

Chapter 2

Histological Evaluation of the Normal Breast

Jose Russo and Irma H. Russo

Keywords Histological evaluation • Procurement of normal breast tissue • Reduction mammoplasty • Surgical tissue • Autopsy material • Needle core biopsies • The stroma of the breast • Cell types in the normal breast • Immunocytochemical markers

2.1 Introduction

The breast is a bilateral organ that in the female undergoes dramatic changes in size, shape, and function in association with infantile growth, puberty, pregnancy, lactation, and postmenopausal regression [1, 2]. The development of the human breast is a progressive process initiated during embryonic life. This process has been discussed extensively in previous publications [3] and will, therefore, not be addressed in this chapter. In this chapter, we describe the parameters that need to be considered in order to evaluate the normality of the human breast. In addition, we address the different morphological patterns observed which depend on the sampling procedures utilized.

2.2 Procurement of Normal Breast Tissue

2.2.1 Reduction Mammoplasty

Reduction Mammoplasty is a surgical procedure of breast reduction by which excess breast fat, glandular tissue, and skin are removed to achieve a breast size in proportion to body size. It is a cosmetic surgery, but also therapeutic for those women who suffer the discomfort associated with overly large breasts.

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Table 2.1 Profile of the population under study

Group	Glandular differentiation	Sample	Age ^a	Parity history			Glandular development ^f
A	Low	1	30	G0 ^b	P0 ^c	A0 ^d	Lobule Type 1
		2	34	G0	P0	A0	Lobule Type 1, alveolar bud, ducts
		3	48	G0	P0	A0	Lobule Type 1, ducts
		4	20	G0	P0	A0	Lobule Type 1
		5	23	G0	P0	A0	Ducts only
		6	36	G0	P0	A0	Lobule Type 1
		7	53	G0	P0	A0	Lobule Type 1, ducts
		8	24	G0	P0	A0	Lobule Type 1, ducts
B	Medium	16 ^e	60	G1	P1	A0	Lobule Type 1, ducts
		17	30	G1	P1	A0	Lobule Types 1, 2, ducts
		18	63	G4	P4	A0	Lobule Type 1, ducts
		19	31	G3	P1	A2	Lobule Type 1, ducts
		20	44	G5	P5	A0	Lobule Types 1, 2
		21	31	G3	P2	A1	Lobule Type 1
		22	36	G3	P2	A1	Lobule Type 1
C	High	9	52	G1	P1	A0	Lobule Types 2, 3
		10	33	G1	P1	A0	Lobule Type 3
		11	31	G3	P3	A0	Lobule Type 3
		12	30	G3	P3	A0	Lobule Type 3
		13	39	G2	P2	A0	Lobule Type 3
		14	18	G0	P0	A0	Lobule Type 3
		15	50	G2	P2	A0	Lobule Types 2, 3

^aAge in years at time of surgery^bG, gravidity, number of pregnancies^cP, parity, number of deliveries^dA, pregnancies ending in abortion^ePatients who developed infiltrating ductal carcinoma in contralateral breast^fLob lobule, AB alveolar bud**Table 2.2** Histopathological criteria of normality for acceptance of breast tissue in this study

Presence of ductal structures alone or in combination with ductule forming lobules surrounded by identifiable intralobular loose connective tissue
The interlobular connective tissue must contain fat and connective tissue but not fibrosis
The ductal and ductular structures must be covered by an identifiable cuboidal or low cuboidal epithelium resting on basement membrane and a discontinuous layer of myoepithelial cells
Breast tissues that contained hyperplastic epithelium in the ductal structures, lobular hyperplasia, cystic formation, apocrine metaplasia, or other metaplastic changes, or another morphological indication of derangement of the normal structure as described above were deleted from this study

We have used 22 reduction mammoplasties performed on patients whose clinical, obstetric, and gynecological histories were available (Table 2.1) [4]. All tissues were morphologically evaluated following the criteria outlined in Table 2.2 [4]. These criteria are what determine the normality of breast tissue by its ductal and

lobular composition and state that no pathological conditions are present in the tissue under study (Table 2.2). The histological criteria were correlated with the age and pregnancy history and scored on three levels of glandular differentiation: low, medium, and high (Table 2.1). In our experience, working in conjunction with a plastic surgeon is vital for obtaining this tissue. Every reduction mammoplasty specimen obtained weighed between 300 and 500 g and the tissues for morphological examination were taken from ten different areas of the gland, fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 5 μ m in thickness, and stained with hematoxylin and eosin (H&E) [4].

The breast tissue from premenopausal nulliparous women is mainly characterized by the presence of fat tissue, separating areas of interlobular stroma composed of fibro connective tissue. The glandular or parenchyma is formed mainly by Lobules Type 1 (Fig. 2.1a) as opposed to the breast tissue from premenopausal parous women in which the amount of fat is significantly less prominent and the interlobular stroma separates Lobules Type 3 (Sample 9 from Table 2.1 and Fig. 2.1b). In general, the glands of nulliparous women are mainly composed of Lobules Type 1 and ducts (Samples 3 and 8 in Table 2.1 and Fig. 2.1c, d), whereas the breast tissue of parous women is mainly formed by Lobules Type 3 (Table 2.1) (see also Chap. 1).

2.2.2 *Surgical Tissue Adjacent to Benign and Malignant Lesions*

We have used normal breast tissue samples from 15 women who underwent surgery for benign and suspicious breast lesions [5]. Excision biopsies were performed and provided 2–6 g of tissue. The normal breast tissue samples were dissected 2 cm distant to the lesion. The donors ranged in age from 21 to 55 years and were subdivided into 2 groups: young women, composed of 5 premenopausal women ranging in age from 21 to 32 years (26.6+5 years), and older women composed of 10 women, 4 premenopausal, 4 postmenopausal, and 2 of menopausal status unknown, ranging in age from 42 to 57 years (49.6+5.5 years). Table 2.3 depicts the age and reproductive profile of the population under study [5]. The morphological profile of each of these samples depends significantly on the reproductive history and the age at the time of sample collection (Fig. 2.2). The breast tissue from premenopausal parous women contains islands of fat tissue separating areas of interlobular stroma composed of fibro connective tissue. The glandular or parenchyma is formed by Lobules Type 1, 2, or 3 in premenopausal women with history of pregnancy (Fig. 2.2a, b, d) (Samples 1, 2, and 4 in Table 2.3). Whereas the normal architecture of the breast is clearly defined from the benign lesions as depicted in Fig. 2.2a or d, it is better to follow the rule of 2 cm from any kind of lesion when the tissue is planned to be used as a normal control either for tissue microarray or for laser capture micro-dissection (see Chaps. 3 and 4). The breast tissue from premenopausal nulliparous woman (Sample 5 in Table 2.3) is also composed of interlobular stroma formed by fibro connective tissue made up of collagen and elastic fibers and fibroblasts. In the specific

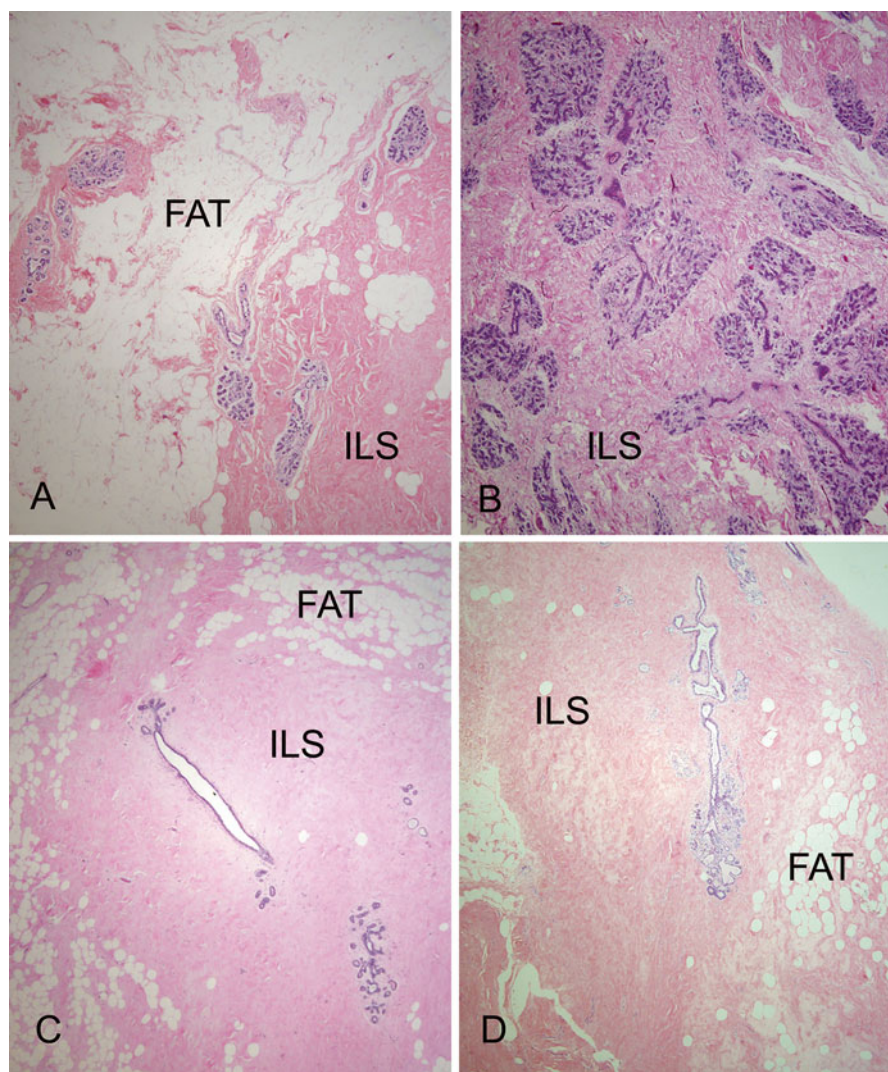


Fig. 2.1 Histological sections of reduction mammoplasty specimens. Stained with hematoxylin and eosin, $\times 2$. (a) Breast tissue from a premenopausal nulliparous woman. Fat tissue (FAT) is separating areas of interlobular stroma (ILS) composed of fibro connective tissue. The glandular or parenchyma is formed by Lobules Type 1. (b) Breast tissue from a premenopausal parous woman. The interlobular stroma (ILS) composed of fibro connective tissue is separating Lobules Type 3. (c, d) Breast tissue from premenopausal nulliparous woman. Fat tissue (FAT) is separating areas of interlobular stroma (ILS) composed of fibro connective tissue. The glandular or parenchyma is formed by Lobules Type 1

case of Sample 5 the adjacent lesion was in an intraductal proliferation that was found 2 cm from the margin of resection. Fresh tissues obtained from excisional biopsies are excellent material for in vitro study either for developing primary culture [4] or for performing organ culture in which cell kinetics studies are performed [5].

Table 2.3 Age, menopausal status, and reproductive histories of the population under study^a

Group	Patient No.	Age, year ^b	Menopausal status	Last menstrual period	Full-term pregnancies
Young women	1	21	Premenopausal	1 day	2
	2	31	Premenopausal	14 days	1
	3	27	Premenopausal	14 days	1
	4	32	Premenopausal	14 days	4
	5	22	Premenopausal	13 days	0
Older women	6	50	Premenopausal	10 days	3
	7	46	Premenopausal	12 days	1
	8	42	Premenopausal	1 day	1
	9	55	Postmenopausal	1 year	3
	10	42	Premenopausal	1 day	1
	11	47	NA	NA	NA
	12	53	Postmenopausal	5 years	0
	13	56	Postmenopausal	12 years	3
	14	57	Postmenopausal	15 years	2
	15	48	NA	NA	NA

^aNA, information not available
^bMean age ± SD for young women was 26.6±5.0 years; it was 49.6±5.5 years for older women

2.2.3 Autopsy Material from Accidental Death

We have utilized breast tissues obtained from 16 accident victims whose autopsies were performed by the medical examiner [6]. These tissues were provided by Dr. S. Bartow who obtained them under NCI-RFP No 1-CB-84231/NO1-CN-23928. The breasts were removed at autopsy by subcutaneous mastectomy. Five of these samples contained invasive ductal carcinomas (Table 2.4). Upon removal from the body the tissue was fixed in 10 % neutral buffered formalin and embedded in paraffin for histological examination. Figure 2.3a–c depicts the histological appearance of Samples 3, 6, and 13 (Table 2.4), which were taken from premenopausal nulliparous women and contained a fat stroma, or combination, with connective tissue mainly formed by collagen fibers. Another sample, also belonging to a premenopausal nulliparous woman (Sample 14, Table 2.4), was characterized by an interlobular stroma composed of fibro connective tissue surrounding Lobules Type 1 and ductal structures (Fig. 2.3d). The breast tissue obtained from autopsy material is excellent for either performing morphological studies by whole mounts, as described in Chap. 1, or histological observations. However, in our hands it did not result as optimal for immunocytochemical studies, due to the long storage in formalin.

