. epithelial and connective tissue other than fibrous tissue (e.g., cartilage or bone). No sign of malignancy.
ADENOCARCINOMA ARISING IN FIBROADENOMA: Adenocarcinomatous change within fibroadenoma.

Comments: "Tumor, mixed, malignant" summarizes all possible mixtures of epithelial and mesenchymal tumor cell components, at least one of which has to be malignant. According to well established diagnostic criteria, "adenocarcinoma arising in fibroadenoma" is regarded as a separate entity. The former "carcinosarcoma type" is not listed as a subtype of the mixed malignant tumor, because it might be a highly pleomorphic epithelial or myoepitelial tumor.

II. Zymbal's gland

The Zymbal's or auditory sebaceous gland is most commonly described in rats. However, a small auditory sebaceous gland is also present in mice (Seely and Boorman, 1999). In rats, it is 3-5 mm in diameter and located anterior-ventral to the ear canal. It is composed of 3-4 lobules, each with an intralobular duct emptying into an excretory duct which empties into the ear canal. There are two smaller sebaceous glands underlying the epithelium of the ear canal which are included as part of the Zymbal's gland by some. These are holocrine glands where the secretion is formed by mature acinar cells degenerating to become the secretory product (Haines and Eustis, 1990, Nielsen, 1978). Spontaneous tumors are occasionally seen and have also been induced by chemical carcinogens.

Degenerative, inflammatory, vascular, and other non-neoplastic changes observed in Zymbal's gland are largely similar to those observed in the mammary gland. Specific examples of these non-neoplastic changes are displayed in **Figures 25-26**.

A. Growth Disturbances

Non-neoplastic

Acinar atrophy : **Zymbal's gland**

Pathogenesis/cell of origin: Acinar cells

Diagnostic features:

- Degeneration of sebaceous cells
- Acini diminished in size
- Consist only of a single layer of cuboidal cells or squamous cells
- Individual acinar cells have reduced amount of cytoplasm can contain yellow-brown granular pigmentation interstitial collagen increased with scattered inflammatory cells
- Ducts can be dilated and filled with inspissated secretory material and inflammatory cells
- Only ductal dilatation and cyst formation observed in the mouse

Hyperplasia, sebaceous cell: **Zymbal's gland (Figure X)**

Pathogenesis/cell of origin: Sebaceous cells

Diagnostic features:

- Lobular pattern retained
- Slight compression of adjacent tissue
- Affected acini enlarged/ partially fused
- Normal maturation sequence from periphery to centre may be less apparent
- Cytoplasm more basophilic and less foamy than that of normal cells
- Nuclei can be enlarged and contain one or more prominent nucleoli
- Proliferation of sebaceous cells with preserved, normal architecture of sebaceous glands
- Can be associated with hypertrophy

Differential Diagnoses

- Adenoma, sebaceous cell: Sebaceous adenomas may contain a large number of germinative (basaloid) cells. The regular architecture of sebaceous glands is distorted.

Comments: Mostly focal; diffuse hyperplasia has been observed with administration of 3,3 – dimethoxybenzidine

Hyperplasia, squamous cell: **Zymbal's gland**

Pathogenesis/cell of origin: Duct epithelium

Diagnostic features:

- Focal increase in thickness of the squamous epithelium with formation of folds and short papillary projections into the duct lumen
- Often rete ridges formation

Differential diagnoses:

- Papilloma, squamous cell: Arborizing pattern of papillae, stratified squamous epithelium overlies cores of connective tissue

Neoplastic

Adenoma, sebaceous cell: **Zymbal's gland (Figure 27)**

Pathogenesis/cell of origin: sebaceous cells

Diagnostic features

- Structural resemblance to normal gland, but regular sebaceous gland architecture is not maintained.
- Well defined but not encapsulated
- Lobulated structure.
- Exophytic and endophytic growth characteristics occur.
- Most tumors consist of a mixture of basaloid cells, cells transitional in character between basaloid cells and mature sebaceous cells.
- Many of the tumor cells show the prominent cytoplasmic vacuolation characteristics of sebaceous gland cells.
- There are a certain number of actively mitotic germinative (basaloid) cells which may predominate in some lobules.
- Cystic areas are often observed derived from intense production of sebum
- Size equal or greater in size than an individual lobule
- Mitotic figures may be present
- Cells are usually small and nuclei are partly pyknotic

Differential Diagnoses

- Hyperplasia, sebaceous cell: Characterized by normal architecture, an increased number of mature glandular cells, and only a small number of germinative (basaloid) cells.
- Carcinoma, sebaceous cell: Characterized by poorly differentiated sebaceous gland cells, invasive growth pattern, and cellular and nuclear atypia.
- Carcinoma, basal cell: (Mixed) basal cell carcinomas show only few areas of sebaceous differentiation and a predominantly non-glandular pattern with a clear predominance of basaloid tumor cells.

Papilloma, squamous cell: **Zymbal's gland**

Pathogenesis/cell of origin: Ductal cells

Diagnostic features

- Arise from main duct epithelium
- Complex arborizing structure
- Stratified squamous epithelium overlies cores of connective tissue
- Papillary growth of cornified squamous epithelium into duct lumina
- No cellular atypia
- Usually associated with glandular proliferation

Differential diagnoses

- Carcinoma, squamous cell: presence of invasive growth and of cellular or nuclear atypia

Carcinoma, sebaceous cell: **Zymbal's gland**

Pathogenesis/cell of origin: sebaceous cells

Diagnostic features

- Often ulcerated
- Large irregular acini
- Usually ductless
- Can contain cystic cavities with sebum, keratin, necrotic cells
- Papillary projections of squamous epithelium can occur in larger cystic cavities
- Central portion can show cystic changes with sebaceous material, degenerated cells, leukocytes
- Cells in basal layer small with hyperchromatic cytoplasm
- Upper layers pleomorphic
- One to two nucleoli in round to oval nuclei
- Cytoplasm light, granulated or vacuolated
- Frequent mitoses
- Stroma shows proliferation of fibroblasts which can be pleomorphic and invade into adjacent tissue
- Squamous metaplasia of sebaceous epithelium possible
- Varying proportions of squamous and sebaceous cells

Differential diagnoses

- Carcinoma, squamous cell: No sebaceous differentiation
- Adenoma, sebaceous cell: Absence of invasive growth and of cellular and nuclear atypia.

Comment: Sebaceous cell carcinomas can be induced by various compounds including cupferron, chloroprene, 1, 3-butadiene, benzene, benzidines, aminostilbenes (list in (Turusov and Mohr, 1979) (and from Pathology of the mouse, 1999 and Pathology of the Fischer rat, 1990).

Carcinoma, squamous cell: **Zymbal's gland**

Pathogenesis/cell of origin: duct epithelium

Diagnostic features

With or without keratinization

- Penetration of basal lamina by invasive growth Invasion of the dermis and striated muscle by nests or cords of squamous cells
- Varying cellular differentiation of squamous cells
- Malignancy is furthermore characterized by variation of size and staining of nuclei, atypical mitotic figures and loss of intercellular bridges

III. Preputial/Clitoral Gland

Preputial and clitoral glands are found in both rats and mice. They are paired modified sebaceous glands located in the inguinal region adjacent to the penis and vagina respectively. In males, the preputial gland empties into the preputial cavity and in females, the clitoral gland duct empties into the clitoral fossa. The growth and secretory activity of preputial and clitoral glands are regulated primarily by testosterone and the pituitary hormones, adrenocorticotropic hormone, growth hormone, and prolactin. Administration of testosterone, but not estrogens, to male or female rats causes hypertrophy and hyperplasia of acinar cells. Castration of adult male rats results in atrophy. In rats, large intracytoplasmic eosinophilic granules

are a prominent feature. These granules contain contain pheromones (aliphatic alcohols) and beta-glucuronidase and are not present in mice. Degenerative, inflammatory, vascular, and other non-neoplastic changes observed in preputial/clitoral glands are largely similar to those observed in the mammary gland. Specific examples of these non-neoplastic changes are displayed in **Figure 28 and 29**. Dilated ducts containing keratin-like material with accompanying inflammatory changes is the most common lesion observed. Tumors are occasionally seen as spontaneous lesions but can be induced by a number of chemical carcinogens.

A. Growth Disturbances

Non-neoplastic

Atrophy: **Preputial/Clitoral gland**

Pathogenesis/cell of origin: Acinar cells

Diagnostic features

- Acini diminished in size
- Degeneration of sebaceous cells
- Consist only of a single layer of cuboidal cells or squamous cells
- Individual acinar cells have reduced amount of cytoplasmic can contain yellow-brown granular pigmentation
- Interstitial collagen increased with scattered inflammatory cells
- Ducts can be dilated and filled with inspissated secretory material and inflammatory cells

Hyperplasia: **Preputial/Clitoral gland**

Modifier: Acinar; Ductular

Pathogenesis/cell of origin: Acinar/ductular epithelium.

Diagnostic features:

Acinar:

- Hyperplastic acinar cells are similar to normal acinar epithelium.
- Central round nucleus with one or rarely two large nucleoli.
- Condensation of heterochromatin on the nuclear envelope.
- Cytoplasm contains small vacuoles, and typical eosinophilic hyalinized granules.
- Focal hyperplasia, foci are surrounded by dark flat basal cells.
- Foci show lobulation similar to normal glands.