2.2.4 Needle Core Biopsies

Breast needle core biopsies must be obtained by a qualified physician and specimens obtained after an authorization for use and disclosure of protected health information for research approved by the Institutional Review Board (IRB) in compliance

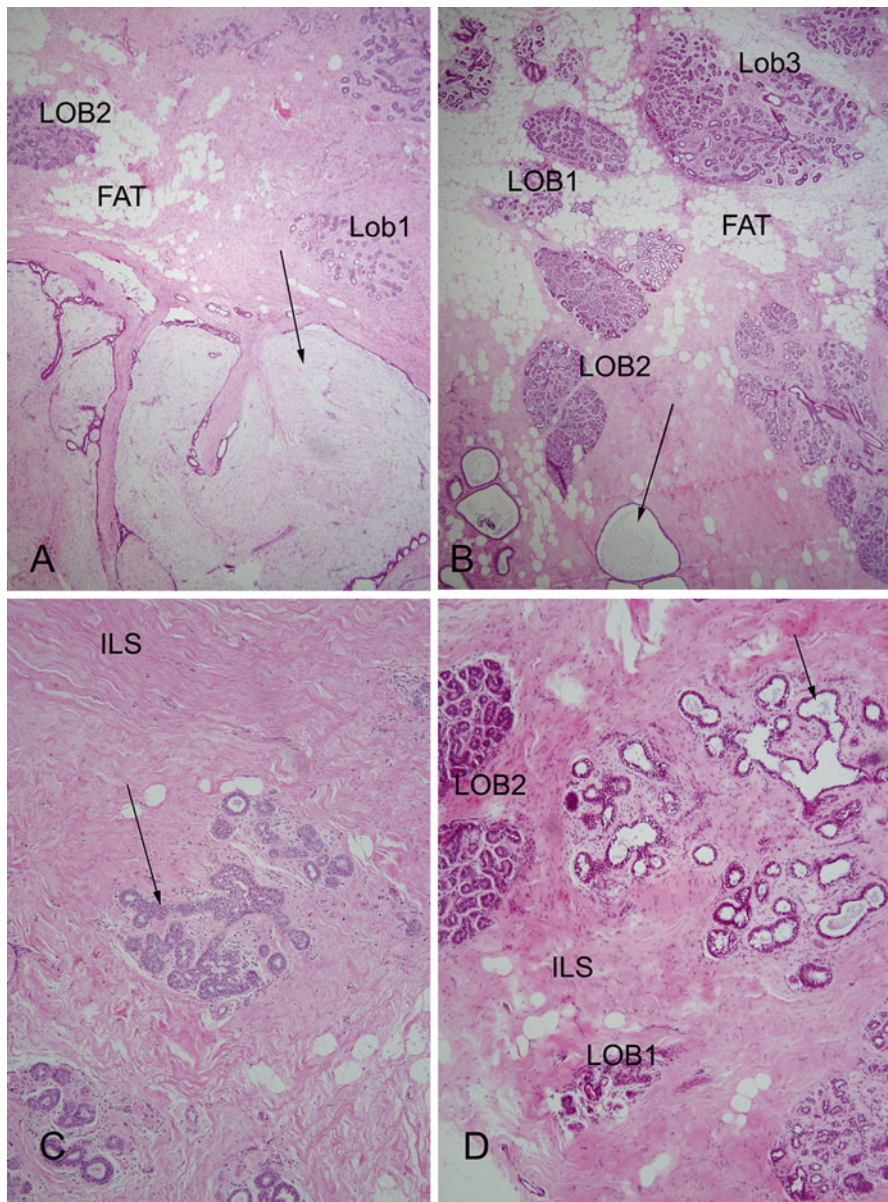


Fig. 2.2 Histological sections of tissue obtained from surgical specimens containing cancerous or benign diseases of the breast. Stained with hematoxylin and eosin, $\times 2$. (a) Breast tissue from a premenopausal parous woman. Fat tissue (FAT) is separating areas of interlobular stroma composed of fibro connective tissue. The glandular or parenchyma is formed by Lobule Types 1 and 2 (LOB1 and Lob2). The *arrow* points toward a fibroadenomatous lesion. (b) Breast tissue from a premenopausal parous woman. The fat (FAT) and interlobular stroma are separating Lobule Types 1, 2, and 3 (LOB1, LOB2, and LOB3). The *arrow* points toward cystic structures. (c) Breast tissue from premenopausal nulliparous woman. The interlobular stroma (ILS) is composed of fibro connective tissue. The *arrow* is pointing an intraductal proliferation that was found 2 cm from the margin of resection of an invasive ductal carcinoma. (d) Breast tissue from premenopausal parous woman. The interlobular stroma (ILS) is composed of fibro connective tissue and separating LOB1 and LOB2. The *arrow* is pointing an LOB1 with cystic changes

Table 2.4 Characteristics of the patient population^a

Patient group	Sample No.	Age	Patient's race ^b	Parity history	Breast side	Cancer	Tumor dimensions (cm) ^c	Tumor location
I	5	20	C	N	Left	No		
	13	21	B	N	Left	No		
	6	30	C	N	Left	No		
	16	61	C	N	Right	No		
II	14	39	C	N	Left	Yes	3.0	UOQ
	15	48	C	N	Right	Yes	4.0	UOQ
III	1	20	C	P	Left	No		
	2	25	C	P	Left	No		
	3	28	C	P	Left	No		
	4	30	C	P	Left	No		
	7	37	C	P	Right	No		
	9	46	C	P	Left	No		
	12	63	C	P	Left	No		
IV	8	39	C	P	Right	Yes	4.0	UOQ
	10	51	B	P	Left	Yes	3.0	LOQ
	11	58	C	P	Left	Yes	5.5	LOQ

^aGroup I, nulliparous females free of breast pathology; Group II, nulliparous females with infiltrating ductal carcinoma; Group III, parous females free of mammary pathology; Group IV, parous females with infiltrating ductal carcinoma

^bC Caucasian, B black, N nulliparous, P parous

^cTumor's largest diameter

with USA HIPAA regulations. All breast core biopsies and corresponding participant data must be de-identified following the safe harbor method recommended by the institution's De-Identification of Protected Health Information policies [7–9]. In the protocol used in our studies, four core biopsies with a 10-gauge biopsy needle were taken from the upper outer quadrant (UOQ) of the right or the left breast, preferentially sampling dense or gritty areas in the breast tissue which may be appreciated on the mammogram for each of the 23 women who entered in the study. From the four core biopsies collected, the first one is preserved in 70 % ethanol for histopathological evaluation; the second, third, and fourth passes are separately preserved in an RNA-preserving fluid (RNAlater®, Ambion) for genomic analysis (see Chaps. 7–9).

For histopathological analysis the first pass is expelled from the needle into a prelabeled 15 mL tube containing 10 mL of 70 % ethanol. The needle is washed in isotonic sodium chloride solution before the next use. Each specimen is labeled with a number and stored for up to 4 weeks at 4° centigrade in the original collection tube until ready for processing. Cell clusters or tissue fragments are tightly positioned in a tissue cassette, which is then dehydrated and embedded in paraffin and sectioned at a thickness of 4 µm and stained with H&E, or processed for immunocytochemical studies utilizing an automated cell stainer (Optimax Plus Automatic Consolidated Cell Stainer, Biogenex, San Ramon, CA) following standard procedures. Figure 2.4a, b depicts the core breast biopsies of postmenopausal nulliparous women with prominent interlobular stroma composed of fibro connective tissue and

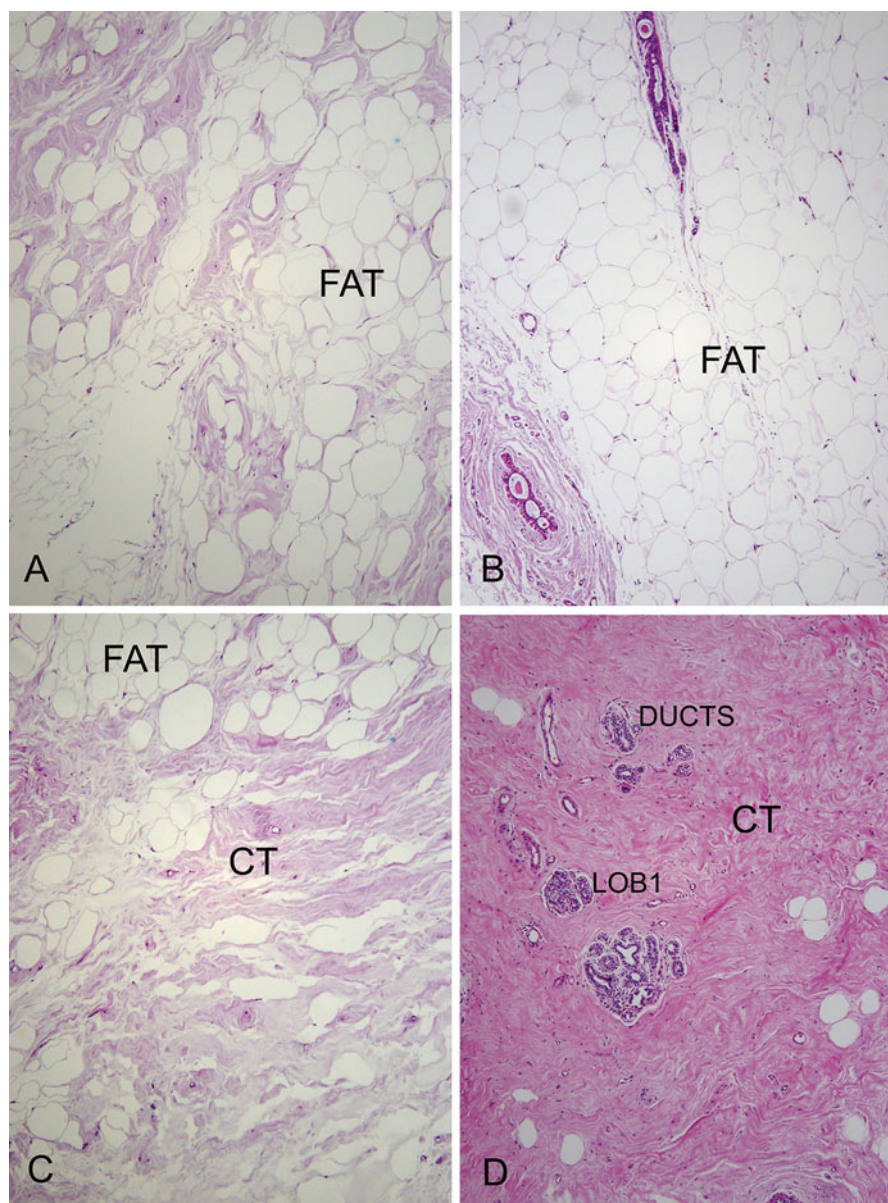


Fig. 2.3 Histological sections of postmortem specimens. Stained with hematoxylin and eosin, $\times 2$. (a–c) Breast tissue from a premenopausal nulliparous woman. Fat tissue (FAT) is separating areas of fibro connective tissue (CT). (d) Breast tissue from a premenopausal nulliparous woman. Interlobular stroma composed of fibro connective tissue (CT) is the main component of the breast tissue and isolated LOB1 and ducts (DUCTS)