Ductular:

- May occur focal, multifocal or generalized.
- Acanthosis and hyperkeratosis of ductular squamous epithelium frequently secondary to inflammation.
- Epithelium consists of 3 – 7 flat layers of squamous epithelium.

Differential Diagnoses

- ADENOMA: Compression and multilayering; focally well delineated, loss of normal structure and lower degree of differentiation.
- PAPILLOMA, SQUAMOUS CELL: Compression of surrounding duct structure and/or destruction of ducts, complex arborizing structure.

Comments: This lesion occurs spontaneously, mainly in older animals. It may be misdiagnosed as mammary gland lesion especially if anatomical location at necropsy is misleading. In older mice, this lesion is associated with dilatation of ducts and chronic inflammation. The lesion may be uni- or bilateral. It is not considered a pre-neoplastic change in these older mice but rather a reactive hyperplasia and there is no evidence of progression. Squamous cell hyperplasia is also associated with squamous cell papilloma but has not been studied as precursor for neoplasia.

Neoplasic

Adenoma: **Preputial/Clitoral Gland**

Synonyms: Cystadenoma

Pathogenesis/cell of origin: Acinar cells

Diagnostic features:

- Masses on lower abdominal wall near genitalia
- Usually solid growth mimicking normal glands
- Architecture not maintained
- Differentiation towards both sebaceous and squamous cells
- Eosinophilic granules in tumor cells
- Stratified acinar epithelial formations with dark-staining sebaceous cells with little cellular or structural atypia
- Solid, cystic and papillary cystic types
- No invasive growth or high degree of atypia and mitosis
- Cystadenomas contain cystic cavities filled with necrotic material

Subtypes:

Solid

- No regular border, infiltrates the subcutaneous tissue.
- Forms nests and loses the typical acinar structure.
- Mitotic activity is high, the cell structure is not changed.
- Some of the adenocarcinomas are surrounded by a thick fibrous capsule which is often infiltrated by acinar cells.
- The duct system in glands containing solid adenocarcinomas shows squamous cell hyperplasia, dilatation, and is filled with inflammatory cells and necrotic cell debris.

Cystic:

- Contains multifocal cystic cavities of different sizes. These cavities represent dilated lumina of acini or small ducts.
- Shows local infiltration into the cutis or pseudocapsule.
- The cytology is similar to the solid adenocarcinoma.

Papillary:

- The acinar cells form irregular papillary structures within grossly enlarged glands.
- The mitotic activity is usually high.
- In some areas a basal cell involvement (basal cell hyperplasia) is noticeable.

Papillary/cystic:

- Has the same cellular composition as the cystic adenocarcinomas.
- The difference is a formation of papillary tumor parts which grow into the cystic cavities.

Differential diagnoses

- Mixed Cell Carcinoma: Mixture of sebaceous and squamous cells Invasion of surrounding tissue (skin, mammary gland); cellular atypia, frequent mitoses
- Carcinoma, squamous cell: Derived from ductal cells, no acinar involvement, rare
- Hyperplasia: uniformity of cells, no capsule (glandular or ductal tissue)

Comment

Spontaneous adenomas are extremely rare, but they can be induced by several compounds (list from Pathology of the Fisher rat, 1990). Cystic and papillary growth variants as seen in the rat have not been described in the mouse.

Papilloma, squamous cell: **Preputial/Clitoral Gland**

Pathogenesis/cell of origin: Ductal cells

Diagnostic features

- Arise from main duct epithelium
- Complex arborizing structure
- stratified squamous epithelium overlies cores of connective tissue
- Papillary growth of squamous epithelium into duct lumina, can be keratinizing or non-keratinizing
- No cellular atypia
- Usually associated with glandular proliferation

Differential Diagnosis

- Carcinoma squamous cell: Destruction of basal membrane; invasion into surrounding acinar tissue; cellular atypia and disorientation; frequent mitosis
- Adenoma acinar cell: shows sebaceous differentiation

Adenocarcinoma: **Preputial/ Clitoral Gland (Figure 30-33)**

Synonyms: Cystadenocarcinoma

Pathogenesis/cell of origin: acinar cells

Diagnostic features

- Irregular acinar cell nests surrounded by thin connective tissue
- Some differentiation to squamous cells can be present
- Small sometimes cystic duct-like structures lined with squamous epithelium can be present
- Cystic cavities can contain keratin and holocrine secretions
- Solid, cystic and papillary cystic types
- Nuclear/cellular atypia
- Hyperchromasia,
- Loss of acinar organization
- Mitotic figures
- Local invasiveness

Subtypes:

Solid

- No regular border, infiltrates the subcutaneous tissue.
- Forms nests and loses the typical acinar structure.
- Mitotic activity is high, the cell structure is not changed.
- Some of the adenocarcinomas are surrounded by a thick fibrous capsule which is often infiltrated by acinar cells.
- The duct system in glands containing solid adenocarcinomas shows squamous cell hyperplasia, dilatation, and is filled with inflammatory cells and necrotic cell debris.