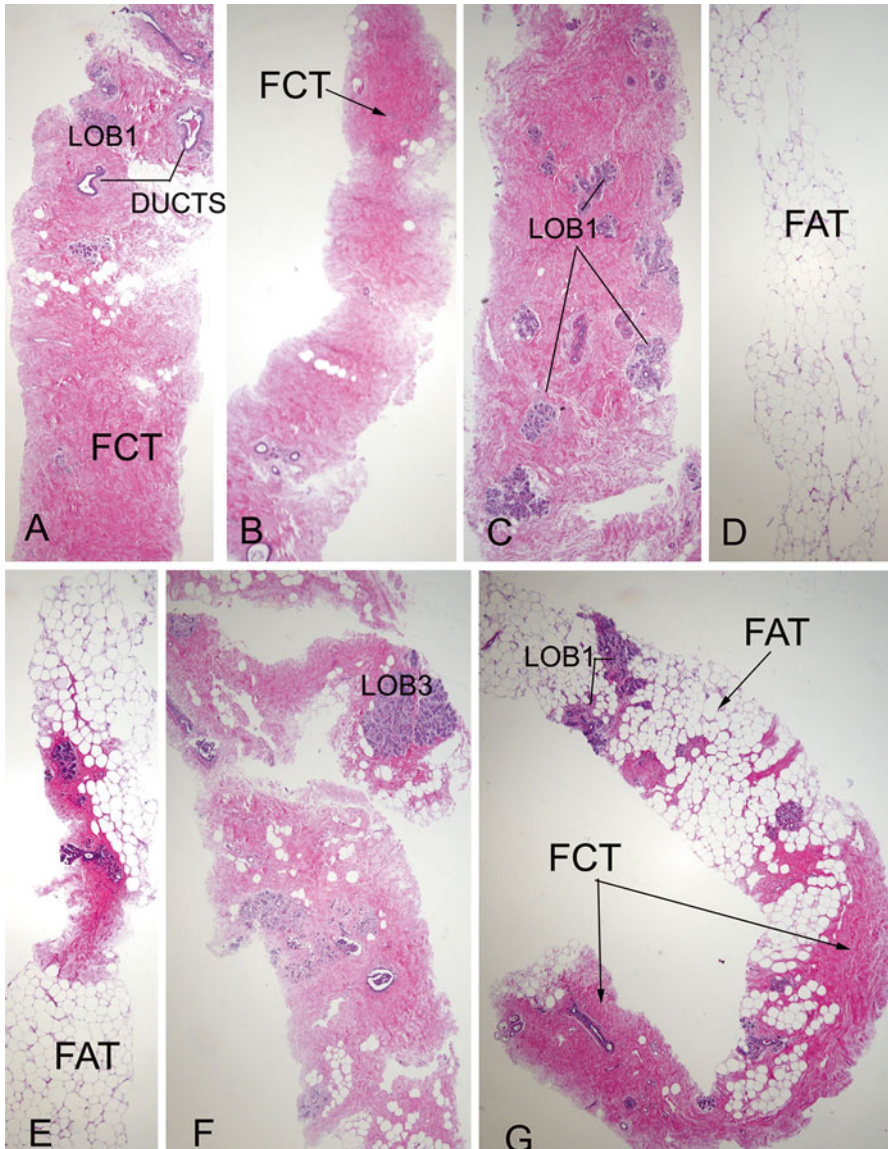


Fig. 2.4 Histological sections of core biopsies. Stained with hematoxylin and eosin, $\times 2$. (a, b) Breast tissue from postmenopausal nulliparous women. Interlobular stroma composed of fibro connective tissue (FCT). The glandular or parenchyma is formed by Lobules Type 1 and ducts (DUCTS). (c) Breast tissue from a postmenopausal parous woman. The interlobular stroma composed of fibro connective tissue separating Lobules Type 1. (d) Breast tissue from a premenopausal nulliparous woman containing small amount of glandular tissue and surrounded by fat tissue (FAT). (f) Breast tissue from premenopausal parous women. Interlobular stroma composed of fibro connective tissue surrounds a well-preserved Lobule Type 3 (LOB3) and Lobules Type 3 in involution. (g) Breast tissue from a premenopausal nulliparous woman containing Lobules Type 1 and surrounded by fat tissue (FAT) and fibro connective tissue (FCT)

the glandular or parenchyma formed by Lobules Type 1 and ducts. In the parous women Lobules Type 1 are separated from dense interlobular stroma. The postmenopausal breast will have involuted considerably and the majority of the tissues will have been replaced by fat (Fig. 2.4d), or a small amount of fibro connective tissue surrounding Lobules Type 1 (Fig. 2.4e). In the premenopausal breast the amount of Lobule Types 1, 2, and 3 depends on the number of years after pregnancy as it is depicted in Fig. 2.4f. Instead, in the nulliparous breast of postmenopausal women Lobule Types 2 and 3 are less common than in the premenopausal breast (Fig. 2.4g) [3, 10].

2.3 The Histological Appearance of the Premenopausal Breast

2.3.1 *The Architecture of the Ductal and Lobular Structure*

We have described in detail the architecture of the lobular structures of the human breast [3, 5, 10]. Basically, the alveoli or alveolar buds cluster around a terminal duct, forming the Lobule Type 1, or virginal lobule, and each cluster is composed of approximately 11 alveoli or alveolar buds. Terminal ducts and alveoli are lined by a two-layered epithelium formed by the luminal and basal cells; these latter ones are intermingled with myoepithelial cells. Lobule formation in the female breast occurs within 1–2 years after onset of the first menstrual period. Full differentiation of the mammary gland forming Lobule Types 2, 3, and 4 is a gradual process taking many years, and in some cases, if pregnancy does not supervene, is never attained [2, 3, 10]. The transition from Lobule Type 1 to Type 2, and of Type 2 to Type 3, is a gradual process of sprouting of new alveoli. In Lobule Type 2 and Type 3, these are now called ductules; they increase in number from approximately 11 in Lobule Type 1 to 47 and 80 in Lobules Type 2 and Type 3, respectively (see Chaps. 1, 2, 3, and 10). The increase in the number of ductules results in a concomitant increase in size of the lobules and a reduction in size of each individual structure.

2.3.2 *The Pregnant and Lactating Breast*

During pregnancy, the breast attains its maximum development. This occurs in two distinctly dominant phases characteristic of the early and late stages of pregnancy [2, 3, 10]. The early stage is characterized by the proliferation of the distal elements of the ductal tree, resulting in the formation of ductules that at this stage can be called acini, thus progressing a Lobule Type 3 into a Lobule Type 4. The intensity of budding and degree of lobule formation go beyond what have been observed in the virginal breast. By the third month of pregnancy, the number of well-formed lobules exceeds the number of primitive buds; however, primitive buds are still

found. In newly formed lobules, the epithelial cells composing each acinus not only increase greatly in number due to active cell division but also increase in size mainly because of cytoplasm enlargement [2]. In the middle of pregnancy, the lobules are further enlarged and increase in number. They surround the duct from which their central branch proceeds so thickly that the chief duct, the terminal or intralobular terminal duct, can no longer be recognized. The transition between the terminal ducts and the budding acini is gradual, making the histological distinction between the two difficult, since both show evidence of early secretory activity. The definitive structure of the ductal tree is essentially settled by the end of the first half of pregnancy; the mammary changes that characterize the second half of pregnancy are chiefly a continuation and accentuation of the secretory activity. Further progressive branching continues with less prominent bud formation. At this time, the formation of secreting units or acini, the differentiated structures, becomes increasingly evident. Proliferation of new acini is reduced to a minimum, and the luminae of those already formed become distended by accumulation of secretory material or colostrum [4]. The epithelium is vacuolated due to the accumulation of lipids. Under the electron microscope the mammary epithelia show numerous lipid droplets and proteinaceous material. In the Lobule Type 4, or lactating breast, the reactivity against milk fat globule protein is highly expressed [3]. The secretory acinus formed during pregnancy is a terminal outgrowth that marks the end of glandular differentiation.

During lactation, the process of growth and differentiation may be observed in the same lobule type, side by side with the process of milk secretion [1, 2, 11, 12]. No major morphological changes of the mammary gland are observed during lactation. The mammary lobules are enlarged and the acini have a dilated lumen filled with granular, slightly basophilic material admixed with fat. There is a significant variation in lobule size throughout the gland, suggestive of a variation in lactogenic activity from lobule to lobule. Milk is synthesized and released into the mammary acini and ductal system, although it can be stored for up to 48 h before the rate of milk synthesis and secretion begins to decrease. As long as milk is removed regularly from the mammary gland, the alveolar cells continue to secrete milk [2, 12, 13].

The accumulation of milk in the ductoacinar lumina and within the cytoplasm of the lactogenic epithelial cells that occurs after weaning has an inhibitory effect on further milk synthesis. This effect is followed by a series of involutional changes in the mammary gland consisting of a multifocal asynchronous process of reduction in volume of the secretory epithelial cells and further inhibition of their secretory activity.

It is considered that post-lactational regression is due to two complementary mechanisms: cell autolysis, with collapse of acinar structures and narrowing of the tubules accompanied by the appearance of round cell infiltration and phagocytes in and about the disintegrating lobules; and finally, regeneration of the periductal and perilobular connective tissue with renewed budding and proliferation in the terminal tubules. Until menopausal involution sets in, the parous organ shows more glandular tissue than if pregnancy or pregnancy and lactation had never occurred [2, 12, 13].

2.3.3 *The Stroma of the Breast*

Both the intralobular and the interlobular stroma are affected simultaneously during development, pregnancy, lactation, and involution [3]. These processes occur, in turn, in a synchronous manner in response to specific hormonal and growth factor stimuli [14]. Two major mechanisms are considered involved in the interaction of the stroma and epithelial cells, the production of soluble growth factors and a modification of the composition of the extracellular matrix. This interaction seems to be bi-directional such that epithelial cells are also capable of influencing stromal cell behavior and governing gene expression [15, 16]. Several hypotheses and molecular pathways have been postulated for explaining the mechanism of organogenesis of the terminal structures and its relation with the stroma [17]. However, there is not significant information available as to how this process is applied or takes place in the human breast. The study of the stroma-parenchyma ratio in the breasts of pubertal, postpubertal, parous, and pregnant women shows that the relationship between parenchyma and stroma is a dynamic process. At puberty, almost 90 % of the mammary gland is made up of stroma: the intralobular stroma, that represents 17 % of the total, consists of the connective tissue that surrounds each individual alveolar bud, and the interlobular stroma, composed of fat and connective tissue, which separates one lobule from another. The parenchyma of these glands, representing 10 % of the mammary area, is made up almost exclusively of Lobule Type 1 and ductal structures. In the glands of postpubertal and young nulliparous women, the parenchyma increases from 10 to 30 % of the total area of the gland (0-10 % is composed of Lob 1, 10-18 % of Lob 2, and 1-3 % of Lob 3) [2, 3, 10]. The intralobular stroma of these breasts represents about 28 % of the total [2, 10]. Parity induces significant differences in mammary gland development. The breasts of parous women are mostly composed of Lobule Type 3 with a markedly reduced proportion of Lobule Type 1. During the involution of the breast of parous women at menopause it is frequently observed that the intralobular stroma is composed of connective tissue, blood vessels, and moderate amount of lymphocytes intermingled with ductules of a Lobule Type 2 in regression (Fig. 2.5a, b). However, it is very unusual to see, even in postmenopausal parous women, the intralobular stroma infiltrated with lymphocytes intermingled with ductules of Lobule Type 2 in regression to Lobules Type 1 (Fig. 2.5c, d).