Cystic:

- Contains multifocal cystic cavities of different sizes. These cavities represent dilated lumina of acini or small ducts.
- Shows local infiltration into the cutis or pseudocapsule.
- The cytology is similar to the solid adenocarcinoma.

Papillary:

- The acinar cells form irregular papillary structures within grossly enlarged glands.
- The mitotic activity is usually high.
- In some areas a basal cell involvement (basal cell hyperplasia) is noticeable.

Papillary/cystic:

- Has the same cellular composition as the cystic adenocarcinomas.
- The difference is a formation of papillary tumor parts which grow into the cystic cavities.

Differential diagnoses

- Adenoma/Cystadenoma; Hyperplasia: more regular structure of acini, no invasion or atypia
- Hyperplasia: uniformity of cells, no capsule (glandular or ductal tissue)

Tumor, basal cell, malignant: **Preputial/Clitoral gland**

Synonym(s): Basal cell carcinoma

Pathogenesis/cell of origin: Basal cells.

Diagnostic features:

- Shows darker basophilic staining cells with elongated to oval nuclei and a high mitotic rate.
- Cells are arranged in circumscribed nests with bundles of mesenchymal tissue between them.
- Acinar cells and ducts are located around these tumors.

Differential diagnoses:

- Carcinoma, squamous cell: Non-keratinizing squamous cells with dark stained cytoplasm; many mitotic Figure Xs.
- Tumor, basal cell, malignant basal cell tumor of the skin: Lacks adjacent Preputial/Clitoral glandular structures and eosinophilic granules; anatomical location helpful.

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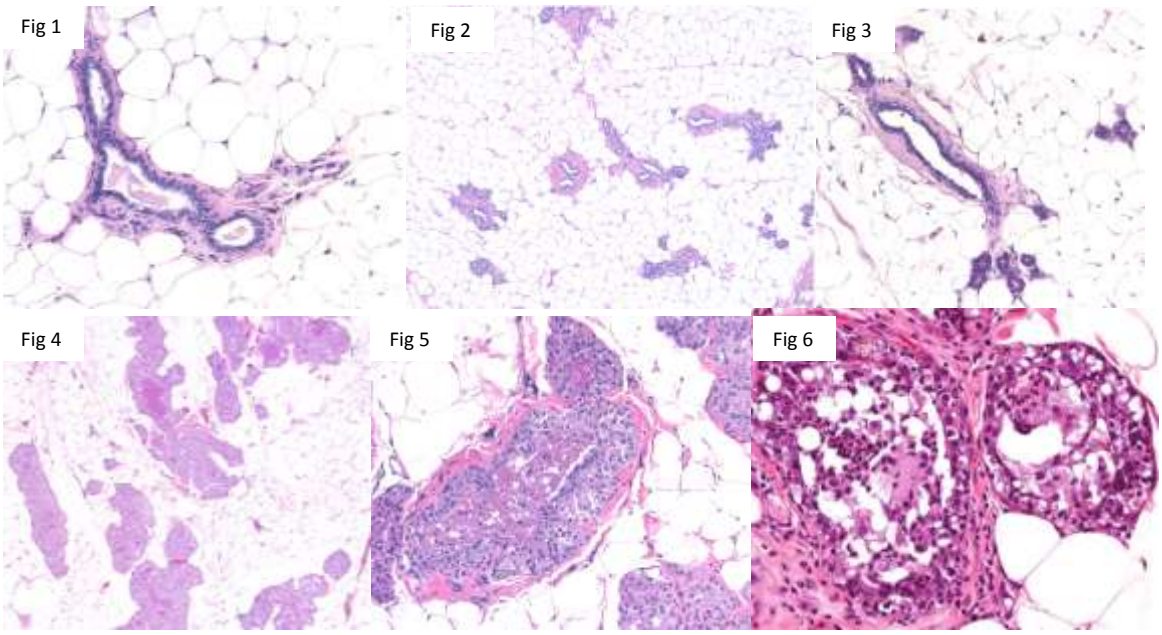
Mammary Gland



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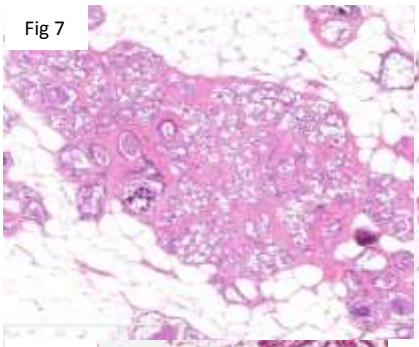

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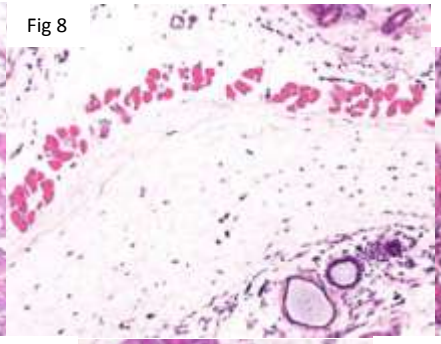

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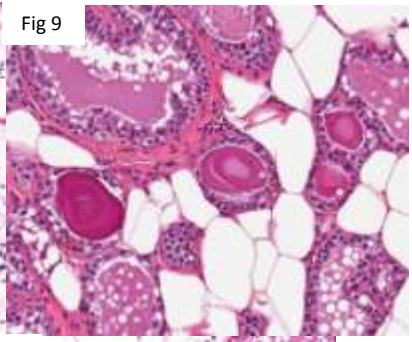

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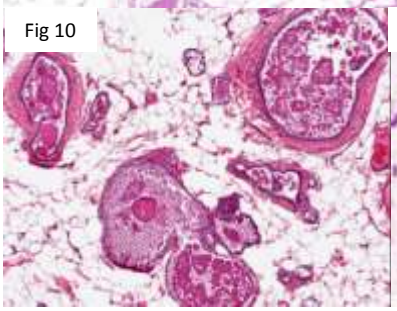

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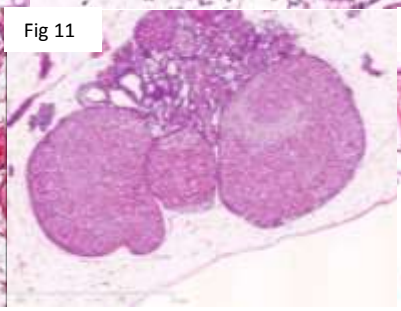

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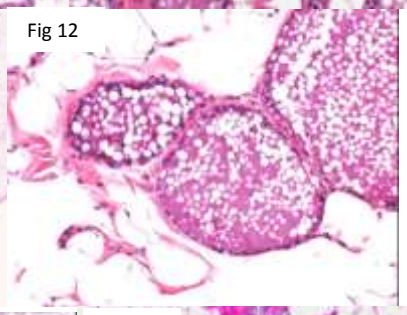

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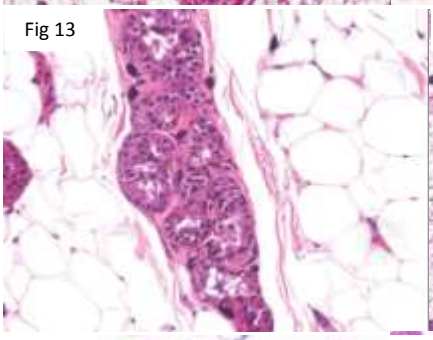

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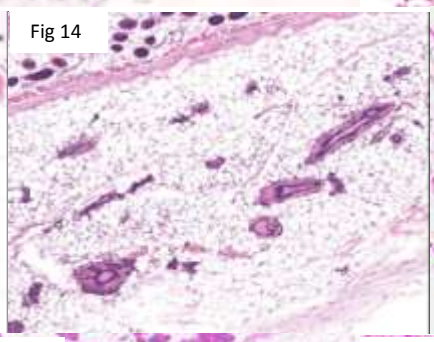

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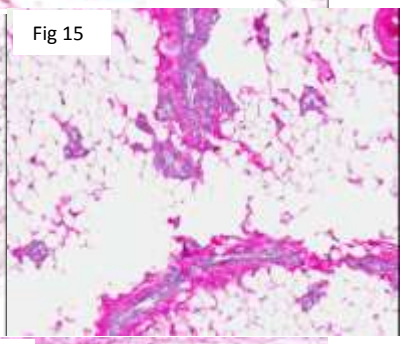

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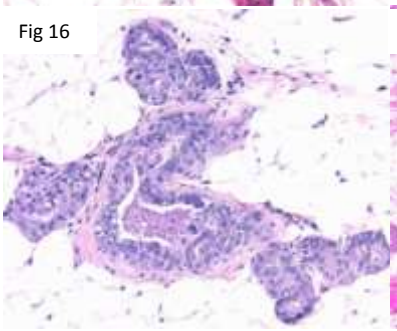