2.4 The Histological Appearance of the Postmenopausal Breast

Menopause supervenes as the consequence of the atresia of more than 99 % of the 400,000 follicles that are present in the ovaries of a female fetus at a gestational age of 5 months. Gonadotropin-releasing hormone secretion is also implicated in this

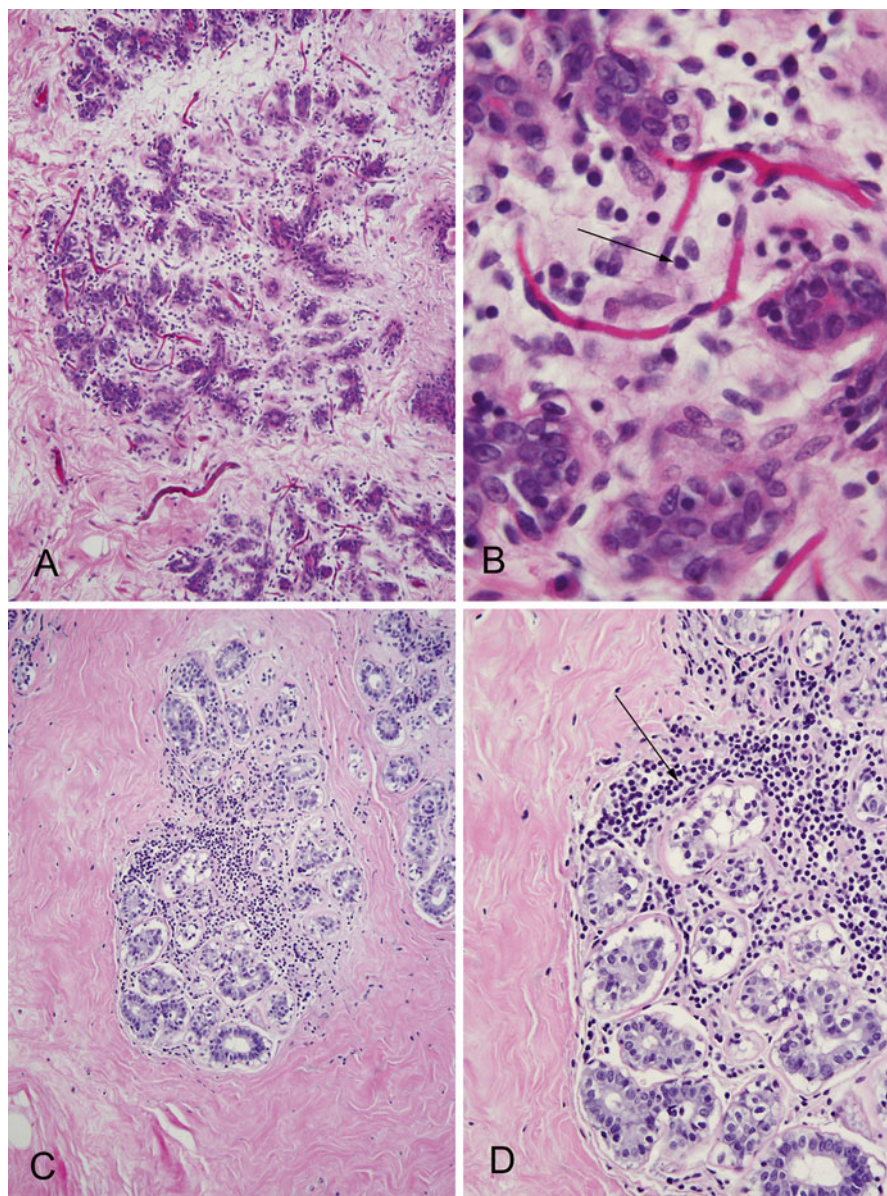


Fig. 2.5 Histological sections of core biopsies. Stained with hematoxylin and eosin. (a, b) Breast tissue from postmenopausal parous women. Intralobular stroma composed of connective tissue, blood vessels, and moderate amount of lymphocytes (*arrow*) intermingled with ductules of a Lobule Type 2 in regression. Magnification $\times 10$ and $\times 40$, respectively. (c, d) Breast tissue from postmenopausal parous women. Intralobular stroma composed of connective tissue, and significant amount of lymphocytes (*arrow*) intermingled with ductules of a Lobule Type 2 in regression. This type of lymphocytic infiltration is extremely unusual. Magnification $\times 10$ and $\times 40$, respectively

phenomenon, indicating that a hypothalamic process is involved in the development of menopause. After menopause, the breast undergoes a regressive phenomenon both in nulliparous and parous women. This regression is manifested as an increase in the number of Lobules Type 1, and a concomitant decline in the number of Lobules Type 2 and Lobule Type 3. At the end of the fifth decade of life, the breast of both nulliparous and parous women contains Lobule Type 1 [2, 3, 10]. In the breast of nulliparous women, the predominant structure is the Lobule Type 1, which comprises 65–80 % of the total lobule type components and their relative percentage is independent of age. Second in frequency is Lobule Type 2, which represents 10–35 % of the total. The least frequent are Lobules Type 3, which represent only 0–5 % of the total lobular population. As depicted in Fig. 2.6a, b, the intralobular stroma is composed of connective tissue and a moderate amount of lymphocytes intermingled with the ductules of a Lobule Type 1 in regression.

In the breast of premenopausal parous women on the other hand, the predominant lobular structure is the Lobule Type 3, which comprises 70–90 % of the total lobule component (see Chap. 1). Only after menopause does it decline in number, and the relative proportion of the three lobule types present approaches that observed in nulliparous women.

Even though during the postmenopausal years in the breast of both parous and nulliparous women the preponderant structure is the Lobule Type 1, only nulliparous women are at high risk of developing breast cancer, whereas parous women remain protected [10, 18]. Since ductal breast cancer originates in Lobule Type 1 (TDLU) [18], the epidemiological observation that nulliparous women exhibit a higher incidence of breast cancer than parous women [1, 2] indicates that Lobule Type 1 in these two groups of women might be biologically different, or exhibit different susceptibility to carcinogenesis [4, 19–21]. The presence of Lobule Type 1 in the breasts of parous women has also been interpreted as a failure of the mammary parenchyma to respond to the influences of pregnancy and lactation [10, 13]. It is possible to postulate that unresponsive lobules that fail to undergo full differentiation under the stimuli of pregnancy and lactation are responsible of cancer development, despite the parity history of a woman. If this were the case, then this unresponsive Lobule Type 1 would be as sensitive to carcinogenesis as the lobules found in the breasts of nulliparous women. We have reported the presence of intralobular hyalinization and lower proliferative activity in the Lobule Type 1 of the parous woman's breast (Fig. 2.6c, d), whereas hyalinization is absent and cell proliferation is higher in the Lobule Type 1 of the nulliparous woman's breast.

2.5 Defining the Normal Breast Histology

In summary, the human breast undergoes a complete series of changes from intra-uterine life to senescence. These changes can be divided into two distinct phases; the developmental phase and the differentiation phase [3]. The developmental phase

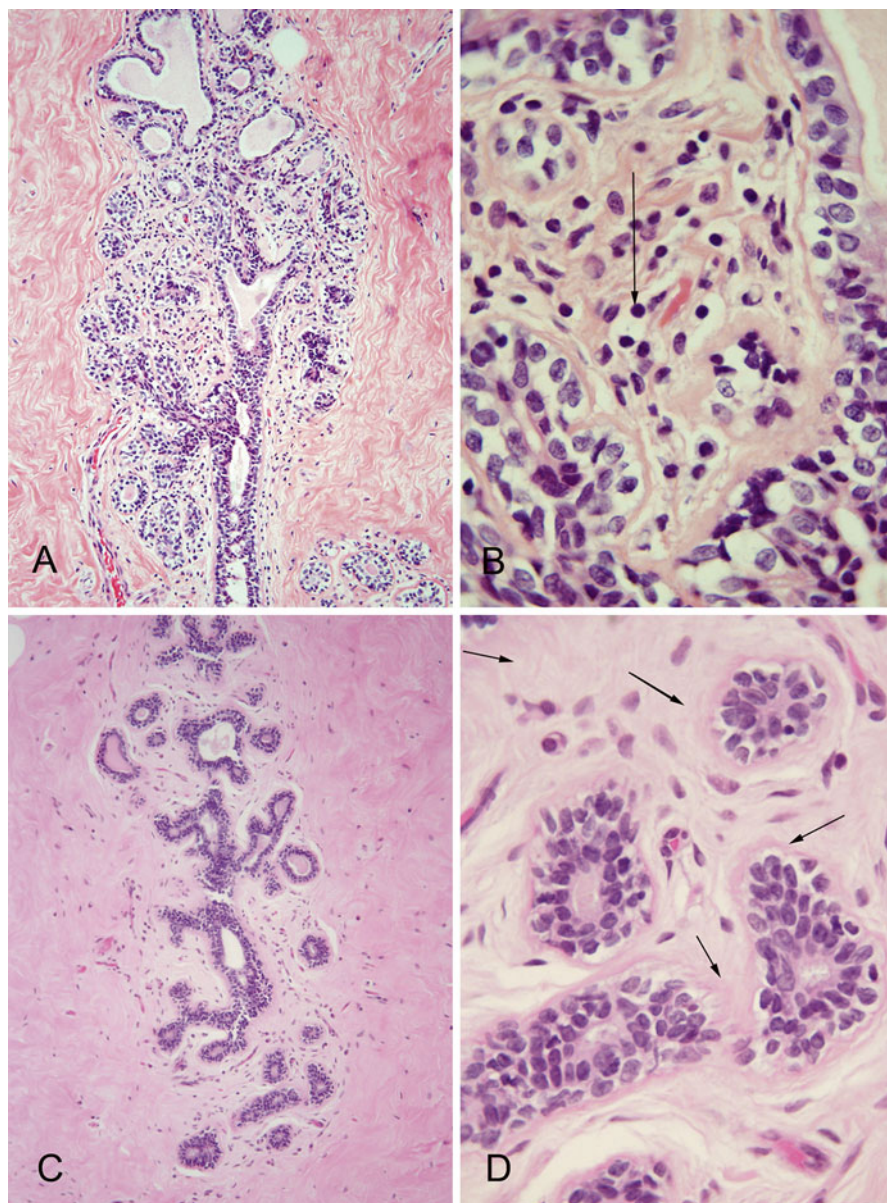


Fig. 2.6 Histological sections of core biopsies. Stained with hematoxylin and eosin. (a, b) Breast tissue from postmenopausal nulliparous women. Intralobular stroma composed of connective tissue and moderate amount of lymphocytes (*arrow*) intermingled with ductules of a Lobule Type 1 in regression. Magnification $\times 10$ and $\times 40$, respectively. (c, d) Breast tissue from postmenopausal parous women. Intralobular stroma composed of hyalinized connective tissue (*arrows*) in a Lobule Type 1. Magnification $\times 10$ and $\times 40$, respectively

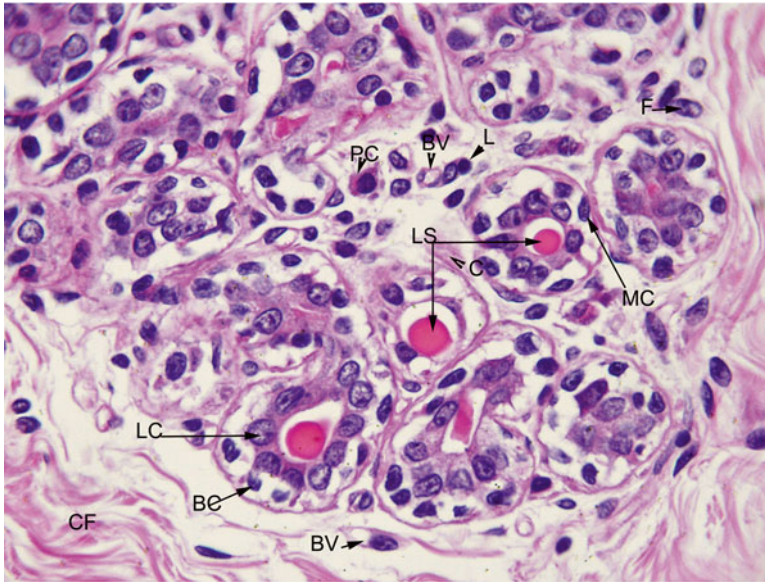


Fig. 2.7 Histological sections of a Lobule Type 2 from a postmenopausal parous woman. The lobule is formed by ductules formed by three cell types: basal cells (BC), luminal cells (LC), and myoepithelial cells (MC). The luminal cells are surrounding a lumen that may contain secretory material (LS). The intralobular stroma is made up of connective tissue formed by collagen fibers (CF) and fibroblasts (F), blood vessels (BV), lymphocytes (L), and plasma cells (PC). There is a well-defined intensity in the amount of collagen fibers (CF) around the lobular structure separating the intra- from interlobular stroma. Stained with hematoxylin and eosin, $\times 40$

includes the early stages of gland morphogenesis, from nipple epithelium to lobule formation. In lobule formation, both processes, development and differentiation, take place almost simultaneously. For example, the progressive transition of Lobule Type 1 to Types 2, 3, and 4 requires active cell proliferation, to acquire the cell mass necessary for the function of milk secretion. This later process implies differentiation of the mammary epithelium. Therefore, the presence of Lobule Type 4 is the maximal expression of development and differentiation in the adult gland, whereas the presence of Lobule Type 3 could indicate that the gland has already been developed. It is important to point out that the presence of proteins that are indicative of milk secretion, such as α -lactalbumin, casein, or milk fat globule type membrane protein, also indicates cellular differentiation of breast epithelium. However, only when all the other components of milk (such as lactose, α -lactalbumin, casein, and milk fat) are coordinately synthesized within the appropriate structure can full differentiation of the mammary gland be acknowledged [2].