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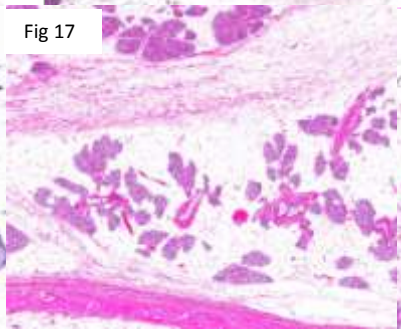

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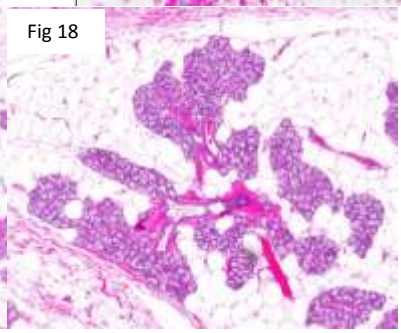

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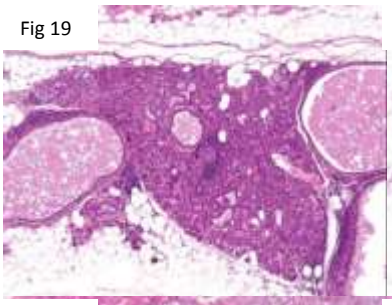

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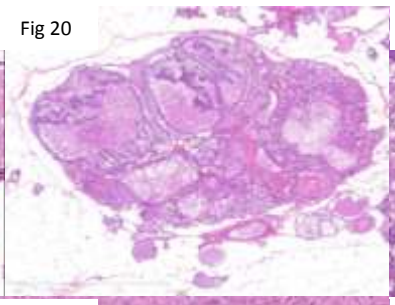

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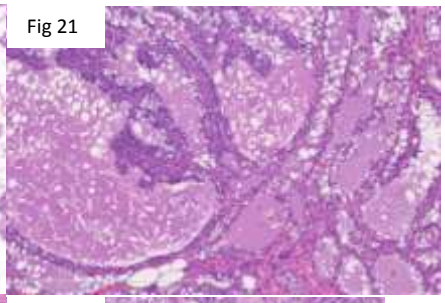

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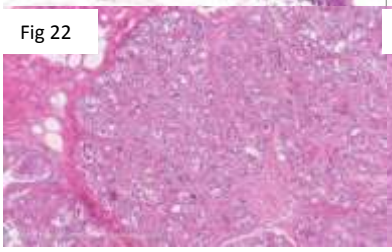

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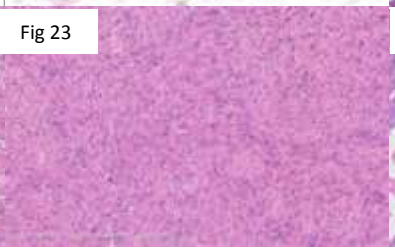

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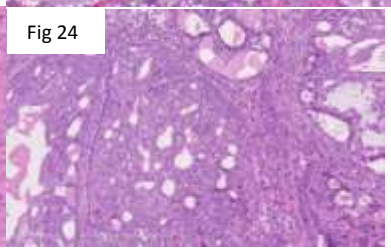
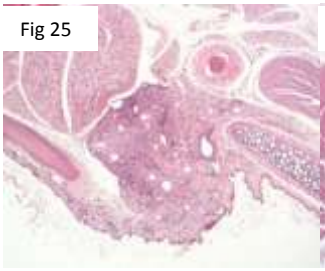


Zymbal's, Preputial, and Clitoral Glands

Fig 25



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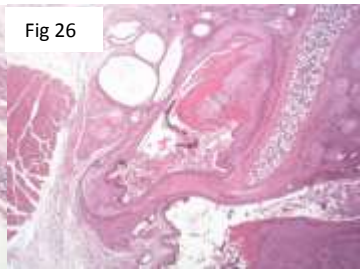

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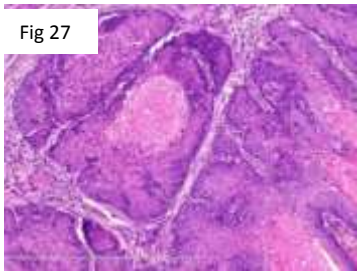

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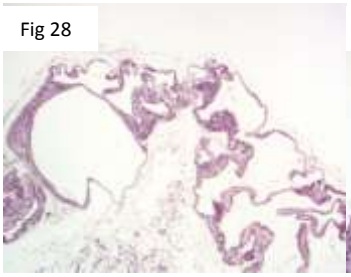