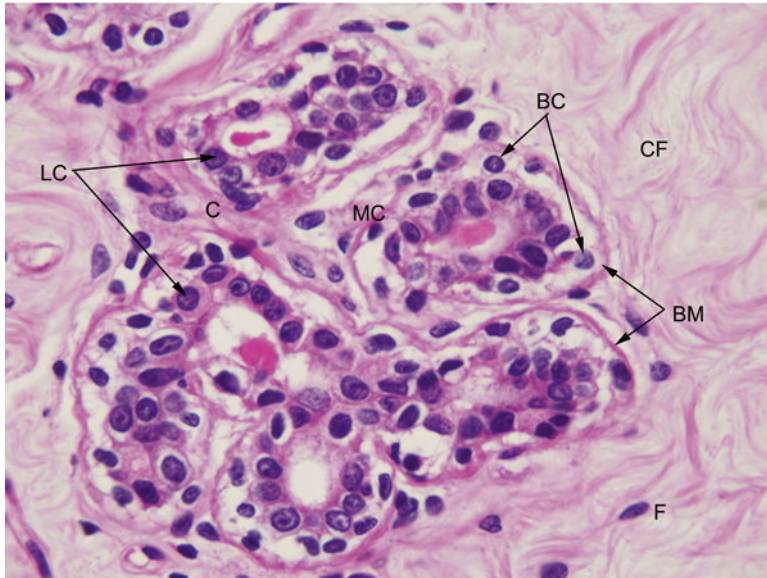


Fig. 2.8 Histological sections of a Lobule Type 1 from a postmenopausal parous woman. The lobule is formed by ductules formed by three cell types: basal cells (BC), luminal cells (LC), and myoepithelial cells (MC). The luminal cells are surrounding a lumen that may contain secretory material and lying in an eosinophilic basal membrane (BM). In the intralobular stroma it is shown connective tissue formed by collagen (C) and fibroblasts (F). The interlobular stroma is clearly demarcated and made up of abundant collagen fibers (CF) and fibroblast (F). Stained with hematoxylin and eosin, $\times 40$

2.5.1 The Cell Types in the Normal Breast

The lobules of the human breast are formed by ductules containing three cell types: basal cells (BC), luminal cells (LC), and myoepithelial cells (MC). The luminal cells surround a lumen that may contain secretory material and lie in an eosinophilic basal membrane (BM). The intralobular stroma is made up of connective tissue formed by collagen and fibroblasts, blood vessels, lymphocytes, and plasma cells. The intensity and amount of collagen fibers around the lobular structure separating the intra- from the interlobular stroma are well defined (Figs. 2.7 and 2.8). In some cases, the basal lamina of the ductular structures is in contact with the fat cells (Fig. 2.9).

Frequently the luminal cells are separated from the basal membrane by an infranuclear vacuolization (Fig. 2.10), whereas the myoepithelial cells remain attached to basal membrane. The nucleus of the myoepithelial cells is more triangular and flatter than the basal cells (Fig. 2.11). The infranuclear vacuolization allows for an appreciation of the difference between the basal cells (BC) and myoepithelial cells (MC) (Fig. 2.12). When these histological sections react with an antibody that recognizes the estrogen receptor alpha (E2R) (Fig. 2.13), it is clear that the receptors are localized in the luminal cells of the breast epithelium whereas the basal and myoepithelial cells are negative for E2R.

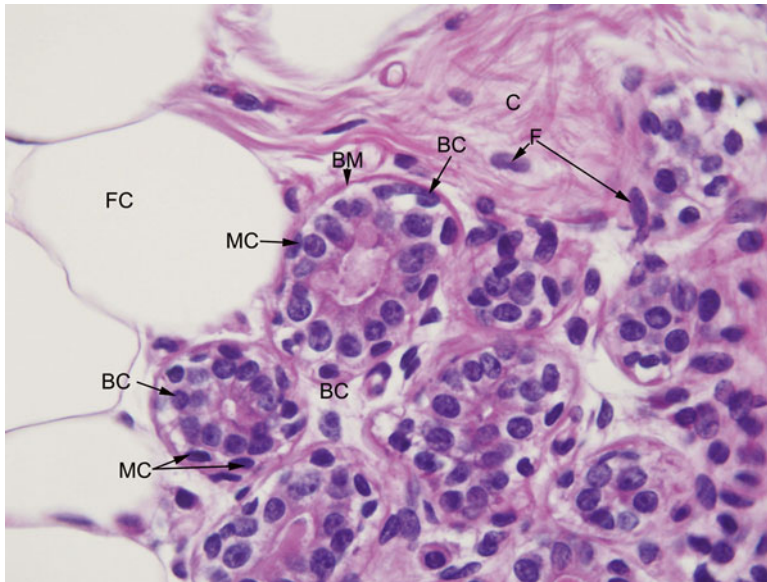


Fig. 2.9 As described in Fig. 2.8 the Lobule Type 1 is formed by ductules formed by three cell types: basal cells (BC), luminal cells (LC), and myoepithelial cells (MC). The luminal cells are surrounding a lumen that may contain secretory material and lying in an eosinophilic basal membrane (BM). In the interlobular stroma it is shown connective tissue formed by collagen (C) and fibroblasts (F), and in some cases the basal lamina of the ductular structures is in contact with the fat cells (FC). Stained with hematoxylin and eosin, $\times 40$

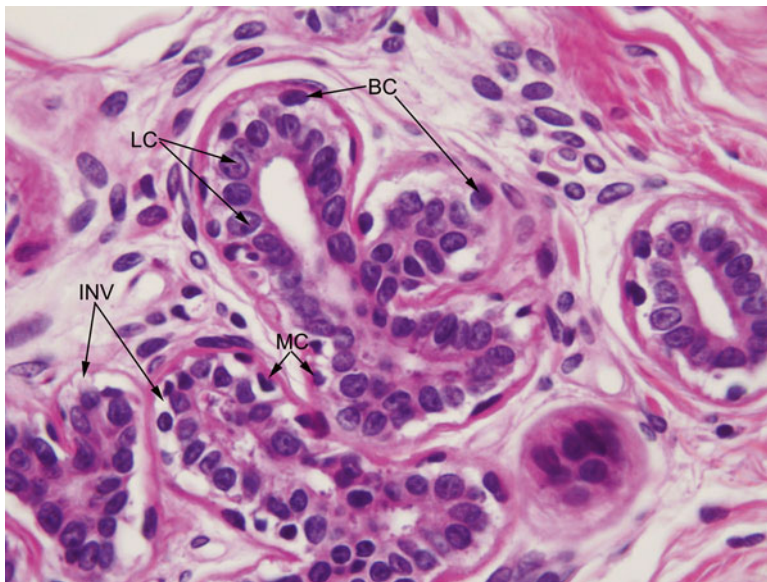


Fig. 2.10 As described in Figs. 2.8 and 2.9 the Lobule Type 1 is formed by ductules formed by three cell types: basal cells (BC), luminal cells (LC), and myoepithelial cells (MC). Frequently the luminal cells are separated from the basal membrane by an infranuclear vacuolization (INV). Stained with hematoxylin and eosin, $\times 40$

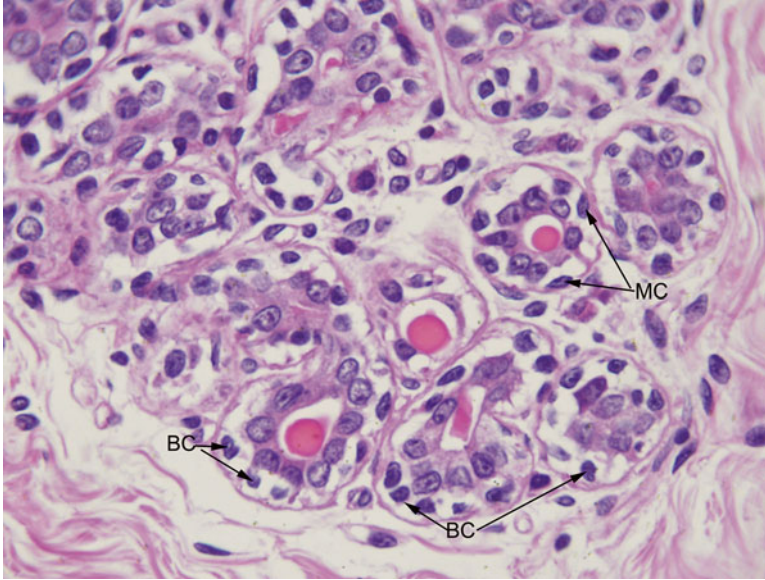


Fig. 2.11 Frequently the luminal cells are separated from the basal membrane by an infranuclear vacuolization whereas the myoepithelial cells remain attached to basal membrane (MC). The nucleus of the myoepithelial cells is more triangular and flatter than the basal cells (BC). Stained with hematoxylin and eosin, $\times 40$

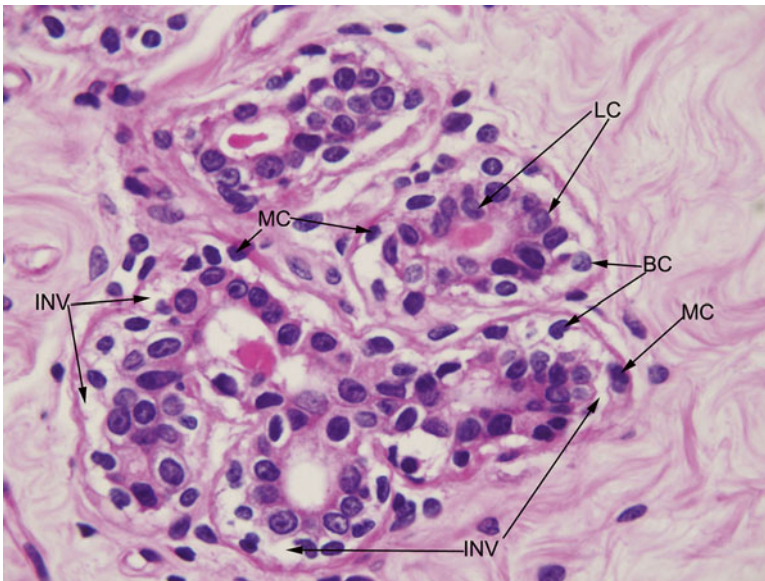


Fig. 2.12 Lobule Type 1 showing luminal cells (LC) separated from the basal membrane by an infranuclear vacuolization (INV); this separation allows appreciating the difference between the basal cells (BC) and myoepithelial cells (MC). Stained with hematoxylin and eosin, $\times 40$

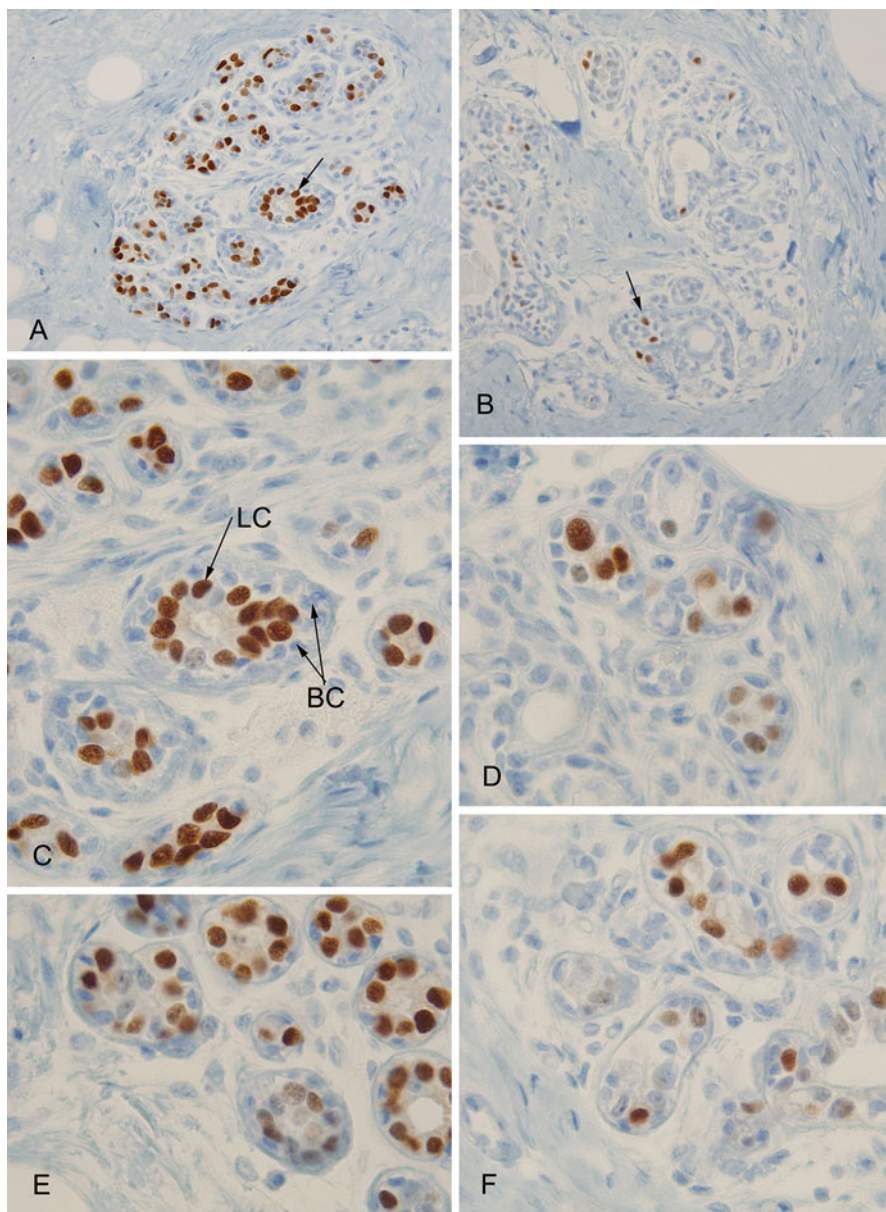


Fig. 2.13 (a–f) Histological sections of a Lobule Type 1 from a postmenopausal parous woman. The histological sections have been reacted with an antibody that recognizes the estrogen receptors (E2R). The receptors are localized in the luminal cells (LC) of the breast epithelium. Basal cells (BC) are negative for E2R. As it is depicted in the figures the number of cells reacting against the antibody vary in intensity (a–c) as well as the number of cells reacting or containing E2R vary in the same breast from lobule to lobule (compare (a) vs. (b) and (c–e) vs. (d–f)). We have used the antibody against estrogen receptor α that is a monoclonal mouse antihuman (clone 1D5) at a dilution 1:50, from DAKO an Agilent Technologies Company. Visualization of the immunocytochemical reactions was performed using the DAB from Biogenex, San Ramon, CA. Cat #: HK124-7KT. The reaction was performed with appropriate negative and positive controls; incubation and staining procedures were performed utilizing the i6000 automated staining system from Biogenex

2.5.2 The Use of Immunocytochemical Markers for Differentiating the Cell Types of the Human Breast

There are several publications which use an immunocytochemical approach to define the different cell types and the role of each as a stem cell in the human breast [22–24]; however, there is a lack of agreement, mainly regarding the use of cytokeratins as a marker of stemness. We will refer to this specific topic in Chap. 6 of this book. In the present chapter, we want to discuss our own results using the same techniques used by other authors in paraffin-embedded tissue.

Using an antibody that recognizes the cytokeratin 5/6 (K5/6) we have identified that there is a difference in reactivity in the breast, mainly in the Lobule Type 1, depending on the parity history of the woman (Figs. 2.14 and 2.15). In the postmenopausal nulliparous woman, the K5/6 reacted positively in the basal cells as shown in Fig. 2.14a, b, d. K5/6 also reacts in the myoepithelial cells (Fig. 2.14c) and is also present in the luminal border of the luminal cells as well as in the lateral border of these cells (Fig. 2.14c). In the postmenopausal parous woman the K5/6 reacts in the luminal border of the luminal cells as well as in the lateral border of these cells (Fig. 2.15a–c). K5/6 is negative in the myoepithelial cells and basal cells (Fig. 2.15d, e). A more specific staining for the myoepithelial cells is the smooth muscle antigen or SMA. The SMA reacts in all the cells that contain actin filaments like in the blood vessels (Fig. 2.16a). It is difficult to separate those that are basal from myoepithelial cells using this technique (Fig. 2.16b–d).

The cytokeratin 5 (K5) has been considered a marker of basal cells as shown in Fig. 2.17a–c. K5 could also react in the myoepithelial cells but does not react with the same intensity as SMA as shown in Fig. 2.16. The luminal cells are completely negative for K5.

A double staining to differentiate the basal and luminal cells can be applied using a cocktail containing antibodies against keratin 5 (K5) and keratin 19 (K19). The K5 reacts in the basal cells as shown in Fig. 2.18a–d. This antibody against K5 seems to be more specific for basal cells and it does not react in the myoepithelial cells as compared to SMA, as shown in Fig. 2.16. The luminal cells are positive for K19 in the luminal border. This double staining procedure was performed using a primary monoclonal antibody against cytokeratin 5 in rabbit combined with a mouse monoclonal antibody against cytokeratin 19. The different colors, brown for the basal cells and purple for the luminal cells, were obtained using a secondary antibody cocktail of anti-mouse polymer-AP and anti-rabbit polymer-HRP.

Another cytokeratin that can be used for detecting luminal cells is keratin 6 (K6). An antibody against K6 reacts in the luminal cells (Fig. 2.19a–c) and is weakly reactive in the basal and myoepithelial cells in sporadic regions of the same breast as it is shown in Fig. 2.19d. Keratin 18 (K18) reacts in the luminal cells (LC) and is not reactive in the basal and myoepithelial cells (Fig. 2.20a, b).

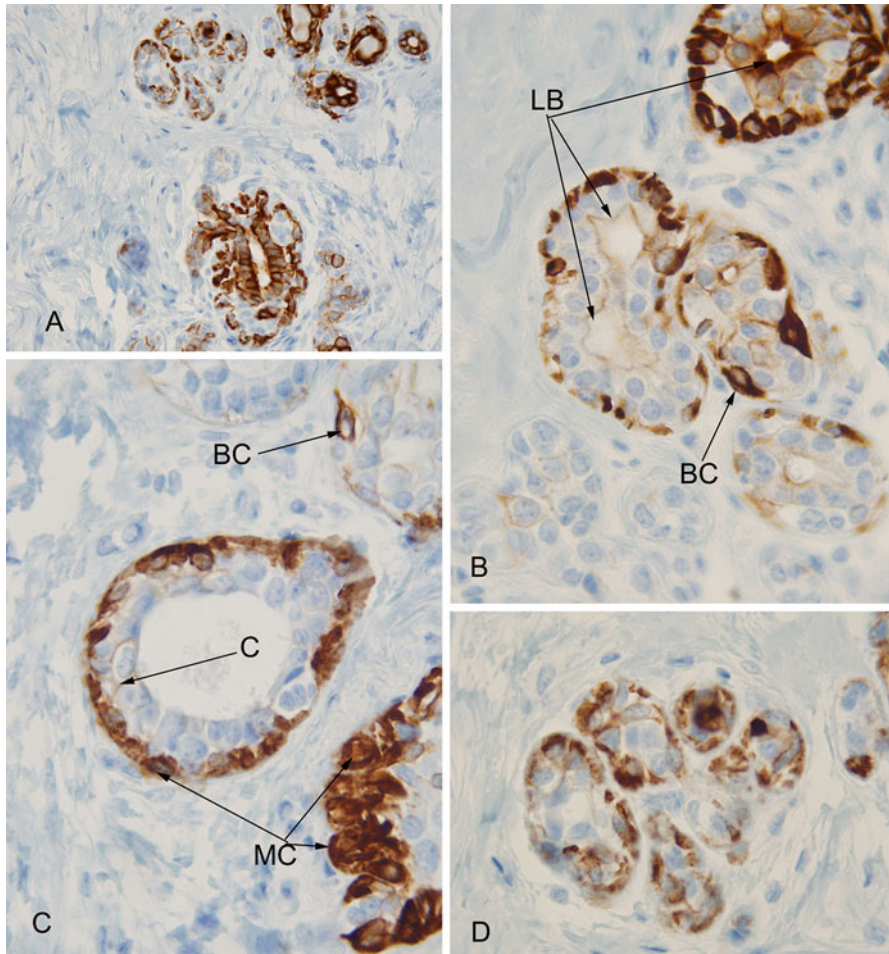


Fig. 2.14 (a–d) Histological sections of a Lobule Type 1 from a postmenopausal nulliparous woman. The histological sections have been reacted with an antibody that recognizes the cytokeratin 5/6 (K5/6). The K5/6 reacted positively in the basal cells as shown in (b). K5/6 also reacts in the myoepithelial cells (MC) as it is shown in (c). K5/6 also is present in the luminal border (LB) of the luminal cells as well as in the lateral border of these cells (C). We have used the monoclonal mouse antihuman cytokeratin 5/6, Clone E6/17 C5, at a dilution of 1:50 (DAKO an Agilent Technologies Company). Visualization of the immunocytochemical reactions was performed using the DAB from Biogenex, San Ramon, CA. Cat #: HK124-7KT. The reaction was performed with appropriate negative and positive controls; incubation and staining procedures were performed utilizing the i6000 automated staining system from Biogenex

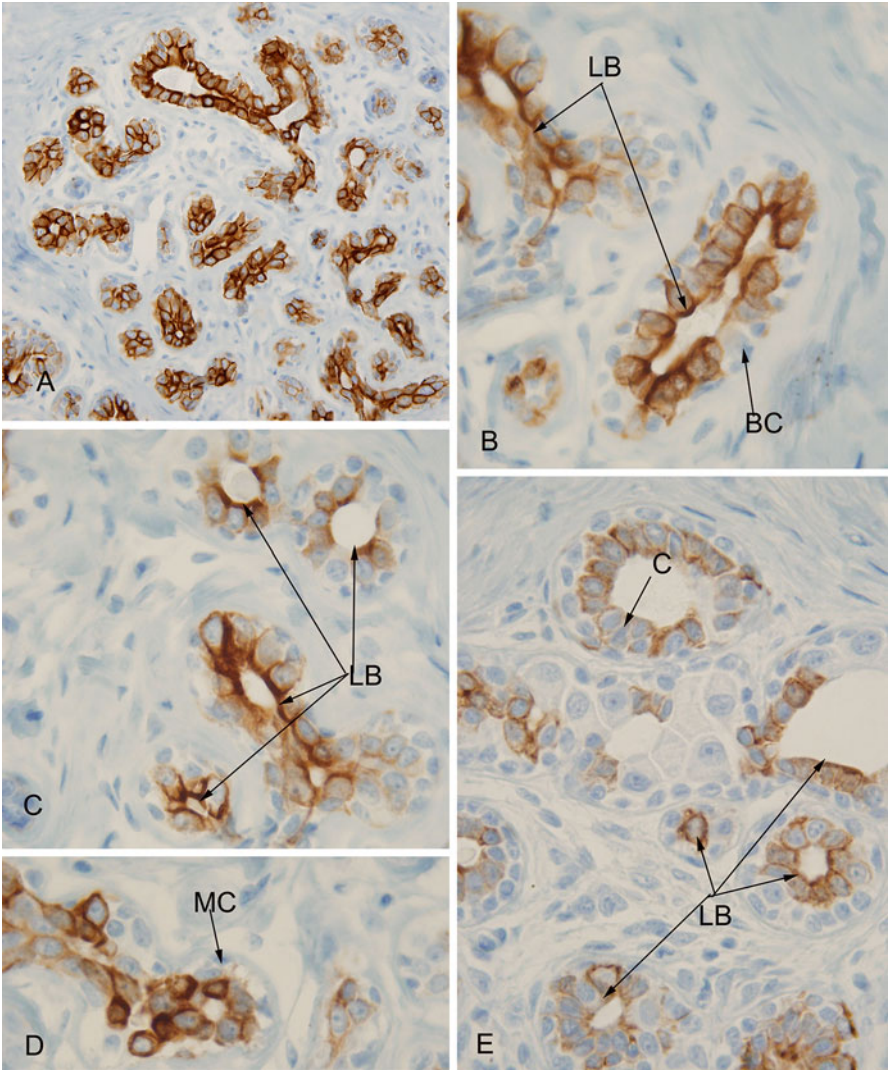


Fig. 2.15 (a–e) Histological sections of a Lobule Type 1 from a postmenopausal parous woman. The histological sections have been reacted with an antibody that recognizes the cytokeratin 5/6 (K5/6). The K5/6 when reacted in the parous breast tissue presents a different location (a–e) than the one shown in Fig. 2.14. K5/6 reacts in the luminal border (LB) of the luminal cells as well as in the lateral border of these cells (C) but is negative in the myoepithelial cells (MC) in (d) and basal cells (BC) are negative. We have used the monoclonal mouse antihuman cytokeratin 5/6, Clone E6/17 C5, at a dilution of 1:50 (DAKO an Agilent Technologies Company). Visualization of the immunocytochemical reactions was performed using the DAB from Biogenex, San Ramon, CA. Cat #: HK124-7KT. The reaction was performed with appropriate negative and positive controls; incubation and staining procedures were performed utilizing the i6000 automated staining system from Biogenex