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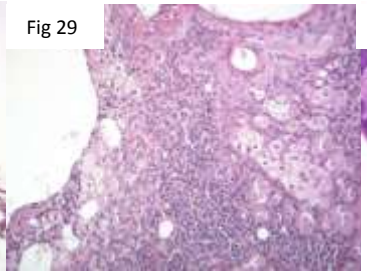

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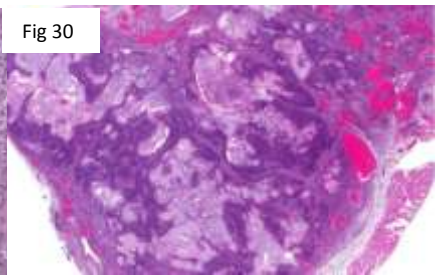

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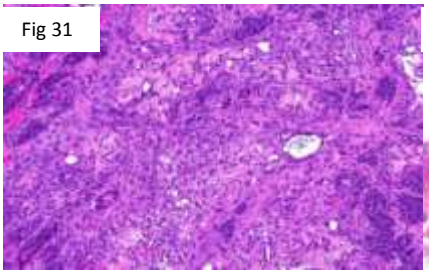

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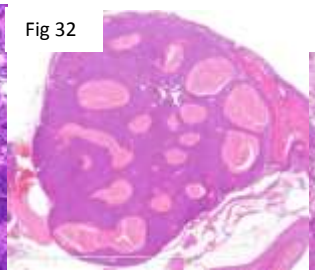

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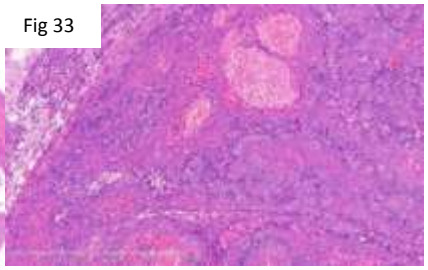
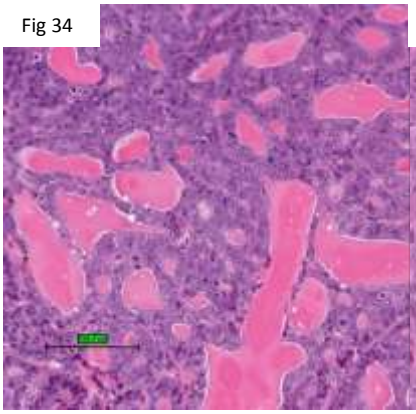


Genetically Modified Mouse Models

Fig 34



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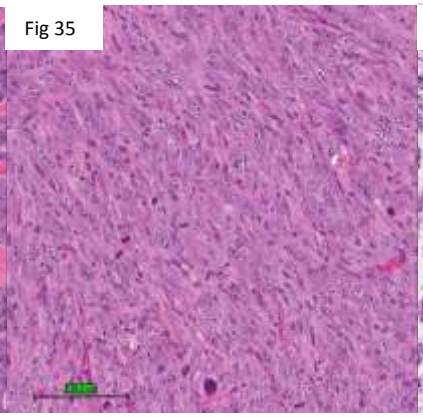

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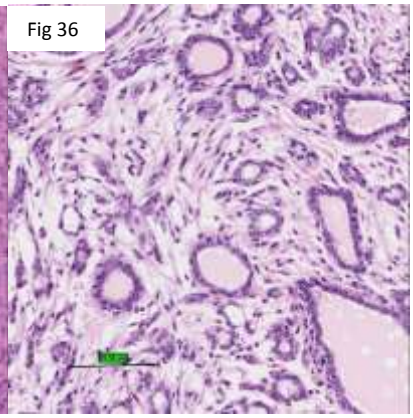

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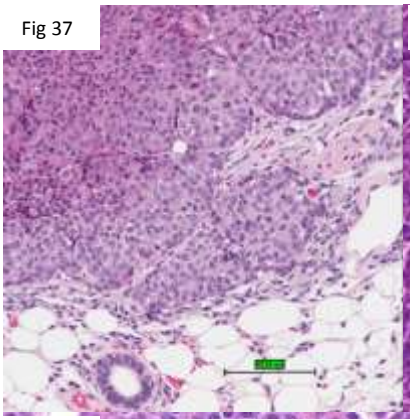