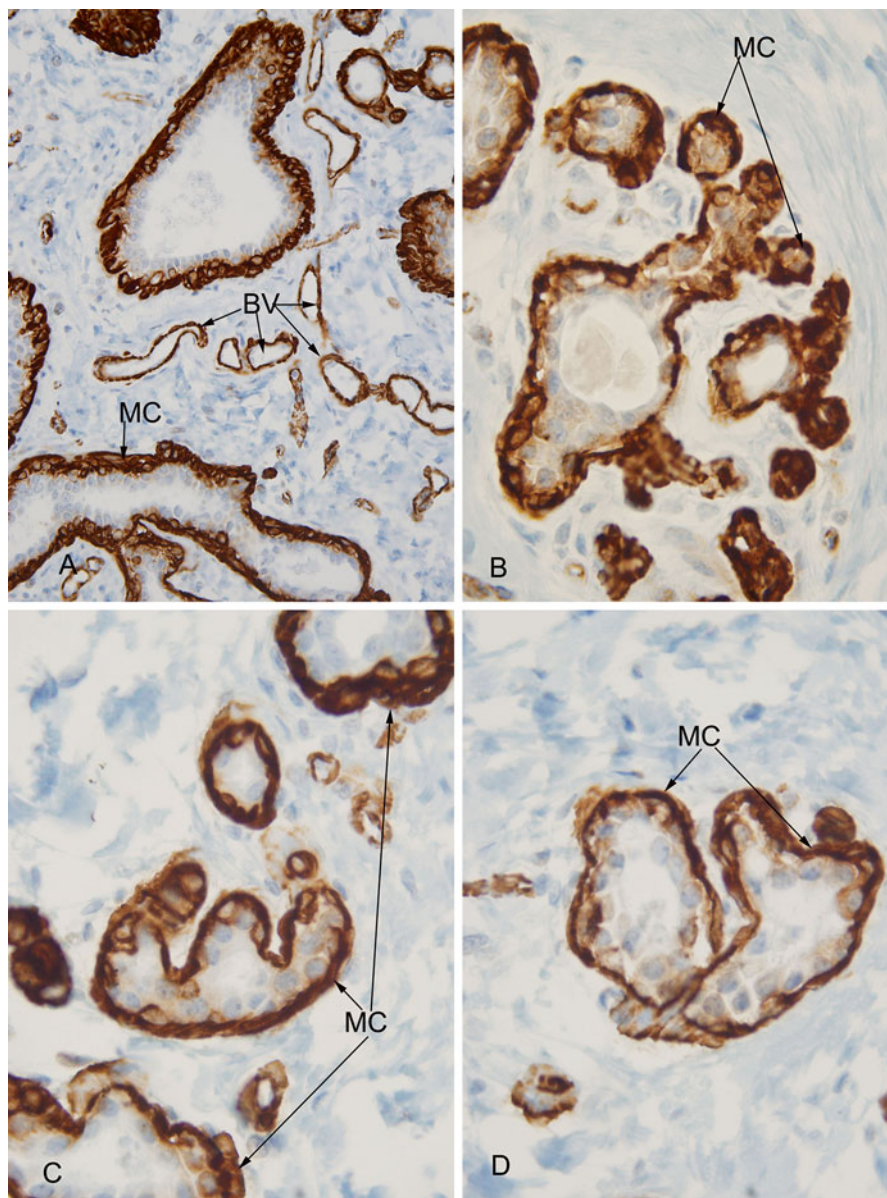


Fig. 2.16 (a–d) Histological sections of a Lobule Type 1 from a postmenopausal parous woman. The histological sections have been reacted with an antibody that recognizes the smooth muscle antigen or SMA. The SMA has been considered a marker of myoepithelial cells (MC). It reacts in all the cells that contain myosin like in the blood vessels (BV) in (a). It is difficult to separate those that are basal cells from myoepithelial cells using this technique. We have used a monoclonal mouse antihuman actin (Smooth Muscle) clone 1A4 at a dilution of 1:50 from DAKO an Agilent Technologies Company. The reaction was developed using DAB from Biogenex, San Ramon, CA. Cat #: HK124-7KT. The sections were counterstained with Mayer's hematoxylin (Biogenex, San Ramon, CA. Cat #: HK100-9K)

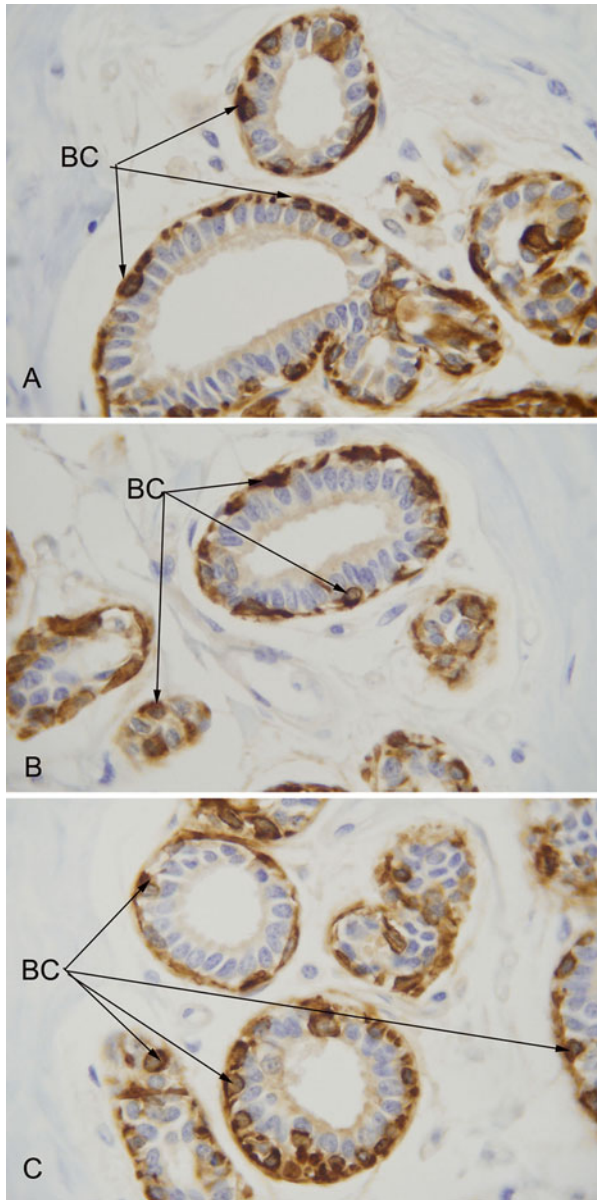


Fig. 2.17 (a–c) Histological sections of a Lobule Type 1 from a postmenopausal nulliparous woman. The histological sections have been reacted with an antibody that recognizes the keratin 5 (K5). The K5 has been considered a marker of basal cells as shown in (a)–(c). K5 also reacts in the myoepithelial cells (MC) but does not react with the same intensity than SMA as shown in Fig. 2.16. The luminal cells are completely negative with this antibody. The primary antibody against cytokeratin 5 [EPR1600Y], rabbit monoclonal antibody, was obtained from Biogenex, Cat #: AN494-5M, that is ready to use as prediluted concentration. The secondary antibody is also from Biogenex, Cat #: HK519-YAK, Polymer HRP. Both antibodies were incubated during 30 min at room temperature. The reaction was developed using DAB from Biogenex (Cat #: HK124-7KT). The sections were counterstained with Mayer's hematoxylin (Biogenex, Cat #: HK100-9K)

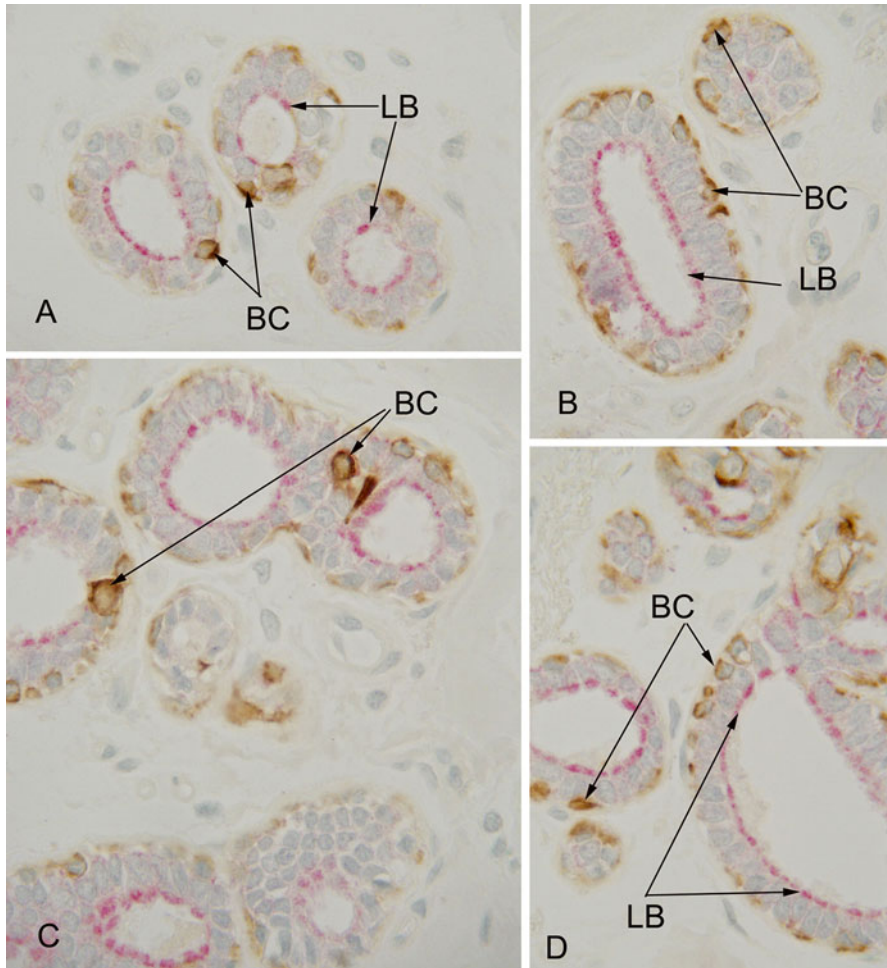


Fig. 2.18 (a–d) Histological sections of a Lobule Type 1 from a postmenopausal parous woman. The histological sections have been double reacted with an antibody that recognizes the keratin 5 (K5) and keratin 19 (K19). The K5 reacts in the basal cells as shown in (a)–(d). This antibody against K5 seems to be more specific for basal cells and is not reacted in the myoepithelial cells as compared with SMA as shown in Fig. 2.16. The luminal cells are positive for K19 in the luminal border (LB). This double staining procedure was performed using a primary antibody against cytokeratin 5 [EPR1600Y], rabbit monoclonal antibody, from Biogenex, Cat #: AN484-5M, that is ready to use as prediluted concentration. The primary antibody against cytokeratin 19 [RCK108], mouse monoclonal antibody, was from Biogenex, Cat #: AN484-10M, that is ready to use as prediluted concentration. The secondary antibody was a cocktail of anti-mouse polymer-AP and anti-rabbit polymer-HRP detection cocktail from Biogenex, Cat #: QS-200-60K. The incubation time for the first antibody was 60 min and for the secondary antibody 30 min, both at room temperature. The reaction was developed using Fast red (Biogenex, Cat #: HK 181-7K) and DAB (Biogenex, Cat #: HK124-7KT). The sections were counterstained with Mayer's hematoxylin (Biogenex, Cat #: HK100-9K)

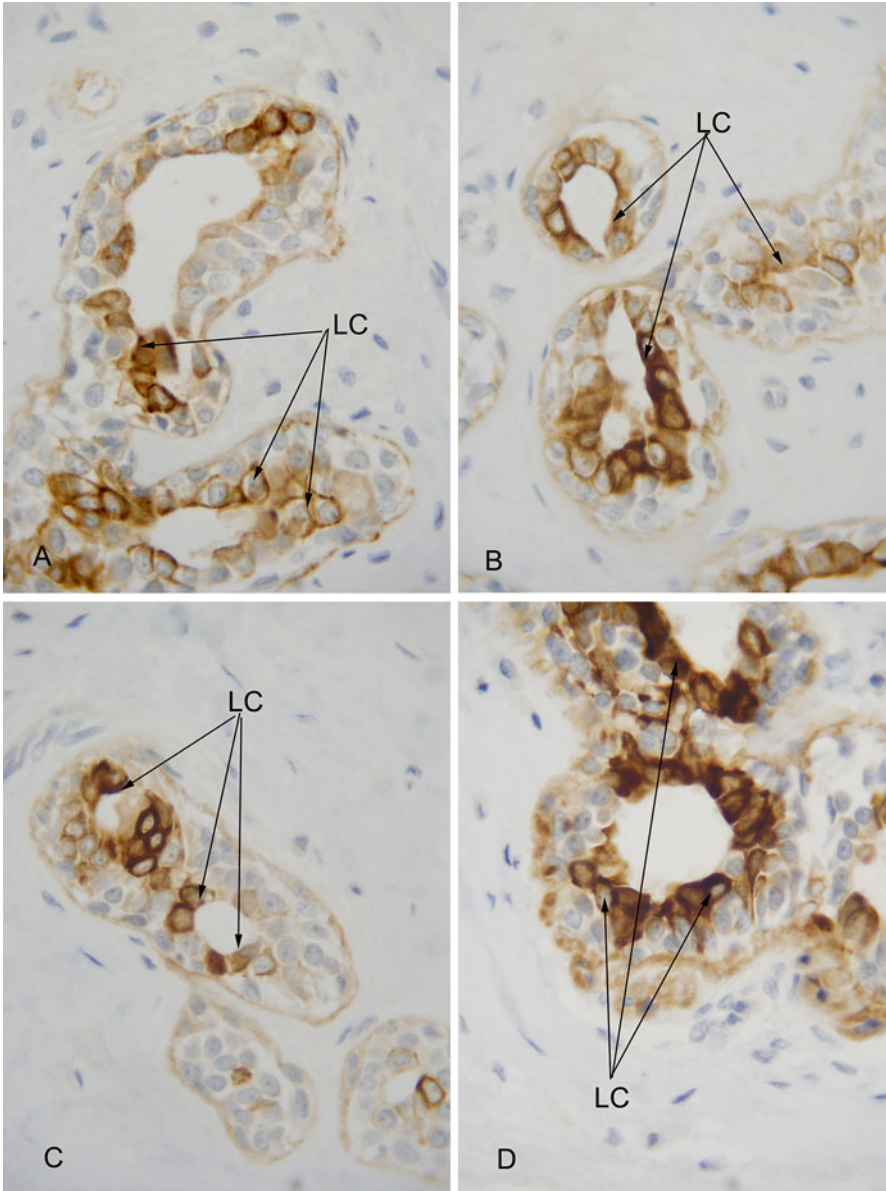


Fig. 2.19 (a–d) Histological sections of a Lobule Type 1 from a postmenopausal parous woman. The histological sections have been reacted with an antibody that recognizes the keratin 6 (K6). This antibody against K6 reacts in the luminal cells (LC) and is weakly reactive in the basal and myoepithelial cells in sporadic regions of the same breast as it is shown in (d). We have used the monoclonal mouse antihuman rabbit monoclonal antibody, Cat #: AN500-10M, from Biogenex, San Ramon, CA. Visualization of the immunocytochemical reactions was performed using the DAB from Biogenex, San Ramon, CA. Cat #: HK124-7KT. The reaction was performed with appropriate negative and positive controls; incubation and staining procedures were performed utilizing the i6000 automated staining system from Biogenex

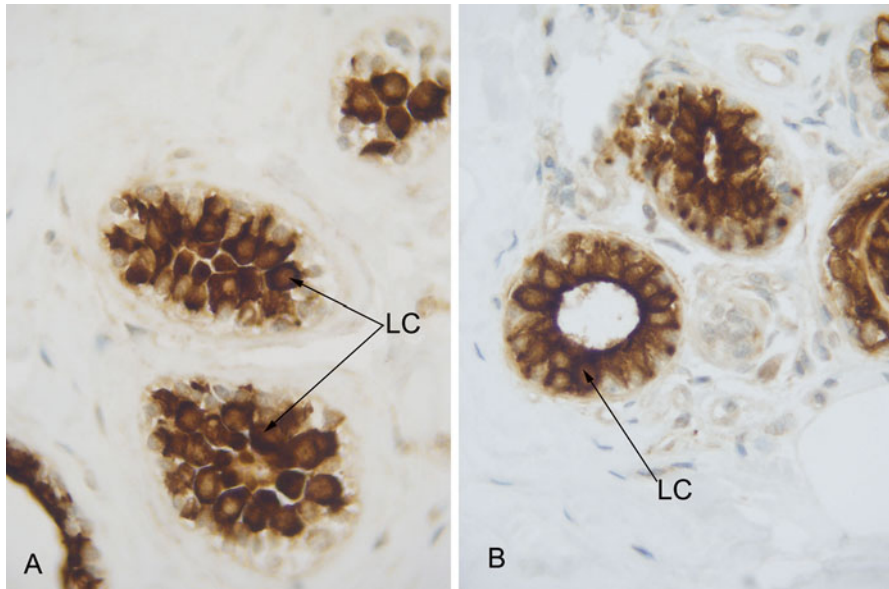


Fig. 2.20 (a, b) Histological sections of a Lobule Type 1 from a postmenopausal nulliparous woman. The histological sections have been reacted with an antibody that recognizes the keratin 18 (K18). This antibody against K18 reacts in the luminal cells (LC) and is not reactive in the basal and myoepithelial cells. We have used the monoclonal mouse antihuman cytokeratin 18, from Biogenex, San Ramon, CA. Visualization of the immunocytochemical reactions was performed using the DAB from Biogenex, San Ramon, CA. Cat #: HK124-7KT. The reaction was performed with appropriate negative and positive controls; incubation and staining procedures were performed utilizing the i6000 automated staining system from Biogenex

Literature Cited

1. Russo J, Russo IH (1998) Development of the human breast. In: Knobil E, Neill JD (eds) Encyclopedia of reproduction. Academic, New York
2. Russo J, Russo IH (1987) Development of the human mammary gland. In: Neville MC, Daniel C (eds) The mammary gland. Plenum, New York, pp 67–93
3. Russo J, Russo IH (2004) Biological and molecular basis of breast cancer. Springer, Heidelberg
4. Russo J, Reina D, Frederick J, Russo IH (1988) Expression of phenotypical changes by human breast epithelial cells treated with carcinogens in vitro. *Cancer Res* 48:2837–2857
5. Russo J, Calaf G, Roi L, Russo IH (1987) Influence of age and gland topography on cell kinetics of normal human breast tissue. *J Natl Cancer Inst* 78:413–418
6. Russo J, Romero AL, Russo IH (1994) Architectural pattern of the normal and cancerous breast under the influence of parity. *Cancer Epidemiol Biomarkers Prev* 3:219–224
7. Peri S, de Cicco RL, Santucci-Pereira J, Slifker M, Ross EA, Russo IH, Russo PA, Arslan AA, Belitskaya-Levy I, Zeleniuch-Jacquotte A, Bordas P, Lenner P, Ahman J, Afanasyeva Y, Johansson R, Sheriff F, Hallmans G, Toniolo P, Russo J (2012) Defining the genomic signature of the parous breast. *BMC Med Genomics* 5:46–57

8. Russo J, Santucci-Pereira J, de Cicco RL, Sheriff F, Russo PA, Peri S, Slifker M, Ross E, Mello ML, Vidal BC, Belitskaya-Levy I, Arslan A, Zeleniuch-Jacquotte A, Bordas P, Lenner P, Ahman J, Afanasyeva Y, Hallmans G, Toniolo P, Russo IH (2011) Pregnancy-induced chromatin remodeling in the breast of postmenopausal women. *Int J Cancer* 131:1059–1070
9. Belitskaya-Levy I, Zeleniuch-Jacquotte A, Russo J, Russo IH, Bordas P, Ahman J, Afanasyeva Y, Johansson R, Lenner P, Li X, de Cicco-Lopez RL, Peri S, Ross E, Russo PA, Santucci-Pereira J, Sheriff FS, Slifker M, Hallmans G, Toniolo P, Arslan AA (2011) Characterization of a genomic signature of pregnancy identified in the breast. *Cancer Prev Res* 4:1457–1464
10. Russo J, Rivera R, Russo IH (1992) Influence of age and parity on the development of the human breast. *Breast Cancer Res Treat* 23:211–218
11. Russo I, Russo J (1994) Role of hCG and inhibin in breast-cancer (review). *Int J Oncol* 4:297–306
12. Russo J, Tay LK, Russo IH (1982) Differentiation of the mammary gland and susceptibility to carcinogenesis. *Breast Cancer Res Treat* 2:5–73
13. Russo J, Hu YF, Silva ID, Russo IH (2001) Cancer risk related to mammary gland structure and development. *Microsc Res Tech* 52:204–223
14. Russo IH, Russo J (1996) Mammary gland neoplasia in long-term rodent studies. *Environ Health Perspect* 104:938–967
15. Xie J, Haslam SZ (1997) Extracellular matrix regulates ovarian hormone-dependent proliferation of mouse mammary epithelial cells. *Endocrinology* 138:2466–2473
16. Petersen OW, Ronnov-Jessen L, Weaver VM, Bissell MJ (1998) Differentiation and cancer in the mammary gland: shedding light on an old dichotomy. *Adv Cancer Res* 75:135–161
17. Sternlicht MD, Kouros-Mehr H, Lu P, Werb Z (2006) Hormonal and local control of mammary branching morphogenesis. *Differentiation* 74:365–381
18. Russo J, Gusterson BA, Rogers AE, Russo IH, Wellings SR, van Zwieten MJ (1990) Comparative study of human and rat mammary tumorigenesis. *Lab Invest* 62:244–278
19. Hu YF, Russo IH, Zalipsky U, Russo J (1996) Lack of involvement of bcl2 and cyclin D1 in the early phases of human breast epithelial cell transformation by environmental chemical carcinogens. *Proc Am Assoc Cancer Res* 37:1005a
20. Russo J, Russo IH (1997) Role of differentiation in the pathogenesis and prevention of breast cancer. *Endocr Relat Cancer* 4:7–21
21. Russo J, Hu Y-F, Yang X, Russo IH (2000) Developmental, cellular, and molecular basis of human breast cancer. *J Natl Cancer Inst Monograph* 27:17–38
22. Clarke CL, Sandle J, Parry SC, Reis-Filho JS, O'Hare MJ, Lakhani SR (2004) Cytokeratin 5/6 in normal human breast: lack of evidence for a stem cell phenotype. *J Pathol* 204:147–152
23. Bocker W, Hungermann D, Decker T (2009) Anatomy of the breast. *Pathologe* 30:6–12
24. Bocker W, Hungermann D, Weigel S, Tio J, Decker T (2009) Immunohistochemistry in breast pathology: differential diagnosis of epithelial breast lesions. *Pathologe* 30:13–19

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