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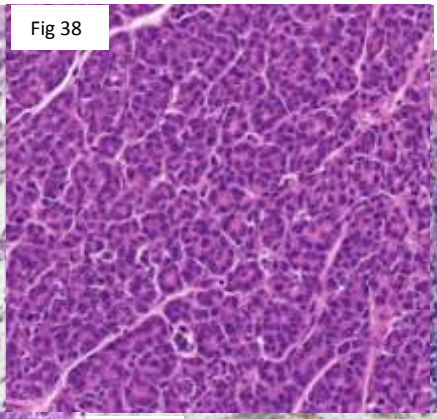

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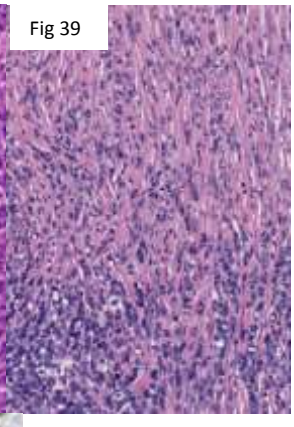

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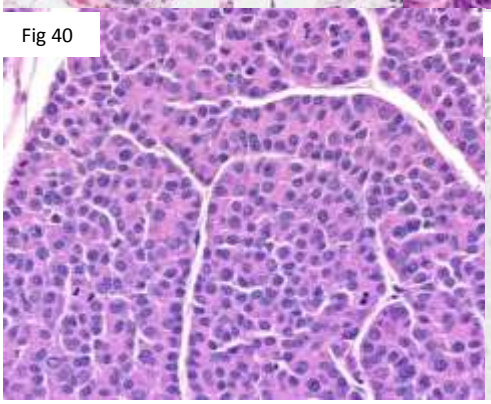

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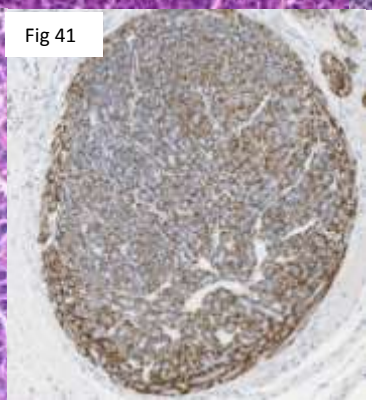

Figure Legends

Fig 1: Normal mammary gland (female mouse)

Fig 2-3: Normal mammary gland (female rat)

Fig 4-5: Normal mammary Gland (male rat):

Fig 6: Mammary gland: Acute inflammation female (rat)

Fig 7: Mammary gland: Periductular fibrosis and mineralization (female rat)

Fig 8: Mammary gland: Edema, interstitial (female rat)

Fig 9: Mammary gland: Corpora amylacea (female rat)

Fig 10-12: Mammary gland: Ductal and alveolar dilation with secretions and/or vacuolation (female rat)

Fig 13: Mammary gland: Pigmentation, epithelial (female rat)

Fig 14: Mammary gland: Atrophy (female rat)

Fig 15: Mammary gland: Atrophy (feminization in male rat)

Fig 16: Mammary gland: Hypertrophy, lobulo-alveolar (masculization in female rat)

Fig 17-18: Mammary gland: Hyperplasia, lobular (female rat)

Fig 19: Mammary gland: Adenoma and ductular dilation (female rat)

Fig 20-21: Mammary gland: Adenoma (female rat)

Fig 22: Mammary gland: Fibroadenoma (female rat)

Fig 23: Mammary gland: Fibroma (female rat)

Fig 24: Mammary gland: Adenocarcinoma (female rat)

Fig 25: Zymbal's gland: Inflammation, chronic (rat)

Fig 26: Zymbal's gland: Hyperplasia, ductal; cyst (rat)

Fig 27: Zymbal's gland: Adenoma (rat)

Fig 28: Preputial gland: Cyst (mouse)

Fig 29: Preputial gland: Inflammation, chronic (mouse)

Fig 30-33: Clitoral gland: Carcinoma (rat)

Fig 34: Typical Myc-induced adenocarcinoma. Note the blue-purple cytoplasm and large pleomorphic nuclei with prominent nucleoli (GEM)

Fig 35: EMT Tumor showing spindle cells (GEM)

Fig 36: Papilloma of a typical PTEN-induced adenomyoepithelioma (GEM)

Fig 37: Large ERbB2/cNeu-induced nodular solid tumor with pushing border (GEM)

Fig 38: MMTV-induced microacinar carcinoma (Type A) (GEM)

Fig 39: E-CaderinXp53 null tumor. Note the lobular carcinoma type of single file infiltrates (GEM)

Fig 40: Small oval cell type typical of Ras-induced mammary tumors. Note the lack of zonation as seen in the cNeu tumors (GEM)

Fig 41: Microacinar Type A tumor in a Tg(MMTV-Wnt1) mouse. Note the pattern of the myoepithelium surrounding the acini (GEM)